The regulation of feed intake in broiler chickens in sickness and in health

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BSc Biology (2016)

A thesis submitted for the degree of Doctor of Philosophy (PhD)

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January 2022

Abstract

There is an increased interest in the use of alternative feed ingredients in broiler production systems on the grounds of food security and reduced environmental impact. Such ingredients are typically bulky in their nature and consequently lead to feeds whereby the energy and nutrient composition is diluted in comparison to more traditional feeds. Therefore, interest is growing in the ability of broiler chickens to cope with bulky ingredients. Understanding such an ability and the limiting factors of such feeds is vital for the development of accurate simulation models that predict broiler performance and the environmental impact. As broilers are challenged by ubiquitous pathogens during their growth, additional understanding of how birds deal with bulky feeds under such challenge is also relevant to such predictions.

There are concerns that modern broilers have lost their ability to regulate their energy intake and that genetic selection for carcass yield has limited the size of their gastrointestinal tract (GIT), thus limiting their capacity to cope with energy dilution. The first two chapters of this thesis investigated the capacity of a modern broiler strain to deal with increasing levels of various bulky ingredients and aimed to identify a feed bulk dimension responsible for limiting feed intake. These experiments also allowed the investigation of the capacity and rate of adaptation of the gastrointestinal tract on these bulky feed ingredients. The results of these experiments showed: 1) Birds showed a remarkable ability to regulate energy intake when feed energy content was reduced up to a point, which was presumed to reflect the maximum capacity for bulk. 2) Further feed dilution with bulky ingredients limited feed intake and penalised performance. 3) The Water holding capacity of the feeds was able to predict the feed intake of birds not previously adapted to bulky feeds, i.e. in the short term. 4) Birds adapted very rapidly on the bulky feeds and the rate of adaptation depended on the bulkiness of the feed, i.e. the bulkier the feed the longer the adaptation.

Infection with coccidia was used as the infectious model to investigate the interaction between feed bulkiness and infection. In the third experiment, infected birds were given access to feeds which were progressively diluted with a bulky ingredient, lignocellulose. In uninfected birds feed intake was reduced as feed dilution increased and performance decreased, whereas in infected birds feed intake increased as feed bulkiness increased, and performance was unaffected by feed bulkiness. In the final experiment, the protein content of the feed was diluted by substituting an ingredient with a high protein content for one with a low protein content, whilst maintaining the energy contents of the feeds. In both uninfected and infected birds feed intake increased as the protein level of the feed increased, and performance

increased. The results from these experiments show that performance during infection is indeed sensitive to feed composition and it may altogether be absent when broilers are offered feeds diluted with bulky ingredients, such as lignocellulose.

The findings of this thesis facilitate the development of models to predict the feed intake and performance of broiler chickens offered feeds with alternative, bulky ingredients. Unravelling how feed intake is regulated during *Eimeria* infection will help to understand how these birds should be fed during the critical stages of infection.

Declaration

This thesis has been composed by me and has not been submitted as part of any previous application for a degree. All sources of information have been specifically acknowledged by means of referencing.

J. D. Taylor

Acknowledgements

First and foremost, I am greatly thankful to all my supervisors, Professor Ilias Kyriazakis, Dr Panagiotis Sakkas and Dr Lucy Asher for their invaluable advice, continuous support, and patience throughout my PhD. I am extremely grateful to have had three of the most hardworking and attentive supervisors. I will be forever grateful to Ilias and Panos for providing me with the opportunity to work as a research technician during my undergraduate degree and encouraging me to pursue a PhD. I owe an enormous debt to Ilias for his insight into the interpretation of this thesis and my development as a researcher. Equal thanks go to Panos for his dedication and effort throughout my PhD, especially during those long days at the farm, the success of my experiments would not have been possible without you. Anytime I needed them, Ilias and Panos were always on hand to offer me scientific and personal advice, their guidance has matured me both professionally and personally. Lucy, you have been an immense support in the last 18 months, thanks for reviewing my work and providing me with a laptop at such short notice when mine gave up!

I also wish to express my sincere gratitude to Sheralyn Smith who offered unrivalled support, effort and humour in the lab and at the farm. Huge appreciation also goes to Debra Patterson, who never stops to think before going above and beyond to help in any way that she can, even if it was to assist in the sampling days! Both Sheralyn and Debra were always on hand for a coffee and a chat to lift my spirits, your kindness and sarcasm were always gratefully received. I am thankful to Professor Damer Blake for the preparation of the infection doses in Chapters 4 and 5, and for his consult and training in laboratory methods. I would like to thank Dr Joao Felipe for his expert statistical advice. I am thankful to Dr Ilias Giannenas, Dr Stephanie Matheson, Dr Idiegberanoise Oikeh, Dr Craig Tallentire, Dr Anne Hunting, Dr Adam Clark, and the personnel at Cockle Park Farm for their assistance in conducting the experimental trials.

I would like to thank my family and friends for their continuous support and encouragement. Special mention goes to: Jack, Mac, Josh and Caroline who have been hugely supportive throughout the last four years. I would not be completing this thesis without the belief and confidence you instilled in me.

Finally, and most importantly, I would like to thank each of the 2,367 chickens from my experiments for their sacrifice. Without you, this thesis would not be possible, my only regret is that the cost of this thesis was paid with your freedom.

Publications and conference presentations

The following publications have been produced from the research in this thesis and appear in peer reviewed journals:

- **Taylor, J**., Sakkas, P. and Kyriazakis, I., 2021. What are the limits to feed intake of broilers on bulky feeds? *Poultry Science*, 100:100825. (Chapter 2)
- **Taylor, J**., and Kyriazakis, I., 2021. Towards the prediction of feed intake capacity of modern broilers on bulky foods. *Poultry Science*, 100:101501. (Chapter 3)
- **Taylor, J**., Sakkas, P. and Kyriazakis, I., 2022. Starving for nutrients: Anorexia during infection with parasites in broilers is affected by diet composition. *Poultry Science*, 101:101535. (Chapter 4 and 5)

The following work has been presented at conferences:

- **J. Taylor**, P. Sakkas, D. Blake, I. Kyriazakis. Is pathogen induced anorexia in broilers infected with *Eimeria maxima* influenced by dietary crude protein content? *In:* 15th European Poultry Conference, September 2018, Dubrovnik, Croatia.
- **J. Taylor**, P. Sakkas, D. Blake, I. Kyriazakis. Is pathogen induced anorexia in broilers infected with *Eimeria maxima* influenced by crude protein content of the feed? *In:* World Poultry Science Association UK Annual Spring Meeting, April 2019, Edinburgh, Scotland.
- **J. Taylor**, P. Sakkas, I. Kyriazakis. Is selection for improved performance limiting the gastrointestinal capacity of modern broilers? *In:* 26th World Poultry Congress, August 2021, Paris, France.

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List of Abbreviations

AA	Amino acids
ADCPI	Average daily crude protein intake
ADEI	Average daily energy intake
ADF	Acid detergent fibre
ADFI	Average daily feed intake
ADG	Average daily gain
ADL	Acid detergent lignin
AME	Apparent metabolizable energy
BW	Body weight
BWp	Mean body weight of each stage of infection
CD	Crypt depth
CF	Crude fibre
cFCR	Corrected feed conversion ratio
CP	Crude protein
d	Day
DM	Dry matter
EAA	Essential amino acids
EBW	Empty body weight
ECW	Empty carcass weight
FCR	Feed conversion ratio
GIT	Gastrointestinal tract
GLM	General linear model
GM	Grass meal
NDF	Neutral detergent fibre
OH	Oat hulls
OPG	Oocyst per gram
PCA	Principal component analysis
pi	post-infection
SBP	Sugar beet pulp
TiO_2	Titanium dioxide
VCR	Villi length to crypt depth ratio
VL	Villi length
WB	Wheat bran
WHC	Water-holding capacity

Chapter 1. General introduction

1.1 Research Context

As the global human population grows, the importance of producing sustainable, economical meat sources is also increasing (FAO, 2018; Tallentire *et al.*, 2018a). In order to become more sustainable and reduce the environmental impact and economic costs of meat production, there is growing interest in the inclusion of alternative feed ingredients, including local feed resources and ingredients resulting from a circular bioeconomy, in animal feeds (Tallentire *et al.*, 2018b; El-Deek *et al.*, 2020). Since soybean meal is the most common protein source in animal feeds and is also one of the most expensive and environmentally costly ingredients for poultry feeds, most research has been conducted on finding alternative protein sources (Van Krimpen *et al.*, 2013; Khan, 2018). However, there is also interest in the use of co- and by-products of food production which are not desirable to humans. Such ingredients include rapeseed meal (Amerah *et al.*, 2015; Recoules *et al.*, 2019) wheat middlings, rice bran, palm kernel meal (Ravindran, 2013) and oat hulls (Scholey *et al.*, 2020). These alternative ingredients are typically lower in energy (Robert *et al.*, 1997; Sundu *et al.*, 2006) and higher in their fibre (or bulk) content due to their physico-chemical properties, such as water-holding capacity (Jiménez-Moreno *et al.*, 2009a). Therefore, the inclusion of such ingredients results in feeds which are lower in energy and have a higher bulk content than more traditional feeds.

The prevailing view is that animals will eat for the first limiting nutrient resource in their feed (Emmans, 1997). However, there are concerns about the ability of modern broiler genotypes to cope with feeds of a lower energy content (Classen, 2017; Scholey *et al.*, 2020), which arise from experiments where broilers are unable to cope with feeds diluted with bulky, fibrous ingredients, i.e. the birds are unable to increase feed intake to accommodate for the reduction in energy content. In such experiments, broilers are unable to adjust their feed intake to cope with feeds of different nutrient compositions (Sahraei and Shariatmadari, 2007; Latshaw, 2008; Delezie *et al.*, 2010; Cho, 2011; Krás *et al.*, 2013). Krás *et al.* (2013), Sahraei and Shariatmadari (2007) and Latshaw (2008) showed that broilers were unable to adjust feed intake when given low energy feeds that were diluted with wheat bran and oat hulls, wheat bran and sand or oats, wheat middlings and alfalfa meal, respectively. The experiments by Delezie *et al.* (2010) and Cho (2011) offer further support to the notion that modern broilers are unable to adjust feed intake when feed energy content is reduced. On the other hand, there is some evidence to suggest that this may not be the case and in fact, modern broilers do indeed retain the ability to increase their feed intake when offered bulky, low energy feeds (Deaton and Lott,

1985; Panigrahi and Powell, 1991; Leeson *et al.*, 1996a; Dozier III *et al.*, 2006a; Amerah *et al.*, 2009; Linares and Huang, 2010). However, the extent of this ability to modify feed intake in response to dietary energy is not well understood. For example, Linares and Huang (2010) observed an increase in feed intake when the energy content of the feed was reduced by diluting the feed with 5% rice bran, however, feed intake decreased when energy content was reduced by 10%. These results are inconsistent with those of Leeson *et al.* (1996a), where broilers were able to increase their feed intake when the energy content was reduced by 20% when feeds were diluted with a mixture of oat hulls and sand. The inconsistencies in the results of experiments investigating broilers ability to cope with reductions in dietary energy content suggest that the feed intake response to dietary energy can be constrained by another factor. The primary difference in these experiments is in the ingredients used to reduce the energy content of the feed. It is therefore likely that the different physical or chemical properties of the bulky ingredients are responsible for the different responses observed across experiments. As the use of alternative, bulky ingredients grows it is important to identify the property responsible for constraining feed intake in various experiments.

Throughout their life cycle, broilers are continuously challenged by pathogens, even in cases where there is a high biosecurity (Millman *et al.*, 2017; Ball *et al.*, 2018). Such diseases impair performance and productivity of the broilers and as a result there are significant economic losses to the industry (Bennett, 2003; Blake *et al.*, 2020). This reduction in performance can largely be attributed to a voluntary reduction in feed intake of infected broilers (Kyriazakis, 2010). This voluntary reduction in feed intake during infection is called pathogen-induced anorexia (Kyriazakis *et al.*, 1998). This phenomenon has been observed across a wide range of animal species including insects (Povey *et al.*, 2014), birds (Kipper *et al.*, 2013) and mammals (Kyriazakis and Doeschl-Wilson, 2009). The evolutionary advantages of pathogen-induced anorexia are well demonstrated, most notably in the experiment by Murray and Murray (1979). These authors observed a reduction in mortality when mice infected with *Listeria monocytogenes* were allowed to develop the anorexic response, whereas infected mice who were force-fed an equal amount of feed as the uninfected control mice succumbed to infection more rapidly. Similarly, mortality was reduced during a *Salmonella* infection in *Drosophila* allowed to develop anorexia compared to those denied anorexia (Ayres and Schneider, 2009).

Despite the range of animals shown to exhibit pathogen-induced anorexia, there remains a dearth of information on the effect of feed composition on pathogen-induced anorexia in broiler chickens. This could be considered surprising since anorexia has long been associated with enteric diseases afflicting poultry, such as necrotic enteritis and coccidiosis (Reid and Pitois, 1965; Helfer *et al.*, 1969). The characteristics of anorexia (onset, duration and extent) are

dependent on factors such as host resistance and the pathogen type. However, whether anorexia is affected by feed composition is yet to be determined (Kyriazakis, 2010). Previous evidence on the effect of feed composition on anorexia is often conflicting with some studies showing a significant effect of feed composition (Webel *et al.*, 1998a; Coltherd *et al.*, 2009) and others showing no effect (Van Heugten *et al.*, 1994; Webel *et al.*, 1998b). Kyriazakis (2010) suggested that this confusion arises from a lack of a systematic approach in such experiments. One such approach will be presented in this first chapter. Unravelling the effect of feed composition on the characteristics of pathogen-induced anorexia may lead to a greater understanding of how to feed infected hosts during the different stages of infection.

In this first chapter, the factors which affect the feed intake of both healthy and parasitized broilers will be discussed, and how they have been addressed throughout this thesis have been outlined. The following subsections provide an overview of the effect of feed characteristics (such as feed form and bulk), housing environment and bulky feeds on the regulation of feed intake. Thereafter, the problem of predicting voluntary feed intake of broilers offered bulky feeds will be outlined and the gastrointestinal tract (GIT) adaptation of broilers to such feeds is summarised. The final subsections describe the paradox of pathogen-induced anorexia and the development of a systematic approach to investigate anorexia.

1.2 Factors affecting voluntary feed intake

The factors that have the potential to affect voluntary feed intake arise by definition from the animal, its feed composition and the environment it is kept in (Emmans, 1981). The sex of a broiler chicken is well demonstrated to affect feed intake, with male broilers consuming more than females at the same weight (Abdullah *et al.*, 2009; Benyi *et al.*, 2015). The intensive genetic selection of chickens depend upon the desired breeding goals which aim to produce highly specialised strains of birds to maximise certain traits such as improved growth rate and feed conversion ratio (Buzala and Janicki, 2016). As such there are inherent differences in the feed intake in chickens bred for different purposes, for example, the feed intake of broiler chickens has been shown to be 2- to 3-fold greater than the feed intake of laying hens (Nir *et al.*, 1993; Saneyasu *et al.*, 2011). Indeed, such differences in feed intake can be extended beyond productive purpose (e.g. laying hens vs broilers) to the different broiler genotypes (e.g. fast vs slow growing broilers). The feed intake of fast growing broiler is significantly greater compared to slow growing broilers in order to facilitate their faster growth rate (Quentin *et al.*, 2003; Fanatico *et al.*, 2008; Torrey *et al.*, 2021; Tůmová *et al.*, 2021).

The physical form in which feed is offered is a factor which has been shown to significantly affect feed intake and growth performance of broiler chickens. Whether pelleting improves the digestibility of different nutrients in the feed remains subject to debate (Svihus and Hetland,

2001; Svihus and Zimonja, 2011; Abdollahi *et al.*, 2013). There is evidence showing that starch digestibility is reduced in pelleted feeds compared to mash (Svihus and Hetland, 2001; Barua *et al.*, 2021) and on the other hand it has been suggested that protein digestibility is improved in pelleted feeds compared to mash (Thomas *et al.*, 1998; Massuquetto *et al.*, 2018). Nonetheless, it is clear within the literature that when broilers are fed pellets performance is improved compared to broilers given mash (Svihus and Hetland, 2001; Amerah *et al.*, 2007b; Naderinejad *et al.*, 2016). It has been suggested that the improvements in performance of pellet-fed broilers is due to increases in voluntary feed intake (Nir *et al.*, 1995; Lemme *et al.*, 2006; Serrano *et al.*, 2012) and reductions in energy expenditure during feeding (Jensen, 2000; Amerah *et al.*, 2007b). In addition, pelleting of the feed causes the particle size to decrease (Mateos *et al.*, 2012) which allows an increase in feed intake due to a reduction in the transit time of the feed in the digestive tract (Amerah *et al.*, 2007a). Based on the above, it is clear that feed form should be carefully considered when designing experiments to investigate the regulation of feed intake.

The environment that an animal is living in also affects their voluntary feed intake. For example, ambient temperature (Abu-Dieyeh, 2006; Liu *et al.*, 2020) and stocking density (Feddes *et al.*, 2002; Thomas *et al.*, 2004) have been shown to significantly affect feed intake and growth performance in broiler chickens. In the experiment by Abu-Dieyeh (2006), broilers exposed to an ambient temperature of 35˚C consumed significantly lower amounts of feed compared to birds maintained at 20˚C over a four-week period. Furthermore, the systematic review and meta-analysis by Liu *et al.* (2020) collated information from 12 experiments investigating the effect of heat stress and found that high temperature caused a significant reduction in feed intake in 11/12 studies. These results are consistent with Emmans and Fisher (1986) who suggested that the inability of an animal to lose sufficient heat to its environment would impede performance. At higher stocking densities, feed intake and growth performance are significantly reduced compared to lower stocking densities due to increased competition for access to feeders (Feddes *et al.*, 2002; Thomas *et al.*, 2004; Dozier III *et al.*, 2006b). The presence of disease could also be considered as an environmental factor which greatly affects feed intake and performance. A voluntary reduction in feed intake during infection is named pathogen-induced anorexia, the extent of such anorexia is by definition dependent upon pathogen type and load (Kyriazakis and Doeschl-Wilson, 2009). The post-infection period can be separated into three stages: pre-patent, acute and recovery (Figure 1.1). The pre-patent stage reflects the lag time between inoculation and the recognition of the pathogen by the host's immune system (Plata-Salaman, 2001). Anorexia is observed in infected hosts during the acute stage of infection, the duration of which can vary between a few days to several weeks

depending on the pathogen type and pathogen load (Kyriazakis, 2010). The recovery stage reflects the rate of reduction of pathogen load within the host and it is at this time where feed intake returns to the level of the uninfected controls (Kyriazakis, 2010). In broiler chickens, there are many diseases that to lead to anorexia, such as necrotic enteritis (McDevitt *et al.*, 2006) and coccidiosis (Kipper *et al.*, 2013). Currently, there is a knowledge gap in the effects of feed composition on the characteristics of pathogen-induced anorexia (Kyriazakis, 2010). Chapter 4 will assess whether the reduction in feed intake of parasitized broilers is affected by feed composition, and Chapter 5 will investigate whether protein content affects the reduction in feed intake of parasitized broilers.

The nutritional composition of the feed is also well documented to affect voluntary feed intake (Forbes, 2005; Ferket and Gernat, 2006; Mbajiorgu *et al.*, 2011). The theory of feed intake regulation states that animals will eat to meet their requirements for the most limiting nutrient (Emmans and Fisher, 1986). The response of broilers given feeds where crude protein is the most limiting factor is controlled by the amino acid (AA) balance rather than crude protein content per se (Summers *et al.*, 1992; Sklan and Plavnik, 2002; Sterling *et al.*, 2006). Swatson *et al.* (2002) showed that feed intake significantly increased with CP content when lysine was maintained to CP ratios and other AA were maintained in relation to lysine. Similarly, Sterling *et al.* (2006) showed that feed intake was significantly increased when lysine content was increased in both Ross and Cobb broilers. However, more recent evidence shows that feed intake decreases, but growth performance increases when digestible lysine is increased in feeds when other essential AA (EAA) were maintained to a constant ratio to lysine (Belloir *et al.*, 2019). Hilliar *et al.* (2020) showed that reducing dietary CP content caused a reduction in feed intake, however there was an increase in feed intake when the feeds were supplemented with glycine. The level of glycine supplementation used in the experiment was chosen to reflect the amount of glycine in the basal feed, the fact that an increase in feed intake was observed when the feeds were supplemented shows the importance of AA balance in the regulation of feed intake. Chrystal *et al.* (2020) maintained ratios of EAA to lysine and observed a linear increase in feed intake as protein content reduced. It is evident that there is great importance of AA balance in experiments offering low protein feeds and this should be taken into consideration during feed formulation.

However, when energy content is the most limiting factor, there is some disagreement in the literature. Plumstead *et al.* (2007) did not observe any compensatory increases in feed intake when energy content was reduced through dilution with vermiculite. Similarly, Li *et al.* (2010) observed no compensatory increases when nutrient density was reduced by exchanging rapeseed oil and fishmeal for corn. On the other hand, as previously mentioned, there is

evidence showing that broilers are able to increase feed intake as the energy content of the feed decreases (Leeson *et al.*, 1996a; Ferket and Gernat, 2006; Linares and Huang, 2010). The extent to which the broilers are able to cope with reductions in energy content varies across studies. One factor which may explain the varied results in the literature is gut capacity. It is known that gut capacity is an important factor in preventing an animal from meeting its desired feed intake because once maximum gut capacity is reached the animal is biologically limited from meeting its desired nutrient resource intake (Emmans, 1987). Therefore, the different responses observed in the above experiments could be explained by the maximum gut capacity being reached at different rates for one of two reasons: 1) the experiments were conducted 14 years apart and it has been suggested that the gut capacity of modern broilers has become reduced due to the effects of genetic selection for increased carcass yield (Rougière *et al.*, 2009; De Verdal *et al.*, 2010), 2) each of the experiments used ingredients which vary in their physicochemical properties, and so a physicochemical property of the diluents could be responsible for determining the maximum gut capacity at different rates. In Chapter 2 and Chapter 3, the gut capacity of a modern broiler strain and their ability to cope with and adapt to feeds of reduced energy content (through dilution with bulky ingredients) will be investigated.

1.3 Characteristics and definition of bulky feeds

A bulky ingredient offers little to no nutritive value to the feed and contains a high dietary fibre content. The term 'dietary fibre' can be defined with either a physiological or chemical definition. The physiological definition states that dietary fibre is the dietary components resistant to degradation by mammalian enzymes, whereas chemically it is classified as the sum of non-starch polysaccharides (NSPs) and lignin (Knudsen, 2001). NSPs can be classified into two distinct groups, soluble and insoluble (Farran *et al.*, 2016). Ingredients such as wheat bran, citrus pulp and sugar beet pulp contain large amounts of soluble NSPs (Choct *et al.*, 2010), whereas ingredients such as oat hulls, cellulose and sawdust primarily contain insoluble NSPs (Hetland *et al.*, 2004). NSPs are largely indigestible in monogastric animals and as such reduce the nutritive value of the feed by their inclusion (Choct, 2015). Bulky feeds are characterised by their physical bulking properties, such as water-holding capacity and feed density, and also their chemical properties such as: acid-detergent fibre, neutral-detergent fibre, crude fibre, acid-detergent lignin, and indigestible matter (Blackwood *et al.*, 2000). Brachet *et al.* (2015) demonstrated the vast differences in the physicochemical properties of a range of bulky ingredients. The authors showed that there was a weak correlation between physical properties (such as water-holding capacity) and chemical properties (such as fibre content), which is in agreement with results of Kyriazakis and Emmans (1995), where the water-holding capacity of

citrus pulp was shown to be greater than that of wheat bran, whereas the reverse was the case for their neutral-detergent fibre content.

1.4 The regulation of feed and energy intake when given feeds diluted with bulky ingredients

The expected relationship between feed intake and the bulk content of the feed is an initial increase in feed intake as bulk content increases, up until a point where feed intake begins to decrease due to the constraints of bulk. This critical point is thought to represent the maximum gut capacity for bulk, and thus the maximum feed intake (Nascimento *et al.*, 2020) (Emmans, 1987). If one considers the above relationship in terms of energy intake, rather than feed intake, the relationship between energy intake and bulk content would remain constant until the point at which feed intake is constrained and energy intake begins to decrease. This relationship is logical since an increase in the bulk content of a feed simultaneously reduces the feed energy content, which when considered in relation to the constraining effect of bulkiness on feed intake, ultimately leads to a reduction in energy intake.

As previously mentioned, there are contradictions in the effect of bulky feeds on feed and energy intake in broiler chickens. For example, Thompson (1980) and Shakouri *et al.* (2006) observed a reduction in feed intake when broilers were offered feeds diluted with rice bran and carboxymethyl-cellulose, respectively. However, in the Shakouri *et al.* (2006) experiment, there were improvements in feed intake and weight gain when the broilers were offered feeds diluted with the pectin or cellulose. Similarly, improvements in feed intake and weight gain were observed when birds were offered feeds diluted with oat hulls (30%) or unmolassed sugar beet pulp (30%) (Savory *et al.*, 1996) or coconut meal (40%) (Farrell, 2005). However, at higher levels of dilution with sugar beet pulp (60%) or coconut meal (60%) the consequence of diet dilution was that feed intake was constrained and consequently weight gain was reduced. The fact that performance was only impeded at such high levels of diet dilution in these two experiments is peculiar considering a dilution with 10% rice bran was sufficient in constraining feed intake in the experiment by Linares and Huang (2010). It is therefore reasonable to suggest that the regulation of feed intake is not simply controlled by the energy content of a given feed and in fact there is a factor, presumably a characteristic of the feed, which is reaching the maximum gut capacity and constraining feed intake.

Past evidence has shown the ability of broilers to maintain energy intake when given diluted, bulky feeds because the birds were able to adapt the size of their GIT relative to body weight (Jørgensen *et al.*, 1996a; González-Alvarado *et al.*, 2010). In these experiments the feeds were diluted with pea fibre, wheat bran or oat bran (Jørgensen *et al.*, 1996), oat hulls or sugar beet pulp (González-Alvarado *et al.*, 2010). In response to the inclusion of bulky ingredients,

Jørgensen *et al.* (1996a) observed an increase in the weights of the small intestine, caeca and colon. Accompanying these digestive adaptations were increases in feed intake and body weight gain when the feeds were diluted with up to 37.5% of each fibre source. In the experiment by González-Alvarado *et al.* (2010) feed intake and body weight gain were not significantly different between the birds given the basal feed and those given the diluted feeds. The weight of both the gizzard and full GIT of birds given the diluted feeds were greater than the basal fed birds, which suggests that the digestive tract adaptations to the feeds allowed performance to be unaffected by the lower energy content of the feeds. However, more recent evidence suggests that modern broiler genotypes have lost this ability to adapt their GIT to cope with bulky, low energy feeds and maintain energy intake, and consequently performance (Krás *et al.*, 2013; Pauwels *et al.*, 2015). Krás *et al.* (2013) offered feeds diluted with 14% wheat bran and 4% oat hulls and although feed intake was not affected by dietary fibre content, weight gain was significantly reduced in the high fibre treatment, which suggests that the birds reached their maximum gut capacity and were unable to accommodate any further increases in feed intake to maintain performance. In another study, Pauwels *et al.* (2015) compared a commercial feed with a scavenger fed composed primarily of Austru 2 Growth (Versele-Laga, Deinze, Belgium), which contains a large amount of fibrous ingredients such as rice bran, corn bran and rapeseed. Similar to the experiment by Krás *et al.* (2013), the birds offered the scavenger feed were able to increase their feed intake compared to those on the commercial feed, however their weight gain was depressed. If the capacity of the GIT in broilers is indeed nearing its biological limits, then the capacity for maximum daily feed intake is also nearing its maximum (Tallentire *et al.*, 2018a). Given the contradictory evidence on broilers ability to cope with low energy, bulky feeds it seems logical that there is a characteristic of bulky ingredients which quickly achieves the maximum gut capacity and is responsible for the constraining effect on feed intake. Identifying such a characteristic may account for the different responses observed in experiments that offered feeds diluted with bulky ingredients.

There is evidence to suggest that genetic selection in broilers has indirectly reduced the capacity of the GIT in order to improve carcass yield (Mitchell and Smith, 1991; McEntee *et al.*, 2003; Rougière *et al.*, 2009; De Verdal *et al.*, 2010; Lumpkins *et al.*, 2010; De Verdal *et al.*, 2011; Alshamy *et al.*, 2018). Mitchell and Smith (1991) compared three lines of broilers subjected to different degrees of selection pressure for improved growth rates. The results expressed relative to body weight showed that intestine weight was significantly reduced in highly selected birds compared to the unselected line. Similarly, De Verdal *et al.* (2011) compared two broiler lines selected or unselected for digestive efficiency and found that the small intestine weight relative to body weight of birds selected for digestive efficiency was

significantly lower in the selected line compared to the unselected line. More recently, Alshamy *et al.* (2018) showed that the relative weight of the small intestine was reduced in Ross 308 birds compared to the Lohmann dual-purpose broiler line. On the other hand, there is also evidence to suggest that the opposite is true, Zuidhof *et al.* (2014) found that there was a slight increase in the relative organ measurements of females from a 2005 broiler strain compared to females of a 1978 strain, although there was no significant difference between both males and females of the 2005 and the 1958 strain. Furthermore, when a modern broiler was compared with a heritage line, it was found that GIT measurements (when expressed per unit of body weight) were greater in the broiler than the heritage line (Schmidt *et al.*, 2009). Similar results were obtained by Jackson and Diamond (1996), who compared the growth and organ development of a broiler strain with red jungle fowl and observed an increase in the wet mass of the broiler small intestine as a percentage of overall body weight. It has been suggested that the specific selection criteria of different broiler strains are responsible for the differences in how broilers respond to low energy feeds. For example, Pym (2005) proposed that broilers which are selected for increased appetite or growth rate alone may be eating to GIT capacity, whereas birds selected for improved nutrient utilisation retain the ability to modify feed intake in response to nutrient composition. If this is indeed true, the unintended consequences of artificial selection on the GIT capacity should be carefully considered as the industry moves towards the feeding of alternative, bulky ingredients.

1.5 The problem of predicting voluntary feed intake

The starting point for any growth simulation model is the accurate prediction of feed intake (Emmans, 1987). If nutrient requirements have not been met and the environmental temperature is not limiting performance, then it can be expected that the birds will consume feed until they reach the maximum gut capacity (Nascimento *et al.*, 2020). There is strong evidence in pigs that bulk characteristics can define gut capacity and constrain feed intake. In these experiments, the authors offered feeds diluted with a wide variety of bulky ingredients such as: sugar-beet pulp, soya hulls, grass meal, wheat bran, sawdust, sunflower husks and maize cobs. The results showed that bulk characteristics such as water-holding capacity, neutral-detergent fibre and acid-detergent fibre constrained feed intake, these characteristics were also shown to define gut capacity in the pigs (Kyriazakis and Emmans, 1995; Tsaras *et al.*, 1998; Whittemore *et al.*, 2001b; Ndou *et al.*, 2013) and have also been used to accurately predict the voluntary feed intake scaled per unit of body weight in pigs offered bulky feeds (Ndou *et al.*, 2013). Scaling performance relative to body weight is important when offering feeds of different nutrient contents because of relationship between feed composition, growth and feed intake. As would be expected, a larger animal will consume more feed than a smaller animal due to their physical

size and so it is difficult to interpret whether the feed composition has affected feed intake or whether the effect is simply due to the differences in growth rate. Accounting for size by scaling data to body weight overcomes this issue and allows conclusions to be drawn on the effects of feed composition on feed intake. The disadvantage to using body weight as a scalar is that it also includes the gut fill, which is increased when feeding bulky feeds. Although scaling to empty body weight would overcome this limitation since the gut fill is removed, emptying the digestive tract is a laborious task which cannot always be completed during intensive sampling days. It was for this reason that performance data were scaled to body weight throughout this thesis.

Determining the bulk characteristics which define maximum gut capacity in broilers would improve the accuracy of predicting feed intake in broilers given feeds diluted with bulky ingredients. Moreover, improving the accuracy in estimating gut capacity of broilers may lead to further improvements in optimising the nutrient density of feeds for broilers. The nutrient density, or energy levels, of a feed should be formulated to optimise the economic returns (Fisher *et al.*, 1974). If the constraining effect of bulk is not accurately assessed, then nutrient densities may be under- or over-estimated and so the broilers may be unable to consume sufficient quantities of feed to grow to their estimated potential (Nascimento *et al.*, 2020). The use of alternative, bulky ingredients could then be more accurately assessed once the maximum gut capacity has been defined and related to a characteristic of bulk.

1.6 Gastrointestinal adaptation to bulky feeds

One explanation for the initial reduction in feed intake when an animal is given a feed of high bulk content could be neophobia. Neophobia is a behaviour defined as a reduction in feed intake when offered an unfamiliar feed (Marples and Kelly, 1999; Bertin *et al.*, 2010), which has been suggested to confer evolutionary advantages, such as avoidance of potentially harmful substances. On the other hand, the initial reduction in feed intake may simply reflect the fact that the GIT has not yet fully adapted to the bulkiness of the feed (Starck, 1999). The rate of gastrointestinal adaptation to feeds with a high bulk content will have direct implications on feed intake and performance as feed intake increases during the adaptation period until the new feed intake equilibrium is reached (Whittemore *et al.*, 2003b). Kyriazakis and Emmans (1995) showed that in pigs, the GIT adapts to increasing levels of bulk when feeds were composed of up to 97% wheat bran. In this experiment, the absolute weights of the stomach and large intestine were significantly heavier in the pigs fed high levels of wheat bran which suggests that these organs play an important role in the adaptation of pigs to bulky feeds. Initially, the scaled feed intake of the pigs fed the highest dilution of wheat bran was significantly reduced when they were first introduced to the feeds. However, at the end of the experiment, once the

gastrointestinal adaptation to the bulky feed was achieved, the pigs given the bulky feeds were consuming more feed per unit of body weight than the basal fed pigs.

Similarly, Jørgensen *et al.* (1996a) observed increases in the GIT of broiler chickens offered bulky feeds. Although the author did not present feed intake, the performance (in terms of empty body weight) of birds given bulky feeds was improved compared to the control birds so it is not unreasonable to assume feed intake was increased for the birds given bulky feeds. However, the differences in empty body weight between the birds offered the basal and diluted feeds may be due to the inadequate production of the basal feed. For example, if the basal feed was deficient in lysine then this could account for the reduced performance of the birds given the basal feed (Sterling *et al.*, 2006). On the other hand, the improvements in performance may be due to the adaptations of the GIT (which are detailed below) facilitating a greater maximum feed intake. Therefore, the rate and extent of adaptation must be accounted for in any simulation model with the aim of accurately predicting feed intake and performance of broilers offered bulky feeds.

In poultry, the role of the crop in the adaptation to bulky feeds is not well explored, the reason for this is likely because of the wide agreement that chickens do not utilise the crop due to *ad-libitum* feeding since the crop acts as a storage organ for feed (Svihus, 2014). However, it has been proposed that the inclusion of structural ingredients (i.e. fibrous or bulky ingredients) to the animals feed could result in an increase in feed intake and as a consequence the development of the crop may be stimulated (Kierończyk *et al.*, 2016). The size of the proventriculus has been shown to increase when broilers are offered feeds diluted with bulky ingredients (Jørgensen *et al.*, 1996a; González-Alvarado *et al.*, 2007). Similarly, there is an abundance of evidence showing that the size of the gizzard increases when broilers are offered bulky feeds (Hetland and Svihus, 2001; Hetland *et al.*, 2005; Amerah *et al.*, 2009; Rougière *et al.*, 2009). Evidence suggests that inclusion of insoluble fibre stimulates gizzard development relative to body weight to a greater extent than soluble fibre (Jiménez-Moreno *et al.*, 2009a; Jiménez-Moreno *et al.*, 2009b). However, the fresh contents of the gizzard expressed as a percentage of gizzard weight was observed to be higher in birds fed soluble rather than insoluble fibre sources (Jiménez-Moreno *et al.*, 2009a; Jiménez-Moreno *et al.*, 2009b). By definition, this effect is due to the higher water retention and bulking properties, such as water-holding capacity, of soluble fibre sources compared to insoluble sources (Eastwood, 1973). The inclusion of bulky ingredients has also been shown to stimulate the development of the small intestine relative to body weight (Jørgensen *et al.*, 1996a; Jiménez-Moreno *et al.*, 2013b; Jiménez-Moreno *et al.*, 2019). Caeca development relative to body weight has also been shown to increase when broilers are offered bulky feeds (Jørgensen *et al.*, 1996a; González-Alvarado

et al., 2007), although this typically occurs when the bulky ingredient is soluble in nature and the greater caeca development can be attributed to increases in fermentation in the caeca. A criticism of the above experiments, with the exception of Jørgensen *et al.* (1996a), is that the effect on the large intestine is often overlooked. Jørgensen *et al.* (1996a) found that the weight of the large intestine relative to body weight increased when broilers were given feeds diluted with bulky ingredients. The increases in GIT development of broilers given bulky feeds by definition increases the maximum gut capacity. It can therefore be assumed that the adaptation of the GIT to bulk allows feed intake to increase and growth performance to recover to the same level of the birds given a basal feed.

1.7 The paradox of pathogen-induced anorexia

As previously mentioned, pathogen-induced anorexia is a common consequence of infection (Kyriazakis *et al.*, 1998). The characteristics of pathogen-induced anorexia (onset, extent and duration) will now be discussed in relation to the different stages of infection, which are shown in a conceptual diagram in Figure 1.1. The first stage of infection is known as the pre-patent, which is assumed to reflect the lag time between infection and recognition of a pathogen by the host's immune system. Following this is the acute stage which represents the peak of infection and is where the onset and greatest extent of pathogen-induced anorexia occurs. By definition, the onset of anorexia is the time at which the reduction in feed intake begins and the extent of anorexia represents the greatest reduction in feed intake during the course of infection. The extent of the reduction in feed intake is believed to be affected by different pathogen loads, which can vary from a small reduction (5-10%) to a complete avoidance of feed (Kyriazakis, 2010). The recovery stage of infection defines the duration of anorexia, as this is when feed intake returns to levels of uninfected control animals.

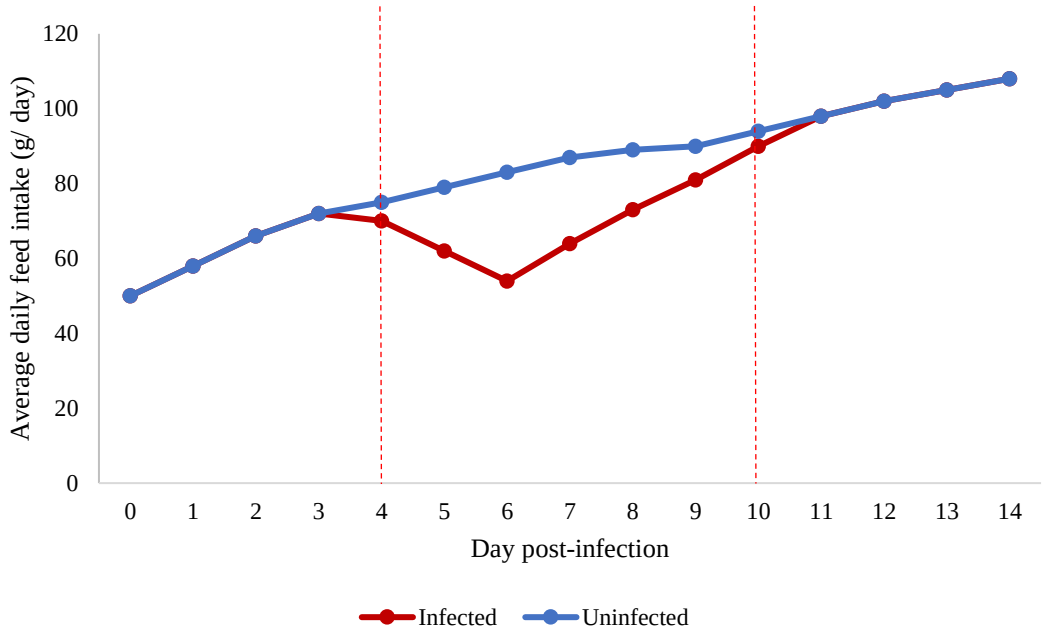


Figure 1.1. A conceptual diagram showing the different characteristics of anorexia (onset, duration and extent) across the different stages of infection separated by red-dotted lines (pre-patent, acute and recovery, respectively).

The phenomenon of pathogen-induced anorexia has been observed across a wide range of animal species such as pigs (Houdijk *et al.*, 2007), mice (Coltherd *et al.*, 2009), humans (Stephenson *et al.*, 2000) and chickens (Webel *et al.*, 1998a) and has been observed in a variety of infections, including macroparasites such as the nematode, *Heligmosomoides bakeri* (Tu *et al.*, 2007) and microparasites such as the bacterium, *Clostridium perfringens* (McDevitt *et al.*, 2006). Furthermore, in the case of broiler chickens, pathogen-induced anorexia has been observed during coccidiosis infection (Kipper *et al.*, 2013) which is caused by a genus of apicomplexan protozoans, *Eimeria* (Blake and Tomley, 2014).

Pathogen-induced anorexia is paradoxical in its nature since it occurs at times when host requirements for nutrient resources are increased, rather than decreased (Hite and Cressler, 2019). Metabolic and nutritional demand is increased in parasitized hosts during pathogen-induced anorexia, therefore growth and productivity are impaired, and reproductive ability is reduced to allow resources to be reallocated towards the immune response (Kyriazakis *et al.*, 1998). For parasites that infect the intestinal tract, the impairment on growth and productivity can largely be attributed to endogenous losses of protein, damage of gut tissue and increased mucoprotein secretions (Sykes, 1983; Poppi *et al.*, 1986; Brown *et al.*, 1991). Pathogens also divert resources away from their host and in response to infection hosts initiate resource-demanding processes, such as the immune response and the repair of the damage caused by the pathogen (Sandberg *et al.*, 2007). Therefore, conventional wisdom would dictate that a

parasitized host would increase their feed intake to overcome the costly effects of parasitism, rather than the observed anorexia.

It has been hypothesised that pathogen-induced anorexia confers evolutionary advantages, usually to the host (Kyriazakis *et al.*, 1998; Rao *et al.*, 2017). Indeed, the experiment by Murray and Murray (1979) illustrated the advantages to host survival during infection since mortality increased in infected mice that were force fed to increase their intake compared to mice that were allowed to develop the anorexic response (Murray and Murray, 1979). The review by Exton (1997) further supports the idea that anorexia is an active host defence mechanism and offers a comprehensive insight into the immunological basis of pathogen-induced anorexia. The author discusses the link between fasting animals and various immune mediators (such as T lymphocytes, macrophages and cytokines) and relates this to infected hosts.

1.8 Developing a model to assess pathogen-induced anorexia

To investigate pathogen-induced anorexia, it is critical to develop a model where there are clear changes in the voluntary feed intake of the infected animals over the different stages of infection. The animals should eventually be able to overcome their infection, so that anorexia is no longer observed, and the different stages of anorexia can be clearly separated. One such disease is coccidiosis which commonly infects broiler chickens and is caused by different species of the genus *Eimeria*. There are 7 species of *Eimeria* that affect broiler chickens and there is great variation in the pathogenicity of each of these genera (Györke, *et al.,* 2013). There is little information on the characteristics of *Eimeria*-induced anorexia. Of the available literature, *Eimeria maxima* has been shown to induce replicable anorexia following a single experimental infection with 7,000 sporulated oocysts and the characteristics of anorexia were easily distinguished (Sakkas *et al.,* 2018; Oikeh *et al.,* 2019). Since there is little information on the necessary dose to induce replicable anorexia in other *Eimeria* species (such as *E. tenella*, *E. acervulina*), *E. maxima* was identified as the ideal species to test the hypotheses. Furthermore, given that the *E. maxima* model was previously developed within the lab to which I am attached (Sakkas *et al.,* 2018), *E. maxima* seemed the ideal parasite to test the hypotheses. The advantages of using this model are as follows: the feed intake of growing chickens changes rapidly over time, the duration of coccidiosis is relatively short in comparison with other gastrointestinal parasites, there is a clear reduction in feed intake during the acute stage of infection, and the birds are eventually able to overcome their infection by which time anorexia is no longer observed (Kipper *et al.*, 2013; Sakkas *et al.*, 2018; Oikeh *et al.,* 2019).

To investigate the effects of feed composition on *Eimeria*-induced anorexia, it is important to formulate feeds where the nutritional composition is progressively reduced without altering the energy to nutrient ratios. In accordance with the theory of feed intake regulation (Emmans

and Fisher, 1986), broilers offered such feeds would attempt to consume such feeds to meet requirements for the most limiting nutrient, unless the birds could not lose sufficient heat to their environment or were limited in their capacity for bulk. Therefore, an inert, bulky material (lignocellulose) seemed an ideal ingredient to utilise for two reasons: 1) lignocellulose offers no nutritive value to the feed (Oikeh *et al.*, 2019), so the energy to nutrient ratios would not be altered; 2) lignocellulose should limit the feed intake of uninfected broilers through its bulkiness once gut capacity has been reached, whereas during the acute stage of infection, when anorexia is observed, the infected broilers are no longer limited by the bulkiness of the feed as they have reduced their voluntary feed intake from the maximum, and as such may increase their feed intake on the bulky feeds in attempt to regulate nutrient intake. Since both nutrients and energy would be reduced by dilution with an inert ingredient, any change in feed intake from the infected animals would indicate that they are attempting to regulate nutrient or energy intake during anorexia. Whereas if the pattern of feed intake is comparable to the uninfected animals, then it could be concluded that feed composition has no effect on pathogen-induced anorexia.

It is important to consider the method of measuring oocyst excretion when giving feeds diluted with an inert, bulky ingredient since this will cause an increase in the amount of excreta produced and consequently dilute oocyst excretion. For example, the excretion of oocysts may not differ between birds given a basal and a diluted feed, but the latter may produce more excreta, which would suggest that the diluted feed improved host resistance. For this reason, oocyst excretion will be scaled to faecal dry matter (number of oocysts per gram; OPG) to prevent misinterpretation of the results.

1.9 Thesis aims and hypotheses

The overarching aim of this thesis was to investigate the ability of modern broiler genotypes to cope with diluted, bulky feeds in sickness and in health, and to identify a physical measure of a feed which is responsible for the constraining effect of bulk on voluntary feed intake in healthy broilers. To achieve this goal, the following specific objectives were targeted to test the hypotheses:

- **Chapter 2:** To investigate the ability of a modern broiler strain to cope with feeds which were progressively diluted with bulky ingredients which differ in their bulk characteristics, and to identify a bulk property which might be responsible for limiting feed intake. It was hypothesized that broilers offered feeds diluted with sugar beet pulp will be limited in their performance further than those offered feeds diluted with the same amount of oat hulls, due to differences in the bulk properties of the two ingredients, such as the WHC. The second objective was to identify a bulk property

which might be responsible for limiting feed intake and use it for future predictions of feed intake on bulky feeds.

- **Chapter 3:** To identify a bulk property which can be used in the prediction of feed intake of modern broiler genotypes fed bulky feeds that were diluted with a range of bulky ingredients which vary widely in their bulk characteristics, and to determine the rate of adaptation of broilers to bulky feeds. It was expected that broilers given bulkier feeds will undergo a longer period of adaptation than those given less bulky feeds. Furthermore, it was hypothesised that feed intake on mixtures between bulky ingredients will result from the principle of additivity for bulkiness, and that previous experience on bulky feeds will improve the ability of birds to cope with a bulkier feed.
- **Chapter 4:** To determine the effect of diluting dietary energy content on pathogen-induced anorexia in *Eimeria maxima* infected broilers, and to indicate whether infected broilers are attempting to regulate their nutrient resource intake. It was hypothesized that healthy broilers would not be able to increase their feed intake when offered feeds diluted with an inert ingredient, as feed intake responses to diet dilution would be constrained by the bulk properties of the feed. Furthermore, it was hypothesized that, in the presence of infection, birds offered diluted feeds would show a reduction in the extent of anorexia as the feed is limiting in energy and nutrients; the basis of the hypothesis is that any further reduction in feed intake is likely to impact on host resistance to the parasite with potentially detrimental consequences to the host.
- **Chapter 5:** Aimed to assess whether pathogen-induced anorexia is sensitive to graded dilutions of feed crude protein content (while energy content was maintained) during infection with *E. maxima* and investigate the impact of such feeds on infection outcomes. It was hypothesised that the feed intake of healthy broilers would increase to a plateau as dietary protein content increases. In the presence of infection, it was hypothesised that infected birds offered a high CP feed would show the greatest extent of anorexia as they reduce feed intake in an attempt to avoid an excess of protein intake. Furthermore, the consequences of CP content on the outcomes of the infection were assessed, i.e. the extent of the damage caused by the parasite, the number of parasites excreted by the host, and the rate of recovery. It was expected that the infection outcomes would be less severe in birds fed a high CP feed as they have more available protein to facilitate an increase in resistance, this will be shown by a reduction in oocyst excretion and histological damage.

Chapter 2. What are the limits to feed intake of broilers on bulky feeds?

2.1 Abstract

The view that genetic selection for carcass yield has limited the size of the gastrointestinal tract (GIT) of modern broilers has sparked concerns that their capacity to cope with energy dilution or bulk is also limited. We investigated the capacity of male Ross 308 broilers to deal with increasing levels of bulk and aimed to identify a feed bulk dimension responsible for limiting feed intake (FI). 528 day-old broilers were allocated to 48 pens and offered a common starter feed until day 8, and 1 of 7 feeds from day 8-36 of age: a basal control (B), which was diluted to three levels (15%, 30% or 45%) with either oat hulls (OH) or sugar beet pulp (SBP). FI was measured daily and birds were dissected for GIT measurements at day 15, 22 and 36. FI increased in birds offered OH15 (135 g/ day), OH30 (140 g/ day) and SBP15 (138 g/ day) compared to birds offered the B feed (106 g/ day; SEM 2.4). By increasing FI, birds were able to compensate for the lower energy content of their feeds. The greatest increase in FI was seen on OH30: its energy content (2273 kcal/ kg) was 26% lower than the B feed (3081 kcal/ kg). There was evidence of adaptation on the bulky feeds, as during the last week only birds on SBP45 were limited in FI and performance. The relative weights of the GIT were greater in the SBP than OH series, suggesting that the former needed to accommodate a higher bulk intake. For the OH series the increase in the relative GIT weights was confined to gizzard and small intestine; whereas for the SBP series the increase was extended to proventriculus and large intestine. Because only SBP45 was limiting FI, we were unable to identify a bulk dimension to be used to predict FI. The data reject the suggestion that modern broilers have a reduced ability to cope with reductions in feed energy content.

2.2 Introduction

There is growing interest in using alternative feed ingredients in livestock production due to the competition between food and feed, the need to reduce the environmental impact of livestock production and reduce the costs associated with importing non-native ingredients (Madeira *et al.*, 2017; Tallentire *et al.*, 2018b). As far as poultry are concerned, such alternative ingredients include co- and by-products of food production which are not desirable to humans, such as wheat middlings, rice bran, palm kernel meal (Ravindran, 2013) and oat hulls (OH) (Scholey *et al.*, 2020). Such ingredients are lower in energy (Robert *et al.*, 1997; Sundu *et al.*, 2006) and possess a higher bulk content than traditional ingredients due to their physicochemical properties, such as water holding capacity (WHC) (Jiménez-Moreno *et al.*, 2009a). Studies in pigs have shown that properties of bulk, such as 'fibre' content and WHC can accurately predict feed intake capacity on bulky feeds (Tsaras *et al.*, 1998; Ndou *et al.*, 2013). Such bulk characteristics may have the potential to predict the feed intake of poultry (Kyriazakis *et al.*, 1994). However, there is a dearth of information as to whether this is indeed the case.

At the same time there have been increased concerns about the ability of modern poultry, especially broiler genotypes to cope with bulk intake and a reduction in feed energy content (Mbajiorgu *et al.*, 2011; Gous, 2013; Classen, 2017; Scholey *et al.*, 2020). Historically, there has been an abundance of evidence showing that the GIT of broilers can adapt and increase in size relative to BW when the birds are offered feeds with bulky ingredients (Griffiths *et al.*, 1977; Deaton and Lott, 1985; Leeson *et al.*, 1996a). More recently, however, Linares and Huang (2010) observed a compensatory increase in feed intake when dietary energy was reduced by 5%; however when energy was reduced by 10% there was no further increase in feed intake and the performance of the birds was penalised. Classen (2017) has suggested that intensive artificial selection has caused the modern broiler to lose the ability to adjust feed intake in response to reductions in dietary energy and that care needs to be taken when including alternative ingredients in broiler feeds. Several studies showing that broilers are unable to adjust feed intake when offered feeds of different nutrient compositions support this suggestion (Sahraei and Shariatmadari, 2007; Swennen *et al.*, 2007; Latshaw, 2008; Delezie *et al.*, 2010; Cho, 2011; Krás *et al.*, 2013). Pym (2005) suggested that broilers which are selected for increased appetite or growth rate alone may be eating to GIT capacity, whereas birds selected for improved nutrient utilisation retain the ability to modify feed intake in response to nutrient composition.

The ability for animals to cope with feeds of a high bulk content is constrained by the physical capacity of the GIT (Kyriazakis and Emmans, 1995). Tallentire *et al.* (2018a) suggested that the capacity of the gastrointestinal tract (GIT) in broilers is nearing its biological limits and as such, so is the capacity for maximum daily feed intake. An explanation for this has been suggested to be that selection for increased carcass yield has occurred at the expense of the size of the GIT (Mitchell and Smith, 1991; McEntee *et al.*, 2003; Rougière *et al.*, 2009; De Verdal *et al.*, 2010; Lumpkins *et al.*, 2010; De Verdal *et al.*, 2011; Alshamy *et al.*, 2018). The opposite view has also been put forward (Zuidhof *et al.*, 2014), as there is evidence to suggest that the GIT of modern broilers has increased compared with a heritage line when GIT measurements were expressed per unit of BW (Schmidt *et al.*, 2009). As the interest in alternative, bulky ingredients grows, it is important to understand the physical limits of the broiler GIT and their ability to cope with bulky feeds.

The aim of this experiment was twofold: 1) to assess the ability of a modern broiler strain to cope with feeds which were progressively diluted with the bulky ingredients oat hulls (OH) or sugar beet pulp (SBP). I hypothesized that broilers offered feeds diluted with SBP will be limited in their performance further than those offered feeds diluted with the same amount of OH, due to differences in the bulk properties of the two ingredients, such as the WHC; 2) to identify a bulk property which might be responsible for limiting feed intake and use it for future predictions of feed intake on bulky feeds.

2.3 Materials and methods

2.3.1 *Feed formulation*

All birds were fed the same conventional starter feed from day (d) 0 until d8 of age. They were offered the experimental feeds from d8-36 of age (Table 2.1). Three grower feeds were formulated (Table 2.1): a basal feed (B), which was subsequently diluted with 45% of either Oat Hulls (OH) or Sugar Beet Pulp (SBP) to produce two bulky feeds. These diluents were selected because of their stark differences in physicochemical properties, such as the water holding capacity (WHC), which is higher in SBP than in OH, whereas the converse is the case for their crude fibre contents (González-Alvarado *et al.*, 2010). Each bulky feed had the same calculated digestible protein to AME ratio, and all other nutrient to AME ratios were the same as in the basal feed. Nutrient ratios were maintained by increasing or decreasing synthetic amino acids, limestone and monocalcium phosphate inclusion, where appropriate.

Mixtures of the basal feed and the two bulkiest feeds were created to produce three dilutions of bulky feeds for each diluent. The first mixture was one-part basal and two-parts bulky feeds to produce a 30% dilution of the basal feed, and the second mixture was two-parts basal and one-part bulky feed to produce a 15% dilution, so all resulting feeds had the same nutrient

to AME ratios. The experimental feeds were offered in pellet form. Each of the bulky feeds and their dilutions were replicated in 7 pens, whilst there were 6 replicate pens for the basal treatment.

Table 2.1. Ingredient, calculated and analysed chemical composition of the grower feeds offered from day 8 to day 36 of age to broiler chickens. The basal (B), high density feed was diluted with 15%, 30%, or 45% of either Oat Hulls (OH) or Sugar Beet Pulp (SBP).

Ingredients (%)	B	OH15	OH30	OH45	SBP15	SBP30	SBP45
Ground maize	10.0	8.50	7.00	5.50	8.50	7.00	5.50
Ground wheat	54.4	44.1	33.8	23.6	44.2	34.0	23.8
Soybean meal (48% CP)	22.0	19.0	15.9	12.9	19.0	15.9	12.9
Soybean meal (70% CP)	1.00	1.33	1.67	2.00	1.33	1.67	2.00
Full fat soya	5.00	4.62	4.23	3.85	4.62	4.23	3.85
Soya oil	3.30	3.03	2.77	2.50	3.03	2.77	2.50
Oat hulls (OH)	-	15.0	30.0	45.0	-	-	-
Sugar beet pulp (SBP)	-	-	-	-	15.0	30.0	45.0
Limestone	1.45	1.27	1.10	0.93	0.97	0.48	-
Monocalcium phosphate	1.25	1.12	1.00	0.87	1.35	1.46	1.56
L-Lysine HCL	0.30	0.40	0.51	0.61	0.42	0.54	0.67
DL-Methionine	0.35	0.30	0.26	0.21	0.32	0.28	0.25
L-Tryptophan	-	0.01	0.02	0.03	0.02	0.04	0.06
L-Threonine	0.15	0.20	0.25	0.30	0.21	0.27	0.34
L-Arginine	-	0.12	0.23	0.35	0.13	0.26	0.39
Valine	-	0.09	0.17	0.26	0.08	0.17	0.25
Iso-leucine	-	0.08	0.16	0.24	0.09	0.17	0.26
Salt	0.25	0.22	0.19	0.16	0.22	0.18	0.15
Sodium bicarbonate	0.15	0.14	0.13	0.12	0.13	0.11	0.08
Vitamin and mineral premix	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Titanium dioxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Ronozyme [a]	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Total	*100*	*100*	*100*	*100*	*100*	*100*	*100*
Chemical composition (%) [b]							
Metabolizable energy (kcal kg^{-1}) (calculated)	3009	2759	2502	2244	2916	2815	2713
Crude protein (CP)	25.4	23.3	21.2	19.1	23.6	21.7	19.8
Digestible crude protein (dCP, calculated)	22.0	19.9	17.9	15.9	20.9	19.9	18.9
Lysine (calculated)	2.01	1.84	1.67	1.49	1.94	1.87	1.80
Digestible Lysine	1.88	1.74	1.56	1.39	1.82	1.79	1.69
Threonine (calculated)	1.30	1.19	1.07	0.96	1.26	1.21	1.17
Ether extract (oil A)	6.39	6.04	5.16	5.19	5.50	5.35	5.96
Total oil (oil B)	6.94	6.77	5.96	5.66	6.04	6.11	6.70
Ash	6.20	5.40	5.30	5.40	7.90	7.90	9.00
Calcium (calculated)	0.98	0.89	0.81	0.73	0.97	0.97	0.97
Available Phosphorus (calculated)	0.41	0.36	0.32	0.27	0.42	0.43	0.44
Crude fibre	2.70	5.90	8.70	10.7	5.80	6.20	6.50
Neutral detergent fibre	9.70	16.2	24.2	28.5	14.7	15.8	15.7
Acid detergent fibre	3.01	7.48	12.2	13.3	7.57	9.96	8.34
Acid detergent lignin	0.73	1.69	2.29	2.49	1.24	1.26	1.27
Insoluble dietary fibre	10.2	18.4	26.0	37.5	19.3	22.4	30.5
Soluble dietary fibre	1.60	2.40	2.00	1.30	4.30	6.20	8.60
Total dietary fibre	11.8	20.8	28.0	38.8	23.6	28.6	39.1
Feed density (g/ ml)	1.28	1.21	1.29	1.38	1.39	1.51	1.66
Water Holding Capacity (g/ g DM)	2.65	2.53	2.74	3.09	3.46	4.73	6.01

[a] Blend of amylase and beta-glucanase

[b] Analysed composition unless otherwise stated

2.3.2 *Experimental design and birds*

All procedures were conducted under the UK Animals (Scientific Procedures) Act 1986 and were approved by the Animal Welfare and Ethical Review Body (AWERB) of Newcastle University no. 7332/2018. A total of 528 male Ross 308 chicks were obtained at d0 of age from a commercial hatchery and were housed in a thermostatically controlled building with 48 pens, each with an area of 0.85m^2. Pens were equipped with feeders and drinkers, with wood shavings used as litter at a depth of 5 cm. All birds were wing tagged upon arrival and randomly distributed into the pens with an initial stocking density of 11 birds per pen. The birds had free and continuous access to feed and water throughout the trial. The temperature at pen level was monitored daily and maintained to meet Aviagen recommendations for spot brooding, starting at 34°C at chick arrival and gradually lowered to 20°C by d25 of age. The lighting schedule was 23L:1D for the first 7 days and was amended to 18L:6D for the course of the trial, whilst light intensity at pen level ranged from 80-100 lux.

Birds were weighed at arrival (d0), prior to treatment allocation (d8) and then twice per week until the end of the trial. After weighing the birds on d8, the stocking density was reduced from 11 to 10 birds per pen. Chicks were then distributed between pens so that there were no significant differences in the mean BW of each treatment. Pen feed intake was measured from d0 to d8, and then daily until the conclusion of the experiment on d36.

2.3.3 *Sampling*

On d15, 22 and 36 of age, two birds per pen with a BW close to the pen average were culled by intravenous lethal injection with sodium pentobarbital (Euthatal®, Merial Harlow, United Kingdom). Following euthanasia, the full GIT was removed and the small intestine was isolated from the point of entry of the bile ducts to the ileo-caecal junction. The jejunum was isolated, weighed and the length was quickly recorded before digesta was collected from the upper 1/3 of the jejunum and stored at -80°C pending viscosity analysis. The empty carcass weight was obtained by weighing the carcass with the GIT removed. The GIT was then separated into individual components: crop, proventriculus, gizzard, duodenum, jejunum, ileum, caeca and colon. The length of the duodenum and ileum were recorded, and the components of the GIT were weighed full. Following this the crop, gizzard, duodenum, jejunum and ileum were carefully emptied of their contents with gentle finger stripping to obtain empty weights.

2.3.4 *Sample analysis*

2.3.4.1 *Viscosity*

Jejunal digesta samples were thawed in a water bath at 40°C for 15 minutes. Following that, the samples were vortexed for 5 seconds and then centrifuged at 10,000 RPM at 20°C degrees for 10 minutes. After centrifugation, the supernatant was carefully removed and pipetted into a

2ml Eppendorf tube. The tubes were then placed in a water bath at 40°C for 5 minutes. Finally, 0.5ml of the supernatant was pipetted into the cone of a Brookfield Model DV-III digital Rheometer (Brookfield Engineering Laboratories, Corp. Stoughton, MA, USA) with a CPE-40 spindle viscosity measurement fitted with a circulating water bath. Readings were obtained at 6 RPM, with a shear rate of 42.5/s at 40°C. Viscosity measurements are reported in centipoise (Cps).

2.3.4.2 *Fibre analysis*

All classical descriptors of feed bulk used traditionally in livestock research were measured. Some of these descriptors are highly correlated as they essentially measure the same bulk characteristics through different measurements (Brachet *et al.*, 2015). Samples of all feeds were analysed for crude fibre (Test method: Commission Regulation (EC) No.152/2009), acid detergent fibre (Test method: AOAC 973.18-1977), neutral detergent fibre (Test method: AOAC 2002.04-2005), acid detergent lignin (Test method: AOAC 973.18-1977), insoluble dietary fibre, soluble dietary fibre and total dietary fibre (Test method: AOAC 2011.25-2011) at a UKAS accredited commercial laboratory to the internationally recognised standard for competence (Sciantec Analytical Services, Cawood, UK).

2.3.4.3 *Water holding capacity*

Analysis was performed in triplicate, using an adaptation of the Robertson and Eastwood (1981) method. A 1 g feed sample was soaked in 250 ml of H_2O at room temperature for 24 hours. Subsequently, the samples were filtered through a Whatman no. 1 filter paper and the wet weight of the samples recorded, before the samples were placed into an oven at 105 °C overnight and the dry weight was recorded. WHC was then calculated as g of water/ g of DM.

2.3.4.4 *Feed density*

Feed density was determined in triplicate by the method described by Kyriazakis and Emmans (1995). First, 100 ml of distilled water at 37°C was placed in a 250 ml flask and a 50g sample of feed was added. After mixing, a further 50 ml of water was added, and the contents allowed to equilibrate for 15 minutes before a final 50 ml of water was added. The sample was left to equilibrate for a further 15 minutes before the flask was filled to volume with water with a burette. The total amount of water contained in the flask was subtracted from 250 ml.

2.3.5 *Calculations and statistics*

To account for mortalities the feeder was weighed when mortalities were found and the average feed intake (g/ bird) was calculated and the assumed intake of the dead bird was subtracted from the pen feed intake. Pen average daily energy intake (ADEI, kcal/ day) was calculated by multiplying pen ADFI (g/ day) by the calculated AME content of the feed. ADFI was calculated over weekly periods, as well as over the whole experimental period (d8-36).

ADEI, average daily gain (ADG) and feed conversion ratio (FCR) were also calculated over the whole period. To account for *a-priori* differences in growth rate due to feed composition performance data were scaled relative to either the mean weekly BW or the mean BW over the whole experimental period (d22) (Whittemore *et al.*, 2001b). Furthermore, when ADFI was calculated over weekly periods, the data were scaled relative to the mean BW of each week. Organ measurements were expressed relative to the empty carcass weight (ECW) of the bird (g/ kg BW) to account for the differences in performance (Oikeh *et al.*, 2019).

All statistical analysis was performed in the nlme package in R (Team, 2013) using its lm and anova functions (Cezaro *et al.*, 2018). All data were analysed with the GLM procedure with feed as a fixed factor. Additional polynomial contrasts were performed on both performance data and organ measurements to study the linear and quadratic responses to diet dilution for OH and SBP separately. For all statistical procedures, the normality of the residuals was assessed with qq-plots and the Shapiro-Wilk test. When significant differences were detected, treatment means were separated and compared by the Tukey's multiple comparison test. Significance was determined at $P < 0.05$. Data are presented as model-predicted least square means with their pooled SEM.

2.4 Results

2.4.1 *Feed analysis*

As shown in Table 2.1, all measured characteristics of feed bulk increased with diet dilution in comparison to the B feed, with the exception of the WHC of OH15. The crude fibre, neutral detergent fibre, acid detergent fibre and insoluble dietary fibre were greater in the OH series compared to the corresponding dilution of the SBP series. The acid detergent fibre content and insoluble dietary fibre were greater in SBP15 than OH15, but the fibre values were greater in the OH30 and OH45 than the SBP30 and SBP45 feeds, respectively. The WHC, feed density and soluble dietary fibre were greater in SBP feeds than OH feeds.

2.4.2 *Mortalities, Feed and Calculated Energy Intake*

There were 7 mortalities (one bird per treatment) from d14 to d33 of age, the cause of mortality was unknown. The progression of daily feed intake from d8-35 of age is shown in Figure 2.1A and 2.1B. The birds offered the OH series or SBP15 feeds were able to increase feed intake above that of the B birds throughout the experiment ($P < 0.05$). In contrast, SBP30 birds were only able to increase their feed intake above that of the B birds from d22 onwards ($P < 0.05$), and the SBP45 birds maintained a similar feed intake to the B birds throughout the experiment ($P > 0.05$).

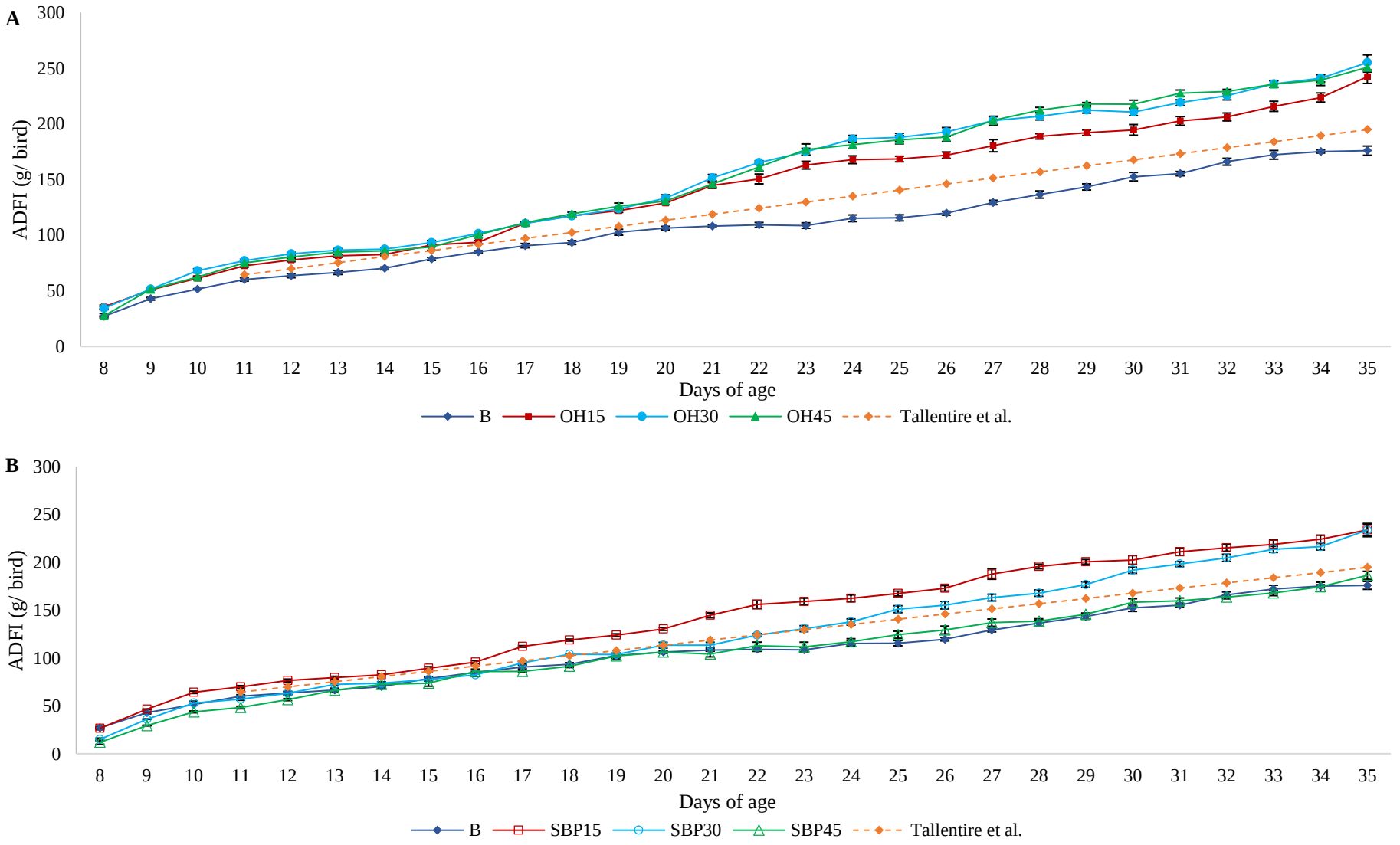


Figure 2.1. Average daily feed intake (ADFI; g/ bird) of broiler chickens given access to a basal feed (B), or the basal feed diluted with 15, 30 or 45% of either a) Oat Hulls (OH) or b) Sugar Beet Pulp (SBP) from d8-35 of age, and the potential average daily feed intake defined by the apparent biological limit suggested by Tallentire et al (2018).

ADFI, ADEI and scaled ADFI calculated over the whole period are presented in Table 2.2. There were no significant differences in ADFI between the birds offered the B and SBP45 feeds ($P > 0.05$). The ADFI of birds on the OH dilution series was significantly greater than that of the birds on the SBP30 and SBP45. The ADFI of the OH series increased linearly as diet dilution increased ($P < 0.01$). Furthermore, quadratic effects of OH and SBP dilutions were observed in ADFI ($P < 0.01$). ADEI of birds offered OH45, SBP30 and SBP45 was significantly lower than B ($P < 0.01$). Quadratic and linear effects of OH and SBP dilution were observed in ADEI ($P < 0.01$). There were no significant differences in ADEI between birds offered OH15, OH30 or B ($P > 0.05$), whereas the SBP15 birds significantly increased ADEI compared to B ($P < 0.01$). When ADFI was scaled to BW, there was a linear increase in scaled ADFI with diet dilution in both the OH and SBP series; quadratic effects were also observed for the SBP dilution ($P < 0.01$). Scaled ADFI was greater in SBP birds than the OH15, OH30 and the B feeds ($P < 0.05$).

Table 2.2. The average daily feed intake (ADFI, g/ day), average daily energy intake (ADEI, kcal/day) average daily gain (ADG, g/ day), and feed conversion ratio (FCR) of broiler chickens given access to a basal feed, or the basal feed (B) diluted with 15, 30 or 45% of either Oat Hulls (OH) or Sugar Beet Pulp (SBP) from day 8-36 of age. ADFI and ADG scaled relative to body weight (BW) at day 22 (mean point of experimental period; g/ kg BW/ day) are also given. Data are presented as LS means and SEM.

	ADFI (g/ day)	ADEI (kcal/ day)	ADFI/ BW (g/ kg BW/ day)	ADG/ BW (g/ kg BW/ day)	FCR
B	106^{a}	329^{c}	109^{a}	78.3^{bc}	1.32^{a}
OH15	135^{bc}	344^{c}	122^{b}	94.5^{f}	1.39^{ab}
OH30	140^{c}	323^{c}	130^{b}	92.7^{ef}	1.53^{bc}
OH45	142^{c}	283^{b}	144^{c}	83.6^{cd}	1.71^{cde}
SBP15	138^{c}	372^{d}	129^{b}	86.3^{de}	1.59^{cd}
SBP30	126^{b}	290^{b}	148^{c}	73.1^{b}	1.73^{de}
SBP45	106^{a}	204^{a}	148^{c}	53.1^{a}	1.87^{e}
SEM	2.4	7.1	2.5	1.87	0.045
			Probabilities		
Feed	<0.001	<0.001	<0.001	<0.001	<0.001
Linear OH	<0.001	<0.001	<0.001	0.341	<0.001
Linear SBP	0.422	<0.001	<0.001	<0.001	<0.001
Quadratic OH	<0.001	0.001	0.994	<0.001	0.572
Quadratic SBP	<0.001	<0.001	<0.001	<0.001	0.483

[a-f]Means within a column that do not share a common superscript are significantly different ($P < 0.05$)

Abbreviations: ADFI, average daily feed intake; ADEI, average daily energy intake; BW, body weight; ADG, average daily gain; FCR, feed conversion ratio.

During each of the four weekly periods, the birds offered the OH series showed a linear increase in scaled ADFI as dilution increased (Figure 2.2). Furthermore, there was a quadratic effect of OH dilution in weeks 1 and 3 ($P < 0.05$). The OH birds consumed significantly more feed per unit of BW than B birds throughout the experiment ($P < 0.05$). This was not the case for birds on the SBP series. During week 1, there was no significant difference in the scaled ADFI of birds offered B, SBP15 and SBP45 feeds ($P > 0.05$). Meanwhile the birds offered SBP30 consumed significantly more than those offered B ($P < 0.05$); as a consequence, there was a quadratic effect on scaled ADFI during week 1 ($P < 0.05$). However, during weeks 2-4 there was a linear and quadratic effect of SBP dilution on scaled ADFI ($P < 0.05$). During these time periods SBP45 birds increased their scaled ADFI above that of the B birds, so that birds in all of the diluted treatments consumed significantly more than the B birds throughout the rest of the experiment ($P < 0.05$).

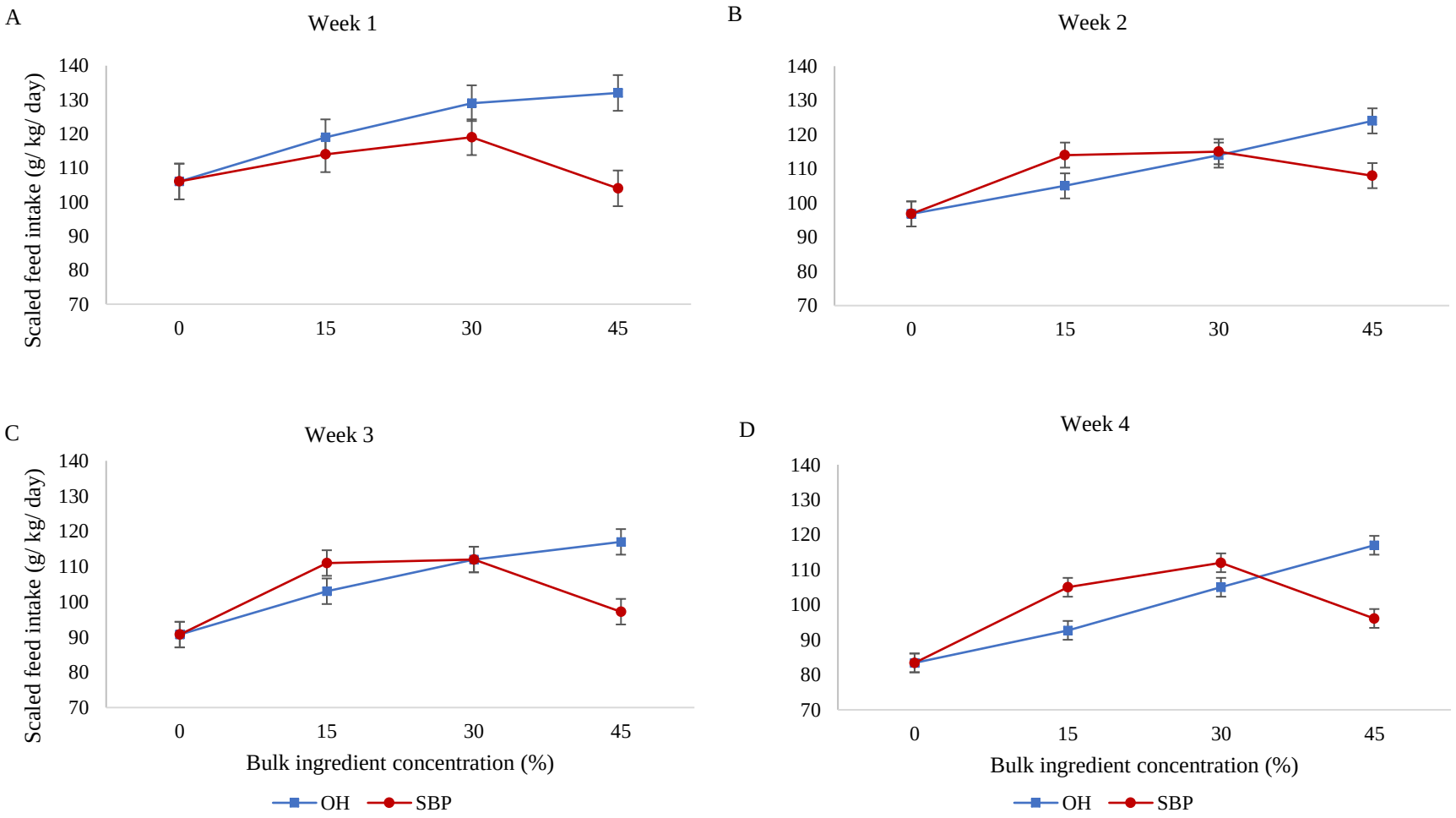


Figure 2.2. Average daily feed intake of broiler chickens for each experimental week, expressed relative to the average body weight of each week (g/ kg/ day), when offered feeds which were diluted with 0%, 15%, 30%, or 45% of either Oat Hulls (OH) or Sugar Beet Pulp (SBP); (**a**) week 1; (**b**) week 2; (**c**) week 3; (**d**) week 4. Data are presented as LS means and SEM.

2.4.3 *Average Daily Gain and FCR*

The progression of body weight from d8-36 of age is shown in Figure 2.3A and 2.3B. Over the whole experimental period, ADG relative to BW of the birds offered OH15, OH30 and SBP15 was significantly greater than birds offered the B ($P < 0.05$) (Table 2.2). There was a linear decrease in relative ADG of the birds given the SBP feeds ($P < 0.01$) but only the performance of the birds offered SBP45 was significantly reduced compared to B ($P < 0.05$). Moreover, a quadratic effect was observed for both the OH and SBP series in relative ADG ($P < 0.01$). FCR increased linearly with diet dilution in both the OH and SBP series ($P < 0.01$). There was no significant difference in FCR (Table 2.2) over the whole experimental period between birds on the B and OH15 feeds ($P > 0.05$). However, all remaining treatments significantly increased FCR compared with the B birds ($P < 0.05$).

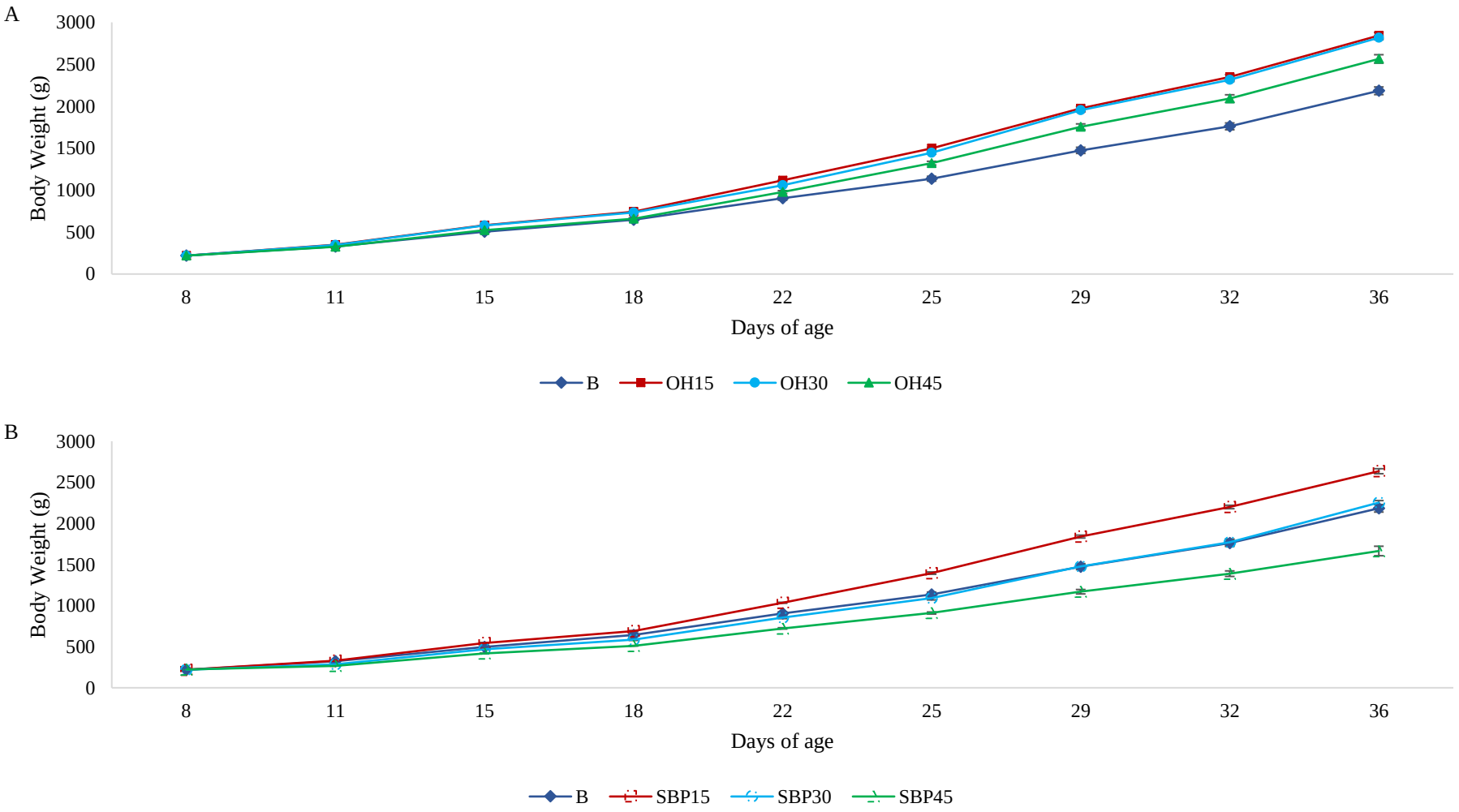


Figure 2.3. The progression of body weight (g) from d8-36 of broilers chickens given access to a basal feed (B), or the basal feed diluted with 15, 30 or 45% of either A) Oat Hulls (OH) or b) Sugar Beet Pulp (SBP).

2.4.4 *Organ Measurements and Viscosity*

Empty carcass weights (ECW) and organ measurements scaled relative to ECW from d15, d22 and d36 are presented in Tables 2.3, 2.4 and 2.5 respectively. There were significant linear and quadratic effects of SBP dilution on ECW at all slaughter points ($P < 0.01$). Whereas there were significant quadratic effects of OH dilution on ECW at all slaughter points, a linear effect on ECW was only observed with OH dilution on d36 ($P < 0.01$). On d15 and 22, the ECW of OH15, OH30 and SBP15 birds were significantly greater than B birds, whilst the SBP30 and SBP45 birds were significantly lighter than B birds ($P < 0.01$). On d36 the ECW was significantly greater in B birds than the SBP series and OH45 birds ($P < 0.01$). There were no significant differences between OH15, OH30 and B birds ($P > 0.05$).

There were no significant differences in the relative full crop and relative full caeca weights on d15 between any of the dietary treatments ($P > 0.05$). On d15, there was a significant quadratic effect of OH dilution on the relative weight of the empty crop ($P < 0.01$). On d22, there was a significant linear effect of OH dilution on the relative full crop weight ($P < 0.05$) and a significant linear effect of SBP dilution on the relative empty crop weight ($P < 0.01$); whereas, on d36 there were significant linear effects of SBP dilution on the relative full and empty crop weight ($P < 0.01$), and a significant quadratic effect of SBP dilution on the relative full crop weight ($P < 0.01$). On d15 there were no significant differences in relative full and empty crop weights between B birds and birds given the diluted feeds ($P > 0.05$), whereas on d22, the relative weight of the full and empty crop was significantly increased in OH30 birds compared to B birds ($P < 0.01$). The relative full and empty crop weight of birds on d36 were significantly increased in SBP45 birds compared to birds on all other treatments ($P < 0.01$).

There was a significant linear increase in relative weight of the full proventriculus at all slaughter points for SBP birds ($P < 0.01$). Relative proventriculus full weight was significantly greater in SBP birds compared with birds offered the OH series or the B feed at all slaughter points ($P < 0.01$). The exception to these effects was the significantly lower full proventriculus weight of OH15 compared to B birds on d22.

There were significant linear effects of OH and SBP dilution on relative full and empty gizzard weight at all slaughter points ($P < 0.01$); furthermore, significant quadratic effects of SBP dilution were observed in relative full and empty gizzard weight on d22, and full gizzard weight on d36 ($P < 0.01$). On d36 there was also a significant quadratic effect of OH dilution on the relative empty gizzard weight ($P < 0.01$). Relative full gizzard weight was significantly increased in SBP and OH45 birds compared to B birds at all slaughter points ($P < 0.01$).

Relative empty gizzard weight was also significantly greater in SBP and OH45 birds at all slaughter points ($P < 0.01$), with the exception of SBP15 birds on d15 and d36 ($P > 0.05$).

There were significant linear effects caused by feed dilution on the relative full and empty weights of the small intestine from birds on both the OH and SBP series at all slaughter points ($P < 0.05$). There were quadratic effects of feed dilution on the relative full weight of the small intestine from birds on the OH series on d22 and d36 ($P < 0.01$). Furthermore, on d36 there were quadratic effects of SBP dilution on relative full small intestine weight and quadratic effects of OH dilution on relative empty weight of the small intestine ($P < 0.01$). As a consequence, there were no significant differences in the relative weight of the full and empty small intestine of birds offered the B feed or the OH series ($P > 0.05$) at any of the slaughter points, with the exception of OH45 birds, where relative empty small intestine weight was increased at d15 and d22, and relative full small intestine weight increased at d22 ($P < 0.01$). In contrast, the full and empty relative weights of the small intestine were significantly greater in SBP birds compared to B birds at all slaughter points ($P < 0.01$).

There were quadratic effects of OH dilution on the relative lengths of the small intestine (Table 2.6) on d15 and d22 ($P < 0.01$) and linear effects of OH dilution on d36 ($P < 0.01$). In contrast, linear effects of SBP dilution were observed on d15 ($P < 0.01$) and linear and quadratic effects of SBP dilution on the relative lengths of the small intestine ($P < 0.01$). There were no significant differences in the relative lengths of the sections of the small intestine between birds offered the B feed and the OH series at all slaughter points ($P > 0.05$), with the exception of the relative jejunum length of OH45 birds, which was significantly longer than that of the B birds on d36 ($P < 0.01$). In contrast, the SBP series yielded significantly longer intestinal segments than B at all slaughter points ($P < 0.01$), with the exception of SBP15 birds at d22 (duodenum, jejunum and ileum) and d36 (duodenum), which were not significantly different from B birds ($P > 0.05$).

There were no significant differences in relative full caeca weight between any dietary treatments at d15 ($P > 0.05$), although there was a linear effect of SBP dilution ($P < 0.05$). Relative full caeca weight on d22 linearly increased with OH dilution ($P < 0.05$) but were not statistically different from B birds ($P > 0.05$). Furthermore, there was a quadratic effect of SBP dilution on relative full caeca weight on d22 ($P < 0.05$), where there was a significant reduction in relative caeca weight of SBP15 birds compared to B birds ($P < 0.01$). There were linear and quadratic effects of SBP dilution on the relative full caeca weight on d36 ($P < 0.05$), which was significantly greater in SBP45 birds compared to those in all other treatments ($P < 0.01$).

There was a quadratic effect of OH dilution on the relative full large intestine weight at d22 ($P < 0.05$), although there were no significant differences between B and OH birds at any of the slaughter points ($P > 0.05$). The relative full large intestine weight linearly increased with SBP dilution at all slaughter points ($P < 0.01$). This led to significantly greater relative full large intestine weights at all slaughter points for SBP birds compared to B birds ($P < 0.01$), with the exception of the SBP15 birds at d22 and 36 ($P > 0.05$).

The effect of diet dilution on jejunal viscosity (cP) was similar across slaughter points, so I will report d36 effects only. Jejunal viscosity increased linearly with SBP dilution: SBP15, 2.82 (CI 2.61 – 3.03); SBP30, 3.57 (CI 3.31 – 3.84); SBP45, 4.11 (CI 3.86 – 4.35) ($P < 0.01$). This was not the case for OH dilution, as there were no significant differences between the OH and B birds: OH15, 1.98 (CI 1.69 – 2.27); OH30, 2.05 (CI 1.83 – 2.27); OH45, 2.37 (CI 2.14 – 2.59); B, 2.18 (CI 1.95 – 2.40).

Table 2.3. Organ measurements expressed relative to empty carcass weight (ECW) of birds slaughtered on day 15 of age. Broiler chickens were offered feeds diluted with 0 (B), 15, 30, or 45% of either Oat Hulls (OH) or Sugar Beet Pulp (SBP) from day 8 of age.

	ECW (g)	Crop (g/ kg ECW)		Proventriculus Full (g/ kg ECW)	Gizzard (g/ kg ECW)		Small intestine (g/ kg ECW)		Caeca Full (g/ kg ECW)	Large intestine Full (g/ kg ECW)
		Full	Empty		Full	Empty	Full	Empty		
B	402^{c}	19.4	7.00^{ab}	8.38^{a}	48.2^{a}	32.7^{a}	89.7^{a}	58.0^{a}	15.4	9.78^{a}
OH15	477^{e}	14.5	5.83^{a}	8.29^{a}	48.3^{a}	33.2^{a}	89.8^{a}	58.6^{a}	17.2	8.59^{a}
OH30	464^{e}	12.3	5.31^{a}	7.34^{a}	54.1^{ab}	38.1^{ab}	92.5^{a}	62.8^{ab}	15.6	9.85^{a}
OH45	400^{c}	14.8	7.42^{ab}	8.18^{a}	65.8^{c}	45.6^{c}	104^{ab}	67.6^{b}	16.7	12.5^{ab}
SBP15	432^{d}	12.7	7.56^{ab}	9.84^{b}	64.3^{bc}	38.7^{ab}	119^{b}	68.8^{bc}	14.8	14.3^{b}
SBP30	350^{b}	17.2	8.33^{b}	9.89^{b}	71.3^{cd}	44.0^{bc}	138^{c}	76.1^{c}	19.3	15.8^{b}
SBP45	306^{a}	14.6	8.77^{b}	10.8^{b}	77.5^{d}	49.2^{c}	154^{c}	84.9^{d}	19.4	16.6^{b}
SEM	7.1	2.96	0.542	0.319	2.44	1.54	3.82	1.83	1.44	0.951
Probabilities										
Feed	<0.001	0.624	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.107	<0.001
Linear OH	0.952	0.558	0.979	0.587	<0.001	<0.001	0.002	<0.001	0.971	0.069
Linear SBP	<0.001	0.838	0.125	<0.001	<0.001	<0.001	<0.001	<0.001	0.021	<0.001
Quadratic OH	<0.001	0.539	0.002	0.358	0.046	0.059	0.122	0.629	0.994	0.111
Quadratic SBP	<0.001	0.871	0.996	0.800	0.191	0.992	0.405	0.943	0.987	0.179

[a-d]Means within a column that do not share a common superscript are significantly different ($P < 0.05$)

Abbreviations: ECW, empty carcass weight; BW, body weight.

Table 2.4. Organ measurements expressed relative to empty carcass weight (ECW) from day 22 of age. Broiler chickens were offered feeds diluted with 0% (B), 15%, 30%, or 45% of either Oat Hulls (OH) or Sugar Beet Pulp (SBP).

	ECW (g)	Crop (g/ kg ECW)		Proventriculus Full (g/ kg ECW)	Gizzard (g/ kg ECW)		Small intestine (g/ kg ECW)		Caeca Full (g/ kg ECW)	Large intestine Full (g/ kg ECW)
		Full	Empty		Full	Empty	Full	Empty		
B	782^{c}	17.3a	6.15a	7.37^{bc}	36.4^{a}	23.1^{a}	80.3^{a}	48.5^{ab}	17.1^{bc}	10.8^{ab}
OH15	957^{f}	18.7^{a}	7.01^{ab}	6.06^{a}	37.3^{a}	26.1^{a}	75.8^{a}	46.7^{a}	13.9^{ab}	7.50^{a}
OH30	879^{e}	43.1^{b}	8.80^{b}	6.20^{ab}	47.2^{b}	32.5^{b}	84.8^{ab}	53.0^{bc}	13.6^{ab}	8.82^{a}
OH45	810^{cd}	30.3^{ab}	7.84^{ab}	6.55^{ab}	49.6^{b}	36.4^{c}	94.8^{b}	56.2^{cd}	13.2^{ab}	9.59^{ab}
SBP15	857de	17.9^{a}	6.84^{ab}	7.87^{c}	52.1^{b}	30.2^{b}	111^{c}	59.2^{d}	10.9^{a}	12.7^{b}
SBP30	666^{b}	16.3^{a}	7.93^{ab}	10.0^{d}	66.7^{c}	36.8^{c}	133^{d}	66.1^{e}	13.4^{ab}	16.6^{c}
SBP45	524^{a}	27.5^{ab}	8.43^{ab}	10.2^{d}	69.7^{c}	38.3^{c}	172^{e}	75.1^{f}	19.5^{c}	21.1^{d}
SEM	14.9	4.12	0.571	0.282	1.47	0.94	2.49	1.27	0.95	0.893
Probabilities										
Feed	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.036	<0.001	<0.001	<0.001
Linear OH	0.999	0.019	0.079	0.014	<0.001	<0.001	<0.001	<0.001	0.041	0.886
Linear SBP	<0.001	0.260	0.008	<0.001	<0.001	<0.001	<0.001	<0.001	0.140	<0.001
Quadratic OH	<0.001	0.374	0.417	<0.001	0.887	0.922	<0.001	0.074	0.403	0.024
Quadratic SBP	<0.001	0.413	0.997	0.947	0.005	0.049	0.569	0.934	<0.001	0.512

[a-f]Means within a column that do not share a common superscript are significantly different ($P < 0.05$)

Abbreviations: ECW, empty carcass weight; BW, body weight.

Table 2.5. Organ measurements expressed relative to empty carcass weight (ECW) from day 36 of age. Broiler chickens were offered foods diluted with 0% (B), 15%, 30%, or 45% of either Oat Hulls (OH) or Sugar Beet Pulp (SBP).

	ECW (g)	Crop (g/ kg ECW)		Proventriculus Full (g/ kg ECW)	Gizzard (g/ kg ECW)		Small intestine (g/ kg ECW)		Caeca Full (g/ kg ECW)	Large intestine Full (g/ kg ECW)
		Full	Empty		Full	Empty	Full	Empty		
B	2518^{d}	7.44^{a}	4.25^{a}	1.68^{a}	24.5^{a}	17.4^{a}	47.1^{ab}	32.2^{ab}	11.2^{a}	8.24^{ab}
OH15	2610^{d}	13.3^{a}	4.90^{a}	1.47^{a}	26.4^{a}	18.2^{a}	44.4^{a}	29.2^{a}	8.69^{a}	7.83^{ab}
OH30	2498^{d}	8.75^{a}	5.05^{a}	1.47^{a}	29.0^{ab}	19.6^{a}	47.1^{ab}	31.9^{ab}	10.4^{a}	6.64^{a}
OH45	2211^{c}	9.71^{a}	4.83^{a}	1.47^{a}	34.0^{b}	23.9^{b}	55.5^{bc}	35.0^{bc}	9.93^{a}	9.00^{ab}
SBP15	2262^{c}	8.63^{a}	5.40^{a}	1.73^{a}	34.0^{b}	20.7^{ab}	62.7^{c}	38.8^{c}	9.33^{a}	11.5^{b}
SBP30	1876^{b}	12.0^{a}	6.24^{a}	2.23^{b}	49.4^{c}	24.0^{b}	87.3^{d}	46.7^{d}	11.6^{a}	16.3^{c}
SBP45	1301^{a}	29.7^{b}	9.69^{b}	2.70^{c}	71.3^{d}	30.4^{c}	137^{e}	58.4^{e}	15.4^{b}	21.8^{d}
SEM	34.8	2.59	0.715	0.066	1.51	1.17	3.01	1.06	1.079	1.277
Probabilities										
Feed	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Linear OH	<0.001	0.994	0.894	0.176	<0.001	<0.001	0.001	0.039	0.919	0.987
Linear SBP	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<.0001	0.007	<0.001
Quadratic OH	<0.001	0.529	0.871	0.213	0.224	0.041	0.002	0.004	0.438	0.203
Quadratic SBP	<0.001	<0.001	0.409	0.004	0.016	0.432	<0.001	0.104	0.017	0.757

[a-e]Means within a column that do not share a common superscript are significantly different ($P < 0.05$)

Abbreviations: ECW, empty carcass weight; BW, body weight.

Table 2.6. Duodenum, jejunum and ileum lengths expressed relative to empty carcass weight (ECW) of birds slaughtered on day (d) 15, 22 and 36 of age. Broiler chickens were offered feeds diluted with 0 (B), 15, 30, or 45% of either Oat Hulls (OH) or Sugar Beet Pulp (SBP) from day 8 of age.

	d15			d22			d36		
	Duodenum length (cm/ kg ECW)	Jejunum length (cm/ kg ECW)	Ileum length (cm/ kg ECW)	Duodenum length (cm/ kg ECW)	Jejunum length (cm/ kg ECW)	Ileum length (cm/ kg ECW)	Duodenum length (cm/ kg ECW)	Jejunum length (cm/ kg ECW)	Ileum length (cm/ kg ECW)
B	48.8^{ab}	133^{a}	126^{ab}	27.4^{ab}	83.8^{b}	76.4^{ab}	11.7^{ab}	29.7^{a}	29.4^{ab}
OH15	44.5^{a}	125^{a}	112^{a}	24.9^{a}	70.6^{a}	66.0^{a}	11.3^{a}	31.6^{a}	28.5^{a}
OH30	42.7^{a}	126^{a}	115^{ab}	27.8^{ab}	76.0^{ab}	70.0^{ab}	12.0^{ab}	33.2^{a}	31.0^{ab}
OH45	49.4^{ab}	140^{a}	128^{b}	30.4^{b}	81.1^{b}	75.8^{ab}	13.4^{bc}	39.3^{b}	34.1^{bc}
SBP15	49.7^{ab}	136^{a}	124^{ab}	29.2^{ab}	79.4^{ab}	79.0^{b}	12.7^{ab}	38.4^{b}	35.8^{c}
SBP30	56.6^{bc}	156^{b}	144^{c}	36.1^{c}	95.7^{c}	95.6^{c}	15.0^{c}	45.7^{c}	42.7^{d}
SBP45	59.8^{c}	170^{b}	157^{c}	44.9^{d}	129^{d}	121^{d}	22.6^{d}	64.1^{d}	63.2^{e}
SEM	2.11	3.6	3.5	1.05	2.56	2.38	0.48	1.03	1.29
					Probabilities				
Feed	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Linear OH	1.000	0.424	0.888	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Linear SBP	<0.001	<0.001	<0.001	0.006	0.988	0.992	0.025	<0.001	0.002

[a-f] Means within a column that do not share a common superscript are significantly different ($P < 0.05$)
Abbreviations: ECW, empty carcass weight.

2.5 Discussion

The experiment was designed to test the ability of a modern fast-growing broiler to cope with increasing levels of diet dilution with bulky ingredients. The feeds were formulated so that energy was the first limiting nutrient resource, because the prevailing view is that animals eat for the first limiting nutrient resource in their feed (Emmans and Fisher, 1986; Gous, 2007). The CP content of the basal feed was formulated to be slightly above Ross 308 nutrient recommendations (Aviagen Ltd., Edinburgh, UK). In doing so, the aim was to avoid deficiencies in specific amino acids, such as threonine, for which demand is increased to cope with an increase in epithelial cell turnover as a result of the high dietary fibre contents of the diluted feeds (Montagne *et al.*, 2003; Bortoluzzi *et al.*, 2018). Based on this assumption, the experiment was able to investigate broiler capacity for bulk as the energy content of the feed was reduced, while the bulk content of the feed increased. To test the hypotheses, OH and SBP were selected as diluents because of distinct differences in their bulk properties (such as fibre content, solubility, WHC and feed density) which were expected to affect feed intake and GIT development in different ways (Jørgensen *et al.*, 1996b; Savory *et al.*, 1996; Jiménez-Moreno *et al.*, 2013b).

The suggestion has been put forward that modern strains of broilers are no longer able to regulate their feed intake when they are given access to feeds of different energy contents (see reviews by Classen (2017) and Mbajiorgu *et al.* (2011)). The results of the present study are inconsistent with this suggestion. For the OH series, birds were able to increase linearly their feed intake, in a manner directly related to the linear reduction in energy intake. For example, the feed energy content of OH30 (2273 kcal/ kg feed) was 26% lower than the B feed (3081 kcal/ kg feed), which led OH30 birds to increase ADFI by 22% compared to B birds. This resulted in a similar ADEI by birds in these two treatments. The energy content of the OH30 feed was lower than in studies which did not observe an increase in feed intake (Plumstead *et al.*, 2007; Latshaw, 2008; Delezie *et al.*, 2010; Li *et al.*, 2010; Krás *et al.*, 2013) and experiments where feed intake was increased (Deaton and Lott, 1985; Leeson *et al.*, 1996a; Dozier III *et al.*, 2006a; Linares and Huang, 2010). This suggests that provided that the bulkiness of the feed does not limit feed intake of the birds, birds will respond to the dilution of energy content of the feed in a manner consistent with the principle of the regulation of feed intake (Emmans, 1986; Gous, 2007; Gous, 2013). Whether birds would continue to adapt to reduced feed energy content, will depend on the ability of their digestive tract to adapt to the bulkiness that accompanies such a dilution.

Tallentire *et al.* (2018a) suggested that modern broilers are reaching the limits of feed intake. The highest feed intake previously seen by broilers has been observed by Leeson *et al.* (1996a), who showed that broilers were able to increase feed intake by 25% without negatively affecting performance when offered feeds diluted with 3.75% each of OH and sand, compared to birds offered a basal feed. Tallentire *et al.* (2018a) assumed that this is the maximum capacity of the GIT for intake in broilers. On the basis of this, they then estimated how much the feed intake capacity of modern strains could increase. Figure 2.1 shows the ADFI (g/ day) from the current experiment plotted alongside the estimated daily feed intake calculated by Tallentire *et al.* (2018a). Over the period of 22-36 days, when it may be assumed that the adaptation of the GIT had reached an equilibrium (see below), the ADFI of the birds on OH30 was 101g. This is 20% higher than the 'maximum' feed intake suggested by Tallentire *et al.* (2018a) over the same live weight range for this broiler strain.

It was hypothesized that broilers offered feeds diluted with SBP will be limited in their performance further than those offered feeds diluted with OH due to differences in the bulk properties of the two ingredients. When the performance of the birds over the whole experimental period was considered, all birds on the diluted feeds had a higher ADFI than the birds on the B feed, with the exception of birds on SBP45. Given that dilution of the B feed with bulky ingredients resulted in reduction of the energy content of the feeds, birds on OH45, SBP30 and SBP45 consumed less ADEI than birds on B throughout the experiment and as a consequence their growth performance was penalised. One therefore, might be tempted to suggest that these three feeds were limiting energy intake through their bulkiness. However, the long-term picture (35 days) is likely to be masking two separate effects: 1) the relationship between ADFI and BW (Whittemore *et al.*, 2003a) and 2) any adaptation that might be occurring over time on the bulky feeds (Zubair and Leeson, 1996; Tsaras *et al.*, 1998; Whittemore *et al.*, 2001a). For this reason, ADFI was scaled per unit of BW and then analysed the scaled ADFI for each of the experimental weeks (week 1-4). These calculations suggest that whilst scaled ADFI might have been limited in some feeds during weeks 1 and 2, by week 4 it was only the SBP45 feed that appeared to be limiting scaled ADFI (and thus energy intake) of the birds: the scaled ADEI of week 4 was 187 (SE 2.85) and 251 (SE 3.37) (kcal/ kg BW/ day) on feeds SBP45 and B respectively ($P < 0.01$). As a consequence of the increased scaled ADFI, birds offered the diluted feeds (other than SBP45) were able to maintain performance to that of birds given the B feed over the whole experimental period. The objective of reducing performance in the SBP birds further than the OH birds was therefore partially fulfilled, although only the SBP45 birds were limited.

As expected and consistent with ADFI, the relative weights of the components of the GIT increased as the amount of the bulky ingredients in the feeds increased, thus demonstrating the great phenotypic plasticity of the broiler GIT (Klasing, 1999). For the OH series the increase in the relative weights GIT components was confined to the gizzard and small intestine. Gizzard development was more pronounced in the SBP series than the OH series, which is inconsistent with studies that have shown the converse and whose authors ascribe the effect to the greater insoluble fibre content of OH (Jiménez-Moreno *et al.*, 2013b). Svihus (2011) suggested that the grinding capability of the gizzard may be limited when birds are fed high fibre feeds. On the contrary, the present results show that the size of the gizzard responded linearly to diet dilution at each of the slaughter points and therefore was not limited in its development nor responsible for limiting feed intake. This is consistent with other authors who also observed a linear increase in gizzard development when offering feeds with increasing levels of dietary fibre (Summers and Leeson, 1986; Jiménez-Moreno *et al.*, 2011; Miya *et al.*, 2019; Okrathok and Khempaka, 2020).

Typically, gizzard development and retention time have been investigated in relation to insoluble fibre sources (Amerah *et al.*, 2009; Sacranie *et al.*, 2012), which are often offered in mash form (Adibmoradi *et al.*, 2016; Abdollahi *et al.*, 2019) or crumble (Svihus *et al.*, 2002; Svihus *et al.*, 2004; Hetland *et al.*, 2005). Furthermore, Jiménez-Moreno *et al.* (2019) showed that the effect of diet dilution with insoluble fibre sources on GIT development is greater when the feeds are offered as mash rather than pellets, with the exception of the crop. It is therefore possible that the differences in gizzard development between this experiment and that of Jiménez-Moreno *et al.* (2013b) are due to differences in feed form. To that end, it is important that feed form should be considered when assessing the adaptation and capacity of the GIT to bulky feeds.

The remaining organ measurements from this experiment were greater in the SBP rather than OH series, consistent with the findings of Jiménez-Moreno *et al.* (2013b). For the OH series, the increase in the relative GIT weights was confined to the gizzard and small intestine. For the SBP series, the increase in the relative GIT weights was also extended to the proventriculus and the large intestine. SBP is rich in soluble non-starch polysaccharides such as pectin and therefore has a high WHC, which results in wetter faeces (Mossami, 2011) and this physical distension of the GIT (or bulk increase) is carried throughout the digestive tract (González-Alvarado *et al.*, 2010). Consistent with the results of this experiment, the relative weight of the proventriculus has been shown to increase when broilers are offered feeds diluted with bulky ingredients (Amerah *et al.*, 2009). However, the role of the large intestine in the accommodation of bulky feeds is often overlooked (Amerah *et al.*, 2009; Jiménez-Moreno *et*

al., 2009a; Jiménez-Moreno *et al.*, 2013b; Adibmoradi *et al.*, 2016; Abdollahi *et al.*, 2019), although there is some evidence showing an increase in the relative weight of the large intestine when broilers given feeds diluted with pea fibre, wheat bran or oat bran (Jørgensen *et al.*, 1996a).

The initial reduction in feed intake when an animal is offered a bulky feed, or inability to increase feed intake to maintain energy intake, reflects the fact that the GIT may not have yet fully adapted to the bulky feed (Starck, 1999; Whittemore *et al.*, 2003b). There was some evidence that this was the case for the birds given the OH30, OH45, SBP15 and SBP30 feeds. For the first three feeds the change in the rate of ADFI seems to have taken 8-10 days, whereas it was around 14-16 days for the SBP30 feed. This is also reflected in the weekly scaled ADFI (Figure 2): birds on SBP15 increased their relative ADFI from week 2. It was only by week 4 that the relationship between ADFI on B, SBP15 and SBP30 became linear. Finally, the higher relative ADFI on the SBP as opposed to OH series (with the exception of SBP45) by week 4 is consistent with the lower energy content of the SBP feeds, and the need to further compensate for this. As the current experiment was conducted on birds of a relatively young age (8 days), it is possible that different adaptation responses would have occurred if the birds were offered the bulky feeds at a later age (Mateos *et al.*, 2012). Evidence shows that when broilers are not introduced to the diluted feeds until d35 of age they are unable to increase feed intake to compensate for the reduction in energy content (Leeson *et al.*, 1996b; Sahraei and Shariatmadari, 2007). This is logical when one considers the developmental plasticity of a newly hatched chick and the dramatic relative increases in GIT development in the first 10 days of life (Sklan, 2001). These suggestions are of relevance when considering the age at which to introduce lower energy or bulky feeds to growing broilers.

The second objective of the experiment was to identify a bulk property that may be used to predict the feed intake of broilers on bulky feeds. As discussed previously, the only feed that appeared to be limiting ADFI throughout the experiment was SBP45; additionally, birds fed the SBP series had greater relative weights of the GIT than birds on the OH series, suggesting that birds given SBP needed to accommodate a higher bulk intake. Out of the several measurements of bulk analysed, SBP feeds had a higher WHC, feed density and soluble dietary fibre content than OH feeds at the same level of inclusion. Some of these bulk characteristics are expected to be highly correlated (such as WHC and soluble fibre content), as they relate to the same physicochemical properties (Brachet *et al.*, 2015). Although the digestibility of the feeds was not measured in this experiment, other work (Jiménez-Moreno *et al.*, 2013a) has suggested that the digestibility of OH and SBP feeds is similar for the same levels of inclusion. The Kyriazakis group (Kyriazakis and Emmans, 1995; Tsaras *et al.*, 1998; Whittemore *et al.*,

2003b) has suggested that for pigs the WHC of the feeds is a good predictor of scaled feed intake on bulky feeds. The relationship appears to be of the form: scaled ADFI (g/ kg/ d) = *a* * 1/WHC (g feed/ g water). If it is assumed that the same relationship applies for broilers, then the value of *a* for the SBP30 and SBP45 feeds would be 700 (SE 10.5) and 889 (SE 11.3), during the last week of the experiment. The critical WHC value of the feed that determines maximum capacity for bulk would be between 4.47 and 6.01 (g/ g DM). Clearly this hypothesis needs to be tested on a wider range of feed ingredients and on feeds that would certainly limit the feed intake of the birds throughout the experimental period.

In conclusion, the results do not offer support to the suggestion that modern broiler strains are no longer able to control their feed intake when they are given access to feeds of different energy contents (Classen (2017) and Mbajiorgu *et al.* (2011)). Birds responded to the dilution of the basal, high quality feed in a manner expected from the principle of regulation of energy intake. In fact, the extent of the increase in feed intake is well beyond the increases previously seen in any experiment addressing the capacity of broilers for bulky feeds (Leeson *et al.*, 1996a; Dozier III *et al.*, 2006a; Linares and Huang, 2010). Given the fact that the levels of inclusion of OH and SBP in the feeds are well above what one might envisage in practice when incorporating bulky ingredients, the concerns about the energy content of modern broiler feeds (Linares and Huang, 2010; Gous, 2013; Classen, 2017; Tallentire *et al.*, 2018a) may be unwarranted. Although only one broiler strain was used in this study (Ross 308), some generalisation about these findings may be justified due to its global popularity. Identifying a property of feed ‘bulkiness’ that will allow the prediction of feed intake and gut capacity of broilers given bulky feeds remains a challenge and will be explored further in Chapter 3.

Chapter 3. Towards the prediction of feed intake of modern broilers on bulky feeds

3.1 Abstract

The use of alternative, often bulky ingredients is becoming more widespread in poultry feeds as the industry seeks to reduce its economic and environmental costs. Consequently, there is an increased need to accurately predict the performance of birds given such feeds and identify their maximum capacity for bulk. Feeds diluted with a range of bulky ingredients were offered to male Ross 308 broilers to assess their capacity for bulk and identify a bulk characteristic responsible for limiting intake. 495 day-old broilers allocated into 45 pens, were offered a common starter feed until day (d) 8, and 1 of 9 grower feeds from d8-29 (Period 1). Each of the grower feeds was diluted with either 30 or 60% of oat hulls (OH), wheat bran (WB) or grass meal (GM), or a mixture of two bulky ingredients at an inclusion level of 30% each (OHWB, OHGM, WBGM). From d29-43 (Period 2), all birds were offered the bulkiest feed (GM60). A number of bulk characteristics were measured on the feeds. Feed intake was measured daily, and birds were dissected on d29 and 43 for organ and carcass measurements. During d8-14 water-holding capacity (WHC) was more consistent in predicting feed intake when scaled per unit of body weight than any other feed bulk characteristic. However, this was no longer the case during d15-18. In Period 2, the response and adaptation to the bulkiest feed was determined by previous experience to bulk. Birds offered a more bulky feed during Period 1, were better able to adapt the size of their digestive organs and increase scaled feed intake, such that there were no differences between these birds and those offered the GM60; the converse was the case for birds on the least bulky feeds (e.g. OH60). It was concluded that WHC is able to predict maximum intake on bulky feeds in unadapted birds. Adaptation to bulky feeds can be very fast, so that their high bulk content no longer limits feed intake and performance.

3.2 Introduction

Prediction of voluntary feed intake is the basis of any simulation model that aims to account for bird performance under different management conditions (Kyriazakis and Emmans, 1999; Gous, 2007). The inclusion of alternative ingredients in poultry feeds is becoming increasingly common to achieve sustainable food production and ensure global food security (Morgan and Choct, 2016; Tallentire *et al.*, 2018b; Tufarelli *et al.*, 2018; Wyngaarden *et al.*, 2020), as such there is an urgent need to understand and predict the ability of modern broiler genotypes to cope with such alternative ingredients, which are typically bulky in nature (Ravindran, 2013; Morgan and Choct, 2016; Scholey *et al.*, 2020).

There have been concerns over the ability of modern broilers to modify their intake as the energy content of the feed is diluted (Gous, 2013; Classen, 2017). However, there is now evidence to suggest that modern broiler genotypes have indeed retained the ability to regulate energy intake, as the energy content of the feed is reduced (Linares and Huang, 2010; Nascimento *et al.*, 2020; Taylor *et al.*, 2021). Clearly this ability is not infinite and holds only up to a maximum feed intake, which presumably relates to the maximum capacity of the gastrointestinal tract (GIT) for volume or bulk. The ability of broilers to cope with feeds that are diluted with fibrous or bulky materials remains somewhat unclear. Two recent experiments provide contradictory evidence in quantifying a feed characteristic at which the maximum capacity for bulk and hence feed or energy intake is reached (Nascimento *et al.*, 2020; Taylor *et al.*, 2021). Nascimento *et al.* (2020) offered feeds diluted with a variety of bulky ingredients including cellulose fibre, rice husks, sawdust, vermiculite and sand. They suggested that the water holding capacity (WHC, g water/g) of a feed could predict the maximum feed intake capacity of broilers, with the maximum scaled feed intake achieved on feeds with approximately 2.6 g/g WHC. On the other hand, in Chapter 2 (Taylor *et al.*, 2021), it was suggested that the maximum scaled capacity for bulk could lie between 4.47 and 6.01 g/g WHC, but it was not possible to define the maximum scaled intake on feeds that were progressively diluted with oat hulls or sugar beet pulp. In addition, Nascimento *et al.* (2020) did not find any adaptation to the bulky ingredients over time (for 45 days), whereas the results from Chapter 2 suggested that adaptation to bulky feeds took approximately 15 days, although this depended on the nature of the bulky ingredient.

Any model that aims to accurately predict feed intake on bulky feeds must be able to account for the rate of GIT adaptation to the bulky feeds, as this will have direct implications on feed intake and by extension on growth performance (Whittemore *et al.*, 2003b). It is during this period of adaptation that feed intake and performance will be depressed to the

greatest extent (Taylor *et al.*, 2021). The rate of adaptation is seemingly dependent upon the physicochemical characteristics of the bulky ingredient (Taylor *et al.*, 2021) and likely dependent upon the age at which birds are first introduced to the bulky feed (Jørgensen *et al.*, 1996b; Leeson *et al.*, 1996; Sahraei and Shariatmadari, 2007). The ability to predict the extent to which intake and performance will be reduced, and for how long the period of adaptation will last after introduction to a bulky feed, will allow more accurate feed intake prediction for bulky feeds to be developed, and may help to develop feeding strategies that will account or even minimise the effects of adaptation.

The objectives of this study were 3 fold: 1) to determine the capacity of a modern broiler genotype for a variety of bulky ingredients, which vary widely in their bulk characteristics, 2) to define a physical or chemical measure of bulkiness that accounts for the constraining effect of bulk on voluntary feed intake, 3) to determine the rate of adaptation to bulky feeds. The expectation is that broilers given bulkier feeds will undergo a longer period of adaptation than those given less bulky feeds. Furthermore, it was hypothesised that feed intake on mixtures of bulky ingredients will result from the principle of additivity for bulkiness, and that previous experience on bulky feeds will improve the ability of birds to cope with a bulkier feed.

3.3 Materials and methods

3.3.1 *Bird management*

All procedures were conducted under the UK Animals (Scientific Procedures) Act 1986 and approved by the AWERB of Newcastle University (no. 7332/2018). A total of 495 male Ross 308 chicks were obtained at day (d) 0 of age from a commercial hatchery and were housed in a thermostatically controlled building with 45 pens, each with an area of 0.85m^2. All birds were wing tagged upon arrival. Pens were equipped with feeders and drinkers, with wood shavings used as litter at a depth of 5 cm. The birds had free and continuous access to feed and water throughout the trial. The pen temperature was set to 34°C at arrival and was gradually reduced to 20°C by d25 of age. The lighting schedule was 23h Light (L):1h Darkness (D) for the first 7 days and was amended to 18L:6D for the course of the trial, whilst light intensity at pen level ranged from 80-100 lux.

Birds were individually weighed at arrival (d0), prior to treatment allocation (d8) and then twice per week until the end of the trial (every 3 and 4 days post allocation). After weighing the birds on d8, the stocking density was reduced from 11 to 10 birds per pen. Chicks were then distributed between pens in a randomised block manner, so that there were no significant differences in the mean body weight (BW) between treatments. Pen feed intake was measured from d0 to d8, and then daily until the conclusion of the experiment on d43.

3.3.2 *Feed formulation and experimental design*

All birds were fed the same conventional starter feed from d0 until d8 of age, when they were then offered one of nine experimental feeds from d8-28 of age (Period 1; Table 3.1). The starter feed followed breeder's recommendations and was identical to what was used in Chapter 2. At d29 all birds were switched to the same experimental feed (see below), until the conclusion of the experiment on d43 (Period 2).

A basal feed appropriate for the growing phase was formulated in Chapter 2 and was diluted with 60% of either Oat Hulls (OH), Wheat Bran (WB) or Grass Meal (GM) to produce three bulky feeds: OH60, WB60, GM60. The three bulky ingredients were chosen for their substantial differences in the physical and chemical properties, consistent with experimental objectives. Each bulky feed had the same calculated digestible protein to AME ratio, and all other nutrient to AME ratios were the same as in the basal feed. Nutrient ratios were maintained by increasing or decreasing the inclusion of synthetic amino acids, limestone and monocalcium phosphate inclusion, where appropriate. To increase palatability and binding of the pellets, Lignobond pellet binder was included at 1%. All feeds contained titanium dioxide (0.5%) as an indigestible marker.

Mixtures of the basal feed and the three bulkiest feeds were created to produce a further 6 bulky feeds, so all resulting feeds had the same nutrient to AME ratios. The first mixture series was one-part basal and one-part bulky feed to produce a 30% dilution for each diluent: feeds OH30, WB30, GM30. The second mixture produced the final three feeds by mixing two of the 60% level of inclusion feeds: feeds OHWB, OHGM, WBGM. Thus, the resulting mixtures contained 30% of each of the bulky ingredients. All experimental feeds were offered in pellet form.

Each of the nine bulky feeds were replicated in 5 pens during Period 1 (d8-28). After removal of birds for sampling on d29, all birds where switched to feed GM60, which was the bulkiest of the feeds offered during Period 1. They remained on this feed until d43, which was the conclusion of the experiment (Period 2).

Table 3.1. Ingredient, calculated and analysed chemical composition of the grower feeds offered from d8 to d28 of age to broiler chickens. The feeds included either 30% or 60% of one of the bulky ingredients Oat Hulls (OH), Wheat Bran (WB) and Grass Meal (GM). Three additional feeds were formulated by mixing two of the 60% bulky feeds at a time: feeds OHWB, OHGM and WBGM.

Ingredients (%)	OH30	OH60	WB30	WB60	GM30	GM60	OHWB	OHGM	WBGM
Ground maize	7.00	4.00	7.00	4.00	7.00	4.00	4.00	4.00	4.00
Ground wheat	35.2	17.4	35.6	22.4	32.8	12.7	19.9	15.0	17.6
Soybean meal (48% CP)	16.3	10.7	15.1	4.9	17.4	12.9	7.81	11.8	8.91
Full fat soya	4.20	2.40	4.20	2.40	4.20	2.40	2.40	2.40	2.40
Oat hulls (OH)	30.0	60.0	-	-	-	-	30.0	30.0	-
Wheat bran (WB)	-	-	30.0	60.0	-	-	30.0	-	30.0
Grass meal (GM)	-	-	-	-	30.0	60.0	-	30.0	30.0
Limestone	1.09	0.71	1.20	0.95	1.28	1.08	0.83	0.90	1.02
Monocalcium phosphate	0.90	0.57	1.43	1.60	1.48	1.72	1.09	1.15	1.66
L-Lysine HCL	0.31	0.32	0.30	0.30	0.48	0.65	0.31	0.49	0.48
DL-Methionine	0.30	0.24	0.29	0.23	0.38	0.41	0.24	0.33	0.32
L-Threonine	0.16	0.17	0.13	0.10	0.26	0.36	0.14	0.27	0.23
Valine	0.06	0.13	0.00	0.00	0.15	0.30	0.06	0.21	0.15
Soya oil	2.31	1.32	2.31	1.32	2.31	1.32	1.32	1.32	1.32
Salt	0.18	0.10	0.18	0.10	0.18	0.10	0.10	0.10	0.10
Sodium bicarbonate	0.11	0.06	0.11	0.06	0.11	0.06	0.06	0.06	0.06
Vitamin and mineral premix[a]	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Titanium dioxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Ronozyme	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Lignobond pellet binder	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*
Chemical composition (%) [b]									
Metabolizable energy (kcal kg^{-1}) (calculated)	2483	1960	2698	2385	2457	1846	2172	1903	2115
Crude protein (CP)	15.6	12.5	17.0	15.0	18.5	17.7	13.8	15.1	16.4
Lysine (calculated)	0.82	0.84	0.89	0.82	1.12	1.08	0.90	0.97	1.00
Ether extract (oil A)	5.21	6.14	6.10	5.76	5.13	4.93	6.06	5.83	4.51
Total oil (oil B)	5.64	0.84	6.13	7.02	5.67	5.42	6.54	6.40	5.09
Ash	4.60	5.20	7.90	8.10	9.50	11.50	7.00	7.80	7.10
Dry Matter	89.7	91.0	89.3	89.0	90.1	92.0	90.3	90.6	92.1
Calcium (calculated)	0.72	0.56	0.77	0.67	0.84	0.80	0.62	0.68	0.73
Available Phosphorus (calculated)	0.50	0.40	0.54	0.46	0.58	0.56	0.43	0.48	0.51
DM digestibility	-	67.4	69.7	67.6	65.9	64.3	62.1	60.3	63.7
Crude fibre	6.70	12.2	6.40	9.30	12.3	16.7	10.7	14.6	13.4
Neutral detergent fibre	17.8	27.6	22.0	28.9	23.9	32.8	28.1	31.1	30.7
Acid detergent fibre	7.76	13.6	6.47	9.70	13.9	20.1	11.6	17.3	15.4
Acid detergent lignin	2.51	3.64	2.05	2.65	2.12	3.92	3.09	3.74	3.28
Feed density (g/ ml)	1.25	1.47	1.19	1.39	1.43	1.75	1.41	1.62	1.58
Water Holding Capacity (g/ g DM)	2.71	3.15	3.55	4.38	4.16	5.94	3.94	4.43	5.02

[a] Blend of amylase and beta-glucanase

[b] Analysed composition unless otherwise stated

3.3.3 *Sampling*

On d29 and d43 of age, two birds per pen with a BW close to the pen average were culled by intravenous lethal injection with sodium pentobarbital (Euthatal, Merial Harlow, United Kingdom). Immediately following euthanasia all birds were weighed. The full GIT of each of the two birds per pen was removed and weighed in full. The lengths of the duodenum, jejunum and ileum were recorded, and a sample of digesta from each bird was obtained from the lower 2/3 of the ileum for digestibility analysis. The GIT was then separated into its individual components: crop, proventriculus, gizzard, duodenum, jejunum, ileum, caeca and large intestine. Each of the components were weighed full, before being carefully emptied of their digesta contents by gentle finger stripping to obtain empty weights. Empty carcass weight (ECW) was obtained by weighing the carcass with the GIT removed. Empty body weight (EBW) was then calculated as BW (g) – gut fill (g).

3.3.4 *Feed analysis*

All classical descriptors of bulk used traditionally in livestock research were measured. Some of these descriptors are highly correlated as they essentially measure the same bulk characteristics through different methods (Brachet *et al.*, 2015). Samples of all feeds were analysed for crude fibre (Test method: Commission Regulation (EC) No.152/2009), acid detergent fibre (Test method: AOAC 973.18-1977), neutral detergent fibre (Test method: AOAC 2002.04-2005) and acid detergent lignin (Test method: AOAC 973.18-1977) at a UKAS accredited commercial laboratory to the internationally recognised standard for competence (Sciantec Analytical Services, Cawood, UK).

WHC analysis was performed in triplicate, using an adaptation of the Robertson and Eastwood (1981) method. A 1 g feed sample was soaked in 250 ml of H_2O at room temperature for 24 hours. Subsequently, the samples were filtered through a Whatman no. 1 filter paper and the wet weight of the samples recorded, before the samples were placed into an oven at 105 °C overnight and the dry weight was recorded. WHC was then calculated as g of water/ g of DM.

Feed density was determined in triplicate by the method described by Kyriazakis and Emmans (1995). First, 100 ml of distilled water at 37°C was placed in a 250 ml flask and a 50g sample of feed was added. After mixing, a further 50 ml of water was added, and the contents allowed to equilibrate for 15 minutes before a final 50 ml of water was added. The sample was left to equilibrate for a further 15 minutes before the flask was filled to volume with water with a burette. The total amount of water contained in the flask was subtracted from 250 ml.

3.3.5 *Sample analysis*

3.3.5.1 *Digestibility*

Analysis of digesta for titanium dioxide (TiO_2) concentration were performed according to the method of Short *et al.* (1996). Digesta samples were freeze-dried for 4 days before being stored at 4°C, pending analysis. A sub-sample of 0.1 g was placed in a microwave furnace at 600°C for 1 hour until the sample was ashed and the ash weight was recorded. Following ashing, 10 ml of 7.4 M H_2SO_4 was added to the sample and placed in screw cap tubes and then microwave digested (CEM, MARS-5) for a further 1 hour. Samples were then filtered through a Whatmann no.2 filter paper into 100 ml volumetric flasks, before 20 ml of hydrogen peroxide was added. The solution was then topped up to 100 ml with deionised water and shaken thoroughly. Then 3.5 ml of the solution was aliquoted to a curvette and ran through a spectrophotometer (Biochrom Libra S12) at 410 nm to measure the amount of light absorbance of the solution. Using a standard curve, the absorbance indicated the amount of TiO_2 in the solution, which was then used to calculate the digestibility coefficient by the following equation:

DM digestibility (%) = 100-100 × TiO_2 in feed DM/ TiO_2 in digesta DM

3.3.6 *Calculations and Statistics*

To account for mortalities the feeder was weighed when mortalities were found and the average feed intake (g/ bird) was calculated and the assumed intake of the dead bird was subtracted from ADFI. ADFI, ADG and FCR were calculated over weekly intervals; data for Period 1 (weeks 1-3) and Period 2 (weeks 4-5) were analysed separately. To account for *a-priori* differences in growth rate due to feed composition, ADFI and ADG data were scaled relative to the mean weekly BW (Whittemore *et al.*, 2001b). The scaled feed intake and daily gains were then transformed by the natural logarithm to ensure that the residuals were normally distributed and avoid statistical bias (Allison *et al.*, 1995). A repeated-measures mixed model was implemented to analyse the transformed scaled feed intake for each week of Period 1, to assess whether broilers adapted to the different feeds over time. A significant increase in the scaled maximum intake of each successive period would indicate that the birds were adapting to the given feed. The model included feed and week as fixed factors, the two-way interaction between feed and week, and pen as a random factor. For consistency, the transformed scaled ADG from Period 1 was also analysed in a repeated measures model which included feed and week as fixed factors and the two-way interaction between feed and week.

Similarly, a repeated measures mixed model was implemented to analyse the transformed scaled feed intake, transformed daily gains and FCR for each week of Period 2; the model

included feed during Period 1 and week as fixed factors, and the two-way interaction between previous feed and week. For both Period 1 and 2 analyses, covariance structures were chosen based on the lowest value for the Akaike and Bayesian information criteria.

Organ measurements were expressed relative to the EBW of the bird (g/ kg EBW) at the end of either Period 1 (d29) or Period 2 (d43) to account for differences in growth performance (Oikeh *et al.*, 2019). These data were analysed with the general linear mixed (GLM) procedure with feed as a fixed factor.

To evaluate which feed characteristics had the highest correlation with scaled feed intake, a principal component analysis (PCA) was used. For the PCA 11 variables (feed characteristics) were considered: crude protein, Oil A, Oil B, dry matter, crude fibre, neutral detergent fibre, acid detergent fibre, acid detergent lignin, feed density, water-holding capacity, and indigestible matter (1-digestibility).

All statistical analysis was performed in R (Team, 2013), using the *factoextra* package and *prcomp* function to perform the PCA, and the *nlme* package and *lm* and *anova* functions for both the repeated measures model and GLM procedures (Pinheiro *et al.*, 2014). For all statistical procedures, the normality of the residuals was assessed with qq-plots and the Shapiro-Wilk test; data did not need any further transformation. When significant differences were detected, treatment means were separated and compared by the Tukey's multiple comparison test. Significance was determined at $P < 0.05$.

3.4 Results

3.4.1 *Feed analysis*

All measured characteristics of bulk increased when dilution levels increased from 30% to 60%. The crude fibre, neutral detergent fibre, acid detergent fibre, acid detergent lignin, feed density and WHC were greater in the GM60 feed compared to all other feeds. The lowest crude fibre, acid detergent fibre, acid detergent lignin and feed density were found in the WB30 feed, whereas the lowest neutral detergent fibre and WHC were found in the OH30 feed. DM digestibility was greatest in the WB30 feed and lowest in the OHGM feed. There was additivity in all bulk characteristics when the feeds were diluted with two bulky ingredients.

3.4.2 *Period 1*

3.4.2.1 *Mortalities, Feed intake, average daily gain and FCR*

There were 9 mortalities (2 birds from WB30 and 1 bird from each of the remaining treatments) from d14 to d37 of age, the cause of mortality was unknown. Some birds on OH30 developed severe feather pecking early on in the experiment (by day 18) and this

treatment was discontinued on welfare grounds; the observation was unique to this treatment, which will not be considered any further. The progression of daily feed intake from d8-28 of age is shown in Figure 3.1 and the back transformed scaled feed intake (g/ kg/ day), scaled daily gains (g/ kg/ day) and FCR over week 1 and weeks 2-3 are presented in Table 3.2. There were no differences in the direction of the change in scaled feed intake between weeks 2 and 3, and for this reason these weeks were considered together. There was a significant interaction between feed and week on the log transformed scaled feed intake ($P < 0.001$). The interaction was due to the differences in the direction of the change in the transformed scaled feed intake between treatments over time (week 1 vs weeks 2-3): whilst scaled feed intake decreased for the majority of the treatments over time, this was not the case for GM60, where scaled feed intake increased from week 1 to weeks 2-3.

There was a significant interaction between feed and week on the log transformed scaled daily gains ($P < 0.05$). The scaled daily gains of the GM30 birds were significantly higher than all other treatments in week 1, but were no longer significantly different from OH60, WB30, OHWB, OHGM and WBGM birds in weeks 2-3 ($P > 0.05$). There was no interaction observed on FCR ($P > 0.05$), but values in week 1 were significantly lower ($P < 0.001$) than values in weeks 2-3 across all treatments.

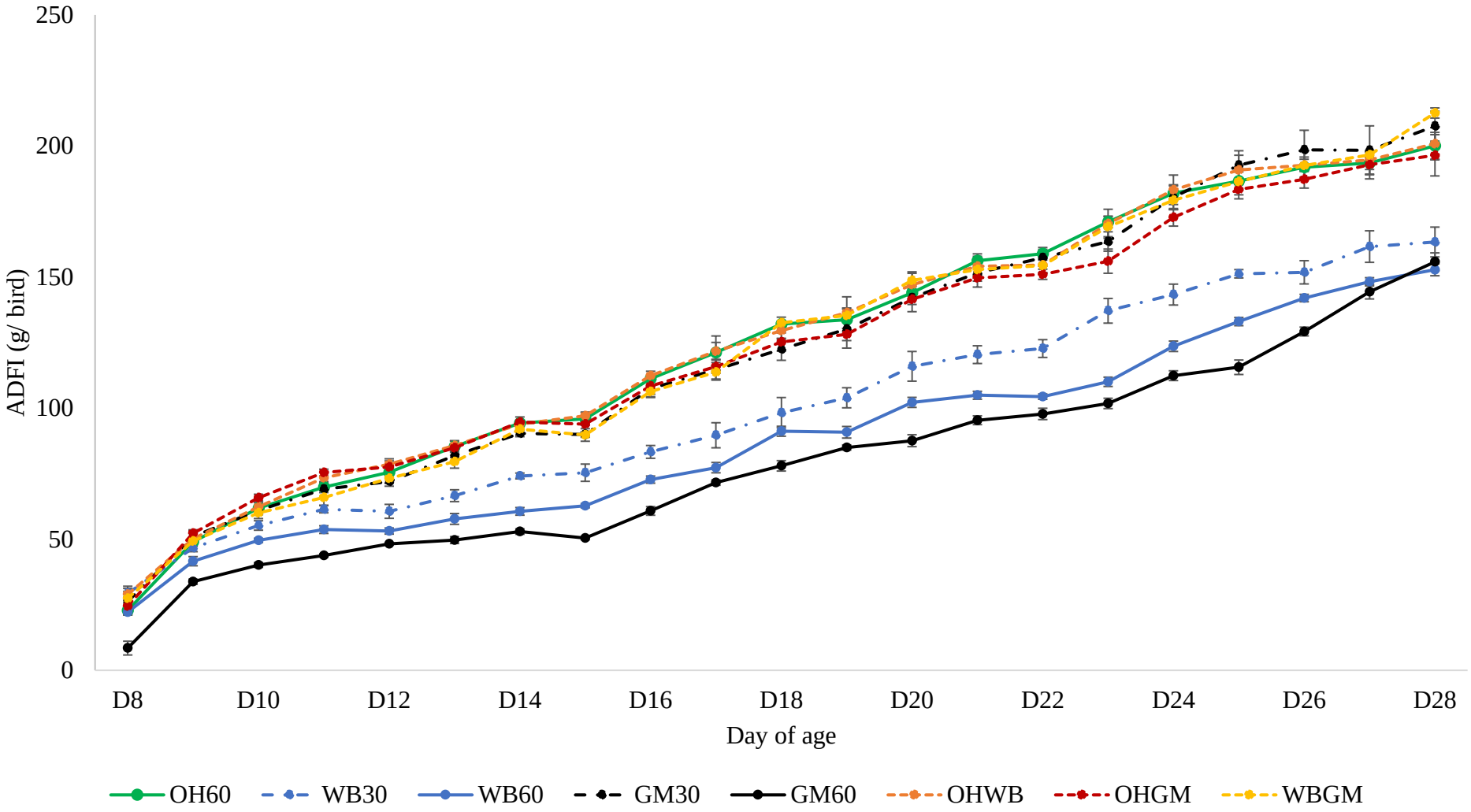


Figure 3.1. Average daily feed intake (ADFI; g/ bird) of broiler chickens given access to feeds containing Oat Hulls (OH at 60%), Wheat Bran (WB at either 30 or 60%), Grass Meal (GM at either 30 or 60%), or feeds containing a mixture of two bulky ingredients at an inclusion level of 30% each (OHWB, OHGM, or WBGM), from d8-28 of age.

Table 3.2. Average daily feed intake and average daily gain expressed relative to the mean body weight of the time period (g/ kg/ day), and FCR calculated over d8-14 (week 1) and d15-28 (week 2-3). Broiler chickens were given access to feeds containing Oat Hulls (OH at 60%), Wheat Bran (WB at either 30 or 60%), Grass Meal (GM at either 30 or 60%), or feeds containing a mixture of two bulky ingredients at an inclusion level of 30% each (OHWB, OHGM, or WBGM). Variables were analysed after transformation by natural logarithms and are presented here as back transformed means with confidence intervals. Means that do not share a common superscript are significantly different ($P < 0.05$) and represent the interaction between previous feed and week.

	ADFI/ BW (g/ kg/ day)		ADG/BW (g/ kg/ day)		FCR	
	W1	W2-3	W1	W2-3	W1	W2-3
OH60	198^{ef} (185-214)	162^{bcd} (151-174)	101^{e} (94.6-108)	79.8^{cd} (74.4-85.6)	2.00 (1.57-2.42)	2.02 (1.60-2.45)
WB30	178de (165-191)	140^{a} (129-150)	105^{e} (98.5-112)	75.9^{bc} (70.8-80.6)	1.92 (1.50-2.34)	2.10 (1.67-2.52)
WB60	164^{cd} (153-176)	164^{cd} (153-176)	85.6^{d} (79.8-91.8)	64.1^{a} (59.7-68.7)	1.94 (1.52-2.37)	2.08 (1.65-2.50)
GM30	181de (169-198)	140^{a} (130-150)	119^{f} (112-128)	79.8^{cd} (75.2-85.6)	1.76 (1.34-2.18)	2.38 (1.96-2.81)
GM60	144^{ab} (134-154)	154^{abc} (144-166)	80.6^{cd} (74.4-88.2)	68.7^{ab} (64.7-73.7)	1.84 (1.41-2.26)	2.00 (1.58-2.43)
OHWB	214^{f} (198-233)	166^{cd} (153-178)	100^{e} (93.7-107)	81.5^{cd} (75.9-87.4)	1.81 (1.39-2.24)	1.99 (1.57-2.42)
OHGM	212^{f} (196-230)	162^{cd} (151-176)	99.0^{e} (92.8-106)	78.3^{cd} (73.0-83.9)	2.12 (1.70-2.54)	2.23 (1.80-2.65)
WBGM	209^{f} (192-224)	169^{cd} (156-181)	105^{e} (97.5-111)	81.5^{cd} (75.9-86.5)	1.64 (1.21-2.06)	1.88 (1.46-2.31)
			Probabilities			
Feed	<0.001		<0.001		0.257	
Week	<0.001		<0.001		<0.001	
Feed × Week	<0.001		<0.001		0.325	

3.4.2.2 *Relationship between Scaled Feed Intake and bulk characteristics*

Figure 3.2 shows the projections of the scaled feed intake and bulk characteristics on the first two dimensions of the PCA during week 1. The analysis identified that PC1 accounted for 61.9 % of the total variation and PC2 for a further 19.8% of the variation in the dataset. All fibre-related feed characteristics (CF, ADF, NDF, ADL), WHC and feed density accounted for equal amounts of the variation within PC1, but the variable which was most highly correlated with scaled feed intake was WHC (- 0.483; $P < 0.05$). This relationship between scaled feed intake and WHC is shown in Figure 3.3a. The correlation between WHC and most fibre characteristics was high and positive (+ 0.711 to +0.739; $P < 0.05$), but moderate between WHC and ADL (+ 0.477; $P < 0.05$).

Using data from weeks 2-3, PC1 accounted for 60.2% of the total variation whilst PC2 accounted for a further 20.9% of the variation in the dataset (PCA projections not shown). Fibre characteristics accounted equally for the variation within PC1; however, the correlation between scaled feed intake and WHC was weak and positive (+ 0.155; $P > 0.05$), shown in Figure 3.3b.

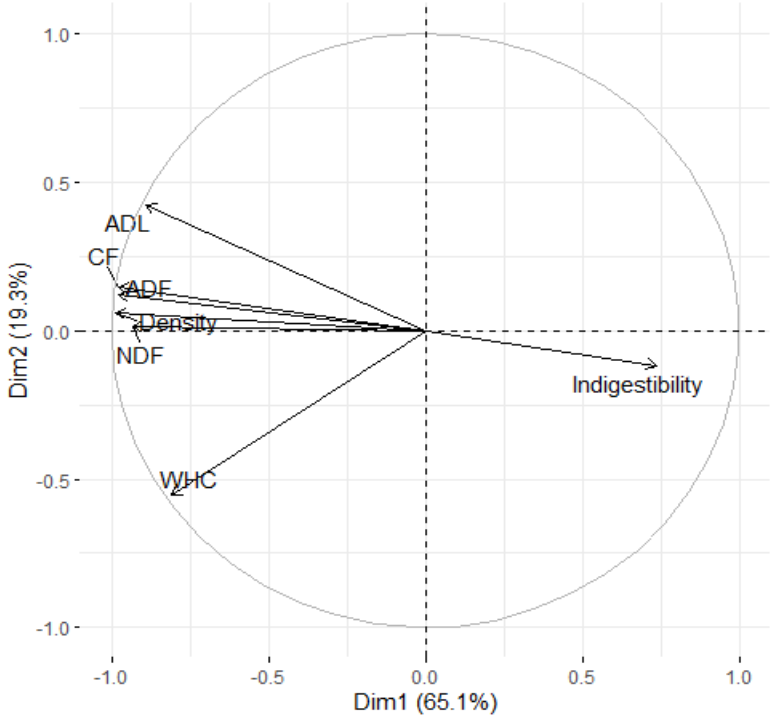
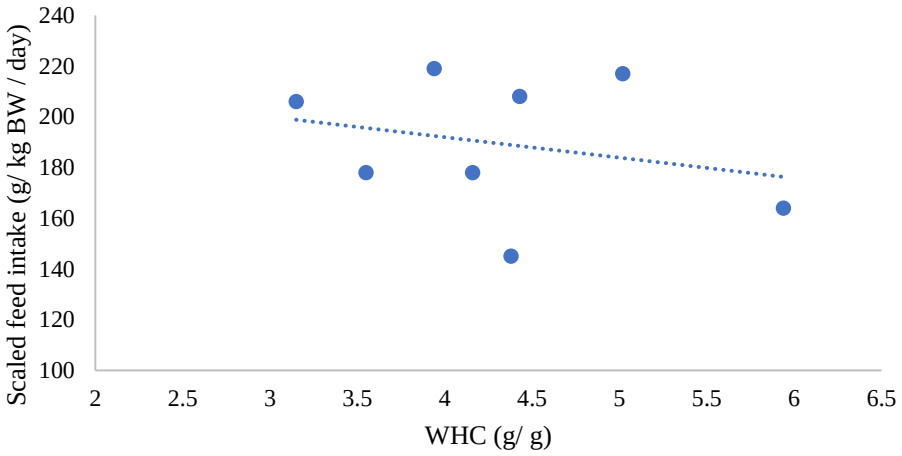


Figure 3.2. The projections of the scaled feed intake and bulk characteristics on the first two dimensions of the Principal Component Analysis (PCA) during week 1 of the experiment. The bulk charactristics were: Crude Fibre (CF), Acid Detergent Fibre (ADF), Neutral Detergent Fibre (NDF), Acid Detergent Lignin (ADL), Water Holding Capacity (WHC), Density and Indigestibility.

a



b

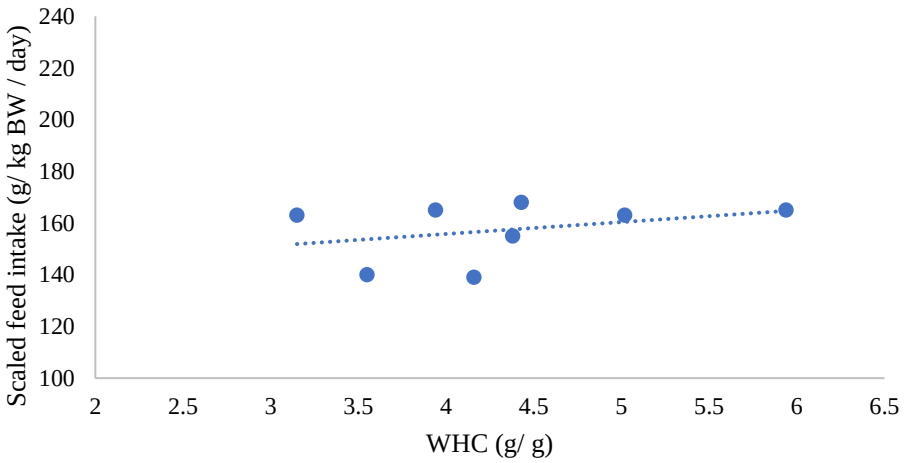


Figure 3.3. The scaled feed intake (g/ kg BW/ day) during the first week of Period 1 (a) and during weeks 2-3 of Period 1 (b) against their water-holding capacity (g/g) of the feeds offered.

3.4.2.3 *Body weight, empty body weight, empty carcass weight and gut fill*

The BW (g) of the dissected birds and their corresponding EBW (g), ECW (g), gut fill (g) and gut fill expressed relative to EBW (g/ kg) on d29 are presented in Table 3.3; with the exception of gut fill, all other measurements were affected significantly by feed ($P < 0.001$). The lowest BW were seen on the WB60 and GM60 treatments, and the highest BW seen on the GM30 and OHWB treatments ($P < 0.05$); the BW of the latter two treatments were not significantly different between them ($P > 0.05$). BW on the remaining treatments lied between these extremes and were not always significantly different between them.

Consistent with BW, birds given WB60 and GM60 had the lowest EBW and ECW ($P < 0.05$), and birds on GM30 had the highest EBW and ECW ($P < 0.05$), although their ECW was not significantly different from OHWB birds ($P > 0.05$). Birds on treatments OH60, WB30, OHGM and WBGM had intermediate EBW and ECW, which were not significant between them ($P > 0.05$). Although there were no significant differences in gut fill between any of the treatments ($P > 0.05$), scaled gut fill relative to EBW was different between treatments: scaled gut fill of GM60 birds was significantly greater than birds on any other treatment ($P < 0.05$) and scaled gut fill was greater in birds given WB60 than those offered OH60, GM30, and OHWB feeds ($P < 0.05$). There were no further significant differences in scaled gut fill ($P > 0.05$).

Table 3.3. Body weight (BW), empty body weight (EBW), empty carcass weight (ECW) and gut fill on d29 of age of broiler chickens given access to feeds containing Oat Hulls (OH at 60%), Wheat Bran (WB at either 30 or 60%), Grass Meal (GM at either 30 or 60%), or feeds containing a mixture of two bulky ingredients at an inclusion level of 30% each (OHWB, OHGM, or WBGM). Gut fill was also expressed relative to EBW (g/ kg). Results are presented as LS means with SEM. Means within a column that do not share a common superscript are significantly different ($P < 0.05$).

	Body weight (g)	Empty body weight (g)	Empty carcass weight (g)	Gut fill (g)	Gut fill (g/ kg EBW)
OH60	1530^{bc}	1410^{bc}	1249^{c}	121	77.3^{a}
WB30	1366^{b}	1245^{b}	1111^{bc}	121	97.7^{ab}
WB60	998^{a}	881^{a}	797^{a}	115	134^{b}
GM30	1719^{d}	1613^{d}	1417^{d}	106	66.8^{a}
GM60	931^{a}	786^{a}	648^{a}	144	184^{c}
OHWB	1560^{cd}	1436^{c}	1262^{cd}	124	86.3^{a}
OHGM	1383^{b}	1253^{b}	1080^{b}	127	103^{ab}
WBGM	1493^{bc}	1360^{bc}	1174^{bc}	131	96.4^{ab}
SEM	38.4	38.9	33.2	11.2	9.02
			Probabilities		
Feed	<0.001	<0.001	<0.001	0.229	<0.001

3.4.2.4 *Empty organ measurements scaled relative to empty body weight*

Organ measurements scaled relative to EBW (g/ kg) on d29 are presented in Table 3.4. Feed offered affected significantly relative GIT organ weight ($P < 0.001$). Birds on GM60 had significantly higher relative weights for any section of the GIT compared with birds on any other treatments ($P < 0.05$), with the exception of relative crop and caeca weights which were arithmetically, but not always significantly higher than all other treatments ($P > 0.05$). The treatment with the second highest relative organ weights was WB60, but its values were not always significantly different from all other treatments. The treatment with the lowest values of relative organ weights was GM30, whose values for all measurements were significantly lower than those of GM60 and significantly lower than those of WB60 for crop, gizzard, jejunum and ileum relative weights only ($P < 0.05$).

Table 3.4. Organ (empty) weights on d29 of age expressed relative to empty body weight (EBW, g/ kg). Broiler chickens were given access to feeds containing Oat Hulls (OH at 60%), Wheat Bran (WB at either 30 or 60%), Grass Meal (GM at either 30 or 60%), or feeds containing a mixture of two bulky ingredients at an inclusion level of 30% each (OHWB, OHGM, or WBGM). Results are presented as LS means with SEM. Means within a column that do not share a common superscript are significantly different ($P < 0.05$).

	Crop empty (g/ kg EBW)	Proventriculus empty (g/ kg EBW)	Gizzard empty (g/ kg EBW)	Duodenum empty (g/ kg EBW)	Jejunum empty (g/ kg EBW)	Ileum empty (g/ kg EBW)	Caeca empty (g/ kg EBW)	Large intestine empty (g/ kg EBW)
OH60	6.98^{abc}	5.98^{a}	27.1^{ab}	8.83^{ab}	17.3^{ab}	14.6^{ab}	7.65^{ab}	10.1^{a}
WB30	6.43^{ab}	8.09^{a}	26.9^{ab}	9.71^{b}	19.9^{ab}	15.3^{ab}	7.03^{a}	10.4^{a}
WB60	9.11^{bc}	8.21^{a}	30.4^{bc}	9.55^{ab}	23.7^{b}	17.8^{b}	8.38^{ab}	12.2^{a}
GM30	4.61^{a}	6.13^{a}	18.8^{a}	6.59^{a}	13.6^{a}	10.7^{a}	5.87^{a}	8.19^{a}
GM60	9.25^{c}	12.7^{b}	42.4^{d}	15.7^{c}	33.5^{c}	25.9^{c}	10.4^{b}	19.2^{b}
OHWB	7.22^{abc}	6.15^{a}	27.3^{ab}	8.53^{ab}	18.9^{ab}	14.1^{ab}	6.36^{a}	10.4^{a}
OHGM	8.35^{bc}	7.46^{a}	28.1^{abc}	8.98^{ab}	20.7^{ab}	15.8^{ab}	7.56^{ab}	10.9^{a}
WBGM	7.16^{abc}	7.38^{a}	26.6^{ab}	9.63^{b}	20.0^{ab}	15.9^{ab}	7.65^{ab}	11.6^{a}
SEM	0.585	0.559	2.33	0.636	1.65	1.36	0.724	1.046
				Probabilities				
Feed	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

3.4.3 *Period 2*

3.4.3.1 *Feed intake, average daily gains and FCR*

The progression of daily feed intake from d29-42 of age is shown in Figure 3.4 and back transformed scaled feed intake (g/ kg/ day), back transformed scaled daily gains (g/ kg/ day) and FCR over week 4 and week 5 are presented in Table 3.5. There was a significant interaction between previous feed and week during Period 2 on the log transformed scaled feed intake ($P < 0.05$). The interaction was due to differences in the direction of the change in transformed scaled feed intake between treatments over time (week 4 vs weeks 5): whilst scaled feed intake did not change from week 4 to week 5 for the birds previously offered GM60, this was not the case for the remaining treatments, where scaled feed intake increased. The transformed scaled feed intake of the birds previously offered GM60 was significantly different from all other previous treatments during weeks 4 and 5 ($P < 0.05$), with the exception of the intake during week 5 of birds previously offered WB60 ($P > 0.05$). There were no further significant differences in transformed scaled ADFI during week 5 ($P > 0.05$). There was no interaction between feed and week on the log transformed scaled daily gains ($P > 0.05$) or FCR ($P > 0.05$), and no significant effect of previous feed on FCR in either week ($P > 0.05$). The lowest log transformed scaled daily gains were seen in GM30 during week 4 and in WB30 during week 5 ($P < 0.001$).

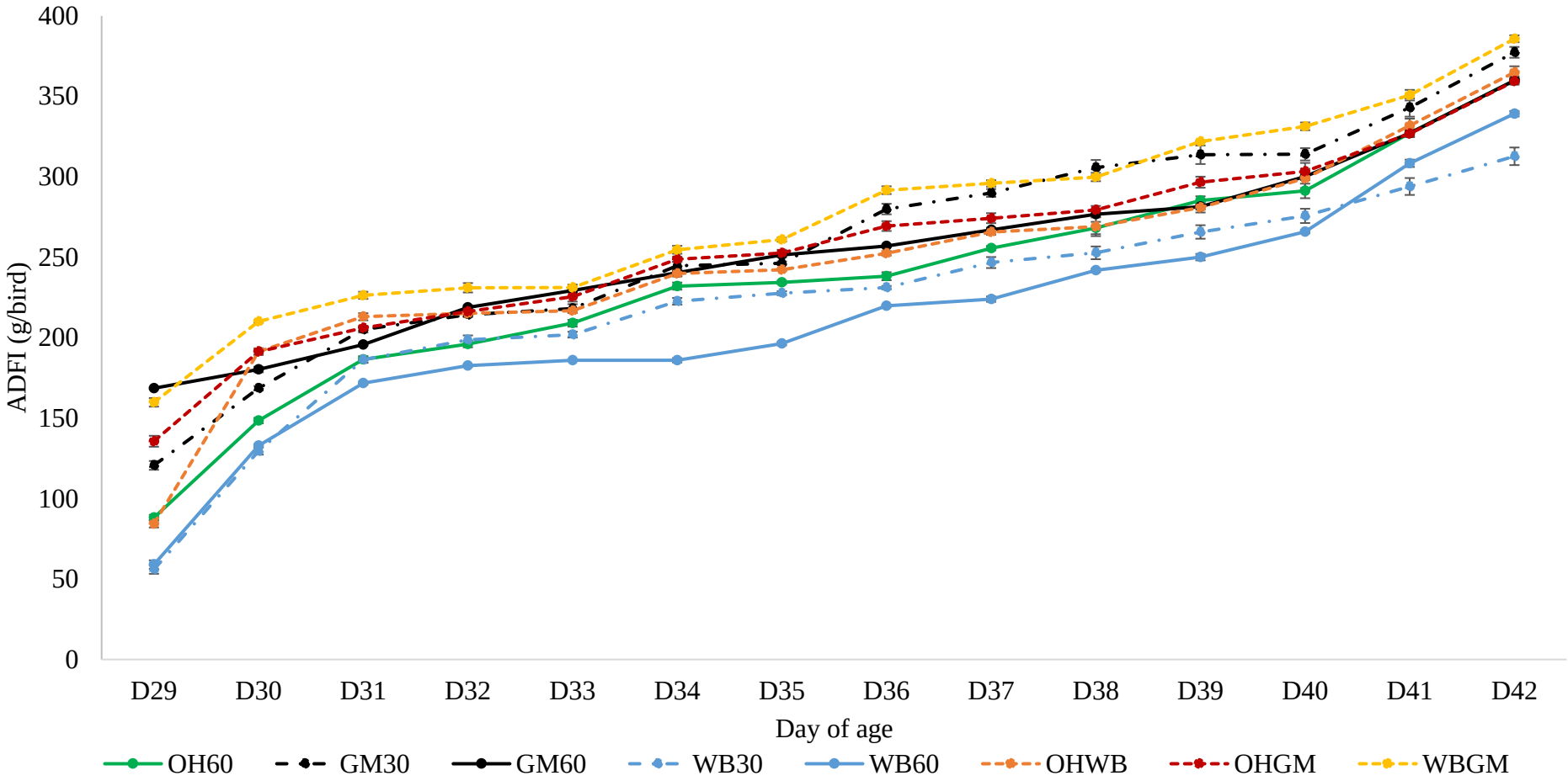


Figure 3.4. The effect of previous feed on average daily feed intake (ADFI; g/ bird) of broiler chickens given access to a feed containing 60% grass meal (GM) from d29-43 of age. Broiler chickens were previously given access to feeds containing Oat Hulls (OH at 60%), Wheat Bran (WB at either 30 or 60%), GM (at either 30 or 60%), or feeds containing a mixture of two bulky ingredients at an inclusion level of 30% each (OHWB, OHGM, or WBGM). from d8-28 of age.

Table 3.5. Average daily feed intake and average daily gain expressed relative to the mean body weight of the period (g/ kg/ day), and FCR calculated over d29-35 (week 4) and d36-42 (week 5). Broiler chickens were given access to a feed containing 60% grass meal (GM) from d29-43 of age. Broiler chickens were previously given access to feeds containing Oat Hulls (OH at 60%), Wheat Bran (WB at either 30 or 60%), GM (at either 30 or 60%), or feeds containing a mixture of two bulky ingredients at an inclusion level of 30% each (OHWB, OHGM, or WBGM) from d8-28 of age. Variables were analysed after transformation by natural logarithms and presented here as back transformed means with confidence intervals. Means within a column that do not share a common superscript are significantly different (*P < 0.05*) and represent the interaction between previous feed and week.

Previous feed	ADFI/ BW (g/ kg/ day)		ADG/BW (g/ kg/ day)		FCR	
	W4	W5	W4	W5	W4	W5
OH60	106^{a} (98.5-114)	128^{bcd} (118-140)	44.0 (37.3-51.9)	36.2 (31.2-42.1)	2.81 (2.00-3.61)	3.52 (2.72-4.33)
WB30	107^{a} (96.5-117)	126^{bcd} (114-137)	51.0 (42.5-62.2)	34.1 (28.8-40.4)	2.85 (2.05-3.66)	3.68 (2.87-4.49)
WB60	128^{bcd} (118-140)	159^{ef} (147-172)	40.0 (34.5-46.5)	30.3 (26.0-35.2)	2.93 (2.12-3.74)	3.83 (3.02-4.64)
GM30	105^{a} (97.5-112)	129^{bcd} (119-140)	56.0 (48.9-65.4)	36.6 (31.5-42.5)	2.35 (1.55-3.14)	3.29 (2.49-4.10)
GM60	174^{f} (159-189)	172^{f} (159-187)	37.0 (31.8-42.9)	30.6 (26.6-35.5)	2.83 (2.03-3.64)	4.04 (3.24-4.85)
OHWB	113^{ab} (104-123)	129^{cd} (119-141)	43.0 (37.0-49.9)	39.3 (34.1-45.6)	2.99 (2.18-3.79)	3.84 (3.04-4.65)
OHGM	125^{bcd} (114-136)	136^{d} (125-148)	40.0 (34.1-47.9)	35.5 (30.6-41.3)	2.79 (1.99-3.60)	3.59 (2.79-4.40)
WBGM	130^{cd} (120-140)	140de (128-151)	47.0 (40.4-54.6)	37.3 (32.1-42.9)	3.38 (2.58-4.19)	3.38 (2.58-4.19)
			Probabilities			
Previous feed	<0.001		<0.001		0.180	
Week	<0.001		0.001		<0.001	
Previous feed × Week	0.004		0.062		0.922	

3.4.3.2 *Body weight, empty body weight, empty carcass weight and gut fill*

The BW (g) of the dissected birds and their corresponding EBW (g), ECW (g), gut fill (g) and gut fill expressed relative to EBW (g/ kg) from d43 are presented in Table 3.6; with the exception of gut fill, all other measurements were affected significantly by previous feed ($P < 0.001$). The BW of birds previously on WB60 and GM60 were significantly lower than those previously on any other feeds ($P < 0.05$) and the highest BW were seen on the birds previously fed GM30 and OHWB treatments, although these were not significantly different from the other birds ($P > 0.05$). Consistent with the BW, birds previously given WB60 and GM60 had the lowest EBW and ECW ($P < 0.05$) and birds previously on GM30 had the highest EBW and ECW ($P < 0.05$) although the latter were not significantly different from the other birds ($P > 0.05$). Although there were no significant differences in gut fill ($P > 0.05$), scaled gut fill relative to EBW was significantly different. The scaled gut fill was greatest in birds fed previously on GM60 ($P < 0.05$). However, there were no significant differences in scaled gut fill between the other treatments ($P > 0.05$).

Table 3.6. Body weight (BW), empty body weight (EBW), empty carcass weight (ECW) and gut fill on d43 of age. Gut fill was also expressed relative to BW, EBW and ECW (g/ kg). Broiler chickens were given access to a feed containing 60% grass meal (GM) from d29-43 of age. Broiler chickens were previously given access to feeds containing Oat Hulls (OH at 60%), Wheat Bran (WB at either 30 or 60%), GM (at either 30 or 60%), or feeds containing a mixture of two bulky ingredients at an inclusion level of 30% each (OHWB, OHGM, or WBGM) from d8-28 of age. Results are presented as LS means with SEM. Means within a column that do not share a common superscript are significantly different ($P < 0.05$).

Previous feed	Body weight (g)	Empty body weight (g)	Empty carcass weight (g)	Gut fill (g)	Gut fill (g/ kg EBW)
OH60	2556[b]	2301[b]	2062[b]	255	111[a]
WB30	2543[b]	2304[b]	2056[b]	240	105[a]
WB60	1988[a]	1763[a]	1566[a]	219	127[a]
GM30	2647[b]	2369[b]	2132[b]	284	120[a]
GM60	2008[a]	1717[a]	1493[a]	291	169[b]
OHWB	2591[b]	2337[b]	2112[b]	253	108[a]
OHGM	2380[b]	2138[b]	1919[b]	241	113[a]
WBGM	2515[b]	2239[b]	1990[b]	276	124[a]
SEM	63.5	60.4	54.9	17.8	8.7
			Probabilities		
Previous feed	<0.001	<0.001	<0.001	0.079	<0.001

3.4.3.3 *Empty organ measurements scaled relative to empty body weight*

Organ measurements scaled relative to EBW (g/ kg) on d43 are presented in Table 3.7. Previous feed affected all relative GIT organ weights with the exception of relative crop weight ($P > 0.05$). Birds that continued on the GM60 feed throughout the experiment had arithmetically, but not always significantly higher, relative weights of sections of the GIT ($P > 0.05$). The second highest relative organ weights were observed in the birds previously fed WB60, but the values were not always significantly different from the other birds. The lowest values of relative organ weights were observed in the birds previously fed WB30, with the exception of relative crop, duodenum and caeca weights, which were lowest in the birds previously fed OHWB, GM30 and OHWB, respectively.

Table 3.7. Organ (empty) weights from d43 of age expressed relative to empty body weight (EBW, g/ kg). Broiler chickens given access to a feed containing 60% grass meal (GM) from d29-43 of age. Broiler chickens were previously given access to feeds containing Oat Hulls (OH at 60%), Wheat Bran (WB at either 30 or 60%), GM (at either 30 or 60%), or feeds containing a mixture of two bulky ingredients at an inclusion level of 30% each (OHWB, OHGM, or WBGM) from d8-28 of age. Results are presented as LS means with SEM. Means within a column that do not share a common superscript are significantly different ($P < 0.05$).

Previous feed	Crop empty (g/ kg EBW)	Proventriculus empty (g/ kg EBW)	Gizzard empty (g/ kg EBW)	Duodenum empty (g/ kg EBW)	Jejunum empty (g/ kg EBW)	Ileum empty (g/ kg EBW)	Caeca empty (g/ kg EBW)	Large intestine empty (g/ kg EBW)
OH60	7.92	6.38^{a}	23.2^{abc}	7.98^{a}	17.7^{a}	14.3^{ab}	5.70	10.4^{ab}
WB30	6.78	6.56^{ab}	19.7^{a}	8.12^{a}	19.0^{ab}	14.5^{ab}	5.77	8.67^{a}
WB60	7.81	7.49^{ab}	26.5^{bc}	9.93^{ab}	23.8^{ab}	16.8^{ab}	6.81	12.9^{b}
GM30	7.62	6.73^{ab}	20.6^{a}	7.86^{a}	19.7^{ab}	13.7^{a}	4.98	9.73^{ab}
GM60	8.21	8.52^{b}	27.2^{c}	10.8^{b}	24.2^{b}	17.5^{b}	6.85	13.3^{b}
OHWB	6.71	6.58^{ab}	21.5^{ab}	8.11^{a}	20.1^{ab}	13.9^{ab}	4.95	10.6^{ab}
OHGM	7.70	7.35^{ab}	23.3^{abc}	8.20^{a}	19.3^{ab}	14.5^{ab}	5.62	11.7^{ab}
WBGM	7.18	6.90^{ab}	21.4^{a}	8.36^{a}	19.2^{ab}	14.8^{ab}	5.29	12.1^{ab}
SEM	0.510	0.448	1.08	0.42	1.28	0.905	0.457	0.819
				Probabilities				
Previous feed	0.362	0.023	<0.001	<0.001	0.008	0.012	0.053	0.001

3.5 Discussion

We used three bulky ingredients (oat hulls, wheat bran and grass meal) to investigate the capacity of a modern broiler strain for bulk, with the overarching objective of reaching a prediction of maximum capacity of the birds for bulky feeds. This is of particular relevance in informing models and predictions of broiler feed intake and performance (Nascimento *et al.*, 2020), especially now that there is an increased interest in the use of alternative, potentially bulky ingredients in broiler feeds (Morgan and Choct, 2016; Scholey *et al.*, 2020). Quantifying the capacity for bulk will define the level of inclusion of such ingredients and the energy density of a feed that will not penalise bird performance. The three bulky ingredients and their levels of inclusion covered a wide range of physiochemical properties, and as far as we are aware, they represent the highest level of inclusion (maximum 60% of the feed) of bulky ingredients used for broilers in the literature. We do not know which of the experimental feeds limited feed intake and consequently the performance of the birds through its bulk, because the experimental design did not include a basal, non-limiting feed against which the intake and performance of the birds could be compared to. However, the performance measured, either as ADG or EBW, at the end of Period 1, was lower on all feeds compared to birds on GM30. During the same period FCR was higher on all feeds compared to GM30, with the exception of the WBGM mixture. Therefore, it can be safely assumed, that all feeds, perhaps with the exception of GM30, limited intake and performance.

As expected, several of the physicochemical properties of the feeds were highly correlated, as they essentially measured the same properties of the 'fibre' components of the feeds (Brachet *et al.*, 2015). In some cases, the correlations between the measurements between the 'fibre' component of the feed and physicochemical properties were broken down, as was the case between the ADL and WHC. Consistently with the results of Nascimento *et al*. (2020) for broilers, the WHC of the feeds was the physical characteristic of the feed most highly correlated with scaled feed intake. This correlation was moderate and is depicted on Figure 3.3a. The correlation did not improve when the GM30 feed was excluded from the analysis. We have assumed, therefore, that the WHC of the feed is the physical characteristic of the feed that best represents its bulkiness. This is consistent with the suggestion made by Kyriazakis and Emmans (1995) and Tsaras *et al.* (1998) about the property of bulky feeds best able to predict the maximum feed intake of pigs.

The WHC of a feed denotes the capacity of its fibrous component to trap water in its matrix, swell and form gels with high water contents (Eastwood, 1973; Ndou *et al.*, 2013). In this respect, our results are consistent with the experiment of Nascimento *et al.* (2020) who

suggested that the high retention of water by bulky ingredients can act as a limiting factor in various parts of the GIT. In terms of the range of the WHC covered by our experiment (2.71 g/g to 5.94 g/g for the OH30 and GM60 respectively), this is similar to the range covered in Chapter 2 for the same age and bird strain. The linear relationship between these two variables during week 1 was:

$$\text{Scaled feed intake (g/ kg/ day)} = 768 \text{ (s.e. 23.7)} \times (1/\text{WHC})$$

$$\text{Residual Standard Deviation } 34.3$$

Given the negative correlation between scaled feed intake and WHC, it is suggested that the relationship between the two variables maybe of the form proposed by Tsaras *et al.* (1998) for pigs, which implies that the WHC can allow for accurate predictions of voluntary feed intake on bulky feeds only when the feed has a constraining effect.

However, the above relationship between WHC and SFI did not hold during weeks 2 and 3 of Period 1 of the experiment, as there was essentially no relationship between the two variables (Figure 3.3b). It is possible that this was due to the adaptation of the birds on the feeds offered, which may no longer have been limiting feed intake, at least for a number of the feeds. This implies that birds were able to adapt at least to some of the feeds relatively quickly and within a space of less than a week. This is logical when one considers the age at which the birds were introduced to the bulky feeds and the greater plasticity of the GIT of young birds (Sklan, 2001). These outcomes are consistent with those in Chapter 2, which suggest that in the same bird strain (Ross 308) birds were able to adapt to bulky feeds based on oat hulls and sugar beet pulp, relatively rapidly, even after a week feeding on bulky feeds that limited their intake and performance. In both Chapter 2 and the current Chapter, the rate of adaptation to the bulky feed depended on the 'bulkiness' of the feed, in the former experiment birds offered a feed diluted with a high level of sugar beet pulp and high WHC, were unable to completely adapt to the feed by the end of the experiment.

On the other hand, Nascimento *et al.* (2020) gave Cobb 500 broilers access to feeds which were progressively diluted by a variety of bulky ingredients, with a widely varying WHC (ranging from 2.38 to 8.38 g/g). They suggested that their birds did not adapt to the high levels of inclusion of the bulky ingredients over a 45-day period, with the exception of the birds offered feeds diluted with sand, which was one of the ingredients with the lowest WHC used. It is difficult to offer and explanation for the discrepancy between the experiment of Nascimento *et al.* (2020), Chapter 2 and the present experiment, other than the experiments

were conducted on different bird strains and in the former case some of the birds were fed individually, whereas in the latter they were group-housed. However, the contrasting results between the experiments have significant implications: in the case of the Nascimento *et al.* (2020) they suggest that gut capacity of modern broiler genotypes may be only marginally extended when the nutrient density of a given feed is diluted, something also previous suggested by Tallentire *et al*. (2018), whereas Chapter 2 and the current experiment suggest that this is not the case.

It was tested whether there was an interaction between treatment and week to investigate the extent of adaptation to the feeds. An interaction showing a significant increase in the scaled feed intake from week 1 to weeks 2-3 would indicate that the birds were indeed adapting to their respective feeds, which was the case here. There was no difference in the scaled feed intake from week 1 to weeks 2-3 in the WB60 birds; meanwhile there was an increase in scaled feed intake in the GM60 birds, whereas there was a reduction in scaled feed intake for the remaining treatments. This indicates that the birds on these two treatments (WB60 and GM60) were undergoing a prolonged adaptation to the feeds in comparison with the remaining treatments, which was expected as these were considered the two bulkiest feeds. The adaptation period, in terms of scaled feed intake, was reflected in the relative GIT development of the birds. The highest dilution with grass meal (GM60) led to the highest relative GIT organ weights compared to all other treatments at the end of Period 1 (d29); this was the case for every GIT component considered and it is consistent with the observation that this was the most limiting feed in relation to feed intake and performance. The GIT development of the WB60 and GM60 birds accommodated their respective increases in scaled feed intake from week 1 to weeks 2-3, as the birds consumed feed to their evolving maximum bulk capacity. Consistent with the previous Chapter, the weight of the colon and the caeca were also significantly increased in the bulky treatments, confirming the frequently overlooked role of the large intestine in the accommodation of bulky feeds (Amerah *et al.*, 2009; Abdollahi *et al.*, 2019). The relative weights of the GIT were higher in birds given WBGM and OHGM rather than those given OHWB, showing that there was an additive effect of bulkiness on the development of the GIT. In the previous Chapter, the relative GIT organ weights responded linearly to progressive diet dilution with either oat hulls or sugar beet pulp; this could not be tested in the present experiment.

Following the change to the bulkiest feed (GM60) during Period 2, the relative weights of the crop and caeca of birds that were previously offered one of the other seven feeds in Period

1 were no longer different to the those birds that were offered the GM60 feed throughout both Period 1 and 2. It has been established that the physical use of the crop in chickens is reduced in modern broilers, since there is continuous access to feed in poultry systems (Classen *et al.*, 2016). Furthermore, it has been suggested that the addition of structural components to the feed stimulates the crop and increases its development (Kierończyk *et al.*, 2016). It is therefore possible that the birds in our experiment began to utilise their crop to a greater extent in Period 2 than Period 1, as they had to cope with a bulkier feed (GM60) than their original feeds in Period 1. By the end of Period 2 (d43 of age), the relative GIT organ weights were no longer different between birds previously on GM60 and WB60. Similarly, the scaled feed intake of birds on these two treatments were no longer significantly different. This suggests that the bulkiness of a previous feed can define how quickly the GIT adapts to a switch to a bulkier feed, as was the case here. Whittemore *et al.* (2003b) suggested that the initial reduction in feed intake when an animal is first offered a bulky feed is a reflection that the GIT has not yet adapted to the new feed. Once adaptation has been reached and the GIT is able to accommodate increased gut fill or the increased involvement of the parts of the GIT involved in fibre digestion, a new feed intake equilibrium can be reached. It is therefore important to improve our understanding of the rate of adaptation to bulky feeds and how this is affected by factors such as age and prior experience to bulky ingredients.

Birds sometimes consume less when they are offered an unfamiliar feed, a behaviour termed as neophobia (Marples and Kelly, 1999; Bertin *et al.*, 2010), which may confer evolutionary advantages, such as avoidance of a potentially harmful substance. The birds in the current experiment were switched over to the bulky feeds after feeding on a starter feed for 8 days which did not contain any of the subsequent bulky ingredients. The switch to the bulky feeds was abrupt, so it is possible that the feed intake on the bulky feeds could be a response to their novelty. However, the expectation would be that a neophobic response to novel feeds should not be associated with their composition per se; any such response should not be associated with the amount of a bulky ingredient in the feed, which was the case here where feeds contained different amounts of the same bulky ingredient. Most of the birds (on 7 out of the 8 treatments) were also switched over from their bulky feeds to a novel, bulkier feed (GM60) during Period 2 of the experiment. Their intake on this feed increased almost instantaneously (in some cases within a day) and reflected the degree of the prior adaptation of the birds on the bulky feeds. Therefore, the concept of neophobia cannot account for the initial response of the birds on the ‘novel’ bulky feeds in this experiment.

It can be concluded that at least in the short term, WHC does seem to be a good predictor of SFI when birds are initially introduced to a bulky feed, consistent with Nascimento *et al.* (2020). However, it seems that once the birds have adapted to the bulkiness of the feed, one can no longer use WHC to predict feed intake since the feed is no longer limiting. Furthermore, since the plasticity of the GIT is seemingly greater in younger birds, the age at which broilers are introduced to a bulky feed may also have implications on the accurate prediction of feed intake, although this warrants further investigation. Identifying links between hydration capacities, such as WHC, with both physical and chemical characteristics of feeds could be used to further improve the accuracy of predictions of voluntary feed intake and performance. It should be noted that the levels of inclusion used in this experiment are far greater than what would be used under practical situations, as they were chosen to develop predictive relationships under a wide range of circumstances. The results suggest that there would be some considerable flexibility in the use of alternative ingredients that may affect the feed intake of birds through their nutrient density. It should be noted that only one genotype was used in both Chapter 2 and 3, therefore, future studies should look into the ability of different genotypes to cope with bulky ingredients to better understand the practicality of offering bulky feeds to broilers in. Furthermore, such experiments should dilute the feeds using other bulky ingredients, such as rapeseed meal, as only 4 different bulky ingredients were assessed.

Chapter 4. Starving for what? Is pathogen-induced anorexia during infection affected by feed composition?

4.1 Abstract

Pathogen induced anorexia (a voluntary reduction in feed intake) is a common consequence of parasitic infections. The hypothesis that pathogen-induced anorexia is independent of feed quality was addressed. This was tested by reducing feed quality through diet dilution with an inert, fibrous ingredient (Arbocel lignocellulose) in uninfected and *Eimeria* infected birds. It was hypothesised that diet dilution would reduce absolute feed intake of non-infected birds. On the other hand, birds subjected to a coccidian infection and offered diluted feeds would show a lower degree of pathogen induced anorexia (compared to infected birds offered non-diluted feeds), as a further reduction in nutrient intake would no longer serve a benefit to the host. A total of 384 male Ross 308 day old broilers were distributed into 48 pens and offered a common starter feed for 6 days. Birds were offered the full dietary treatment on d10 of age. The experimental feeds were produced by mixing a basal feed (No fibre, NF) with 5% (Low fibre, LF), 10% (Medium fibre, MF), or 15% (High fibre, HF). At d13 (d0pi; post infection) half of the pens were infected with 7,000 sporulated *E. maxima* oocysts. Feed intake was measured daily until d15pi and faeces were collected from d4pi until d10pi to measure oocysts per gram (OPG). In the absence of infection, diet dilution resulted in the expected dose dependent reduction in absolute ADFI ($P < 0.05$). Whereas, in the presence of infection, there were no significant differences in absolute ADFI between the infected birds ($P > 0.05$). However, when expressed relative to body weight, ADFI significantly increased with diet dilution in both the infected and uninfected birds ($P < 0.05$). To that end, relative ADEI was significantly greater in the HF infected birds compared to the NF infected birds, whereas the reverse was the case in the uninfected birds ($P < 0.001$). Moreover, there were no significant differences in OPG excretion between the different dietary treatments from d5-9pi ($P = 0.997$). In conclusion, the results suggest that infected birds fed a diluted feed do not suffer anorexia to the same extent as birds fed a non-diluted feed, as if birds were trying to regulate their energy or nutrient intake. Therefore, it could be concluded that the characteristics of pathogen-induced anorexia are dependent on feed composition.

4.2 Introduction

A voluntary reduction in feed intake is a common characteristic of infections, in a variety of host-pathogen systems (Adelman and Martin, 2009; Laurenson *et al.*, 2011; Hite and Cressler, 2019). This reduction in feed intake is defined as pathogen-induced anorexia (Kyriazakis *et al.*, 1998). Pathogen-induced anorexia constitutes a paradox because it occurs during a time when host requirements for nutrient resources are increased (Kyriazakis *et al.*, 1998; Hite and Cressler, 2019). Pathogens divert resources away from the host productive functions, to facilitate the increased nutrient and metabolic requirements for the activation and maintenance of cell mediated immune responses and the repair of damaged tissue (Sheldon and Verhulst, 1996; Klasing, 2007; Sompayrac, 2019). Therefore, one would expect the infected host to increase their feed intake to cope with the increased nutrient demands of the immune system (Kyriazakis *et al.*, 1998). However, parasitized hosts typically reduce their feed intake during this critical time (Ayres and Schneider, 2009; Adamo *et al.*, 2010). Pathogen-induced anorexia is a 'hard-wired' behaviour across a wide range of infected animals, including humans, and therefore it has been hypothesised that it confers evolutionary advantages, usually to the host (Kyriazakis *et al.*, 1998; Rao *et al.*, 2017). Murray and Murray (1979) demonstrated the advantages of anorexia to host fitness in an experiment where mice were infected with *Listeria monocytogenes* and either fed *ad-libitum* or force-fed the same daily feed intake of their uninfected controls. Indeed, infected mice forced to increase their feed intake succumbed to the infection more rapidly and to a greater extent than those allowed to develop the anorexic response.

It is well documented that the characteristics of anorexia (the onset, duration and extent) depend on the pathogen type. For example, infections with macro-parasites tend to last several weeks (Sandberg *et al.*, 2007; Kyriazakis and Doeschl-Wilson, 2009); whereas infections with micro-parasites last a few days (Sakkas *et al.*, 2018). Macro-parasites (e.g. helminths) do not replicate within the host, whereas micro-parasites (e.g. Coccidia) do indeed reproduce within their host (Henken *et al.*, 1994). However, as identified by Kyriazakis (2010), there is dearth of information about the effect of feed composition on anorexia in broiler chickens.

An alternative way of viewing pathogen-induced anorexia is to suggest that infected hosts are actually targeting a specific nutrient intake through their feed intake, which poses the question, what is being regulated? Thus, anorexia may not be an unavoidable consequence of infection, but rather the outcome of an interaction between the host and their feed composition. During coccidiosis the feed intake of growing chickens changes very rapidly over a short period of time; a clear reduction in feed intake during the infection is observed (~20% of the non-infected

birds), and birds are eventually able to overcome their infection, by which time anorexia is no longer observed (Kipper *et al.*, 2013; Sakkas *et al.*, 2018; Oikeh *et al.*, 2019). Hence, coccidiosis was identified as an ideal infection model to address the hypotheses. To test the hypotheses, a nutrient dense feed was progressively diluted with an inert material. The consequences of such a dilution are that the energy content of the feed declines, while the energy to nutrient ratio of the feeds remains constant. The expected response in uninfected birds is that absolute feed intake will decrease as diet dilution increases since the birds will be constrained by the bulkiness of the feed. This enables an investigation into whether infected birds attempt to regulate nutrient resources during anorexia since by definition, anorexia reduces feed intake so these birds will no longer be constrained by the bulkiness of the feed.

In the present study it was hypothesized that healthy broilers would not be able to increase their feed intake when offered feeds diluted with an inert ingredient, as feed intake responses to diet dilution would be constrained by the bulk properties of the feed. Furthermore, in the presence of infection birds offered diluted feeds would show a reduction in the extent of anorexia as the feed is limiting in energy and nutrients; the basis of the hypothesis is that any further reduction in feed intake is likely to impact on host resistance to the parasite with potentially detrimental consequences to the host. Understanding which, if any, nutrient resources are targeted by infected hosts and the consequences of this on the outcomes of the infection will have implications on how hosts should be fed during the critical stages of an infection.

4.3 Materials and methods

4.3.1 *Husbandry, birds and feeds*

All procedures were conducted under the UK Animals (Scientific Procedures) Act 1986 and EU Directive 2010/63/EU for animal experiments and carried out under Home Office authorization (P441ADF04). Furthermore, all procedures were approved by the Animal Welfare and Ethical Review Body (AWERB) of Newcastle University. Birds were housed in a windowless, thermostatically controlled building in 48 pens with an area of 0.85m^2. Pens were equipped with tube feeders and bell-drinkers, with wood shavings used as litter at a depth of 5 cm. The birds had *ad libitum* access to feed and water throughout the trial. The pen temperature was set to 34°C for chick arrival and was gradually reduced to 20°C by day (d) 25 of age. The lighting schedule was 23L:1D for the first 7 days and was amended to 18L:6D for the remainder of the trial, whilst light intensity at pen level ranged from 80-100 lux.

A total of 336 male Ross 308 day old chicks were obtained from a commercial hatchery and were wing tagged upon arrival. All birds were offered the same conventional starter feed from

d0-7 of age and were then offered a mixture of start feed and one of four experimental feeds from d8-10 of age, from d11-28 the birds were given the full experimental treatment (Table 4.1). A basal grower feed was formulated (No fibre; NF) and used to produce a further three feeds, by diluting the basal feed with either 5% (Low fibre; LF), 10% (Medium fibre; MF), or 15% (High fibre; HF) Arbocel RC Fine lignocellulose (JRS PHARMA, Rosenberg, Germany). All energy to nutrient ratios were maintained as Arbocel RC Fine is an inert ingredient. Arbocel is a natural lignocellulose produced from fresh spruce trees (*Picea* species) by JRS PHARMA. All feeds were offered as a coarse mash passed through a 5mm screen.

Table 4.1. Ingredient, calculated and analysed chemical composition of the common (starter) feed offered from day 0 to day 7 of age, and the experimental feeds offered from day 8 to day 28 of age. The basal, high density feed (NF) was diluted with 5%, 10%, or 15% Arbocel lignocellulose to result in the LF, MF and HF feeds, respectively.

		Lignocellulose inclusion (%)			
Ingredients (%)	**Common Starter**	**NF (0)**	**LF (5)**	**MF (10)**	**HF (15)**
Ground Maize	10.0	10.0	9.50	9.00	8.50
Ground Wheat	51.5	53.9	51.2	48.5	45.8
Soybean meal (48% CP)	26.0	23.0	21.9	20.7	19.6
Arbocel	-	-	5.00	10.0	15.0
Full fat Soya	5.00	5.00	4.75	4.50	4.25
Limestone	1.25	1.25	1.19	1.13	1.06
L-Lysine HCL	0.40	0.30	0.29	0.27	0.26
DL-Methionine	0.40	0.35	0.33	0.32	0.30
L-Threonine	0.15	0.15	0.14	0.14	0.13
Soya oil	3.00	3.50	3.33	3.15	2.98
Monocalcium phosphate	1.50	1.25	1.19	1.13	1.06
Salt	0.25	0.25	0.24	0.23	0.21
Sodium bicarbonate	0.15	0.15	0.14	0.14	0.13
Premix	0.40	0.40	0.38	0.36	0.34
Titanium dioxide	-	0.50	0.50	0.50	0.50
Total	100	100	100	100	100
Nutrient and chemical composition (%)[1]					
Metabolizable energy (kcal kg^{-1}) (calculated)	3057	3105	2938	2794	2627
Gross energy (MJ kg^{-1})	16.9	17.0	17.0	17.0	17.0
N × 6.25 (Crude protein; CP)	21.4	19.4	18.5	17.6	16.9
Crude fibre	2.30	2.87	4.51	7.23	10.7
Ether extract (oil A)	5.55	6.05	5.75	5.45	5.14
Total oil (oil B)	6.52	7.32	6.86	6.51	6.30
Ash	6.40	7.60	5.00	5.20	5.40
Water Holding Capacity (g/ g DM)	-	2.40	2.85	3.17	3.64
T-lysine (calculated)	1.43	1.26	1.20	1.13	1.07
Av-lysine (calculated)	1.34	1.17	1.11	1.06	1.01
Methionine (calculated)	0.70	0.63	0.60	0.57	0.53
Methionine+ cysteine (calculated)	1.03	0.94	0.89	0.84	0.80
Threonine (calculated)	0.91	0.85	0.80	0.76	0.72
Tryptophan (calculated)	0.25	0.23	0.22	0.20	0.19
Calcium (calculated)	0.95	0.93	0.88	0.83	0.79
Phosphorus (calculated)	0.69	0.65	0.62	0.59	0.55
Av-Phosphorus (calculated)	0.47	0.42	0.40	0.38	0.36
Salt (calculated)	0.31	0.31	0.29	0.28	0.26
Sodium (calculated)	0.18	0.17	0.17	0.16	0.26

[1] Analysed nutrient composition (%) unless otherwise stated.

4.3.2 *Experimental design and inoculations*

Birds were weighed at arrival (d0), at the end of the starter period (d7), and then every three days until the end of the trial. To reduce the risk of refusals and starve outs the birds were offered a mixture of one-part common starter and one-part experimental feed between d8 and d10 of age, and the experimental feed from d11. Pen feed intake was measured over the starter period (d0-d7) and then daily until the conclusion of the experiment. At d7 of age birds were weighed and randomly allocated to pens which were previously assigned to one of the four feeding treatments. On d13 of age (d0 post-infection), birds were orally inoculated with either a single dose of 0.5 ml of H_2O (control), or 7000 sporulated *Eimeria maxima* oocysts of the Weybridge laboratory reference strain suspended in H_2O (infected), so that within a dietary treatment 6 pens contained control and the other 6 pens contained infected birds.

4.3.3 *Sampling*

On d6 and 12pi, one bird from each pen with a body weight (BW) close to the pen average was culled by intravenous lethal injection with sodium pentobarbital (Euthatal, Merial Harlow, United Kingdom); the sampling times corresponded to the acute and recovery stages of the infection. All birds were immediately weighed after euthanasia. During necropsy, the full gastrointestinal tract (GIT) was removed, weighed and separated into individual segments of interest; duodenum, jejunum, and ileum. After measuring the length of the duodenum, jejunum and ileum, a small section (1-2cm) of duodenum and jejunum was collected and stored in 10% neutral buffered formalin for morphometrical analysis. The duodenal sample was collected from the middle of the duodenal loop; and the jejunal sample from halfway between the point of entry of the bile ducts, and Meckel's diverticulum.

Following intestinal tissue sampling; a probe was then inserted directly into the lumen of the gizzard, caeca, duodenum, jejunum and ileum to measure the pH, using a Jenway 3505 pH/mV/Temperature Meter. Afterwards an incision was made in the gizzard to remove the contents, and the empty weight was recorded. Finally, the remaining caecal contents were removed by finger stripping to obtain the empty caeca weight.

At day 15 pi, two birds per pen with a BW close to the mean BW of the pen were culled by lethal injection with sodium pentobarbital (Euthatal, Merial Harlow, United Kingdom). The gastrointestinal tract was removed as previously described.

4.3.4 *Sample analysis*

4.3.4.1 *Oocyst production*

Excreta were collected from d5-10 post-infection by placing polyethylene sheets on top of the wood shavings of each pen for 90 minutes. Upon sheet removal, excreta were pooled per

pen into screw cap pots and stored at 4°C, pending analysis. The modified McMaster technique was used to estimate excretion of daily oocysts per gram (Kaufmann, 1996).

The excreta were thoroughly mixed before a 3 g sample was removed. The sample was mixed with 42 ml of water and passed through a sieve. The solution was then transferred to a glass test tube and centrifuged at 1500 RPM for 2 minutes at room temperature. The supernatant was carefully siphoned off and the pellet vortexed until it was fragmented. Then 10 ml of saturated NaCl was added and the solution thoroughly mixed. A sample of the solution was taken from the centre of the tube before being carefully transferred to the McMaster counting slide. Slides were left undisturbed for 10 minutes to allow the oocysts to rise to the top of the slide, before being read at 10 × magnification. The sum of the oocyst count of each chamber was calculated and multiplied by 50 to give oocysts per gram of excreta.

4.3.4.2 *Histology*

Intestinal segments fixed in formalin from the duodenum and jejunum from one bird per pen (6 birds per treatment) were subjected to a series of graded ethanol baths followed by xylene in a Shandon Excelsior Es Tissue Processor (Thermo Fisher Scientific Inc., Waltham) in order to dehydrate the tissue. The samples were then embedded in paraffin wax, sectioned at 4 µm and stained with haematoxylin/eosin (HE). After staining, the histological sections were scanned using the Leica SCN400 slide scanner system (Leica Microsystems, Buffalo Grove, IL, USA) and images were captured using the Leica SCN400 image viewer software. Morphometric features of the intestinal structure were observed at 10 × magnification. Villus height and crypt depth measurements were ascertained using Aperio ScanScope CS (Aperio Technologies, Vista, CA). The vertical distance from the villus tip to the villus-crypt junction of 10 villi was used to determine mean villus height, whilst crypt depth was the vertical distance from the villus-crypt junction to the lower limit of the crypt of 10 corresponding crypts. Villi length to crypt depth ratio (VCR) was calculated as the usual measure of cell proliferation, which reflects the extent of intestinal damage (Kettunen *et al.*, 2001).

4.3.4.3 *Water holding capacity*

Sample analysis was performed in triplicate, using an adaptation of the Robertson and Eastwood (1981) method. A 1 g feed sample was soaked in 250 ml of H_2O at room temperature for 24 hours. Subsequently, the samples were filtered through a Whatman no. 1 filter paper and the wet weight of the samples recorded, before the samples were placed into an oven at 105 °C overnight and dry weight recorded. WHC was then calculated as g of water/ g of DM.

4.3.5 *Calculations and statistics*

To account for mortalities the feeder was weighed when mortalities were found and the average feed intake (g/bird) was calculated and the assumed intake of the dead bird was subtracted from ADFI. Pen (n = 6) was considered the experimental unit for all data and all statistical analysis was performed using the nlme package in R (Team, 2013) using lm and anova functions from the nlme package (Pinheiro *et al.*, 2013). For all statistical procedures, the normality of the residuals was assessed with qq-plots and the Shapiro-Wilk test. When significant differences were detected, treatment means were separated and compared by the Tukey's multiple comparison test. Significance was determined at $P < 0.05$. Data are presented as model-predicted least square means with the SEM.

Average (mean) daily crude protein intake (ADCPI, g/ day) and pen average (mean) daily energy intake (ADEI, kcal/ day) were calculated by multiplying average (mean) daily feed intake (ADFI, g/ day) by the CP level and the energy content of the feed respectively. A repeated-measures mixed model was implemented to analyse daily feed intake. The model included feed composition, infection, and day as fixed factors, two-way interactions between feed composition and infection, feed composition and day, infection and day and the three-way interaction between feed composition, infection, and day. Covariance structures were chosen based on the lowest value for the Akaike and Bayesian information criteria. Further analysis of variance was carried out on time points when the three-way interaction between feed composition, infection and day was significant. ADFI, ADEI and ADCPI were not statistically affected by the infection until d4 pi and recovered from d9 onwards. Therefore, the periods d0-3pi, d4-8pi and d9-14pi, were considered to represent the pre-patent, acute and recovery stages of the infection respectively. ADFI, ADEI and ADCPI were then calculated over the different stages of the infection. Similarly, corrected feed conversion ratio (cFCR) was calculated over the different stages of infection. As the diluent used is inert and offers no nutritional value, cFCR was calculated by subtracting the level (%) of diet dilution from feed intake.

Two analyses were performed on the feed, resource intake and daily gain data: 1) Based on the day that a reduction in feed intake was observed and the day that feed intake recovered in infected compared to control birds, absolute ADFI, ADEI, ADCPI and ADG data were calculated over the pre-patent, acute and recovery stages of infection and were analysed with general linear mixed (GLM) models with feed composition and infection as fixed factors and their interactions. 2) These actual intakes and their corresponding ADG data calculated per stage of infection were also scaled relative to the mean body weight of each stage of infection (BWp) and log transformed. The scaling allowed to account for the different growth trajectories

arising from feed composition, as it has been shown that different conclusions can be drawn when feed intake is analysed on a body weight as opposed to time basis (Kyriazakis *et al.*, 1991; Allison *et al.*, 1995). The log transformation ensured that the residuals were normally distributed and avoided statistical bias (Pinheiro *et al.*, 2013). Log transformed relative ADFI, ADEI, ADCPI and ADG were analysed with general linear mixed (GLM) models with feed composition and infection as fixed factors and their interactions.

Histological measurements from one bird per pen were obtained at day 6 and day 12 post-infection, these were expressed relative to the body weight of the bird at the point of euthanasia (µm/ kg BW) to account for a priori differences in performance (Kyriazakis *et al.*, 1991). The following organ measurements were log transformed to obtain normal residual distribution: d6pi ileum length (cm/ kg BW); d15pi full small intestine weight (g/ kg BW).

The measured dry matter (DM) of daily excreta was used to express oocyst excretion per g DM, to account for differences in the quantity of the faeces produced. Oocyst excretion was analysed by a repeated-measures mixed model with feed composition, day and their interactions as fixed factors and pen as a random factor. Oocyst per g DM data also needed to be log transformed prior to statistical analysis, as the residuals were not normally distributed; the means were then back-transformed for data presentation.

4.4 Results

4.4.1 *Absolute daily performance*

There were no mortalities from d11 of age until the conclusion of the experiment. The evolution of ADFI (g/ bird), ADEI (kcal/ bird) and ADCPI (g/ bird) from the start of the infection (d0 pi) until the end of the experiment is shown in Figure 4.1a, 4.1b and 4.1c, respectively. Feed composition significantly affected absolute ADFI (d0-14pi), ADEI (d0-14pi) and ADCPI (d0-14pi) as birds offered the diluted feeds consumed less than those offered the basal feed ($P < 0.05$). There was a significant interaction between feed composition and infection for absolute ADFI on d6 and 7pi ($P < 0.05$), as infected birds offered diluted feeds showed a reduction in the extent of anorexia compared with NF birds. An interaction was also observed on absolute ADEI and ADCPI from d6-8pi ($P < 0.05$).

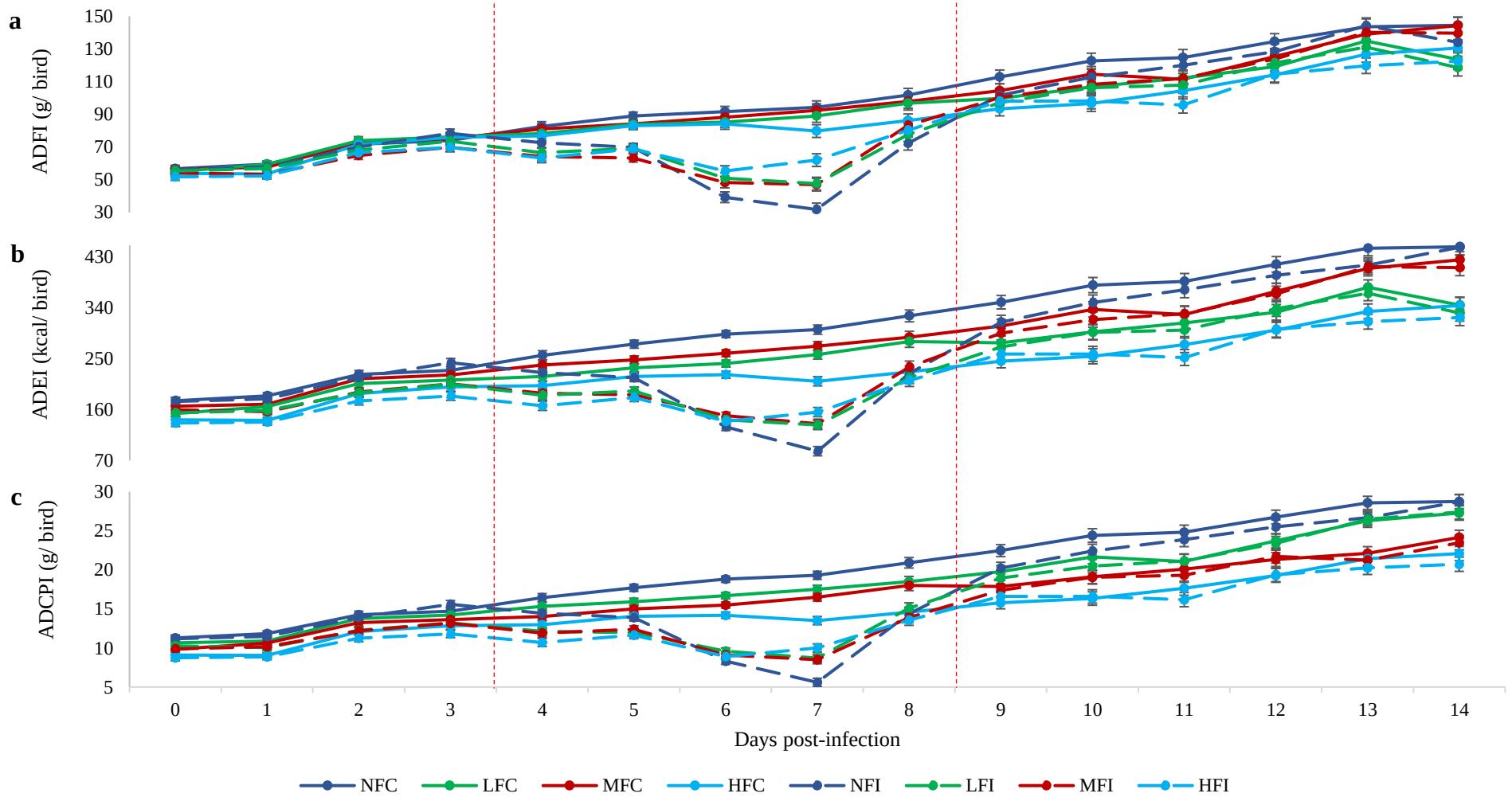


Figure 4.1. **(a)** Average daily feed intake (ADFI; g/ bird); (**b**) Average daily energy intake (ADEI; kcal/ bird); (**c**) Average daily crude protein intake (ADCPI; g/ bird) over the post-infection period (d0-14pi) of broiler chickens who were inoculated with either 0 (C), or 7×10^3 (I) sporulated *E. maxima* oocysts and offered feeds diluted with 0% (NF), 5% (LF), 10% (MF), or 15% (HF) Arbocel lignocellulose. Dotted line indicates the separation into the periods of infection (d0-3, 4-8, 9-14pi).

4.4.2 *Feed and nutrient resource intake during pre-patent, acute and recovery stages*

Table 4.2 shows the absolute ADFI, ADEI and ADCPI during each stage of infection. During the acute stage of infection, infected birds showed significant reductions ($P < 0.001$) in absolute ADFI (g/ day), ADEI (kcal/ day) and ADCPI (g/ day). There were also significant differences in the absolute ADEI and ADCPI between infected birds and control birds during the pre-patent and recovery stage of infection ($P < 0.001$). Diet dilution significantly reduced absolute ADFI during the recovery stage only ($P < 0.001$) but caused significant reductions in absolute ADEI and ADCPI throughout the experiment ($P < 0.001$). There was an interaction between feed composition and infection on absolute ADFI during the acute stage of infection only ($P < 0.05$). This was caused by the different effect of feed composition on control and infected birds; in the control birds absolute ADFI decreased as diet dilution increased, whereas in the infected birds, absolute ADFI increased with diet dilution.

ADFI, ADEI and ADCPI calculated per period of infection were also scaled relative to the mean body weight of the period of infection and were then transformed by the natural logarithm. The transformed relative ADFI, ADEI and ADCPI intakes during each stage of infection are shown in Table 4.3. During the acute stage of infection, there was a significant effect of infection on relative ADFI (log ADFI, g/ kg/ day), ADEI (log energy intake, kcal/ kg/ day) and ADCPI (log protein intake, g/ kg/ day), with the infected birds consuming less than their uninfected counterparts ($P < 0.05$). The effect of feed composition caused an increase in relative ADFI ($P < 0.05$) but relative ADEI and ADCPI intake were significantly reduced as diet dilution increased ($P < 0.05$). There was a significant interaction between infection and feed composition on relative ADEI during the acute stage of infection ($P < 0.001$). This interaction was because there was no significant difference in relative ADEI of HF infected birds and their uninfected counterparts, while there was a significant reduction in relative ADEI between the remaining infected and uninfected treatments. Contrary to the actual intakes, these data show that during the recovery stage, infected birds significantly increased their relative ADFI, ADEI and ADCPI intake compared to control birds ($P < 0.05$). There was also an interaction between infection and feed composition on relative ADEI and ADCPI during the recovery stage ($P < 0.05$), because the HF infected birds significantly increased their nutrient resource intake compared to the uninfected HF birds.

Table 4.2. ADFI (g/ day), ADEI (kcal/ day), and ADCPI (g/ day) of the infected and the corresponding uninfected control birds during infection with *Eimeria maxima* oocysts. Data are presented over the pre-patent (d0-3pi), acute (d4-8pi), and recovery stages (d9-14pi). Birds were offered feeds diluted with 0% (NF), 5% (LF), 10% (MF), or 15% (HF) Arbocel lignocellulose. (LS means with SEM).

		ADFI (g/ day)			ADEI (kcal/ day)			ADCPI (g/ day)		
		Pre-Patent	Acute	Recovery	Pre-Patent	Acute	Recovery	Pre-Patent	Acute	Recovery
Feed composition										
	NF	65.5	73.5	127^{z}	210^{z}	231^{z}	324^{z}	13.5^{z}	14.9^{z}	20.8^{z}
	LF	63.0	75.0	124^{yz}	191^{y}	211^{y}	299^{yz}	12.3^{y}	13.6^{y}	19.2^{yz}
	MF	64.8	74.5	116^{xy}	185^{y}	203^{xy}	274^{xy}	11.9^{y}	13.1^{xy}	17.6^{xy}
	HF	61.9	74.0	111^{x}	167^{x}	190^{x}	246^{x}	10.7^{x}	12.2^{x}	15.8^{x}
	SEM	1.40	1.84	2.8	4.2	4.8	7.5	0.27	0.31	0.48
Infection										
	Control	65.2	86.8	121	195	235	304	12.5	15.1	19.5
	Infected	62.4	61.7	118	181	183	268	11.7	11.8	17.2
	SEM	0.99	1.30	2.0	3.0	3.4	5.3	0.19	0.22	0.34
Feed composition × Infection										
Uninfected	NF	65.5	89.9^{b}	131	213	261	356	13.7	16.8	22.9
	LF	65.5	88.8^{b}	125	202	242	317	13.0	15.5	20.4
	MF	66.1	86.6^{b}	118	191	225	290	12.3	14.5	18.6
	HF	63.7	82.0^{b}	112	175	211	252	11.2	13.5	16.2
Infected	NF	65.6	57.2^{a}	124	207	201	292	13.3	12.9	18.8
	LF	60.5	61.2^{a}	122	180	181	282	11.6	11.6	18.1
	MF	63.4	62.4^{a}	114	178	181	259	11.5	11.6	16.6
	HF	60.1	65.9^{a}	110	160	169	241	10.3	10.9	15.5
	SEM	1.98	2.60	3.9	6.0	6.7	10.6	0.38	0.43	0.68
Source						*Probabilities*				
Feed composition		0.266	0.950	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Infection		0.054	<0.001	0.189	0.002	<0.001	<0.001	0.002	<0.001	<0.001
Feed composition × Infection		0.622	0.021	0.961	0.620	0.334	0.113	0.620	0.334	0.113

[a-d]Means within a column that do not share a common superscript are significantly different ($P < 0.05$).
Abbreviations: ADFI, average daily feed intake; ADEI, average daily energy intake; ADCPI, average daily crude protein intake; pi, post-infection.

Table 4.3. Relative ADFI (log(g/ kg BW/ day)), ADEI (log(/ kg BW/ day)), and ADCPI (log(g/ kg BW/ day)) of the infected and the corresponding uninfected control birds during infection with *Eimeria maxima* oocysts. Data are presented over the pre-patent (d0-3 pi), acute (d4-8 pi), and recovery stages (d9-14 pi). The data were scaled relative to the mean body weight of each stage of infection (BWp) and were then transformed by the natural logarithm. Birds were offered feeds diluted with 0% (NF), 5% (LF), 10% (MF), or 15% (HF) Arbocel lignocellulose. (LS means with SEM).

		ADFI/ BWp (log(g/ kg/ day))			ADEI/ BWp (log(kcal/ kg/ day))			ADCPI/ BWp (log(g/ kg/ day))		
		Pre-Patent	Acute	Recovery	Pre-Patent	Acute	Recovery	Pre-Patent	Acute	Recovery
Feed composition										
	NF	5.02^{x}	4.56^{x}	4.66^{x}	6.38^{z}	6.02^{z}	6.12^{z}	3.61^{z}	3.28^{z}	3.34^{z}
	LF	5.07^{xy}	4.66^{y}	4.71^{xy}	6.37^{yz}	6.07^{z}	6.09^{yz}	3.59^{yz}	3.31^{z}	3.39^{z}
	MF	5.10^{yz}	4.71^{y}	4.72^{y}	6.34^{xy}	6.03^{z}	6.04^{y}	3.56^{xy}	3.30^{z}	3.38^{z}
	HF	5.15^{z}	4.80^{z}	4.78^{z}	6.32^{x}	5.92^{y}	5.91^{x}	3.55^{x}	3.15^{y}	3.16^{y}
	SEM	0.015	0.021	0.014	0.010	0.015	0.020	0.010	0.015	0.014
Infection										
	Control	5.10	4.79	4.89	6.38	6.14	6.00	3.59	3.39	3.30
	Infected	5.07	4.58	4.93	6.32	5.88	6.08	3.57	3.14	3.34
	SEM	0.011	0.015	0.010	0.007	0.010	0.014	0.007	0.010	0.010
Feed composition × Infection										
Uninfected	NF	5.03	4.70	4.60	6.40	6.23^{c}	6.11^{bc}	3.62	3.48^{c}	3.39^{cd}
	LF	5.07	4.77	4.66	6.39	6.21^{c}	6.07^{bc}	3.60	3.44^{c}	3.38^{bcd}
	MF	5.13	4.82	4.69	6.39	6.20^{c}	6.00^{b}	3.60	3.50^{c}	3.41^{d}
	HF	5.16	4.87	4.76	6.35	5.92^{b}	5.82^{a}	3.56	3.13^{ab}	3.02^{a}
Infected	NF	5.01	4.43	4.73	6.36	5.81^{a}	6.14^{c}	3.61	3.07^{a}	3.30^{bc}
	LF	5.07	4.55	4.75	6.34	5.92^{b}	6.11^{bc}	3.58	3.19^{b}	3.40^{d}
	MF	5.08	4.61	4.76	6.29	5.86^{ab}	6.09^{bc}	3.53	3.10^{ab}	3.35^{bcd}
	HF	5.13	4.72	4.79	6.29	5.93^{b}	6.00^{b}	3.54	3.13^{ab}	3.30^{b}
	SEM	0.021	0.030	0.019	0.014	0.021	0.028	0.014	0.021	0.019
Source		*Probabilities*								
Feed composition		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Infection		0.105	<0.001	<0.001	<0.001	<0.001	<0.001	0.007	<0.001	0.012
Feed composition × Infection		0.667	0.216	0.142	0.211	<0.001	0.039	0.269	<0.001	<0.001

[a-e, x-z] Means within a column that do not share a common superscript are significantly different ($P < 0.05$).

Abbreviations: ADFI, average daily feed intake; ADEI, average daily energy intake; ADCPI, average daily crude protein intake; BWp, mean body weight of the period of infection; pi, post-infection.

4.4.3 *ADG, relative ADG, cFCR*

Body weights throughout the infection are given in Figure 4.2. The effects of feed composition, infection, and their interaction on absolute ADG (g/ day), relative ADG (log(g/ kg/ day)) and cFCR calculated over the post-infection period are presented in Table 4.4. Infection significantly reduced absolute ADG at the acute stage of infection only *(P < 0.05)*. Diet dilution caused a linear reduction in absolute ADG in control birds throughout all stages of infection, with HF showing the slowest relative gain compared to the other dietary treatments *(P < 0.05)*. There was an interaction between feed composition and infection during the acute stage of infection on absolute ADG *(P < 0.05).* The nature of the interaction was due to the similarities in performance of the infected birds, whereas the performance of the control birds was affected by their feed composition. Furthermore, the infected HF birds showed the ability to maintain similar performance as their uninfected counterparts *(P < 0.05).* Infection caused an increase in cFCR during the acute stage of infection *(P < 0.05).* There was no significant effect of diet dilution on cFCR *(P > 0.05)*, however cFCR linearly increased in the control birds during the acute stage of infection *(P < 0.05).*

Infection significantly affected relative ADG during the acute and recovery stages of the infection (*P < 0.05*); where the performance of infected birds was lower than that of control birds during the acute stage of the infection, the reverse was the case during the recovery stage. There was a significant interaction between infection and feed composition (*P < 0.05*) on relative ADG during the acute stage of the infection. There were no significant differences in the relative ADG of the infected and control birds offered the LF and HF feeds, whereas the performance of the infected NF and MF birds was significantly reduced compared to their uninfected counterparts.

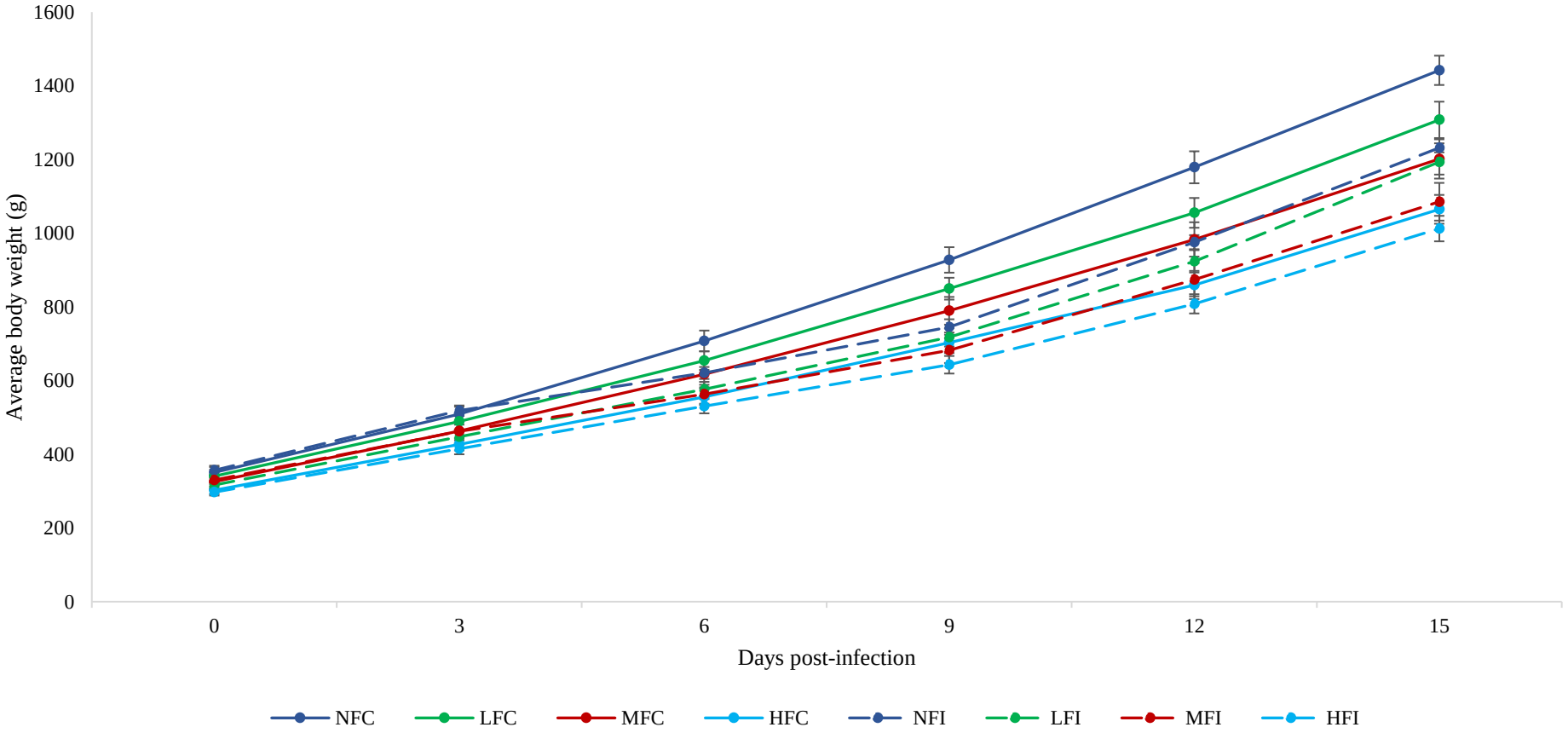


Figure 4.2. Average body weight (g) of the infected (I) and the corresponding uninfected control birds (C) at the start (d0pi) of infection with *Eimeria maxima* oocysts until the end of the experiment (d15pi). Birds were offered feeds diluted with 0% (NF), 5% (LF), 10% (MF), or 15% (HF) Arbocel lignocellulose.

Table 4.4. Average daily gain (ADG; g/ day), average daily gain/ body weight at the start of the period daily gains (log(g/ kg BW/ day)), and corrected feed conversion ratio (cFCR) of the infected and the corresponding uninfected control birds during infection with *Eimeria maxima* oocysts. Data are presented over the pre-patent (d0-3pi), acute (d4-8pi), and recovery stages (d9-14pi), and ADG data were also scaled relative to the mean body weight of each stage of infection (BWp) and were then transformed by the natural logarithm. Birds were offered feeds diluted with 0% (NF), 5% (LF), 10% (MF), or 15% (HF) Arbocel lignocellulose. (LS means with SEM).

		ADG (g/ day)			ADG/ BWp (log(g/ kg/ day))			cFCR		
Period		Pre-Patent	Acute	Recovery	Pre-Patent	Acute	Recovery	Pre-Patent	Acute	Recovery
Feed composition										
	NF	52.5^{c}	54.5^{b}	71.5^{a}	4.52^{z}	4.41^{yz}	4.24	1.31	1.53	1.84
	LF	47.1^{bc}	53.3^{b}	66.7^{a}	4.47^{yz}	4.49^{z}	4.24	1.30	1.54	1.80
	MF	44.3^{ab}	46.1^{a}	58.3^{b}	4.44^{y}	4.39^{y}	4.18	1.30	1.57	1.84
	HF	40.4^{a}	42.3^{a}	52.3^{b}	4.43^{y}	4.41^{yz}	4.17	1.31	1.58	1.83
	SEM	1.47	1.68	1.88	0.019	0.023	0.023	0.027	0.043	0.040
Infection										
	Control	48.2	58.3	62.4	4.48	4.55	4.16	1.29	1.45	1.85
	Infected	46.2	39.8	62.0	4.45	4.30	4.26	1.31	1.60	1.80
	SEM	1.04	1.19	1.33	0.014	0.016	0.016	0.019	0.030	0.028
Feed composition x Infection										
Uninfected	NF	54.1	70.5^{d}	73.5	4.52	4.63^{e}	4.18	1.29	1.37	1.84
	LF	50.8	61.1^{cd}	65.5	4.49	4.57^{de}	4.17	1.28	1.42	1.85
	MF	47.2	55.0^{bc}	58.9	4.47	4.53^{de}	4.14	1.28	1.46	1.84
	HF	41.8	46.6^{ab}	51.7	4.44	4.47^{cd}	4.14	1.31	1.53	1.88
Infected	NF	49.0	38.6^{a}	69.5	4.53	4.19^{a}	4.29	1.34	1.69	1.84
	LF	44.5	45.4^{ab}	68.0	4.45	4.42^{cd}	4.32	1.32	1.65	1.74
	MF	41.5	37.2^{a}	57.6	4.42	4.25^{ab}	4.23	1.33	1.68	1.84
	HF	39.1	37.9^{a}	52.8	4.41	4.35^{bc}	4.21	1.31	1.63	1.79
	SEM	2.08	2.37	2.65	0.027	0.033	0.032	0.038	0.061	0.056
Source		Probabilities								
Feed composition		<0.001	<0.001	<0.001	0.005	0.013	0.076	0.301	0.052	0.863
Infection		0.453	<0.001	0.823	0.142	<0.001	<0.001	0.202	0.001	0.204
Feed composition x Infection		0.661	<0.001	0.630	0.732	<0.001	0.621	0.321	0.062	0.650

[a-d] Means within a column that do not share a common superscript are significantly different ($P < 0.05$)

Abbreviations: ADG, average daily gain; BWp; mean BW of the period; cFCR, corrected feed conversion ratio; BW, body weight day 0 pi; C, Uninfected; I, Infected; pi, post-infection.

4.4.4 *Oocyst per gram (OPG)*

There was no evidence of infection amongst the uninfected birds, as there were no oocysts present in the faeces from their pens. Infected birds began to excrete oocysts from d5 post-infection and were virtually zero by d10 post-infection (Figure 4.3). There was no effect of diet or an interaction between diet and time on oocyst excretion/ g DM ($P > 0.05$); the across time back transformed means and confidence intervals for oocysts/ g DM were 2,392 (1,164 to 4,915), 2,515 (1,212 to 5,167), 2,592 (1,249 to 5,324), and 3,533 (1,703 to 7,332) for dietary treatments NF, LF, MF and HF respectively.

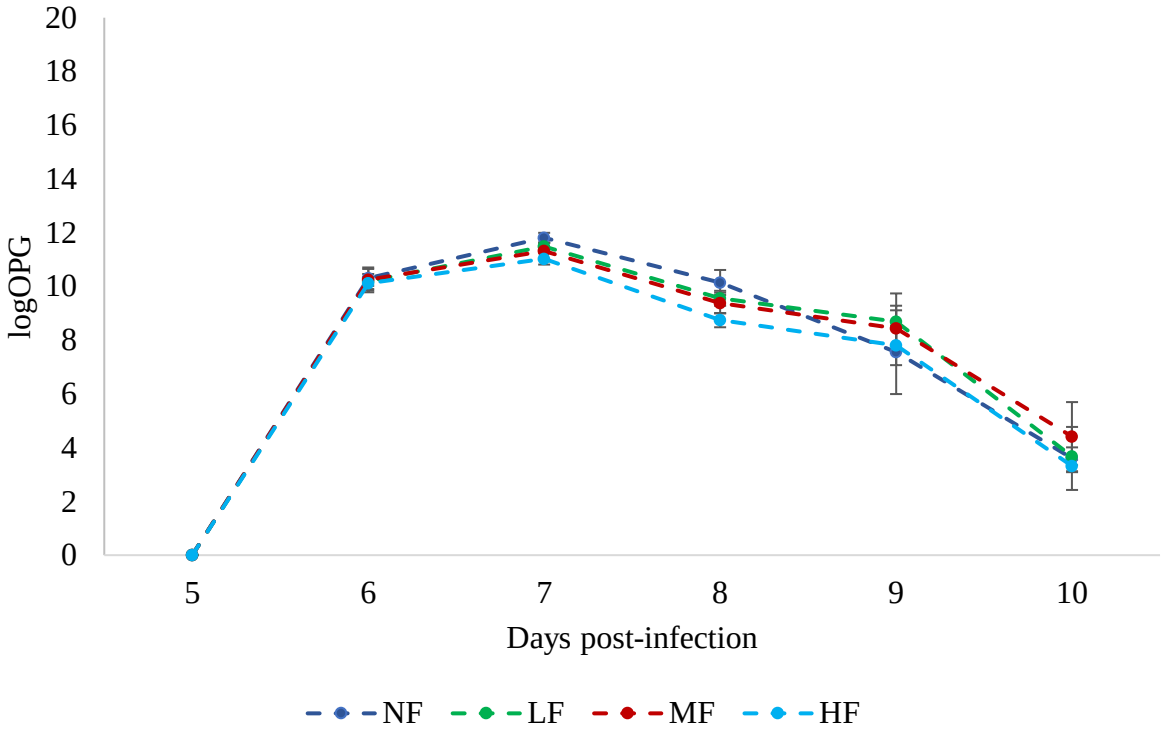


Figure 4.3. Number of oocysts per gram of faecal DM (OPG) on a logarithmic scale, for birds infected with 7000 *Eimeria maxima* oocysts offered diets with the same ME:CP ratio, diluted with 0% (NF), 5% (LF), 10% (MF), or 15% (HF) Arbocel lignocellulose

4.4.5 *Histology, organ measurements and pH*

Sections of the duodenum and jejunum were collected from one bird per pen at d6 (Table 4.5) and d12 (Table 4.6) post-infection. Infection significantly affected ($P < 0.05$) crypt depth, but not villi length, in the duodenum and jejunum (µm/ kg BW) at d6 post-infection; infected birds had deeper crypts in comparison to control birds. Similarly, at d12 post-infection duodenal and jejunal crypts were deeper in infected birds than controls ($P < 0.05$). Infection significantly increased villi length in the jejunum at d12 post-infection ($P < 0.05$). Villi length to crypt depth ratio was significantly reduced in infected birds in the duodenum and jejunum at both d6 and d12 post-infection ($P < 0.05$). Jejunal villi length was significantly affected ($P < 0.05$) by diet dilution on both d6 and d12 post-infection; birds fed diluted feeds had longer villi than NF fed birds.

The length of intestinal segments of the infected birds were greater compared to control birds at d6 and d12 post-infection ($P < 0.05$) (Table 4.7). Moreover, on d6 post-infection, an interaction between feed composition and infection was observed in the relative lengths of the duodena, jejuna, and ilea ($P < 0.05$). Infected birds fed diluted feeds developed their small intestine to a greater extent than infected NF birds, whereas in the absence of infection there were no differences in intestinal development between the dietary treatments ($P > 0.05$). The greater intestinal growth of the infected birds resulted in a significant effect of feed composition on the duodena, jejuna, and ilea at d6 post-infection, and duodena at d12 post-infection ($P < 0.05$). The pH of the gizzard, small intestine, and caeca are presented in Table 4.8; however, there was no significant effect of feed composition or infection *($P > 0.05$).*

Table 4.5. Villi length (VL; µm/ kg BW), crypt depth (CD; µm/ kg BW), and villi length to crypt depth ratio (VCR) of the duodenum and jejunum, during the acute stage (d6pi) of the infected and the corresponding uninfected control birds during infection with *Eimeria maxima* oocysts. Birds were offered feeds diluted with 0% (NF), 5% (LF), 10% (MF), or 15% (HF) Arbocel lignocellulose.

		Duodenum			Jejunum		
		VCR	VL (µm/ kg BW)	CD (µm/ kg BW)	VCR	VL (µm/ kg BW)	CD (µm/ kg BW)
Feed composition	NF	9.51	1918	239	5.43	1029[a]	223
	LF	8.33	2290	302	5.21	1318[ab]	307
	MF	9.21	2118	272	5.79	1417[b]	270
	HF	9.23	2171	285	6.12	1344[b]	264
	SEM	0.663	121.6	17.6	0.431	79.7	23.6
Infection	Control	12.2	2181	184	7.39	1234	172
	Infected	5.99	2068	364	3.88	1320	360
	SEM	0.468	86.0	12.5	0.305	56.3	16.7
Feed composition × Infection							
Uninfected	NF	13.3	2078	159	7.04	1002	155
	LF	10.2	2085	208	6.98	1238	181
	MF	11.9	2284	195	7.22	1359	193
	HF	13.1	2277	174	8.32	1338	160
Infected	NF	5.71	1758	318	3.81	1056	292
	LF	6.45	2496	396	3.44	1399	434
	MF	6.50	1952	349	4.35	1475	348
	HF	5.31	2066	395	3.92	1351	368
	SEM	0.937	171.9	24.9	0.610	112.7	33.3
Source				*Probabilities*			
Feed composition		0.627	0.196	0.090	0.472	0.007	0.111
Infection		<0.001	0.359	<0.001	<0.001	0.287	<0.001
Feed composition × Infection		0.116	0.114	0.530	0.637	0.917	0.311

[a-b]Means within a column that do not share a common superscript are significantly different ($P < 0.05$).
Abbreviations: VL, villi length; CD, crypt depth; VCR, villi length to crypt depth ratio

Table 4.6. Villi length (VL; µm/ kg BW), crypt depth (CD; µm/ kg BW), and villi length to crypt depth ratio (VCR) of the duodenum and jejunum, during the recovery stage (d12pi) of the infected and the corresponding uninfected control birds during infection with *Eimeria maxima* oocysts. Birds were offered feeds diluted with 0% (NF), 5% (LF), 10% (MF), or 15% (HF) Arbocel lignocellulose.

		Duodenum			Jejunum		
		VCR	VL (µm/ kg BW)	CD (µm/ kg BW)	VCR	VL (µm/ kg BW)	CD (µm/ kg BW)
Feed composition	NF	13.0	1535	126	6.45	771[a]	127[ab]
	LF	12.9	1571	125	8.24	972[ab]	123[a]
	MF	11.4	1701	158	7.27	1003[b]	152[ab]
	HF	12.1	1718	144	7.09	1066[b]	158[b]
	SEM	0.93	101.5	8.9	0.713	58.0	10.5
Infection	Control	13.8	1573	119	8.33	879	107
	Infected	10.9	1689	158	6.19	1027	173
	SEM	0.63	68.7	6.0	0.489	41.0	7.2
Feed composition × Infection							
Uninfected	NF	16.2	1547	96.4	7.47	635	83.0
	LF	13.7	1434	110	8.91	921	105
	MF	12.8	1710	139	8.53	923	117
	HF	12.4	1602	131	8.43	1035	123
Infected	NF	9.82	1524	157	5.44	906	170
	LF	12.1	1708	141	7.57	1023	140
	MF	9.91	1692	176	6.02	1082	187
	HF	11.8	1833	157	5.76	1098	193
	SEM	1.31	143.5	12.6	1.104	89.8	16.3
				Probabilities			
Feed composition		0.628	0.408	0.053	0.330	0.009	0.041
Infection		0.002	0.291	<0.001	0.003	0.012	<0.001
Feed composition × Infection		0.131	0.593	0.500	0.900	0.608	0.323

[a-b] Means within a column that do not share a common superscript are significantly different ($P < 0.05$).
Abbreviations: VL, villi length; CD, crypt depth; VCR, villi length to crypt depth ratio

Table 4.7. The relative lengths of the duodenum (DL), jejunum (JL), and ileum (IL) (cm/ kg body weight (BW) at the point of euthanasia) during the acute (d6 post-infection) and recovery stages (d12 post-infection), and the relative weight of the full small intestine (SIF) (g/ kg BW) (d15 post-infection) of the infected and the corresponding uninfected control birds during infection with *Eimeria maxima* oocysts. Birds were offered feeds diluted with 0% (NF), 5% (LF), 10% (MF), or 15% (HF) Arbocel lignocellulose.

		D6pi			D12pi			D15pi
		DL (cm/ kg BW)	JL (cm/ kg BW)	IL * (cm/ kg BW)	DL (cm/ kg BW)	JL (cm/ kg BW)	IL (cm/ kg BW)	SIF * (g/ kg BW)
Feed composition								
	NF	31.6^{y}	81.5^{x}	4.41^{y}	26.0^{yz}	64.9	64.7	4.44^{y}
	LF	34.6^{yz}	91.3^{xy}	4.47^{y}	23.0^{y}	63.9	69.2	4.57^{z}
	MF	38.9^{z}	101^{yz}	4.68^{z}	27.1^{z}	69.2	70.5	4.57^{z}
	HF	39.0^{z}	111^{z}	4.70^{z}	28.1^{z}	71.3	75.3	4.64^{z}
	SEM	1.87	2.96	0.030	0.94	3.27	2.82	0.025
Infection								
	Control	33.1	84.2	4.46	23.9	61.3	64.2	4.50
	Infected	38.9	108	4.67	28.1	73.4	75.6	4.60
	SEM	1.32	2.32	0.03	0.67	2.31	2.00	0.016
Feed composition × Infection								
Uninfected	NF	31.2^{a}	83.7^{a}	4.39^{a}	22.8	56.8	54.4	4.39
	LF	32.6^{ab}	78.1^{a}	4.46^{a}	21.3	57.7	64.7	4.52
	MF	35.1^{abc}	84.9^{a}	4.44^{a}	25.9	68.4	69.3	4.49
	HF	33.6^{abc}	90.0^{a}	4.57^{a}	25.7	62.2	68.5	4.60
Infected	NF	32.1^{a}	79.3^{a}	4.44^{a}	29.1	73.0	74.9	4.49
	LF	45.3^{c}	125^{b}	4.90^{b}	24.6	70.2	73.8	4.61
	MF	34.1^{abc}	97.7^{a}	4.51^{a}	28.4	69.9	71.6	4.64
	HF	44.3^{bc}	132^{b}	4.84^{b}	30.5	80.4	82.2	4.67
	SEM	2.64	4.37	6.15	1.33	4.63	3.99	0.032
					Probabilities			
Feed composition		0.019	<0.001	<0.001	0.003	0.343	0.081	<0.001
Infection		0.003	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
Feed composition × Infection		0.028	<0.001	0.002	0.518	0.289	0.153	0.622

a-c, x-z Means within a column that do not share a common superscript are significantly different ($P < 0.05$).

* indicates data were log transformed for analysis.

Table 4.8. The pH of the gizzard, duodenum, jejunum, ileum and caeca from d6 and 12pi of the infected and the corresponding uninfected control birds during infection with *Eimeria maxima* oocysts. Birds were offered feeds diluted with 0% (NF), 5% (LF), 10% (MF), or 15% (HF) Arbocel lignocellulose.

		D6pi					D12pi				
		Gizzard pH	Duodenum pH	Jejunum pH	Ileum pH	Caeca pH	Gizzard pH	Duodenum pH	Jejunum pH	Ileum pH	Caeca pH
Uninfected	NF	2.05	5.93	6.09	6.34	6.14	2.00	6.00	5.96	6.56	6.38
	LF	2.10	6.17	6.14	5.94	6.37	1.94	6.11	5.96	6.61	5.76
	MF	1.78	5.83	5.92	6.33	6.23	1.74	6.20	6.13	6.50	6.14
	HF	1.99	5.92	5.83	5.95	6.01	1.95	6.23	6.13	6.85	5.86
Infected	NF	1.64	6.12	5.93	6.49	6.08	2.44	6.27	6.12	7.06	6.23
	LF	1.90	5.98	5.84	6.41	6.26	1.79	6.14	6.00	6.61	5.83
	MF	1.90	6.13	6.03	6.24	6.60	1.99	6.25	6.12	6.59	5.97
	HF	1.72	5.96	6.04	6.13	6.45	1.60	6.25	6.16	7.18	5.99
	SEM	0.201	0.166	0.141	0.171	0.183	0.328	0.095	0.092	0.192	0.074
		Probabilities									
Feed composition		0.877	0.653	0.913	0.179	0.270	0.709	0.509	0.400	0.487	0.887
Infection		0.602	0.651	0.601	0.204	0.246	0.908	0.085	0.429	0.208	0.586
Feed composition × Infection		0.490	0.419	0.358	0.640	0.479	0.658	0.215	0.365	0.910	0.210

4.5 Discussion

The experimental feeds were formulated to test the hypothesis that pathogen-induced anorexia is sensitive to feed composition. To do this, it was important to ensure that such feeds would constrain feed intake in the uninfected birds to identify any changes in the direction of the effect of feed composition between the uninfected and infected birds. By definition, pathogen-induced anorexia reduces feed intake and as such this means that the infected birds would no longer be constrained by their feed composition during this period and would be capable of modifying their feed intake to meet their desired nutrient intake during anorexia. In order to ensure that energy was the first limiting nutrient and all nutrient to energy ratios were accurately maintained, lignocellulose was identified as a suitable diluent since it is an inert, bulky material and would not provide any nutritional value to the feeds. Furthermore, lignocellulose should constrain feed intake in the uninfected birds due to its bulkiness, in line with the results from Chapter 2 and Chapter 3 which showed that the bulkiness of feeds can constrain feed intake in healthy broilers when energy was the first limiting nutrient.

The literature surrounding the responses of uninfected birds to diluted feeds is inconsistent (Leeson *et al.*, 1996b; Savory *et al.*, 1996; Hetland and Svihus, 2001; Shakouri *et al.*, 2006). The feeds offered in the current experiment were first limiting in energy, but there was no increase in feed intake in birds offered the diluted feeds as in the experiment by Leeson *et al.* (1996b). Instead, the results were more comparable with those of Savory *et al.* (1996), since in this experiment feed intake was reduced as dilution increased. As dilution with the inert material increased, ADFI of the uninfected birds initially increased and then decreased, presumably because at the highest dilutions the birds were limited by their capacity for bulk (Kyriazakis and Emmans, 1995); lignocellulose has a high WHC (Chen *et al.*, 2019) which is one of the determinants of bulk (Ndou *et al.*, 2013). Therefore, in this study it seems that the bulkiness of the diluted feeds prevented the birds from showing compensatory feed intake to achieve the targeted energy intake. It can be suggested that the confusion surrounding diet dilution and its effects on feed intake arises from differences in the bulk properties of ingredients used to dilute the feeds.

The effect of feed composition on the feed intake of broiler chickens infected with the coccidian parasite, *E. maxima* was investigated. It was hypothesised that feed composition would affect the extent of pathogen-induced anorexia, and as such, broilers offered diluted feeds who were exposed to *E. maxima* would show a lesser extent of anorexia compared to

those offered a basal feed. As the diluted feeds are reduced in nutrient density, it was expected that feed intake would be increased during anorexia on these feeds compared to the basal feed to enable the host to consume a specific nutrient intake. This contrasts with the prevailing view that anorexia is dependent *only* on the host and the pathogen (Kyriazakis, 2014), or the suggestion that during subclinical infections the extent of anorexia is consistent across hosts and pathogens: ~20% reduction in the feed intake of their uninfected counterparts (Sandberg *et al.*, 2006).

Consistent with the hypothesis, the results suggest that infected hosts attempted to regulate their nutrient resource intake during infection and thus the extent of anorexia was affected by feed composition. The infected birds increased their ADFI proportionally to the dilution, to maintain nutrient intake during the acute stage of infection, so that there were no differences in energy or protein intake amongst any of the infected treatments. In fact, the relative nutrient intake on the most diluted feed (HF) was similar between the infected and non-infected birds during the acute stage of infection, suggesting complete absence of pathogen-induced anorexia. As far as we know, this is the first such manipulation of pathogen-induced anorexia.

A consequence of these effects on feed intake was that the outcomes of the infection were unaffected by feed composition. There were no differences in ADG, the number of excreted oocysts or the damage to the GIT between the infected birds on the different feeds. As these traits correspond to resistance (oocysts) and resilience (ADG and VCR) to infection, it could be concluded that this strategy did not influence resistance and resilience to *Eimeria* infection. This finding is in contradiction to Kyriazakis (2010), who proposed that infected hosts given access to a feed of high nutrient content would express immunity earlier, and thus recover from infection faster, than infected hosts given a feed of low nutrient content. However, the results from the present experiment support the idea of Kyriazakis (2010) that the extent of anorexia would be greater when infected hosts are offered a feed of high nutrient content compared with infected hosts given a feed of low nutrient content. This suggests that parasitized animals are tightly regulating nutrient intake to meet specific targets during anorexia to help them overcome infection. However, it was not possible to identify the targeted nutrient resource due to the nature of the feed formulation. These results support the hypothesis proposed by Kyriazakis *et al.* (1998), that the underlying mechanism behind anorexia in parasitized animals is a functional, costly, adaptive behaviour.

During recovery (from d9 post infection), previously infected birds consumed more feed per unit of body weight than their uninfected counterparts. This was accompanied by increases in both relative energy and protein intake, and relative growth rate. However, the degree of compensatory growth depended on feed composition, with birds on the most diluted feed, showing the least ability to compensate. Such increases are consistent with the compensatory intake seen during the recovery from infections in several species (Banfield and Forbes, 2001; Coltherd *et al.*, 2009; Pastorelli *et al.*, 2012), and is consistent with the idea that animals aim to return to a desired body weight and composition after a period of limitation in their growth (Kyriazakis and Houdijk, 2007). The fact that the compensatory intake was seen whilst the digestive tract was still recovering from the infection (the effects on both the jejunum and ileum were still evident by day 14 of the infection), suggests that there may be improved absorption of nutrients during recovery from infection (Turk, 1973; Banfield *et al.*, 2002). The results offer support to the experiment by Turk (1973), who investigated the effects of a coccidian infection on calcium absorption in broiler chickens. He observed compensatory increases in nutrient absorption from d10pi in broiler chickens infected with either; *E. acervulina, E. necatrix, E. brunetti* or *E. tenella*. Furthermore, Banfield *et al.* (2002) found an increase in small intestine weight of birds fed feeds containing carboxymethyl cellulose (CMC), which they suggested to be a rapid attempt to increase the absorptive surface area of the GIT due to the reduced diffusion rates caused by the inclusion of CMC which increased digesta viscosity. In the present study, the lengths (d6 and 12pi) and weight (d15pi) of the small intestine were significantly higher in the infected birds offered diluted feeds. These results suggest that the absorptive area of the GIT was increased in birds offered diluted feeds in response to the effect of lignocellulose inclusion on digesta viscosity, in agreement with Banfield *et al.* (2002). Contrary to expectation, there were no significant effects of feed composition or infection status on pH of the GIT. Diet dilution with cellulose has been shown to reduce digesta pH throughout the GIT (Jiménez-Moreno *et al.*, 2009a); similarly pH of the GIT has been shown to reduce during coccidiosis infection (Ruff and Reid, 1975).

In conclusion, this study demonstrates that the characteristics of anorexia, specifically *Eimeria*-induced anorexia in broiler chickens, can be affected by feed composition. It appears that the birds alter their feed intake in response to feed composition during anorexia as they attempt to attain a specific nutrient intake by changes in their feed intake, however it was not possible to identify which nutrient was being regulated. The results support the view that anorexia is an adaptive, functional behaviour of the host which comes at a short-term cost.

Unravelling the targets of infected hosts during pathogen-induced anorexia will shed light on how infected animals, and even humans, should be fed during the critical stages of infection. Considering the importance of dietary crude protein in the expression of immunity (Korver, 2012; Calder, 2013; Clough *et al.*, 2016) it is reasonable to assume that the crude protein content of feed will also affect the characteristics of anorexia; considering the effect of feed protein content on the immune system, and the intrinsic link between pathogen induced anorexia and the immune system. Therefore, in the following Chapter, the effect of protein dilution on pathogen-induced anorexia in broilers infected with *E. maxima* will be investigated.

Chapter 5. Is pathogen-induced anorexia sensitive to feed crude protein content?

5.1 Abstract

The hypothesis that pathogen-induced anorexia depends on feed composition was addressed by formulating four iso-energetic feeds which differed in their crude protein (CP) content (12%, 16%, 20%, 24%), and offered to uninfected and birds infected with *Eimeria maxima*. It was hypothesised that the duration of anorexia would be reduced in infected birds fed high CP feeds with the same digestible limiting EAA to lysine, as high CP may increase resistance in parasitized hosts. A total of 480 male Ross 308, day old broilers were distributed into 48 pens and offered a common starter feed until day (d) 8 of age. A gradual feed changeover was implemented from d8-10 of age, and birds were offered the full dietary treatment on d10 of age. At d13 of age (d0 post infection; pi) half of the birds were infected with 7,000 sporulated *E. maxima* oocysts. Feed intake was measured daily until d15pi and faeces were collected from d5pi until d11pi to measure oocysts per gram (OPG) of excreta. In the presence and absence of infection absolute average daily feed intake (ADFI) increased to a plateau as protein content increased ($P < 0.05$). There was no significant interaction between protein content and infection on both absolute and relative ADFI throughout the experiment ($P > 0.05$). However, there was an interaction during the acute stage of infection between protein content and infection on absolute average daily crude protein intake (ADCPI) ($P < 0.05$) but not relative ADCPI ($P = 0.091$). This interaction was due to the infected birds offered the 20% CP feed showing no significant difference in absolute ADCPI compared to their uninfected counterparts, whereas there were significant reductions in absolute ADCPI between the remaining infected birds and their respective controls ($P = 0.01$). Furthermore, there was no significant difference in absolute and relative ADCPI between infected birds offered the 20% and 24% CP feeds. The fact that the infected birds offered the 12% and 16% feeds were unable to reach the ADCPI of the 20% and 24% birds likely reflects their inability to cope with the excess energy intake associated with reaching such ADCPI since the feeds were iso-energetic. This idea is supported by the lack of significant differences in the relative average daily energy intake between birds given the 12% and 16% feeds ($P > 0.05$), which were significantly greater than the birds given the 20% and 24% feeds. On d9pi, birds fed the 12% CP feed excreted significantly more oocysts compared to birds fed 20% CP ($P < 0.05$) confirming the negative effect of low protein feeds on the resistance to infection. In conclusion, during pathogen-induced anorexia, the birds

appeared to target protein intake during the acute stage of infection although this warrants further investigation.

5.2 Introduction

Causal hypotheses for pathogen-induced anorexia are in abundance (Sarraf *et al.*, 1997; Plata-Salaman, 1998; Langhans, 2000), however these hypotheses fail to address the functionality and purpose of such anorexia. In the past, proposed hypotheses to explain the paradoxical occurrence of pathogen-induced anorexia include: 1) anorexia is a costly, behavioural adaptation by the host, and 2) anorexia is an unavoidable consequence of infection. However, more recently Kyriazakis (2014) proposed that both approaches can be combined to make pathogen-induced anorexia more predictable by appreciating what an infected animal is trying to achieve through its feeding behaviour. Here, the author suggested that it may be possible to make predictions about pathogen-induced anorexia by linking the host immune response to anorexia. Furthermore, he suggested that the characteristics of anorexia could become more predictable if we gain a better understanding of what the infected animal is trying to achieve through its feeding behaviour.

Indeed, in Chapter 4, the results suggested that broiler chickens infected with *Eimeria maxima* were modifying their feed intake in an attempt to regulate either their energy or nutrient resource intake during the acute stage of infection. Broilers were infected with *E. maxima* and offered feeds which were progressively diluted with an inert material (lignocellulose), the consequences of such a dilution result in feeds of reduced energy and nutrient composition whilst the ratios of energy to nutrients were maintained. The results showed that infected birds offered feeds with a low energy content reduced the extent of anorexia compared with infected birds offered high energy feeds, which resulted in similar energy and nutrient resource intakes between the two treatments during the acute phase of infection. Despite there being clear differences in the extent of anorexia, the onset, duration and recovery of anorexia did not differ between treatments lasting from d4 until d8 post infection (pi). However, due to the nature of the feeds, it was not possible to determine if either energy or nutrient intake were being targeted during pathogen-induced anorexia and so the question remains, what is being targeted during infection?

It is known that protein is important in the expression of immunity, since most of the effector mechanisms of the immune system are proteinaceous in nature (Coop and Kyriazakis, 1999). Therefore, since feed composition affects the characteristics of pathogen-induced anorexia, it seems logical to investigate protein as a potential targeted resource during anorexia. There is some old evidence (Sharma *et al.*, 1973) suggesting that high levels of crude protein (CP)

increase the severity of caecal coccidiosis in chickens. This has been ascribed to higher levels of trypsin secretion caused by a high dietary protein level, since trypsin is considered responsible for excystation of a larger proportion of infective doses of oocysts (Britton *et al.*, 1964). However, as this effect appears to be observed in other infections (e.g. with *Salmonella gallinarum* (Hill and Garren, 1961; Boyd and Edwards Jr, 1963) and Newcastle disease virus (Boyd and Edwards Jr, 1963) and Marek's disease virus (Proudfoot and Aitken, 1969), there seems to be a generalised advantage to avoid excess protein intake during the acute stages of the infection. Kyriazakis *et al.* (1991) suggested that in healthy animals at least, such an avoidance of excess protein intake was associated with preventing the overloading of the metabolic processes in dealing with it. However, from the view of an infected animal, this could be interpreted as the host reducing the risk of a more severe infection. Diseases such as coccidiosis are known to be pre-disposing factors for secondary infections, such as necrotic enteritis (NE) (Collier *et al.*, 2008; Prescott *et al.*, 2016). Furthermore, it has been shown that an excess dietary protein is another significant factor which increases the risk of the host to succumbing the NE (Drew *et al.*, 2004; Wu *et al.*, 2014).

In uninfected broilers, the response to the protein content of the feed largely depends upon the ratios of amino acids (AA) to crude protein content (Summers *et al.*, 1992; Sterling *et al.*, 2003; Sterling *et al.*, 2006). In cases where there is an AA imbalance, the feed intake has been shown to decrease linearly as CP increases (Sklan and Plavnik, 2002; Sterling *et al.*, 2006). However, if the ratios of AA to CP are maintained, then feed intake and weight gain increase before reaching a plateau (Sklan and Plavnik, 2002). Therefore, it is important to maintain AA to CP ratios when formulating feeds to assess the effect of crude protein content on performance in both the presence and absence of infection.

The effect of dietary protein content on anorexia in monogastric animals is somewhat contradictory. Williams *et al.* (1997) showed that the extent of anorexia was reduced in pigs fed lysine deficient feeds. Similarly, Webel *et al.* (1998a) observed a tendency for the effect of lipopolysaccharide administration on growth performance to be greater in chicks offered feeds supplemented with higher levels of arginine. The results from these two experiments are in agreement with the effect of AA balance on performance in healthy broilers described above (Summers *et al.*, 1992; Sterling *et al.*, 2003; Sterling *et al.*, 2006). In parasitized hosts, the experiment by Coltherd *et al.* (2009) showed that there was no significant effect of dietary protein content when amino acids were maintained relative to protein content on the extent of anorexia in mice infected with *Heligmosomoides bakeri*. On the other hand, Tu *et al.* (2007; 2009) observed in two experiments that there was an immediate reduction in feed intake post-

infection of mice infected with *H. bakeri* when given access to protein-sufficient feeds, whereas the feed intake of the infected mice given protein-deficient feeds remained unaffected. However, there was delayed expulsion of the parasite in the protein-deficient mice, indicating a reduced resistance to infection in these mice compared to the protein-sufficient mice. It should be noted that in the two Tu experiments, it is unclear as to whether the feeds were formulated in such a manner that the AA to CP ratio was kept constant and this may account for the different feed intake responses observed between these experiments and the experiment by Coltherd *et al.* (2009).

Studies investigating the effect of dietary protein content on coccidiosis in poultry often suffer one major criticism, which is the period in which feed intake data are presented. This criticism arises because the onset, duration and recovery of anorexia during coccidiosis occur within a few days (Kipper *et al.*, 2013; Sakkas *et al.*, 2018; Oikeh *et al.*, 2019), therefore, infrequent measurements or data presented as a weekly average can overlook any subtle differences in the characteristics of anorexia. For example, Sharma *et al.* (1973) investigated the effect of dietary crude protein content on intestinal and caecal coccidiosis. Unfortunately, it is not possible to make inferences on the patterns of anorexia from the Sharma *et al.* (1973) experiment because the performance data were presented from d0-8 and d8-14 post-infection. More recently, the presentation of feed intake and growth performance has been overlooked completely in favour of immunological measurements (Arczewska-Włosek *et al.*, 2017; Arczewska-Włosek *et al.*, 2018).

The current experiment addressed whether pathogen-induced anorexia is sensitive to protein supply. This was tested by progressively diluting the nutrient dense feed with a protein-free ingredient (starch); whilst such a dilution maintains the energy content of the feeds, it results in feeds of different protein contents. It was hypothesised that the feed intake of healthy broilers would increase to a plateau as dietary protein content increased. In the presence of infection, it was hypothesised that infected birds offered a high CP feed would show the greatest extent of anorexia as they reduce feed intake in attempt to avoid an excess of protein intake. Furthermore, the consequences of CP content on the outcomes of the infection were assessed, i.e. the extent of the damage caused by the parasite, the number of parasites excreted by the host, and the rate of recovery. It was expected that the infection outcomes would be less severe in birds fed a high CP feed as they have more available protein to facilitate an increase in resistance, this will be shown by a reduction in oocyst excretion and histological damage.

5.3 Materials and Methods

5.3.1 *Husbandry, birds and feeds*

All procedures were conducted under the UK Animals (Scientific Procedures) Act 1986 and EU Directive 2010/63/EU for animal experiments and carried out under UK Home Office authorization (P441ADF04). Furthermore, all procedures were approved by the Animal Welfare and Ethical Review Body (AWERB) of Newcastle University. All chicks were obtained from a commercial hatchery. They were housed in a thermostatically controlled building in 48 pens, each with an area of 0.85m^2. All birds were wing tagged upon arrival. Pens were equipped with feeders and drinkers, with wood shavings used as litter at a depth of 5 cm. The birds had free and continuous access to feed and water throughout the trial. The pen temperature was set to 34°C at arrival and was gradually reduced to 20°C by d25 of age. The lighting schedule was 23h Light (L):1h Darkness (D) for the first 7 days and was amended to 18L:6D for the course of the trial, whilst light intensity at pen level ranged from 80-100 lux.

A total of 432 male Ross 308 day old chicks were used. At d8 of age the birds were allocated to the dietary treatments until d29 of age when the trial was terminated. All birds were fed the same starter feed until d7 of age (Table 5.1). Each dietary treatment was replicated in 12 pens and each pen contained 9 birds at the start of the experiment, which was reduced to 7 birds at d13 of age. Four grower feeds were formulated with different levels of CP (Nitrogen x 6.25; Table 2); 12%, 16%, 20%, and 24%. The feeds were formulated to have constant CP to Lysine ratios and AA to Lysine ratios. Calcium, phosphorus, vitamins and minerals were constant in each of the grower feeds; the nutrient composition of the 20% CP feed was formulated to be identical to the basal feed used in Chapter 1. Experimental feeds were offered as a fine mash.

Table 5.1. Ingredient, calculated and analysed chemical composition of the common (starter) feed offered from day 0 to day 7 of age, and the experimental feeds offered from day 8 to day 28 of age. The basal, high density feed (24%) was diluted with corn starch in exchange for corn gluten meal, to result in iso-energetic feeds with crude protein contents of 20%, 16% and 12%.

		Crude protein content (%)			
Ingredients (%)	**Common Starter**	**24**	**20**	**16**	**12**
Ground Maize	10.0	41.1	40.4	39.7	39.1
Ground Wheat	51.5	-	-	-	-
Corn starch	-	10.0	16.7	23.3	30.0
Corn gluten meal	-	32.6	26.4	20.2	14.0
Soybean meal (48% CP)	26.0	-	-	-	-
Full fat Soya	5.00	-	-	-	-
Powdered cellulose (Solka-floc)	-	8.43	8.71	8.98	9.26
Limestone	1.25	1.34	1.34	1.35	1.35
L-Lysine HCL	0.40	1.15	0.97	0.78	0.60
DL-Methionine	0.40	0.02	0.03	0.03	0.04
L-Threonine	0.15	0.14	0.13	0.11	0.10
L-Tryptophan	-	0.10	0.08	0.07	0.05
L Arginine	-	0.64	0.54	0.44	0.34
Valine	-	0.00	0.01	0.03	0.04
Iso-leucine	-	0.04	0.05	0.06	0.07
Soya oil	3.00	2.00	2.17	2.33	2.50
Monocalcium phosphate	1.50	1.45	1.50	1.55	1.60
Salt	0.25	0.25	0.25	0.25	0.25
Sodium bicarbonate	0.15	0.35	0.35	0.35	0.35
Premix	0.40	0.40	0.40	0.40	0.40
Titanium dioxide	-	0.50	0.50	0.50	0.50
Total	100	100	100	100	100
Nutrient and chemical composition (%)					
Metabolizable energy (kcal kg-1) (calculated)	3057	3104	3104	3104	3104
Gross energy (MJ kg-1)	16.9	17.0	17.0	17.7	18.1
N × 6.25 (Crude protein; CP)	21.4	25.0	21.9	14.8	12.6
Crude fibre	2.90	8.94	9.11	9.28	9.45
Ether extract	5.55	4.20	4.27	4.33	4.40
Ether extract (oil A)	5.55	4.89	4.74	5.10	5.41
Total oil (oil B)	6.52	5.76	5.81	6.61	7.18
Ash	6.40	4.70	4.20	4.20	4.70
T-lysine (calculated)	1.43	1.41	1.18	0.96	0.73
Av-lysine (calculated)	1.34	1.34	1.13	0.92	0.70
Methionine (calculated)	0.70	0.64	0.54	0.44	0.34
Methionine+ cysteine (calculated)	1.03	1.06	0.89	0.73	0.56
Threonine (calculated)	0.91	0.94	0.79	0.65	0.50
Tryptophan (calculated)	0.25	0.21	0.18	0.14	0.11
Calcium (calculated)	0.95	0.89	0.90	0.91	0.92
Phosphorus (calculated)	0.69	0.60	0.58	0.56	0.54
Av-Phosphorus (calculated)	0.47	0.44	0.44	0.44	0.44
Salt (calculated)	0.31	0.30	0.31	0.31	0.32
Sodium (calculated)	0.18	0.21	0.21	0.21	0.21

[a] Provided per kilogram of feed: Vitamin A (vitamin A acetate), 13.5kIU; Vitamin D (cholecalciferol), 5.0kIU; Vitamin E (dl-α tocopherol acetate), 100mg

* Analysed composition

5.3.2 *Experimental design and inoculations*

Birds were weighed at arrival (d0), at the end of the starter period (d7), and then every three days until the end of the trial. To reduce the risk of refusals and starve outs the birds were offered a mixture of one-part common starter and one-part experimental feed between d8 and d10 of age, and the experimental feed from d11. Feed intake was measured between d0-7 and then daily until the conclusion of the experiment. Feed intake was measured over the starter period (d0-d7) and then daily until the conclusion of the experiment. At d7 birds were weighed and randomly allocated to one of the four feeding treatments. On d13 of age (d0 post-infection), birds were orally inoculated with either a single dose of 0.5 ml of H_2O (control), or 7000 sporulated *Eimeria maxima* oocysts of the Weybridge laboratory reference strain suspended in H_2O (infected), so that within a dietary treatment 6 pens contained control and the other 6 pens contained infected birds. *E. maxima* was chosen as it enables us to address the objectives of the experiments. The pathogen induces replicable anorexia on standard feeds; such anorexia is relatively short in duration and is eventually overcome (Sakkas *et al.*, 2018; Oikeh *et al.*, 2019).

5.3.3 *Sampling*

On day 6 and 12pi, one bird from each pen with a body weight (BW) close to the pen average was culled by intravenous lethal injection with sodium pentobarbital (Euthatal, Merial Harlow, United Kingdom); the sampling times corresponded to the acute and recovery stages of the infection. All birds were immediately weighed after euthanasia. During necropsy, the full gastrointestinal tract (GIT) was removed and separated into individual segments of interest; duodenum, jejunum, and ileum. After measuring the length of the duodenum, jejunum and ileum, a small section (1-2cm) of duodenum and jejunum were collected and stored in 10% neutral buffered formalin for morphometrical analysis. The duodenal sample was collected from the middle of the duodenal loop; and the jejunal sample from halfway between the point of entry of the bile ducts, and Meckel's diverticulum.

On d15pi, 3 birds per pen were selected, euthanised, weighed, and dissected as previously described. All remaining birds were culled by lethal injection with sodium pentobarbital (Euthatal®, Merial Harlow, United Kingdom).

5.3.4 *Sample analysis*

5.3.4.1 *Oocyst production*

Excreta were collected from d5-10 post-infection by placing polyethylene sheets on top of the wood shavings of each pen for 90 minutes. Upon sheet removal, excreta were pooled per pen into screw cap pots and stored at 4°C, pending analysis. The modified McMaster technique was used to estimate excretion of daily oocysts per gram (Kaufmann, 1996).

The excreta were thoroughly mixed before a 3 g sample was removed. The sample was mixed with 42 ml of water and passed through a sieve. The solution was then transferred to a glass test tube and centrifuged at 1500 RPM for 2 minutes at room temperature. The supernatant was carefully siphoned off and the pellet vortexed until it was fragmented. Then 10 ml of saturated NaCl was added and the solution thoroughly mixed. A sample of the solution was taken from the centre of the tube before being carefully transferred to the McMaster counting slide. Slides were left undisturbed for 10 minutes to allow the oocysts to rise to the top of the slide, before being read at 10 × magnification. The sum of the oocyst count of each chamber was calculated and multiplied by 50 to give oocysts per gram of excreta.

5.3.4.2 *Histology*

Intestinal segments fixed in formalin from the duodenum and jejunum from one bird per pen (6 birds per treatment) were subjected to a series of graded ethanol baths followed by xylene in a Shandon Excelsior Es Tissue Processor (Thermo Fisher Scientific Inc., Waltham) in order to dehydrate the tissue. The samples were then embedded in paraffin wax, sectioned at 4 µm and stained with haematoxylin/eosin (HE). After staining, the histological sections were scanned using the Leica SCN400 slide scanner system (Leica Microsystems, Buffalo Grove, IL, USA) and images were captured using the Leica SCN400 image viewer software. Morphometric features of the intestinal structure were observed at 10 × magnification. Villus height and crypt depth measurements were ascertained using Aperio ScanScope CS (Aperio Technologies, Vista, CA). The vertical distance from the villus tip to the villus-crypt junction of 10 villi was used to determine villus height, whilst crypt depth was the vertical distance from the villus-crypt junction to the lower limit of the crypt of 10 corresponding crypts. Villi length to crypt depth ratio (VCR) was calculated as the usual measure of cell proliferation, which reflects the extent of intestinal damage (Kettunen *et al.*, 2001).

5.3.5 *Calculations and statistics*

To account for mortalities the feeder was weighed when mortalities were found and the average feed intake (g/bird) was calculated and the assumed intake of the dead bird was subtracted from ADFI. Pen (n = 6) was considered the experimental unit for all data and all statistical analysis was performed using the nlme package in R (Team, 2013) using lm and anova functions from the nlme package (Pinheiro *et al.*, 2013). For all statistical procedures, the normality of the residuals was assessed with qq-plots and the Shapiro-Wilk test. When significant differences were detected, treatment means were separated and compared by the Tukey's multiple comparison test. Significance was determined at $P < 0.05$. Data are presented as model-predicted least square means with the SEM.

Average (mean) daily crude protein intake (ADCPI, g/ day) and average (mean) daily energy intake (ADEI, kcal/ day) were calculated by multiplying average (mean) daily feed intake (ADFI, g/ day) by the CP level and the energy content of the feed respectively. A repeated-measures mixed model was implemented to analyse daily feed intake. This analysis was done to separate the different stages of the infection (pre-patent, acute and recovery stage). The model included protein content, infection, and day as fixed factors, two-way interactions between protein content and infection, protein content, and day, infection and day and the three-way interaction between protein content, infection, and day. Covariance structures were chosen based on the lowest value for the Akaike and Bayesian information criteria. Further analysis of variance was carried out on time points when the three-way interaction between protein content, infection and day was significant.

Two analyses were performed on the ADFI, ADEI, ADCPI and ADG data: 1) Based on the day that a reduction in feed intake was observed and the day that feed intake recovered in infected compared to control birds, actual ADFI, ADEI and ADCPI and ADG data were calculated over the pre-patent, acute and recovery stages of infection and were analysed with general linear mixed (GLM) models with protein content and infection as fixed factors and their interactions. 2) These actual intakes and their corresponding ADG data calculated per stage of infection were also scaled relative to the mean body weight of each stage of infection (BWp) and log transformed. The scaling allowed to account for the different growth trajectories arising from feed composition, as it has been shown that different conclusions can be drawn when feed intake is analysed on a body weight as opposed to time basis (Kyriazakis *et al.*, 1991; Allison *et al.*, 1995). The log transformation ensured that the residuals were normally distributed and avoided statistical bias (Pinheiro *et al.*, 2013). Log transformed relative ADFI, ADEI and ADCPI and ADG were analysed with general linear mixed (GLM) models with protein content and infection as fixed factors and their interactions.

Histological measurements from one bird per pen were obtained at d6 and d12 post-infection, these were expressed relative to the body weight of the bird at the point of euthanasia (µm/ kg BW) to account for a priori differences in performance (Oikeh *et al.*, 2019).

The measured dry matter (DM) of daily excreta was used to express oocyst excretion per g DM, to account for differences in the quantity of the excreta produced. Oocyst excretion was analysed by a repeated-measures mixed model with protein content, day and their interactions as fixed factors. Oocyst per g DM data also needed to be log transformed prior to statistical analysis, as the residuals were not normally distributed; the means were then back-transformed for data presentation.

5.4 Results

5.4.1 *Absolute daily performance*

There were no mortalities from d11 of age until the conclusion of the experiment. The evolution of ADFI (g/ bird), ADEI (kcal/ bird) and ADCPI (g/ bird) from the start of the infection (d0 pi) until the end of the experiment are shown in Figure 5.1a, 5.1b and 5.1c, respectively. ADFI, ADEI and ADCPI were not statistically affected by the infection until d4pi and recovered from d9pi onwards. Therefore, the periods d0-3pi, d4-8pi and d9-14pi, were considered to represent the pre-patent, acute and recovery stages of the infection respectively. Protein content significantly affected absolute ADFI (d0-14pi), ADEI (d0-14pi) and ADCPI (d0-14pi), as reducing CP reduced feed intake ($P < 0.05$). There was a significant interaction between protein content and infection for absolute ADFI on d6 and 7pi ($P < 0.05$), as the extent of anorexia was less on low protein feeds compared to the high protein feeds.

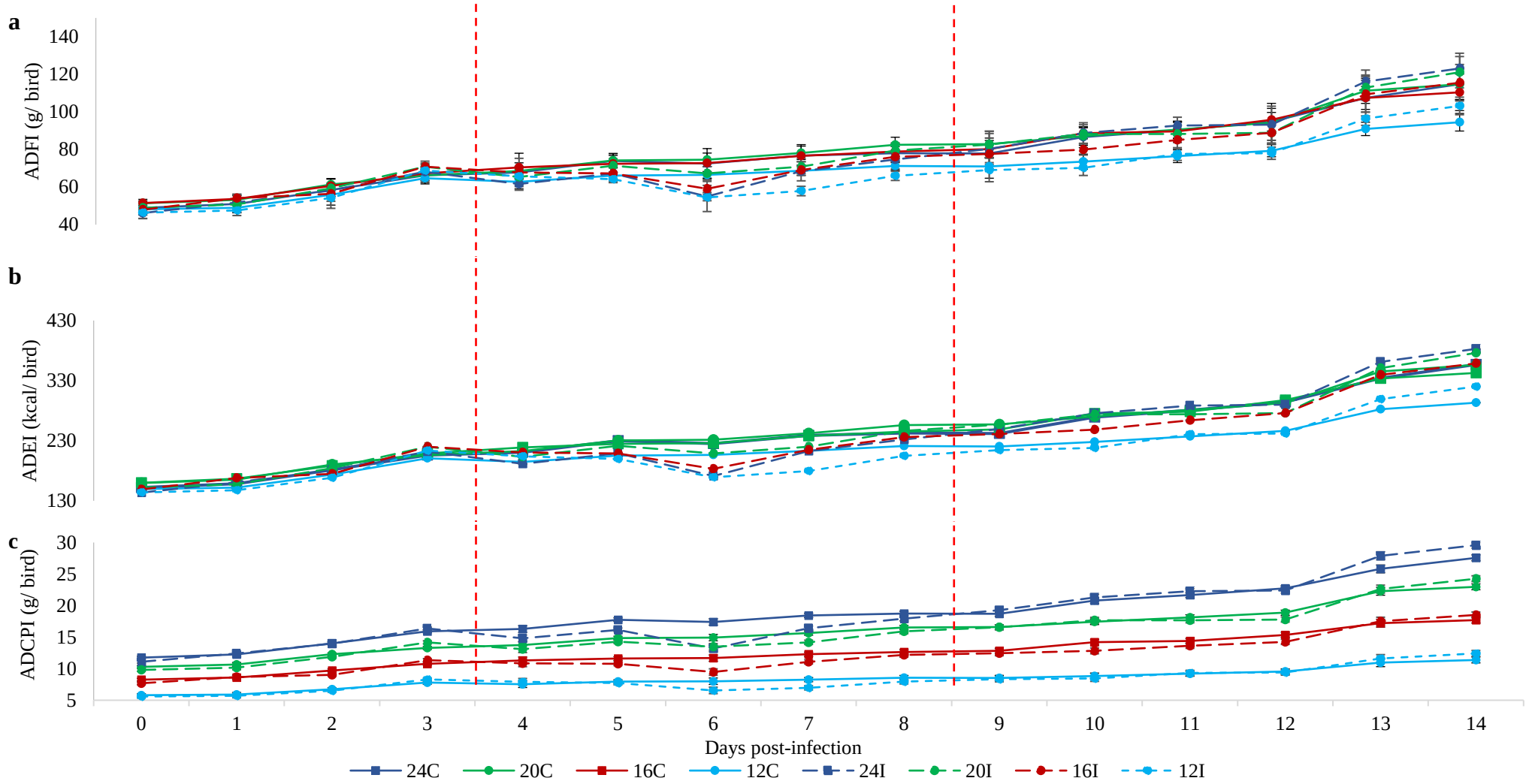


Figure 5.1. **(a)** Average daily feed intake (ADFI; g/ bird); (**b**) Average daily energy intake (ADEI; kcal/ bird); (**c**) Average daily crude protein intake (ADCPI; g/ bird) over the post-infection period (d0-14pi) of broiler chickens who were inoculated with either 0 (C), or 7×10^3 (I) sporulated *E. maxima* oocysts and were offered feeds with 24%, 20%, 16%, or 12% CP. Dotted line indicates the separation into the periods of infection (d0-3, 4-8, 9-14pi).

5.4.2 *Feed and nutrient resource intake during pre-patent, acute and recovery stages*

Table 5.2 shows the actual ADFI, ADEI and ADCPI during each stage of infection. During the acute stage of infection, infected birds showed significant reductions ($P < 0.001$) in ADFI (g/ day), ADEI (kcal/ day) and ADCPI intake (g/ day). There were also significant differences in the ADFI of infected birds compared to the control birds during the pre-patent stage of infection ($P < 0.001$), but this effect was absent during the recovery stage of the infection. Reducing the CP content of the feed caused a significant reduction in ADFI, ADEI and ADCPI throughout all stages of infection ($P < 0.001$). There was an interaction between protein content and infection during the acute stage of infection on ADCPI ($P < 0.001$). This was because there were no significant differences in ADCPI between the infected and control birds offered the 20% CP feed, whereas the remaining infected treatments significantly reduced ADCPI compared to their uninfected counterparts.

The transformed relative ADFI, ADEI and ADCPI during each stage of infection are shown in Table 5.3. Throughout all stages of infection, infected birds showed significant changes in their relative ADFI (log feed intake, g/ kg/ day) and ADEI (log energy intake, kcal/ kg/ day) and ADCPI (log protein intake, g/ kg/ day) ($P < 0.05$). The direction of the effect was different between the three stages of infection: the relative intakes of the infected birds were significantly lower than the control birds during the pre-patent and acute stage whereas the reverse was the case during the recovery stage of the infection ($P < 0.001$). Contrary to the actual intakes, relative ADFI and ADEI increased, and relative ADCPI decreased as the CP content of the feeds decreased throughout all stages of infection ($P < 0.001$). There was no interaction between infection and protein content on relative ADFI, ADEI and ADCPI during any of the stages of the infection. However, there was a tendency ($P = 0.09$) for an interaction between infection and protein content on the relative ADCPI during the acute stage of the infection. This was due the infected birds on the 24% CP feed showing a greater reduction in feed and nutrient intake compared to infected birds on the other treatments.

Table 5.2. ADFI (g/ day), ADEI (kcal/ day), and ADCPI (g/ day) of the infected and the corresponding uninfected control birds during infection with *Eimeria maxima* oocysts. Data are presented over the pre-patent (d0-3 post-infection), acute (d4-8 post-infection), and recovery stages (d9-14 post-infection). Birds were offered feeds with 24%, 20%, 16%, or 12% CP (LS means with SEM).

		ADFI (g/ day)			ADEI (kcal/ day)			ADCPI (g/ day)		
		Pre-Patent	Acute	Recovery	Pre-Patent	Acute	Recovery	Pre-Patent	Acute	Recovery
Protein content										
	24	57.3^{yz}	70.3^{z}	97.2^{z}	174^{yz}	216^{z}	302^{z}	13.9^{z}	16.8^{z}	23.2^{z}
	20	58.8^{z}	74.1^{z}	96.9^{z}	179^{z}	227^{z}	301^{z}	12.0^{y}	15.0^{y}	19.8^{y}
	16	59.0^{z}	71.5^{z}	94.1^{z}	179^{z}	221^{z}	292^{z}	9.63^{x}	11.5^{x}	15.1^{x}
	12	55.2^{y}	64.9^{y}	81.7^{y}	169^{y}	200^{y}	253^{y}	6.78^{w}	7.75^{w}	9.83^{w}
	SEM	0.69	1.00	1.26	2.2	3.0	3.9	0.10	0.14	0.17
Infection										
	Control	58.9	74.0	92.1	176	226	286	10.6	13.5	16.9
	Infected	56.3	66.4	92.8	175	206	288	10.5	12.1	17.1
	SEM	0.49	0.71	0.89	1.6	2.1	2.8	0.07	0.11	0.12
Protein content × Infection										
Uninfected	24	58.5	75.3	95.3	175	229	296	14.0	18.1^{f}	22.9
	20	60.1	77.3	96.8	180	235	301	12.1	15.5^{e}	19.7
	16	60.7	75.2	95.4	181	231	296	9.73	12.0^{d}	15.3
	12	56.1	68.2	81.0	169	208	251	6.76	8.20^{b}	9.70
Infected	24	56.1	65.4	99.0	174	203	308	13.7	15.6^{e}	23.5
	20	57.5	70.8	97.0	178	220	301	12.0	14.6^{e}	20.0
	16	57.3	67.9	92.7	178	211	288	9.52	10.9^{c}	14.9
	12	54.2	61.7	82.4	168	191	256	6.80	7.30^{a}	9.90
	SEM	0.98	1.41	1.78	3.1	4.3	5.5	0.18	0.20	0.24
Source						Probabilities				
Protein content		0.001	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	<0.001
Infection		0.001	<0.001	0.577	0.520	<0.001	0.576	0.290	<0.001	0.402
Protein content × Infection		0.883	0.577	0.355	0.968	0.557	0.355	0.657	0.001	0.215

[a-f, x-z] Means within a column that do not share a common superscript are significantly different ($P < 0.05$).

Abbreviations: ADFI, average daily feed intake; ADEI, average daily energy intake; ADCPI, average daily crude protein intake; pi, post-infection.

Table 5.3. Relative ADFI (log(g/ kg BW/ day)), ADEI (log(/ kg BW/ day)), and ADCPI (log(g/ kg BW/ day)), and daily gains (log(g/ kg BW/ day)) of the infected and the corresponding uninfected control birds during infection with *Eimeria maxima* oocysts. Data are presented over the pre-patent (d0-3 post-infection), acute (d4-8 post-infection), and recovery stages (d9-14 post-infection). The data were scaled relative to the mean body weight of each stage of infection (BWp) before being transformed by the natural logarithm. Birds were offered feeds with 24%, 20%, 16%, or 12% CP (LS means with SEM).

		ADFI/ BWp (log(g/ kg/ day))			ADEI/ BWp (log(kcal/ kg/ day))			ADCPI/ BWp (log(g/ kg/ day))		
		Pre-Patent	Acute	Recovery	Pre-Patent	Acute	Recovery	Pre-Patent	Acute	Recovery
Protein content										
	24	5.06^{x}	4.86^{x}	4.84^{y}	6.20^{x}	5.99^{x}	5.97^{y}	3.67^{z}	3.44^{z}	3.40^{z}
	20	5.13^{y}	4.95^{y}	4.88^{y}	6.26^{y}	6.08^{y}	6.01^{y}	3.56^{y}	3.37^{y}	3.29^{y}
	16	5.18^{z}	5.00^{yz}	4.95^{z}	6.32^{z}	6.14^{yz}	6.08^{z}	3.39^{x}	3.18^{x}	3.12^{x}
	12	5.16^{yz}	5.02^{z}	4.98^{z}	6.29^{yz}	6.16^{z}	6.12^{z}	3.07^{w}	2.91^{w}	2.87^{w}
	SEM	0.013	0.014	0.014	0.013	0.014	0.014	0.010	0.012	0.011
Infection										
	Control	5.15	5.00	4.89	6.28	6.13	6.02	3.44	3.27	3.15
	Infected	5.12	4.92	4.93	6.25	6.05	6.06	3.41	3.18	3.19
	SEM	0.009	0.010	0.010	0.009	0.010	0.010	0.007	0.009	0.008
Protein content × Infection										
Uninfected	24	5.07	4.92	4.81	6.21	6.05	5.94	3.69	3.51	3.38
	20	5.15	4.98	4.86	6.28	6.11	5.99	3.58	3.39	3.27
	16	5.20	5.04	4.94	6.34	6.17	6.07	3.41	3.22	3.10
	12	5.17	5.06	4.96	6.30	6.20	6.09	3.08	2.96	2.84
Infected	24	5.06	4.80	4.87	6.19	5.93	6.00	3.65	3.37	3.43
	20	5.11	4.92	4.89	6.25	6.06	6.03	3.55	3.34	3.32
	16	5.16	4.97	4.96	6.29	6.10	6.09	3.37	3.14	3.13
	12	5.14	4.98	5.00	6.28	6.12	6.14	3.07	2.85	2.89
	SEM	0.018	0.020	0.020	0.018	0.020	0.020	0.014	0.017	0.015
Source						*Probabilities*				
Protein content		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Infection		0.027	<0.001	0.006	0.027	<0.001	0.006	0.005	<0.001	<0.001
Protein content × Infection		0.826	0.452	0.889	0.826	0.452	0.889	0.612	0.091	0.894

$^{x-z}$ Means within a column that do not share a common superscript are significantly different ($P < 0.05$).

Abbreviations: ADFI, average daily feed intake; ADEI, average daily energy intake; ADCPI, average daily crude protein intake; BWp, mean body weight of the period of infection; pi, post-infection.

5.4.3 *ADG, relative ADG, and FCR*

Body weights throughout the infection are given in Figure 5.2. The effects of feed, infection, and their interaction on ADG and FCR calculated over the post-infection periods are presented in Table 5.4. Infection significantly reduced daily gains during the acute stage of the infection ($P < 0.001$), as the performance of the infected birds was significantly reduced in comparison to the control birds. As dietary CP content was reduced there was a significant reduction in ADG throughout the experiment ($P < 0.001$). There was no interaction observed between protein content and infection throughout the experiment ($P > 0.05$).

Infection significantly affected relative ADG during the acute and recovery stages of the infection ($P < 0.05$). Whilst the performance of the infected birds was lower than that of the control birds during the acute stage of the infection, the reverse was the case during the recovery stage. There was an interaction between protein content and infection on relative ADG during the acute stage of infection ($P = 0.009$). There were no significant differences in the relative ADG of the infected and control birds offered the 12%, 20% and 24% CP feeds, whereas the performance of the infected 16% birds was significantly reduced compared to their uninfected counterparts.

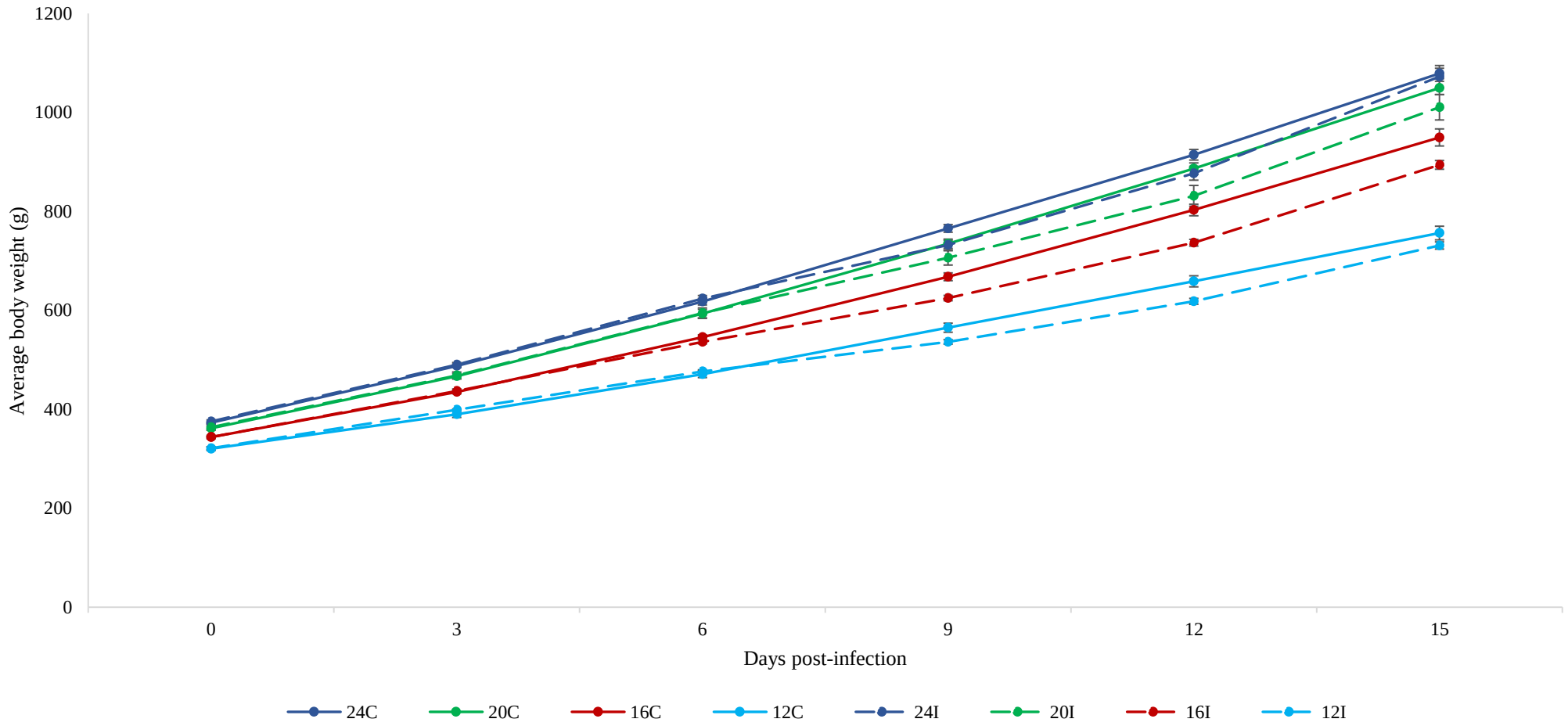


Figure 5.2**.** Average body weight (g) of the infected (I) and the corresponding uninfected control birds (C) at the start (d0pi) of infection with *Eimeria maxima* oocysts until the end of the experiment (d15pi). Birds were offered feeds with 24%, 20%, 16%, or 12% CP.

Table 5.4. Average daily gain (ADG; g/ day), average daily gain/ body weight at the start of the period daily gains (log(g/ kg BW/ day)), and feed conversion ratio (FCR) of the infected and the corresponding uninfected birds during infection with *Eimeria maxima* oocysts. Data are presented over the pre-patent (d0-3pi), acute (d4-8pi), and recovery stages (d9-14pi). ADG data were also scaled relative to the mean body weight of each stage of infection (BWp) and were then transformed by the natural logarithm. Birds were offered feeds with 24%, 20%, 16%, or 12% CP who were inoculated with 0, or 7×10^3 sporulated *E. maxima* oocysts.

		ADG (g/day)			ADG/ BWp (log(g/ kg/ day))			FCR		
		Pre-Patent	Acute	Recovery	Pre-Patent	Acute	Recovery	Pre-Patent	Acute	Recovery
Protein content										
	24	39.7^{d}	43.2^{c}	46.7^{c}	4.20^{z}	3.86^{z}	4.09^{z}	1.42^{d}	1.62^{d}	2.09^{c}
	20	36.8^{c}	42.1^{c}	44.2^{c}	4.15^{yz}	3.86^{z}	4.08^{z}	1.57^{c}	1.75^{c}	2.19^{c}
	16	31.8^{b}	35.1^{b}	39.3^{b}	4.08^{y}	3.76^{y}	4.07^{z}	1.82^{b}	2.04^{b}	2.40^{b}
	12	25.0^{a}	25.9^{a}	27.6^{a}	3.94^{x}	3.60^{x}	3.91^{y}	2.18^{a}	2.52^{a}	2.97^{a}
	SEM	0.54	0.65	0.88	0.019	0.013	0.015	0.028	0.033	0.031
Infection										
	Control	33.1	40.2	39.3	4.08	3.79	4.01	1.77	1.85	2.41
	Infected	33.5	32.9	39.6	4.10	3.75	4.07	1.72	2.11	2.42
	SEM	0.38	0.46	0.62	0.013	0.009	0.010	0.020	0.023	0.022
Protein content × Infection										
Uninfected	24	39.5	46.9	44.7	4.20	3.85^{c}	4.04	1.42	1.57^{a}	2.13
	20	36.9	45.1	45.0	4.15	3.86^{c}	4.08	1.58	1.68^{ab}	2.15
	16	32.0	39.2	40.2	4.07	3.81^{c}	4.06	1.82	1.90^{c}	2.38
	12	24.1	29.6	27.3	3.88	3.63^{ab}	3.88	2.27	2.27^{d}	2.97
Infected	24	39.8	39.4	48.6	4.19	3.87^{c}	4.15	1.41	1.67^{ab}	2.05
	20	36.7	39.1	43.5	4.14	3.86^{c}	4.09	1.57	1.82^{bc}	2.24
	16	31.6	30.9	38.5	4.09	3.71^{b}	4.09	1.82	2.19^{d}	2.41
	12	25.9	22.3	27.8	3.99	3.56^{a}	3.94	2.10	2.78^{e}	2.97
	SEM	0.76	0.92	1.25	0.027	0.019	0.021	0.040	0.046	0.044
Source						*Probabilities*				
Protein content		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Infection		0.507	<0.001	0.748	0.165	0.006	0.001	0.104	<0.001	0.815
Protein content × Infection		0.482	0.657	0.103	0.126	0.009	0.100	0.130	<0.001	0.292

[a-c] Means within a column that do not share a common superscript are significantly different ($P < 0.05$).

Abbreviations: FCR, feed conversion ratio; BWp, mean body weight of the period; CP, crude protein; C, Uninfected; I, Infected; pi, post-infection.

5.4.4 *Oocyst per gram (OPG)*

There was no evidence of infection in control pens, as there were no oocysts observed in the excreta from these pens. Infected birds began excreting oocysts from d6 post-infection and were virtually zero by d9 post-infection (Figure 5.3). There was a statistical effect of protein content ($P < 0.001$) and a significant interaction between protein content and time ($P < 0.05$) on oocyst excretion/ g DM. The across time back-transformed means and confidence intervals for oocyst excretion/ g DM were 1,380 (620 to 3,041), 5,014 (2,208 to 11,384), 6,374 (2,893 to 14,186), 9,997 (4,403 to 22,697) for the 24%, 20%, 16% and 12% dietary treatments respectively. The interaction between day and diet was due to birds offered the 12% CP treatment excreting significantly more oocysts/ g DM at d9pi, compared to those fed the 20% CP treatment ($P < 0.05$).

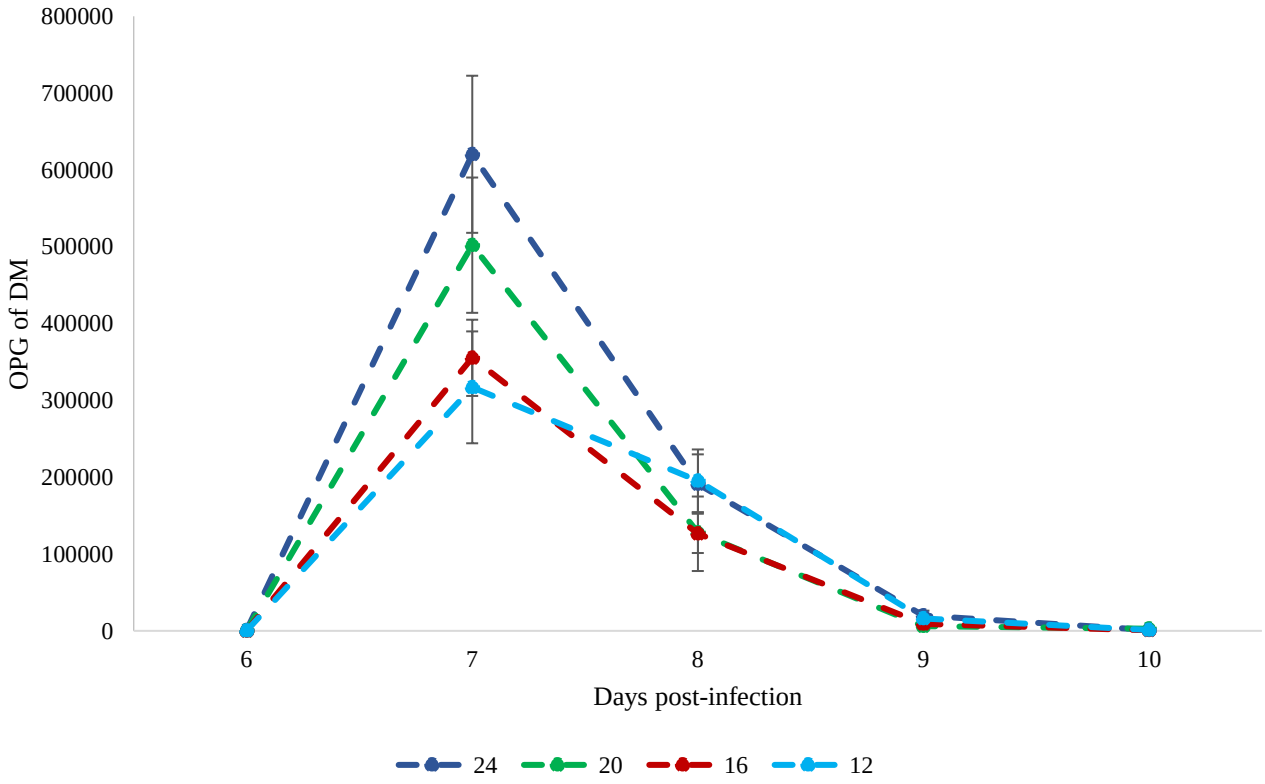


Figure 5.3. Number of oocysts per gram of faecal DM (OPG) on a logarithmic scale, for birds infected with 7000 *Eimeria maxima* oocysts offered isoenergetic diets (3105 kcal/ kg ME) with 24%, 20%, 16%, or 12% CP.

5.4.5 *Histology and organ measurements*

Sections of the duodenum and jejunum were collected from one bird per pen at d6 and d12 post-infection. Infection caused significant increases in crypt depth at both time points, in both the duodenum and jejunum ($P < 0.05$). At d6 post-infection (Table 5.5), infection caused significant reductions in villi length and villi length to crypt depth ratio in the duodenum and jejunum ($P < 0.05$). At d12 post-infection (Table 5.6), villi length to crypt depth ratio remained significantly lower in infected birds ($P < 0.05$). Reducing dietary CP caused significant increases in villi length to crypt depth ratio and villi length only in the duodenum at d6 post-infection ($P < 0.05$). At d12 post-infection, reducing dietary CP increased villi length in the duodenum and jejunum ($P < 0.05$), and crypt depth in the jejunum ($P < 0.05$). There was an interaction between infection and protein content at d12 post-infection, as infected birds on the 12% CP feed had larger crypt depths than their uninfected counterparts ($P < 0.05$).

The growth of intestinal segments of the infected birds were greater compared to control birds at d6 and d12 post-infection ($P < 0.05$) (Table 5.7). No interaction between protein content and infection was observed at either time point in any of the measurements ($P > 0.05$). Reducing CP content caused an increase in the relative lengths of the duodena, jejuna, and ilea at both time points ($P < 0.05$). Birds offered the 12% CP feed yielded significantly larger relative intestinal segment lengths than all other dietary treatments ($P < 0.05$).

Table 5.5**.** Villi length (µm/ kg body weight (BW) at the point of euthanasia), crypt depth (µm/ kg BW), and villi length to crypt depth ratio of the duodenum and jejunum, during the acute stage (d6 post-infection) of the infected and the corresponding uninfected control birds during infection with *Eimeria maxima* oocysts. Birds were offered feeds with 24%, 20%, 16%, or 12% CP.

		Duodenum			Jejunum		
		Villi length to crypt depth ratio	Villi length (µm/ kg BW)	Crypt depth (µm/ kg BW)	Villi length to crypt depth ratio	Villi length (µm/ kg BW)	Crypt depth (µm/ kg BW)
Protein content	24	7.49^{y}	2186^{y}	352	5.88	1531	300
	20	8.76^{yz}	2361^{y}	323	5.51	1482	298
	16	8.71^{yz}	2547^{y}	360	5.52	1504	323
	12	9.68^{z}	2891^{z}	361	5.51	1676	362
	SEM	0.530	95.7	26.3	0.420	90.6	18.5
Infection	Control	12.3	2786	232	7.81	1699	225
	Infected	5.01	2207	467	3.41	1397	417
	SEM	0.375	67.7	18.6	0.297	64.1	13.1
Protein content × Infection							
Uninfected	24	10.8	2545	244	8.16	1687	214
	20	12.3	2569	212	7.24	1480	206
	16	12.4	2789	230	7.87	1697	227
	12	13.7	3240	241	7.97	1931	251
Infected	24	4.15	1828	461	3.61	1375	386
	20	5.20	2152	435	3.78	1483	391
	16	5.01	2305	491	3.17	1311	418
	12	5.70	2542	481	3.06	1421	474
	SEM	0.751	135	37.2	0.595	128	26.2
Source				*Probabilities*			
Protein content		0.048	<0.001	0.715	0.900	0.435	0.067
Infection		<0.001	<0.001	<0.001	<0.001	0.002	<0.001
Protein content × Infection		0.860	0.606	0.937	0.624	0.241	0.796

$^{x-z}$ Means within a column that do not share a common superscript are significantly different ($P < 0.05$).

Table 5.6. Villi length (µm/ kg body weight (BW) at the point of euthanasia), crypt depth (µm/ kg BW), and villi length to crypt depth ratio of the duodenum and jejunum, during the acute stage (d12 post-infection) of the infected and the corresponding uninfected control birds during infection with *Eimeria maxima* oocysts. Birds were offered feeds with 24%, 20%, 16%, or 12% CP.

		Duodenum			Jejunum		
		Villi length to crypt depth ratio	Villi length (µm/ kg BW)	Crypt depth (µm/ kg BW)	Villi length to crypt depth ratio	Villi length (µm/ kg BW)	Crypt depth (µm/ kg BW)
Protein content	24	10.2	1851^{x}	190	7.15	1133^{y}	162^{y}
	20	10.2	1915^{xy}	200	6.68	1113^{y}	176^{y}
	16	10.8	2107^{y}	217	6.77	1216^{y}	187^{yz}
	12	12.1	2523^{z}	226	7.51	1499^{z}	217^{z}
	SEM	0.696	59.9	10.1	0.409	41.0	10.4
Infection	Control	13.1	2094	164	8.04	1271	162
	Infected	8.51	2104	252	6.01	1210	209
	SEM	0.492	42.4	7.15	0.289	29.0	7.3
Protein content × Infection							
Uninfected	24	11.7	1811	158	7.41	1163	162^{a}
	20	12.5	1948	159	7.84	1093	143^{a}
	16	13.7	2091	159	7.68	1260	167^{a}
	12	14.6	2525	181	9.25	1567	176^{a}
Infected	24	8.70	1891	221	6.89	1103	163^{a}
	20	7.85	1882	241	5.52	1134	208^{ab}
	16	7.85	2122	275	5.86	1172	208^{ab}
	12	9.64	2520	271	5.77	1432	259^{b}
	SEM	0.984	84.7	14.3	0.578	57.9	14.6
Source				*Probabilities*			
Protein content		0.185	<0.001	0.066	0.462	<0.001	0.004
Infection		<0.001	0.870	<0.001	<0.001	0.148	<0.001
Protein content × Infection		0.549	0.850	0.318	0.097	0.488	0.043

[a-b, x-z] Means within a column that do not share a common superscript are significantly different (P < 0.05).

Table 5.7. The relative lengths of the duodenum (DL), jejunum (JL), and ileum (IL) (cm/ kg body weight (BW) at the point of euthanasia) during the acute (d6 post-infection) and recovery stages (d12 post-infection), and the relative weight of the full small intestine (SIF) (g/ kg BW) (d15 post-infection) of the infected and the corresponding uninfected control birds during infection with *Eimeria maxima* oocysts. Birds were offered feeds with 24%, 20%, 16%, or 12% CP.

		D6pi			D12pi			D15pi
		DL (cm/kg BW)	JL (cm/kg BW)	IL (cm/kg BW)	DL (cm/kg BW)	JL (cm/kg BW)	IL (cm/kg BW)	SIF (g/kg BW)
Protein content								
	24	34.1[a]	89.7[a]	78.6[a]	30.3[ab]	70.8[ab]	64.4[a]	56.4[a]
	20	35.0[a]	91.6[a]	88.0[b]	27.9[a]	67.2[a]	60.5[a]	57.3[a]
	16	36.7[a]	97.1[ab]	87.3[ab]	31.3[b]	75.7[b]	67.5[a]	62.9[b]
	12	40.8[b]	108[b]	101[c]	36.3[c]	92.5[c]	86.0[b]	68.5[c]
	SEM	1.38	3.49	2.39	0.81	1.67	2.18	1.40
Infection								
	Control	35.6	91.1	88.2	28.2	67.6	62.5	55.1
	Infected	37.8	102	89.5	34.7	85.5	76.7	67.5
	SEM	0.98	2.47	1.69	0.57	1.18	1.54	0.99
Protein content × Infection								
Uninfected	24	34.1	86.6	77.4	26.6	61.3	56.5	49.2
	20	33.6	83.0	87.5	26.3	60.9	57.6	50.1
	16	35.7	96.4	86.5	27.7	67.4	59.0	57.2
	12	38.9	98.5	101	32.3	80.8	76.8	63.8
Infected	24	34.2	92.8	79.8	34.1	80.3	72.4	63.7
	20	36.5	100	88.6	29.5	73.5	63.4	64.5
	16	37.7	97.9	88.1	35.0	84.1	76.0	68.7
	12	42.7	118	102	40.3	104	95.1	73.1
	SEM	1.95	4.93	3.38	1.14	2.36	3.08	1.98
					Probabilities			
Protein content		0.007	0.003	<0.001	0.007	<0.001	<0.001	<0.001
Infection		0.119	0.003	0.567	0.119	<0.001	<0.001	<0.001
Protein content × Infection		0.806	0.233	0.993	0.806	0.165	0.181	0.509

[a-c] Means within a column that do not share a common superscript are significantly different ($P < 0.05$).

Abbreviations: BW, body weight at dissection; DL, duodenum length; JL, jejunum length; IL. Ileum length; SIF, small intestine full weight; cm/kg BW, centimetres per kilogram of BW at dissection; g/kg BW, grams per kilogram of BW at dissection

5.5 Discussion

The primary objective of the study was to investigate the effects of feed CP content on the characteristics of pathogen-included anorexia. The hypothesis that broilers offered high CP feeds whilst exposed to *E. maxima* would shower a greater extent of anorexia compared to those offered low CP feeds was tested. An established infection model (Sakkas *et al.*, 2018) was implemented to induce anorexia in infected birds. To formulate feeds appropriate for testing the hypotheses a variety of factors had to be considered. Sklan and Plavnik (2002) showed the importance of maintaining AA to CP ratios in studies investigating the effect of reduced dietary CP on broiler performance. The authors observed a linear decrease in feed intake as dietary CP increased when AA levels were constant across feeds, whereas when the AA to CP ratios were fixed, feed intake increased with dietary CP level to a plateau. Similar results were reported by Sterling *et al.* (2003), when birds were offered feeds with additional lysine supplementation there was an increase in feed intake compared to those offered lysine deficient feeds. Therefore, the past evidence showing a reduction in feed intake as dietary CP increases (Parsons and Baker, 1982; Fancher and Jensen, 1989; Smith and Pesti, 1998) is likely a result of an imbalance in AA to CP ratios in the experimental feeds offered to the animals. To that end, it was important to ensure that AA to CP ratios were maintained in the present experiment. Furthermore, it was important that energy content was maintained in the experimental feeds to ensure that the responses observed were due to CP content of the feed. Hence, the feeds were formulated to contain different amounts of corn starch (high in energy, low in protein) and corn gluten meal (high in energy, high in protein) to manipulate CP content, whilst maintaining the energy content of the feed. Ratios of essential amino acids, and minerals were maintained at constant ratios to CP across the feeds by supplementing with synthetic AA, or limestone and monocalcium phosphate. This allows accurate conclusions to be drawn on the effect of CP content in the feed on the characteristics of anorexia.

It was important to account for *a priori* differences in performance to allow meaningful comparisons between dietary treatments. Therefore, when data were split into the different periods of infection, performance variables were scaled relative to the mean body weight of each period (Kyriazakis *et al.*, 1991; Allison *et al.*, 1995). To that end, the results from absolute ADFI support the hypothesis that feed intake would increase to a plateau as CP increases in the absence of infection. However, when ADFI was expressed per unit of BW, the results no longer support the hypothesis as there was a reduction in relative ADFI as CP increased. The hypothesis that birds offered a high CP feed would show the greatest extent of anorexia is supported by the results from this study as the greatest reduction in ADFI was observed in the

birds offered the 24% feed. However, unlike in Chapter 4 where the birds offered the least nutrient dense feed (HF) showed the lowest extent of anorexia, in this experiment the lowest extent of anorexia was not observed in the least nutrient dense feed (12% CP) but in the 20% feed. There was a significant interaction between protein content and infection on absolute ADCPI, which was caused by the fact that there was no significant difference in ADCPI between infected and uninfected birds given the 20% CP feed, whereas the remaining infected treatments significantly reduced ADCPI when compared to their uninfected counterparts. This could be interpreted as an attempt by the birds to target a protein intake during infection, however the birds on the 16% and 12% CP feeds were unable to achieve this target. The relative feed intakes of the birds provide a clue why this was the case: as the protein content of the feeds was decreased the relative intake of the birds increased. This resulted in an increase in relative energy intake, but compensation in relative protein intake was not achieved. It has been suggested that this is because animals are limited in dealing with the excess energy intake that accompanies this response, due to their inability to dissipate it as heat in the environment (Kyriazakis *et al.*, 1999). For smaller sized birds, such as the infected birds this effect would be even more acute and may be accentuated by pyrexia (Heinrich, 1977). The results are consistent with the 'protein leverage theory', which suggests that for non-infected animals dietary protein is a primary driver of feed intake (Raubenheimer and Simpson, 2019).

Unlike in Chapter 4, the resilience and resistance of the infected birds was affected by feed composition. During the acute stages of the infection, especially moving from the 20% to the 16% and 12% CP levels, average daily gain was decreased, and oocyst excretion was increased as the dietary CP content was decreased. On the 12% CP feed, the increase in oocyst excretion was 7 times that of the birds on the 20% CP feed. These effects were the outcome of the decreased CP intake and are consistent with the suggestions that the immune response is compromised by lower protein intake in several species infected by macro or macro-parasites (Korver, 2012; Calder, 2013; Clough *et al.*, 2016). Several of the immune mediators activated during primary *Eimeria* infections (cytokines, the acute phase protein response, lymphocyte proliferation, goblet cells etc) are proteinaceous in nature and as such they are expected to rely on protein intake. The fact that there were no differences in the damage caused by the coccidia between the different CP feeds, further suggests that birds minimised the consequence of the infection. This is consistent with Coltherd *et al.* (2009), who suggested that such (maintenance) functions are prioritised during primary infections. Maintenance of tissues associated with survival, such as the integrity of the gastrointestinal tract, ensures survival in the longer term (Garcia *et al.*, 2018).

There is an increasing amount of evidence that (healthy) animals and humans are targeting a specific protein intake when they are given a choice between different feeds (Kyriazakis and Emmans, 1999; Raubenheimer and Simpson, 2019). The mechanisms by how this regulation is achieved are currently being elucidated (Hill *et al.*, 2020). Chapter 4 and Chapter 5 not only extend these suggestions to infected animals, but they also suggest that the birds also avoided excess protein intake. Whilst it is clear that a certain level of protein intake during infection with *E. maxima* is beneficial both in terms of resilience and resistance, the question is why excess of protein intake, as in the case of the 24% CP feed, is detrimental? Old evidence suggests that high levels of CP increase the severity of coccidiosis and other infections in broiler chickens (Sharma *et al.*, 1973; (Hill and Garren, 1961; Boyd and Edwards Jr, 1963; Proudfoot and Aitken, 1969). This suggests that there may be a generalised advantage to avoid excess of protein intake during the acute stages of the infection. The infected animal may be attempting to reduce the risk of a secondary infection since diseases such as coccidiosis can be pre-disposing factors for more severe infections, such as necrotic enteritis (NE) (Prescott *et al.*, 2016). Increased amounts of undigested protein favour the proliferation of protein fermenting pathogenic bacteria, such as *C. perfringens* (Drew *et al.*, 2004; Wu *et al.*, 2014), which challenge gastrointestinal health and may lead to local and systemic inflammation.

During the recovery stage of infection, there were no significant differences in absolute feed or nutrient resource intake. However, when the data were scaled relative to the mean BW during the recovery stage of infection, previously infected birds consumed more feed per unit of body weight than their uninfected counterparts. This was accompanied by increases in relative energy intake, protein intake and the relative weight of the small intestine at d15pi. Such increases are consistent with the concept of compensatory intake seen during the recovery from several infections in several species (Banfield and Forbes, 2001; Coltherd *et al.*, 2009; Pastorelli *et al.*, 2012), and are consistent with the idea that animals aim to return to a desired body weight and composition after a period of limitation in their growth (Kyriazakis and Houdijk, 2007). The fact that the relative compensatory intake was accompanied by increases in relative small intestine weight, and occurred whilst the digestive tract was still recovering from the infection (the effects on the villi to crypt ratio in both the duodenum and jejunum were still evident by d12pi), suggests that there is improved of absorption of nutrients during the recovery from infection (Turk, 1973; Banfield *et al.*, 2002). Turk (1973) observed compensatory increases in nutrient absorption from day 10 until day 35 of a coccidian infection. Furthermore, an increase in the expression of nutrient transporters in the jejunum of chickens is often observed following a challenging stimuli (e.g. recovering from infection, or heat stress) (Garcia *et al.*, 2018).

However, since nutrient absorption was not measured in the current experiment, this requires further investigation.

Consistent with the effect on absolute feed and nutrient resource intakes, the absolute growth rate of previously infected animals was reduced during the recovery period, whereas the relative intakes showed that there was an increase in the relative growth rate of the previously infected animals. This can be seen consistent with the idea of compensatory growth post nutritional limitation. However, the degree of compensatory growth depended on feed composition, with birds on the 12% CP feed showing the least ability to compensate in their relative growth. This is in agreement with the view that the nutritional environment after a period of limitation will affect the rate of compensation (Kyriazakis and Houdijk, 2007). As far as we are aware this is the first demonstration of the principle in the context of recovery from an infection.

In conclusion, this study further supports the view that pathogen-induced anorexia is indeed affected by feed composition. However, it was not possible to determine the target nutrient of the animals during anorexia from this experiment nor from the previous experiment in Chapter 4. Future work could involve offering broilers infected with *E. maxima* feeds of reduced energy content, whilst maintaining CP content of the feed at a constant level. As Chapter 4 reduced both energy and CP content whilst maintaining the ratio of energy to CP, and the current study reduced CP content whilst maintaining energy content, and ratios of EAA to CP. This, or similar work is critical in identifying the nutritional targets of infected hosts, with the aim of being able to assist the animals in meeting these targets through proper and thorough nutrition.

Chapter 6. General discussion

6.1 Introduction

The sustainability of livestock production has come under intense scrutiny in recent years. One avenue currently being explored to achieve sustainable meat production is the use of alternative feed ingredients (Leinonen and Kyriazakis, 2016; Tallentire *et al.*, 2018b; El-Deek *et al.*, 2020). Ingredients such as rapeseed meal are being considered as alternative protein sources in European production systems to replace economically and environmentally costly ingredients such as soybean (Van Krimpen *et al.*, 2013; Khan, 2018). To that end, there are also attempts to utilise undesirable feed co- and by-products from human food production, such as rice bran and palm kernel meal (Ravindran, 2013). These ingredients are typically fibrous, bulky and lower in energy and nutrient content than the ingredients they are replacing in animal feeds. Therefore, the inclusion of alternative feed ingredients results in feeds lower in energy and nutrient density. There is a lack of consensus in the literature investigating the effect of reducing feed energy content with bulky ingredients on the regulation of feed intake in broiler chickens. In fact, concerns of modern broiler genotypes to cope with a reduction in dietary energy content have being growing (Classen, 2017; Scholey *et al.*, 2020). Chapter 2 and Chapter 3 of this thesis attempted to investigate the ability of a modern broiler genotype to cope with feeds of reduced energy content by diluting feeds with bulky ingredients. The evidence from these two chapters suggest that broilers do indeed retain the ability to modify their feed intake in response to dietary energy under the experimental conditions tested, although this depended on the properties of the bulky ingredient used to reduce the energy content of the feeds.

This thesis also addressed a knowledge gap on the effect of feed composition on pathogen-induced anorexia in poultry, in Chapters 4 and Chapter 5. The former chapter assessed how the energy content of feed affects pathogen-induced anorexia, whilst the latter investigated how crude protein content affects pathogen-induced anorexia. Indeed, the evidence from these experiments suggest that the extent of pathogen-induced anorexia is greatest in feeds of high energy and protein composition, and lowest in feeds of low energy and protein composition. Understanding what the infected animal is trying to achieve through its feeding behaviour will have implications on how hosts should be fed during the critical stages of infection.

In this final chapter, the findings from the preceding experimental chapters will be synthesised and their overall relevance critically discussed. The penultimate section of this chapter addresses the scope for future research.

6.2 Broiler capacity for bulky feeds

6.2.1 *Feed intake and growth performance*

In controlled environmental conditions, the maximum feed intake of an animal is ultimately limited by its biological limits in gut capacity. Recent suggestions state that the gut capacity of modern broilers has become reduced due to artificial selection for improved growth rate and feed efficiency (Pym, 2005). Consequently, it has been suggested that the maximum capacity for feed intake is nearing its biological limits. If this notion is indeed true, then by definition the maximum capacity for feed intake will be reduced. The results from Chapter 2 and 3 contradict this suggestion and in fact suggest that the maximum feed intake capacity is yet to be reached.

To accurately predict feed intake on bulky feeds it is important to understand all of the factors that constrain feed intake. To that end, the literature shows that feed intake responses to dietary energy content are not simply dependent upon the energy content of the feed and may instead be constrained by other factors such as feed bulkiness. The bulk properties of the ingredients used to reduce the energy content of feeds in such experiments could account for the differences observed in the literature. For example, feed intake is often shown to increase in studies where energy content is reduced due to the addition of bulky ingredients to the feeds and in these experiments the alternative ingredients used have relatively low bulk properties (such as WHC and feed density) (Leeson *et al.*, 1996a; Ferket and Gernat, 2006; Linares and Huang, 2010). To that end, the greatest increase in feed intake observed in the literature was by Leeson *et al.* (1996a), who diluted feeds with oat hulls and sand, both of which have low bulk properties. The reductions in dietary energy content between the diluted feeds and basal feeds are comparable between the Leeson *et al.* (1996a) experiment and Chapter 2 (22% and 26%, respectively), however the maximum feed intake was 20% greater in Chapter 2 compared with the maximum feed intake observed by Leeson.

It is well established that developmental plasticity of the GIT is greater in younger chickens (Sklan, 2001). Indeed, there is evidence showing that broilers are unable to adapt feed intake to bulky feeds when they are introduced to the feeds at 35 days of age (Leeson *et al.*, 1996b; Sahraei and Shariatmadari, 2007; Salami and Odunsi, 2017). Leeson *et al.* (1996b) observed a significant reduction in energy intake from day 35-42 when feeds were diluted

with 20% oat hulls and 20% sand. Sahraei and Shariatmadari (2007) did not calculate energy intake, however since body weight was significantly reduced in birds offered feeds diluted with 14% wheat bran and 14% sand from day 35-45, it can be assumed that these birds were unable to adapt to diluted feeds to maintain energy intake and performance. Similarly, Salami and Odunsi (2017) found that broilers were unable to maintain energy intake when given feeds of reduced energy content at 35 days of age. The lack of adaptation in these studies presumably reflects the lower plasticity of the gastrointestinal tract (GIT) in older broilers, which consequently reduces the adaptation to the bulky feeds of broilers at this age. It is likely that different responses would have been observed had the experimental feeds been offered at a later age. Indeed, the results from Chapter 3 offer some insight into this. In Period 1 (d8-29), adaptation to each of the bulky feeds was achieved by the end of the second week (d22). This was concluded since there were no significant differences in the direction of the change in relative feed intake between weeks 2 and 3 and therefore the performance data were analysed over weeks 2 and 3 of the experiment. However, during Period 2 (d29-42, when all the birds were offered feed diluted with 60% grass meal (GM), only the birds previously offered the feed diluted with 60% wheat bran were able to achieve adaptation to the novel bulky feed at the end of the two-week period. To that end, the results of this thesis, Leeson *et al.* (1996b), Sahraei and Shariatmadari (2007) and Salami and Odunsi (2017) demonstrate that the age at which the birds are introduced to the experimental feeds is an important factor to be considered when offering broilers bulky feeds.

6.2.2 *Effects of bulky feeds on the gastrointestinal tract*

The effect of bulky feeds on the development of the GIT in broiler chickens is widely demonstrated (Jørgensen *et al.*, 1996a; González-Alvarado *et al.*, 2010) (Jiménez-Moreno *et al.*, 2009a; Jiménez-Moreno *et al.*, 2009b). The relative weight of the gizzard is consistently shown to increase when broilers are offered bulky feeds although the effect is dependent on the solubility of the bulky ingredient (González-Alvarado *et al.*, 2010; Svihus, 2011; Jiménez-Moreno *et al.*, 2019). Indeed, Chapter 2 and Chapter 3 are consistent with the literature as the weight of the gizzard relative to body weight was more pronounced when the fibrous ingredient was soluble (e.g. sugar beet pulp, SBP and grass meal, GM birds) compared to insoluble ingredients (e.g. oat hulls, OH) (González-Alvarado *et al.*, 2008; Jiménez-Moreno *et al.*, 2013b; Jiménez-Moreno *et al.*, 2019). To that end, in Chapter 2, the gizzard weight relative to body weight linearly increased with diet dilution which is in agreement with several authors (Summers and Leeson, 1986; Jiménez-Moreno *et al.*, 2011; Miya *et al.*, 2019; Okrathok and Khempaka, 2020). Similarly, in Chapter 2 the relative length of the small

intestine was increased in birds given the bulky feeds, although this effect was more pronounced in the SBP birds, which is consistent with Jiménez-Moreno *et al.* (2013b), who also offered feeds diluted with OH and SBP, albeit the dilution levels used were drastically lower than in Chapter 2. However, the effects of bulk on all of the components of the GIT are not always presented in the literature. Chapter 2 and Chapter 3 provide evidence that the large intestine plays an important role in the adaptation to bulky feeds, as the physical distension of the GIT caused by bulky feed ingredients is carried throughout the digestive tract. However, this effect was restricted to ingredients with greater bulk properties (SBP and GM) which is consistent with the findings of Jørgensen *et al.* (1996a), where the relative weight of the large intestine was greater in birds given feeds diluted with pea fibre compared to those diluted with wheat bran or oat bran. The greater effect of soluble fibre on the size of the large intestine compared can be ascribed to physical distension throughout the GIT (González-Alvarado *et al.*, 2010)

In Chapter 2, digesta samples for viscosity analysis were collected to account for the effect of digesta viscosity on broiler performance (Lázaro *et al.*, 2003). The inclusion of soluble fibre sources in broiler feeds has been shown to increase digesta viscosity and negatively affect performance and nutrient digestibility (Jiménez-Moreno *et al.*, 2009a). Indeed, there was no difference in jejunal viscosity between OH birds and basal birds, whereas there was a linear increase in jejunal viscosity in the SBP birds, supporting the conclusions of Jiménez-Moreno *et al.* (2009a). To that end, the increased viscosity of the SBP birds offers an alternative explanation for the effects of feed bulkiness on broiler performance and the adaptation of their GIT due to the physical distention (González-Alvarado *et al.*, 2010) and reduced passage rate (Owusu-Asiedu *et al.*, 2006) caused by soluble fibre sources. Previously, it has been suggested that there is a rapid attempt to increase the absorptive surface area of the GIT to limit the effect of reduced diffusion rates when digesta viscosity is increased by the inclusion of bulky ingredients (Banfield *et al.*, 2002).

6.2.3 *Effects of feed form*

The feeds in Chapter 2 and Chapter 3 were offered in pelleted form. However, a preliminary experiment identical to that of Chapter 2 was conducted where the feeds were offered as mash. The results of this preliminary experiment are not presented in this thesis as the feed intake data were unreliable due to feed spillage. During this experiment, the height of the feeders was increased and the flow of feed from the feeder was reduced in attempt to limit the extent of feed spillage. However, it was not possible to eliminate this effect until the final week of the experiment. Furthermore, as the feeds were offered in mash form, it was observed that the birds

were actively avoiding the bulky ingredients and selecting for the other ingredients. It is possible that this selective eating was the cause of the feed spillage as the birds overzealously searched through the feed for the desired ingredients. Therefore, to overcome these problems, the experiment was then repeated with the feeds offered as pellets to avoid selective eating and reduce feed spillage. In retrospect, this change in experimental design was beneficial in achieving the objectives of identifying the maximum capacity for feed intake since there is consistent evidence showing feed intake is increased by feeding diets in the pellet form (Svihus and Hetland, 2001; Amerah *et al.*, 2007b; Jiménez-Moreno *et al.*, 2016; Naderinejad *et al.*, 2016; Abdollahi *et al.*, 2018). Furthermore, Abdollahi *et al.* (2018) showed that reducing nutrient density interacts with feed form. There were significant improvements in feed intake and growth performance in the birds given pelleted feeds of low nutrient density compared with the equivalent feed offered as mash. After all, one of the primary aims of pelleting has been to convert fibrous, bulky and poor quality ingredients to a compact form that are easy to ingest (Abdollahi et al., 2019).

Due to the unreliable data from the preliminary experiment, it would be expected that the absolute feed intake of birds from Chapter 2 would be greater than those in the preliminary experiment. However, the feed intake data scaled relative to body weight from the final week of the preliminary experiment (d23-39 of age) was actually greater than that of Chapter 2 (Figure 6.1). Although the greater feed intake of the mash-fed birds was not reflected in relative daily gains (Figure 6.2), demonstrating the unreliability of the feed intake data from the preliminary experiment.

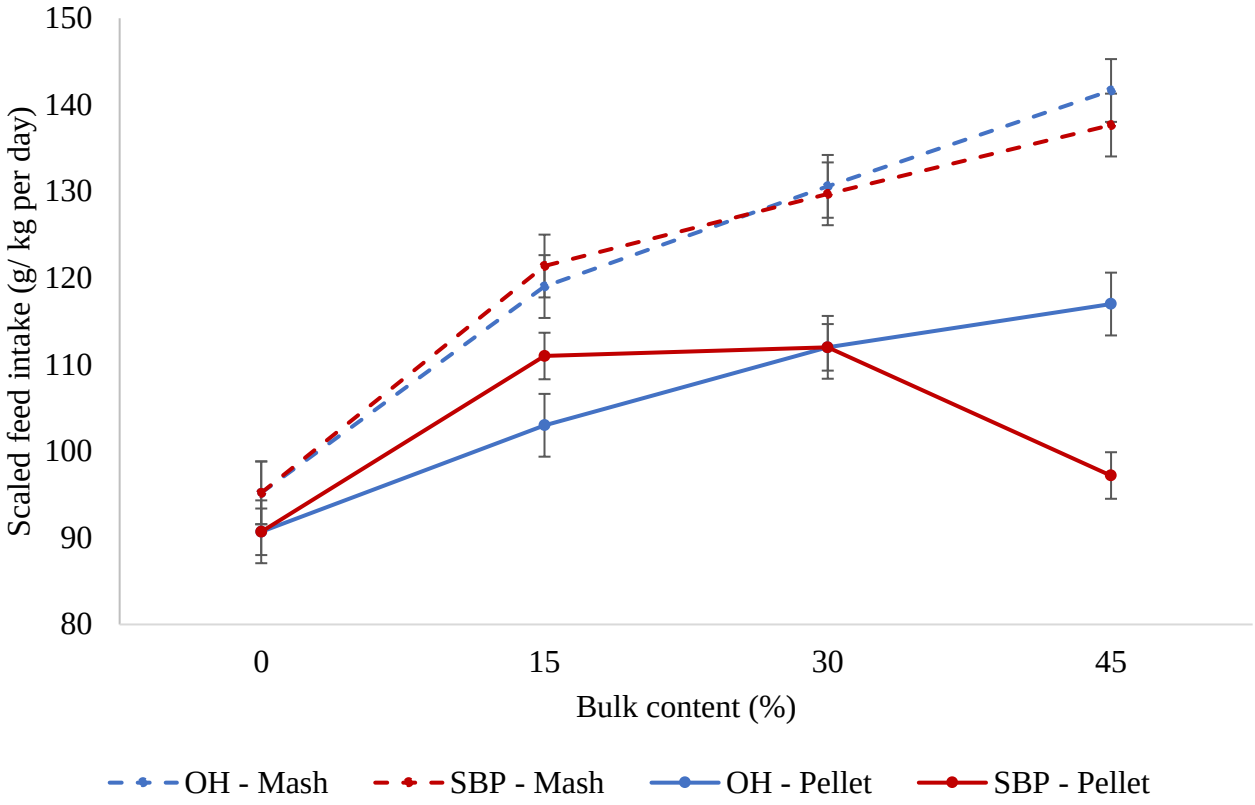


Figure 6.1. Average daily feed intake of broiler chickens from d23-29, expressed relative to the average body weight from d23-29 (g/ kg/ day), when offered feeds which were diluted with either 0%, 15%, 30%, or 45% of either Oat Hulls (OH) or Sugar Beet Pulp (SBP) as either mash or pellets.

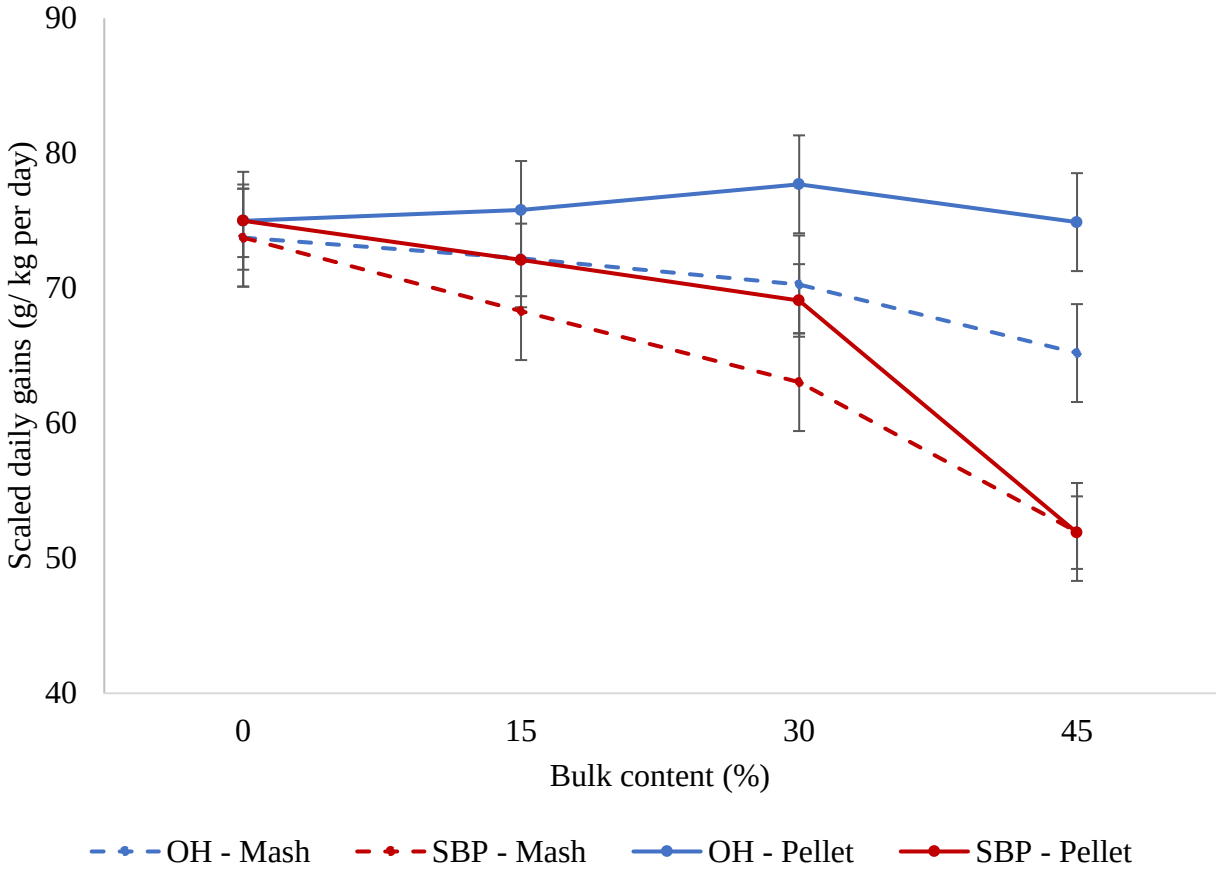


Figure 6.2. Average daily gains of broiler chickens from d23-29, expressed relative to the average body weight from d23-29 (g/ kg/ day), when offered feeds which were diluted with either 0%, 15%, 30%, or 45% of either Oat Hulls (OH) or Sugar Beet Pulp (SBP) as either mash or pellets.

6.3 Effects of feed composition on pathogen-induced anorexia and infection outcomes

6.3.1 *Reducing dietary energy content*

Chapter 4 suggests that infected hosts attempted to regulate their nutrient resource intake during anorexia, the feed intake of the infected birds increased with diet dilution, whereas in the control birds feed intake decreased as diet dilution increased. When the energy and nutrient resource intake data were scaled relative to BW, the relative energy and protein intake on the most diluted feed (15% lignocellulose) was similar between the infected and non-infected birds, and the energy and protein intakes of the infected birds was similar. These findings are consistent with Kyriazakis (2010), who suggested that the disruption in feed intake regulation caused by infection may be modulated by feed composition.

It has been hypothesised that the addition of a soluble fibre source (pectin) during infections would modulate the intestinal environment of broilers infected with coccidiosis due to the inhibiting effect of increased digesta viscosity on pathogen colonisation in the GIT

(Liévin-Le Moal and Servin, 2006; Wils-Plotz *et al.*, 2013). Therefore, it may be beneficial to include soluble fibre sources in feeds to limit the invasion of pathogens in the GIT. Indeed, an effect of soluble fibre inclusion on the invasion of *E. maxima* schizonts in ileal enterocytes was observed, suggesting that pectin did in fact play a role in resilience to the parasite (Wils-Plotz *et al.*, 2013). In contrast, there were no effects of feed composition on the resilience to *E. maxima* infection in Chapter 4. Evidently, these are two different measures of resilience, however Wils-Plotz *et al.* (2013) also measured ileal VCR and found no significant effect of pectin inclusion, consistent with the findings of Chapter 4. Therefore, had the invasion of schizonts in the intestinal enterocytes been counted in Chapter 4, there may indeed have been an effect of feed composition on resilience to *E. maxima*.

As previously mentioned, pelleting aims to convert fibrous, bulky and poor quality ingredients to a compact form (Abdollahi et al., 2019) and results in greater feed intake and performance compared to mash feeds (Abdollahi *et al.*, 2018). Therefore, it would be interesting to investigate whether the same responses in both the control and infected birds would occur had the experimental feeds been offered as pellets. Furthermore, lignocellulose is an insoluble fibre source and so the comparison with the experiment by Wils-Plotz *et al.* (2013) is tenuous. However, lignocellulose is recognised for its bulking properties (Oikeh *et al.*, 2019) and so may have a similar effect to pectin in modulating the intestinal environment. Therefore, future work should compare the effect of a soluble fibre source with lignocellulose on the resilience to *Eimeria* infection and measure both VCR and the invasion of schizonts in the GIT to confirm the suggestions.

6.3.2 Reducing dietary protein content

In Chapter 5, there was a greater extent of anorexia observed in high CP feeds compared to low CP feeds. This could be expected due to the intrinsic link between anorexia and the immune response, and the link between dietary protein and the immune response. Many studies have shown that increasing dietary protein has a positive effect on infection outcomes and host fitness, which is logical considering many of the effector mechanisms of the immune system such as antibodies, immunoglobulins and mucins are proteinaceous in nature. In addition amino acids are used to cover the requirements of expanding immune cell populations (Cohen *et al.*, 2017; Sadiku *et al.*, 2021). It is clear that a certain level of protein intake during infection with *E. maxima* is beneficial in terms of resistance and resilience. However, as the old adage goes ‘too much of a good thing is never good’, and over-supplementation of dietary protein during an infection has been shown to be detrimental to the host, but the question remains: why is this the case? Sharma *et al.* (1973) demonstrated the

ambiguity of this interaction perfectly with chickens inoculated with *E. acervulina* and *E. tenella*, who were offered feeds varying in CP content. The birds offered feeds with a high CP content were more tolerant to the infection, shown by a reduction in weight loss during the infection compared with birds offered feeds low in CP content. However, resistance was penalised in birds offered the feeds with a higher CP content shown by increases in oocyst production and mortality, which suggests that high levels of CP increase the severity of coccidiosis. As previously mentioned, this has been ascribed to the higher levels of trypsin secretion, which is caused by an increased dietary protein level, and is considered responsible for excystation of a larger proportion of infective dose of oocysts (Hill et al, 1963). The experiment conducted by Sharma *et al.* (1973) supports previous findings where birds offered feeds with no, or low levels of CP showed a reduction in mortality and caecal lesions compared with birds offered feed with a 20% CP content (Britton *et al.*, 1964). These findings are surprising since the expectation would be that the immune response is penalised by low protein feeds due to the inherent relationship between protein supply and the immune response.

In contrast to the above, there is evidence to suggest that there is reduced immunocompetence of broilers given low protein feeds (Laika and Jahanian, 2017). The effect of low protein feeds on immunocompetence reflects the importance of AA in the immune response. An increasing number of studies are evaluating essential AA requirements of broilers in the face of intestinal pathogenic challenges (Star *et al.*, 2012; Tan *et al.*, 2014; Luquetti *et al.*, 2016; Laika and Jahanian, 2017; Castro *et al.*, 2020). By definition, an excess or lack of specific AA can lead to imbalances and such imbalances affect AA metabolism. There is an increased energy expenditure when AA are metabolized in addition to the production of ammonia generated by uric acid degradation, which damages the health of chickens and increases the environmental impact of broiler production (Khajali and Wideman, 2010). It has been repeatedly shown that inflammation and enteric disease increases the requirement for AA as their utilization is prioritized over maintenance functions for immune-related functions and intestinal recovery from infection (Star *et al.*, 2012; Tan *et al.*, 2014; Laika and Jahanian, 2017; Lai *et al.*, 2018; Castro *et al.*, 2020). Studies have mainly focused on Arginine (Laika and Jahanian, 2017; Castro *et al.*, 2020), Methionine (Lai *et al.*, 2018) and Threonine (Star *et al.*, 2012) while an increased supplementation with non-essential AA such as glutamine may also improve infection outcomes (Luquetti *et al.*, 2016; Bortoluzzi *et al.*, 2018). One way of reconciling the contradictions of low protein feeds on the immune response is to suggest that mortality and lesions were greater in the experiments by Sharma *et*

al. (1973) and Britton *et al.* (1964) because the greater amount of undigestible protein facilitated the proliferation of more pathogenic bacteria, such as *C. perfringens*. Therefore, the greater mortality and lesion scores in the birds fed high CP may not have been a result of *Eimeria* infection. This explanation would also account for the greater reduction in the extent of anorexia in the birds given the high CP feeds in Chapter 5, as they target a protein intake to prevent secondary infections. Furthermore, the reduced resistance of the birds given the low CP feeds can be accounted for by the importance of essential and non-essential AA in the immune response.

The negative effect of high levels of dietary protein on infection outcomes is not unique to coccidiosis and has been observed in chickens infected with *Salmonella gallinarum* (Hill and Garren, 1961; Boyd and Edwards Jr, 1963), Marek's disease (Proudfoot and Aitken, 1969) and Newcastle disease virus (Boyd and Edwards Jr, 1963). From an infected animal's perspective, this could be an attempt at reducing the risk of a secondary infections. It is known that increased undigestible protein and diseases such as coccidiosis can be pre-disposing factors for more severe infections, such as necrotic enteritis (NE) which is caused by the pathogenic bacterium *Clostridium perfringens* (Prescott *et al.*, 2016). To that end, since the protein source used in Chapter 5 were relatively digestible, the adverse effects of high dietary protein content may be greater when using more common, and less digestible, protein sources. Indeed, as these protein sources have a lower proportion of digestible protein, they likely impose a greater threat to intestinal integrity and function due to excessive protein fermentation (Gilbert *et al.*, 2018). Therefore, avoiding an excess of protein intake during the acute stages of infection appears to be a generalised mechanism of infected hosts to improve host fitness and survival. This may be the reason for the greater extent of anorexia in the birds given the 24% CP feed in Chapter 5.

6.4 Scope for future research

Throughout the course of this project, several potential future research objectives for understanding the regulation of feed intake in broiler chickens were identified:

- It is important to note that only one broiler genotype was assessed in the experiments presented in this thesis. Therefore, future research should investigate whether these findings can be applied to other broiler genotypes, particularly in slow growing genotypes as there is increased interest in the production of slow growing birds to improve bird welfare (Davies, 2019; Dixon, 2020). As there are inherent differences in the feed intake, growth performance and GIT development of slow and fast-growing

genotypes (Picard *et al.*, 1999; Singh *et al.*, 2021), it is likely that there will be differences in the rate of adaptation to bulky feeds between slow and fast-growing genotypes. Furthermore, it seems logical that there would be differences in the characteristics of anorexia between genotypes, although information is scarce on the comparison of resistance to coccidiosis in slow and fast-growing genotypes (Giles *et al.*, 2019). In an experiment comparing the resistance and tolerance to *E. maxima* infection (Sakkas *et al.*, 2018), there was no significant difference between fast and slow growing broiler genotypes in feed intake scaled to body weight over the acute stage of infection. However, in that experiment the birds were not offered feeds of different compositions. It would therefore be of interest to assess the effect of feed composition on the characteristics of anorexia between different genotypes, as fast-growing genotypes have been shown to increase their feed intake in response to reductions in energy and nutrient content of the feed, whereas slow-growing genotypes were unable to increase feed intake when given such feeds (Fanatico *et al.*, 2008). It could be expected that the extent of anorexia would be greater in slow-growing genotypes given feeds of reduced energy and nutrient content compared to fast-growing genotypes.

- Future studies should compare how the day at which the birds are allocated to bulky feeds affects their rate of GIT adaptation to the feeds. Whilst it is known that developmental plasticity is greatest in the first 10 days of life (Sklan, 2001), it is not known whether the rate of adaptation would increase when the birds were introduced to the feeds at an increasingly younger age. However, as it has been shown that early nutrient supply is essential in increasing intestinal development, it is unlikely in a commercial setting to offer alternative feeds during the starter period (Sklan, 2001; Prabakar *et al.*, 2016). Therefore, the rate of GIT adaptation to alternative feeds should be determined at a variety of ages since it is during the grower and finisher period that alternative feeds would be offered to commercial broilers.
- It has been suggested that chronic intestinal inflammation is the key driver of reduced performance when feeds include soluble fibre sources (Cardoso Dal Pont *et al.*, 2020), therefore future studies should measure the degree of intestinal inflammation as this may account for the reduced performance of birds given bulky feeds diluted with soluble fibre, such as those in Chapter 2 and Chapter 3.
- This thesis presents evidence that pathogen-induced anorexia is affected by feed composition and the results suggest that during the acute stage of infection infected

hosts attempt to tightly regulate their nutrient intake. Broiler chickens infected with *E. maxima* were shown to reduce their energy and nutrient intake during the acute stage of infection, however due to the nature of the feed formulation it was not possible to identify whether energy or nutrient resources were being regulated. Therefore, future work should seek to identify whether energy or nutrient resources are being tightly regulated during pathogen-induced anorexia. This could be done by formulating feeds of reduced energy content whilst crude protein is kept constant. Alternatively, the experiment could be designed with choice feeding where the birds have access to a variety of feeds so that they are better able to regulate their feed and nutrient intakes. Unravelling the targets of infected hosts during pathogen-induced anorexia will shed light on how infected animals, and possibly even humans, should be fed during the critical stages of infection. Future studies investigating the effect of feed composition on pathogen-induced anorexia, should also measure the immunological responses (such as cytokines, the acute phase protein response, lymphocyte proliferation and goblet cells) of the animal. Due to the importance of protein in the resistance to infection, it would be interesting to also make such immunological measurements during experiments where the protein content of the feed is reduced. Indeed, there was reduced resistance in the low protein feeds in Chapter 5 which suggests that there were differences in the immunological responses. It would also be interesting to know if the same principles apply to different pathogens, and to the interactions between different hosts and pathogens, including co-infections. Since *E. tenella* is considered the most pathogenic species (Patra *et al.*, 2010), It could be expected that infection with this species would induce more severe pathogen-induced anorexia than the other *Eimeria* species, although this clearly requires further investigation.

- There was no effect of infection or an interaction between feed composition and infection on the (absolute) food intake and gain of birds during the recovery stage of infection. However, when these quantities were considered relative to body weight of the bird, birds on the more nutrient dense feeds were able to increase relative food intake, which resulted in compensatory growth during recovery. Therefore, access to different feeds may need to be considered during the acute and recovery stages of infection. As poultry management is moving away from reliance on antimicrobials, understanding how to feed animals to cope with and recover from infection becomes increasingly important. The results from Chapter 4 and Chapter 5 suggest that during the acute stage of infection it may be beneficial to offer feeds of reduced energy or

nutrient composition, whereas the reverse appears to be the case during the recovery stage of infection. Therefore, future work should investigate whether this suggestion is indeed true by offering feeds of different compositions during the different stages of infection.

- There would be merit in assessing the microbiota composition in the small and large intestine, and the caeca during the infection when given feeds of different protein contents. As it has been shown that fermentation of undigested protein alters the gut microbiota and could lead to bacterial dysbiosis (Apajalahti and Vienola, 2016; Bryan *et al.*, 2019). It is possible that this occurred in the high protein feeds in Chapter 5, which may offer an alternative explanation for the greater extent of anorexia seen in these birds.

6.5 Conclusions

The findings from this thesis improve the understanding of the regulation of feed intake in broiler chickens. In Chapter 2 and Chapter 3, the regulation of feed intake of healthy broilers given feeds diluted with bulky ingredients was assessed. The results from Chapter 2 supported the hypothesis that broilers given feeds diluted with SBP would be limited in their performance further than those given feeds diluted with the same amount of OH due to differences in the bulk properties of the two ingredients. Furthermore, there was a substantial increase in feed intake relative to body weight in the broilers given the bulky feeds. In fact, this increase was beyond the increases previously seen in experiments addressing broilers' capacity for bulky feeds. The increase in feed intake was accompanied by increases in GIT measurements, which were greater in the SBP birds than the OH birds, suggesting the former needed to accommodate a greater bulk intake. Unfortunately, the objective of identifying a bulk property responsible for limiting feed intake that could be used to predict feed intake on bulky feeds was not achieved. However, this objective was partially achieved in Chapter 3, where feeds were diluted with OH, GM, WB or a mixture of two of the bulky ingredients. It was found that WHC was a good predictor of scaled feed intake during the period when the birds were initially introduced to the bulky feed. The results suggest that once the birds were adapted to the bulky feed, WHC was no longer able to accurately predict scaled feed intake since there was no longer a constraining effect of the bulky feed. The rate of this adaptation was dependant on the bulkiness of the feed, with bulkier feeds (e.g. GM60) requiring a longer adaptation period than less bulky feeds (e.g. WB30). The results supported the hypothesis that feed intake on mixtures of bulky ingredients will result from the principle of additivity for bulkiness and that previous experience to bulky feeds will improve the ability of birds to

adapt to a bulkier feed. All birds were given the GM60 feed on d29 until the conclusion of the experiment and their rate of adaptation reflected the bulkiness of the feeds they were given from d8-29.

Chapter 4 and Chapter 5 investigated the regulation of feed intake in broilers parasitised with the coccidian, *E. maxima*. The objective of Chapter 4 was to investigate whether dietary energy content affects pathogen-induced anorexia and to provide evidence that infected birds are attempting to regulate their nutrient resource intake. The results showed that this was indeed the case, as there were differences in feed intake, but not energy or protein intake in the infected birds. In contrast, in the uninfected birds there was a decrease in feed, energy and protein intake as dietary energy content was reduced. It remained unclear which nutrient resource was being targeted during pathogen-induced anorexia from this experiment. Chapter 5 was therefore designed to address the uncertainty over which nutrient is being regulated, by offering isoenergetic feeds with different protein contents. In healthy birds, the results supported the hypothesis that feed intake would increase to a plateau. The same pattern was observed in the infected birds which was contrary to the expectation that birds given the highest CP feed would show a greater extent of anorexia. However, in terms of protein intake, the results supported this hypothesis as there was a greater reduction in protein intake in the birds given the high CP feed. It was expected that the outcomes of infection would be more severe in birds given low CP feeds. Indeed, this was observed as oocyst excretion was greater in birds given the low CP feed.

The results from these experiments show that performance during infection is indeed sensitive to feed composition and it may altogether be absent when broilers are offered feeds diluted with bulky ingredients, such as lignocellulose. The findings of this thesis facilitate the development of models to predict the feed intake and performance of broiler chickens offered feeds with alternative, bulky ingredients. Unravelling how feed intake is regulated during *Eimeria* infection will help to understand how these birds should be fed during the critical stages of infection.

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