Development of improved disease monitoring tools and management strategies to promote health in finishing pigs

By

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For my parents

ABSTRACT

This thesis investigated strategies to improve detection and control of disease in finishing pigs. It was found that a disinfection routine in finisher pig housing reduced bacteria on pen surfaces. All-in/all-out systems had lower concentrations of airborne bacteria and ammonia than continuous flow systems, however pig health and productivity did not differ between systems. Serum acute phase proteins (APPs) were significantly related to a reduced average daily gain (ADG) of pigs in the two weeks before slaughter.

The lifetime growth of >700 pigs was monitored to explore consequences of early growth on lifetime health and performance. The probability of pigs showing illness later in life was associated with litter size and early growth rate. Early stage differences existed between the growth rate of light and heavy birth weight groups.

Oral fluid (OF) was utilised for measurement of APPs, to determine the presence of sub-clinical disease in pigs. C-reactive protein (CRP) and Haptoglobin (Hp) in the OF of individual pigs was negatively related to their ADG over the finishing period and lifetime respectively. CRP within a pooled OF was negatively related to pen finishing ADG.

To collect pooled OF samples from groups of pigs, a single length of rope in a pen of ≤ 25 pigs generated >80% chewing, in 60 minutes. A higher proportion of pigs chewed the rope when housed in a fully slatted system than in a straw system. An interaction occurred between the housing system and the number of ropes provided.

Daily water use patterns of finisher pigs were evaluated for early disease detection. The mean daily quantity of water consumed per pig within a given week differed in relation to the severity of scour observed in the following week at a level approaching significance.

These findings demonstrate possible techniques to identify disease in the sub-clinical stage.

Declaration

This thesis has been composed by myself and has not been accepted in any previous application for a degree. The work of which this is a record has been done by myself unless otherwise stated. All sources of information have been specifically acknowledged by means of referencing.

Yolande Maria Seddon

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Conferences and abstracts

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- Seddon, Y.M., Farrow, M., Guy, J.H. and Edwards, S.A (2011) Real time monitoring of finisher pig water consumption: Investigation at pen level. Proceedings of the XVth International Society for Animal Hygiene congress, Vienna, Austria, 3-7th July 2011, pp. 95-98. Theatre presentation.
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Chapter 1: The importance of disease in the UK pig industry

1.1 Introduction

Escalating production costs pose a real threat to the future of the UK pig industry as a market-dependant sector of British agriculture. With many costs outside the control of producers, twice in recent years (late 2008 and 2011) pig producers within the UK and throughout Europe operated at a commercial loss. The most recent example has been as a result of the global increase in grain prices, which has had predictably negative financial consequences, (BPEX, 2010a).

In 2009, the cost of producing pigs in Great Britain was 131.7p/kg deadweight, a 4% reduction from 2008 (BPEX, 2010a). The performance of British finishing pigs has been improving steadily since 2003 when the average daily gain was 627g/day, to 2009 when it was 819g/day. This was 7% above the EU average but the best EU competitor industries are performing at 898g/day (BPEX, 2010a). The feed conversion ratio (FCR) of British finishing pigs, at 2.77 in 2009, is one of the lower FCRs of recorded countries. UK slaughter weights are generally lower than EU competitors producing heavier pigs with a slightly lower (2.66) FCR (BPEX, 2010a). This suggests that there is some scope for improving the FCR of British pigs.

The positive improvements in performance made by the UK producers in recent years must be maintained in order to reduce the gap in performance between the UK and other countries and to increase market competitiveness (BPEX, 2010a). This involves improving the physical performance of the pigs, reducing feed costs through improved feed efficiency and increasing throughput through increased daily live weight gain.

Poor health negatively impacts all the performance parameters of finishing units; reducing feed efficiency and daily live weight gain, and increasing mortality (Dijkhuizen, 1989). Poor health in British herds is, in part, held accountable for their reduced performance. Therefore, the improvement of pig health has become one of the single most important factors that will determine the sustainability of the industry over the next 10 years, as outlined in the British Pig Executive (BPEX) Health and Welfare Strategy

(BPEX, 2003). Disease will be an ongoing challenge to the British pig industry as it continues to evolve.

As a result of a combination of poor health status, poor management and lack of investment, the British pig producer is unable to benefit from the genetic potential of the pigs (Richardson, 2004), altogether resulting in inefficient production. Research into pig health management is therefore of continual importance in order to develop strategies to deal with these challenges.

1.2 The disease status of the UK finishing pigs and cost to the industry

Disease has been defined as "a bodily abnormality or failure to function properly," (Concise Medical Dictionary, 1998). Disease can result not only from pathogenic organisms (e.g. viruses, bacteria), but also malfunctions of physiological systems within an individual. Due to the short life of pigs produced for slaughter, disease caused by infectious pathogenic organisms is a major cause of concern within the industry and is the main focus of this study.

Poor health within the UK herd has been estimated to cost the British pig industry £50 million per year (BPEX, 2003). However, this estimate is rather rudimentary since, at the time of writing the report, there was little real knowledge of the health and disease status of the UK industry and the actual cost is likely to be far higher. Disease results in a number of deleterious effects for both the welfare and the production of growing animals. The stunting of growth and a reduction in feed efficiency are two of the main effects, with both directly increasing costs of production. Stunting in growth is in part due to the failure of sick animals to eat (Kelley et al., 1993), but also a result of the pathology of the infection and the subsequent immune and metabolic response which results in a redistribution of nutrients away from growth and towards the mounting of immune response (Colditz, 2004). In addition, there will be further costs for veterinary treatment and any resulting mortality. Pigs with chronic immune activation not only take longer to reach slaughter weight, but also produce a poor carcase composition, with a higher fat to lean tissue ratio (Williams et al., 1997a), introducing the potential for producers to incur further losses through price deductions at slaughter as carcases fail to make the grade anticipated

Sweden and Denmark have a mandatory system for disease surveillance, with producers required to record *all* disease on their unit (Olsson et al., 2001). In the UK, however, producers need only inform officials of notifiable diseases. As it currently stands within the UK there is no national herd health classification scheme to address all the diseases of economic importance. However, due to the potential risk to human health posed by zoonotic diseases, the UK does have a Zoonoses National Control Programme for Salmonella in pigs. Established by BPEX in 2002, national salmonella testing on meat juice is performed monthly on pigs sent to abattoirs. In addition, all units are expected to have an on-farm salmonella control strategy regardless of their current salmonella status (BPEX , 2010b).

Investigation of the extent of infection with non-notifiable diseases within the UK is largely conducted through the collation of information provided on diagnostic samples submitted by farmers and vets to disease surveillance centres such as the Veterinary Laboratories Agency (VLA), now merging with Animal Health to form the Animal Health and Veterinary Laboratories Agency (AHVLA, 2011) within England and Wales, and the Scottish Agricultural College for Scotland. Information is collated and disseminated by the AHVLA via updates to their website and quarterly disease reports. Some monitoring of the prevalence of disease on a sample of farms is also carried out by veterinarians participating in the National Animal Disease Information Service (NADIS, 2011). In addition, there is the British Pig Health Scheme (BPHS) (BPEX, 2011a) which relays health information to individual farms based on a selection of pathological lesion scores of their pigs at slaughter.

Whilst these schemes provide information on the diseases currently present within the UK, and allow detection of newly emerging diseases (providing the diagnostic tests are capable), they provide no accurate indication of the prevalence of each disease, and therefore the results provided are likely to not be fully representative of the UK pig population (Stärk and Nevel, 2009). Endemic diseases are likely to be under reported (Strachan, 2004). In addition, because the UK does not have a routine testing scheme, there is also little knowledge of the rate of re-infection of herds.

To overcome this deficiency, beginning in 2009 the UK industry developed a series of regional health surveillance and elimination groups to map out the disease status of regional holdings. A further step will then be to combine regional data and provide a picture of the prevalence of disease at national level. Surveillance has begun with the Yorkshire and Humberside Health for pig herds (YHH), with action soon to follow from two further groups, East Midlands Pig Health (EMPH) and Eastern Pig Health (EPH), (BPEX, 2011b). All are part of the English pig industry's pig health improvement strategy, involving a team consisting of BPEX, DEFRA and the local Regional Development Agency, such as Yorkshire Forward. Other industry bodies such as the National Pig Association (NPA) have lent additional support. The target of the YHH is to enable farmers to reduce the production lost as result of disease, thus reducing costs by a target of £8 per pig (BPEX 2011b).

The major diseases of economic concern in the UK finishing herd are briefly described below:

1.2.1 Porcine Reproductive and Respiratory Syndrome virus

Porcine reproductive and respiratory syndrome virus (PRRSv) can cause significant losses to production in both breeding sows and growing pigs and thus has fast become one of the most economically important diseases affecting pigs since its discovery in Europe in 1991 (Corzo et al., 2010). PRRSv is spread worldwide, and believed to be endemic in nearly all pig producing countries (Benfield et al., 1999), however, only the USA produces accurate reports on the national prevalence of PRRSv (Albina, 1997).

The true prevalence of PRRSv in the UK remains largely unknown but the prevalence of PRRSv in all infected countries is believed to be high (Albina, 1997). As an indication, 56% of 356 samples submitted for testing in Britain during 2001-2003 were positive for PRRSv, although regional variation was apparent with fewer sero-positive herds where outdoor production predominated (Richardson, 2004). Data now incoming from the YHH health improvement programme gathered from 233 herds willing to disclose disease status, shows that 58% were positive for PRRSv (BPEX, 2010c).

It has been calculated that the greatest proportion of economic loss resulting from PRRSv occurs in the grower-finisher phase, followed by the nursery stage and the least

proportion of annual loss results from abortions and stillbirths in the breeding stage. Neumann et al., (2005) calculated the distribution of the annual national economic loss resulting from PRRSv to be 52.2% of loss occurring in the grower-finisher, 35.9% in the nursery and 11.9% in the breeding stage. The respective costs of lost production in each phase for the US swine herd, in millions of dollars and pounds sterling at the exchange rate at the time of writing (May 2011) is: $292.23m (£177.25m), $201.34m (£122.13m) and $66.75m (£40.48m) (Neumann et al., 2005). Per pig, the total increased cost of production for the US nursery and grower/finisher phase was calculated at $6.01 (£3.65) and $7.67 (£4.65) (Neumann et al., 2005). In growing pigs these losses primarily arise from the decreased average daily gain, reduced feed efficiency and an increase in mortality and unmarketable pigs.

1.2.2 Enzootic Pneumonia

Mycoplasma Hyopneumoniae (*M. Hyo*), the primary pathogen of enzootic pneumonia, is the most common pathogen affecting grower-finisher units worldwide; epidemiological data indicate that 95-97% of intensive piggeries are *M. Hyo* positive (Llopart et al., 2002). The disease is presumed as endemic across the UK. In a well managed and relatively clean production environment the effect of the disease can be minimal. However, in combination with secondary pathogenic invaders, *M. Hyo* can create huge economic losses due to pulmonary lesions and pulmonary immunosuppression (Ciprian et al., 1988). *M. Hyo* is a chronic disease, gradually occurring, usually affecting growing pigs of three to six months of age, and can persist for a number of weeks or months with or without the clinical signs of coughing (Ross, 1999). It is these characteristics that account for the large economic cost of the disease. It can produce high morbidity with chronically underproductive pigs, often leading to growth retardation yet with no real clinical signs of illness (Ross, 1999). Mortality is often associated with secondary bacterial infections, and pigs often die in the later stage of the finishing cycle incurring high cost penalties.

1.2.3 Swine Dysentery

Swine dysentery is an important disease that affects growing and finishing pigs. *Brachyspira hyodysenteriae* is the pathogen responsible, causing inflammation of the large intestine. Swine dysentery is an economically devastating disease due to the reduced growth rates of affected individuals, medication costs and loss of business as a result of

the sale of infected weaners (Taylor, 1979). Clinically affected individuals display watery diarrhoea ranging in colour from grey to yellow or dark with blood streaks. They become gaunt and emaciated, often hunching up and arching their backs. Death of clinically infected individuals is often a result of dehydration, acidosis and hyperkalemia (Harris et al., 1999). Occasionally individuals will die after acute infection, often with no signs of diarrhoea. Their cause of death is unknown.

Sub-clinically affected pigs show no signs until a stressor results in a breakdown and clinical emergence of the disease. This sequence of events makes incoming stock a primary source for the spread and infection of swine dysentry. Losses as a result of mortality in growing pigs are less common than they used to be as a result of the quick recognition of the disease and the prompt application of antibiotics by stockpeople (Taylor, 1979).

1.2.4 Porcine Circovirus Type - 2

Porcine Circovirus Type-2 (PCV-2) is a widespread virus within the global pig industry. The PCV-2 virus is linked with a range of pig diseases; PCV-2 associated pneumonia (porcine respiratory disease complex) (Kim et al., 2003), Porcine Dermatitis and Nephropathy Syndrome (PDNS), PCV-2 associated enteritis, and also as a viral component of Post Weaning Multisystemic Wasting Syndrome (PMWS) (Chae, 2005). In the UK, PCV-2 created most problems through PWMS, a disease causing large financial losses from poor, wasting pigs and mortality. In the UK, the once high mortality from the disease has now changed to a chronic form of illness (Burch, 2007a). The introduction of a circovirus vaccine to the UK in 2007 has allowed the industry to gain better control over the disease.

1.2.5 Salmonella

The ability to colonize a wide variety of environments has led to Salmonella becoming a widespread pathogen. Whilst pigs can be clinically infected with disease causing serotypes it is not commonly a problem. The principal cause for concern with salmonella is the widespread infection of pigs with non- disease causing serotypes that represent a food safety issue for the general public (Schwartz, 1999). *S. Typhimurium* accounts for the majority of cases of salmonellosis in pigs.

Whilst individual diseases result in lost production it is apparent that nearly all of the economically significant endemic diseases in the finishing herd have synergistic relationships with other pathogens making their combined effect more potent. For example, *Mycoplasma* enhances the pathological effects of PRRSv (Thacker et al., 1999). Results from the initial YHH survey revealed that 91% of producers wanted to target Swine Dysentery, 88% to target PRRSv, 86% EP and 45% Sarcoptic mange (Lister, 2010).

1.3 Animal welfare and health

The concept of animal welfare has arisen from ethical concern over the treatment of animals (Duncan and Fraser, 1997). The widespread use of the term amongst businesses, politicians, consumers and veterinarians alike (Hewson, 2003) demonstrates the continued and increasing importance of animal welfare to our society. Debate continues in literature over a true definition of animal welfare (see for example Duncan and Fraser, 1997, Fraser and Weary, 2004). However, a common attribute of the most widely accepted definitions of animal welfare sensibly incorporates both the mental and physical wellbeing of an individual (see definition by Duncan, 1993). Both these aspects are considered in the framework for EU legislation.

For the purpose of this study welfare shall be defined as an individual's "state as regards its attempts to cope with its environment" (Fraser and Broom, 1997 p. 391). Coping refers to the balanced state of an individual "having control of mental and bodily stability" (Fraser and Broom, 1997 p. 386).

Animal welfare within the UK is often assessed according to the Five Freedoms – the needs of an animal as defined by the Farm Animal Welfare Council (FAWC) who modified the earlier Five Freedoms given in the 1966 Brambell report (Webster 2005a). The FAWC Freedoms are as follows:

1) Freedom from hunger and thirst – by ready access to fresh water and a diet to maintain full health and vigour.

2) Freedom from discomfort – by providing an appropriate environment, including shelter and a comfortable resting area.

3) Freedom from pain, injury or disease – by prevention, or rapid diagnosis and treatment.

4) Freedom to express normal behaviour – by providing sufficient space, proper facilities and company of the animal's own kind.

5) Freedom from fear and distress – by ensuring conditions and treatment which avoid mental suffering.

Commercial pig production presents many challenges to meeting all of the five freedoms. Within pig production the first two freedoms should more or less always be adequately met. However, depending on the production system and health status of the farm, the ability to meet the remaining three freedoms will vary.

Pig producers are often concerned about the animal welfare movement, and the requirements it could bring for changes to production systems, often raising the cost of production. Legislation and the adoption of Assurance Schemes, both contain elements designed to focus on and improve the welfare of farmed pigs in Britain. Producers often have little choice but to comply with standards of both market and government in order to maintain the market access which the Assurance Schemes provide (Hubbard et al., 2007). Compared to other European countries, the UK currently has one of the highest pig welfare standards (Bock and Van Huik, 2007). Though standards have been set, real pig welfare status on farms is dependent on the actions of the producers themselves. Currently pig farmers differ in their perception of what constitutes good animal welfare. Bock and van Huik (2007) identified two main groups: those that base their welfare on the ability of the animal to express natural behaviour (which can give rise to good psychological welfare) and those who define welfare in terms of the provision of the animal's basic biological needs (food, water, climatic conditions, good health), all of which can be measured in zoo-technical performance. Ideally, producers should be considering both sets of requirements.

Disease is regarded as one of the most important causes of animal suffering, and it often goes unrecognised as such (Gregory, 2004). A pig in poor health is experiencing sub-optimal welfare and therefore sick pigs need prompt treatment and to be provided with a suitable environment; an example being a hospital pen for very sick and bullied individuals who are clearly not coping within the standard pen environment. Discussion continues that a number of practices designed to improve the health of the pigs, e.g. slatted floors for hygiene, reduce another aspect of the welfare as psychological welfare is compromised by housing in a barren environment (see Kilbride et al., 2009, Guy et al., 2002a, Scott et al., 2006a). However, in general, the actions taken to improve the health of pigs will improve the overall welfare of the pig and if the UK industry can improve the health status of the UK herd it will greatly improve the welfare of a large number of pigs.

1.4 Conclusion

Steps taken to prevent disease, improve detection and thus better control its management within pig units are extremely important for the profitability and sustainability of the British pig industry and will simultaneously bring a greatly improved welfare for the pigs concerned. The diseases highlighted in literature as most costly to the industry are also nearly all endemic within the UK herd. Collectively the industry is now taking steps to work together for the eradication of specific diseases within areas of the UK; responsibility now falls to all individual parties to be diligent for success and vigilant of re-infection. An investigation into the practices that the industry and individual producer can adopt to improve the reduction, monitoring, management and detection of disease is the subject of this thesis.

Chapter 2: Review of disease mechanisms, risk factors and control measures

2.1 Host defence mechanisms

The important role of host defence and how it is affected by various factors in the environment is central to an understanding of how disease and disease progression occur.

2.1.1 The immune system

Immunity is 'the ability of the body to resist infection by a disease-causing organism or to overcome the organism if it succeeds in invading the body' (Torrance et al., 1995 p. 49). The activity of the immune system will determine the ability of individuals to resist and cope with disease. In terms of pig production lost production following disease challenge is strongly linked to the individual animal's immune system reactivity.

The immune system is a network of cells, tissues and organs that work together to defend the body against antigens. Antigens can be anything that is a non-self invader of the body such as bacteria, viruses, fungi, and parasites (NIH, 2003). Two types of immune defence exist: innate and acquired. Innate immunity, comprising of several non-specific defence mechanisms (Torrance et al., 1995), is present within an individual from birth and is the first rapid line of defence against invasion by micro-organisms (Salak-Johnson and McGlone, 2007). Components of the innate immune system include skin sebum, stomach acid, ciliated cells of the respiratory tract, mucus membranes of the lungs and gut, monocytes, macrophages, and the chemical messenger cytokines (Sompayrac, 2008).

Acquired immunity results from an antigen-specific response (Salak-Johnson and McGlone, 2007), that develops throughout the lifetime of an individual as various pathogens are encountered (Torrance et al., 1995). The organs of the immune system, often termed the lymphoid organs, are hubs for the production of immune cells. Positioned throughout the body, the lymphoid organs are joined via a system of vessels, together making up the lymphatic system. The lymphatic vessels lie close to veins and arteries allowing cells and fluid to be easily exchanged from the blood and the lymphatic

vessels and also enabling the blood to be continuously monitored for foreign invaders (NIH, 2003). Although the majority of immune cells are produced in the lymphoid organs, immune reactions are not confined to these organs and also take place in non-lymphoid organs such as the lung (Pabst, 1996).

Acquired immunity produces a highly specific response, each focused on one particular antigen. The cells that form this specific response are lymphocytes, commonly known as white blood cells (WBC). T and B-lymphocytes (or T and B-cells for short) form a large part of the specific immune response. All lymphocytes originate from unspecialised cells in the bone marrow. Some pass onto the thymus, where self-replicating colonies of T-cells are produced. B-cells are produced in a variety of other lymphoid organs, but their name derives from the bone marrow they originated from (Torrance et al., 1995).

The acquired response arises from a number of other lymphocytes as part of the cell-mediated response, together helping to bring about the recognition of the antigen. On infection with a micro-organism microbial proteins released inside the host cell are displayed on the cells surface and act as antigens. Lymphocytes known as T-helper cells recognise these antigens as non-self and activate the B-cells, macrophages and cells known as killer T-cells. Killer T-cells act directly on antigens in a cell-mediated response by recognising the antigens as foreign and destroying the host cell through chemical release that destroys the cell by lysis, (Torrance et al., 1995).

2.1.2 Cytokines

Cytokines are protein hormones (Petersen et al., 2004), produced and released by a variety of defence-orientated cells in response to immune stimulation (Langhans and Hrupka, 1999). Cytokines act as chemical messengers and orchestrate non-specific and specific immune reactions within the body (Langhans and Hrupka, 1999), helping to limit the spread of an invading micro-organism through orchestrating immune defence cells into action (Fossum, 1998). Cytokines are one of the very first responses to the onset of disease. Studies characterising the response of cytokines to infectious challenge in pigs have found levels of IL-1 and IL-6 were elevated within 24 hours of experimentally induced *Actinobacillus pleuropneumonaie* infection (Murtaugh et al., 1996). Employing a more frequent sampling routine has characterised the response more precisely and found that, following injection with low-dose lipoplysaccharide (LPS), levels of TNF-α have

been seen to rise to maximum levels within two hours before returning to baseline levels within 12 hours (Moya et al., 2006). Cytokines are important to the discussion of pig health as they are largely responsible for the reduced growth that accompanies pigs experiencing immune activation.

There are at least 15 different types of cytokines reported (Gruys et al., 2005), the response of each of which is influenced by the specific pathogen challenge (Salak-Johnson and McGlone, 2007). Distinguishable groups of cytokines exist; Interleukins (IL) are one such and consist of monokines and lymphokines. These chemicals are secreted by monocytes, macrophages and lymphocytes and act to direct and regulate the immune response (NIH, 2003). Interferons (INF) are another group of cytokines that alter the physical properties of immune cells, stimulating anti-viral immune responses (NIH, 2003).

The principle pro-inflammatory cytokines are, interleukin-6 (IL-6), interleukin 1-alpha (IL-1α), interleukin 1-beta (IL-1ß), interleukin-8 (IL-8) and tumour necrosis factor-alpha (TNF-α) (Murtaugh et al., 1996). These are collectively known as the pro-inflammatory cytokines because they are released by macrophages, the cell representing the first line of immune defence (Johnson, 1997). These are the main cytokines involved in the synthesis of acute phase proteins (APPs). As the magnitude of immune response is proportional to the intensity of invasion (Sompayrac, 2008), this is reflected in the duration of the cytokine response. The rapid return of cytokines to baseline levels found by Moya et al., (2006) is likely due to the use of LPS. A major component of the outer cell wall of Gram-negative bacteria (Murtaugh et al., 1996), LPS is not a live micro-organism and therefore can be cleared by the body in a relatively short time period. In comparison, experimentally infecting pigs with live *Actinobacillus pleuropneumonaie* and withholding antibiotic treatment produced elevated levels of IL-6 up to four days after infection (Fossum et al., 1998), evidence that the duration of cytokine response is linked to the presence of infectious micro-organisms within the host.

2.1.3 The systemic role of cytokines and consequences for pig production

Whilst acting locally to induce a cellular immune response, the effect of cytokines has been found to extend systemically, bringing about a profound behavioural, metabolic and neuroendocrine response in the individual (Johnson, 1997). The changes are believed to

be a result of cytokines acting on the CNS (Dantzer and Kelley, 1989). The manifestation of results is believed to serve a protective role in the body, helping to control disease spread within an individual and promote behaviour that would optimise recovery (Dantzer, 2004). Such behavioural changes include a loss of appetite, decreased reproductive drive, reduced social interaction, increased sleepiness (Rabin, 1999), and a reduction in water intake (Dantzer, 2004), characteristically referred to as 'sickness behaviour'(Kelley et al., 1993). A detailed theory behind the adaptive behavioural response to infection can be found in Dantzer (2004).

Acting on the preoptic anterior hypothalamus, IL-1, IL-6 and TNF-α alter thermoregulatory properties, inducing a state of fever in affected individuals (Kluger et al., 1995, cited in Rabin, 1999), unfavourable for the growth of many pathogens (Dantzer, 2004). A number of the main pro-inflammatory cytokines, including IL-1, IL-6, IL-8 and TNF-α are known to be responsible for activating fever (Kelley et al., 1993), and producing anorectic effects (Langhans and Hrupka, 1999) in immunologically challenged individuals. This action of cytokines upon the body is responsible for the reduction in performance of pigs.

2.1.4 Acute phase proteins

Acute phase proteins (APPs) are a group of blood proteins considered to be non-specific innate immune components. Pro-inflammatory cytokines act as messengers stimulating hepatocytes to synthesise and release APPs into the bloodstream altering blood serum concentrations (Murata et al., 2004). The changing concentrations of APPs act to restore homeostasis in individuals subjected to external or internal challenge helping to restrain microbial growth before the individual develops acquired immunity to the challenge and can repair tissue damage (Murata et al., 2004). APPs are categorised into positive and negative proteins and, in response to challenge, their concentrations in the body increase or decrease respectively (Murata et al., 2004).

In contrast to the relatively short life of circulating cytokines, the response of APPs is of a much longer duration. A single stimulus will commonly cause APPs to remain elevated for 24 hours, with levels starting to decrease after 48 hours (Gruys et al., 2005). Upon chronic immune activation APPs continue to be secreted (Eckersall, 2004) and levels have been found still elevated at 2 weeks post infection (Sorensen et al., 2006).

The response of certain APPs has been characterised and a number of the positive APPs can be listed in three groups depending on the magnitude of response. i) Those with a 50% increase in concentration; ii) those with a two-three fold increase, including Haptoglobin (Hp) and fibrinogen; iii) those with a rapid 5-fold to 1000-fold increase, including C-reactive protein (CRP), Serum Amyloid A (SAA) and Pig major acute phase protein (PigMAP) (Gruys et al., 2005). With APP values rising days before specific antibodies to the triggering disease can be found (Parra et al., 2006), there is potential for APPs to aid disease surveillance in pig health management (discussed in section 2.6.4). Knowledge of the characteristic responses of individual APPs is important in order to accurately measure the response. A large amount of work has been conducted examining the APP response to specific diseases in the field on a range of conditions including viral, bacterial and acute inflammation (see Parra et al., 2006).

2.1.5 The endocrine system and the stress response

It is important to consider stress with regards to the health of the pig, as it can directly affect the ability to resist and the extent to which an individual will succumb to disease.

Stress can be defined as 'the biological response elicited when an individual perceives a threat to its homeostasis' (Moberg, 2000 p. 1). The disturbance to the normal physiological and mental equilibrium of an individual is often referred to as the 'stressor.' Stress forms a part of everyday life and all organisms are equipped with the mechanisms to cope. Individuals have two responses to stress, the autonomic response and the endocrine response.

The autonomic response is involuntary, originating from the autonomic nervous system (ANS) which comprises two systems: the sympathetic and parasympathetic systems. These are responsible for the control of involuntary responses, control of the endocrine glands, the cardiovascular, respiratory and gastrointestinal systems (Roberts, 1971). A stressor activates the sympathetic system, increasing heart rate, respiration rate and reduces gastrointestinal movement, altogether preparing the individual for the "fight or flight" response as proposed by Cannon (1929, cited in Moberg, 2000).

The endocrine system is a series of glands located throughout the body that secrete hormones, chemical messengers that act upon different target organs of the body. When homeostasis is threatened, a response is elicited by virtually every endocrine system in the body (Squires, 2003). The hypothalamic-pituitary-adrenal-axis (HPA) controls one of the main responses to stress and, on the perception of a threat, nerve cells in the hypothalamus release corticotrophin releasing factor (CRF). The CRF stimulates the anterior pituitary gland, generating the release of adrenocorticotrophic hormone (ACTH). ACTH stimulates the adrenal cortex to secrete glucocorticoid hormones (Moberg, 2000). Glucocorticoids stimulate the liver to convert fat and protein to metabolites for the further conversion to a readily available energy resource, glucose (Moberg, 2000). In pigs, the glucocorticoid hormones cortisol and corticosterone are produced (Broom and Johnson, 1993), and their measurement is often used to provide a physiological measure of stress (Grandin, 1997).

High levels of glucocorticoid hormones within the blood have a negative feedback mechanism on the hypothalamus, reducing production of CRF, which consequently reduces the concentration of ACTH and thus the concentration of glucocorticoids (Moberg, 2000). Glucocorticoid receptors have also been identified in the hippocampus of the brain, which play a part in the braking structure to the HPA (Dantzer, 2001).

The stress response is non-specific and thus the same response can be elicited to a variety of stimuli perceived as a stressor by the individual. A chronic stress response sustained over time can induce changes that directly affect the biological functions of an individual. Chronic stress has been found to attenuate the glucocorticoid negative feedback mechanism designed to prevent the occurrence of chronic stress (Mizoguchi et al., 2003). The prolonged action of the glucocorticoids on mobilising energy reserves can have a pronounced effect on the productivity of livestock, and also pathological consequences. This is termed the biological cost of stress (Moberg, 2000).

The effect of stressors is believed to be additive with multiple concurrent stressors having a negative and linear effect on growth performance in pigs (Hyun et al., 1998). On this principle, it suggests that the removal of just one of the accumulative stressors will provide benefits for the pig (Hyun et al., 1998).

2.1.6 The cross-talk between the immune and endocrine system

Stress can have profound effects on the immune system, and subsequent health of an individual, since the immune and endocrine systems do not function as separate physiological systems but work in conjunction with one another. It is important to be both aware of, and to understand, the mechanism of communication between the two systems. It is this cross-talk between the immune and endocrine systems that is responsible for altering the disease susceptibility of animals (Kelley, 1988). This concept is not new, and many previous experiments have contributed to the body of knowledge that has shown that, whilst lymphoid cells are affected by hormones from the neuroendocrine system, immune components from the lymphoid system also affect the activity of the neuroendocrine system (Kelley, 1988).

The stress hormones released following activation of the HPA axis all have effects on aspects of the immune system (Salak-Johnson and McGlone, 2007). Leukocyte distribution and specific neutrophil function in pigs is significantly modulated by stress-related hormones (Salak-Johnson et al., 1997). At physiological concentrations, cortisol was not directly involved with the immune suppression of natural killer (NK) cell activity, but associated suppression did occur at pharmacologically low or high concentrations of cortisol, for which the mechanism is unknown (Salak-Johnson et al., 1996). A review by Salak-Johnson and McGlone (2007) discusses various evidence for the cross-talk between the immune and endocrine system, concluding that the relationship is complex and, depending on the stress, the immune system of an individual can be suppressed, enhanced, or unaffected by stress.

2.2 Risk factors for disease

The vast majority of risk factors for disease stem from the pig's environment. There is evidence that host genetics could play a role in the susceptibility of some breeds of pigs to infection (Opriessnig et al., 2009). However, this review will focus on risks stemming from the environment of the pig. The term environment is very broad and refers to all factors that impact on the pig, both physical and biological (Gonyou et al., 1999). Biological risk factors relate to the presence of specific pathogens and diseases that can affect the herd. Whereas the physical factors may not be infectious agents their presence in the environment can still trigger and exacerbate clinical and sub-clinical disease in

pigs, reducing performance and overall welfare. The following section reviews a number of the risks commonly present within the production environment.

2.2.1 Infected pigs

The greatest risk for the transmission and continued infection of animals within the herd is the presence of another sick individual. Contact between infected individuals and non-infected individuals originates most commonly from the diseases brought in by new stock, which can introduce new diseases into the herd. Transmission of disease from direct contact between pigs can take place via vertical (sow to offspring) and horizontal (from an infected individual to a naive individual) transmission. Pathogens already established within the breeding herd, without proper control measures in place, will continue to affect each new generation of the growing offspring.

Pathogens have been found to be expressed via a variety of routes; for example, PRRSv is secreted in nasal secretions, urine (Rossow et al., 1994), semen (Yaeger et al., 1993), oral fluids (OF), and the faeces of infected individuals (Wills et al., 1997). The direct contact of infected with uninfected pigs generally produces a rapid disease transmission, whilst indirect contact (such as infected pigs within the same airspace) will, in general, lead to a slower spread of infection, as has been found for *M. Hyo* (Fano et al., 2005).

Pigs exhibiting clinical signs of infectious disease are easily recognisable as a risk for disease transmission to other individuals. Yet, where clinical signs are absent, sub-clinical and carrier states of pigs can also exist, posing a risk which might not be fully recognised. Having recovered from *M. Hyo* infection pigs can remain as asymptomatic carriers for up to 200 days post infection and be able to infect other pigs (Pieters et al., 2008).

The routes of disease transmission between pigs highlight the importance of having control methods in place for the finishing herd, serving variously to reduce contact between infected and susceptible groups, reduce the shedding of the infection, or to protect susceptible animals (Fano et al., 2005) all aiming to prevent what could otherwise turn into a perpetual continuation of the disease.

2.2.2 Alternate routes of transmission of infectious organisms

Providing the environmental conditions are optimal to support pathogen survival, infectious pathogens are known be transmitted by a number of other routes, highlighting the need for effective bio-security measures to protect the herd and prevent infection. Dee et al. (2002) demonstrated the ability of PRRSv to be spread via fomites (boots, containers, vehicles and personnel) in cold weather (< 0°C). Contamination of stockmen's boots has been linked to the re-infection of herds with swine dysentery (Windsor and Simmons, 1981). Mycoplasmal pig diseases have been isolated from flies (Fischer et al., 2001), and PRRSv from mosquitoes (Otake et al., 2002).

Reports of repeated re-infection of farms by PRRSv, despite strict control measures, have led to consideration that transmission of certain pathogens may be possible on the wind (Lager et al., 2002). *M. Hyo* and PRRSv have been detected 4.7km from the source of infection (Dee et al., 2009) supporting the hypothesis that aerial transport of swine pathogens can occur, and over relatively long distances.

Biosecurity is the term used for all measures put in place to protect the herd from infection from agents entering from outside the herd. The varied potential routes of infection reinforce the need for strict biosecurity measures to be upheld, and the importance of working for the collaborative simultaneous eradication of diseases within certain regions to avoid recontamination of herds from neighbouring farms.

A recent meeting of the YHH pig health scheme concluded that the 2006-2007 UK outbreak of swine dysentery was accountable for by the following routes of disease transmission in varying proportions:

•Pig movement 44.8%
• Management 13%
• Local spread 10%
• Pig transport 10%
• Birds 7%
• Contractor 3%
• Dead pig transport 3%

• Feed lorry 3%
• Unknown 3%

Source: (Waddilove, 2009).

2.2.3 Herd size

Herd size is regularly listed as a risk factor for disease in growing pigs (Bäckström and Håkan, 1978, Done, 1991, Goldberg et al., 2000). However, large herds differ from small herds in many ways apart from just the number of pigs. Those producers with larger herd sizes might be expected to have greater controls in place via the management and housing system adopted, and automated controls and feed supplies to mitigate the increased risk that an increasing herd size can bring. By contrast, the smaller herds may impose less strict disease preventative management routines, and be run with less professional input. This has been reported for smaller swine herds in the USA, with more frequent use of trucks for a multiple purpose, introducing disease risks (Gardner et al., 2002).

Gardner et al., (2002) have explored the many interlinking factors to herd size and provide an excellent review of a large number of studies examining herd size as a risk factor for disease. They indicate that the relationship between herd size and disease varies, being positive for respiratory diseases and protective for others such as milk spot in livers. Therefore, whether herd size is a risk factor may ultimately depend on the disease in question. Diseases which can be spread via airborne transmission present problems largely outside the producer's control. In this sense, the larger herd sizes could be associated with a greater opportunity for disease transmission resulting from the number of susceptible individuals within a localised airspace. It is on this basis that increased numbers of pigs within buildings and pens are associated with an increased risk of disease (Done, 1991).

2.2.4 Stress

The intricate relationship between the immune and endocrine system (as explained in section 2.1.6), makes sources of on-farm stress a risk factor for animals to succumb to disease already present within the farm. On-farm stressors for the pig primarily originate from three sources; the social environment, the climatic environment, and the interaction

of the pig with stockpeople. Together, these stressors could be grouped into physical (heat and cold) and emotional (fear, social distress) stressors (Kelley, 1980).

2.2.4.1 Social Stress

To accommodate requirements for modern production, growing pigs are subjected to a number of socially stressful situations throughout their life, which result from the mixing of unfamiliar pigs at various stages of production, high stocking densities and large group size.

2.2.4.2 Mixing and hierarchy

Pigs are a social species which establish a dominance hierarchy in groups. The abrupt disruption of the social group leads to aggressive interactions between unfamiliar pigs to re-establish a social hierarchy (Puppe, 1998). Mixing is easily observable as stressful to the pigs; visible injuries often result from fights, and the extent of bodily lesions has been found to relate to the number and duration of fights experienced by individual growing pigs (Stukenborg et al., 2011). Cortisol, a recognised physiological indicator of stress, is often found to be elevated in individuals following mixing (Merlot et al., 2004).

The stress of mixing can have effects on production, as has been demonstrated with pigs reared in 'specific stress free systems' (SSFS) involving no mixing and no transport. Pigs reared in SSFS had a higher daily growth rate and weight at 143 days of age than pigs that had been transported and mixed at weaning, and then further mixed and transported at 25kg, as is the practice in multi-site production of pigs (Ekkel et al., 1995). Grower pigs, having been mixed five times, had a lower daily gain than unmixed control pigs (Coutellier et al., 2007). In this study, the period of mixing that resulted in weight loss also corresponded to the period during which cortisol levels were significantly raised in the mixed pigs. It should also be noted that Coutellier et al. (2007) found that the pigs appeared to habituate to mixing a further seven times, with no significant differences between weight gain and cortisol levels of the two groups for the remainder of the trial. However, in a commercial setting pigs would not be repeatedly mixed this number of times, and so the effects seen after mixing for fewer (five) times would be more applicable to the production setting. In addition, Coutellier et al. (2007) only mixed together two pigs at one time, whereas in a commercial pen a larger number of pigs would be fighting, with some experiencing multiple fights (found to range from 0 – 139

fights per pig per pen) (Stukenborg et al., 2011). Therefore, the effect of the stress and a reduction in growth could be more extreme than reported here.

The repeated mixing of pigs prior to slaughter is a practice often performed by producers who hold back the light weight pigs rather than send them to slaughter and risk penalties and lost profit. Whilst there are some articles on marketing strategies for producers having to deal with growth variation (see Vansickle, 2004) there is little research in the literature on the effect of re-grouping on the expression of disease, and this is an area that requires further investigation. There are already penalties arising from small, slow growing pigs, but it may be the case that there are greater overall penalties on the farm if the slower growing pigs, which could also be stunted in growth as a result of harbouring disease, are held back and stressed through re-mixing to optimise pen space.

Records of incidence of disease following the stress of mixing in pigs are poorly reported in literature. One study by Hessing et al. (1994) found a higher rate of morbidity amongst subordinate than dominant pigs when mixed whilst infected with Aujesky's virus. The proliferation of pathogens has been found in pigs subjected to stressful events, and mixing of pigs has been found to increase faecal shedding of salmonella by pigs (Callaway et al., 2006).

The majority of studies examining social stress have examined immune suppressing and modulating effects, rather than disease prevalence per se. However, the direction of response has been found to be related to the gender and dominance status of individuals. In one study only mixed barrows showed lower lymphocyte proliferation, IgM and INF-γ and IL-10 cytokine production, in response to vaccination challenge. This response was less in dominant rather than subordinate barrows, whilst no differences were detected in gilts (De Groot et al., 2001).

Individual differences are known to exist in pigs (Erhard et al., 1999). There is evidence to suggest that there are large differences in the immune reactivity (Bolhuis et al., 2003) and disease susceptibility of individuals. These appear to be related to social status, with the more dominant pigs having a higher cell mediated immunity and, in one study, better resistance to Aujzesky disease virus (Hessing et al., 1994). Although this is largely beyond the producer's control, individual differences between pigs may explain why

some pigs within the herd succumb to disease and others do not. If this could be linked to phenotypic characteristics it may be possible for producers to highlight potentially susceptible pigs in advance.

2.2.4.3 Weaning

Weaning is a particularly important time for the health management of pigs, encompassing a combination of stressors: removal of the sow, removal of the protective immunoglobulins from the sows milk before the piglet is immunologically developed (Wallgren and Melin, 2001), movement to an unfamiliar environment, mixing with unfamiliar individuals and change from a high fat - low carbohydrate, to a high carbohydrate - low fat diet. The effect of stressors is known to be additive (Broom and Johnson, 1993), and the weaning and allocation of piglets is commonly associated with the onset of disease. The effect of the combination of stressors experienced by piglets is believed to contribute to this process through alterations to immune function (Wattrang et al., 1998). The majority of pig producers in the UK wean at 26-28 days of age. At this point, the functions of active innate and cell-mediated immunity are still immature, whilst pathogens passed from the sow begin to establish. Combined with the grouping of many individuals into one airspace, there is clear potential to create a reservoir for pathogens (Done, 2001a). As a result, a range of enteric and respiratory diseases can break out in piglets, a comprehensive list of which is provided by (Done, 2001a). Failing to establish the weaner could have dire consequences for the performance of the pigs later in life. Whether a low weight post weaning has detrimental effects on the health of the pig later in life is poorly reported in the literature.

2.2.4.4 Relationship of the pig with stockpeople

Psychological stressors are regarded as one of the leading stressors for animals (Gray, 1987) and, of the psychological stressors, the impact of fear is one of the greatest (Mason, 1971). A large component of fear in livestock is neophobia, the fear of novelty, the unpredictable and unknown. This is an important survival instinct held by all sentient animals and serves as a guard against danger (Webster 2005b).

The stress response of pigs to handling is directly influenced by the level of fear the animals have of humans (Hemsworth and Coleman, 1998). Growing pigs often receive little contact from humans in comparison to breeding pigs. This is also evident in data

from farm labour studies. Madsen and Kristensen (2005) report data from the Danish Agriculture Advisory Service stating an average labour input of 10-12 minutes per finisher pig produced. Calculations taken from a pig industry labour study of UK pig farms detailing husbandry procedures that involve human-pig interaction, such as feeding, bedding down, moving pigs etc, reveals similar results; an average of 15 minutes of labour contact per finisher pig produced, compared to 20 minutes per piglet produced from breeding sows (Webster and Haprer, 2006). For human-animal interaction with sows, this equates to 196 minutes per litter produced per sow, based on weaning an average of 9.56 piglets per litter (BPEX, 2010c). This shows that the development of good human-animal relationships is likely to be far greater in breeding pigs receiving a lot of care and attention to optimise reproduction, than in growing pigs. In addition it is unfortunate that the little human contact experienced by growing pigs is often to perform potentially negative operations, such as injecting and moving, and thus a chronic fear of humans can remain.

A relatively short stressful experience of handling has the ability to generate adverse conditions for health in the pig. Pigs that experienced increased handling (herded and weighed for eight consecutive days) shed a larger number of *E.coli* and total coliforms compared to control groups (handled twice) (Dowd et al., 2007). The increased shedding of bacteria is indicative that the pig is experiencing stress and demonstrates how stressful procedures may enhance the spread of disease. This is of particular relevance when animals are transported; for example the stress of transport is found to increase faecal shedding of Salmonella in pigs that have not had feed withheld 24 hours prior to slaughter (Isaacson et al., 1999).

Information from research suggests that more emphasis should be placed on the influence of psychological stressors on animals, and that possibly even enriching the environment of the animal to make life a more positive and enjoyable experience could have benefits for pig health and resistance to disease. Creating a positive environment for growing pigs, with enrichment that stimulated and rewarded the pigs, positively altered concentrations of a number of immune parameters (significantly higher concentrations of IgG and *in vitro* T–cell proliferation), and gave an increased speed of wound healing (Ernst et al., 2006).

2.2.5 The climatic environment of the pig

The quality of the environment provided, if not managed correctly, presents a number of risk factors for introducing disease, aggravating existing conditions and increasing the susceptibility of individuals to disease.

2.2.5.1 Temperature

Temperature has been described as the predominant component of the climatic environment to affect pigs (Le Dividich and Herpin, 1994). Changes in the thermal environment can affect the immune system and disease susceptibility of individual pigs via the generated stress response and alteration to the host resistance.

Temperature fluctuations have been associated with a high incidence of post-weaning diarrhoea in piglets (Le Dividich et al., 1980). The highly variable temperatures that can occur within the UK at certain times of year mean that optimum climatic control systems are necessary for indoor, bedding-free piggeries. Incidentally, automatic temperature control was associated with a reduced incidence of post-weaning diarrhoea (Laine et al., 2008).

An experiment by Shimizu et al. (1978) demonstrated that eight to twelve week old pigs were resistant to virulent transmissible gastroenteritis when raised in rooms at a constant 30°C. Yet when temperatures decreased shortly before or after virus inoculation severe disease occurred in all those exposed. In addition, pigs subject to a fluctuating air temperature from 20 - 4°C every 24 hours developed profuse diarrhoea, and maintained clinical signs far longer than those maintained at consistent low temperatures. This study is useful for clearly demonstrating that, under the correct environmental conditions, pigs were less susceptible to disease.

Miniature pigs subjected to heat stress at 35°C had a reduced CD4/CD8 ratio, indicative of immune suppression (Xiang-hong et al., 2011). The ratio of CD4 and CD8 cells is a predictor of immune function in mammals, and can be used to determine the level of immune suppression. However, groups of pigs heat stressed at 24°C and 32°C experienced no negative effects to the immune response to PRRSv infection (Sutherland et al., 2007). This may have been largely due to the age of the pigs (seven weeks), when

the environments maintained would have been within the pigs thermo-neutral zone, and thus not particularly stressful.

In the majority of experiments examining temperature stress the temperatures studied are fairly extreme. Whether pigs in reality will experience such extreme fluctuations of temperature in production will depend on the nature of the production system. Outdoor produced pigs and those in straw yard systems may experience such variations in temperature more regularly than would be expected in an artificially ventilated indoor piggery. However, it should also be considered that, in the production setting, there are a larger number of factors (as discussed previously) combining that will also impinge on the pig to lower resistance. Therefore, a small degree of fluctuation in temperature may disproportionately increase the susceptibility to disease in individuals. Whilst numerous studies have contributed to the development of recommended temperatures for housing pigs within the thermoneutral zone for optimal growth, there are few studies available that have assessed, under commercial conditions, what increase or decrease in temperature could lead to a reduced resistance to disease in pigs. A cross sectional study of 143 French farms revealed a mean temperature in the fattening system below 23°C was associated with an increased risk of pleuritis in pigs (C. Fablet, personal communication, 13th July 2011).

2.2.5.2 Aerial pollutants

With the intensification of pig production, the quality of air within enclosed spaces is of particular importance in preventing and reducing health problems. It is well known that spending sufficient periods of time within environments of poor air quality can produce a number of negative effects on human health, which has prompted copious research by organisations such as the Institute of Occupational Health (IOH) and World Health Organisation (WHO) into occupational hazards. The pig industry is a particular focus of this research. Pig confinement buildings are notorious for having poor air quality (Robertson, 1994) and high levels of aerial contaminants, creating occupational health issues for the staff who have to work within them. There is substantial evidence to suggest that poor air quality in pig buildings is responsible for reduced productivity in pigs as well as respiratory complaints in farm workers (see Dosman et al., 2000, Donham, 1991, Black, 2003).

Black (2003) concludes that the critical components of air quality to be monitored and controlled are the concentrations of viable bacteria, respirable dust, endotoxins and organisms pathogenic towards pigs. However, even the lower risk components (the inhalable dust fractions) acting synergistically with other aerial pollutants pose significant health risks responsible for declines in pig health and performance, as highlighted by Done (1991).

2.2.5.2.1 Dust

Of the various livestock production systems, piggeries and poultry houses produce the highest quantities of dust (Wathes, 1994). Dust in pig barns is a combination of organic and inorganic material. There is a lower volume of inorganic material, mainly consisting of concrete dust, ash and soil. Organic matter comprises the majority of dust containing animal skin and dander, feed and bedding particles, grain mites, dried faeces, aerosol droplets produced from pigs sneezing, coughing and urinating, viable and non-viable bacteria, bacterial cell wall components, fungal hyphae and spores and viruses (Black, 2003). With a high organic content, piggery dust is biologically active (Gonyou et al., 1999), making it more liable to have adverse effects on humans and animals than other types of dust (Donham et al., 1989; cited in Gonyou et al., 1999). In humans, prolonged dust exposure is a contributing factor in the development of allergies and lung hypersensitivity (Rylander, 1989; cited in Wathes, 1994). Dust exposure has increased levels of the pro-inflammatory cytokines TNF-α and IL-6 in farm workers, and is believed to be responsible for the decline in lung function commonly seen in piggery workers (Wang et al., 1996; cited in Black, 2003). As the respiratory immune system of pigs is considered to serve as a model for respiratory disease in humans (Pabst, 1996) it seems reasonable to assume that any environmental conditions that adversely affect humans could also be affecting the pig in similar ways. Perhaps even more so, for in intensive indoor production the pig is exposed to these conditions constantly. However, it has been shown that concentrations of specific aerial pollutants do differ between the pig and the farmer's breathing zone (Kim et al., 2007a).

There is a growing body of evidence surrounding the effects of dust inhalation on the health of the pig. Piggery dust has been found to contribute to the aetiology of respiratory disease in pigs (Jericho and Harries, 1975) and is a causal factor of bronchitis, increased coughing and lung lesions (Gonyou et al., 1999).

The composition of dust is recognised as important (see Demmers et al., 2003), and it is believed to largely determine the extent of health risk posed, for whether dust is inhaled or not depends upon the particle size. Particle sizes are classified as follows:
Inhalable: particle sizes of 100µm or smaller, hazardous when deposited within the upper airway system (throat).
Thoracic: particle sizes of 10µm or smaller, hazardous when deposited within the lung airways.
Respirable: particle sizes of 4µm or smaller, hazardous when deposited within the gaseous exchange region of the lungs (SKC, 2011a).

It is reported that particles smaller than 1µm pose the greatest risk to health as such particles are small enough to penetrate the delicate lining of the lungs causing permanent damage (Gonyou et al., 1999), yet particles of feed ranging from 15µm to 30µm have been found in the alveoli and lymph nodes of pigs (Jericho and Harries, 1975).

2.2.5.2.2 Micro organisms
Since the inhalation of infectious aerosols is a route of disease transmission (Stärk, 1999), concentrations of airborne viable bacteria within the environment of the pig are believed to be the single most important factor affecting health and performance as influenced by air quality (Black, 2003).

It is difficult to compare results across studies as the concentrations of airborne bacteria are affected by many uncontrolled variables such as ventilation rate and management factors, both of which influence aerosol generation and decay (Stärk, 1999). Concentrations of airborne microorganisms also differ between pig and human breathing zones (Kim et al., 2007a) and therefore comparisons cannot be reliably made between studies that compare or summarise the potential effect of bacterial levels measured in different breathing zones. In the breathing zone of the pig (0.3m from the floor), total dust has been found to be positively correlated to total airborne bacteria (Kim et al., 2007a), indicating that the microorganisms are likely to have originated from dry matter in the piggery (Stärk, 1999). Therefore, many of the factors that affect the concentration of dust will also affect the concentration of viable microorganisms, and vice versa (Black, 2003).

The majority of studies measure microorganism levels as total viable count, however, there is an argument that the species present should also be identified (Cormier et al., 1990). Whilst it is rational to consider that bacteria not pathogenic to pigs pose no threat to pig health, viable non-pathogenic bacteria are also found to stimulate the immune system of the pig leading to depressed growth rate through chronic immune activation (Williams, 1998). Negative correlations have been found between mean growth rates across production units and concentrations of viable bacteria (Murphy et al., 2000; cited in Black, 2003). The chance of infection developing is dependent on the pathogenicity of the infectious organism and the susceptibility of the individual. Even in this relationship, the chance of infection is in turn affected by the concentration of organisms in the air and the efficiency of clearance by the respiratory tract (Stärk, 1999). Therefore it is logical to infer that measurements of total viable count provide a good estimation of the bacterial load being encountered by pigs. If it is known which respiratory pathogens are present on the farm, having some measure of the bacteria load in the air will provide an insight into how much challenge the pigs have to encounter, not only from the pathogens but from respiratory clearance in general.

The concentration of airborne microorganisms in indoor pig houses is affected by a number of things. However, particular correlations that have emerged showing that the concentration of viable airborne bacteria is correlated with the shed hygiene (Magwood and Marr, 1964) and the total number of airborne microorganisms is significantly correlated to housing density of pigs at both weaner (Pavičić et al., 2008) and fattener stages of production (Pavičić et al., 2006). Therefore it seems logical to assume that, if good shed hygiene is maintained with acceptable stocking densities, then levels of airborne bacteria should be minimised. Following a review of the current research concerning air quality in piggeries commissioned by Australian Pork Limited, Black (2003) recommends that within all pig housing systems the maximum concentration that viable bacteria should reach is 50,000CFU/m^3.

2.2.5.2.3 Gases

Various gases exist within a piggery. The decomposition of excreta is the primary emission source (Stombaugh et al., 1969). Concentrations of gases are considered of greater importance in confined pig buildings where slurry is stored beneath the pigs. Ammonia, hydrogen sulphide, methane, carbon dioxide and carbon monoxide are

highlighted as the gases which pose a threat to pig health and production (Gonyou et al., 1999). The most studied and prevalent gas within piggeries is ammonia. It is discussed here because it is the one most often linked to respiratory disease.

2.2.5.2.3.1 Ammonia

Ammonia (NH_3), a highly toxic, corrosive and irritant gas (Hamilton et al., 1998), is reported as the aerial pollutant that is found in the highest concentration in pig houses (Drummond et al., 1980). Being extremely soluble in water, ammonia is rapidly concentrated in the aqueous layer of the mucus membranes of the eyes, nose and throat of exposed individuals (Doig and Willoughby, 1971; cited in Hamilton et al., 1998). Surveys of ammonia levels in commercial piggery buildings provide a rough idea of ranges to be expected, with levels being recorded in a range between 9.1 – 15.9ppm (Seedorf and Hartung, 1999). However, depending on the farm management system, ventilation and floor type there will be concentrations outside this range.

There is evident concern in the literature over the effects of ammonia on animal health, yet studies to determine and evaluate the role played by this gas in development of disease have largely proved inconclusive. Four weeks of exposure to high concentrations of ammonia (100 and 150ppm) has pathological consequences on the lungs of the pig, with acute inflammation of the tracheal epithelium and exudate in the turbine lumen (Drummond et al., 1980). Investigations into the higher concentrations of ammonia on pig health provide an important insight into what symptoms may appear and what levels can be tolerated by pigs for prolonged periods of time. They do unfortunately lack relevance to the ammonia levels experienced by pigs in a commercial situation. Hamilton et al. (1996) conducted far more relevant work in testing and discovering that ammonia concentrations as low as 5 and 10ppm significantly contributed to the etiology and severity of Atrophic Rhinitis by facilitating the growth and survival of *Pasturella multocida* (*P. multocida*) in the upper respiratory tract of the pig. *P. multocida* is poor at colonising the lung and/or nasal cavity unless predisposing tissue damage has occurred (Ciprian et al., 1994, Hamilton et al., 1998), which suggests, rather concerningly, that aerial ammonia concentrations as low as 5 and 10ppm are capable of causing damage to the mucosal lining of the upper respiratory tract in pigs, thereby allowing *P.multocida* to proliferate.

Evidence of the colonisation of nasal turbines with *P. multocida* has been found with higher levels of ammonia exposure (50-100ppm), although these concentrations demonstrated no influence on the severity of turbine atrophy (Andreasen et al., 2000). This is in contrast to the findings of Hamilton et al., (1998) who found evidence of turbine atrophy at concentrations of 10ppm of ammonia.

Despite the fact that ammonia facilitated the development of *P. multocida* infection in the upper respiratory tract of the pig, ammonia alone was found to have no effect on the disease development of *M. hyo* (Andreasen et al., 2000), of which *P. multocida* is an important secondary pathogen. At concentrations of 50 and 100ppm of ammonia, an increase in immune cells (white blood cells (WBC), lymphocytes and monocytes), cortisol and Hp has been recorded in young pigs (von Borell et al., 2007).

Apart from the effect of ammonia on disease progression, behavioural tests suggest that spending time in an environment polluted with ammonia may induce a feeling of sickness. Jones et al. (1996) found that pigs choose to spend a significantly lower proportion of time, and consume less feed, in chambers polluted with ammonia compared to chambers with fresh air. A reduction in feed intake is a behaviour associated with a general feeling of malaise (Johnson, 1997) and numerous studies looking at the effect of ammonia on pigs have observed a reduction in feed intake (Stombaugh et al., 1969, von Borell et al., 2007) and depressed growth (Drummond et al., 1980, Stombaugh et al., 1969), findings which are of importance for both animal welfare and production.

However, it is the synergistic effects of ammonia in combination with other aerial pollutants that may be of greater importance.

2.2.5.2.4 Synergistic effects

It is the chronic and simultaneous exposure to several aerial pollutants that have the greatest effect on animal health as a result of synergistic effects (Wathes, 1994). The severity of atrophic rhinitis has been associated with the levels of aerial pollutants (dust, ammonia, total bacterial counts) in piggeries (Robertson et al., 1990). Unpublished research by T. Murphy (cited in Black, 2003), found that pigs which were intranasally inoculated with cultured viable general airborne bacteria, predominantly of *Streptoccus* origin, developed non-specific bacterial infections of the lungs when combined with 20

minute bouts of increased exposure to ammonia, with growth rate depression related to the severity of the lung infection.

However, in some circumstances when these situations are created experimentally, the results are not clear cut. One of the largest studies evaluating the synergistic role of ammonia and dust on weaning pig health was unable to establish a link, finding the concentrations tested to have no effect on pig health (Done et al., 2005). Often when situations are recreated, the magnitude of the actual response can be lost. In addition, in an attempt to be consistent, the study by Done et al., (2005) used manufactured dust. This could also demonstrate that the synergistic effects brought about by combining dust and bacteria are required to have aggravating effects. However, it was discovered that inhalable dust concentrations of 5.1 or 9.9mg/m^3, combined with ammonia concentrations ranging from 0.6 to 37.0ppm, adversely affected the performance of weaned pigs (Wathes et al., 2004).

2.3 Health management for the control of disease

The ultimate goal of any pig health management programme is to prevent disease challenge. A number of approaches can be taken in order to control, limit and prevent or eradicate disease, but choosing which one to take is dependent on the nature of the diseases present within a herd (Harris, 1988). The main principles of disease control centre around ensuring that the infectious agent is eliminated, by either colostral anti-bodies or therapeutics and management, before removal of pigs from one site/building to the next (Harris, 1988). The role of various health management strategies including production approaches, antimicrobials and prophylactic measures are outlined and discussed.

2.3.1 Biosecurity

If a new herd can be established with high health status, prevention of disease ingress by application of good biosecurity measures is the best form of health management.

The objective of biosecurity is to prevent the entry onto a farm of pathogens that could cause disease (Thorp, 2003).

A mail survey of 1889 farms, with replies representing 25% of the UK herd, showed:

- More than 50% of units allowed livestock vehicles on their premises

- 15% of farms had shower facilities, but for only 30% of these farms was use strictly enforced.

To accompany the survey, an audit of 40 UK pig farms provided an insight into the level of biosecurity on UK pig farms – where it was working correctly, and where it was not (see Robertson et al., 2004). As a brief summary:

Weak points

- 5% of farms were sharing equipment
- 12.5% of farms had no requirement for pig free status
- Of farms purchasing stock (55%), only 7.5% were kept informed about the health issues of the source stock from their suppliers.
- Over 60% of farms had no means of preventing intruders, and 7% were completely open to any live animals entering.

Positive points

- Percentage of farms successfully controlling rats (65%), mice (45%), flies (55%), birds (52.5%).
- 53% of farms regularly carrying out vermin control audits, and 40% occasionally.
- 60% of farms had a "no entry" sign which was enforced and obeyed.

The general conclusion was that many farms had some measures in place to practise good biosecurity, but only certain specific precautions rather than comprehensive measures (Robertson et al., 2004). For an effective biosecurity programme everything needs to be covered. A farm`s biosecurity 'is only as good as its weakest point' (Thorp, 2003 p. 101).

Individual perceptions vary as to what constitutes a risk. It may be that individuals place more emphasis on certain procedures believed to be more critical than others. The cost of implementing and upgrading the farm to improve biosecurity may be a contributing factor in these decisions. Unfortunately, the lack of investment in biosecurity from a financially stretched industry could have deleterious consequences at the herd and national level (Robertson et al., 2004). There is a higher probability that a herd will test Salmonella positive if the biosecurity measures of the herd are poor (Baptista et al., 2010).

It is reported that calls have been made to see if key areas of biosecurity can be targeted instead (Robertson et al., 2004). The danger of this approach is that a single area not targeted may allow a significant ingress of disease.

2.3.2 Antimicrobials

For many years antimicrobial growth promoters (AGPs) were used to improve the growth of pigs. The AGP activity alters the composition of intestinal microflora, preventing the proliferation of pathogens and leading to enhanced growth (Laine et al., 2004). Concerns over resistance to antimicrobials led to an EU ban on all AGPs in food producing animals from 1st January 2006 (VMD, 2005). However, antimicrobials played an important part in pig health and only since the banning of AGPs has it become clear just how much of an important prophylactic role these drugs played (Casewell et al., 2003).

The removal of AGPs has been linked to increased incidences of diarrhoea, weight loss and mortality due to *Escherichia coli* and *Lawsonia intracellularis* in post-weaned pigs (Casewell et al., 2003). Following the 1986 ban of AGPs, Sweden saw significant enteric problems emerge in piglet production, resulting in a subsequent four year increase in the use of therapeutic antimicrobials in 75% of farms to overcome problems (Wierup, 2001).

Figures published by the Veterinary Medicines Directorate (VMD) show that total sales of antimicrobials within the UK have been relatively static between 1998-2003, although with a lower percentage of market share than in previous years (VMD, 2005). Between 1998 – 2001 antimicrobials for pigs accounted for 24% of the total market sales for food producing animals (VMD, 2003). Prior to the ban in 2005, the largest quantity of antimicrobials was sold for use in pigs. However, this was far lower than in previous years at only 14% of the market (VMD, 2006). Table 2.1 displays the quantity of antimicrobials sold for pigs in from 2005 to 2009.

Table 2. 1 Sales of therapeutic antimicrobials for pigs only

	2005	2006	2007	2008	2009
Quantity ('000 tons)*	56	71	66	62	62

* = Tonnes of active ingredient. (Source: VMD, 2010).

Since the 2006 ban, sales of antimicrobials for pigs have risen. In 2006, an increase of 15 tonnes (to 71 tonnes of active ingredient) was sold compared to 2005. Since 2006, this

has fallen, but the quantity sold is still above the levels in use before the ban. The 2010 VMD report states the largest single species sales of antimicrobials is still for use in pigs, now with over 18% of the market (VMD, 2010). The quantity of antimicrobials sold in 2009 for did not differ from the quantity sold in 2008. In 2009 there was also increasing swine dysentery in pigs (VMD, 2010).

Growth improvements from antibiotic usage indicate that there is disease challenge present within the pigs' environment. The experiences of Sweden demonstrate that, with improvements to disease management, in-feed antibiotics can be reduced (Wierup, 2001). Indeed, antibiotics are found to have little effect on healthy animals reared in biosecure conditions and fed an adequate diet (Taylor, 1999, Van Lunen, 2003). Farmers appear to be aware of this also, with surveys indicating that those farmers who believed their farm environment could be improved were significantly more likely to use in-feed antimicrobials for their growers and finishers (Stevens et al., 2007). The issues relating to why farmers are choosing to use antimicrobials rather than addressing the environmental problems should be investigated further. However, much is undoubtedly attributable to the decline of the UK pig industry and the situation, in recent years, of poor competitive status (Burch, 2005). Reducing the use of antimicrobial drugs in livestock production is an objective of the VMD. In order to achieve this in UK production, better control over health management and housing improvements are required.

2.3.3 Vaccination

Vaccination involves the administration of antigenic materials to an individual, causing a natural immune response to develop (Hurst and Murphy, 1996). Vaccination is regarded as the preventative measure of choice (Done and Burch, 2000), and has been successful in offering producers control over a range of porcine diseases. For respiratory disease vaccination offers excellent protection, reducing the extent of clinical disease and prevalence of lesions at slaughter (Done and Burch, 2000, Maes et al., 1999). Vaccinating pigs against *M. hyo* has resulted in improvements in growth rate and feed intake (Scheidt et al., 1994). Done and Burch (2000) report lower circulating levels of cytokines in vaccinated pigs, a consequence of reduced immune challenge, offering a plausible explanation for the improved growth and feed intake seen by Scheidt et al. (1994). Whilst some trials have found no effect of *M. hyo* vaccines on FCR (see Scheidt et al., 1994), others trials have reported a benefit (Maes et al., 1999). Yet, testing the efficacy of *M. hyo*

vaccination in Continuous Flow (CF) and All-in/All-out (AIAO) facilities, Diekman et al., (1999) found vaccinating did not reduce the prevalence of lung lesions or influence growth rate in either environment; the actual production method, rather than the vaccine, having a more pronounced effect.

Vaccination of sows rather than the piglets can offer protection from diseases via the passive immunity conferred by antibodies produced in the milk. However, for some diseases, for example PRRSv, better protection against the respiratory manifestation of the disease in fatteners is offered by vaccinating the piglets rather than the sows (Kritas et al., 2007), resulting in reductions in morbidity and mortality (Mavromatis et al., 1999). Therefore, careful thought needs to be given to a targeted vaccination programme.

Despite the benefits of vaccination, it should not be relied upon as a method of disease control. It takes time to develop a vaccine and, as has been the case with a number of the emerging diseases (those involved in PMWS for example), identifying the pathogens responsible presents a challenge and further delays the process. In addition, a limit will be reached to the number of diseases that vaccination can protect against, emphasising the need for other protective measures to be constantly researched and developed. Furthermore, vaccination will not always offer the expected protection, for immunity acquired through vaccination can wane over time, allowing individuals to become susceptible to the target pathogen once more (Hurst and Murphy, 1996). Therefore, vaccination should be used where appropriate, but cannot be solely relied upon.

2.3.4 Depopulating

Establishing a herd with high health status is a very good approach to achieving excellent control over disease problems, and should be considered as a first step in disease management (Reeves, 2006). Conducting a total de-stock and re-stock involves depopulating all the existing stock, a thorough cleaning of the whole site and repopulating with breeding stock obtained from herd of known high health status. Producers who have multiple, economically costly diseases present in the herd may choose to take this option as a lasting solution to the problem of disease on their unit (Harris, 1988). In carrying out a full herd de-stock/re-stock, Kingston (2004) estimates producers can expect to see improvements in the finisher herd of a 65-85% reduction in mortality, a 70-90% reduction in veterinary drug usage and a 30-40% increase in daily liveweight gain. Producers must

be able to afford the expenditure, whilst a return on investment is to be expected between 14-26 months (Kingston, 2004). Once high health status is achieved, the second stage is to maintain it, and this presents a far greater challenge to producers (Reeves, 2006).

For this reason it is important to understand the real value of establishing a high health herd in the context of how well it can be maintained. A de-stock/re-stock is a pointless exercise if the farm is poorly isolated from other high disease herds (Harris, 1988). If this is the case, the producer can only opt for other methods of health management to improve the situation. Alternatives are available to eliminate, avoid or control disease at a level that is both manageable and causes minimum disruption to production. These are beneficial for they are effective at a reasonable cost, which is returned in production outcomes (Harris, 1988).

2.3.5 Alterations to production

The type of production system in place on a farm will have a significant impact on the health of the pigs (Kingston, 1999). Poor pig flow is a major cause of disease on pig farms and understanding the principles of manipulating pig flow is an essential element of pig health management (Carr, 1999a). As our knowledge of disease transmission has improved, different methods of production have evolved to minimise the spread and transition of disease, and altogether lower the immune challenge on the pigs. Adopting a production system that minimises the spread of disease is of particular importance for producers that have specialised into raising fattening pigs, where a variety of pigs of different ages may be maintained within one farm.

2.3.5.1 Continuous flow production

The continuous flow (CF) system is worth looking at first as this system of production operates with no measures taken to separate pigs of differing age groups into separate airspaces, and presents the highest immune challenge to growing pigs (Kingston, 1999). This system has the effect of 'recycling' the disease within a herd. Younger pigs entering the CF system develop the endemic diseases of the herd as their colostral antibodies dwindle, and they in turn transfer the disease on to groups of younger pigs introduced into the same airspace (Kingston, 1999). There are obvious flaws in a CF system, and reviews of on-farm risk factors for disease have exposed CF production as a risk factor for the transmission of disease, in particular respiratory disease (Done, 1991). Yet despite this,

CF production remains fairly common in British pig farms principally as a result of many British producers still farming with old buildings produced when CF was a common and acceptable method of pig production. The increased intensity of pig farming and tight profit margins are making alterations to production a necessity in order to regain control over endemic production diseases within herds of growing pigs.

2.3.5.2 All-in-all-out production

All-in-all-out (AIAO) production addresses all the problems that CF brings, and has been described as an essential addition to modern pig farming (Done, 1999). Correctly performed, AIAO production requires all pigs born in one room or building within the same week of farrowing, to be weaned together and housed in a separate room and airspace to adjoining weeks or batches. Pigs are then moved up to grower and finisher accommodation on the same basis (Kingston, 2004). The separation of batches into separate airspaces removes direct contact and largely prevents indirect contact with older pigs, reducing the transmission of disease from older to younger pigs (Dolcic and Bilkei, 2001). Should an outbreak of disease occur within a batch, this system confers protective benefits by containing the disease within one batch of pigs. For maximum benefits, AIAO production should operate a thorough cleaning and disinfecting routine in between batches of pigs.

The AIAO system is reported to offer producers surrounded by farms of poor health status a reasonably low cost and effective way to manage and control a number of common health problems (Kingston, 1999). It has been found to have direct benefits in controlling respiratory disease within a herd (Done and Burch, 2000), with strict AIAO production found to be favourable for low incidence of pneumonia and pleurisy (Bäckström and Håkan, 1978). Under controlled conditions, using a herd with a history of mycoplasma and pasturella pneumonia, Scheidt et al., (1995), demonstrated that adopting AIAO could reduce the incidence of respiratory disease . No clinical signs were seen in AIAO housed pigs, compared to 43% of CF pigs exhibiting clinical signs, and a 54% lower prevalence of lung lesions was detected at slaughter in pigs housed in the AIAO system compared to those in the CF system. Although Diekman et al., (1999) found vaccinating for *M. hyo* had no effect on prevalence of lung lesions in pigs housed in AIAO and CF systems, AIAO production is reported to improve the efficiency of vaccines (Done, 2001b).

A well managed AIAO system can confer indirect health benefits to pigs via a reduction in stress from fewer pig movements, reduced mixing (Done, 2005), and a greater control over optimum temperature ranges for individual batches. AIAO has been found to improve daily gain and FCR, and reduce days to slaughter (Scheidt et al., 1995). However, the full success of these improvements is also dependent on the genetics, nutrition and management of the herd being optimal (Scheidt et al., 1995). Done correctly, the effect of AIAO is reported to be so great that therapeutic health treatments need only be administered when necessary and not as a routine preventative measure, as is commonly employed on many farms (Taylor, 1999).

Despite the advantages of AIAO systems, it was estimated in 2000 that only 25% of UK producers were operating on AIAO production (Done and Burch, 2000). It is recorded that vets have been encouraging producers to adopt AIAO production (Done and Burch, 2000). Practicing vet Kingston (2004) claims that a number of farmers who have tried conversion to AIAO could not justify the health and performance benefits against the extra time spent cleaning and disinfecting and managing a stricter farrowing schedule. However, it is likely that where no significant benefit in AIAO production has been achieved, other confounding factors are hindering performance (Scheidt et al., 1995). Building configuration is reported as a confounding factor (see Kingston, 1999), where poorly situated buildings are at risk from influx of exhaust ventilation from nearby pig buildings. With respiratory disease transmissible on the wind between farms it seems entirely plausible that disease around individual farms can be spread by this method also, and producers considering a conversion to AIAO should consider this risk.

With the above in mind, it is also logical to assume that producers operating AIAO production on a room basis should also be operating a strict closed door policy when entering each room, and working around the farm from youngest growing pig to oldest. Failure to do so will only result in a falling short of achieving the separate airspaces between batches and possible spread of pathogens on boots and clothing. This sort of attention to detail in the day to day management of the farm is reported as overlooked by some farmers and is believed to be a reason why some producers may not get the full benefits returned on adoption of an AIAO management strategy. Speaking of his experiences, Carr (1999b, pp 39) states “many pig farmers claim to do ‘all-in/all-out,’ but

when examined in any detail are found to only give it lip service at best." It is evident that to receive the true benefits of a good AIAO system it must be implemented with diligence, attention to detail and meticulous planning or the time and effort involved is wasted.

2.3.5.3 Batch management

Batch management and AIAO fundamentally operate on the same principle (Kingston, 2004), although in literature batch management often refers to systems where batches of pigs are born with a longer time-scale between batches. The time-scale of farrowing will depend upon the availability of space and the size of the sow herd. Increasing the time-scale between batches confers further health benefits, reflected in performance improvements, lowered mortality and lower drug costs of the two herds demonstrated in Table 2.1.

Table 2. 2 Weekly Vs three weekly batch production

	Weaning Interval		
	Weekly	3 Weekly	Improvement (%)
Gain/day (g)	490	547	12
FCR	2.36	2.26	4
% Mortality (6-90kg)	11.5	6.6	43
Medication (£/pig)	2.19	0.31	48

(Source: Kingston, 2004).

Typically, for an AIAO production system to work efficiently, a sufficiently large number of pigs must be produced to fill rooms and buildings, depending on the system adopted (Richardson, 1999). Planning is critical. Poor planning and a failure to follow a pre-determined strategy has been found to result in the collapse of the system (Hawe, 2001). Where producers are struggling to maintain strict AIAO policies on a weekly batch system, converting to a three week system is a suitable alternative solution (Richardson, 1999). Not only does the system allow producers with smaller herds to produce sufficient numbers of pigs in a batch, it also offers time management benefits, with the bulk of the

work spread each week: a farrowing week, a serving week and a weaning week (Hawe, 2001). This arrangement also allows sufficient time to wash and disinfect the buildings and allow a sufficient downtime between batches of pigs, helping improve disease control (Hawe, 2001). Converting will have its costs, as typically adopting a three week batch system requires extra building space, commonly either an extra weeks farrowing space and/or weaning space (Kingston, 2004). This is likely to be a deterrent to many producers who might consider adopting the system.

2.3.5.4 Segregated early weaning

Segregated early weaning (SEW), or alternatively Isowean, is a disease management practice involving the removal of the piglets from the sow whilst the maternal antibodies are still high (Fangman et al., 1996a) and housing in an area that is physically isolated from the breeding herd (Patience et al., 2000). The theory behind this practice is that the maternally derived passive immunity will prevent vertical transfer of indigenous pathogens, whilst the physical act of removing the pigs to another site for continued production reduces the exposure of pigs to the endemic pathogens of the herd of origin (Fangman and Tubbs, 1997).

SEW typically involves piglets being weaned at ≤ 21 days of age (Fangman and Tubbs, 1997) and, compared to other methods, proves as effective as medication and vaccination protocols for controlling the transmission of pathogens from dams to piglets at weaning (Clark et al., 1994). It is also reported as being a good alternative for resolving major disease breakdowns, providing an alternative for conducting a de-stock/re-stock (Harris and Alexander, 1999).

Investigations such as that carried out by Clark et al., (1994) have found that control over different diseases can be achieved by varying the age at which pigs are weaned. As suggested by Harris (1993, cited in Fangman and Tubbs, 1997), a selection of important porcine diseases and the respective weaning age for control are presented in table 2.2. The different weaning ages represent a safe time period before the disease is transferred from the dam to the offspring (Done and Burch, 2000).

Table 2. 3 Diseases and respective weaning age for control

Organism	Weaning Age
Pseudorabies virus	< 21 days
Actinobacillus pleuropneumoniae (APP)	< 21 days
Mycoplasma hyopneumoniae (M.hyo)	<10 days
Pasteurella multocida (P.multocida)	<10 days
Haemophilus parasuis (HPS)	<14 days
Porcine reproductive and respiratory syndrome virus (PRRSv)	<10 days
Salmonella cholerasuis	<12 days
Transmissible gastroenteritis virus (TGEV)	<21 days

SEW can take place in two-site and three-site systems and provides the basis on which multi-site systems of production developed (Harris and Alexander, 1999). The differences are summarised by Fangman and Tubbs (1997):

- Two-site production: breeding and farrowing facilities separated from the nursery and finishing facilities.
- Three-site facilities: first site containing breeding and farrowing facilities. Second site comprising the nursery stage. At around 23kg, grower pigs moved to a third site for finishing.
- Multi-site: Several sites used for breeding and farrowing. Weaned pigs are transported to a common separate nursery, and later on to a separate finishing site.

There is no point performing SEW if the pigs cannot be grown up to finishing away from the main production site. If returned to the original site production decreases again, as demonstrated by Kingston (1999, pg 89). The overall success of these systems is highly dependent on maintaining a strict AIAO pig flow (Done and Burch, 2000).

Studies focus not only on disease prevention, but how SEW can be production enhancing. Primarily these studies do so by comparing a variety of weaning ages for off-site and on-site production. Improvements are reported for daily gain, FCR and daily feed intake from implementation of the SEW system (Fangman et al., 1996b). The production improvements are believed to occur as a result of less energy expenditure by the pigs in fighting infection and antigen challenge (Harris and Alexander, 1999).

Inconsistent results are a common finding between field trials (see Fangman et al., 1996a; Fangman et al., 1996b), and are likely to be, in part, accountable for by uncontrolled environmental variables such as differences in management, building design, feed, group size etc. The research conducted by Patience et al., (2000) recognised and addressed these factors accordingly and found SEW pigs gained 73g and 75g more growth per day compared to early-weaned pigs housed on site (OSEW) and control pigs weaned at 21 (± 3 days) of age, respectively. In addition, feed intake was higher for the SEW pigs, consuming 52g/day and 72g/day more feed than the OSEW and control pigs. This equated to a 9% improved feed conversion efficiency for SEW pigs compared to OSEW pigs.

Weaning age also appears to influence the performance parameters, with those earlier weaned pigs (11-14 days) having reduced growth, FCR and daily feed intake (Fangman et al., 1996a, Fangman et al., 1996b). However, a deficiency in these two studies is that the performance of pigs was not followed beyond 6 weeks post weaning, providing no information as to whether the reduced performance seen in the younger weaned pigs remained so through to slaughter weight. This information is important. The balance between production benefits gained though disease control and any potential performance lost as a result of early weaning should be investigated. Patience et al., (2000) found piglets weaned at 12 days of age had much better production parameters than those studied by Fangman et al. (1996a and b). Interestingly Drum et al., (1998) found SEW pigs only to have improved growth in the nursery phase. This 'head-start' conferred no benefits through in the finishing phase, with SEW and conventionally weaned pigs reaching slaughter weight in an equal number of days.

There is little available information in literature pertaining to the number of pig production systems operating SEW production within the UK, although Harris and Alexander (1999) report that multiple site production systems operating the isowean principle are being successfully utilised in the UK. A working example of UK multi-site pig production is provided by Willox (2001), reporting on Grampian contract farms.

2.3.5.5 Medicated early weaning

Medicated early weaning (MEW) is an extension of SEW, adding an additional safeguard against the spread of infection. MEW involves sows being farrowed in isolation, medicated both before and after weaning. Piglets medicated during the suckling period and for 10 days after weaning (at 5 days of age) onto isolated sites (Done and Burch, 2000). Medicating sows before and after weaning, and their piglets from birth, is believed to further reduce the excretion rate of viable infectious organisms and the susceptibility of the piglets to infection (Alexander et al., 1980). A variation of this system, called modified medicated early weaning (MMEW) involves sows not being farrowed in isolation and variable early weaning ages depending upon the pathogen to be eliminated (Done and Burch, 2000).

The practice of MEW is not used on a regular commercial basis, and its application is far better tailored towards the production of pigs for breeding herds (Harris and Alexander, 1999). When performed well, the practice of SEW is equally effective for disease control (Clark et al., 1994). Yet there will be times when the periodic implementation of MEW offers a solution for producers to gain control and eradicate diseases endemic within the herd, offering a cost effective regime for improving health and performance in the slaughter generation (Dee, 1994).

2.3.6 Cleaning and disinfecting

Cleaning and disinfection is regarded as one of the most important disease control strategies. Contaminated buildings can harbour infectious agents over long periods of time, continuing to spread infection to new animals in a disease cycle (Quinn and Markey, 2001). Effective disinfection breaks this cycle and removes, or at the very least reduces, the exposure of new batches of pigs to the pathogens of their predecessors (Bowman et al., 1996). Pearce (1999) found disinfecting to be the only husbandry practice that is significantly protective against scour in piglets, whilst application of disinfection through fogging within a building can assist in controlling respiratory disease spread (Waddilove, 2008).

The benefits of disinfection extend further than just pathogen control, and serve to reduce the total antigenic challenge within the pigs' environment (Waddilove and Blackwell, 1997). This reduction in challenge is reflected in production benefits; pigs housed in

cleaned pens displaying 6-14% improvements in growth rate as opposed to those housed in unwashed pens (Cargill and Banhazi, 1996).

Yet for these benefits to be realised, one point is consistently re-stated throughout literature; the need to follow the working steps of a correct disinfection procedure. Devised by the (WHO, 1994), these are as follows:

i) The removal of solid muck and dry matter
ii) The application of a pre-cleanser (de-greaser)
iii) Powerwashing the pen clean
iv) Application of disinfection
v) Drying the building before re-stocking.

These steps are mandatory. Should one step be skipped or carried out poorly, the effectiveness of the disinfection routine is reduced. The failure to remove all organic matter prior to application of the disinfection has a major effect of reducing the efficiency of the disinfection (Thompson et al., 2007). Foot dips have also been found to be ineffective at disinfecting boots, unless scrubbing of the boots prior to or whilst standing in the disinfectant has been carried out to remove all organic matter (Amass et al., 2000). Similarly, failure to completely dry a building prior to replacing pigs is equally detrimental, the wet surfaces providing an ideal environment for bacteria to proliferate.

With this emerging evidence of the rigorous cleaning routine required, there is concern within the industry as to whether producers are implementing effective routines. Of 14 farms surveyed for the effectiveness of their disinfection routines, only one employed the use of a pre-cleanser, and four did not follow up washing with a disinfectant (Mannion et al., 2007). All 14 farms surveyed used a cold pressure wash and no benefits will have been conferred from heat acting to kill microbes. Yet, of rather more concern, Pearce (1999) found only 40% of pig producers employed the use of disinfectant anywhere on their unit. It can only be assumed that lack of time and labour, tight deadlines between moving batches, working with older, less efficient buildings and desires to cut costs are factors that could be contributing to producers not cleaning effectively.

It is imperative that any disease control programme is feasible (Quinn and Markey, 2001). The issue that stands out from these surveys is that farmers are unlikely to be employing adequate disinfection control strategies and, if this is due to a real or perceived lack of time, perhaps steps could be taken to devise suitably less labour intensive cleaning regimes. However, the microbial content of an animal house is so great that, even with the five working steps fully implemented, 10^3 cfu/cm^2 of bacteria will still remain on surfaces following disinfection (Böhm, 1998). Therefore it seems unlikely that a reduction in the cleaning routine to aid time management would still bring worthwhile improvements. Adopting a production system that allows sufficient time for cleaning each building is necessary for cleaning and disinfection to be carried out fully.

Whilst there are many articles available from disinfection system manufacturers demonstrating the cost effectiveness of employing a good routine, there is no evidence available of the amount of money wasted in labour, water and consumables by carrying out a poor disinfection routine. This would be a useful analysis to perform.

2.4 Monitoring health

Monitoring health is the key to being able to make adjustments and gain improvements. There is a range of methods available for monitoring health status at both the individual animal and at herd level. This summary outlines a number of the techniques by which health can be monitored and disease diagnosed.

2.4.1 Direct observation

On farm observation of animals is perhaps the simplest and most basic level of health monitoring, but is also the most easily accessible (Friendship, 2005). It is an important measure, especially when combined with other diagnostic techniques. The prevalence of coughing, diarrhoea, uneven size in groups of pigs, lameness and superficial wounds, as well as symptoms of specific conditions can be assessed visually at both pen and herd level. To do this successfully the observer must have the time to observe the animals properly and also have good knowledge of the signs of specific conditions. Pitfalls of this method are that it takes time to carry out thoroughly and that significant observations can easily be overlooked by farmers who are short of time or working in poor light.

Furthermore, certain conditions may not be rapidly detected, or acted upon, because of the differing perceptions of the observers.

A system for recording coughing has been tested and suggested as an objective and inexpensive method to assess the prevalence of respiratory disease within a herd (Morris et al., 1995). It is not known whether any producers actually utilise this technique systematically on farm to assess respiratory disease in growing pigs, although the EU Welfare Quality project, which has developed the first EU protocols for assessing farm animal welfare, has included recording coughing as one method for assessing the respiratory health of growing pigs (Welfare Quality, 2009).

With regard to controlling infectious disease, when clinical signs are apparent the disease will most likely have already spread to other pigs in the immediate vicinity. Infectious disease present and persisting at a sub-clinical level cannot be visually identified, although growth performance records would be able to inform that the pigs were underperforming. The precise targeting of persistent sub-clinical disease requires a more sensitive approach.

2.4.2 Slaughter checks

Monitoring of pig carcases at slaughter is very useful as a diagnostic aid in determining pig health at herd level. Before the development of reliable lab tests this was the most effective method to monitor health in herds (Friendship, 2005). Visual inspection of the organs of a pig can allow the diagnosis of sub-clinical conditions such as enzootic pneumonia, parasitic infestations and rhinitis (Pointon et al., 1999). Organs are assessed by scoring them against a scale. For accurate assessment it is recommended that palpation of the tissues and organs is done, since visual- only scoring of lesions has been found to give an increased risk of misclassification (Pointon et al., 1999). Scores of organs at slaughter can provide useful information for the farmer on areas of management that may be detrimental to the health and welfare of the pigs. Yet the extent of information to be derived from slaughter check is limited because lesions can heal over time. This results in the carcase lacking representation of the health status of the pig in the earlier stages of production (Friendship, 2005). For these results to be interpreted in full they should be combined with clinical observations by farmers and production records, including indices of outbreaks of clinical signs of disease (Pointon et al., 1999).

2.4.3 Automated monitoring systems – indirect observation

The automated monitoring of livestock offers a continuous system of objective observation 24 hours a day, seven days a week. A list of the different applications of automated monitoring systems for livestock is provided by De Vries and Reneau (2010). For growing pigs raised indoors, systems are commercially available to assist the producer in optimising the environmental control of the building, providing continuous logging of temperature and ventilation, and alerting producers of a change in conditions which could ultimately lead to an increased chance of disease. Automated recording of livestock behaviour is already utilised within the dairy industry (Roelofs et al., 2005). It is standard practice in the poultry industry where it is utilised for the detection of disease, and is recommended for such a purpose in the code of recommendations for the welfare of chickens (DEFRA, 2002). The automated monitoring of water consumption in piglets has shown a change in consumption occurring one day before the outbreak of clinical symptoms of scour (Madsen and Kristensen, 2005), and thus could potentially offer a lead indicator of health changes to follow. A system for the automated recording of water intake has been developed for pigs by the farm systems and energy control services company, Farmex (Crabtree et al., 2008). However, there is relatively little scientific research in literature utilising the automated monitoring of water consumption in pigs, and more should be conducted before advice is given to producers on how changes in water consumption may relate to disease occurrence.

Q-scan, the real-time capture of growth data by visual imaging, developed by Silsoe Research Institute (White et al., 2004) and now marketed in the UK by Innovent, could be utilised for detecting sub-optimal growth within pens of pigs, and where within the system it is occurring. For use in monitoring health changes, research into exactly how quickly the system could alert to a change in growth in relation to the time course of the disease requires investigation. It may be that the use of water data, could be significant for the predictive monitoring of performance in pigs.

Once up and running, a benefit of automated monitoring systems is that they can provide immediate signals to producers, enabling them to investigate potential problems. For this to be achieved, it is important we are knowledgeable and confident as to what signals the pigs are relaying to us.

2.4.4 Diagnostics

Diagnostic tests are used principally by veterinarians to assess the health status of a population of pigs. Such tests can be used to: i) detect pathogens or toxins responsible for disease outbreak and the herd health status; ii) evaluate the infection and exposure status of pigs; iii) determine which sub-groups of pigs within a herd are affected by a particular pathogen/pathogens within the herd; iv) estimate the percentage of the herd with antibodies to specific pathogens; v) monitor the serologic response of the herd to vaccination; vi) monitor the progress and success of disease control and eradication programmes (Gardner and Blanchard, 1999). However, in evaluating herd health in general no one test provides the definitive answer, and the results should always be analysed in the context of additional environmental and production parameters.

There is a wide range of diagnostic tests available, allowing detection of specific pathogens, antibodies, antibiotic resistance of bacteria, and measurement of a selection of biomarkers such as cytokines and APPs. In addition, measurements can be taken from a large range of samples; whole blood, serum, OF (saliva), faeces, urine, meat juice and tissue samples. The choice of sample and diagnostic test to use will depend on the question to be answered, with consideration given to the cost and speed, and also test accuracy (Gardner and Blanchard, 1999).

The precision and standardisation of assays is of particular importance to livestock health control, with large number of animals being transported between farms and countries before entering the human food chain.

An increased emphasis is now being placed on tests that can utilise easily obtainable biological fluid saving time and labour and reducing the stress to the animals involved. The use of a biosecure method assists in the protection of herd health. Samples collected on farm by the producer, such as pooled OF (Prickett et al., 2008a) and colostrum (Nielsen, 1995) and at slaughter (Snary et al., 2010) are three such methods.

One problem of some diagnostic tests is that their application at the level of monitoring required for control of certain diseases can be cost-prohibitive (Prickett et al., 2008b). The use of bulk samples is one way to reduce laboratory costs, accounting for a large number

of individuals in few samples. Reduced laboratory costs also provide for increased frequency of sampling if required, which can often lead to a greater awareness of the disease dynamics within the herd. Bulk sampling of milk is successfully employed by the dairy industry for the detection of mastitis (Madouasse et al., 2010), bovine viral diarrhoea virus, bovine herpesvirus type 1 and Leptospira Hardjo using antibodies (Bishop et al., 2010), and for the monitoring of liver fluke and treatment success (Duscher et al., 2011). For the pig industry, the use of pooled OF could provide a diagnostic medium for the rapid detection of a large population of animals at relatively little cost, and potentially cover a larger number of animals in the sampling process. In addition, it fulfils the desire for non-invasive diagnostic tools. Pooled OF has been collected from a single length of cotton rope presented to groups of pigs. This method has been used successfully to detect the presence of PRRSv and PCV-2 infections in groups of pigs (Prickett et al., 2008b), PCV-2 antibodies (Prickett et al., 2011) and swine influenza virus (Detmer et al., 2011). However, more work is required in order to expand the number of diseases to be screened in porcine OF. A review of the application of OF based diagnostics in veterinary medicine suggests great potential for a range of diseases to be detected (see Prickett and Zimmerman, 2010).

All sampling methods have their pros and cons and these must be weighted when deciding on an appropriate test to use. Collection of samples at slaughter, provides data on the overall health of the pig herd, but with the pigs already killed, such historic data will not provide information on disease dynamics within the farm as it occurs. The ability to collect OF on farm whilst a group of pigs are still alive offers a system to monitor the changing disease dynamics of the farm as it occurs within a sub-population of the farm, thus enabling the producer to address issues while the disease challenge is occurring.

The use of biomarkers in assisting diagnosis of health conditions is an increasingly important area. Screening humans for levels of various APPs has allowed doctors to predict the susceptibility of individuals to specific health complications. CRP has been used to assess the absolute risk of individuals to coronary heart disease (Pearson et al., 2003), and diabetes (Freeman et al., 2002). The success of this line of research has led to recommendations being made for screening of populations, allowing those at risk to receive medical treatment in advance of adverse health risks (Pearson et al., 2003).

This potential is still being realised for animal medicine (Eckersall and Bell, 2010, Shirazi-Beheshtiha et al., 2011, Petersen et al., 2004). As APPs mirror the action of cytokines, their relevance to pig health monitoring is as a potential marker of herd health, offering an opportunity to classify the severity of immune activation posed by sub-optimal conditions (Petersen et al., 2004), especially where sub-clinical disease may be present. As components of the innate immune system, both cytokines and APPs have the potential to act as early markers of disease in veterinary medicine. As the response is closely related to disease progression and recovery (Eckersall, 2004), monitoring the rise and fall of APPs can provide a non-specific tool for determining the health status of individuals. In human medicine APPs have become the chosen biomarkers for monitoring inflammation and infection (Eckersall, 2004). In comparison to cytokines, the longer duration of the APP response is likely to have contributed to their favour. Yet the magnitude of the APP response is believed to be representative of the action of cytokines (Sorensen et al., 2006), and therefore measurement of APPs does provide the ability to simultaneously characterise the cytokine response.

The sensitivity and specificity of the acute phase response can be enhanced by combining the values of positive APPs with those of negative APPs in the acute phase index (API), (Toussaint et al., 1995, cited in Gruys et al., 2005). This technique is able to enhance the detection of unhealthy animals in a population (Toussaint et al., 2004), and this method is often recommended for it allows the detection of chronic as well as acute conditions of disease (Eckersall, 2004). In the paper referenced, Toussaint et al., (2004) used with success the combination of CRP and Hp (positive), and Albumin and Vitamin A, representing retinol binding protein, (negative). Yet to current knowledge, all studies using this method have only applied tests on an individual animal basis (see Toussaint et al., 1995, Toussaint et al., 2004). The feasibility of this method when applied to overall herd health must be investigated.

As one of the first responses of the immune system APPs could be utilised for the early detection of disease, as has been demonstrated (Harding et al., 1997). Upon chronic immune activation APPs continue to be secreted although, as the disease progresses, the relative protein concentrations change (Eckersall, 2004). Being able to characterise this could provide a useful tool for pig health monitoring. APPs can be measured in a variety of body fluids: CRP, Pig-MAP, SAA and Hp have been reliably measured in blood serum

(Parra et al., 2006), Hp in plasma, whole blood and meat juice (Hiss et al., 2003) and CRP and Hp in OF (Gutiérrez et al., 2009b, Gutiérrez et al., 2009a). Before APPs can be employed as biological markers of health, extensive studies are required to provide reference values on the concentration of these proteins in both healthy and sick animals (Piñeiro et al., 2007). The measurement of APPs in OF would provide a low stress method of sample collection. The concentration of APPs in pooled sample of OF has not been explored. However, if this methodology was possible, the reduction in samples could significantly reduce costs.

Hp, one of the most extensively tested APPs, is thought best to reflect the pathological state of an individual (Parra et al., 2006) increasing in concentration in response to bacterial, viral and inflammatory infection in pigs showing clinical signs of the disease and those not (see Parra et al., 2006). Levels of Hp and CRP have been found to be positively correlated in both clinically healthy and diseased pigs (Chen et al., 2003), and in healthy pigs so too have levels of Pig-MAP and Hp (Piñeiro et al., 2007), demonstrating that some APPs respond in a parallel manner to certain conditions.

The use of APPs has been incorporated into rapid point-of-care tests. These rapid diagnostics are widely utilised within the human health care industry providing a medical test that can inform at or near the point of care of the patient. This area is developing rapidly in analytical scope and clinical application (Luppa et al., 2011). In providing a rapid diagnosis, point-of-care testing allows quicker decisions to be made regarding treatments and interventions (Wilkins, 2011). For pigs, rapid tests have a potential for use as pen-side diagnostics, which could be of great assistance in times of disease outbreak and emergency. In addition, the use of such tests at the abattoir could provide an immediate assessment on the suitability of the carcase for human consumption. Currently on the market is the PigMAP stick®, (PigCHAMP Pro Europa S.A., Segovia, Spain), a one-step immunocromatographic method for the detection of elevated levels of Pig-MAP concentration in serum and whole blood. This method is proposed to be used for the rapid assessment of pig health whilst on farm and at slaughter (Barrios et al., 2005).

2.7 Conclusion

Environment and management factors play a central role in the development of infectious disease within pig herds, and will influence the extent to which within-herd disease will affect the productivity. The complex, additive and synergistic interactions of environmental factors on the pig can be the divide between an infected farm having an actual clinical breakdown in disease or not. Effective and timely observation of pigs will provide information on how they are coping. The use of automated monitoring systems allows an additional, continuous and non-subjective level of observation that can also be used to keep historic records on the performance and environment of the pig at very low labour cost. Such systems could be of particular use for large group sizes. Diagnostic tests are an essential part of pig health monitoring to identify and quantify disease and challenge facing pigs. With the current financial pressures on the UK industry, disease needs to be reduced within the UK herd. But a system of diagnostic tests that could utilise a pooled fluid sample could offer a viable lower cost option of obtaining biological fluid for diagnostic testing.

It is the subject of this thesis to investigate a series of technologies and tools that could assist producers in improved disease monitoring, detection and management strategies for the promotion of improved health in finishing pigs.

Chapter 3: Use of disinfection in two pig finishing systems: effects on air quality, pig health and performance

3.1 Introduction

Measures to safeguard animal health are of great importance, especially the application of strict hygiene programmes in preventative and protective roles (Hartung, 2005). Cleaning and disinfection is regarded as one of the most important and effective protective methods of disease control. Contaminated buildings can harbour infectious agents over long periods of time, continuing to spread infection to new animals in a disease cycle (Quinn and Markey, 2001). Effective disinfection breaks this cycle by stopping or reducing the exposure of new batches of pigs to the pathogens of their predecessors (Bowman et al., 1996). Pearce (1999) found disinfecting to be the only husbandry practice that was significantly protective against scour in piglets, whilst Cargill and Banhazi (1996) found pigs housed in disinfected buildings had improved growth rates in comparison to those housed in unclean housing.

Aerial transmission of disease is a particular problem in animal housing systems (Wathes, 1994). Aside from the dangers of infection by specific porcine pathogens, levels of aerial contaminants such as dust and viable non-specific bacteria are often the cause of respiratory conditions in pigs, such as bronchitis, increased coughing and lung lesions (Gonyou et al., 1999). Cleaning a building prior to a batch entering reduces levels of total dust, respirable dust and total airborne bacteria (Cargill and Banhazi, 1996), although only for a period of time. It seems that the benefits of disinfection could stretch much further than surface hygiene. Furthermore, (Magwood and Marr, 1964) found a direct relationship to exist between the concentration of bacteria on surfaces and the amount of bacteria in the air of poultry houses. To date there appears to be no available information concerning the effect of disinfection on reducing aerial contaminants and on pig health.

The benefits of disinfection are greatest when used in combination with an all-in-all-out (AIAO) management system, where it is a highly effective way of breaking the disease cycle, reducing losses to disease and improving performance (Bown, 2006). AIAO is known to be superior to Continuous Flow (CF) as a method of pig production (Ice et al., 1997), and having a CF system does reduce the opportunity for buildings to be cleaned

thoroughly (Cargill and Banhazi, 1996). In suggestions for improving herd health and production, it is regularly recommended that producers should convert from a CF to an AIAO batch management system. However, this is not always a practical option. Many producers do little more than investigate cost-benefit appraisals of building refurbishment due to concerns about minimising capital costs. Therefore many producers find themselves having to cope with old building designs and reduced production until such times as they are able to alter building design to incorporate AIAO and/or construct new, purpose-built AIAO housing.

Quite separately from the presence of infectious disease, the dusts and moulds found within a dirty environment can be enough to elicit immune system activation in pigs, generating synthesis of cytokines and acute phase proteins (APPs) (Williams, 1998). Raising pigs in a dirty environment can produce a chronic immune activation which reduces growth and feed efficiency from the action of the cytokines, which repartition nutrients away from tissue deposition. Pigs raised in conditions with high infection pressure have raised APPs, reflecting the activation of an immune response, with a negative effect on the growth rate of pigs (Franek and Bilkei, 2004).

With the knowledge that disinfection can reduce health problems by reducing direct contact with pathogens and improving air quality, this piece of research was designed to investigate the potential for cleaning of pens between groups of pigs to reduce the health problems and increase pig performance in a CF system. Therefore, the objectives of this study were to explore the comparative effects of cleaning and disinfection on aerial hygiene, pig health and performance in three systems: a CF system with no disinfection, a CF system with disinfection over time and an AIAO system with disinfection. In addition, immune activation within the pigs housed in these conditions was assessed through the measurement of APPs.

3.2 Methodology

3.2.1 Experimental facility

This research was performed at the Newcastle University research farm, Cockle Park, UK, a commercially run 200 sow farrow to finish herd. Previous serological tests had confirmed that the farm had *Mycoplasma hyopneumoniae* (*M. hyo*), Porcine Reproductive and Respiratory Syndrome virus (PRRSv), *Actinobacillus pleuropneumonia* (APP) and

Porcine Circo Virus Type-2 (PCV2) actively circulating within the grower/finisher herd. In addition, *Haemophilus parasuis* was present, and active as a secondary opportunistic pathogen. All pigs were vaccinated for pneumonia (*M. hyo*) at weaning, but outbreaks of respiratory disease were seen later in the finishing stages.

Pigs were housed in a part-slatted finishing house consisting of three adjacent rooms, identical in size and layout. Rooms were equipped with separate ventilation systems with the ventilation adjusting in response to a thermostat set to maintain room temperature at 19˚C.

Each room measured 46.m^2 (7.60 x 6.10m) and contained four pens (3.07 x 3.26m). Pens were divided with solid divisions preventing nose to nose contact between pigs and had a layout comprising of a concrete lying area (3.05 x 2.49m) and a slatted dunging area (3.05 x 0.74m), over which two nipple drinkers were located. A twin space feed hopper, providing 0.30m feeding space per pig, was secured to the wall in the lying area, into which an *ad-libitum* supply of pelleted finisher pig feed (Crude protein 18.50%, Lysine 1.12%, 12.7 MJ/DE/kg), (Feedco, UK) was provided. The quantity of feed provided was recorded. Excrement was stored in a slurry pit running under the length of the building.

Prior to the start of the study, the drinker system and header tanks were cleaned and disinfected with VirkonS (Dupont Animal Health, Suffolk, UK) at the manufacturers recommended concentration of 1:200. The flow rate of all drinkers was checked and adjustments made so all drinkers ran at a flow rate of 0.9-1L/minute. The slurry pit was emptied via a suction hose and this was repeated thereafter when required (at approximately fortnightly intervals). In each room, all surfaces were swept out to remove excess dust and an industrial vacuum was used to remove dust and cobwebs from the ceiling, lighting, vents, extractor fan and temperature control boxes.

3.2.2 The animals

Three replicates ran from February – September 2008 in which a total of 432 Large White x Landrace pigs were selected between 50-55kg and allocated by weight, litter and gender to form pens of 12 pigs. Each pen was balanced for gender and initial weight (53.2 ± 2.89kg, mean ± S.D). Upon selection, all pigs were ear-tagged with an individual identity number. All experimental pigs were sourced from grower houses on the farm. The aim

was for the experimental pigs to be sourced from all-in/all-out grower rooms. However, this was not always possible and a number of pens of grower pigs had to be selected from continuous flow systems. Therefore, experimental pigs may have been infected with *M. hyo* before entering the study. However, upon selection, no pigs were exhibiting any symptoms of pneumonia (coughing, listless, laboured breathing). Pigs were sent to slaughter on an individual basis at an average weight of 90.8 ± 11.5kg (mean ± S.D), which resulted in pens being cleared over a number of weeks.

3.2.3 The treatments

Three treatments were tested, simulating different scenarios of pig production. Each system was simulated with one of the three rooms as performed in previous studies (Scheidt et al., 1995, Ice et al., 1997). The rooms were maintained as separate entities as far as possible by keeping the doors closed, and when moving between rooms closing the doors once personnel had walked through. Treatments were as follows:

Treatment one: All-in/All-out system (AIAO)

Located in room one, with the whole room cleaned, disinfected and dried prior to pigs arriving. Per replicate, all four pens were filled, with a total of 48 pigs within the same batch. One replicate ended when the whole room had cleared for slaughter; the whole room did not empty at once as it was desirable to explore the total time pigs took to reach slaughter weight. No new pigs entered the room until the whole batch had left. Following each batch the room was cleaned, disinfected and dried before refilling with the next batch.

Treatment two: Continuous flow with the use of disinfection (CFD)

Located in room two and run as a continuous flow system (CF) with pens disinfected between each batch of pigs. At the start of the experiment, the CF room was initially set up to create a natural model for disease transmission between older and younger pigs. To begin, the CFD room was seeded with 36 pigs of nine, eight and six weeks older than the experimental pigs, sourced from a CF finisher building with a history of respiratory disease caused by *M. hyo* and PRRSv. Upon selection many of the seeder pigs were exhibiting clinical signs (coughing) and were presumed to be naturally infected with *M. hyo*. It was assumed that the pneumonia would spread from the older pigs to the younger pigs, in a natural model of staggered ages to facilitate disease spread (Scheidt et al.,

1995). Upon initial room formation, the seeder pigs filled three pens in each CF room, creating staggered ages of pigs housed within one room, with a total age spread in the CF room of nine weeks. Throughout the three replicates of the study, the CF room was never empty of pigs. Upon pens clearing for slaughter, the pen was cleaned, disinfected and dried before another pen of pigs was selected and re-filled the empty pen, maintaining a population of staggered ages of pigs. This system rolled on throughout the run of three replicates, and where possible a population of 48 pigs was maintained per room. Each pen of pigs chosen to fill the CF room was from a separate batch, ensuring staggered age groups.

Treatment three: Continuous flow with no use of disinfection (C)
Located in room three, treatment three replicated treatment two in design, but acted as a negative control group, with no washing and disinfection of pens between batches of pigs.

For treatments one and two disinfection was carried out in accordance to a protocol developed from recommendations for standard on-farm practice by the World Health Organisation (WHO, 1994). Initially, feed hoppers were emptied, solid muck removed and pen surfaces swept clean. This was followed by all surfaces of the pen and feed hopper being soaked in a water/detergent mixture (Kick-off, R S Hygiene Ltd, UK) at the manufacturers recommended concentration of 1:250 using a cold power-washer with a flow rate of 11L/minute, until all surfaces were thoroughly soaked. For practical reasons, a one hour soaking time was allowed for the water/detergent mixture to soften hard soiling, after which all surfaces were power-washed with cold water. A peracetic acid plus hydrogen peroxide disinfectant (Oxsan, RS Hygiene Ltd, UK), compatible with the pre-cleanser, was applied to the surfaces at the recommended dose of 1:125, and the surfaces left to dry over night. All concentrations of product and solution were made in a barrel with litres marked on the side ensuring correct dilutions were measured. 25L of the concentrations were sprayed per pen via a suction hose attached to the power-washer.

In an attempt to control outbreaks of scour, small adjustments to the feeding regime were made per replicate. During replicate one, all experimental pigs were fed the pelleted finisher feed from day one. During replicate two, this was altered so that pens coming on trial were fed for a week on medicated home mixed meal produced by the farm, after which the pelleted finisher feed was gradually merged in. The experimental pigs had been

receiving this medicated grower mix, prior to being selected for trial, and hence it was added to smooth the transition. This feeding regime was repeated for the third replicate to create a smooth transition on to the finisher pellets. However, for the third replicate the grower mix had been produced unmedicated due to a planned change in the veterinary management of the pigs on the farm.

3.2.4 Measurements

3.2.4.1 Pig health and production

All pens were checked daily for signs of ill health. Individuals displaying clinical signs were treated accordingly, with the pig identification number, clinical signs and treatments administered recorded. A humane endpoint, similar to that used by Done et al. (2005), was established for the need to remove an animal from trial. For a pig to be removed from trial it had to meet one of the following criteria:

i) Not responding to treatment following administration of the full course
ii) Prolapsed anus
iii) Wasting: continuously losing weight.

All pigs removed from trial were placed in a hospital pen and no longer monitored. Pigs that had slow growth but were showing no overt clinical symptoms, or that responded to treatment, were retained on trial to explore the effect of disease on the growth rate of pigs, the extent of lost production, and the time taken to reach slaughter.

A series of health score assessments were conducted on all pens once per week, on the same day each week and between the hours of 13.00-14:30. To score, the observer entered the room, the pigs were immediately roused and a stopwatch was started. If any pens pigs which failed to stand they were encouraged to do so by the observer slapping the pen with the palm of their hand. Each pen was observed for a period of five minutes during which the following observations were made:

1) *Cough score:* The number of coughs heard from a pen within a five minute period and, where possible, the individuals seen coughing as an indicator of the onset of pneumonia (Morris et al., 1995).

2) *Sneeze score:* The number of sneezes heard from a pen as above, to provide an indication of any upper respiratory complaints based on the protocol used by Ekkel et al. (1995).

3) *Scour score:* Fresh defecation on the pen floor was scored for consistency on the following scale (Wellock et al., 2006).

1 = Firm
2 = Soft, spreads slightly
3 = Very soft, spreads readily
4 = Watery, liquid consistency

4) *Pen health score:* A score was assigned to the pen based on the number of pigs showing clinical signs, providing an overview assessment of pen health based on that used by Wellock et al., (2006)

1 = 0 pigs showing signs of ill health
2 = 1 pig showing signs of ill health
3 = 2 pigs showing signs of ill health
4 = 3 pigs showing signs of ill health
5 = 4 pigs showing signs of ill health
6 = ≥ 5 pigs showing signs of ill health

5) *Cleanliness score:* Each pen of pigs was assessed for cleanliness and assigned a score as indicated below, based on that used by Wellock et al. (2006).

1 = Clean, all pigs free from faecal contamination
2 = ≤ 50% of the pen of pigs contaminated with faecal matter on part of body
3 = All pigs contaminated with a small amount of faecal matter
4 = All pigs heavily contaminated with faecal matter.

6) *Pen hygiene:* The percentage of solid floor covered in faeces was estimated, adapted from the score used by Rantzer and Svendsen (2001a).

0% = No faecal matter covering the floor.

25% = One quarter of the floor covered in faeces

50% = Half of the floor covered in faeces.

100% = All of the solid lying area covered in faeces.

Every two weeks all pigs were individually weighed and the quantity of feed remaining in the hopper (refusals) taken per pen. From this data the growth rate (average daily gain, ADG), feed consumption and feed conversion efficiency were calculated for all pens. During weighing of pigs, farm personnel moved from the 'clean' AIAO group, to the progressively 'dirty' C. Final live weight was taken prior to slaughter. Oral fluid (OF) samples were collected from each pig via a large cotton swab (Millpledge Veterinary, UK), whilst in the weigh crate. Blood was collected from pigs at exsanguination and lungs scored for *M. Hyo* using the 55 point scale developed by (Goodwin et al., 1969). The backfat thickness at P_2 position and cold carcase weight for each pig were obtained from abattoir carcase reports.

All blood was brought back to the university laboratory, allowed to clot and then spun in a centrifuge at 2000g for 10 minutes to separate the serum. Serum was extracted and frozen at -20ºC for later analysis.

3.2.4.2 Acute phase protein analysis

Serum samples from 90 pigs were analysed for the determination of Haptoglobin (Hp) and C-reactive protein (CRP). Samples were selected from one replicate (replicate two), and spread equally across all treatments (30 per treatment), with a cross section of pigs chosen on the basis of whether the pig had a good or poor growth rate in the final two weeks prior to slaughter. Good growth was classed as 750g/day or above, and the pig must have either maintained good growth in the weeks prior to slaughter or had an increase in growth rate leading up to slaughter. Pigs that showed a reduction in growth rate in at least the last two weeks prior to slaughter were classed as "poor doers". Selected pigs were then categorised as to whether clinical signs (coughing, obvious lethargy) had been seen in those individuals in the final weeks before slaughter.

Determination of APPs was performed via time resolved immunofloretic assay, at Murcia Veterinary University, as described by (Gutiérrez et al., 2009a, Gutiérrez et al., 2009b).

3.2.4.3 Environmental measurements

The maximum and minimum temperatures of each system were recorded daily by thermometer. Beginning at the start of replicate two, a series of environmental measurements were collected from each treatment to provide information on the level of contaminants within the microenvironment of the pigs. Environmental measures were collected on the same day that weekly health scores were performed, allowing measures of pen hygiene to be related to the environmental measurements.

3.2.4.3.1 Surface samples

As a measure of surface bacterial contamination, and to test the efficiency of disinfection, each pen was sampled before and after cleaning (where appropriate) using swab samples.

Sterile cotton swabs encased in sterile plastic tubes (Fisher Scientific, UK), were used for the sampling. To increase microbial pick-up (Crook, 1996), swabs were moistened with 2ml of buffered sodium chloride (NaCl) peptone water (Oxoid, UK), and added to the tubes aseptically within a class two safety cabinet prior to transport to the farm.

AIAO and CFD pens were swab sampled before and after cleaning and disinfection. For individual pens, following the removal of pigs, pre samples were taken before any soiling had been removed from the pen surfaces. After cleaning and disinfection, post-samples were taken when pens were dry, prior to new pigs entering. Pre-samples were taken from all AIAO, CFD and C pens. Although not washed, C pens were sampled to establish an idea of the bacterial levels in pens repeatedly used, but not washed.

Per pen, a total area of 15cm^2 was sampled, taken from three designated sites (5cm^2/site): the inside base of the feed hopper, the concrete lying area and the slats. These areas were chosen to provide a representative sample of the variety of surfaces to be cleaned in a pig pen, and some which can prove problematic to clean. The 5cm^2 swab area was controlled by a paper template, a fresh template used for each sample, preventing cross contamination between samples.

Following collection, all samples were refrigerated and transported back to the laboratories on cold packs. Samples were refrigerated upon arrival and, due to the volume of work, processed 24 – 96 hours after collection (Amass et al., 2007).

Samples were agitated for a few seconds via a vortex mixer prior to dilution and culture. Serial ten-fold dilutions were performed in replicate, from 10^0 – 10^8, by transferring a 10µl aliquot of each sample into 90µl of buffered sodium chloride peptone water. A 20µl aliquot of the original sample and each subsequent dilution were plated onto 90mm triple vent petri dishes containing Tryptone soya agar (Oxoid, UK) previously prepared. All samples were plated in duplicate.

Samples were incubated at 37°C for 24 hours, after which plates containing between 30-400 colonies were enumerated by eye (ocular enumeration) over a light box.

3.2.4.3.2 Aerial contaminants

Ammonia, dust and airborne bacteria, three of the main important aerial contaminants generated and released in pig production (Kim et al., 2005), were measured on a weekly basis.

Ammonia

Concentrations of aerial ammonia were measured using a portable gas monitor configured to ammonia with a sampling range of 0-100ppm (Crowcon, Oxfordshire, UK). Spot concentrations were measured per room at a height of 1.5m, in the centre of the room. This was within the air stream created by the ventilation fan, following evidence that gases within the pig building followed the air stream formed by the ventilation (Kim et al., 2007b), to obtain a representative sample of the ammonia concentrations within the room. The ammonia sampler was turned on outside the piggery in clean air and allowed to stabilise. The researcher then moved between each room individually measuring spot concentrations of ammonia in the designated spot of each room. The monitor was allowed to stabilise in each room for 30 seconds, and the reading taken. At times the monitor would not settle, in which case an average of the fluctuating range was taken. The ammonia sampling was conducted on the same day each week as the health scores, between 13:00 – 14:00. Humidity was measured at the time of sampling with a digital humidity monitor and the temperature noted by thermometer.

Total and respirable dust

Each week active dust samples were collected from each room over a 24 hour period. A manifold system made from 6.4mm diameter transparent nalgene tubing (Fisher Scientific, UK), was constructed running along the length of piggery within the three rooms. Two sections branched off per room to which IOM sample heads (SKC, Dorset, UK) were connected via a critical orifice specifically manufactured to allow air flow through at a rate of 2L/min. Air was drawn through the length of the tubing network using a 25L/minute pump (Brey, D-8940, Memmingen, Germany). Sample heads were hung at a height of 1.5cm, and located within the air stream created by the ventilation fan to obtain a representative sample of the room dust concentrations. Total and respirable dust were measured using the gravimetric method. Glass fibre filters (25mm diameter, 1.6µm pore size, Whatman, UK), and multidust foams (4µm cut point, SKC Ltd, UK), were placed together in a sample cassette, allowing simultaneous multifraction sampling, and loaded into an IOM sample head (SKC Ltd, UK), as shown in Fig. 3.1, allowing the collection of total and the respirable dust to be measured simultaneously. Prior to sampling and filter loading, all cassettes were cleaned and disinfected with 70% ethanol solution.

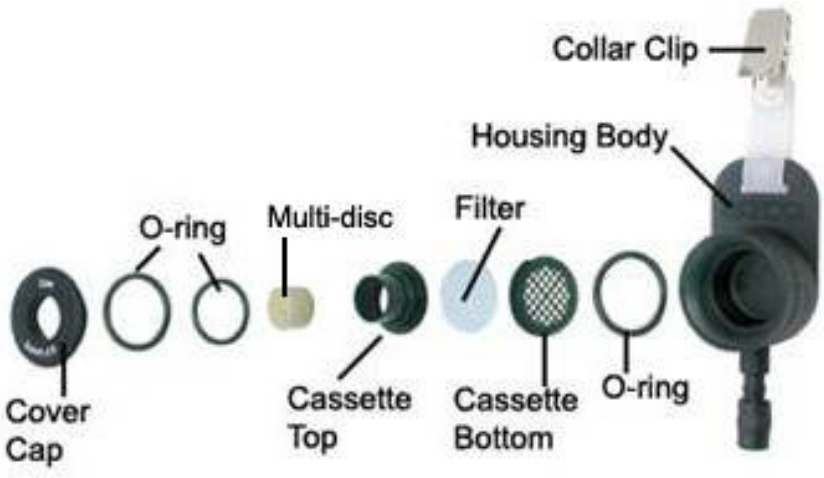

Figure 3. 1 I.O.M multidust sampler in exploded view (Source: SKC Ltd).

Pre and post sampling, the weight of the filter, foam and cassette were taken together, followed by the weight of the filter and cassette together. All filters were weighed in a controlled atmosphere, set at 21ºC and 55% humidity to avoid hydroscopy. Filters were allowed to adjust in the room for several hours prior to weighing. With the study running

over the course of the summer months, at times the humidity in the weighing room unavoidably exceeded 55%. In such an event, the new filters were weighed at a higher humidity, and the higher humidity created for the following week. Any samples that had previously been weighed at a lower room humidity in the week before were not weighed until the room humidity had reduced.

Each week, sampling began after all work had been conducted in the building. The flow rate through each sample head was checked at the start and end of sampling with a rotameter (SKC Ltd, UK). A cut off range was accepted between 1.7 – 2.1L/min.
Control filters were brought to the sample site, exposed, but not subjected to sampling, and weighed using the same procedure. Humidity was recorded at the start and end of the sampling over 24 hours with a digital humidity monitor and the max/min of temperature taken by thermometer.

Filter weighing strategy

Filters were stored in a room with temperature controlled to 21˚C. Prior to weighing the humidity was set to a controlled level (52 ± 2%) and the filters placed in a clean petri-dish with vents, allowing the filters to settle for two hours, after which they were weighed using a microbalance (0.0001g, Mettler AC 100, Salter, UK), previously tested for accuracy with standard weights. Practice tests revealed that allowing the filters to settle for two hours in this controlled temperature and humidity allowed controlled weighing and prevented hydroscopy of the filters far better than other methods (desiccation etc). Blank control filters, which were brought to the sample site and exposed, but not subjected to sampling, were also weighed to gauge a control on the level of any hydroscopy taking place.

Following collection, dust was weighed on a delayed schedule of one week. Because of the time taken to prepare the room to the correct humidity to allow samples to be re-weighed, this delayed schedule was chosen for practicality and to ensure consistency in the re-weighing of the samples. During this one week time delay, the collected samples remained stored in the sample cassettes and IOM heads, in a sealed box, out of direct sources of heat or sunlight. Taking a pragmatic view, this method of storage ensured that the dust was retained in as natural a state as possible and that the live cells were stable

and dormant, and is likely to give a representative sample of the level of bacteria within the dry dust (Brian Crook, personal communication, 13th May 2008).

Airborne micro-organisms

Cultures were made from the total dust collected in the IOM cassettes as a measure of the total airborne micro-organisms per m^3 air. Post-weighing, dust filters were removed with tweezers, placed in individual sterile plastic containers and transported back to the laboratories.

In a class two safety cabinet, containers were individually opened and the filters cut up into small pieces using sterile tweezers and scissors. This was done because early tests had shown the respirable foam retained dust, preventing it from being washed through, if not cut open. Tweezers and scissors were sterilised between each filter. Following this, 10ml of PBS, with 0.01% w/v Tween 20 added to help break up clumps, was added to each filter set and mixed for 1 minute with a Vortex machine. From the solution a dilution series up to 10^3 was performed, and used to inoculate plates in replicate, with 20µl per half plate. Plates were incubated at 37˚C for 24 hours, after which those plates with 30 – 400 colonies were enumerated by eye, with the use of a lightbox. Exposed controls were run to check for background contamination.

3.2.4.4 Calculations

Surface sample average bacterial counts

Per sample location, the average count from duplicate plated samples was taken and multiplied by the dilution. The bacterial counts per $5cm^2$ and per cm^3 were used in the analysis to compare the separate sampling areas. To provide total bacterial counts per cm^2 of pen, average bacterial counts were calculated for each of the three sample locations within the pen, similar to multiple counts performed by Amass et al., (2007), and the average number of CFU expressed per cm^2.

Dust samples

The inhalable and respirable dust fractions were calculated as directed in standard operating instructions (SKC, 2011b). With all filters held within individual casing of the sampler, the inhalable fraction was determined by subtracting the total weight of the dust collected on the filter and the foam pre and post sampling. The respirable fraction was

calculated from subtracting the weight of the filter only, pre and post sampling. To eradicate any effects of hygroscopy, any difference in weight of the blank filter was then subtracted from the final dust weight obtained.

From the weight of the respective dust, the quantity of dust per m^3 of air was calculated via the following calculation:

The total weight of dust collected, g/24 hours, multiplied by 1000 to transform to weight in mg/24 hours.

The total volume of air (m^3) to pass through the filter over 24 hours was calculated via:

$$\frac{\text{Average flow rate (L/minute) x the total time the pump was run (hours) x 60}}{1000}$$

The mg of dust collected per sample was divided by the volume of air (m^3) collected in 24 hours to give the total volume of dust per m^3 of air. This sum was used to calculate both the total and respirable dust components. When any change in the flow rate over the 24 hour sampling period occurred, the average flow rate was calculated and used in the final calculation.

Total viable bacterial count

An average total bacterial count was obtained from the duplicate aliquots per sample to obtain an average plate count, and corrected for the inoculum quantity, dilution and wash volume. To determine the total quantity of CFU per m^3 the following calculation was performed:

$$\frac{\text{Total bacterial count per plate}}{\text{Volume of air over 24 hours (L) X 1000}}$$

The average of the two sample heads used per room was taken.

Ventilation

Per system, Ventilation was measured by recording the total number of kilowatts used to power the fans per system. The total m^3 of air to move through the fan over 24 hours was calculated by:

$$\left.\begin{array}{c}\underline{\text{Kwh/24 hours}} \\ 0.05\end{array}\right\} \text{X } 1000$$

This result was divided by 24 to give the m^3 of air moved per hour.

3.2.5 Statistical analysis

Statistical analyses were conducted using the Minitab 15.0 and SPSS 17.0 statistical packages.

Prior to analysis, all data were checked for normality using the Anderson Darling test, and, where possible, non-normal data were transformed. Appropriate parametric or non-parametric tests were then selected.

Missing values in the data from air monitoring existed from occasions where samples were dropped, or blank deductions revealed severe error, rendering them unusable.

The Kruskal-Wallis test was used to assess any differences between the concentrations of bacteria on pen surfaces before and after cleaning and disinfection, and also across the different sampling positions before and after cleaning and disinfection.

Multiple linear regression analysis was performed to determine the principle environmental factors responsible for factors influencing the air quality and a General Linear Model (GLM) to compare the mean concentrations of aerial contaminants between the systems, with the system and the trial week as factors, and with washing activity as a covariate. The health and productivity of the pigs was compared between the systems by use of a GLM, with system and replicate as factors.

Differences in temperature between the systems were analysed via Kruskal-Wallis analysis.

Correlation analysis was used to examine individual relationships between environmental variables and between pig health scores and incidences of disease.

Information on the total number of pigs to have remained healthy throughout the trial, and those which were removed and developed clinical illness was collated, and the ADG of pigs that remained healthy, developed respiratory illness or scour calculated and related to production costs.

Descriptive statistics of the acute phase proteins were produced and a GLM run to compare the concentration of APPs from the individual pigs between the systems. Also included in the model as factors were the health code assigned to the pigs and whether the pig had been treated or not treated, along with interactions between the system and the assigned health code and whether the pig had been treated or not treated.

3.3 Results

3.3.1 Disinfection on pen surface bacteria samples

Of pens that received washing (within AIAO and CFD systems), disinfection significantly reduced the median levels of bacteria within pens: pre vs post CFU 11.5 x 10^3/cm^2 vs 7.8 x 10^3/cm^2, (H = 24.8, d.f. = 1, P<0.001). Before washing, the CFU/5cm^2 of the slats differed significantly from the CFU found on the lying area and the hopper, but there was no significant difference between the CFU of these areas post washing (Table 3.1).

Table 3. 1 CFU/5cm^2 for each pen surface area sampled pre and post washing and disinfection

	Floor	Slats	Hopper	H	P
Pre-wash	12.5x $10^4{}_a$	27.0x$10^4{}_b$	15.7x$10^4{}_b$	7.81	<0.05
Post-wash	103.5	353.0	35.5	3.09	0.213

Quantity of CFU are significantly different (P < 0.05) where subscripts differ.
Pre floor, slats and hopper N = 17 for each, post floor (N = 17), slats (N = 16) and hopper (N = 17), D.F 2 for each test.

3.3.2 Correlations between environmental variables

There was a negative correlation between the ventilation rate and the log of total (r = -0.423; d.f. = 33, P < 0.05) and the log of respirable dust (r = -0.349; d.f.= 33, P < 0.05). The mean pen hygiene score showed a positive correlation with ammonia concentration

(r_s = 0.479; d.f = 63, $P < 0.01$) and a negative relation to airborne bacteria (r_s = -0.316; d.f. = 42, $P < 0.05$), with the latter also being related to humidity level (r_s = 0.607; d.f. = 42, $P < 0.01$).

There were positive correlations between a number of the environmental pollutants (Table 3.2). Ammonia correlated to total dust, total dust to respirable dust and aerial bacteria to respirable dust.

Table 3. 2 Correlations between the environmental pollutants (correlation coefficient and level of significance).

	Ammonia	Total dust	Log 10 Respirable dust
Total dust	0.36*	-	-
Log 10 Respirable dust	NS	0.436**	-
Log 10 Aerial bacteria (cfu/m^3)	NS	NS	0.625***

Where asterisk occur, * = $P < 0.05$, ** = $P < 0.005$, *** = $P < 0.001$. Total dust/ammonia, d.f = 42; Respirable dust/total dust, d.f = 43; Aerial bacteria/respirable dust, d.f = 38.

3.3.3 Effect of disinfection on environmental contaminants

Concentrations of ammonia and airborne bacteria were significantly higher in the CFD and C, compared to the AIAO system. Between the CFD and C system concentrations of ammonia and airborne bacteria were higher in the C. Total and respirable dust did not differ between the systems (Table 3.3). Trial week was significant as a factor for all environmental contaminants ($P < 0.001$ for all), and washing activity was not significant.

Table 3. 3 Concentrations of environmental contaminants per system (mean ± s.e.m).

Measurement	AIAO	CFD	C	*P*
Total dust (mg/m³)	2.37 ± 0.17	2.88 ± 0.17	2.41 ± 0.15	0.289
Respirable dust (mg/m^3)	0.14 ± 0.02	0.21 ± 0.02	0.17 ± 0.02	0.539
Ammonia (ppm)	1.84 ± 0.23^a	2.93 ± 0.25^b	3.11 ± 0.22^b	<0.01
Airborne bacteria (CFU x 10^5/m^3)¥	1.8 ± 1.2^a	3.9 ± 0.8^b	4.3 ± 1.2^b	<0.005

Concentrations within a row are significantly different (P<0.01) where superscripts differ.
¥ - Log 10 of airborne bacteria used in analysis, unlogged values presented here.

Investigation of the determinants of air quality showed that the act of cleaning and disinfection only had an effect on the concentration of ammonia within the system (Table 3.4), with this husbandry procedure linked to an increase in ammonia. In contrast, ventilation rate, the number of pigs per room and the pen hygiene score were the

influential factors on total and respirable dust. A lower ventilation rate was found to increase the concentrations of total and respirable dust, as was a greater number of pigs in the room. In addition, a higher pen hygiene score (indicative of a dirtier pen) was related to a reduced concentration of total dust, a reduced concentration of airborne bacteria, but an increased concentration of ammonia. An increased input of feed during the 24 hours of sampling was related to lower concentrations of airborne bacteria, whilst an increase in humidity was linked to higher concentrations of respirable and airborne bacteria.

Table 3. 4 Factors affecting air quality parameters (regression coefficient and level of significance).

Predictor	Total dust (mg/m^3)	Respirable dust (log mg/m^3)	Airborne bacteria (log CFU/m^3)	Ammonia (ppm)
R^2 (adj)	58.0%	38.6%	50.8%	31.0%
Washing activity	NS	NS	NS	0.969*
Ventilation (m^3/hour)	-0.00075***	-0.00039***	NS	NS
No. pigs/room	0.098***	0.031**	NS	-0.034*
Pen hygiene	-0.036***	NS	-0.040***	0.056***
Feed input in 24 h (kg)	NS	NS	-0.00449*	NS
Temperature (˚C)	NS	0.213*	NS	NS
Humidity (%)	NS	0.035**	0.146***	NS

Where asterisk occur: *** = P<0.001, ** = P<0.01, * = P<0.05.

The minimum and daily variation in temperature differed significantly between the systems, although the actual fluctuation was very little. There was no difference in the maximum daily temperature (Table 3.5).

Table 3. 5 Medium daily maximum, minimum and daily variation in temperature (°C) between the systems

	AIAO (N = 162)	CFD (N = 193)	C (N= 192)	H	*P*
Max	20.0	20.0	20.0	0.62	0.734
Min	18.0_a	17.0_b	17.5_b	13.1	0.001
Variation	2.0_a	2.0_a	3.0_b	16.7	<0.001

Where superscripts differ within a row, values differ significantly ($P < 0.001$)

3.3.4 Pig health and productivity

Pig health measures showed there was a difference in sneezing (median counts/5min) between the rooms, (AIAO: 0.11, CFD: 0.35, C: 0.27; H = 6.47, d.f = 2, $P < 0.05$) but there were no other room differences of pig health or productivity (Table 3.6).

Table 3. 6 Pig production and health between the systems (mean and sem)

Parameter	AIAO	CFD	C	*P*
Average gain per day (kg)	0.70 ± 0.02	0.72 ±0.02	0.72 ± 0.02	0.728
Feed consumed/pig/day (kg)	1.97 ± 0.04	2.05 ± 0.04	2.12 ± 0.04	0.070
FCR	2.81 ± 0.09	2.90 ± 0.09	2.94 ± 0.09	0.628
Pneumonia (pigs/pen)	0.17 ± 0.18	0.50 ± 0.18	0.42 ± 0.18	0.567
Scour (pigs/pen)	3.08 ± 0.53	3.00 ± 0.53	2.42 ± 0.53	0.631
Removed due to illness (pigs/pen)	0.50 ± 0.29	0.58 ± 0.29	0.50 ± 0.20	0.898
Died (pigs/pen)	0.17 ± 0.11	0.17 ± 0.11	0.08 ± 0.08	0.922

Of the population studied, 69% of pigs remained free from disease throughout the finishing period, 23% showed symptoms of enteric disease (scour) as their main health problem, and 4% respiratory problems (respiratory distress/laboured breathing), 2% lost weight with no evidence of clinical signs and 1% suffered other conditions. There was a 1.4% mortality and, of the 432 pigs on trial, 8.3% had to be removed to hospital accommodation. Although free from obvious disease and with a steady growth rate, 28.7% of the pigs termed healthy were spotted coughing whilst on trial.

Lung scores were significantly higher across all systems in replicate one than in replicates two and three (Replicates, 1: 7.89, 2: 3.07, 3: 2.48, s.e.m.1.00; $P = 0.001$), with a significant interaction between room and replicate ($P < 0.05$, Fig. 3.2).

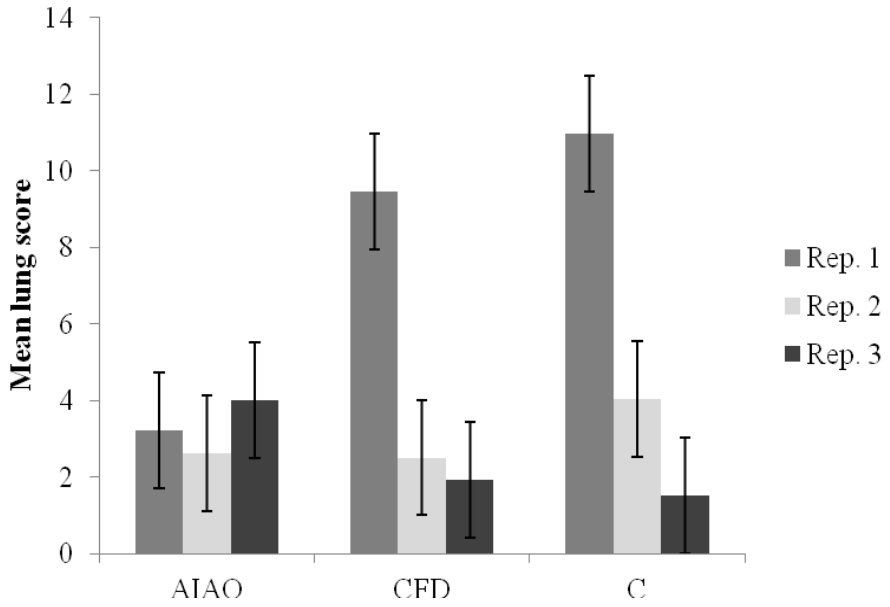

Figure 3. 2 Mean lung score across the systems and over the replicates.

Within two weeks of entering the building, scour and pneumonia struck – infected pigs showing an average reduction in weight gain of 2.3 kg compared to unaffected pigs. After 4 weeks on trial, the depressive effects of disease widened the gap in growth between sick and healthy pigs to 5.7kg.

Scour was seen from the first fortnight on trial. In the first fortnight, the average daily gain of scouring pigs was 128g lower than healthy pigs, and in the second fortnight it reduced further to 160g lower than healthy pigs (Fig. 3.3). On recovery from disease the gain per day of previously scouring pigs improved by 90g/day, between 4 - 6 weeks on trial, resulting in a daily gain only 70g/day lower than healthy pigs at this time. The weight and daily gain of pigs that had initially suffered pneumonia or scours was not significantly different between the 4th and 6th week on trial from pigs that had remained disease free.

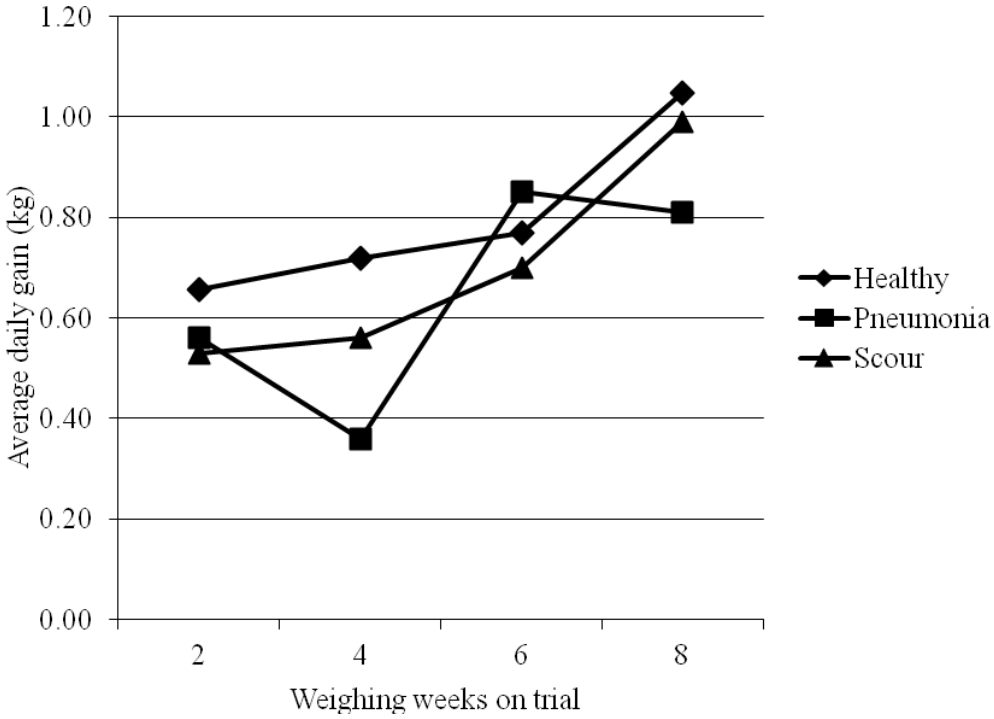

Figure 3. 3 Average daily gain (kg) of healthy, pneumonic and scouring pigs over four fortnightly growth periods.

A broad group analysis of the growth of pigs over the finisher period, including removed live pigs, revealed healthy pigs had an average daily gain of 0.79kg, whilst for those which suffered from respiratory and enteric problems, daily gain reduced to an average of 0.42kg and 0.62kg respectively. Growth rates in relation to specific disease symptoms and outcomes are displayed in Fig. 3.4.

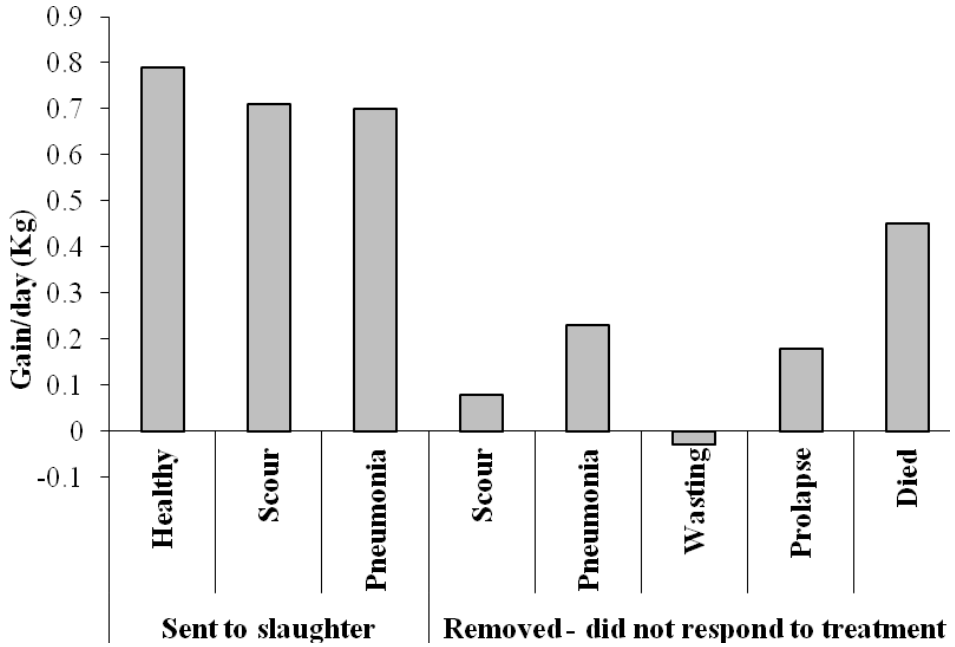

Figure 3. 4 Average daily gain (kg) of healthy pigs and pigs displaying clinical signs of a range of diseases, that were and were not sent to slaughter.

3.3.4.1 Correlations between health scores

At the pen level (N=36), cough scores were positively correlated to the prevalence of pneumonia in live pigs (r_s = 0.549; d.f. = 34, $P < 0.001$), and the log of lung scores at slaughter (r = 0.335; d.f. = 34, $P < 0.05$). A high pen health score (indicating poor health) was negatively correlated to gain per day (r_s = -0.430; d.f. = 34, $P < 0.01$).

3.3.5 Acute phase proteins

There were no significant differences in the concentration of Hp and CRP from pigs between the three systems, the assigned health codes, or whether the pig had been administered veterinary treatment or not in the finisher period. The range in concentration of the APPs between the systems is displayed in figures 3.4 and 3.5.

Concentrations of Hp and CRP were highly correlated (r_s = 0.777, P <0.001), and also negatively correlated to the liveweight gain per day in the final two weeks before slaughter (Hp: r_s = -0.263, P < 0.05; CRP: r_s = -0.246, P < 0.05).

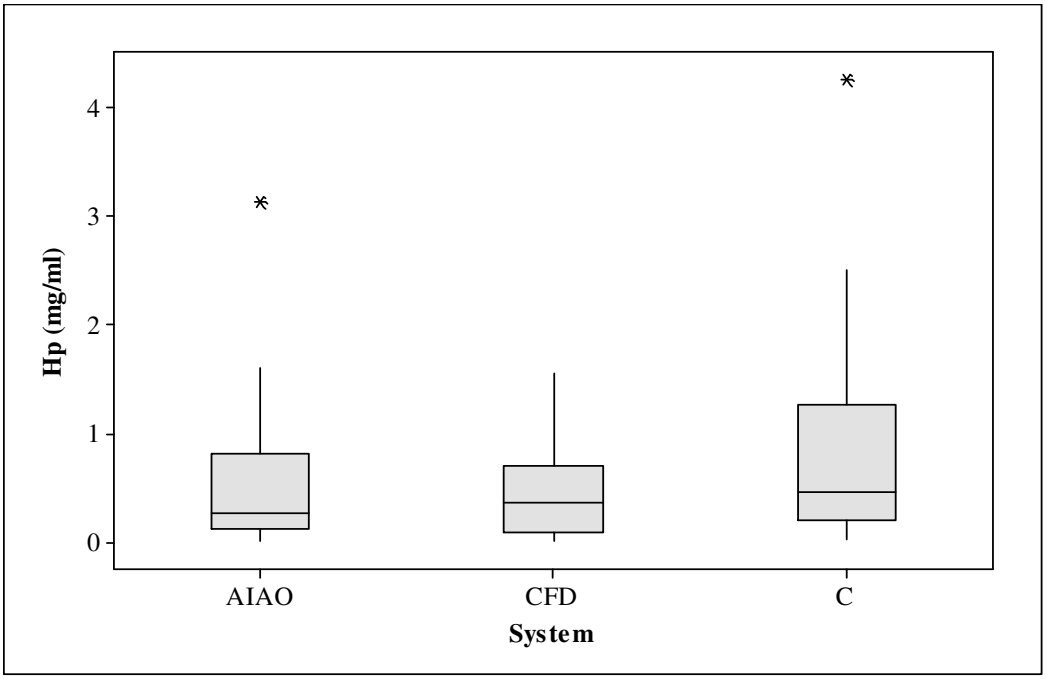

Figure 3. 5 Concentrations of Hp in pigs between the three systems (n = AIAO: 29, CFD: 30, C: 29). The plot displays the median (line within the box), 25th and 75th percentiles (box), 5th and 95th percentiles (whiskers) and outliers (*).

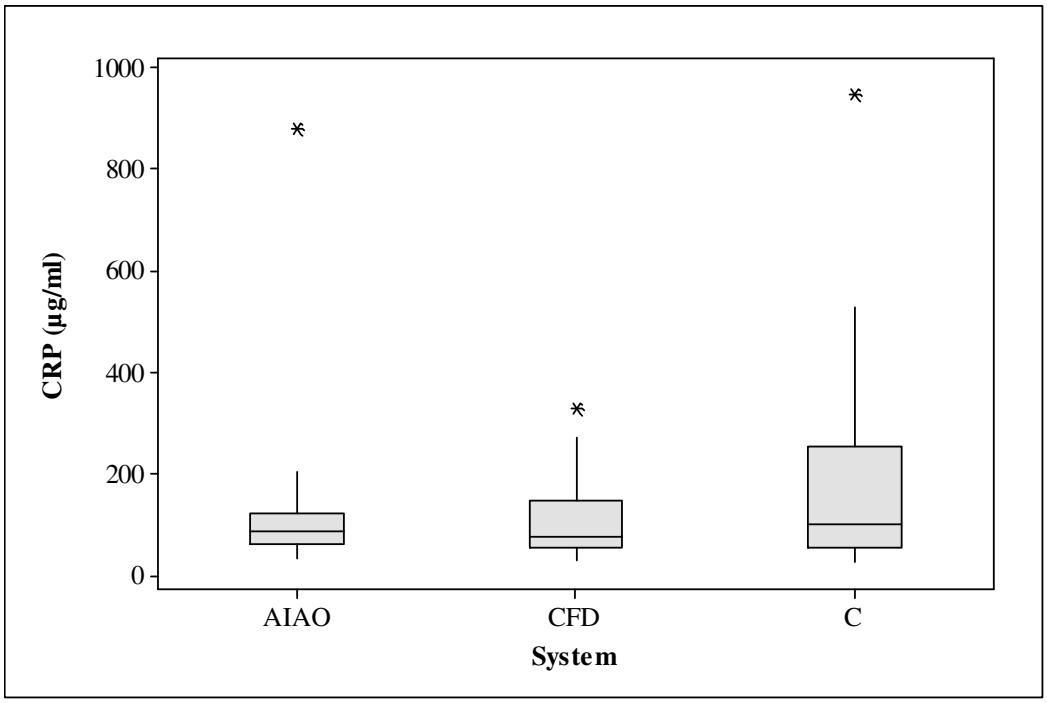

Figure 3. 6 Concentrations of CRP in pigs between the three systems (n = AIAO: 29, CFD: 30, C: 29). The plot displays the median (line within the box), 25th and 75th percentiles (box), 5th and 95th percentiles (whiskers) and outliers (*).

3.4 Discussion

This study compared the use of washing and disinfecting in AIAO and CF systems to determine whether productivity in the CF system could be improved, though alleviating one additive effect of the total environmental challenge on the pigs, and in addition, whether the activity of washing would influence the air quality.

3.4.1 Effect of disinfection on surface samples

The cleaning and disinfection routine conducted in this study was an effective regime, significantly reducing the total colony forming units (CFU) per cm^2 of pen surface and importantly achieving a level below the recommended maximum threshold of 10^3 CFU per cm^2 post disinfection (Böhm, 1998).

A total CFU per cm^2 for unwashed animal houses has been reported as 10^9 CFU/cm^2 (Böhm, 1998). When compared, the total CFU per cm^2 detected within the pen environment in this study is lower than levels found on livestock utility surfaces pre-

disinfection in other studies, where levels ranged from 10^6 - 10^9 (Amass et al., 2007, Rantzer and Svendsen, 2001a, Rantzer and Svendsen, 2001b). However, each of these studies used different culture media which could have influenced the growth of the bacteria, and some a different culture technique, such as deep-cultivated bacteria within the agar (Rantzer and Svendsen, 2001b). Mannion et al. (2007) found more comparable levels of bacterial CFU per cm^2, with levels ranging from 10^2 – 10^5, although these samples were looking for Enterobacteriaceae only. Visual observation indicated that the pen surfaces samples were very dirty pre-disinfection, covered in faeces, dust and feed particles. Therefore, it is not believed that the low CFU obtained in the pre-disinfection sample is an indication that there was low bacterial counts on the surfaces. Instead, it could be an indication that the bacterial pick-up and release into the inoculation was low, as is recognised can be a problem with swabbing (Moore and Griffith, 2007). The lower CFU could also in part be due to the agar medium used. Tryptone soy agar is non-specific and supports the growth of a wide range of organisms, however, the type of bacteria present on surfaces within livestock buildings may be better suited to other broth mediums. Certain strains of bacteria are known to have low viability in certain broth mediums. This could increase bacteria die-off and therefore reduce the number of bacteria viable to be cultured.

3.4.2 Aerial contaminants between the systems

3.4.2.1 Ammonia and total airborne bacteria

The concentrations of ammonia and total airborne bacteria were lowest in the AIAO and highest in the C system. Both the CFD and the C systems differed significantly from the AIAO system. The total number of micro-organisms per m^3 of air can serve as an indicator of aerial hygiene (Pavičić et al., 2006), demonstrating the action of washing pens in a CF system did not exert a large enough influence to significantly improve the air quality. Washing activity, rather had the effect of increasing the concentrations of ammonia in the environment, and did not have a significant effect on any of the other environmental variables. Ammonia being highly soluble within water is likely to have become soluble in the increased water vapour in the air as a result of washing, generating an increased emission of ammonia gas into the air.

In a study by Cargill and Banhazi (1998) the act of not washing between batches of pigs in an AIAO system was less detrimental to air quality than when done in a CF system,

(with only a 13.5% difference in aerial bacterial between washed and unwashed AIAO systems, and a 52.2% between washed AIAO and CF systems). In the current study, there is a 123.5% percentage increase in total aerial bacteria between the AIAO and CFD system, a 141.2% increase between the AIAO and C system, and a 7.9% increased between the C and CFD systems. The percentages given from both studies demonstrate that it is the action of the system, rather than the washing, that is improving the air quality.

The lower concentrations of ammonia and airborne bacteria within the AIAO system demonstrate further benefits of the AIAO system in helping control air quality, which has also been noted by Banhazi (2011). AIAO systems housing pigs of one age group, aid maintenance of an improved temperature control for the age group of pigs. With multiple ages of pigs within one airspace, CF does not accommodate the temperature requirements of all pigs as well as AIAO accommodation can. Inside temperature and the weight of pigs (which influences indoor air temperature) have been found to have a significant effect on pen fouling of hard standing (Ni et al., 1999). The poor maintenance of temperature for a percentage of the pig ages can increase the risk of defecation in wrong areas of the pen increasing the surface area on which manure is spread, leading to increased release of ammonia from the mixing of the enzyme urease in the faeces and urea from the urine (Robertson, 1994). In this study, there was no difference in the mean pen hygiene scores between the systems, although this was a subjective score and so may have some degree of error in classifying how fresh the manure was. Incorrect pen fouling is often associated with increases in building temperature, causing pigs to muck in the lying area, and lie in the mucking area to assist thermo regulation. However, in this study there was no difference in the daily maximum temperature between rooms. Instead, the raised ammonia in the CF systems could be due to the fact that the C system was never washed and thus resulted in a build up of faecal matter within the pens, contributing to a continuous generation of ammonia. In the CFD system, whilst muck was removed from pens between batches, the act of washing was also found to increase levels of ammonia emission as urine and faecal matter remained on the surfaces of other pens. The AIAO system however only received washing when all pigs had been removed and therefore there was no leeway for extra ammonia production within the system.

3.4.2.2 Influences on the aerial contaminants

The primary influences on the concentration of aerial contaminants between the systems were the ventilation rate, the number of pigs present within the room and the level of pen hygiene.

3.4.2.2.1 Ventilation

Ventilation rate had a significant negative relationship with the total and respirable dust concentration. Increasing the ventilation rate reduces the sedimentation of the dust particles allowing them to be removed via the ventilation exhaust. This is one of the principle dust removal mechanisms in piggeries (Robertson, 1994). The ventilation rate has not always been found to affect the dust concentrations; Kim et al. (2007b) found that an increasing ventilation rate decreased respirable dust particles, but not total dust. The principle environmental factors affecting aerial pollutants alter between different piggeries (Banhazi et al., 2008). Whether the ventilation rate affects the total dust concentration is likely to be largely based on the composition of the total dust within individual piggeries, which is likely to differ between piggeries due to the composition and form of the feed, and the humidity of the air. Other research suggests that the respirable dust is more commonly affected by the ventilation rate (Banhazi et al., 2008), since the finer particle size may remain airborne indefinitely (Dawson, 1990), and thus be easily carried on the ventilation air stream.

3.4.2.2.2 Number of pigs

The number of pigs housed within each system had a positive relationship with the total and respirable dust. The concentration of aerial pollutants has been found to be directly proportional to the stocking density (Pavičić et al., 2008, Pavičić et al., 2006), the animals themselves being the main polluters (Wathes et al., 1983). Dust, micro-organisms and gases become airborne through the movement of the animals, breathing, coughing sneezing, excreting, workers in the building and feed inputs, combined with convection of body heat from the animal and cooler surrounding air (Pavičić et al., 2006).

3.4.2.2.3 Pen hygiene

Increasing pen hygiene score (increased soiling of pen floors) was significantly related to increase in ammonia. The concentration of ammonia in the air is regularly found to relate to the degree of pen hygiene (Banhazi et al., 2008). Ammonia is released from excreted faeces (Smith et al., 2000) from excess nitrogen in the manure derived from undigested

proteins (Robertson, 1994). A mixing of faeces and urine, as occurs in the pig pen environment, generates mixing of urea within the urine and the enzyme urease present within the faeces resulting in a rapid breakdown of urea and release of ammonia (Robertson, 1994). The increased floor area over which the urine and faeces is spread, as occurs when the pen hygiene decreases, increases the area for volatilisation, increasing the release of ammonia further (Robertson, 1994). This will be exacerbated by agitation of the faeces/manure combination on the pen surface by the pigs themselves.

Wetter, dirtier pens, giving rise to the increasing pen hygiene score is likely to be responsible for the relationship between increasing pen hygiene score and reducing concentrations of aerial bacteria and total dust concentrations. Airborne bacteria are adsorbed onto the dust within the air and also affected by the quantity of faeces. A damp pen surface arising from increased pen dirtiness from faeces and urine would lead to a reduction in the quantity of dust being formed by dry particle generation being roused from the floor, and thus lead to a reduction in dust and airborne bacteria and thus how this association is formed.

3.4.2.2.4 Temperature and humidity

The positive association between temperature and respirable dust is a general relationship that has been found to occur when the environment of the piggery dries out leading to an increase in particle generation (Pedersen et al., 2001, cited in Banhazi 2011). The lack of association between total dust and the temperature may be down to the composition of the dust within the piggery, and thus its formation.

An increase in humidity has been linked to an increase in endotoxin concentration (Banhazi et al., 2008). In this study, the association between increasing humidity and increased respirable dust and aerial bacteria indicates that the increasing humidity may in part have been due to faecal matter drying out. This in turn generated increased respirable and aerial microbial particles, with particles likely to have been generated from the dust and faecal matter on the pen floor. This has also been noted and suggested by Banhazi (2011).

It should be considered that there is a variety of conflicting evidence on the effects of temperature and humidity on aerial pollutants in livestock housing. The way in which

aerial contaminants operate within different buildings is largely influenced by the management system in question (Banhazi, 2011), and is also in part due to the sampling techniques employed by different studies. Some of the earlier studies carried out have been reviewed by Dawson (1990). A high temperature and increased humidity has been found to lessen pig activity (Kim et al., 2008), which in turn affects the concentration of dust and airborne bacteria concentrations (Dawson, 1990).

3.4.2.2.5 Feed input

The quantity of feed placed into the hoppers within the 24 hours during which dust sampling took place had no effect on the total and respirable dust particles, as may be expected. This could be a result of the feed composition, which is known to influence dust production (Dawson, 1990). Except for the first week on trial for replicates two and three, in which the new pigs were fed meal, the pigs were fed pellets.

The negative relationship between the quantity of feed placed in the feed hoppers within the 24 hours of dust sampling and a lower aerobic bacteria is not one that has been reported elsewhere; the majority of studies do not mention the quantity of feed to go into the hoppers during dust sampling. However, the inclusion of increased dietary fat in feeds has been found to reduce total airborne bacterial counts (Chiba et al., 1987).

3.4.3 Pig health between the systems

In this study there was a difference in the intensity of sneezing between the systems, a greater proportion of sneezing occurring in the two CF systems than the AIAO. However, there were no significant differences in any of the other health measurements, or the productivity of the pigs. It was expected that the AIAO and CF systems would show a difference in the growth rate of the pigs, based on previous studies (Cargill and Banhazi, 1998, Ice et al., 1997). The systems tested are believed to help prevent (AIAO) or contribute (CF) to respiratory disease spread. The figures on the percentage of pigs displaying clinical signs of pneumonia and lung scores indicate there was little effective pneumonia challenge to pigs at this production stage in any of the rooms, and the reduction in lung scores in the CF rooms over the course of the three replicates suggests the pneumonia pressure reduced over time. This could be a problem of trying to create a controlled system on a small scale. However, it also coincides with a seasonal change from winter to summer, giving an increase in ambient temperature and ventilation rate,

which is associated with reduced pneumonia (Ostanello et al., 2007). The high prevalence of scour that occurred may well have eliminated any potential growth differences between the rooms. The outbreaks were related to an existing *Brachyspira* pathogen within the herd, which was exacerbated by the experimental allocation and relocation procedures.

The concentration of APPs can provide a marker for demonstrating the degree of immune activation of the pigs. Differences in concentration of APPs have been found in relation to the hygiene status of the farm (Geers et al., 2003). The lack of significant difference between the median concentration of APPs found between the systems is in agreement with the lack of clinical health issues between the systems, and also indicates that the significant differences in ammonia and aerial bacteria between the CFD and C systems to the AIAO were not sufficient to create an underlying immune response. However, despite this there were still significant negative correlations between the live weight gain of the pigs in the final two weeks before slaughter and the concentration of APPs. This demonstrates a relationship between those pigs growing at a slower rate prior to slaughter having raised immune activation.

Correlations between pig health and productivity suggest a use for the health scores for assessing pig health. There is differing evidence in other studies to the use of health scores. Quantification of coughing has been found to relate to the incidence of pneumonia at slaughter (Morés et al., 2001), as in the current study, although Morris et al., (1995) did not find coughing a sensitive enough indictor of lung lesions at slaughter. Health scores should be continued to be tested to confirm their effectiveness.

3.4.4 Air quality comparison between systems and pig health

The total bacteria CFU/m^3 found in different studies varies considerably, not only due to the type of equipment used to collect the sample, but also a result of the housing system the pigs are in. Of the quantities of aerial bacterial found in this study, levels found in the AIAO system are similar to those found by Cargill and Banhazi (1998). The levels found in CFD and C systems in this study are comparable to the levels found by (Robertson et al., 1990), which (in combination with the other aerial pollutants measured), was found to influence the severity of atrophic rhinitis in pigs. In the current study, only the severity of sneezing was different between the systems. Sneezing can be used as a clinical measure of Atrophic Rhinitis in growing-finishing pigs (Morés et al., 2001), although this was not

present in the current study, and pigs infected with PRRSv infection have also been found to display sneezing (Roberts and Almond, 2003). Ammonia is a respiratory irritant, however it is primarily found to only create problems at concentrations far higher (11ppm) than found in this study (Donham, 1991). The endotoxin content of bacteria has been found to irritate the lining of the respiratory tract, with data suggesting respiratory health could be affected far more by endotoxin content than dust (Zejda et al., 1994). The acute and chronic exposure of pigs to environmental pollutants has been highlighted as contributing to the aetiology of pig respiratory diseases (Wathes et al., 2004). With no difference in other indicators of respiratory disease (coughing, pneumonic pigs) or in the daily gain of the pigs between systems, any effect of aerial bacteria on increased sneezing was not having a measurable detrimental effect on the pigs, but could be an indication of increased respiratory irritation.

The concentrations of total and respirable dust found in this study were similar, to slightly lower in concentration to those found elsewhere (Robertson et al., 1990, Kim et al., 2007a). There will naturally be variation between studies due to the different housing conditions, management and location of the sampling within the piggery. Maximum exposure limits for dust, ammonia and aerial bacteria have been recommended as no greater than 3.7mg/m^3 for total dust, 0.23mg/m^3 for respirable dust, 11ppm for ammonia (120cm above floor level) and no greater than 4.3 x 10^5 cfu/m^3 of total airborne bacteria within the air (Donham, 1991). All the concentrations of aerial pollutants recorded in this study are below these levels, and therefore this could be a contributory factor to the lack of difference in the health or growth of the pigs between the systems. However, the levels of total airborne bacteria recommended by Donham (1991) are higher than that recorded by Robertson et al., (1990) which were linked to increased atrophic rhinitis scores, further adding to the numerous conflicting results surrounding effects of air quality on disease within pig herds.

3.4.5 Production loss from disease

Broad group analysis of the pigs, including removed live pigs, revealed significant growth reductions from scour and respiratory problems. The growth of marketable pigs that scoured was chronically stunted, and after initial evidence of scour was spotted, the optimal growth rate of the pigs did not return.

Assuming a value of 4p/g gain (Richardson, 2011), it was calculated that a pig with visible scour or respiratory distress, but which recovered without hospitalisation, had a reduced gain of 80 - 90g/day, resulting in a £3.20 - £3.60 loss per pig. The 4p/g gain is derived from input costs, sale value, FCR and interest rates as calculated over wean to slaughter (Richardson, 2011), and therefore this cost calculation provides only a rough estimation. Additional to this loss through reduced gain are the costs of drugs and labour for treatment.

3.6 Conclusions

The use of a disinfection routine did significantly reduce the bacterial count within pen surfaces, but did not significantly improve air quality, pig health or productivity in this study. On air quality, the system itself had a more profound effect, with AIAO having a better air quality than CF systems. The relationships between ventilation and pig numbers emphasise the importance of building management to alleviate poor air quality and suggest that maintaining a good quality ventilation system and correct stocking density would manage aerial contaminants better than cleaning in a CF building. Whilst it is recognised that AIAO and CF systems can be protective and contributory to spreading pig sickness respectively, there was no effect of system on pig health in this study. This study found contaminant levels that were comparable with other reports, but not in the concentrations known to cause health problems. Whilst it is recognised that at high concentrations, environmental contaminants can be limiting factors for productivity and negatively affect pig health, this was not evident in this study, suggesting the challenge presented was not great enough in this particular scenario. Substantial reductions in growth can be seen from when pigs begin to display clinical signs of disease. The range of simple health scores utilised in this study could be a useful method to aid producers in monitoring health and productivity within their system, such as simple cough scores can be used by producers to check pneumonia severity.

Chapter 4: Inter-relationships between birth weight, growth profile and health in the lifetime performance of pigs

4.1 Introduction

Variation within a population of individuals is a natural occurrence. Yet, for pig production, minimal variation in the size of similarly aged individuals is desirable, ensuring pen groups of pigs can be fed the most appropriate diet and are within the target slaughter weight range at the same time. Variable growth rates between individual pigs from the same batch increases total days to slaughter. In a continuous flow (CF) system, this affects pen usage, whilst in all-in/all-out (AIAO) systems the effects can range from disrupting rooms to the entire barn usage (Patience and Beaulieu, 2006). Opting to clear pens and send all pigs to slaughter still has the consequence that individual pigs will fall outside the desired carcase weight range resulting in lost revenue. Therefore, variation and lost growth is costly at all stages of pig meat production.

Disease pressure within the rearing environment is believed to be the leading cause for increasing variability in pigs, beyond the inherent factors such as birth weight and suckling nutrition (Patience and Beaulieu, 2006). Herd health status has been found to correlate to the coefficient of variation of bodyweight of growing pigs at all progressive stages to slaughter weight (De Grau et al., 2001, cited in Patience and Beaulieu, 2006).

Individuals differ in their susceptibility to disease and, in a population of growing pigs, a percentage of individuals will fail to thrive. Why certain individuals become unthrifty and express clinical disease whilst others do not, has been a subject of much research. There are large differences in immune reactivity and disease susceptibility in pigs, which appear to be related to an individual's social status in a stable social structure (Hessing et al., 1994). Raised under the same conditions, pigs vary greatly in their level of immune system stimulation, indicating differences between the levels of protection each individual may have (Dionissopoulos et al., 2001). This could be linked to individual differences, which may arise from early life experience and interactions with the level of colostrum intake, social and management factors (Davis et al., 2006).

Relationships between birth weight and physiological competence have been found to exist in pigs (Patience and Beaulieu, 2006). In general those of a higher birth weight have a higher daily gain throughout their growing life up to market weight and vice versa (Quiniou et al., 2002). However, whilst much research has focused on these relationships, there is little available information on whether there is a relationship between birth weight, subsequent performance and susceptibility to disease in later productive life. Gardner and Hind (1990) found a low birth weight (less than 1kg) and younger weaning age (less than 24 days) had small but significant effects on the extent of pneumonic lesions found in pigs slaughtered between 30 - 50kg, and Davis et al., (2006) have looked at weaning age on the growth performance and immunological response of pigs.

The objectives of this current study were to determine whether there is a relationship between early weight and growth performance and subsequent health challenges, and their effect on the subsequent growth of pigs up to slaughter weight. Should relationships exist, it was an aim to establish what, if any, were the characteristics of these susceptible pigs that could make them identifiable at an early stage within the production cycle.

In addition, this study could also serve as a case study to provide information on how poorly growing pigs continue to fare when kept within the main production system, an aspect of pig production rarely, if ever, monitored. In turn, this will enable an estimate of the loss in profit due to this practice and could lead to strategies for management to identify and deal differently with these pigs.

The data collected in this study will provide useful information on the distribution in bodyweight of growing pigs under commercial conditions. Such individual growth data are in short supply due to the increase in workload that weighing pigs places on producers (Patience and Beaulieu, 2006). Pig growth data available from research papers can often provide little information on the true variation within groups of growing pigs due to the pre-selection often required in experiments (Patience and Beaulieu, 2006).

4.2 Materials and methods

This trial ran for 10 months from August 2009 – June 2010, during which the lifetime performance of 744 pigs, originating from 66 litters, was monitored on a 120 sow farrow-to-finish farm. The piglets monitored had been identified and weighed at birth, and again

at weaning as part of a separate study. For this study, the monitoring of the piglets continued subsequent to weaning, upon entering the grower house, entering the finishing house and upon weighing out to slaughter.

Pigs were weaned at four weeks of age and remained in litter groups throughout the weaner stage (4 - 10 weeks of age), in pens measuring 1.67 x 1.84 m. Upon entering the grower stage (10 - 15 weeks of age) two litters were mixed together to form larger pen groups of ≥ 20 pigs and housed in pens of 3.80 x 2.46 m. Pigs remained in these pen groups throughout the finisher stage (15-22 weeks), where pigs were housed in pens measuring 3.00 x 3.80 m.

All accommodation from the weaner through to the finisher was in fully slatted, environmentally controlled and fan ventilated buildings. All pens were provided with a single piece of hanging enrichment to meet legislative requirements. The temperature of the buildings was set via a thermostat which controlled the speed of the ventilation fans in response to the room temperature. At piglet entry the weaner accommodation temperature was maintained at 26°C, and subsequently reduced by 0.2°C each subsequent day for the following 20 days, until reaching 22°C, being then kept at this level until the pigs left the building. The temperatures of the grower and finisher buildings were set at 22°C and 19°C respectively. Throughout each stage feed was provided *ad-libitum* via multi-space hoppers and water supplied freely via two drinker nipples per pen, increased to four in the grower building. Weaner accommodation was in general operated on an AIAO basis, but occasionally different batches would be housed in the same room, rendering it CF. The grower and finisher buildings were operated on a CF system.

At weaning piglets were fed a diet manufactured by A-One feeds Ltd (Thirsk, UK). The weaner diet consisted of Flat Deck 1 (22.0% CP, 1.7% Lysine, 16.0 MJ DE/kg), fed as a creep during the lactation phase and 1kg/piglet fed after weaning, followed by Flat Deck 150 (21.0% CP, 1.6% Lysine, 16.5 MJ DE/kg) fed at 2kg/piglet, followed by Turbo wean (21.0% CP, 1.5% Lysine, 15.8 MJ DE/kg), fed for four weeks post weaning. Following this, a home mixed meal diet produced at the farm was fed in the weaner (20.5% CP, 1.3% Lysine, 14.8 MJ DE/kg) and grower stage (20.0% CP, 1.2% Lysine, 14.0 MJ DE/kg). For the finishing stage, commercially manufactured finisher pellets were fed (18.5% DE, 1.1% Lysine, 12.7 MJ DE/kg, Top grade finisher pellets, Farmway, Durham,

UK). Pigs from 44 of the litters monitored had originated from a nutritional trial looking at the effect of the addition of the omega 3 fatty acid, Docosahexaenoic acid (DHA), derived from algal biomass (AB), in the diet of sows during gestation and lactation. Dietary treatments comprised different levels of inclusion of DHA as follows: 1) a vegetable oil control supplement, 2) DHA inclusion at a low level (0.1 g/kg - commercial test level), 3) DHA inclusion at a high level (1.0 g/kg - positive control inclusion). All feed was available *ad-libitum* in multispace feed hoppers, and water via bite drinkers. All accommodation was fully slatted, with a single piece of hanging enrichment supplied to each pen to meet legislative requirements.

Lifetime health records for all pigs were maintained jointly by farm staff and research staff, with any clinical signs and treatments administered being recorded, however, pens remained under the control and direction of the farm management. When pigs died or were removed from a pen to enter the hospital pen, the weight of the pig was recorded. Weekly pen health scores were conducted on each pen during the grower and finisher phase (methodology for health scores was as described in chapter 3.2.4.1).

Pigs went to slaughter according to the prevailing market requirements, which resulted in pigs leaving for slaughter at bacon weight (100kg) or cutter weight (65-85kg), depending on weekly demand. As pigs went to slaughter, it was common for the pigs remaining in the pens to be mixed to generate space. Records were kept, as far as possible, when individual pigs were subjected to mixing.

4.3 Statistical analysis

Birth and weaning data from the previous records were collated with the subsequent weight records to produce data on the lifetime performance of all pigs. The data analysed were from all pigs that had survived to weaning, and therefore did not include piglets that had been crushed or killed by the sow, nor had died or been euthanized early due to weakness or illness. However, data on the total litter size at birth were included. Calculations were performed to determine the average daily gain (ADG) of all pigs at the various stages of growth, and the total lifetime gain. Due to pigs being moved from pens, and mixing occurring, pen composition did not remain constant. Therefore, data on pen health scores were collated to produce average scores for the grower and finisher accommodation.

Of the 744 pigs originally monitored, data on 714 were used for analysis. This was a result of missing data on a certain proportion of the pigs, leading to the decision to discard these pigs (n = 30) from analysis. A series of lost ear tags in the finisher building resulted in a proportion of the 714 being unidentifiable for slaughter. These are regarded as missing for the slaughter data but were included in some of the earlier analyses and it is made clear when this was the case.

The statistical packages Minitab 15.0 and SPSS 17.0 were used for all analysis of the data. Descriptive statistics of the weight, growth and health of the pigs was collated over the different stages of production leading to slaughter.

All data were checked for normality and, where possible, non-normal data were transformed, or an appropriate non-parametric test chosen. Correlations amongst variables were tested using Spearman's rank correlation due to lack of normality in data.

The effect of gender on whether the pigs displayed clinical signs of disease or later died was examined using Chi-square analysis using the statistical package SPSS, and the effect of gender on the lifetime ADG of the pigs was examined using the Kruskal – Wallis statistic from Minitab.

Multiple regression analysis was used to examine the most influential factors affecting birth weight, birth to wean daily live weight gain, wean weight and subsequent lifetime ADG. Factors (displayed in table 4.1) were placed individually into a single regression analysis, and those found to be significant ($P < 0.05$), were then placed together in a multiple regression analysis. As classed in table 4.1, sow and litter factors were used to determine the main effects on birth weight, sow and litter, age, and weight and growth on wean weight. All factors were considered for their effect on daily live weight gain. This analysis was repeated separately for the pigs born to sows on the DHA nutrition trial to test for long term effects of these treatments.

Table 4. 1 Factors placed individually into a single regression model against pig weight and average daily live weight gain from birth to slaughter.

Factors	Predictor
Sow and litter	Sow parity
	No. Born alive
	No. Stillborn
	No. Mummified
	Total litter size
Age	Weaning age
	Age at entering grower accommodation
	Age at entering finisher accommodation
Weight	Birth weight
	Wean weight
	Weight at entry to grower accommodation
	Weight at entry to finisher accommodation
	Slaughter weight
Live weight gain	Birth- wean ADG
	Wean-grower ADG
	Grower-finisher ADG
Health	Sick in farrowing accommodation (yes/no)
	Sick in weaner accommodation (yes/no)
	Sick in grower accommodation (yes/no)
	Sick in finisher accommodation (yes/no)

Multiple logistic regression analysis was used to investigate whether pre-natal factors and the early weight and/or ADG of piglets could be used to determine whether the pigs were likely to become clinically sick or not in later in life. Tested separately in the model were the sow and litter data (as displayed in table 4.1), and birth weight, wean weight and birth to wean ADG. Significant factors were again added to the model for multilevel analysis.

Of pigs that remained clinically healthy throughout life, factors influencing slow growth were investigated by identifying pigs (with a 1/0 code) with an ADG $\geq$ 2 s.d. below the mean within each production stage. Pigs growing at $\leq$ 2 s.d. below the mean received a score of one (slow growth), and those growing at the mean or above received a zero code (standard growth). Factors contributing to slow growth in the preceding stages (as displayed in table 4.1, except health info) were investigated via multiple binary logistic regression analysis performed using the Minitab statistical package, first individually, and then with significant factors reanalysed in a multilevel analysis. Whether slow growing pigs were likely to be also slow growing in the following stage was examined with the Fisher exact probability statistic, using the cross tabulation test conducted in SPSS.

To explore the effect of birth weight on subsequent performance, all pigs were assigned to three birth weight groups, Light (L <1kg), Medium (M 1-1.59kg) and Heavy (1.6 – 2.5kg) based on the weights regarded as important for survival (Quiniou et al., 2002). This was analysed in a General Linear Model (GLM) with birth weight group as an independent variable and the weight and ADG of pigs at subsequent stages as the dependant variables. Weaning age was added as a covariate when examining the difference in weaning weight between the groups. When examining ADG, weaning age was added as a covariate when exploring birth to wean ADG between the groups, with wean weight and weaning age added as covariates when exploring wean to grower ADG, and slaughter weight added as a covariate when exploring finisher to slaughter ADG.

Data on the effect of mixing the pigs could not be explored as it became impossible to keep an accurate record of mixing within the finisher building. It was observed that the vast majority of pigs were mixed on several occasions.

4.4 Results

4.4.1 Pig performance and health

The range in weight of the pigs at each stage of growth is given in Table 4.2. As the average age of the pigs increased, the increasing standard deviation of weights demonstrated larger variation as the pigs become heavier. However, the coefficient of variation indicates that, when considered in proportion to the size of the pigs, the variation in the weight decreased as the pigs became older.

Table 4. 2 The distribution of body weight (kg) for all pigs monitored at each growth stage*

	Birth	Wean	Grower	Finisher	Slaughter
Average age (days)	0	27.4	74.7	114.1	154.4
Minimum	0.55	1.86	10.60	27.90	53.40
25th percentile	1.31	6.36	25.30	50.20	75.60
Mean	1.56	7.57	29.51	56.7	83.72
Medium	1.58	7.60	29.80	57.20	82.60
75th percentile	1.81	8.85	33.80	63.10	91.30
Maximum	2.52	12.61	50.80	84.00	126.10
Range	1.97	10.75	40.20	56.10	72.70
Standard deviation	0.34	1.77	6.33	9.16	11.40
Coefficient of variation	22.05	23.32	21.45	16.14	13.60

*Pig numbers, birth (n = 714), wean (n = 714), grower (n = 712), finisher (n = 692), slaughter (n = 658).

The range in the ADG of the pigs at each period of the production cycle increased as the pigs got older. A proportion of pigs with poor and good growth rates are apparent in each period of the growth cycle. The mean ADG of the pigs did not differ between the grower and finisher stages, with some pigs experiencing weight loss in both periods (Figs. 4.1 and 4.2).

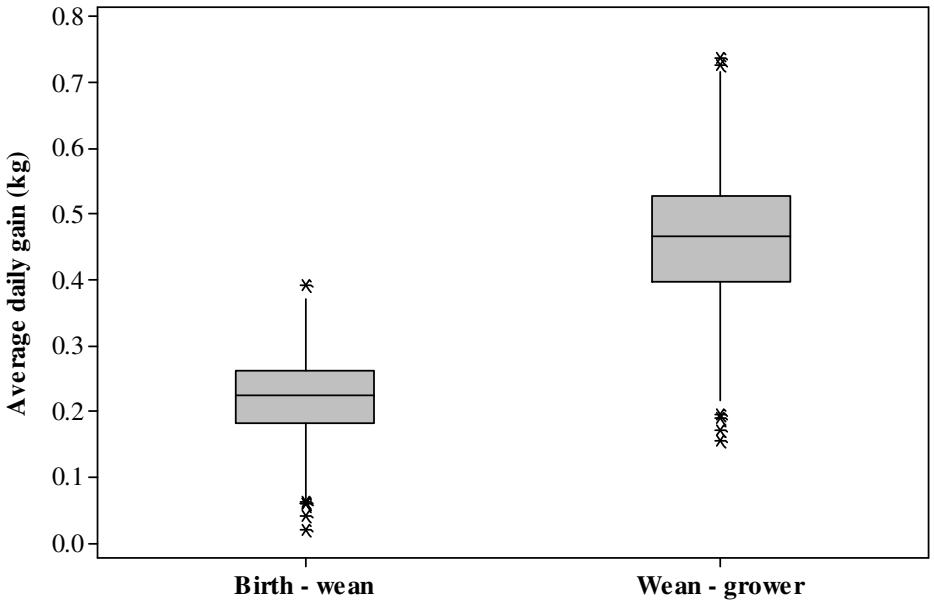

Figure 4. 1 The range in ADG of all pigs during the birth to wean (n = 714) and wean to grower period (n = 712), (median Birth-wean: 0.22, Wean- grower: 0.47, W = 265025.0, $P < 0.001$; median wean-grower: 0.47, grower – finisher: 0.69, W = 295662.0, $P < 0.001$).

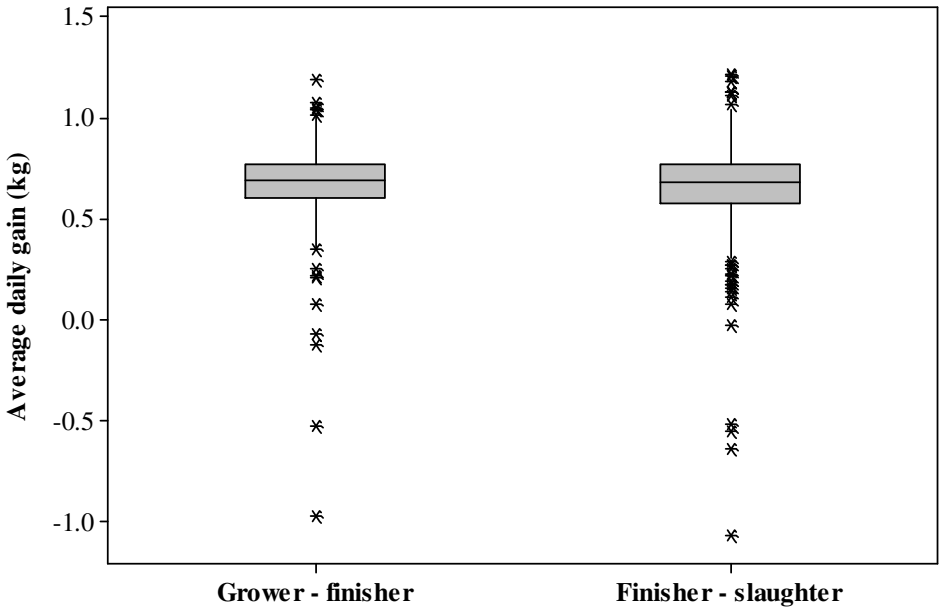

Figure 4. 2 The range in ADG of all pigs during the grower to finisher (n = 701) and finisher to slaughter period (n = 666), (median grower – finisher: 0.69, finisher – slaughter: 0.68, W = 490355.0, $P > 0.05$).

Table 4.3 displays birth and sow information on all the pigs monitored.

Table 4. 3 Details of sow parity and litters from which monitored pigs were derived (mean ± sem, unless stated otherwise)

	Details of all pigs
No. Of litters	66
Average sow parity	3.75 ± 0.09
Born alive	13.82 ± 0.09
Still born	0.88 ± 0.03
Mummified	0.2 ± 0.02
Total litter size	14.89 ± 0.08

Of the 714 pigs monitored, 52 (7.3%) became clinically ill (including those that died) over their lifetime. Table 4.4 shows a breakdown of the division of sick and healthy pigs, the number that did and did not make it to slaughter and the proportion of pigs that went missing/became unrecognisable due to lost tags.

Table 4. 4 Breakdown of health and losses of pigs monitored

Category	No. of pigs	Percentage (%)
Healthy	628	88.0
Clinically ill but later sold for slaughter	30	4.2
Clinically ill and not sold for slaughter	19	2.7
Clinically ill and went missing	3	0.4
Missing	34	4.8
Total	714	100.0

A breakdown in the ailments of the 52 pigs that became clinically ill during their lifetime is displayed in table 4.5. The majority of clinical cases occurred within the grower and finisher phase of production, yet the largest amount of veterinary treatments was administered whilst piglets were still in the farrowing accommodation.

Table 4. 5 Number of pigs and variety of clinical ailments that occurred

	Farrowing accommodation	Weaner	Grower	Finisher
Splay leg	0	1	0	0
Septic toe	4	0	0	0
Pityriasis Rosea	0	1	0	0
Abscess	1	1	0	0
Leg problems (swelling/lame)	2	0	9	9
Joint ill	1	0	0	0
Scour	0	0	1	1
Pneumonia/respiratory	0	0	2	1
Erysipelas	0	0	1	0
Flank bitten	0	0	4	1
Hernia	0	0	0	2
Prolapse	0	0	0	2
Anal Stricture	0	0	0	1
Bullied	0	0	1	0
Tail bitten	0	0	0	1
Lethergy	1	0	0	1
Died/euthanized	0	0	2	4
Total cases*	9	3	20	23
Total veterinary treatments*	11	1	5	0

* Three pigs had multiple ailments over the course of their life, and a number of pigs were administered more than one course of veterinary treatments.

Averages of the pen health scores collected from pens in the grower and finisher stage of production revealed a higher average score for all conditions in the finisher building (Fig. 4.3). However, it was the cough score which rose the greatest, indicating a larger prevalence of respiratory conditions occurring in the finisher accommodation.

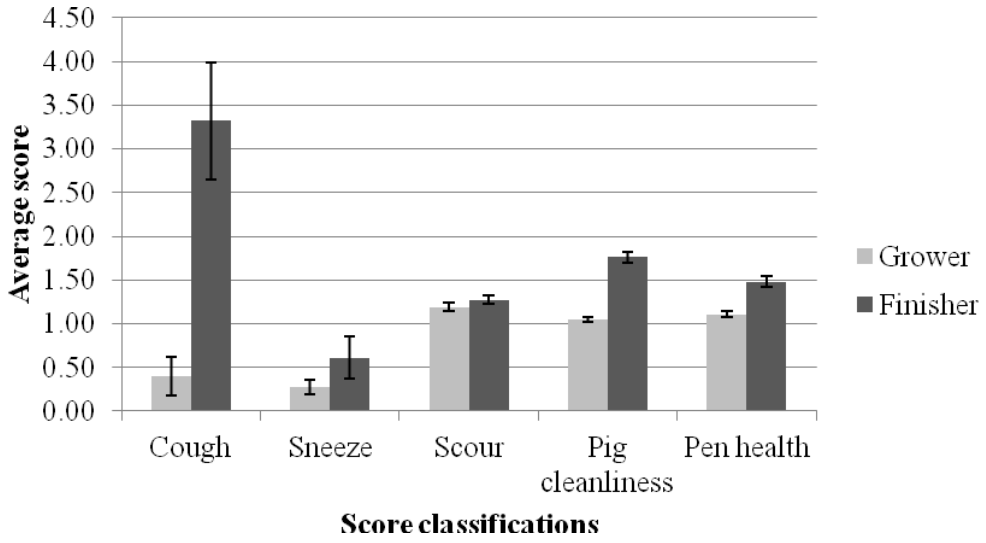

Figure 4. 3 Average health scores taken from pens in the grower and finisher accommodation

The mean lifetime ADG of all pigs was 0.59 ± 0.003kg/day. Analysis of all pigs showed that pigs that had become sick at some point throughout their life had a significantly lower lifetime ADG than those that had not (Healthy: 0.60, Sick: 0.52 kg± 0.01, $P < 0.001$, mean ± pooled sem); birth weight and the number of days on trial (to correct for different marketing strategies) were significant as covariates ($P < 0.001$ and $P < 0.005$ respectively).

Analysis of all pigs, excluding those that were lost in the system (n = 37), revealed no significant differences in the lifetime ADG of pigs that remained healthy throughout their life and those that became sick but were subsequently sold for slaughter. However, pigs that became sick and were not sold for slaughter had a significantly lower lifetime ADG when compared to both other groups, (Fig. 4.4).

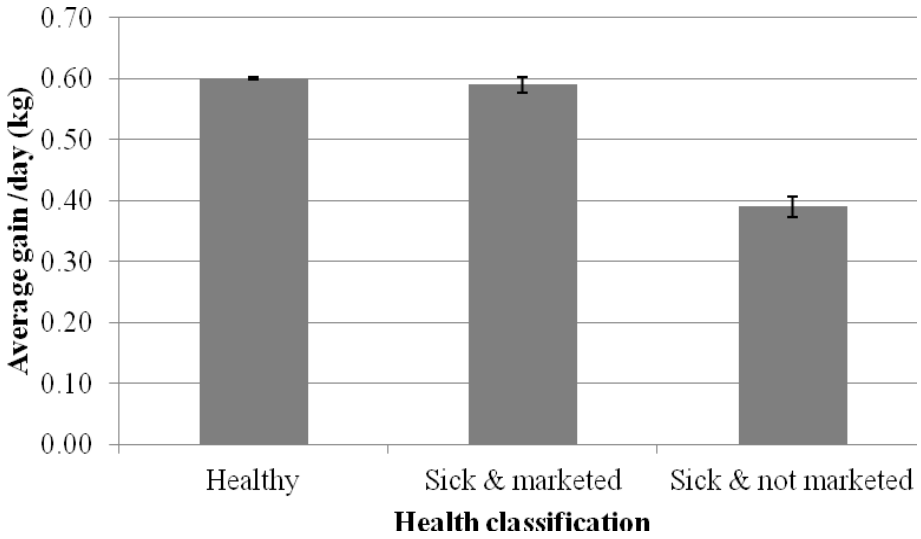

Figure 4. 4 Difference in average daily live weight gain (kg) between healthy (N= 628) and sick pigs that did (N= 30) and did not (N= 19) make it to sale for slaughter.

There was no significant difference between the genders in the number of pigs that became sick or remained clinically healthy (χ^2 (1) = 1.229, P = 0.268). Boars had a slightly better lifetime ADG than gilts, and this was significant; Boar: 0.60, gilt: 0.59 kg, (Median, H = 7.79, d.f. = 1, P = 0.005).

There was great variability in the length of time pigs took to get to slaughter (table 4.6), or were monitored as within the system but not making it to slaughter (birth to end of monitoring).

Table 4. 6 Range in days of the time taken by pigs from birth to slaughter, or to removal from production

	Birth to slaughter (N= 658)	Birth to off monitoring (N= 19)
Range (days)	124 - 223	81 - 264

4.4.2 Factors affecting growth performance from birth to slaughter

Total litter size was negatively but weakly correlated to the weights of the pigs at the different growth stages. Correlations existed between all the pig weights at the various growth stages, however, the strength of the correlations varied (Table 4.7).

Table 4. 7 The relationship between litter size, birth weight and subsequent weights at the different stages of production. (Spearman's rank correlation coefficient)

	Total littersize	Birth Wt	Wean Wt	Grower Wt	Finisher Wt
Birth Wt	-0.192	-	-	-	-
Wean Wt	-0.216	0.533	-	-	-
Grower Wt	-0.156	0.422	0.571	-	-
Finisher Wt	-0.131	0.425	0.450	0.771	-

All relationships significant at the level of $P < 0.01$.

A larger litter size was associated with a reduced birth to wean, wean to grower and lifetime ADG, but did not have a significant relationship with the ADG in the grower and finisher stages (Table 4.8). There were positive but weak correlations between the birth weight and the subsequent performance, with the associations becoming weaker the older the pigs became, as was also the case for the relationship between wean weight and the subsequent performance. Expectedly, stronger correlations existed between the weight of the pigs in the grower section and the growth in the birth to wean and wean to grower stage (expected as the weight of the grower pigs originates from the performance of the pigs up to that point). Likewise, the correlation between finisher entry weight and the ADG at the various stages became progressively stronger the older the pigs were.

Table 4. 8 The relationship between littersize and live weight at each of the production stages and the average daily gain (kg) of the pigs at each of the different stages of growth.

	Total littersize	Birth Wt	Wean Wt	Grower Wt	Finisher Wt
Birth-wean	-0.210	0.398	0.959	0.507	0.380
Wean-grower	-0.100	0.321	0.368	0.917	0.688
Grower-finisher	NS	0.214	0.256	0.403	0.715
Finisher-slaughter	NS	0.084*	0.113	NS	NS
Lifetime	-0.104	0.290	0.336	0.584	0.681

Unless stated otherwise, all relationships significant at the level of $P < 0.01$. Where asterisk occur * = $P < 0.05$.

Results from the multiple regression analysis of data from the pigs that did and did not progress to slaughter, but whose endpoints were known, are presented in table 4.9. Birth weight was negatively related to the numbers of pigs born alive. The ADG from birth to weaning was positively influenced by birth weight, but negatively related to littersize, the number of pigs mummified and the weaning age. Wean weight was strongly determined by the birth weight and, not surprisingly, ADG between birth to weaning. Lifetime ADG was positively influenced by weaning age and the ADG from the weaner stage through to slaughter. However, the age of pigs when moved to the grower and finisher stages was

negatively related to lifetime ADG. Occurrence of clinical illness was a factor with a significant influence on growth in the grower stage, but this was not so for any of the other stages of growth and displaying clinical signs did not affect the overall lifetime live weight gain.

Table 4. 9 Factors significant and not significant in birth and wean weights, and live weight gain at different stages throughout life

Predictor	Birth weight	Birth - wean ADG	Wean weight	Wean - grower ADG	Grower - finisher ADG	Finisher - slaughter	Lifetime ADG
R^2 (adj)	4.09	18.43	95.69	24.76	22.68	26.17	96.23
Sow parity	X	X	X	0.0085***	NS	X	NS
Littersize	NS	-0.00336***	NS	NS	X	NS	NS
No. Born alive	-0.0311***	NS	NS	NS	NS	NS	NS
No. Mummified pigs	X	-0.0112*	NS	X	X	X	NS
No. Stillborn	NS	X	X	0.0148***	NS	X	NS
Birthweight	~	0.0618***	0.957***	NS	NS	NS	NS
Birth-wean ADG	~	~	26.65***	-1.35***	-0.63**	NS	-0.051**
Sick in farrowing	~	X	X	X	X	X	X
Weaning age	~	-0.0042***	X	NS	X	0.0126**	0.00130***
Sick within weaner	~	~	~	X	X	X	X
Wean weight	~	~	~	0.0645***	NS	NS	NS
Wean-grower ADG	~	~	~	~	-1.04**	X	0.279***
Sick in the grower	~	~	~	~	0.360***	X	NS
Grower weight	~	~	~	~	0.03073***	X	NS
Age enters grower	~	~	~	~	-0.0122***	X	-0.00117**
Age enters finisher	~	~	~	~	~	X	-0.00088*
Grower-finisher ADG	~	~	~	~	~	-0.309**	0.228***
Weight into finisher	~	~	~	~	~	X	0.00090*
Sick in the finisher	~	~	~	~	~	NS	NS
Slaughterweight	~	~	~	~	~	0.00667***	0.00130***
Illness within lifetime (yes/no)	~	~	~	~	~	~	NS
Finisher - slaughter ADG	~	~	~	~	~	~	0.2385***

Where asterisks occur, *** = $P < 0.001$, ** = $P < 0.005$, * = $P < 0.05$; NS = not significant. X = term not included in the model as it had not shown a significant influence when running terms individually in a separate model. ~ = term not considered for the model.

4.4.3 Lifetime performance and health of pigs in birth weight classes

The birthweight classifications had, by definition, significantly different mean weights at birth (L: 0.89, M: 1.39, H: 1.92 ± 0.17 pooled S.D., $P < 0.001$), and all remained significantly different throughout the growing life (Figure 4.5). For the wean weight, weaning age was also significant as a covariate ($P < 0.001$).

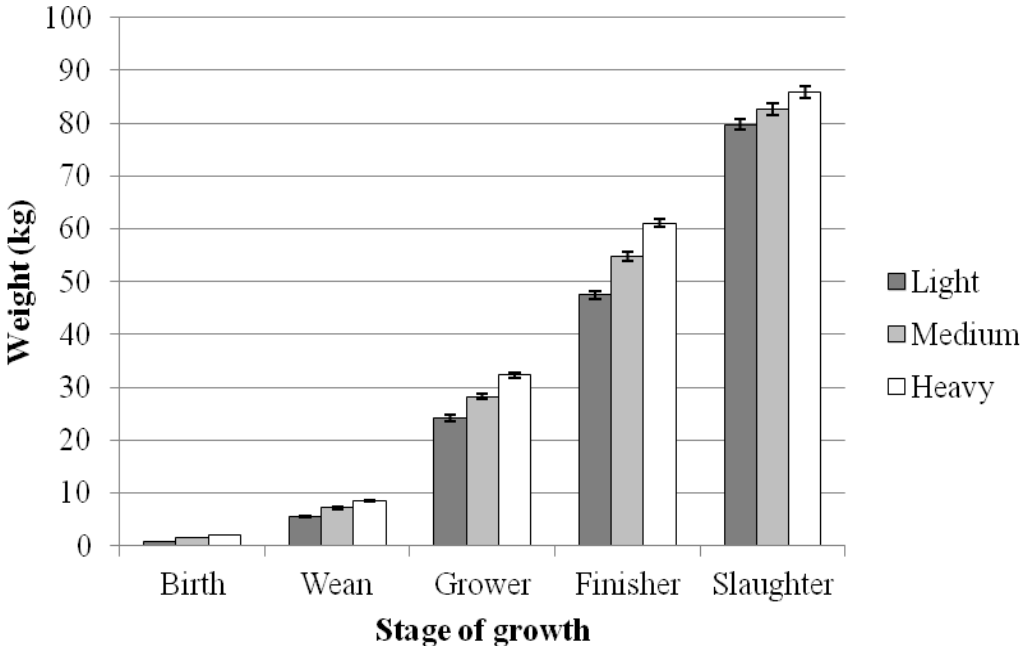

Figure 4. 5 Weight of pigs grouped by initial birth weight at different stages of the production system.

The ADG of the pigs within the different birthweight groups was significantly different in the birth to wean stage of growth, and in the following stages only the heavy group were significantly different from the medium and light groups. However, towards the final stage of production, there was again no significant difference (Table 4.10).

Table 4. 10 The average daily gain of pigs throughout productive life originating from light, medium and heavy birth weight categories (mean ± sem).

	Light	Medium	Heavy	Pooled s.e.m	P
Birth - wean$^{\gamma}$	0.17^{a}	0.21^{b}	0.24^{c}	0.01	<0.001
Wean - grower$^{¥}$	0.42^{a}	0.45^{a}	0.48^{b}	0.01	< 0.005
Grower - Finisher	0.60^{a}	0.66^{a}	0.71^{b}	0.01	< 0.001
Finisher – Slaughter*	0.65	0.68	0.67	0.01	NS

Where superscripts differ within a row, values are significantly different. γ = wean age significant as a covariate (P <0.005), ¥ = wean weight and weaning age significant as covariate (P <0.001), * = slaughter weight was significant as a covariate (P <0.001).

4.4.5 Predictors of later health

Binary logistic regression revealed there were significant relationships between a number of prenatal and early factors and whether or not pigs would become clinically sick later in life. Results of all factors tested are displayed in Table 4.11.

Table 4. 11 Factors tested for whether pigs would become clinically sick later in life

Predictor	Coefficient	SE of coefficient	Z	*P*
Total litter size	-0.31033	0.06826	-4.55	<0.001
Sow parity	-0.0641221	0.0625721	-1.02	0.305
No. Born alive	-0.273268	0.0643753	-4.24	<0.001
Birth weight	0.0612107	0.431186	1.42	0.156
Birth- wean ADG	5.75459	2.46748	2.33	<0.05
Wean weight	0.188366	0.0835376	2.25	<0.05

Factors significant on individual level analysis (Table 4.11) lost all significance when assessed in a multiple level analysis. It is believed this could be due to changes in the degrees of freedom in the analysis. A separate further analysis revealed a significant difference in the total litter size between pigs that became clinically sick later in life and those that remained clinically healthy (Sick: 16.3, Healthy: 14.8, pooled s.e.m: 0.19, P < 0.001).

4.4.6 Factors influencing slow growing pigs

Of all pigs that were not observed as displaying clinical signs of ill health, there was not a significant probability that slow growing (2 s.d below the mean ADG for that period) pigs in the birth to wean period would be growing slowly (2 s.d. below the mean) in the wean to grower phase (Table 4.12).

Table 4. 12 The number pigs observed with standard and slow growth (2 s.d below mean) in the birth – wean and wean to grower phase (N = 628)

	Wean-grower		
Birth-wean	Standard	Slow	Total
Standard	591	19	610
Slow	17	1	18
Total	608	20	P =0.446

There was a significant probability that pigs growing slowly in the wean – grower phase would also grow slowly in the grower-finisher phase (Table 4.13).

Table 4. 13 The number pigs observed with standard and slow growth (2 s.d below mean) in the wean to grower and grower – finisher phase (N = 628)

	Grower-finisher		
Wean-grower	Standard	Slow	Total
Standard	594	14	608
Slow	16	4	20
Total	610	18	P<0.05

However, there was no significant probability that pigs growing slowly in the grower – finisher phase would continue at a reduced growth in the finisher – slaughter phase (Table 14.14).

Table 4. 14 The number of observed with standard and slow growth (2 s.d. below mean) in the grower – finisher and finisher slaughter phase (N = 628)

	Finisher - slaughter		
Grower - finisher	Standard	Slow	Total
Standard	594	16	610
Slow	18	0	18
Total	612	16	P = 1.000

Results of the multiple binary logistic regression model identified a number of factors significant in influencing slow growing pigs in the various growth stages of production (table 4.15).

Table 4. 15 Factors associated with slower growing pigs (coefficient and 95% lower and upper confidence interval)

Predictors	Birth-wean	Wean-grower	Grower-finisher	Finisher-slaughter
Pearson χ^2 (d.f)	435.238 (451), $P = 0.695$	568.291 (620), $P = 0.932$	1258.41 (620), $P = 0.000$	1512.98 (619), $P<0.001$
Sow parity	0.219608*	X	X	X
Littersize	X	NS	X	X
No. Born alive	X	0.584859*	X	X
No. Mummified pigs	X	2.13548***	X	X
No. Stillborn	X	X	X	X
Birth weight	-1.51729*	-2.26192 *	NS	X
Birth-wean ADG	~	NS	NS	X
Wean weight	~	NS	NS	X
Weaning age	X	X	NS	X
Wean-grower ADG	~	~	-17.5395^T	X
Weight into grower	~	~	NS	X
Age enters grower	~	~	X	X
Grower-finisher ADG	~	~	~	28.2332***
Weight into finisher	~	~	~	X
Age enters finisher	~	~	~	0.203365***
Slaughter weight	~	~	~	-0.480686*

Where asterisk occur, *** = P <0.001, ** = P <0.01, * = P <0.05, T = 0.064. ~ = Not considered in the model, X = not significant in individual analysis, NS = not significant in multiple analysis.

With a score of 1 equalling slow growth, and a score of 0 equalling standard growth, a low birth weight was associated with slower growth rate between the birth to wean and wean to grower stage only. Sows with an older parity influenced slow growth in the birth to wean stage. Pigs from litters with a greater number of piglets born alive and a greater portion of mummified piglets were associated with slower growth in the wean to grower stage. There were no significant associations to slow growth in the grower to finisher phase in multiple analysis, but an association at the level of a tendency (P = 0.064) between pigs that grew at standard rate in the wean to grower stage growing at a reduced rate in the grower to finisher phase. Pigs that grew well in the grower to finisher stage and entered the finisher at a higher slaughter weight were also associated with slower growth in the finisher phase. Slower growing pigs were marketed at a lighter slaughter weight.

4.4.7 Effect of DHA supplementation on health and performance

There was no significant difference in the birth and wean weight or the lifetime performance and health of piglets born to sows fed the different supplementation diets (table 4.16).

Table 4. 16 Weight, live weight gain and health of piglets (N= 449) born to sows fed DHA diets (mean and pooled s.e.m, unless stated otherwise)

	DHA diet				
	Control	Low	High	Pooled s.e.m	P
Birth weight	1.63	1.64	1.57	0.03	0.187
Wean weight	7.72	7.75	7.60	0.15	0.555
Grower	31.35	31.00	30.22	0.48	0.243
Finisher	60.05	58.50	57.64	0.75	0.068
Birth- wean ADG*	0.22	0.22	0.22	0.01	0.932
Wean-grower ADG¥	0.48	0.48	0.46	0.01	0.133
Grower - finisher ADG	0.69	0.68	0.66	0.01	0.417
Finisher - slaughter ADGγ	0.66	0.67	0.70	0.01	0.145
No. of pigs sickχ	2%	2%	1.6%	~	0.918

* = weaning age was significant as a covariate (P < 0.001), ¥ = weaning age and wean weight were significant as covariates (P < 0.001 for both), γ = slaughter weight was significant as a covariate (P < 0.001). χ = Chi-squared analysis, χ^2 = 0.712, d.f. 2.

4.5 Discussion

This study has recorded the lifetime growth and health performance of > 700 pigs to determine relationships between weight, live weight gain and health in different growth phases of growing pigs managed on a commercial farrow to finish farm. As pigs remained under the control of the commercial farm management it provides data on how a certain approach to management can affect the performance, health and profitability of the pigs.

4.5.1 The performance and health of the farm

A range of diseases are known to circulate within the farm (as described chapter 3) and the lifetime ADG of the pigs was sub-optimal at 0.59kg/day. The growth of pigs that became clinically sick was 70g lower than this, at 0.52kg/day, and those that remained clinically healthy had an average growth rate 10g higher, at 0.60kg/day. The performance of these pigs was somewhat below the UK average for rearing and feeding herds, of 0.65kg/day (BPEX, 2011c). The ADG of the pigs increased significantly between each stage of production until the finisher building, where the ADG was no different to that shown in the grower stage. The greatest number of clinical health conditions was seen in the finisher stage, although the number of clinical cases was not much more than in the grower stage, with only three more cases noted, and the types of ailment were similar. The severity of health scores was greater in the finisher building than the grower, and altogether this suggests that the pigs were facing a greater immune challenge in the later stages of production.

Examination of the CVs in live weight suggests that the variation remains fairly constant from birth, reducing as the pigs enter the finisher building. Based on a selection of data suggesting 'normal' variation, Patience and Beaulieu (2006) have suggested threshold levels for variability by which a producer can then seek to manage the variability within the herd, advising a CV of live weight at weaning to be around 20%, to be 12 – 15% at entry to the grower phase and 8 – 12% when the first group of pigs leave a batch for slaughter. The CVs found in this study are higher than those suggested as suitable by Patience and Beaulieu (2006), and suggest action could be required to reduce these on this farm. The data found in this study are similar to those reported in a survey of commercial farms in Ontario, where it was found that those with a greater number of diseases and those operating a CF production have lower growth rates and a greater CV in

comparison with those with fewer diseases and those operating AIAO production (Dewey et al., 2001). However, the study by Dewey et al., (2001) also found far larger CVs, of 31% at 7 weeks of age, demonstrating the great variability of performance between individual farms.

4.5.2 Relationships between weight and subsequent growth performance

Piglet birth weight showed negative relationships to the number of pigs born alive, which was closely correlated to the total litter size. A negative relationship between larger litter sizes and birth weight is a common finding and is believed to reflect intrauterine growth retardation (IUGR) (Morise et al., 2008). IUGR naturally occurs in litters of piglets, but at an increased prevalence with larger litter sizes. Piglets that have experienced IUGR are lower in birth weight and have been found to have a longer and thinner intestine at birth, with a modified intestinal adaptation and bacterial colonisation in the immediate post-natal period (D'Inca et al., 2011), and a reduced intestinal villous height at 2 days of age (D'Inca et al., 2010). In addition, IUGR permanently alters foetal muscle fibre development (Tse et al., 2008), which is believed to contribute to poor growth later in life (Wu et al., 2006). These physical differences of a low birth weight piglet, and the competition between littermates for high quality teats, could explain why ADG in the birth – wean period was positively related to birth weight, and negatively to litter size and the number of mummified piglets. Aside from when disease is the cause, a larger litter size increases the risk of mummified piglets being born (Le Cozler et al., 2002), which can result from insufficient space in the uterus to accommodate viable placentae for all foetuses.

An increased age at weaning positively influenced the lifetime ADG of the pigs. Although the farm operated on a weekly batch weaning at 4 weeks of age, the weaning age ranged from 24 – 35 days. This range of variation is possibly due to slight changes in production flow. The difference in weaning age could also represent a management decision by the farmer to keep smaller piglets on the sow for longer before weaning, as appears to be suggested by the negative relationship between weaning age and the birth to wean ADG.

The difference in weaning age (maximum of 11 days) was enough to significantly influence the lifetime ADG of the pigs. An improvement in lifetime ADG and mortality

has been seen in pigs as weaning age increased from 12 to 21 and 15.5 to 21.5 days of age (Main et al., 2004), and a linear improvement in ADG in piglets weaned at 8-13 or 17-21 days of age (Fangman et al., 1996). Linear improvements in ADG are likely to be due to increased weaning weight, which is directly confounded with weaning age in the present study, and hence it is more sensible to report effects of weaning age, as has been discussed by Main et al., (2004). An increased weaning age also means pigs will have an increased physiological maturity at weaning, providing benefits for the pigs. This may be linked to increased gut maturity (Miller et al., 2007), and to increased immunological competence resulting from a further developed immune system (Bailey et al., 2001).

A greater birth weight was associated with a greater birth to wean ADG, which together were significant in contributing to a greater weaning weight of piglets. This relationship has been found previously (Quiniou et al., 2002). However, weaning weight did not significantly influence the lifetime ADG, but weaning age did. A higher birth to wean ADG was negatively associated with growth at later stages of production and negatively influenced the lifetime ADG. Considered together, these relationships appear to reflect the effect of a management decision by the farm, with pigs that had a better birth to wean ADG being weaned earlier, and this later had an effect on reducing the lifetime ADG. In contrast, piglets that had poorer birth to wean ADG, and thus were possibly held back to wean later had a better lifetime ADG. This appears to indicate the pigs were moved to the next stage of production not on the basis of their age, but their size. This is further demonstrated by the negative relationships between the age at which pigs entered the grower and finisher accommodation and their lifetime ADG, suggesting slower growing pigs were held back and moved up the production system later than faster growing pigs. This type of management has negative implications for production as the difference in age spread between pigs housed in the same room increases, and the older, slower growing pigs are being held back in the system contributing to disease spread to the younger pigs.

The positive relationship of birth weight and birth to wean ADG early in life, however, was not a significant determinant of lifetime ADG. Clinical illness was influential on the live weight gain of the pigs in the grower stage but was not influential on the lifetime ADG. However, the observation and recording of clinical health conditions is not able to measure the effect of any sub-clinical health problems that may be circulating in areas of

the production system, affecting the overall performance. It was the performance of pigs in each stage, and the age at which they were moved to following stages (which could be a reflection of the ADG of each stage) which had the greatest influence on the lifetime ADG of the pigs. Health is known to affect the performance of pigs, and this lack of association could be indicative of the presence of sub-clinical disease since only associations to clinical disease could be noted.

As an attempt to investigate any activity of sub-clinical disease, investigation of the factors contributing to pigs growing at a reduced rate (as defined by 2 s.d. from the mean, and not noted for clinical signs) in each production stage demonstrated that a lower birth weight contributed to reduced growth in the postnatal period and early weaner stage, but did not have lasting effects. Other factors influenced slow growth at later stages. Slow growing pigs in the grower to finisher phase were only influenced by the ADG in the wean to grower phase at the level of tendency, and this is in agreement with the significant probability that pigs which grew slowly in the wean to grower phase would also have reduced growth in the finisher phase. Whether pigs grew slowly in the finisher phase was dominated by age and growth rate in the grower to finisher phase. This differed from the results of all pig analysed (table 4.9) and demonstrates that increased age and a slower growth in the grower to finisher period were linked to a slow growth in the finisher to slaughter period.

The weight of the pigs in the birth weight groups remained significantly different; indicating those pigs born light, remain light, which is the same result found by (Rehfeldt and Kuhn, 2006), who classified piglets in similar mean weight groups. The ADG of pigs in the birth weight groups was significantly different in the birth to wean, wean to grower and grower to finisher phase, with no differences in growth rate in the finisher phase. Pigs in all birth-weight groups grew at significantly different rates in the birth-wean phase, however, from the wean to grower phase only the light and medium pigs grew significantly differently from the heavier weight pigs. This result differs from that found by Rehfeldt et al., (2008), who found a prolonged significant difference in postnatal growth between three birth weight groups with similar mean weights to those in this study. Dunshea et al., (2003) found heavier weaned pigs ate more feed than lighter pigs (age for age). The lack of difference in ADG between the lower and medium birth weight

groups could suggest specific effects on the performance of pigs at the farm level, such as disease pressure and social effects.

The difference in the liveweight and ADG of the pigs in different birth weight groups which remained significantly different throughout production, demonstrates that the smaller pigs require extra days to reach slaughter weight simply to make up the difference in weight which existed from birth. The present study cannot demonstrate this due to the differing slaughter weight of the pigs, however, it has been shown by (Beaulieu et al., 2010), who found a difference of 20 days to market for pigs grouped as heavy (1.75 - 2.50kg) and light (0.80 – 1.20kg), which is a substantial difference in time for building turnover. In addition, a higher ADG is highly correlated with feed efficiency (Robson, 1976), and thus further costs could be accumulating from reduced live weight gain of smaller pigs.

To reduce the effects that birth weight and prenatal factors have on lifetime development management to optimise weight at birth and weaning are often applied to try and reduce variability and maximise postnatal performance. Cross-fostering of piglets amongst litters born within 24 hours of each other is often performed to assist low-birth weight piglets in competing only against piglets of a similar size (Cutler et al., 1999), and also to even out the piglet to teat ratio. When recording performance, cross-fostering was found to not affect the weight gain of the pigs nor overall litter survival (Milligan et al., 2001), and rather cross fostering is repeatedly linked to increased risk factors for the spread of disease (Andraud et al., 2009, Elbers et al., 2006). Larger sizes will inevitable reduce availability of teats, which would limit access to milk. Providing litter size does not exceed the number of functional teats, the milk yield of sows has been found to relate linearly to littersize (Auldist et al., 1998), and therefore, providing optimal nutrition to boost sow milk quality and fostering only when the number of piglets exceeds the number of teats may be the best course of action.

Indoor producers will often provide a creep feed prior to weaning to encourage uptake prior to conversion to full time feeding of creep at weaning, with the aim of improving daily gain. Whether creep feed is useful is debatable based on evidence from studies. Provision of a creep feed diet for varying durations has been found to provide no benefit

to the ADG, feed intake and feed efficiency of piglets, with those that did and did not consume the creep feed being equal in performance at weaning at 28 days (Sulabo et al., 2010), whilst others have found the provision of creep feed pre-weaning able to increase feed efficiency and overall weight of the litter (Lynch et al., 1998). Creep intake is known to be influenced by the milk supply of the sow, the diet formulation, age and the management of the pigs (Lynch et al., 1998). In this study, whilst creep intake was not measured, the birth to wean ADG was influential on the lifetime ADG, therefore creep feeding in this period may be a contributing factor to improved performance, potentially through increasing the readiness by which pigs will consume feed, and aiding adaptation of the gut to solid food. This has been found to increase performance in the post-weaning phase, and was found as the only benefit of creep feeding in the particular study by Sulabo et al., (2010). However, farm specific factors are likely to be influencing the differing results between the studies.

In trying to assist the growth of lighter pigs further, feeding a diet higher in lysine pre-weaning (Mróz et al., 1987) and post weaning has been able to significantly increase the ADG of pigs in the immediate feeding period (Lynch et al., 1998). However, no continued benefit in gain was sustained in either study, with the performance of pigs equalling when fed conventional diets. Lynch et al., (1998) concluded that pigs may be able to compensate for stunted growth if fed an adequate diet. It would be worthwhile to investigate whether the increased ADG of pigs periodically fed a high lysine feed could be enough to make up the difference in lost growth between lighter and heavier pigs when it is really required. Feeding a diet higher in certain amino acids could be a solution for farmers to deal with a greater amount of variability; however the increased feed cost may not be a sustainable option, and requires a cost benefit analysis. and the effect at feeding at different stages during production too , if aiming to promote growth, should be investigated for any effect on carcase quality.

With conflicting results in literature, the efforts to maximise postnatal gain may not aid lighter pigs as physiologically they can be different to heavier pigs of the same age, being underdeveloped (Pluske et al., 2003). Where weaning weight is found as a factor, it can be a reflection of birth weight since such correlations were found in this study. Weight at weaning has been found to be poorly associated with post-weaning performance, and rather the age of the pigs at weaning has a significant association with the lifetime

performance (Lynch et al., 1998), as was found in this study. This suggests that there may be over- emphasis on higher weaning weights, when more should be placed on the development of the pig. A need for the economic evaluation of techniques used in commercial piggeries to assist small pigs has been highlighted by Quiniou et al., (2002), which may answer the question as to whether there is a limit to which small pigs should be helped.

The development of the pig at different stages in the production cycle has been found to be significant in affecting performance, as was displayed by age relationships in this study. In newborn piglets, birth weight is critical for survival, and the work of Baxter et al., (2008) highlights the importance of prenatal development in aiding piglet survival. Strategies to improve the prenatal growth development of the pig is an area producers should optimise, through nutrition and management of the sow. However, amongst other factors interactions between nutrition and metabolism of the sow and oocyte quality are all highlighted as playing a role in the prenatal development of pigs, and investigation of these mechanisms is required to move forward the area of research on prenatal programming in pigs (Foxcroft et al., 2009).

4.5.3 Relationship between prenatal and early postnatal factors and clinical health later in life

This study has found a relationship between prenatal and early postnatal factors and subsequent clinical disease on an individual analysis level. Total litter size and the number of piglets born alive, (which will be closely related to the total litter size), played a significant role in whether pigs later suffered clinical cases of disease. This is an important and interesting finding in providing a means by which producers may be able to have a prior awareness of which pigs may be at a greater risk of susceptibility to disease, or may benefit from alternative management. This is especially so as genetic selection for larger litter sizes to maximise sow productivity has been on the increase. A larger litter size is often associated with a reduced birth weight (Morise et al., 2008). In this study a lower birth weight was associated with an increase in the total number of pigs born alive, which was closely related to the number total litter size. The significant effect of total litter size suggests that pre-natal factors could influence health. In humans adverse development early in life (which has included low birth weight, poor postnatal growth and famine in *utero*) has been linked to a range of negative health consequences arising

from alterations to development of the immune and endocrine systems, amongst other things (Lummaa and Clutton-Brock, 2002). The association between total litter size and low birth weight often found suggests that IUGR occurs on a larger scale in larger litter sizes. Piglets that have experienced IUGR have been found to have a longer and thinner intestine at birth, with a modified intestinal adaptation and bacterial colonisation in the immediate post-natal period (D'Inca et al., 2011), and had reduced intestinal villous height at 2 days of age (D'Inca et al., 2010), demonstrating immaturity in colostrum induced intestinal adaption. This has been indicated as having a possible effect on the impairment of immune function in IUGR pigs later in life (Wang et al., 2008, cited in D'Inca et al., 2010). In larger litters, colostrum availability could also be limiting (Boulot et al., 2008), reducing passive immunity of piglets and having possible adverse effects on development of active immunity (Devillers et al., 2011). The ADG of the piglets in the period between birth and weaning was also a significant factor, with piglets of a lower weight gain being linked to the occurrence of clinical sickness later in life. Wean weight was also a significant factor, but this is likely due to the strong correlation with birth to wean ADG. These associations between early weight and weight gain may also be linked to the intake of the pigs colostrum and subsequent milk intake, with Devillers et al., (2011) demonstrating that birth weight and body weight were linked to colostrum intake. Piglets with a lower colostrum intake also had a lower weight gain, and a lower immunoglobulin G (Ig G) concentration.

Few studies have assessed the effect of pre-natal factors on the subsequent health of pigs to slaughter, with the majority of studies focusing on performance. However, in humans adverse development early in life (which has included low birth weight, poor postnatal growth and famine in *utero*) has been linked to a range of negative health consequences, as previously stated (Lummaa and Clutton-Brock, 2002). Gardner and Hird (1990) found a low birth weight (less than 1kg) and younger weaning age (less than 24 days) had small but significant effects on the extent of pneumonic lesions found in pigs slaughtered between 30 - 50kg. In light of the evidence in human research, this is an area that requires further investigation. Selection for a standard littersize equal to the number of functional teats (14), under optimal nutritional management, rather than an increased litter size with similar management may be a more sensible way forward, and should be investigated.

4.5.4 Dietary inclusion of DHA to sow diets

Piglets born to sows fed dietary inclusion of tuna fish oil during gestation have been found to have grown faster in the first 35 days of life than piglets from sows fed a basal diet (Rooke et al., 2001). In terms of pig health there has been an interest in inclusion of DHA in diets for the potential ability for the fats to have an immune modulating influence (Yaqoob, 2010), which may lead to better growth rates through the modulation of the cytokine response. However, in this study, inclusion of increased levels of dietary DHA in the sow diets showed no real benefits for piglet production, and had no effect on lifetime performance and health.

4.5.5 A note on management

A strength of this study is that it provides actual data on the treatment and management of pigs on a commercial farm. Whilst this should be happening on all farms, the records are often sparse in this respect. Medicine record keeping must, by law, note all the treatments given to pigs. The extent of sickness in the grower and finisher stages, however, would not be represented since untreated and sub-clinically ill pigs are not recorded. While this type of data is not well documented in literature it does, however, agree with the generally accepted view that producers dedicate a greater amount of attention to the breeding sows and weaning piglets, important for ensuring reproductive performance, survival, and a good start in life for weaner pigs. This has been suggested by Madsen and Kristensen (2005) who refer to work of the Danish Agriculture Advisory Service proposing that the average labour time invested per finisher pig produced is little at 10-12 minutes.

The reduction in veterinary treatments in the grower phase and lack of them in the finisher phase demonstrate management that does not dedicate enough attention to finisher pigs. However, with performance at its worst in the finisher building, it could also demonstrate a desire not to spend money on veterinary drugs when the pigs are performing poorly and nearing slaughter. The data on the length of time pigs remained in the production system revealed a surprising state of affairs; that one pig remained in production for nine months (264 days). This particular pig had suffered lameness problems, but not of sufficient severity for euthanasia. A pig remaining in the system so long is a guaranteed loss. Since it is likely to have been consuming around ~2kg of feed per day in the finisher phase, and remained in the finisher for 147 days, it would have

consumed an estimated total of 294kg of feed and gained only 53kg in weight (ADG of 0.36kg), which results in a FCR of 5.5. The cost in feed alone for this pig in the finisher stage, at £202.55/tonne (BPEX, 2011c) would be £59.55. Altogether this is a predictable loss and cost the producer a lot of money.

The data from this study demonstrate that refraining from treating pigs with clinical disease, and allowing sick pigs to remain in the system, raises concerns both for the welfare of the pig and health management in general. The sick pig can continue to spread disease, especially respiratory disease such as pneumonia. Removing sick pigs to a hospital pen outside the area of other producing pigs is the recommended option and, with prompt and correct management, the use of hospital pens has been found to cut mortality of sick pigs and contribute positively to farm cash flow (Muirhead, 2000).

4.6 Conclusion

This study provides complete data on the lifetime growth and clinical health of pigs on a commercial farm under the standard management of the farm. The data from this study suggests that many different inter-related factors significantly influenced the growth performance of pigs at different stages of the production system, and their effects should warrant further research. In general, the litter size, numbers born alive, birth weight and early birth to wean ADG was often linked to performance of the pigs in the early stage of production. Smaller litter sizes associated with heavier weights and better ADG. Pigs of a lower birth weight were also associated with growing particularly slow (at 2 s.d. below the mean) in the birth to wean and wean to grower stage, and may reflect that these pigs could be more susceptible to sub-clinical disease. However, these factors did not always show continuing significance towards affecting the growth rate of pigs at later stages of production. Often factors closely related to the following stage were significant as influential on subsequent growth and demonstrate the importance that management at each stage of production has in contributing to the growth and overall lifetime performance of the pig. The relationships found in this study were similar to those found elsewhere and demonstrate that, when assessed in birth weight groups, piglets of lower birth weight are more likely to grower slower throughout life. These pigs were in this respect disadvantaged from birth and could possibly take longer to reach slaughter. The relationships found suggest that physical competency of the pigs is a strong influence on

how well the pigs will perform, with weaning age, rather than weight, being influential on the lifetime growth of the pigs suggesting that physiological maturity at weaning affects lifetime performance. The significant relationships between the litter size and birth to wean ADG and the wean weight of pigs, and whether pigs would become clinically sick or not later in life, are important findings which suggest that the development and early viability of the piglets are connected to influencing long term health. Producers could observe this and it may be that different management of such pigs would be favourable. An optimal litter size may be the best target for producers, rather than selecting for increased litters. Together, these are areas that require further investigation and cost benefit analysis. The information provided from this study on the administration of veterinary treatments to pigs at different stages of the production system, whilst under commercial management, reveal some concerning findings, that may be happening elsewhere, and are negative in terms of pig welfare or health control. Pigs not monitored properly and remaining in the system as slow growers will cause significant losses to production.

Chapter 5: Concentration of Acute Phase Proteins in serum and oral fluid of finishing pigs in relation to age, productivity and health status

5.1. Introduction

Acute phase proteins (APPs) are a group of non-specific innate immune components released by hepatocytes upon immune stimulation (Murata et al., 2004). They provide one of the earliest signs of immune activation; the concentration of positive and negative APPs will increase or decrease respectively in response to inflammatory processes, systemic and localised disease, tissue damage and injury (Heegaard et al., 2011). There is also evidence to suggest that APP production could respond to stress (Piñeiro et al., 2009a).

Haptoglobin (Hp), C-reactive protein (CRP) and Pig Major Acute Phase Protein (Pig-MAP) are considered to be the three major positive APPs in pigs (Niewold et al., 2003). All are highly sensitive (Chen et al., 2003, Heegaard et al., 2005), reacting to acute clinical and sub-clinical experimental infection in pigs (Sorensen et al., 2006), with an up-regulation in concentration of two to threefold, five - 100 fold, and eight – 12 fold respectively in response to an inflammatory stimulus (Niewold et al., 2003, Barrios et al., 2005). The concentrations of APPs closely follow the clinical course of disease (Martín de la Fuente et al., 2010) and the magnitude of the APP response is closely related to disease progression and recovery (Eckersall, 2004). Therefore, by studying changes in the concentration of APPs it has been possible to distinguish between sick and healthy individuals on farm (Gutiérrez et al., 2009c) and at slaughter, with studies showing alterations in APP levels in pigs suffering from respiratory disease (Amory et al., 2007), wasting (Yamane et al., 2006) and a variety of other clinical signs (van den Berg et al., 2007).

In human medicine, APPs have become biomarkers providing predictive signals that highlight individuals at risk of coronary heart disease (Pearson et al., 2003) and diabetes (Freeman et al., 2002). This has led to recommendations for those at risk to receive medical treatment in advance of the condition developing (Pearson et al., 2003). In clinically ill cattle it was possible to differentiate between acute and chronic inflammatory conditions based on the APP response, and with greater accuracy than haematological

tests (Horadagoda et al., 1999). In sheep Hp was a more sensitive and specific marker for the presence of a bacterial infection, and less likely to give false positive and false negative results than haematological tests (Skinner and Roberts, 1994).

Since concentrations of APPs change before the occurrence of antibodies (Parra et al., 2006), their value as a measure for general increased immune activity at certain time-points of the production chain can assist in pinpointing the exact stage of development of a particular disease, that could be circulating undetected. With regular monitoring, APPs could provide a measure for predicting disease progression and recovery throughout a production system. The increased immune activation indicated by APPs has been found to be negatively correlated to weight gain of pigs (Williams et al., 1997b), whilst Hp in particular has been found to be a good marker of weight gain in pigs (Eurell et al., 1992). Therefore, APPs could provide a potential indicator for detecting areas of sub-optimal performance in pig production operations.

It would be of particular interest to determine whether monitoring levels of APPs could provide an objective indicator of the presence of sub-clinical disease. Sub-clinical disease has particularly devastating effects on production since infected animals can remain undetected, making control very difficult. Sub-clinically infected pigs will often have reduced growth rates and higher feed conversion ratios, resulting in lost productivity and increased costs. Currently there are no objective markers to allow the assessment of sub-clinical disease on farms (Athanasiadou et al., 2010). It has been suggested that an APP response in the absence of clinical signs could indicate a sub-clinical state of infection. This has been demonstrated with pigs infected to a sub-clinical level with *Streptococcus suis* (Sorensen et al., 2006), Aujesky's disease (Parra et al., 2006), sub-clinical post weaning colibacillosis (Houdijk et al., 2007), and pigs given a low-dose of lipopolysaccharide (LPS) as a model of sub-acute inflammation (Moya et al., 2006). Induced sub-clinical infections that have been systemic (*Listeria monocytogenes*) and localised (*Enterotoxigenic E.coli*) resulted in up-regulation of APPs and a reduction in growth performance (Athanasiadou et al., 2010).

APPs have been detected in a variety of body fluids, namely whole blood, serum, meat juice and oral fluid (OF) (Hiss et al., 2003, Gutiérrez et al., 2009b). OF is a sample of fluid from the oral cavity collected via an absorbent device (Atkinson et al., 1993) which

contains a mixture of saliva and gingival crevicular fluid (the latter having a composition similar to serum) (Rai, 2007). The measurement of APPs in OF, being low cost and of minimal stress to the animal, enables regular real-time monitoring of levels in pigs. Hp and CRP have now been repeatedly and reliably measured in OF using an adapted time resolved immunofluorometry assay (TR-IFMA) (Gutiérrez et al., 2009b, Gutiérrez et al., 2009a). This method of analysis has been used to compare the concentration of APPs in OF of pigs that were experimentally infected with PRRSv (Gómez-Laguna et al., 2010) and under field conditions comparing the APP values on a Specific Pathogen Free (SPF) farm to one with a known PRRSv infection (Gutiérrez et al., 2009c). These studies found good agreement in the levels of APPs in OF with levels in other biological fluids tested, confirming that monitoring APPs in OF could serve as a complementary, or possibly alternative measure to blood testing. OF has the additional advantage of being easily collected at a group level, offering the possibility for collection of pooled samples and larger scale population sampling for surveillance purposes (Prickett and Zimmerman, 2010).

Before APPs can be employed as markers of sub-clinical disease extensive studies are required to provide reference values for the concentration of these proteins in both healthy and sub-clinically infected animals. Therefore, the objectives of the present study were to evaluate the use of OF in individual pig and pooled pen samples as an alternative to serum, to evaluate the ability of APPs in both fluids to provide a measure of pig herd health and performance, and their potential to elucidate areas of sub-clinical disease occurrence in a group of finisher pigs. With a history of PRRSv on the study farm, a further aim was to investigate the sensitivity with which APPs could detect a sub-clinical state of PRRSv, and the level of immune activation and productivity at the point of sero-conversion. In doing so, this study provided an opportunity for work to be conducted on the detection of the European strain of the PRRSv in OF using PCR. Whilst OF sampling has been successful with the US strain (Prickett et al., 2008a), further work is required to achieve good and consistent levels of detection of the European strain.

5.2. Materials and Methods

5.2.1 Animals, housing and management

This study was conducted at a 120 sow farrow-to-finish unit, as used in the previous experiments (see Chapters 3 & 4). Production data from ~700 pigs tracked and monitored on this farm demonstrated that the finishing herd performance was sub-optimal at 0.59kg/day (see Chapter 4). Information from previous serological tests indicated that a number of diseases were present within the herd (Chapter 3, section 3.2.1) and, with relatively few clinical signs of disease occurrence, the sub-optimal growth rate indicated the presence of sub-clinical diseases.

For this experiment levels of APPs in 80 grower-finisher crossbred pigs ((Large White x Landrace) x (Duroc x Pietrain)) were studied. Lifetime performance of the pigs was monitored as part of a larger study (see Chapter 4). Over a period of four weeks, pigs were consecutively selected at the point of entry to a fully slatted finisher house, producing a cohort of different ages. Two groups of ten pigs were formed per selection, based on weight, to produce a light (mean ± s.e.m 47.1 ± 0.73kg) and heavy group (60.5 ± 0.8kg). The gender in the pens was mixed, but not equalised as it was a greater priority to select within the correct weight range.

Pigs were housed in one room of the two-room finisher house, in pens measuring 1.46 x 3.79m, with open bars allowing nose to nose contact between pigs in adjacent pens. The ventilation in the building was controlled by fan ventilation which adjusted in response to temperature, with the thermostat set at 19˚C. Pigs were fed *ad-libitum* a pelleted finisher diet from a feed hopper with five feeding spaces (for diet details see Chapter 4, section 4.2). All feed inputs were recorded. Water was provided *ad-libitum* via two drinking nipples per pen. Artificial light was provided to the pigs between the hours of 08.00 and 16.30. However, natural light could also enter the finisher house through the ventilation windows.

5.2.2 Production and health measurements

Pigs were weighed once every two weeks and the weight of remaining feed recorded to give an accurate pen feed intake for the period between each weighing. Pigs were checked daily for signs of ill health and any conditions and veterinary treatments recorded. Pigs were removed from trial if they showed no improvement in health

following treatment, or had an ailment that required separation of individual pigs, such as rectal prolapse, in line with the protocol described in Chapter 3, section 3.2.4.1. A health score (as performed in Chapter 3) was conducted once each week as a general overview of the health of the pigs per pen.

Pigs were consigned to slaughter at a live weight between 70-85kg (cutter weight). At slaughter the lungs were scored for pneumonia on 55 point scale (Goodwin et al., 1969) and the stomachs removed. Stomachs were brought to the University laboratory where they were cleaned and the area around the pars oesophagea region scored for keratinisation and ulcers on a scale of 0-5, in accordance with that developed by Potkins et al. (1989). Carcase weight and backfat depth measured at the P_2 position (mm) was obtained for each pig from abattoir records.

5.2.3 Measurement of Acute Phase Proteins

Paired serum and OF samples were collected from all pigs when the oldest group reached slaughter weight, ensuring that live weight and age varied at the point of sampling. Due to the volume of work involved and the availability of technical assistance, OF and blood samples were collected on separate but consecutive days. As the less stressful procedure, collection of OF was performed the day before blood collection to avoid stress resulting from blood collection potentially affecting the levels of APPs.

As there is evidence that the collection material could influence the levels of analyte to be detected (Strazdins et al., 2005), 20 OF samples were collected in duplicate using cotton rope (Outhwaites Ropemakers, Hawes, UK) and synthetic polymer foam swabs (Salimetrics Oral Swab, (SOS), Salimetrics Europe Ltd, UK). To obtain OF from individual pigs, the 18mm diameter cotton rope was cut into lengths of 30cm, and the three strands untwisted to provide thinner strands for ease of collection. Sections of rope were offered to individual pigs to chew. Following collection, pigs were marked with coloured spray to identify those which had been sampled. This method was not quick, taking 30-60 minutes to sample a pen of 10 pigs, but was of minimal stress to the pigs as they remained in their pen group with minimal human intervention.

Where repeat OF collections were made with the use of the SOS, this was done immediately after the cotton rope was removed from the pig's mouth. To obtain the

sample three SOSs were threaded onto a section of 0.6mm steel wire to retain them in place and then passed into the pig's mouth. After removal from the pig each section of rope was placed in individually labelled sample bags and SOS in salivette tubes, sealed and labelled. Following collection from one pen the samples were sent to the laboratory on the farm site to be processed for extraction of OF.

A pooled OF sample was collected from each pen group as described by Prickett et al., (2008b), on the same day as individual sample collection. Each pen was provided with one length of 18mm diameter cotton rope, suspended over the pen via a wooden bar (as described in detail in Chapter 6, section 6.2.3). The length of cotton rope was cut to 60cm, allowing it to hang at pig shoulder height. The rope was presented for a total duration of 60 minutes, after which it was removed, placed in a plastic bag, sealed, and taken to the on-site laboratory for processing. For each piece of rope used, the damp section of rope was cut off and placed in a 14cm x 14cm pouch made from durable autoclave bags (Fisher Scientific, UK). A series of small holes were made in the base of the pouch and the pouch suspended in a 50ml falcon tube (Starstead, UK) and secured with elastic bands. Tubes were then spun at 1,500g for ten minutes and the OF extracted. For some tubes it was necessary to re-spin the tubes for longer to remove the OF.

Blood samples were taken through puncture of the jugular vein into 10ml plastic vacutainer tubes coated with silica as a clot activator (BD Vacutainer, UK). Fresh needles were used for each pig. During sampling pigs were restrained through use of a nose snare. Following collection, blood samples were allowed to stand for one hour until clotted, after which they were spun in a centrifuge at 2000*g* for ten minutes and serum extracted. Following extraction, samples were stored immediately at -20°C. Following completion of processing, samples were transported on ice back to the University laboratory where they were subsequently stored at -80°C. On the day following the completion of blood sampling, samples were separated and shipped on dry ice to: i) the University of Murcia Veterinary Hospital, Spain, for analysis of Hp and CRP in serum and OF; ii) PigCHAMP Pro Europa, Zaragoza, Spain for the detection of Pig-MAP in serum and OF, and iii) the Animal Health and Veterinary Laboratories Agencies (AHVLA) in Weybridge and Penrith, UK for analysis of detection of virus levels and antibodies to PRRSv in serum and OF.

5.2.4 APP determination and serology

Concentrations of Hp and CRP were determined in serum and OF via TR-IFMA as developed at Murcia University (Gutiérrez eta l., 2009a, Gutiérrez et al., 2009b). These assays have a limit of detection of 0.52ng/ml for Hp (Gutiérrez et al., 2009a) and 0.47ng/ml for CRP (Gutiérrez et al., 2009b). (Further details of the TR-IFMA measurement of CRP and Hp can be found in appendices A). Concentrations of Pig-Map were determined in serum by use of the commercially available Pig-MAP stick®, a one-step immunocromatographic method for the detection of elevated levels of Pig-MAP concentration in serum (PigCHAMP Pro Europa S.A., Segovia, Spain). Concentrations of Pig-MAP were detected in OF by use of a porcine specific sandwich ELISA test using two anti Pig-MAP monoclonal antibodies, (PigCHAMP kit ELISA, PigCHAMP Pro Europa S.A., Segovia, Spain). This ELISA test was originally validated for the detection of Pig-MAP in serum (Piñeiro et al., 2009b), and was not validated for use with OF. This was the first attempt to determine Pig-MAP in OF using this ELISA. The analysis procedure was conducted according to Piñerio et al., (2009b). However, in order to increase the sensitivity of the ELISA for OF, the quantity of antibody fixed to the plate was increased and the concentration of conjugate were increased in order to increment the limit of detection. This gave a limit of detection of 50ng/ml (M. Piñerio, personal communication, 23rd August 2011).

Serum samples were analysed for the presence of PRRSv RNA (ORF 7) using a quantitative real-time TaqMan® PCR test for the detection of the European strain (Genotype 1), and for the presence of antibodies against PRRSv using a PRRSv Ab ELISA validated by the AHVLA (test code: TC0412) in accordance with the test protocol of the HerdChek X3 PRRS antibody test kit (IDEXX Laboratories Inc, Maine, USA). Detection of PRRSv in OF samples was conducted by real-time PCR for detection of the European strain adapted from a methodology developed by Kleioboeker et al., (2005). Further details of the analysis for the determination of PRRSv in serum and OF can be found in Appendices B.

5.2.5 Statistical analysis

Statistical analysis of the data was performed with the use of the statistical packages Minitab 15.0 and SPSS 17.0 statistical software. Prior to analysis, all data were checked for normality using the Anderson-Darling test. Data which were not normally distributed

were transformed where possible and an appropriate parametric or non-parametric test selected. Descriptive statistics were calculated to provide the mean, minimum, 25th percentile, median, 75th percentile and maximum of the APP values detected in serum and OF from all individual pigs, along with the pooled OF sample from each pen of pigs. Per pen, the concentration of Hp and CRP in individual OF samples was used to calculate average concentrations of Hp and CRP per pen.

The average daily gain (kg) (ADG) of all pigs sampled was calculated for each of the different production stages from birth through to slaughter and between each fortnightly weighing period in the finishing stage, to relate APPs to long term growth performance of the pigs. To provide a basis by which the APPs could be linked to the immediate productivity of the pigs, the ADG of each individual pig was calculated for the period of daily gain during which sampling took place. In addition to production details, the health data on farm and at slaughter were collated for each pig. A yes/no score was awarded to each pig for the presence of respiratory lesions (Amory et al., 2007). For pen data, the ADG and FCR were calculated together with average scores for the weekly health assessment and lung and stomach scores at slaughter. To establish if the APPs could be linked to weight variability within pens, the standard deviation (s.d.) of pen weights was calculated at entry to the finisher building and at the time before each pen was sampled. A score of whether pens were positive or negative for PRRSv in OF, the percentage of pigs with PRRSv found in serum and the percentage of the pen to have seroconverted to PRRSv was calculated.

Pearson and Spearman's rank correlations were used to examine relationships between the concentration of APPs both within and between individual and pen average serum and OF samples, including the pooled pen OF samples.

A general linear model (GLM) within analysis of variance (Minitab) was used to explore any differences between the health and productivity of pens designated heavy and light pen groups.

Multiple stepwise regression analysis was performed on individual pig and pen data to determine which measures of pig health and performance were associated with the levels of APPs. Residuals were stored and checked for normality. For each APP, to determine

which terms to place in the model, each factor displayed in Tables 5.1 and 5.2 was placed individually with the APP concentrations into a linear regression model. Those factors that had a P value ≤ 0.05 from the single regression model were entered into the multiple regression model.

Table 5. 1 Factors placed individually into a single regression model against the concentrations of Hp, CRP and Pig-MAP detected in the serum and OF of individual pig

	Individual pigs
Production	Age
	Days in the finisher building before sampling
	Finisher entry weight (kg)
	Finisher gain/day (kg)
	Lifetime gain/day (kg) (i.e. from birth to slaughter)
	ADG at time of sampling (kg)
	Individual pig weight at sampling (kg)
Health	Lung score
	Stomach score
	Presence of lung lesion (yes/no)
	PRRSv antibody S/P ratio
	Serum positive/negative to PRRSv antibody
	Serum positive/negative to PRRSv
	OF positive/negative to PRRSv

Table 5. 2 Factors placed individually into a single regression model against the concentrations of Hp, CRP in the pooled OF samples per pen, and against the pen average concentration of Hp, CRP and Pig-MAP detected in the serum and OF.

	Pen data
Production	Age
	Heavy/light group
	Pen start weight (kg)
	S.D of pen start weight
	S.D. Of pen weight at sampling
	Feed conversion ratio
	Feed consumed/day (kg)
	ADG (kg)
	P2 (mm)
Health	Lung score
	Stomach score
	Cough score
	Sneeze score
	Pig cleanliness
	Scour score
	Pen health score
	Cold carcase weight (kg)
	PRRSv in OF (%)
	PRRSv in serum (%)
	% of pen seroconverted to PRRSv

Differences between the APP concentration in OF collected by rope and SOS were explored via the Wilcoxons signed rank matched paired test. Correlations between APP levels found in the OF collected via rope and synthetic swab were made via Spearman's rank correlation and, for the concentration of APPs in the respective serum samples, via Pearson's correlation.

5.3. Results

5.3.1 Concentrations of APPs in serum and OF

The descriptive statistics of the individual concentrations of APPs found in individual serum and OF samples, along with pooled pen OF samples are shown in Table 5.3. The concentrations of all APPs were higher in serum than in OF. There was a greater variability in the concentration seen in OF Pig-MAP, followed by the concentrations of CRP and Pig-MAP in serum and OF CRP. The concentrations of Hp and CRP in the pen pooled OF samples showed less variation in the sample concentration.

Table 5. 3 Descriptive statistics of the APP concentrations in serum and OF from individual pigs and pooled OF samples.

	Hp serum (mg/ml, *n* = 80)	Hp OF (µg/ml, *n* = 80)	Hp pooled OF (µg/ml, *n* = 8)	CRP serum (µg/ml, *n* = 80)	CRP OF (ng/ml, *n* = 77)	CRP pooled OF (ng/ml, *n* = 8)	Pig-MAP serum (mg/ml, *n* = 80)	Pig-MAP OF (µg/ml, *n* = 70)
Mean	1.51	1.32	1.68	127.40	49.18	33.72	0.84	0.33
Minimum	0.04	0.23	0.75	17.30	11.76	25.24	0.32	0.05
1st quartile	0.88	0.69	1.03	65.10	28.97	26.43	0.55	0.05
Median	1.44	1.22	1.77	110.80	39.49	28.83	0.67	0.08
3rd quartile	2.05	1.65	2.18	146.90	57.61	34.15	0.84	0.23
Maximum	3.52	3.81	2.63	458.60	214.79	67.39	3.87	3.85
CV	53.93	60.62	38.84	71.01	69.65	41.44	73.46	0.20
Range	3.48	3.58	1.88	441.3	203.03	42.15	3.55	3.80

5.3.2 Correlations between the APPs in serum and OF

Positive but weak correlations were found between the concentrations of CRP and Hp found in serum and in OF, whereas no correlations were found between the concentration of Pig-MAP in serum and OF (Table 5.4). The concentrations of Hp and CRP within OF and within serum were positively and strongly correlated. In serum, the levels of CRP and Hp were positively correlated to the levels of Pig-MAP. CRP was also correlated to Pig-MAP in OF, but Hp was not. There were no correlations between the average pen CRP and Hp concentrations in serum and the pooled pen CRP and Hp OF concentrations.

Within OF and serum, positive correlations were found between CRP and Pig-Map, and Hp and Pig-MAP in serum only. However, the strength of these correlations differed, with a stronger relationship existing between Hp and Pig-MAP (Table 5.4).

Table 5. 4 Correlation coefficient and level of significance between concentrations of Hp, CRP and Pig-MAP in serum and OF samples from individual pigs. Significance: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

		CRP serum	Hp serum	Pig-MAP serum	CRP OF	Hp OF
Serum	CRP					
	Hp	0.524***†				
	Pig-MAP	0.290**	0.564**			
OF	CRP	0.272*†				
	Hp		0.297**†		0.734***†	
	Pig-MAP			0.063	0.305*	0.234

All correlations performed by Spearman's rank unless stated otherwise. Those marked with † performed by Pearson's correlation using log 10 of data.

The average concentration per pen of individual pig OF CRP showed a strong positive correlation to the concentration of CRP detected in the pooled pen OF ($r_s = 0.786$, $P < 0.05$). However, there was no significant relationship found between the average concentration of OF Hp from individual pigs per pen and the concentration of Hp in the pooled OF pen sample.

5.3.3 Pig health and productivity

Table 5.5 shows the productivity of pigs studied and from which paired serum and OF samples were collected. Out of the 80 pigs, only 4 pigs were seen with clinical signs of early pneumonia (coughing), and one pig had advanced pneumonia (laboured breathing, losing weight) and was subsequently removed from trial. Only one pig was seen to have scour, although liquid scour was seen on the floor of two other pens during weekly health scores. The severity of individual lung lesions ranged from 0 to 40, but the overall average lung score was low. Some 98.8% of pigs had stomach lesions. With the ulceration score ranging from 0 to 5, an average score above 3 indicates that most pigs had moderate to severe parakeratosis of the pars oesophagea region. There was no mortality within the pigs studied and all except the one removed due to pneumonia were marketed at slaughter.

Table 5. 5 Productivity and health ailments of all pigs studied (n = 8, pen mean and range)

	Finisher ADG (kg)	FCR	No. of pigs seen coughing	Cough score	Lung score	Scour score	Stomach score
Mean	0.65	3.31	0.05	1.50	3.30	1.32	3.45
Range	0.52-0.72	2.51-3.93	0-2	0-4.5	0.9-6.5	1-1.8	2.6-4.1

The pens designated as heavy and light groups had significantly different start weights at entry to the finisher building. Between the groups there were significant differences between the ADG and the FCR, with the heavier pen groups performing worse (table 5.6). However, between the groups there was no significant difference the mean pen health, lung and stomach scores, and the average pen concentration of serum and OF CRP and Hp.

Table 5. 6 Health and performance of heavy and light pen groups (N = 8)

	Heavy	Light	Pooled sem	*P*
Start weight (kg)	60.45	47.08	1.30	<0.001
Gain/day (kg)	0.62	0.66	0.02	0.418
FCR	3.69	2.94	0.13	<0.01
Slaughter weight (kg)	78.6	74.3	2.14	0.204
Lung score (0-55 scale)	3.04	3.55	1.13	0.758
Stomach score (0-5 scale)	3.19	3.71	0.24	0.174
Pen health score	1.26	1.38	0.15	0.596
Serum CRP (µg/ml)	122.00	132.70	18.13	0.692
OF CRP (ng/ml)	54.56	43.60	8.94	0.419
Serum Hp (mg/ml)	1.39	1.62	0.22	0.496
OF Hp (µg/ml)	1.23	1.40	0.20	0.590
Serum Pig-MAP (mg/ml)	0.83	0.85	0.08	0.876
OF Pig-MAP (µg/ml)	0.26	0.40	110.10	0.418

5.3.4 PRRSv serology and status and APPs

The majority of pigs tested positive for antibodies to PRRSv in serum and negative for virus (Table 5.7). All OF samples, from both individual pigs and the accompanying pooled pen samples, were negative for PRRSv.

Table 5. 7 Percentage of samples positive and negative for PRRSv

	Antibodies (%)			Viremia (%)	
	Positive	Negative	Inconclusive	Positive	Negative
Serum	91	8	1	7	93
OF	0	100	0	0	100
Pooled OF	-	-	-	0	100

5.3.5 Relationship between concentration of APPs and pig health and performance

Factors related to the concentration of APPs in serum and OF differed between individual and pen data. In addition, where factors showed a relationship with OF samples, this relationship did not consistently occur in the paired serum sample. The relationships

between individual pig health and production factors and the concentrations of APPs are given in Table 5.8. A total of eight factors were found to be associated with the concentrations of CRP, Hp and Pig-Map in serum and OF. However, the number of factors associated with each APP varied from one to seven. Age was the factor most frequently significant in single regression models, but was not a significant factor in the final model. Instead ADG during the finisher stage and the number of days pigs had been in the finisher building prior to sampling were the two factors which most frequently remained in the multiple regression model.

The number of days pigs had been housed in the finisher building prior to sampling was found to be significantly and negatively related with the concentration of OF CRP and OF Hp, yet positively related to the serum Hp. Daily gain of pigs in the finisher stage was significantly and negatively related to the concentrations of OF CRP, serum Hp and serum Pig-MAP. The lifetime gain/day was significantly and negatively related to the OF and serum concentrations of Hp, but no other APPs. A code for the presence of pathological lesions in the lungs at slaughter was positively linked to the concentration of OF Hp and OF Pig-MAP, indicating increased OF APPs when no lung lesions were observed. No factors were found to be associated to serum CRP.

At the pen level (Table 5.9), far fewer predictors were included in the multiple regression model (maximum two), yet from the significant results, the r^2(adj.) values were much stronger. A highly significant negative relationship was found between pooled OF CRP and the pen mean gain/day. However no factors were found to relate to the pooled OF Hp. In contrast to the pooled OF samples, the pen average concentration of OF CRP and Hp from individual pigs was found to be significantly and negatively related to the s.d. of the pen weight at the time of sampling. However no factors were found to relate to the pen average of serum CRP and Hp concentrations, nor for the Pig-MAP serum and OF concentrations (Pig-MAP data not shown).

Table 5. 8 Relationships between pig production factors and the concentration of APPs in serum and OF from individual pigs (N = 80), (regression coefficient and level of significance).

	OF CRP (log 10)	Serum CRP (log 10)	OF Hp (log 10)	Serum Hp	OF Pig-MAP	Serum Pig-MAP
Predictor						
R^2 (adj)	31.10%	no factors significant	28.40%	27.30%	4.7%	13.5%
Age (weeks)	NS	X	NS	NS	NS	X
Days in finisher before sampling	-0.0097***	X	-0.0088***	0.0222**	X	X
Finisher gain/day	-0.52***	X	NS	-1.25*	X	-1.63***
ADG during sampling	NS	X	NS	X	X	X
Weight at sampling	X	X	NS	X	X	X
Lifetime gain/day	X	X	-0.201***	- 4.5**	X	X
Presence of lung lesion (yes/no)	X	X	0.153**	X	341*	X

Significance: *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; NS = not significant. X = term not included in the model as it had not shown a significant effect when running terms individually in a separate model.

Table 5. 9 Relationships between pen production factors and the mean concentration of APPs in serum and OF from individual pigs (N = 8), (regression coefficient and level of significance).

Pen samples	Pooled pen OF samples		Pen mean samples			
	CRP	Hp	OF CRP	Serum CRP	OF Hp	Serum Hp
Predictor						
R^2 (adj)	65.3%	No factors significant	71.8%	No factors significant	67.5%	No factors significant
Days in finisher before sampling	X	X	NS	X	X	X
Finisher gain/day	-176**	X	X	X	X	X
Scour score	NS	X	X	X	X	X
S.D. Of pen weights at time of sampling	X	X	-10.2**	X	-0.217**	X

Significance: *** = $P < 0.001$; ** = $P < 0.005$;* = $P < 0.01$, NS = not significant. X = term not included in the model as it had not shown a significant effect when running terms one at a time in a separate model.

5.3.6 Effect of collection method on OF APP concentrations

The concentration of both CRP and Hp was numerically lower in the OF collected via cotton rope compared to that collected by the SOS, however there was no significant difference between the concentrations of CRP and Hp in the OF collected from either device (Table 5.10).

Table 5. 10 Median concentration of CRP and Hp detected in duplicate samples of OF when collected by cotton rope or synthetic swab (N = 19), interquartile range in brackets.

Acute phase protein	Rope	Synthetic swab	*T*	P
CRP	37.8 (27.4 - 65.2)	51.41 (34.5 - 59.3)	110	NS
Hp	1.19 (0.78 - 2.4)	1.34 (0.87 - 1.69)	102	NS

The concentration of OF Hp collected by rope was positively correlated to the concentration reported for the SOS ($r = 0.523$, $P < 0.05$). However, no relationship was found between the OF CRP collected via rope and that from the SOS. The levels of CRP in OF collected via SOS were positively correlated to the levels found in the respective serum samples ($r = 0.649$, $P < 0.005$), whilst no relationship existed between the concentration of serum CRP and that found in the OF collected via cotton rope. No relationship existed between the Hp concentrations found in serum and OF collected by either the cotton rope or synthetic swab from the 20 pigs.

5.4. Discussion

This study analysed the concentration of APPs in serum and OF to determine whether monitoring APPs in OF could be used as an alternative to serum in the study of pig health and performance, and whether levels of sub-clinical disease could be detected. In addition, it is believed this is this first study to analyse the concentration of APPs in a pooled OF sample and the detection of Pig-MAP in OF.

5.4.1 The concentration and relationship between APPs in serum and OF

Concentrations of Hp and CRP in serum were positively correlated to the levels in OF, however the correlation was weak. Hiss et al., (2003) found weak but significant correlations between serum and OF Hp using an enzyme immune assay. Given that the current study used the same specific reagents, assay methodology and labs as (Gutiérrez et al., 2009a, Gutiérrez et al., 2009b), a similar strength of relationship between APPS in serum and OF was expected. However, the investigation for correlations between Hp in serum and OF by Gutiérrez et al., (2009a, Gutiérrez et al., 2009b) used samples obtained from clinically healthy pigs (no signs of disease and sero-negative for PCV2, PRRSv and pseudorabies), and those suffering a wide range of clinical disease conditions (which included amongst others, wasting syndrome, multiple abscesses, diarrhoea, external injuries to limbs, ears and tails). Sampling pigs from two extreme health states (clinically

healthy, clinically diseased) of a wide range of conditions would produce a more widely distributed range of values, which could explain the stronger correlations obtained.

Further factors that on what may have altered the relationship between the serum and OF APP concentrations shall be briefly discussed. The presence of only weak relationships could indicate interference from pre-analytical factors, such as the presence of feed particles, affecting concentration of APPs in the OF samples. Pre-analytical variables are known to markedly influence the results of proteomic studies and it is an issue that must be addressed for the progression of diagnostics using various fluids and analytes (Ferguson et al., 2007).

It is worth considering that there is evidence that APPs are produced locally in salivary glands (Lecchi et al., 2009). Although there are few reports in the literature on locally expressed APPs in the salivary glands of the pig, extrahepatic expression of APPs has been found in pigs (Skovgaard et al., 2009), and therefore it is logical to assume there is local production of APPs in the salivary glands of the pig. The local production of APPs could influence the concentration of APPs collected in the OF samples to a greater or lesser extent depending on the positioning of the collection device in the mouth of the pig, and this is a consideration for all OF diagnostics.

This study also sampled OF and blood on two separate days for reasons of practicality. This could, in part, be contributing to the lower significant correlation between OF and serum APPs. The concentration of APPs is not believed to be influenced by a circadian rhythm (Otabe et al., 1998). However, there is evidence to suggest that APPs can respond to stress, with elevated concentrations after 24 and 12 hours of a stressor (road transport) (Piñeiro et al., 2009a). The method of OF collection used in the current study was considered to be of low stress for the pigs, especially when compared to stressors such as road transport. However, there was excitement and disturbance in a number of the pens due to the activity created by technicians offering ropes to collect OF. When transport stress has been kept short (1hr 15 mins, with 2hrs lairage), the concentrations of serum APPs (Hp and PigMAP) have been found to be unchanged (Saco et al., 2003). Therefore, exactly how much effect sampling on separate days may have had is unknown. However, it would be useful for repeat studies to try sampling on the same day if possible.

The collection device did not appear to alter the concentration of APPs within the OF in the current study. However, whilst there was no difference in the median of the samples, the lack of correlation between the CRP concentration from OF collected via rope and synthetic swab indicates that there may have been some loss of APP. With the CRP levels lower in the OF collected via rope, it raises the question as to whether the rope could have caused a deterioration in the sample, although not significant.

The lack of correlation between the concentrations of Pig-MAP in serum and OF indicates that OF Pig-MAP concentrations are not a reliable indicator in this study. The concentrations of OF Pig-MAP detected were very low, and had been expected to be of similar concentration to OF HP, in ug/ml (M. Piñeiro, personal communication, 25th March 2010). This suggests that the sensitivity of the ELISA test used for Pig-MAP determination in OF was not sufficient. Further work with an assay of greater sensitivity or using the TR-IFMA when developed for Pig-MAP detection would be worthwhile. The serum Pig-MAP concentrations can be viewed as reliable as the assay used was validated (Barrios et al., 2005).

With regards to the APP concentration in serum, the positive relationships between Hp and Pig-MAP, Hp and CRP, and CRP and Pig-MAP found in this study are similar to those reported elsewhere (Piñeiro et al., 2009c, Diack et al., 2011). Although it is worth noting that these studies did not all perform like-for-like correlation analysis, with Piñerio et al., 2009a assessing linear correlation through the linear regression analysis procedure in the statistical programme SAS. However, Diack et al., (2011) used Pearson correlation which was used for a proportion of the correlation analysis in this study. The strength of correlation between serum Hp and Pig-MAP observed is very similar to that found by Clapperton et al., (2007), who performed Pearson correlation, and the Hp and CRP, and CRP and Pig-MAP correlations are of similar strength to those found by Diack et al., (2011) in plasma. The strong positive correlation between the concentrations of OF CRP and Hp would suggest that, although lower in concentration and of a weaker relationship to serum, the concentrations of OF CRP and Hp are indicative of the same relationship. Therefore the OF may indeed be reflecting what is occurring in the serum, although the APP levels are far lower, and the correlation between serum and OF lower than expected.

5.4.2 Concentration of APPs in individual pigs in relation to pig health and productivity

During the course of the current experiment, relatively few clinical signs were observed in the pigs (coughing/scour/other); the pen health scores were relatively low, there was zero mortality and only one pig requiring treatment. In addition, as over 90% of the pigs were seropositive for PRRSv, there is certainty that there was little to no active infection of PRRSv ongoing at the time of sampling. However, many other infections were known to be present on the farm and therefore the exact disease status of the pigs in this study at the time of sampling was unknown. The daily gain of the pigs and the FCR may be considered to reflect this, and were sub-optimal in comparison to what a commercial pig producer would aim to achieve, and also to the genetic potential of the pigs. This poor growth rate was echoed in a larger group of pigs studied through the finisher building (see Chapter 4), demonstrating that this was a consistent level of performance for this particular farm and not an atypical period of reduced growth. This study found no difference in the growth rate of the heavy and light pen groups. Since animals were in age matched cohorts there was no difference between the age of the groups. The lack of difference in ADG between light and heavy groups upon entering the finisher building provides further evidence that a challenge presented in this building severely restricted the growth of all individuals to the same level, as under normal circumstances, the heavier pigs may be expected to have increased ADG as was found in Chapter 4. It is in such a scenario as this that APPs may provide useful information as to whether the poor growth rate of the pigs is as result of immune activation, possibly as a result of sub-clinical disease, or whether growth could be limited due to poor management factors such as inadequate temperature, nutrition or water supply.

Concentrations of APPs have been inversely related to gain per day and live weight in growing pigs in a number of studies (Franek and Bilkei, 2004, Clapperton et al., 2005). The current study has yielded similar results, with lower concentrations of serum Hp and Pig-MAP, and OF CRP related to an increased finisher pig ADG, and a lower serum and OF HP also related to an increased lifetime ADG of individual pigs. The mechanism of the APP response links their synthesis with a reduced gain per day as a result of cytokine activation, diverting energy away from metabolism and growth and into an immune activation to fight infection (Colditz, 2004). This effect has been demonstrated in experimental models, with immune stimulation via E.coli lipopolysaccaride challenge

producing a reduction in the daily gain of pigs (Jiang et al., 2009). The inverse relationship between APPs and ADG contributes to an explanation for the sub-optimal growth rate of the pigs studied and represents the possible impact of the pigs experiencing chronic immune system activation as suggested by Clapperton et al., (2005).

The relationship between APP concentrations and ADG in the finisher building demonstrates that the 'snap-shot' of immune activity obtained from one sample could offer the potential to assess for the whole pig performance of a particular on-farm production stage, or indeed lifetime performance. The predictive value of Hp for lifetime growth performance has been recognised (Eurell et al., 1992), and it was also found in this study that both serum and OF Hp were related to the lifetime growth of the pigs. With uncertainty about the reliability of OF APP concentrations, given the weak correlation found with serum values, the fact that the relationship with lifetime gain occurred in both serum and OF Hp provides some indication that this may not be one that has occurred by chance alone. APPs are known to display different levels of sensitivity to the same diseases, and different time courses. Some, such as CRP, are better known for responding to acute conditions and others, such as Hp, to chronic conditions (Petersen et al., 2004, Sorensen et al., 2006). Therefore, the simultaneous activation of CRP, Hp and Pig-MAP could suggest concurrent infections challenging the pigs.

In this study, the age of the pigs in each cohort is closely related the time that they had been housed in the finisher building. However, at the individual pig level, despite age and the number of days in the finisher house having been entered into the model as two separate significant input variables, it was the number of days pigs were housed in the finisher building that continued to show a greater relationship to the concentration of APPs. Changes in the concentration of APPs in relation to the length of time pigs had been in the building could indicate that growing pigs encounter varying degrees of challenge as they progress through the production chain.

The conflicting direction of relationship between Hp in serum and OF in relation to the length of time pigs had been in the building is surprising, but could be an indication that the pigs had previously suffered an infection that resulted in haemolytic anaemia, as the concentration of serum Hp is known to decrease upon haemolysis (Tecles et al., 2007). A disease such as PRRSv is known to cause anaemia in pigs (Halbur et al., 2002) and, with

nearly all pigs sero-converted to PRRSv at the time at sampling, the pigs in this study had already encountered and overcome the infection. However, if anaemia had been caused by the PRRSv, the effects could still be present as the pigs would require time to overcome the anaemia. This is in agreement with the positive relationship to the Hp the longer the pigs are in the building. The effect of blood cell haemolysis on serum Hp concentrations suggests that using OF for APP sampling could, in some conditions, provide greater reliability as a diagnostic fluid for APPs. The greater number of associations between OF APPs and production parameters than serum APPs, as found in this study, is in support of this. Gutiérrez et al., (2009c) have also suggested that OF could provide a more sensitive indicator of infection than serum.

Whether or not a pig's lungs had lesions present at slaughter was related to the levels of OF Hp and Pig-MAP. Amory et al., (2007) were the first to find a relationship between serum Hp and whether pathological lung lesions were present in pigs at slaughter. In the current study, with a score of one equalling presence and a score of two equalling absence, the positive relationship suggests that pigs with a higher OF Hp and Pig-MAP concentration had lower prevalence of lung lesions at slaughter. Amory et al., (2007) analysed serum Hp concentrations in samples taken at the time of slaughter, and therefore linked Hp concentrations with concurrent conditions. In the current study, APP concentrations were analysed from different cohorts of pigs throughout the finisher period, and therefore the lung scores measured at slaughter (for all pigs except the oldest group sampled) were many weeks following the APP measurements.

5.4.3 Pen level relationships

At the pen level, the highly significant inverse relationship between the concentration of CRP in the pooled OF sample and pen average gain per day is an encouraging result. The use of a pooled OF sample suggests a potential means by which APPs can be cost effectively incorporated into on-farm monitoring, offering the ability for producers to measure the extent of lost growth as a result of immune activation from sub-clinical disease. This information could in turn be used to aid the formulation of diets to assist pig growth in the face of immune challenge. However, more work is required to investigate the lack of relationship of pig growth with Hp from a pooled sample found in this study. Further work would benefit from studies conducted in a range of environments with

different levels and types of clinical and subclinical disease to test the robustness of this technique.

5.5 Conclusion

This is the first study looking at APPs in OF of pigs under commercial farm conditions that has attempted to elucidate whether concentrations of APPs in OF samples could be related to pig health and productivity status associated with sub-clinical disease. The relationships between the daily live weight gain and the concentration of APPs demonstrate the potential use of APPs to inform on reduced production as a result of immune activation. The measurement in OF could provide a practical, low cost method to objectively assess the level of immune activation associated with sub-clinical disease in pigs, and the relationship between the pooled OF CRP and live weight gain in pens of pigs suggests potential for the measurement at a group level which would reduce costs even further. With the concentrations of APPs in OF being much lower than those in serum, and with positive but weak correlation with serum values, the validity of the results can be questioned. However, this could also highlight some important issues to be considered in regard to sampling technique if OF samples are to be used routinely for diagnostic purposes. Nevertheless, the results demonstrate that there were far more associations between pig health and productivity parameters and the APPs in OF than those in serum, and thus a potential further benefit of using OF. Further research should enable confirmation of whether the associations found here are detected elsewhere, and should explore the lack of relationship between production parameters and the pooled OF Hp sample. Work to assess the degree of lost production relative to a given change in APP concentrations would be a further useful step in developing decision support tools to support farm management decisions.

Chapter 6: Optimising oral fluid collection from groups of pigs: effect of the housing system and the provision of multiple ropes

6.1 Introduction

The human health care and veterinary professions have long sought the ability to assess and monitor the physiological states of individuals through a non-invasive sampling approach. Oral fluid (OF) has been found to reflect, amongst others, the concentration of antibodies Wang et al., (2002), viruses (Okamoto et al., 2010), hormones (Yates et al., 2010) and acute phase proteins (Gutiérrez et al., 2009c) circulating in the blood, and consequently has been described as a 'mirror of the body's health'(Greabu et al., 2009).

The term OF has been used to describe a general sample of fluid from the oral cavity collected via an absorbent device (Atkinson et al., 1993), and is a more appropriate term than saliva since the fluid sample will contain a mixture of both saliva and gingival crevicular fluid, which has a composition similar to serum (Rai, 2007). An extensive review of the developmental history and field use of OF diagnostics in human and domestic animal medicine has been conducted by Prickett and Zimmerman (2010). This review details strong evidence of the potential for oral fluid-based diagnostics for use in veterinary medicine.

The testing of pooled OF samples collected via cotton rope has great potential as a future methodology for conducting health surveillance in livestock. The foremost advantage of a pooled test over individual tests is that more individuals can be represented for a fixed laboratory cost (Christensen and Gardner, 2000). The sampling method is also quick and collection of OF is of low stress to the animals involved, compared to alternative methods such as blood sampling.

During studies developing OF diagnostics for porcine health monitoring, a standard operating procedure (SOP) for the collection of OF samples from pen groups of pigs has been developed at Iowa State University (Prickett et al., 2008a). This sampling technique has been utilised for the surveillance of Porcine Reproductive and Respiratory Syndrome virus (PRRSv) under experimental conditions (Prickett et al., 2008a) and in limited field studies (Prickett et al., 2008b). The SOP collects a pooled OF sample per pen by means of

a single rope suspended at pig shoulder height for 20-30 minutes. Utilised to collect OF from groups of four (Prickett et al., 2008a) to 30 pigs (Prickett et al., 2008b), OF and serum samples achieved ≥ 77% agreement in PRRS virus detection.

Disease surveillance operations use epidemiological calculations based on assumptions of the disease prevalence within a herd to determine the required sample size necessary to achieve at least a 95% level of confidence for detecting at least one positive animal (Thrushfield et al., 2001). In performing a group OF collection there is a level of uncertainty as to whether the sample is representative of the group. Presenting rope to a group of pigs for OF collection relies on the novelty of the rope to elicit exploratory behaviour in the pigs (Van de Weerd et al., 2003), causing them to chew the rope and deposit OF. Previous research on the provision of environmental enrichment for pigs highlights a number of factors that could hinder the success of retrieving the required sample representation from a pen group.

Pigs housed in groups perform synchronised exploratory behaviour (Docking et al., 2008). Competition is likely to arise as a result of the limited quantity, spatial distribution and temporal availability of the rope (Turner et al., 2000) thus decreasing the total number of pigs from which a sample can be obtained. During social competition lower ranking pigs often fail to access resources (O'Connell et al., 2004) and therefore a sampling strategy that provides opportunity for rope interaction from dominant and submissive pigs is important.

The manner of presentation can influence the time pigs will spend chewing a rope (Statham, 2008), and the age of the pigs could influence the latency to interact (Docking et al., 2008). Whether pigs are housed with the provision of bedding may also influence their behaviour towards the rope, since the extent and duration of interaction between pigs and enrichment items is dependent on whether the stimulus properties of the enrichment are of a greater value than those already within the environment (Scott et al., 2007). The effect of these factors must be established to enable the development of robust sampling protocols suitable for use in the differing husbandry systems encountered in current pig farming practice. The sampling regime and method of rope presentation must be practical and adaptable to different pen designs if it is to be used on commercial farms.

Therefore, the aim of this study was to investigate the chewing interaction of pen groups of pigs towards rope presented in their pen, for the collection of a pooled OF sample, and to explore how the representative quality of the OF sample can be optimised. In achieving this aim, specific objectives of the study were to determine, when presenting a pen of pigs with rope for a total duration of 60 minutes: 1) what percentage of the pen group interact with the rope over the course of the presentation period; 2) how the total duration of time chewing the rope per pig changes over the course of the presentation period; 3) what effect an increase in the number of ropes has on the level of pig chewing; 4) whether there is an effect of housing system on rope interaction; and 5) how these factors interact to affect the representative quality of the OF sample.

6.2 Materials and Methods

6.2.1 Experimental design and treatments

The experiment used a Latin Square design to test four different rope provision treatments in each of two housing systems. Four pens of pigs in each housing system received in turn 1, 2, 3, or 4 ropes for a period of 60 minutes in a fully balanced design over a period of two weeks (Table 6.1)

Table 6. 1 Presentation of the rope to pens of pigs in both housing systems

	FS				**SK**			
Rope presentation occasion / Pen number	1	2	3	4	1	2	3	4
	Number of ropes presented over test period							
1	1	2	3	4	1	2	3	4
2	4	3	2	1	4	3	2	1
3	2	4	1	3	2	4	1	3
4	3	1	4	2	3	1	4	2

6.2.2 Animals and housing

This study was conducted at Cockle Park Farm, the Newcastle University commercial farrow to finish pig unit. A total of eight pens, comprising 155 Large White x Landrace x Duroc grower pigs were selected and housed in one of two different systems – fully slatted (FS) accommodation or straw kennels (SK) (4 pens per system). Prior to selection all pigs had been raised in fully slatted accommodation. Pigs to be housed in the SK were moved to this system one week prior to testing. Pens in the FS system measured 3.8 x

2.46m and were provided with enrichment, consisting of a single length of alkathane tubing suspended at pig shoulder height, meeting minimum EU legislative requirements. Those in the SK system had a dunging area of 4 x 2.5m and an enclosed kennelled (covered, low ceiling shelter) lying area measuring 3.66m x 1.67m, with an inset area that was occupied by a feeder of 0.4 x 1.5m. The SK system was cleaned by tractor scraping the dunging passage twice per week, and fresh straw bedding provided to a shallow depth that covered the concrete floor. Pens in both housing systems contained one feed hopper with five head spaces of 0.24m each, through which a home produced meal was fed *ad-libitum*. Water was freely available via two bite drinkers per pen. All groups of pigs selected were previously established commercial pen groups and thus remained representative of commercial practice. As a result, the number of pigs per pen differed (range 17-24 pigs) and pens were not balanced for gender or weight (pen weight range: 42.1 – 62.5kg; mean 52.5kg). All pigs were individually identified at selection via colour code markings applied to their backs.

6.2.3 Rope presentation and OF extraction

All rope presentations were performed between 09:00 and 13:00 hours. To retain novelty and to elicit exploratory behaviour on subsequent presentations, a three day break was left between presentations of new rope (Gifford et al., 2007).

Pure cotton rope (Outhwaites Ropemakers, Hawes, UK) of 18mm diameter was used, cut to lengths of 60cm so that, once hung above the pen, the end was level with pig shoulder height, as recommended by Prickett et al. (2008b). Ropes were suspended from a 2.74m wooden bar secured over each pen, passed through holes drilled into the bar with a minimum inter-rope distance of 40cm, and secured at the top with a knot. This method of rope presentation allowed the rope to be presented to the pigs in a manner that allowed a high level of spatial access, whilst being a convenient method of presentation that could be adopted by pig producers. Each rope presentation was filmed in real time using a video camera secured above the pen. Following 60 minutes of presentation all rope was retrieved, placed in a plastic bag, and the OF extracted by hand as described by Prickett et al. (2008b). To investigate the quantity of OF remaining in the rope following hand extraction, the damp (chewed) section was cut from each length and divided into four smaller sections. These sections were then placed into plastic pouches made from autoclave bag (Fisher scientific, UK) cut into squares of 14 x 14cm with the corners

collected together. A series of puncture holes were made in the base of the pouch using scissors. The pouch was suspended in a 50ml falcon tube (Starstead, UK), with elastic bands to secure the pouch to the top of the tube. Tubes were centrifuged at 1,500g for 10 minutes and the resulting OF extracted and the volume recorded.

6.2.4 Behavioural observations

Video recordings of the pig/rope interaction were observed to record the rope chewing interactions of each pig. With the use of behavioural software (Observer, Noldus, NL), pigs were scored continuously as either chewing the rope (mouth open and clamped around the rope), or 'other,' (not chewing, nosing rope, performing other behaviours in the pen).

6.2.5 Statistical analysis

Behavioural data on chewing interactions of individual pigs were collated to yield mean values per pen. On presentation days where more than one rope was provided per pen, behavioural interactions per rope were merged to give the total interaction with any rope per pen. As the number of pigs differed per pen, the cumulative percentage of pigs to chew rope was calculated. The mean total duration of chewing per pig was calculated from the total chewing duration of all pigs in the pen, including those which did not chew the rope. To calculate the mean latency to interact with the rope per pen, any pigs that had not interacted with the rope over the presentation period were given a latency to interact of 3600 seconds (60 minutes).

Statistical analyses were conducted using Minitab 15.0 and SPSS 17.0 statistical packages. All data were first checked for normality using the Anderson-Darling test. Data which were not normally distributed were transformed where possible, or an appropriate non-parametric test selected.

6.2.5.1 Pig chewing interaction within 60 minutes of rope presentation

For statistical analysis, the total rope provision time (60 minutes) was divided into cumulative time periods of 15 minutes. Using a General Linear Model (GLM) command for analysis of variance, separate analysis of the four time periods incrementing by 15 minutes was conducted. Per time period, the percentage of pigs chewing the rope and the mean total chewing duration per pig were analysed. To assess the representative utility of the OF collected, the percentage of pigs to have chewed the rope for at least 20 seconds

was calculated and analysed within each of the four time periods of the GLM model. This was based on the assumption, extrapolated from previous experience of individual sampling by hand, that any pig which had chewed the rope for this period of time would have deposited a substantial amount of OF onto the rope. The mean latency to interact was only analysed for the 60 minute time period. In each model the number of ropes provided (1 - 4), the system, (FS, SK) and the influence of prior rope experience (sequential observation session number) were analysed, with interactions between all three factors included in the model. All residuals were stored and checked for normality using the Anderson-Darling test.

To examine the effect of increasing presentation time, pair-wise analyses (paired-t or Wilcoxon signed rank depending on distribution of data) were conducted to compare sequential time periods.

6.2.5.2 Chewing behaviour of individual pigs

To continue assessment of factors which might affect the representative utility of the sample collected, Spearman's rank correlations were calculated between the relative weight of the pig within the pen group and its percentage contribution to the total chewing time per pen. This was based on the premise that liveweight can be linked to social dominance (Beilharz and Cox, 1967, Tindsley and Lean, 1984) and carried out to therefore establish whether there was a relationship between the relative weight of the pig and the ability to access the rope and chew.

Calculation of the percentage contribution to the total chewing per pen was as follows:

$$\frac{\text{total chewing of individual pig} \times 100}{\text{sum of the total chewing within pen}}$$

The relative percentage of the weight of each pig was calculated as:

$$\frac{\text{individual pig weight} \times 100}{\text{pen mean weight (kg)}}$$

Per pen, the Kendall coefficient of concordance was calculated to determine the rank consistency of the total chewing time of individual pigs across the four rope presentations.

6.2.5.3 Quantity of OF extracted

To determine whether there was equality of variance between the quantities of OF extracted by hand and by centrifuge, the Levene's test for equal variances was used.

The quantity of OF extracted by hand and by centrifuge, and the total amount obtained, were analysed by GLM with housing system and number of ropes, together with their interaction, included in the model.

To determine the strength of the relationships, Pearson's correlations were performed on transformed data between the quantities of OF gained through extraction by hand, centrifuge and the total quantity obtained per rope, also their relation to the mean total chewing time per pig within 60 minutes of presentation, and the percentage of pigs to chew the rope for 20 seconds. The strength of relationship between the quantity of OF collected by the various means and the percentage of pigs to chew the rope was determined via the Spearman's rank correlation.

For each significant correlation fitted line plots of the original (untransformed) data were performed to establish whether the relationship seen was linear or curvilinear.

6.3 Results

6.3.1 Pig chewing interaction within 60 minutes of rope provision

Provision of the rope for 60 minutes generated over 80% of the pen group chewing the rope (Fig. 6.1).

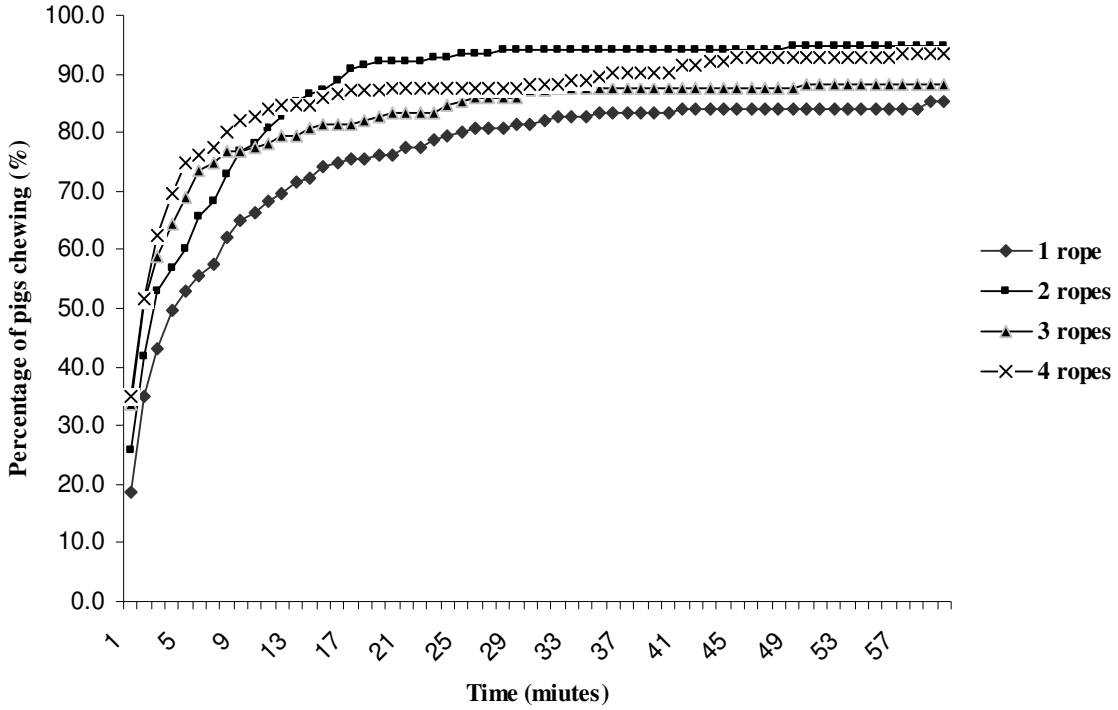

Figure 6. 1 Cumulative percentage of pigs to have chewed rope within a 60 minute period of rope provision (1-4 ropes, in both housing systems).

The greatest increment in the percentage of pigs chewing the rope was from 0-15 mins (0-82.3%). Analysis of the cumulative 15 min time periods revealed further small but significant increases in the percentage of pigs chewing up to 45 mins, 15-30 mins (82.3-88.1% ± 2.3, $P < 0.001$); 30-45mins (88.1-90.1% ± 2.04, $P < 0.01$); with no further significant increase up to 60 minutes (90.1-90.9% ± 1.92, $P = 1.00$).

The mean total duration of chewing per pig continued to increase significantly over the cumulative 15 minute time increments ($P < 0.001$ for all time periods).

6.3.2 Number of ropes provided

There was no effect of the number of ropes provided, or interaction between number of ropes and housing system, on the mean percentage of pigs chewing the rope, nor on the mean latency to interact (Table 6.2). The number of ropes provided did affect the mean duration of chewing time per pig (Fig. 6.2).

Table 6. 2 Effect of the number of ropes provided (1-4) on the mean percentage of pigs to interact over the cumulative time period per system and on the mean latency to interact (s).

Duration		Number of ropes					
(mins)	System	1	2	3	4	sem	*P*
	FS	82	93	97	98		
15	SK	68	81	68	74	5.7	NS
	FS	88	98	98	98		
30	SK	75	90	80	79	4.0	NS
	FS	91	98	98	99		
45	SK	78	90	81	87	4.6	NS
	FS	91	98	98	99		
60	SK	81	92	82	88	4.2	NS
Latency	FS	646	335	217	205		
(s)	SK	1122	657	1007	885	143.8	NS

P is for the rope x system interaction.

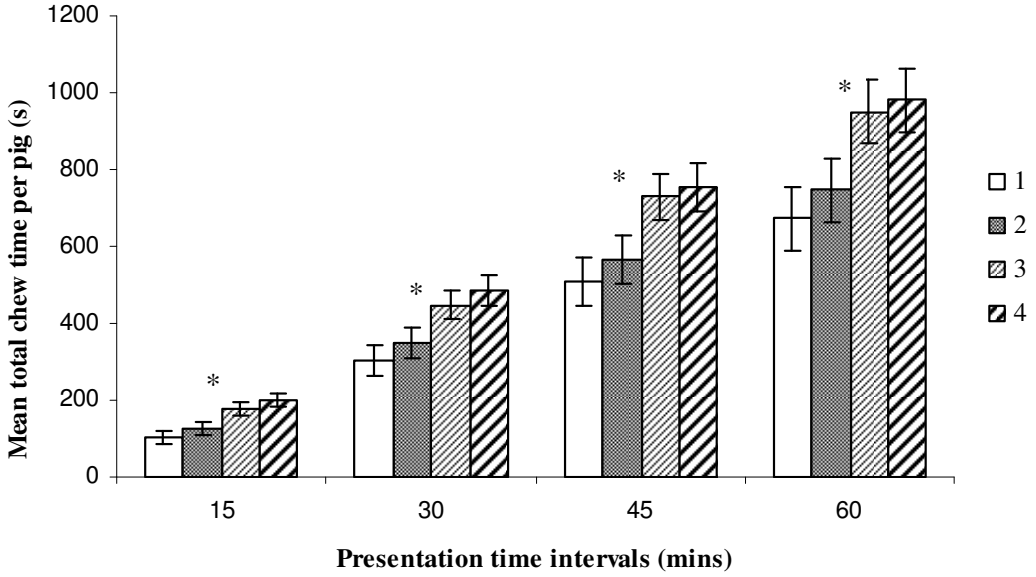

Figure 6. 2 Cumulative mean total chew time per pig when presented with either 1, 2, 3 or 4 ropes. Significance of number of ropes: * = $P < 0.05$.

6.3.3 Effect of housing system

Housing system had a significant effect on level of rope interaction. Pens in the FS system showed a higher percentage of pigs chewing the rope, a longer mean total chew time per pig and a quicker latency to interact than those in the SK system (Table 6.3).

Table 6. 3 Effect of housing system on cumulative pen interaction (mean ± sem).

	% of pigs which chewed rope				Total time chewing/pig (s)			
Duration (min)	FS	SK	sem	*P*	FS	SK	sem	*P*
15	92	73	2.8	<0.001	227	74	12.3	<0.001
30	95	81	2.0	<0.001	595	197	27.6	<0.001
45	96	84	2.3	<0.005	978	302	43.6	<0.001
60	96	86	2.1	<0.01	1290	385	58.4	<0.001

There was an interaction ($P < 0.05$) between housing system and the number of ropes provided on the mean total chewing time per pig (Table 6.4). Increasing the number of ropes increased total chewing time in the FS housing but had no effect in the SK housing.

Table 6. 4 Interaction between the housing system and number of ropes presented on the mean total chew time per pig (s)

	15 min		30 min		45 min		60 min	
No. Ropes	FS	SK	FS	SK	FS	SK	FS	SK
1	135^{a}	73^{ac}	405^{a}	202^{ac}	713^{a}	304^{ac}	953^{a}	391^{ac}
2	177^{a}	70^{ac}	487^{a}	210^{ac}	802^{ab}	331^{ac}	1062^{ab}	434^{ac}
3	268^{ab}	84^{c}	688^{ab}	208^{c}	1141^{ab}	316^{c}	1485^{ab}	413^{c}
4	327^{b}	68^{c}	799^{b}	169^{c}	1255^{b}	256^{c}	1661^{b}	302^{c}
sem	24.5		55.2		87.2		116.7	

Per time period, values within rows and columns lacking a common superscript letter differ significantly ($P < 0.05$).

6.3.4 Prior experience of rope provision

Prior experience of rope provision, as represented by presentation occasion within the Latin Square design of the experiment, did not affect the chewing interactions of the pen groups for any of the dependant variables.

6.3.5 Percentage of pen group to chew rope for 20 seconds

The cumulative percentage of pigs chewing the rope for at least 20 seconds within a given period of time was also strongly affected by housing system (Table 6.5).

Table 6. 5 Cumulative percentage of pigs chewing rope for ≥ 20 seconds between two housing systems over four 15 minute time periods (mean ± sem).

Duration (min)	Fully slatted	Straw kennels	sem	P
15	88	53	3.9	<0.001
30	94	69	2.8	<0.001
45	95	75	3.0	<0.001
60	95	79	2.7	<0.005

6.3.6 Chewing contribution of individual pigs

Fig. 6.3 shows the distribution of chewing times for individual pigs in the two housing systems for the most competitive presentation; one rope. The chewing time of individual pigs showed a high degree of individual variation.

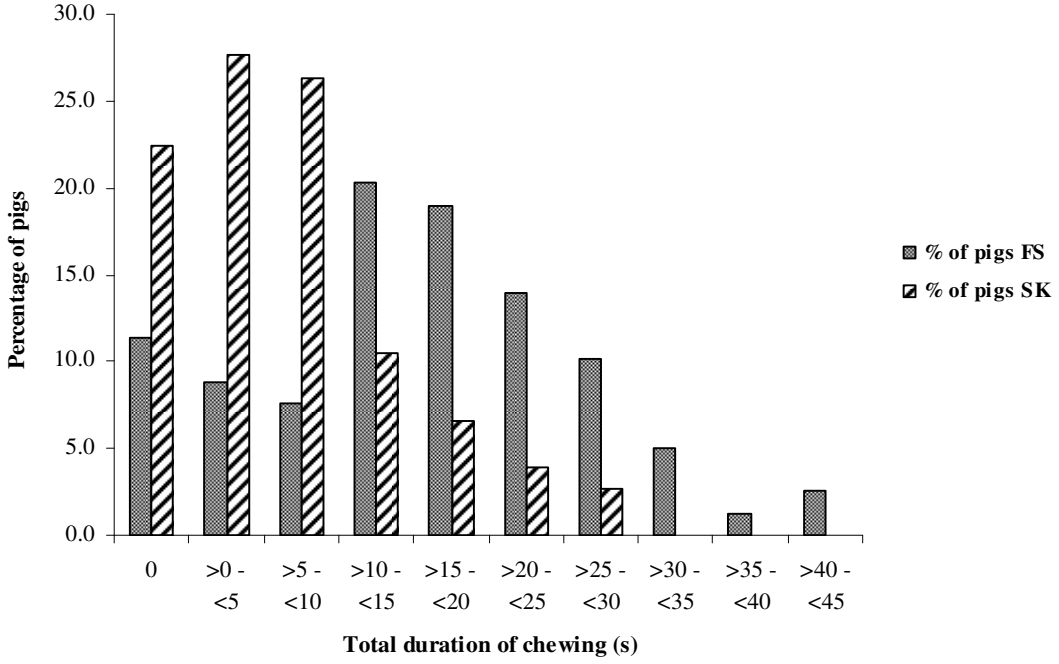

Figure 6. 3 Total duration of rope chewing by all pigs studied, when presented with one rope, in two different housing systems.

The relative weight of individual pigs bore no relationship to their contribution to the total group chewing time, when presented with one (Fig. 6.4), two (r^2 = -0.137); three (r^2 = 0.040) or four ropes (r^2 = 0.088).

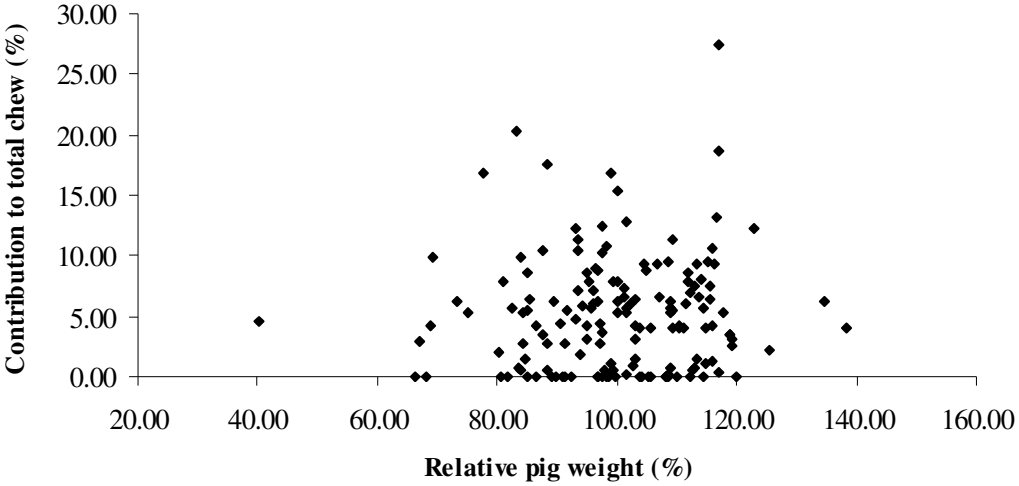

Figure 6.4 Relative percentage weight of individual pigs plotted against the percentage contribution of total pen chewing when presented with one rope, $r^2 = 0.064$.

The Kendall's coefficient of concordance test revealed an association within five pens between the chewing behaviour of individual pigs for each rope presentation (Table 6.6).

Table 6.6 Pens in which there was or was not an association in individual chewing behaviour of pigs over the presentation of the four ropes as shown with Kendall's coefficient of concordance (*W*).

System	Pen	*W*	d.f	χ^2	Asmptotic P
FS	1	0.375	16	24	NS
FS	2	0.422	18	30.4	<0.05
FS	3	0.368	18	26.5	NS
FS	4	0.609	23	56	<0.001
SK	5	0.323	17	21.9	NS
SK	6	0.51	20	40.8	<0.005
SK	7	0.468	19	35.5	<0.05
SK	8	0.411	16	26.3	≤0.05

6.3.7 Quantity of oral fluid collected

The total amount of OF collected per pen was plentiful for diagnostic testing regardless of the number of ropes used, ranging from 12 to 108ml (Fig. 6.5). Low amounts of OF were only extracted from individual ropes that had been presented to the pen in combination with a number of others.

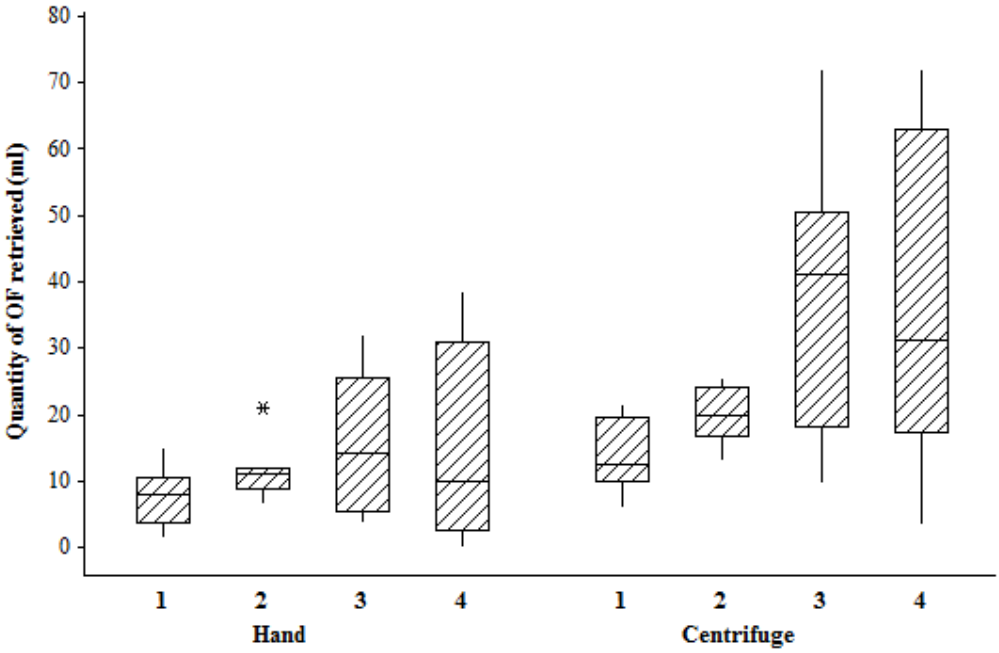

Figure 6. 5 Total quantities of OF collected by hand and centrifuge from the total number of ropes provided in one presentation period (1-4). Median value represented by line inside box, 25th and 75th percentiles, the upper and lower edges of the box respectively, 5th and 95th percentiles, the upper and lower whiskers. Outliers marked by *.

The variance in the amounts of OF collected by hand and centrifuge was not equal (W = 4.76; P < 0.05), indicating that hand extraction (CV 77%) was a less consistent methodology than centrifuge extraction (CV 70%).

The mean quantity of OF extracted by hand was significantly lower than the additional amount obtained by centrifuge; (H: 12.6; C: 26.8 ± 1.6, P < 0.001). There were significant differences between the total quantity of OF collected between systems (FS: 53.4 vs SK: 25.3 ± 3.4 ml, P < 0.001) and with the number of ropes provided (1: 21.6; 2: 31.4; 3: 52.5; 4: 51.9, ± 4.8 ml, P = 0.001), with an interaction between the two (Fig. 6).

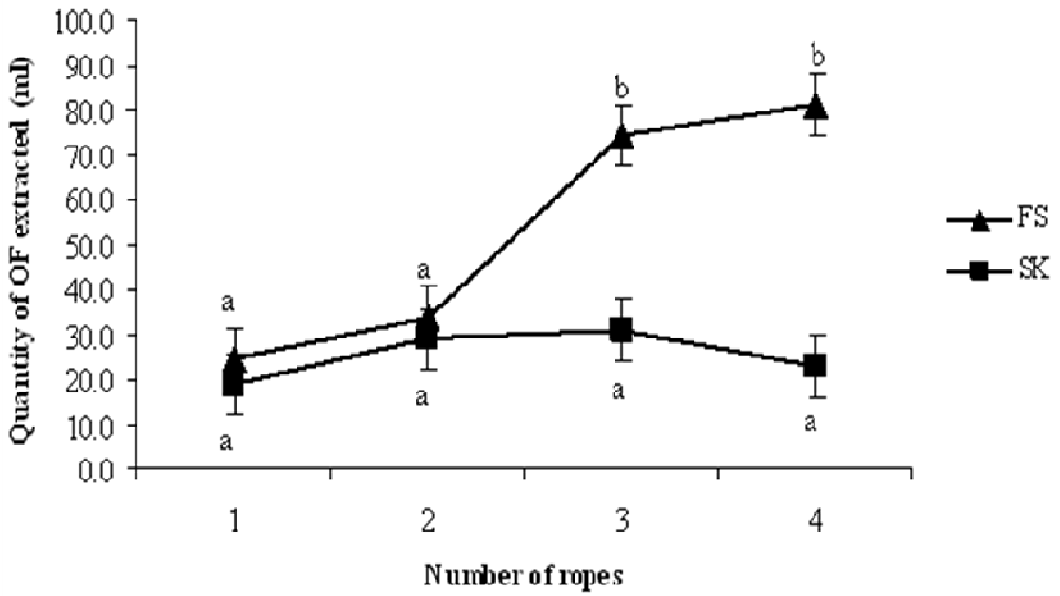

Figure 6. 6 Interaction between the number of ropes provided and housing system on the total quantity of oral fluid extracted from ropes. Means lacking a common superscript differ ($P < 0.001$).

Strong positive correlations were found between the quantities of OF collected by different methods, and also to a number of the pig/rope chewing interactions (Table 6.7). Fitted line plots with regression analysis revealed that the relationships between the methods of OF extraction, and the percentage of pigs chewing: mean total chewing time per pig and the percentage of pigs chewing for 20 seconds were curvilinear, and data were transformed for analyses.

Table 6.7 Correlations between quantities of OF extracted and pig/rope chewing interaction measures

	Hand	Centrifuge	Total
Hand			
Centrifuge	0.625		
Total	0.829	0.952	
% pigs chewing	0.633[†]	0.709[†]	0.729[†]
Mean total chew per pig	0.708	0.773	0.828
% pigs chewing 20s (Log 10)	0.722	0.741	0.806

Unless stated otherwise, correlations performed using Pearsons rank correlations with square root transformation data. All correlations significant at $P < 0.001$

[†] Spearmans rank correlation performed using untransformed data

6.4 Discussion

The present study investigated the chewing interaction of groups of pigs towards rope presented for the collection of a pooled OF sample that could be used in disease surveillance. The results are discussed in line with the objectives listed in the introduction.

6.4.1 Percentage of pen to interact

Presenting the rope for a period of 60 minutes resulted in over 80% of the pen group chewing the rope. In accordance with epidemiological calculations of disease prevalence in group sizes of up to 21 individuals a pooled sample that covers over 80% of the pen population, when sampling from farms that have an estimated disease prevalence of 10% or higher, is more than sufficient to be 95% sure of finding at least one positive individual (De Blas et al., 2000). To achieve the same level of surveillance with pens of lower disease prevalence, in this case 5%, would require a sample with 95% of the pen group accounted for. Whilst the most significant uptake of individuals chewing the rope occurred within the first 30 minutes of presentation, the benefits of increasing the presentation time to 60 minutes are evident, by allowing useful extra time for individual pigs to gain access to the rope resource. With little increase in the percentage of pigs chewing from 45-60 minutes, there is evidence to suggest there is little to be gained through increasing the rope presentation time beyond 60 minutes. Yet, in both housing systems, increasing the duration of presentation did increase the mean total chewing time per pig, which may prove beneficial for the representative quality of the OF.

6.4.2 Effect of housing system

Housing system was the most prominent factor affecting the pig/rope chewing interaction, with pigs in the FS system showing a consistently higher amount of rope chewing interaction than those in the SK systems. The main environmental difference between the systems is the provision of straw bedding in the SK system, and the absence of it in the FS system. A number of behavioural and physiological parameters have been found to differ in pigs housed in barren (FS) or enriched (SK) housing systems, such as fear reactions (Courboulay et al., 2009), circadian rhythm of cortisol (Munsterhjelm et al., 2010), humoral immune response and coping behaviour of individuals (Bolhuis et al., 2003), spatial learning and memory (Jansen et al., 2009) and exploration of the pen environment (Guy et al., 2002b). Therefore, it is highly likely that the provision of straw

is responsible for the differences in rope chewing interaction seen between the two housing systems.

When presented with an enrichment substrate, how attractive the characteristics of the enrichment are is a key factor that determines the extent of object-directed pig exploratory. For pigs, Van de Weerd et al., (2003) found enrichment with attractive characteristics to be chewable, ingestible, deformable, destructible and odorous. Whilst these characteristics are often associated with rootable substances such as straw, hanging objects that had a number of the desirable characteristics were also effective at attracting and maintaining a pig's interest. Hanging rope has a number of the necessary appealing properties, being ingestible, destructible, deformable and chewable (Van de Weerd et al., 2003). In both housing systems, the rope provided an appealing stimulus for oral exploration, most likely because it was novel in the environment of the pig. However, the difference in chewing behaviour between systems demonstrates that the rope provided a stronger stimulus for sustained exploration over the 60 minute period in the FS than in the SK system.

As the most widely studied rooting material for pigs (Studnitz et al., 2007), straw is regarded as providing species-relevant environmental enrichment, occupying the pig in exploratory activities for the greatest period of observed time when compared to other species-relevant enrichment objects (Van de Weerd et al., 2006). When used as bedding straw becomes enrichment material provided over a large area, allowing all members of the pen group to simultaneously explore. This reduces competition and allows for the synchronised exploratory behaviour of the pig (Docking et al., 2008). In the current study, the lower chewing interaction with the rope in the SK system is most likely due to the straw having greater stimulus properties in which many individuals could partake than the presented ropes.

Differences in the level of oral manipulation of rope between systems follows the same pattern of behaviour seen by Scott et al. (2006b) who provided enrichment objects to pigs in FS accommodation and to those already housed on straw bedding. Scott et al. (2006b) found that there was a higher level of oral manipulation towards hanging objects in a FS rather than a straw system, whilst pigs housed on straw maintained a higher level of straw

manipulation which was unaffected by provision of the additional hanging enrichment objects.

The results of the current study provide evidence to support two conclusions. Firstly, pigs will show a greater level of exploratory behaviour towards a rope introduced to the pen when there are fewer alternative objects available to explore, as has been suggested by Scott et al. (2007). Secondly, and in consequence, pigs raised in relatively barren environments will show a greater exploration towards a rope added to their home pen than pigs living in enriched environments (Stolba and Wood-Gush, 1980, Scott et al., 2006b).

6.4.3 Effect of increasing rope provision

The provision of multiple ropes only generated an increased mean total chew time per pig in the FS system. The provision of single lengths of rope for pigs to chew provides a limited point source for exploration. As pigs display synchronised exploratory behaviour (Docking et al., 2008) competition for access to resources is likely to arise when only a limited source of rope is available. It is logical to assume that competition would be greater in the FS system since the rope was one of a very limited number of resources available for the pigs to explore. For the FS system it appears that increasing the number of ropes provided a greater opportunity for pigs to interact with the rope for a longer period of time. The increase in total mean chewing time per pig with the provision of multiple ropes seen in the FS system contrasts to the results of Scott et al. (2007), who found provision of multiple (four) source point enrichment objects did not elicit an increase in oral manipulation of the objects, compared to when only one was provided. However, the stimulus properties of the plastic 'helicopter' enrichment provided in the study by Scott et al. (2007) were low; made of 18mm diameter, 80cm lengths of alkathene pipe, they were only chewable. This is reiterated in a statement made by Scott et al. (2007) that the levels of interest in the helicopter toy were low to the extent that there was no competition for access to the toy in a pen containing 32 pigs. In addition, this comparison should be considered in line with the differing time available for habituation to enrichment objects between the studies since the helicopter toys remained permanently in the pen and had probably lost novelty by the time of observations. Trickett et al. (2009) showed that, whilst newly presented rope attracted a high level of

interaction in weaned piglets, time spent in manipulation reduced to less than half over the course of a week of presentation.

Increased rope provision had no effect on the chewing behaviour of pigs in the SK system, reiterating the idea that when there was an alternative, more appealing substrate to explore (i.e. straw) there was a low level of motivation amongst the pigs to explore the rope. This also provides evidence that the hanging enrichment provided in the FS system to satisfy EU legislation was not satisfactory to maintain the interest of the pigs.

The high number of pigs that chewed when just one rope was provided (over 80%), indicates that this percentage of pigs can only have succeeded in chewing the rope through a considerable degree of movement and rotation between individuals in competition to gain access to the rope. This was observed by the author during the test and when watching the behavioural videos. The results indicate that the provision of one rope, as the smallest amount of resource available, was not limiting in the percentage of pigs able to access the resource in either system with group sizes of <25 pigs. However, in the FS system the provision of multiple ropes might be beneficial to increase the duration of total chewing per pig. Work on larger group sizes is needed to determine the point at which additional ropes become essential to achieve a representative sample and the optimal pig:rope ratios in a wider range of circumstances.

6.4.4 Chewing behaviour of individuals and the representative quality of the pooled OF sample

A real concern of the induced competition for the rope resource was that the resulting oral fluid sample would not be representative of the pen population. It is a widely accepted view that, where competition exists, it is the more socially dominant individuals that will gain access to the resource. It is common for a limited resource to be divided unequally depending on dominance rank, where the most dominant is likely to obtain a large proportion of the resource and little will remain for the lower ranking individuals (Craig, 1986). However, Turner et al. (2000) have questioned whether a higher social rank does actually confer privileged access to resources in finishing pigs. The work of McGlone (1986) demonstrates that submissive animals may also retaliate to defend their position, and therefore to single out dominance as solely responsible for an individual's access to resources is not always justified.

The relative weight of individual pigs within the group revealed no relationship to their percentage contribution to the total time spent chewing rope, suggesting that the larger more dominant pigs were not controlling the resource. Social hierarchy is complex and there is evidence to suggest that social dominance is not always linked to the weight of individuals (O'Connell and Beattie, 1999). However, in the current study live weight was the only possible indicator of social rank available. These results could suggest that whether pigs within the group chewed the rope or not, and for how long, could be largely dependent on their individual motivation to chew. Individual differences are known to exist in pigs (Spoolder et al., 1996). Similarly, competitive success towards a limited resource in dairy cows has been found to vary according to each cow's motivation to access the resource (Val-Laillet et al., 2008).

Calculations showing the percentage of pigs to have chewed the rope for at least 20 seconds revealed encouraging information. Over the course of the 60 minute presentation period, a high percentage of pigs from the both systems had chewed for ≥ 20 seconds, (95% and 79% in the FS and SK systems respectively). This indicates that we can be relatively confident that the sample obtained was representative. The percentages of pigs sampled in the current study would be appropriate for sampling pen populations of 21 pigs with a disease prevalence of 5% in the FS system and 10% prevalence in the SK system, to be 95% sure of detecting viremia in at least one individual (De Blas et al., 2000).

The associations found between the mean total chew time of individual pigs across the four periods within five pens indicates that there was consistency in the relative length of individual chewing time for a certain population of pigs over the course of the consecutive presentations of the rope. This suggests that with repeated sampling, the same representation would be achieved in these groups. A number of individuals in pens did not chew the rope at all over the four rope presentations; these animals showed no signs of clinical abnormality and were not of lower relative weight, suggesting that they were individuals with low exploratory motivation.

These behavioural data provide an initial indication of the representative quality of the pooled OF samples collected via this technique. However, further research is required,

including accompanying behavioural studies, to be confident of the reliability and consistency of this sampling technique. Work in which the level of viremia tested in a pooled sample is compared to the viremia results of the OF samples of individuals within the group, analysed in conjunction with the behavioural knowledge of which individuals had chewed the rope and for what length of time, would provide further reassurance on the reliability of this technique. In addition, it would be beneficial to investigate the effect of extending the rope presentation period on the quality of the sample collected, including subsequent laboratory tests to guarantee representative quality.

6.4.5 Prior experience of rope on the behaviour of the pigs

Prior experience of receiving the rope had no effect on any of the pig/rope chewing interactions in either housing system. This is a positive result as it indicates that each subsequent presentation of rope generated sufficient exploratory interest in the pigs. The delay of three days between presentations, in combination with the short exposure time of the rope per presentation, will have assisted in maintaining the interest of the pigs for subsequent presentations, in accordance with the results of Gifford et al. (2007). New pieces of rope used in each presentation will have renewed exploratory value to the pigs. Being tightly wound at the beginning of the presentation period, the structure of the rope strands will unwind and soften over the course of the presentation. Trickett et al., (2009) noted a significant increase in chewing behaviour when old rope was replaced by new after a two week period of continuous presence.

6.4.6 The quantity of OF collected

The cotton rope yielded large quantities of OF. Per pen, the quantity of OF extracted by hand was significantly lower than the amount obtained by centrifuge. However, the majority of diagnostic tests require no more than 1 - 2ml of biological fluid for testing. Therefore the quantities extracted by hand, per pen, in the current experiment yield a sufficient quantity to cover multiple tests and allow additional OF to be retained for future use if repetition of analysis may be required.

It is highly advantageous to be able to extract OF by hand to produce a solely on-farm collection procedure. It was very difficult to obtain OF from a small number of the ropes in this experiment, and from one particular rope it was not possible to extract any fluid at all. Without doubt the quantity of OF extractable by hand will vary depending on

individual technique, factored by the total amount of OF deposited on the rope. The statistical relationships found between the quantity of OF obtainable, the percentage of pigs chewing the rope, and the mean total chewing time per pig, indicate that where little or no OF could be obtained it is likely that there was a reduction in chewing activity towards these ropes. Whilst it is highly desirable to have a technique that can be performed on farm, there may be situations in which reduced chewing activity generates ropes that do not yield much OF. This study has shown that there is an alternative method that can be used to successfully extract the OF in the laboratory, through the use of a suspended pouch and a centrifuge. It is useful to know that, should any ropes prove difficult to harvest, extraction performed at the laboratory is a feasible option that could be offered as part of the diagnostics package. Such a package must allow for the costs of collection, packing and postage and the speed of return of results.

It is important to consider variance in the technique of individuals extracting OF by hand and the quantity extracted, since there may be consequences for the final representative quality of the OF extracted. In this study the variance in quantities of OF extracted by hand and centrifuge were not equal. Further work needs to be conducted in order to establish whether the representative quality of OF extracted by hand is diagnostically equivalent to that of the greater amount extracted by centrifuge.

The strong positive correlations found between the total quantities of OF extracted by hand, centrifuge and the total amount obtainable, would suggest that the amounts obtained via hand are in some way representative of the amount further obtainable with the aid of the centrifuge. The quantity of OF extracted by hand, centrifuge and the total amount, having strong positive correlations to the mean percentage of pigs to chew, the mean total chewing time per pig, and the percentage of pigs to chew for 20 seconds, together suggest that the oral fluid obtained is representative of the percentage of pigs to chew and that the increased chewing time increases the amount of OF obtained.

The form and direction of all relationships between quantities of oral fluid extracted by the various techniques, and their relation to the chewing behaviours of the pigs were found to be curvilinear. In terms of the quantity of oral fluid extracted in relation to the mean total chewing time of individual pigs, this could in part be as result of the level of absorbency of the rope.

6.5. Conclusions

Provision of rope to a pen of pigs in group sizes of 17-24 is an effective method of obtaining a group OF sample suitable for diagnostic purposes. Based on the findings of this study, for group sizes of ≤ 25 pigs, it would be suitable to use 1 or 2 ropes for 45 minutes to optimise the percentage of pigs chewing the rope. Sample representation is poorer, although still acceptable, in straw bedded housing systems and is not improved by the provision of additional ropes. Presenting the rope for longer periods of time may be useful to improve the representative quality of the sample, especially when sampling in straw housing systems. The difference in exploratory chewing behaviour of the pigs between housing systems warrants further investigation to determine strategies to improve the uptake. Studies trialling the use of rope to collect samples in the more enriched outdoor systems of production should also be carried out.

A plentiful quantity of OF is obtainable from the ropes, and this can be extracted by hand. Additional OF can be extracted by the use of centrifuge where required. Correlations indicate the quantities extracted by hand and centrifuge are representative of the chewing behaviour of the pigs. However, further studies to investigate the extent of the representative quality of the OF collected by this method are required to confirm reliability of this sampling method for disease testing.

Chapter 7: Monitoring water consumption to assist health management

7.1 Introduction

The monitoring of water consumption is considered to offer benefit for the observation and management of health status in pigs (Bird and Crabtree, 2000, Madsen and Kristensen, 2005). In healthy, growing pigs, automatically logged water usage should generate consistent patterns of intake both within and across days (Crabtree et al., 2008). The behaviour of pigs has been found to alter at different stages of disease processes (Krsnik et al., 1999), and drinking behaviour in particular (as measured by total time spent drinking) has been related with more clinical/clinical-chemical variables associated with disease than any other behaviour, including feeding (Reiner et al., 2009). Furthermore, a change in water intake pattern has been found to appear in the sub-clinical stage of infection (Krsnik et al., 1999, Madsen and Kristensen, 2005), before disease symptoms become visually apparent. Therefore, automated monitoring of water intake provides a sensitive and non-subjective extension of observations by stock people, with the continuous data capture of water flow able to provide both real time monitoring and historical measurements which can be consulted to widen the scope of the investigation when problems arise (Smith et al., 2009). It has been reported that the knowledge revealed from such data often contradicts what was originally believed to be the reason for short comings in pig performance and health (Smith et al., 2009).

For the improvement of pig health, utilising water consumption data in real-time could provide a lead indicator of impending health conditions, granting an opportunity to take early action. This could be of particular assistance for the control of circulating endemic disease. An early intervention will improve pig welfare, performance and financial benefits to the farm. Monitoring the water intake of growing and laying birds is routinely used within the poultry industry and is a requirement of the Assured Chicken Production and Lion Quality standards (P. Morgan and A. Joret, personal communication, 20th April, 2011). It is regarded as particularly helpful for detecting upcoming problems, and cited in the Code of Recommendations for Poultry Welfare as an aid to disease detection (DEFRA, 2002).

Despite the perceived benefits of monitoring water use, there has been a relatively low uptake of automated monitoring systems within the pig sector. Whilst the water can be recorded automatically, there is no fully automated system that can download and interpret the data into simple and meaningful messages for producers. Currently, automated interpretation of water data is not possible because the factors contributing to the total amount of variation within the water consumption of groups of pigs remain largely unquantified. This makes it difficult to pinpoint the causative factors responsible for generating specific signal alarms, based on temporal deviations from normality, and currently there would be uncertainty to a given diagnosis with a high risk of both false positives and false negatives (De Vries and Reneau, 2010).

Water intake in pigs is known to be influenced by liveweight, pig growth rate, temperature, feed intake and health status (Brooks and Carpenter, 1993, Madsen and Kristensen, 2005). Research on a variety of farms, with different group sizes, disease status, housing system and feeding type etc, needs to be conducted in order to be able to build a robust statistical model with enough sensitivity to relate changes in water use to specific variables, providing reassurance that any action taken is for the correct cause. In terms of utilising the water consumption of pigs to detect pending health conditions, distinguishing between deviations in pattern attributable to disease and those attributable to other environmental variables is of particular importance to reduce the number of false positives; for example, a deviation in consumption as a result of disturbance to the pigs might be taken to represent a change in the health status.

To date, work has been done to record the drinking behaviour of pigs during disease onset and recovery (Reiner et al., 2009, Krsnik et al., 1999), however in neither of these studies was the quantity of water consumed recorded. Theoretical models have been constructed to predict optimum water intake of pigs under non-limiting conditions (Schiavon and Emmans, 2000) and, whilst there is an increasing number of early-adopter producers who record water intake of their pigs, there is very little published scientific work exploring the automated monitoring of water consumption, and very little in relation to water consumption and pig health. The paper of Madsen and Kristensen (2005) is a notable exception, demonstrating that water intake changes were associated with the early stages of occurrence of an outbreak of post-weaning scour. From practical experience, the staff of the pig housing environmental control specialist company, Farmex Ltd, have published

details of case studies which detail experience of early adopter producers where an outbreak of swine influenza was detected three days prior to outbreak (Bird, 2007). Papers studied have measured the water consumption of groups of pigs from all-in/all-out housing, in which all of the pigs are of the same age and are expected to be at the same level of performance. The measurement and interpretation of water consumption in continuous flow housing may present more challenges for disease detection due to the varying age and health status of the pigs, and therefore the limitations of this technique need to be assessed. Work is required to distinguish the changes that occur in healthy pigs (due to environmental variables), pigs exposed to stressful events and various pathogens, and in different housing systems, diets and conditions of pig flow. To further refine a model, it would be of interest to determine whether infection with specific pathogens can be recognised through changes in water consumption.

For this research, the water consumption of pen groups of pigs housed in a continuous-flow finisher building with a history of ongoing respiratory disease was monitored. The aim was to determine, a) what factors were influencing the variation in water use, and b) whether the water use could be linked to episodes of clinical disease within the group. Whereas other studies have looked at the daily rhythm of water use by large groups of individuals, often at the room or building level, a further aim of this study is to evaluate what can be done through monitoring smaller group sizes, at the single pen level, and using the daily water use as a simple measurement.

7.2 Materials and methods

7.2.1 Animals and housing

This study ran from February to May 2010. Over two replicates, the daily water consumption of 240 (Large White x Landrace) x (Duroc x Pietrain) finisher pigs was monitored throughout their finisher phase (120 pigs per replicate). Pigs were selected upon entry to the finisher house at a mean weight of 52.6 ± 0.51 kg (mean ± sem), and penned in groups of 10. Pigs were consigned to slaughter when they reached 85kg and were selected individually, and therefore the number of pigs in each pen would often reduce gradually.

Pigs were housed in a fully-slatted finisher house with automatically controlled natural ventilation (ACNV). Pens measured 1.46 x 3.79 m^2, giving an initial space allowance per

pig of 0.55 m^2. The temperature of the room was set at 19˚C and was controlled by a thermostat from which ventilation rate was altered – vent opening was adjusted and additional fans could speed up or slow down in response to room temperature. Pelleted finisher diet (Olympic 501 pellets, BOCM Pauls Ltd, UK) was supplied a*d-libitum* to each pen via a five space feed hopper. Water was freely available via two bite drinkers. The Olympic 501 pellets (Protein: 16.5%, Lysine: 1.20%, 13.4 MJ DE/kg) diet was introduced 20 days after the initial pens began trial. 11 pens from replicate one had previously been fed Desert King finisher (Protein; 18.5%, Lysine: 1.12%, 12.7 MJ DE/kg) pellets (FeedCo, UK).

7.2.2 Water consumption and temperature monitoring

A water meter (Titon Enterprises Ltd, Dorset, UK) was fitted into the down-pipe of each drinker to monitor the water flow rate via a pulse meter which logged flow by 5 minute intervals. In addition, two temperature sensors were suspended above the pens at two points in the building, over the middle of the pens on either side of the passageway. The water meter and temperature sensors were linked to an environmental monitoring modem (Barn Report, Farmex Ltd, UK) installed at the farm, from which data could be downloaded via the internet from a computer.

Two drinker types (Monoflo, Monoflo International Inc and Arato, Aratowerk, Germany) were tested in this study, equally distributed across pens. Monoflo drinkers consist of a stainless steel spring pin which the pigs bite, or push to the side to release the water flow. The Arato drinker releases water when a pig bites down on the top of the drinker, and will stop water flow when the pig swallows. The flow rate of water in each drinker was checked every two weeks and the drinkers were cleaned and adjusted to deliver a flow rate of 1,000ml/min. A cut off point of 800ml/min was established for any problematic drinkers. Problematic drinkers occasionally occurred when, despite all efforts, (cleaning of the drinker, changing the head, changing the t-bar etc), the flow rate would not reach 1,000ml/min, and it was concluded that a blockage further up the pipe system may be having an effect.

7.2.3 Production and health monitoring

Upon selection, pigs were individually identified with an ear tag and weighed. All pigs were weighed thereafter at intervals of two weeks. Health scores of each pen were conducted at weekly intervals (see Chapter 3, section 3.2.4.1 for health score

methodology). For the second replicate only, calculations were made of the mean feed intake per pen by recording feed supplied within a given period between pig weighings.

Due to a power failure, twenty days of water data were lost from all pens in replicate two. The data from one pen in replicate two were omitted when only three days of data had been recorded for the whole pen following this power failure. The pig performance and feed intake data from this pen were also omitted.

7.3 Statistical analysis

7.3.1 Production and health data

Per pen, the daily total water consumption, along with the corresponding internal room and external temperatures were downloaded from the Barn report via the 'define batch' setting, allowing the period between entry and exit from the building to be extracted. The fortnightly periods between the weighing of each pen of pigs were numbered as ascending monitoring periods, (1, 2, 3 etc) to allow identification of block periods of water consumption between the weighing of the pigs. An estimated daily weight of the pen was calculated using the start and end weights of each period and incrementing the weight daily by the mean daily gain over that period. The estimated pen weight was adjusted appropriately if any pigs left the pen during that period. The running weight of the pen was divided by the number of pigs present within the pen at that time to give an average running weight per pig.

For the second replicate, the average feed consumed per pen per day was calculated and converted to a per pig basis by dividing by the number of pigs in the pen. In addition, the feed conversion ratio (FCR) was calculated per pen.

Prior to analysis all data were checked for normality using the Anderson-Darling test, and where possible, non-normal data were transformed. The statistical packages Minitab 15.0 and SPSS 17.0 were used for the analysis of these data.

A general linear model (GLM) was used to determine any differences between the start and slaughter weight of the pigs monitored between the replicates, and differences in the ADG of the pigs between the two replicates, with start and slaughter weight as covariates.

Spearman's rank correlation analysis was performed to relate changes in water consumption patterns to temperature and feed consumption from replicate two.

A GLM was used to compare the two drinker types for the quantity of water consumed per pig per day across the pens, with the drinker type and the replicate as factors and the average weight of pigs in each pen as a covariate.

For each pen, each day of monitoring was coded for when ill-health occurrences were seen and when disturbance was caused to the pigs through human interaction, both coded with a yes/no score. As overall health score of the pen was recorded only once weekly, this score was given for modelling purposes to all days within the week in which they were taken.

7.3.2 Multiple regression model

A multiple regression analysis was used to determine which factors were associated with the variation in total daily water consumption of pigs on both a whole pen and mean individual pig level. Regression analyses were performed using the statistical package Minitab 15. Multicollinearity of independent variables had been previously assessed using the statistical package SPSS 17. Multicollinearity was considered to exist in variables were the variance inflation factor was found to be greater than 10. The initial model included: internal and external building temperature (minimum, mean and max temperatures reached per day of water consumption), the number of pigs in the pen, the drinker type, the estimated live weight of the pen and the estimated mean pig average daily live weight gain.

To the result of this model, the number of pigs sick within the pen each day and the occurrence of human disturbance within the building were tested as further possible explanatory factors. In addition, for replicate two only, the FCR for each of the water monitoring periods, the average quantity of feed consumed per pen per day, and the average quantity of feed consumed per pig per day were tested in the model.

7.3.3 Mixed model analysis

A Restricted Maximum Likelihood Model (REML), using the SPSS 17.0 statistical package, was used to compare the water usage per pig per day within given weeks against

the records for the severity of clinical disease and number of cases reported, as defined by the health scores for the corresponding week and the following week. The average weight per pig for the week corresponding to the water consumption data, and the temperatures were added as covariates and, from week three onwards, the number of pigs per pen was added as a covariate. The number of pigs per pen was only included in the model from week three as this is when pig sales first began.

To increase sensitivity of the model, after the first results had been produced, all covariates that were not significant were removed and the model re-run. Water consumption in relation to scour score was investigated up to week five of pigs being in the building, as little scour was observed after this week. Water consumption in relation to pen health and cough score was investigated up to week seven of pigs being in the building.

7.4 Results

7.4.1 Pig Productivity and health

All but six pigs out of the 240 completed the designated trial period to slaughter. Over the course of the trial, three pigs were removed due to rapid weight loss, one suffered a rectal prolapse, one became lame with an infected limb and one died of unknown causes. In addition, two pigs received veterinary treatment but remained on trial, one for body wounds and one for lameness. The range in severity of the pen health scores over the weeks pens were on trial are shown in Fig 7.1.

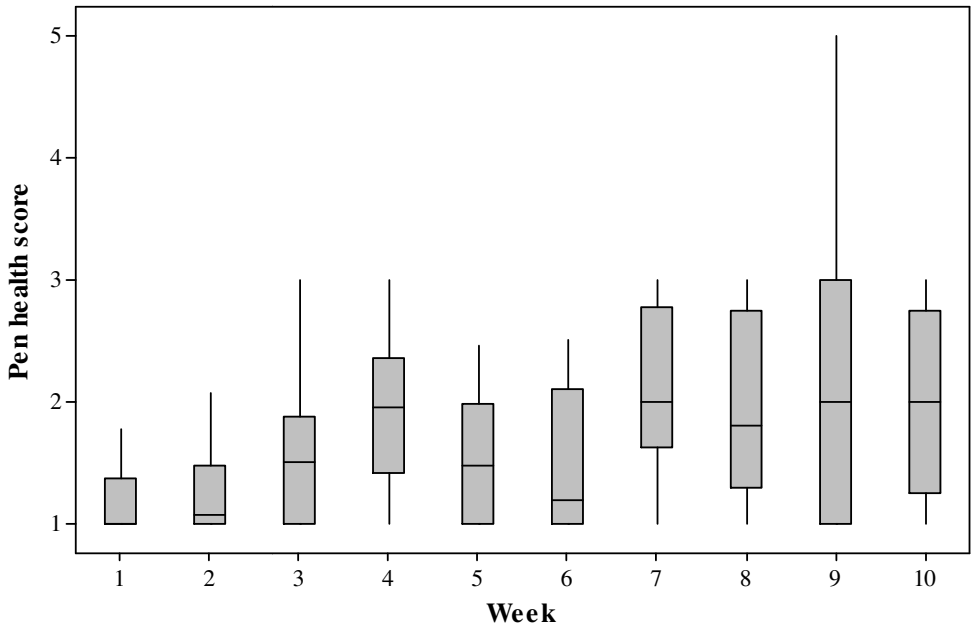

Figure 7.1 Range of the pen health scores across all pens monitored over the weeks in the finisher building.

A mean of the pen health score of all pens over the weeks on trial (Fig. 7.2) demonstrates the gradual increase in health score severity over the weeks.

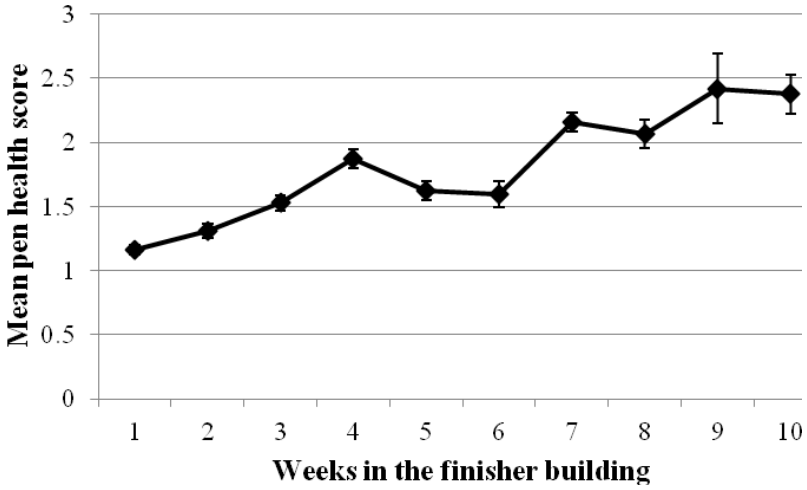

Figure 7.2 Pen health score of all pens for each week in the finisher building (mean ± sem).

The amount of coughing, as recorded by the cough scores, varied greatly over all pens over the weeks, with a number of pens showing high scores from week four and the overall severity of the scores increasing by week seven (Fig. 7.3).

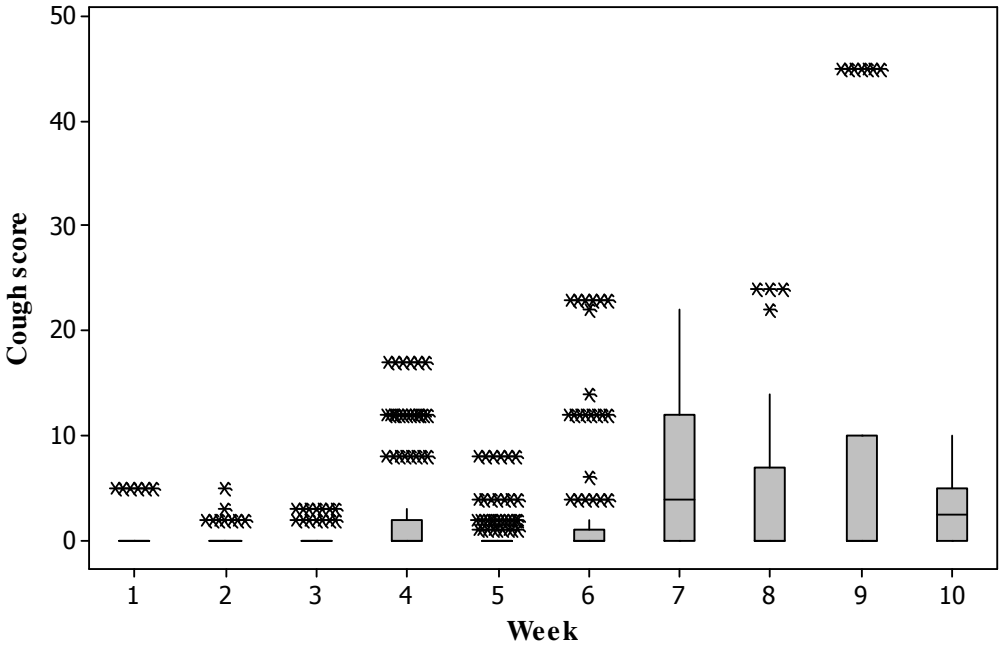

Figure 7.3 Cough scores over the weeks in the finisher building

Outbreaks of scour occurred across numbers of pens from the first week in the building, with the most severe cases seen in week two, decreasing thereafter with no further cases seen until one mild case in week ten (Fig. 7.4).

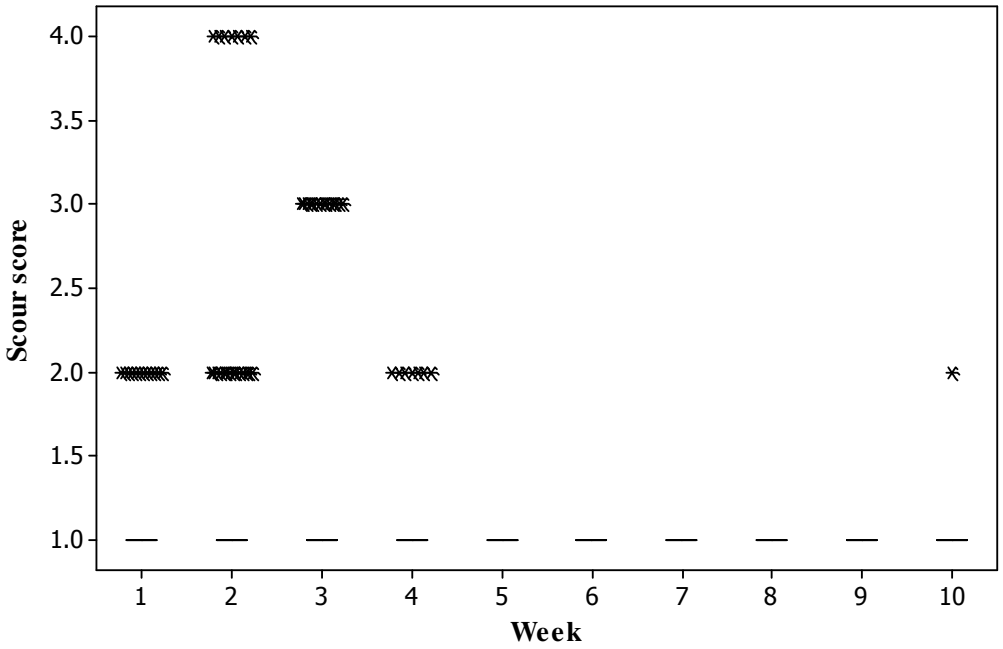

Figure 7.4 Scour score of pens over the weeks in the finisher building

There was no significant difference between the two replicates in the start weight of the pigs (Table 7.1) but, due to changed market demands over the course of the trial, the slaughter weight was significantly reduced between the two replicates. Taking into account the start weight and slaughter weight in the analysis (as covariates), there was no significant difference between replicates in the average daily gain of the pigs to slaughter. There was a difference of 40g in the ADG of pen groups between the drinker types (0.65kg/day Monoflo, 0.69kg/day Arato ± 0.02, P = 0.102), however this was not significant. In this analysis replicate also not significant as a factor.

Table 7.1 Performance data for pigs (N = 230) studied over two replicates

	Replicate			
Measurement	1	2	Pooled S.E.	*P*
Start weight (kg)	53.6	52.0	0.74	0.121
Slaughter weight (kg)*	89.5	81.3	0.59	<0.001
Average daily gain (all pigs to slaughter, kg)*¥	0.70	0.70	0.01	0.923
Days to slaughter*¥	46.5	47.6	0.78	0.362

* = start weight was significant as a covariate ($P < 0.001$), ¥ = slaughter weight was significant as a covariate ($P < 0.001$).

The mean time pigs spent on trial (from entering the finishing house to reaching slaughter weight), was 6.6 weeks. The water intake per pig per day increased linearly over the initial weeks (Fig. 7.5), but had a lower rate of increase as pigs neared slaughter weight. Over the course of the trial there was an average of 7.07L of water consumed per kg of gain.

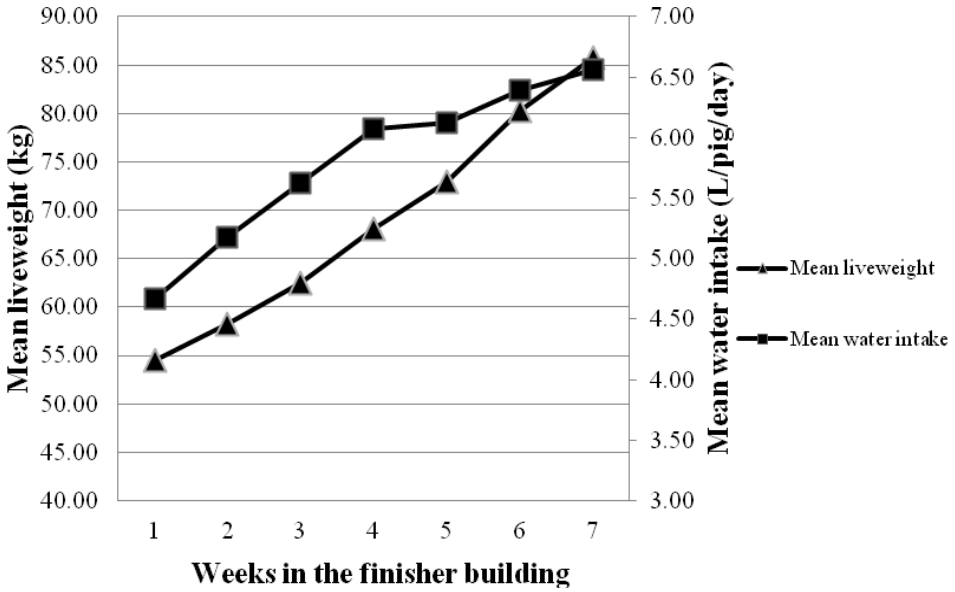

Figure 7.5 Mean live weight and daily water intake per pig over the increasing weeks in the finisher building.

7.4.2 Environmental conditions

During the course of the trial, the internal building temperature varied widely around the set temperature of 19°C, ranging from 8.7 – 26.6ºC. The variation around the mean internal building temperature is displayed in Fig. 7.6.

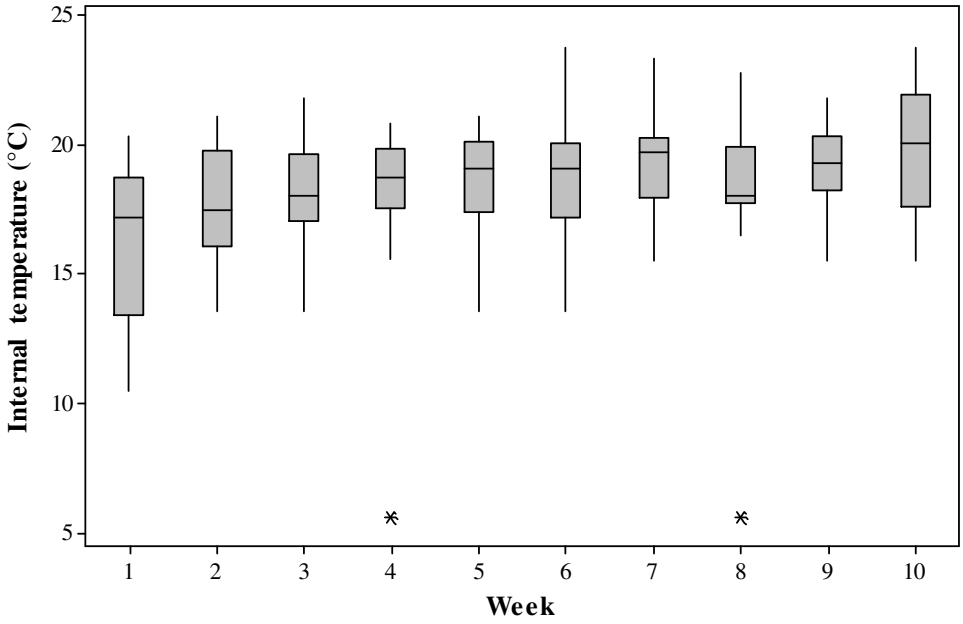

Figure 7.6 Range of the daily mean temperatures recorded on the internal room sensors of the finishing building.

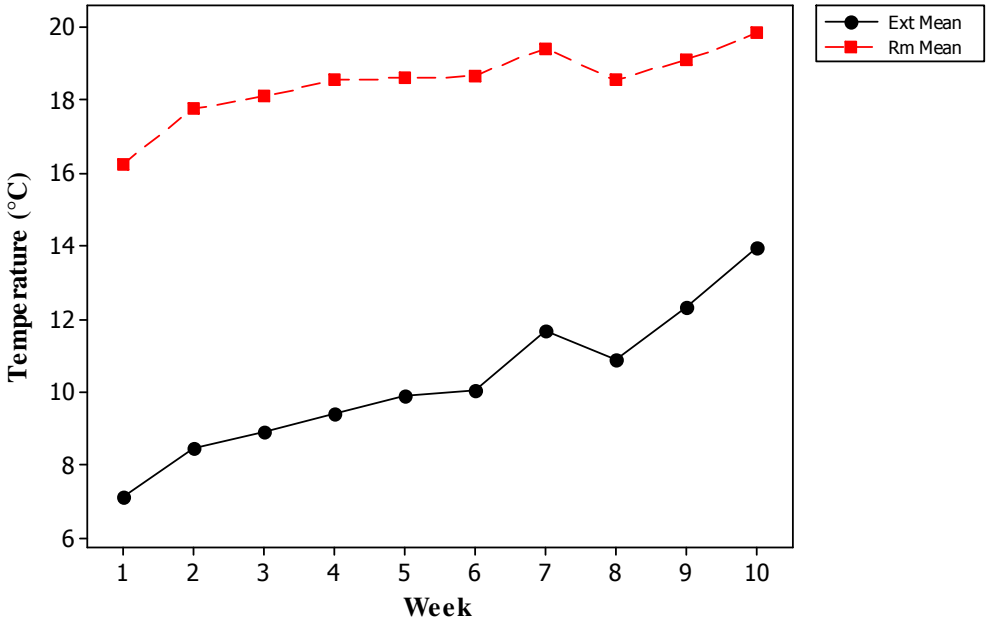

Figure 7.7 Mean daily internal room and external building temperature over the two replicates and weeks on trial

A strong relationship was found between the external and internal room temperature (minimum temperature: $r_s = 0.803$, $P < 0.01$; mean temperature: $r_s = 0.876$, $P < 0.01$; maximum temperature: $r_s = 0.782$, $P < 0.01$). Graphical presentation demonstrates that the internal room temperature tracked the external building temperature (Fig. 7.7).

7.4.3 Factors influencing water usage

The variation in the quantity of water consumed by all pens expressed as per pig per day (L), is displayed in Fig. 7.8.

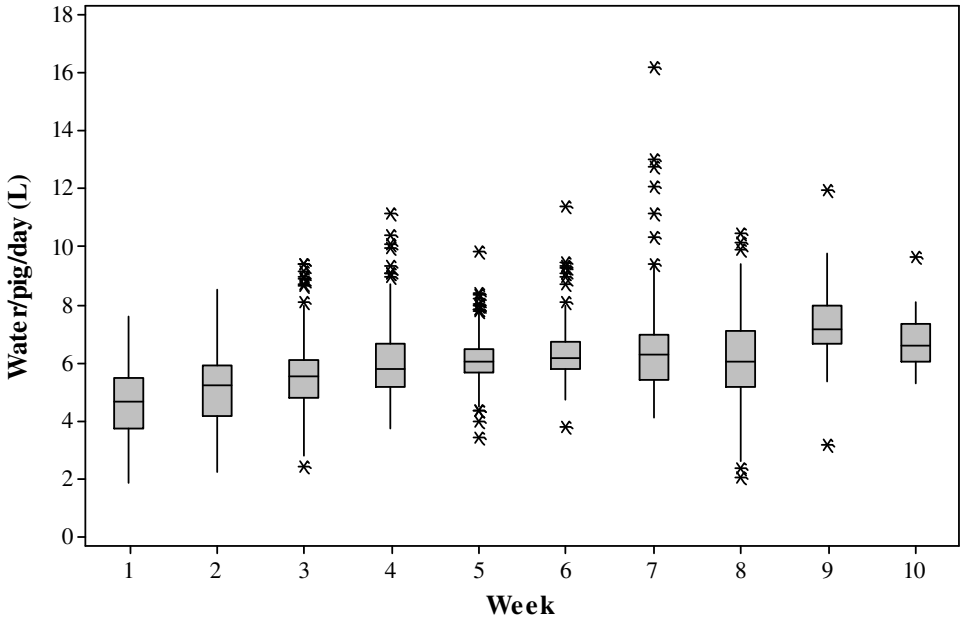

Figure 7.8 Range in water intake by all pens over the weeks on trial

When analysed as a mean value per pen over the whole period, drinker type did not have a significant effect on the quantity of water consumed per pig per day, (Monoflo: 6.0 Arato: 5.6 ± 0.25 L, $P = 0.256$), and replicate as a factor and pig weight as a covariate were not significant.

The external minimum temperature was negatively correlated to the average pen feed intake ($r_s = -0.196$, d.f. = 367, $P < 0.01$), and positively to the FCR ($r_s = 0.200$, d.f. = 367, $P < 0.05$).

Multiple regression analysis of all data over the two replicates revealed the weight of the pigs, the number of pigs within the pen, daily live weight gain, drinker type and the external min and external max building temperatures influenced the water usage at the mean pig and pen level (Table 7.2). A change in the direction of the relationship between water usage and group size occurred depending on whether the use was expressed on a pen or individual pig level.

Table 7.2 Factors associated with the variation in total daily pen water usage and mean water usage per pig and per pen (N = 23), replicates 1 and 2.

Predictors	Individual pig	Per pen
R^2 (adj)	31.70%	47%
Pig weight	0.0367***	0.0397***
No. Pigs	-0.184***	2.24***
Daily live weight gain	1.66***	13.9***
Drinker type	-0.421***	- 2.82***
Ext. min. temp.	-0.118***	- 0.64***
Ext. max. temp.	0.048***	0.244**

Where asterisk occur, *** = P <0.001, ** = P <0.05

Current observation of the number of pigs sick within the pen and daily disruption within the building from human presence had no significant explanatory effect on the daily water consumption.

A separate analysis of replicate two only, which included the feed intake data for the pens over the weeks on trial (table 7.3), showed an effect of the pen FCR and the maximum internal room temperature, whilst the drinker type, external min and the external max were no longer significant factors.

Table 7. 3 Factors associated with the variation in the mean daily water usage per pig and per pen (N = 11) in replicate two

Predictors	Individual pig	Per pen
R^2 (adj)	43.80%	53.73%
Pig weight	0.0450***	0.0740***
No. Pigs	- 0.203***	NS
Daily live weight gain	1.60***	NS
Drinker type	- 0.22^T	NS
Room max. temp	- 0.075*	- 0.75**
FCR	- 0.28*	- 4.62***

Where asterisk occur, *** = P <0.001, ** = P <0.01, T = 0.056

7.4.4 Health scores in relation to water consumption

Analysis of the different symptoms of disease, (as recorded by the health scores), showed a reduction in water consumption in pens suffering from scour at a clinical score of four in week two (Fig. 7.9). However, this reduction was not significant (P = 0.124). Of relevance to sub-clinical disease detection, pens scored for occurrence of scour in the following week (1 week later), had a strong tendency (P =0.067) towards reduced water consumption in the current week (Fig.7.10).

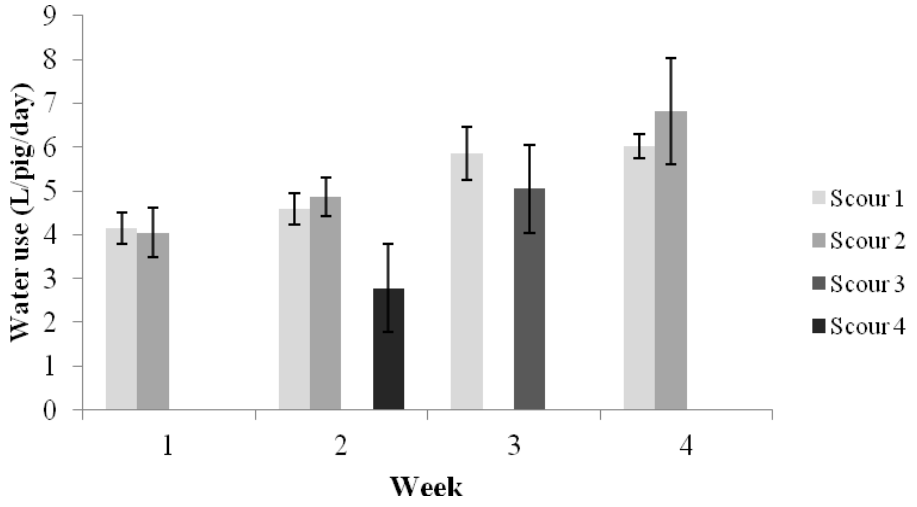

Figure 7. 9 Daily water use per pig in relation to the pen health score of the corresponding week

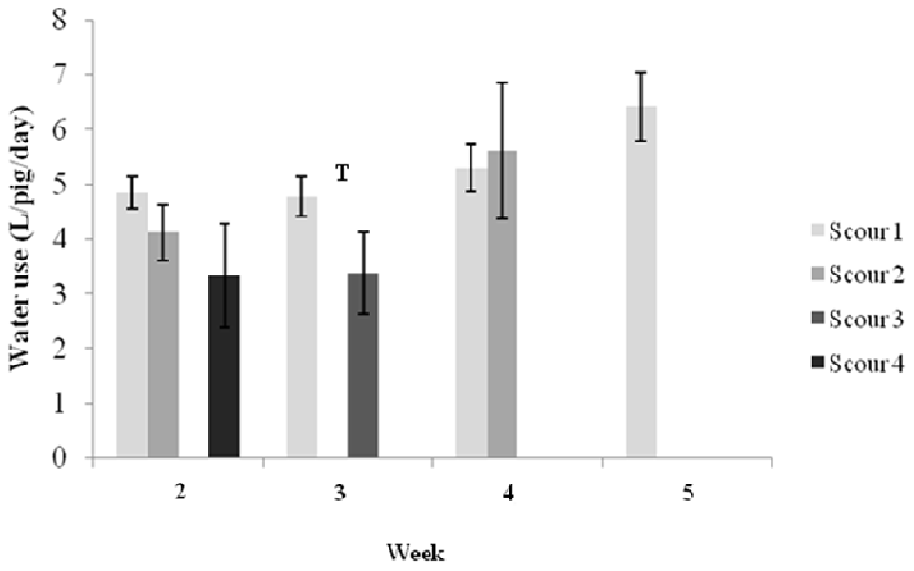

Figure 7. 10 Daily water use per pig in relation to the pen health score given in the following week. T = $P = 0.067$

Significant as covariates in relation to water consumption were; external min and mean, and room mean and min temperature in week one ($P < 0.001$), pig weight in week two ($P < 0.05$), week three ($P = 0.001$) and week four ($P < 0.05$).

There were significant differences in the water consumption in relation to the severity of cough scores in the current and following weeks ($P < 0.001$), but these did not follow any consistent pattern (Data not shown). The REML model was only able to provide mean values for the water consumption in weeks one and three, and therefore no further data are presented.

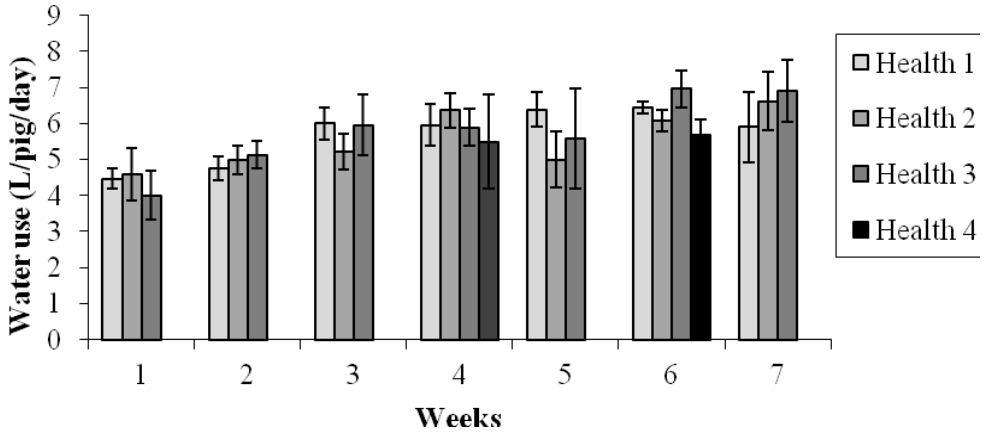

Figure 7. 11 Daily water use per pig per day in relation to the severity of pen health score in the corresponding week

There was no significant difference in the severity of the pen health scores in relation to the water consumption over the water use of the current week (Fig. 7.11), and for the majority of the following weeks (Fig. 7.12). Of the following weeks, the water consumption of week six was significantly reduced in relation to the severity of pen health scores to be seen in week seven (Fig. 7.12). However, the consumption of water did not appear to follow any consistent pattern over the weeks in relation to the severity of the pen health scores.

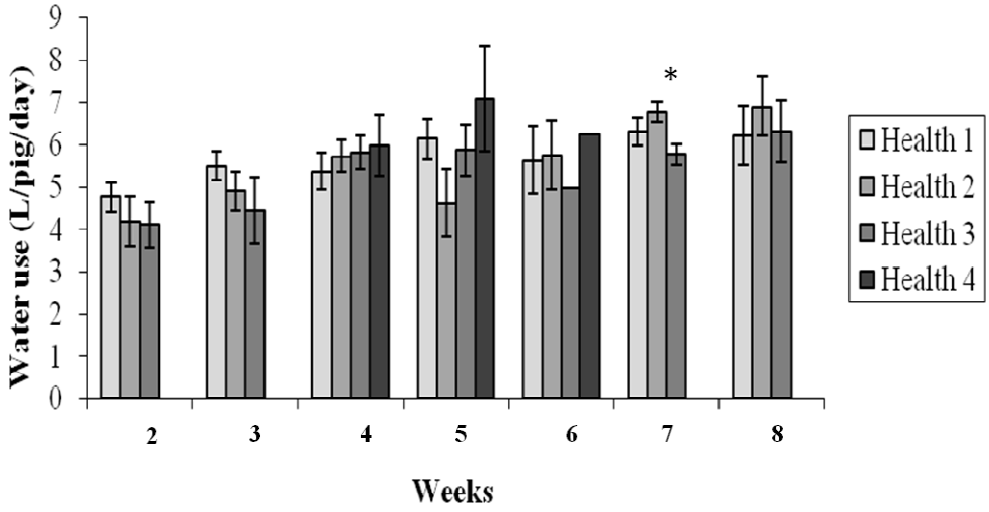

Figure 7. 12 Daily water use per pig in relation to the severity of pen health score in the following week. Where asterisk occur * = (P <0.05).

For the analysis of water consumption in relation to pen health, significant as covariates over the following weeks were; week one, external min and mean, and room mean and min temperature (P <0.001 for all), week two: pig weight (P < 0.05), week three: pig weight (P <0.001), the number of pigs (P < 0.05), week four: pig weight (P <0.05), week six: the number of pigs (P < 0.001).

7.5 Discussion

This study measured the daily water intake by groups of pigs, housed in a continuous flow building with circulating disease, and explored, a) which factors were responsible for the daily variation in the water consumption, and b) whether daily water intake could be linked to episodes of clinical disease within the group. The small group size (10 pigs per pen) had been chosen in order to provide close surveillance of the pigs, and to increase the number of replicates in a limited period of time. It was also of interest to determine, at these smaller group sizes which are likely to increase variability in mean daily intake, what can be meaningfully determined.

Previous studies exploring water usage in relation to disease have picked up changes when there was a definitive outbreak in disease across a larger group of pigs (400 - 900 pigs per group) of similar age (Madsen and Kristensen, 2005). In the current study, the ages of the pigs differed across the pens and clinical outbreaks of disease occurred in a variety of pens at different times within the building, presenting a more diffused onset of disease across the group. Therefore, these data provide information on how the daily water consumption patterns of groups of pigs change under conditions of progressive disease.

The weight and number of pigs within the pen and environmental temperature were main factors associated with water usage. The intake of water steadily increased in relation to the weight of the pigs. The results of the multiple regression analysis demonstrated a positive relationship between the weight of the pigs and the daily live weight gain and the total quantity of water consumed. This is a relationship to be expected, as increased water in required by the pigs for protein deposition and protein turnover as they gain live weight. Water contributes significantly to the development of live weight gain in pigs

(Brooks and Carpenter, 1993), and therefore, noting the quantity of water being consumed at specific stages throughout the growing life of the pig might allow a simple low-labour measure to assess the growth rate at a given stage of growth of the pigs. The narrowing gap between the water intake and the weight increase as pigs neared slaughter weight has been noted by other authors (see Brooks and Carpenter, 1993), and is believed to be due to a reduction in the body protein turnover as the pig begins to reach mature size (Whittemore and Elsley, 1979). The mean quantity of water consumed per pig per day in this trial is much lower than has been reported elsewhere. A model of water intake of growing pigs in non-limiting conditions, as developed by Schiavon and Emmans (2000), suggests that an 80kg pig should be drinking above 8L of water per day. Bird (2006) states that the water intake of growing pigs is dependent on the quantity of feed eaten, regardless of whether the pig is 50kg or 80kg. For a pig growing and eating well, a predictive chart developed by Bird (2006) indicates a pig aged 18 weeks old should be consuming 8L of water per day. In comparison, at 18 weeks of age, the pigs on this study were using between 5-5.5L of water per day. The quantity of water intake by pigs on this trial is much below what is considered normal under optimum conditions, suggesting that there are sub-optimal factors within the environment generating this low intake. It is known that the water intake of pigs will deviate largely from the optimum depending on factors such as diet composition, temperature and disease status of the pigs (Agricultural Research Council, 1981). A case study reported by Smith et al., (2009) demonstrates how comparing daily water intake of two wean to finish buildings on the same farm was able to inform of a health and feed availability problem, where water intake was persistently lower in one building than the other.

This is in agreement with the average growth rate of the pigs over this period; at 0.70kg per day, this is 300g below what the pigs could be achieving at this stage in their growth cycle, indicating substantial influences from within the pigs' environment. The negative relationship between mean water intake per pig and group size suggests this may have affected the ease with which drinkers could be accessed. On a pen level, the greater the number of pigs, the greater quantity of water is used collectively, and therefore, as the number of pigs reduces from the pen, the total water consumption reduces.

The Monoflo and Arato drinkers compared in this study are popular nipple drinkers used for growing pigs. The direct comparison between the quantity of water consumed per pig

per day between the two drinker types in this study yielded no significant difference in the quantity of water used. However, in a previous comparison between the two, an additional 0.5 L of water was found to be used with Monoflo drinkers, a difference largely due to a greater water wastage (Gill, 1988). In this study, however, drinker type became a significant factor when analysed in conjunction with additional variables within the pen (the number of pigs and pig weight) and the immediate environmental (temperature). This is likely to be due to the level of degrees of freedom between the analyses along with influence of better matched covariates.

Temperature is known to affect water consumption due to the intimate relationship between water and the metabolism. The positive relationship between water intake and maximum temperatures reached suggests that, as the temperature increased above the set level, pigs would consume more water, perhaps in order to replenish evaporative loss through increased respiration rate. In hot environments, increased evaporation leads to an increase in water consumption (Schiavon and Emmans, 2000). An optimum temperature range to house finishing pigs is between 18 – 21ºC (Kouba and Sellier, 2011). The internal building temperature in this study regularly dropped below the thermoneutral zone, following closely the pattern of the external temperature, rather than maintaining a set temperature as the external building temperature decreased. When temperatures fall below the thermoneutral zone, the pigs will increase feed consumption in order to raise their metabolism and generate body heat (Infram and Legge, 1974). The negative relationship between minimum daily temperature and water intake might reflect the fact that, during this cold winter period, low temperature stimulated feed intake and entrained water consumption. The water intake of dry fed pigs is closely related to quantity of food eaten (Bigelow and Houpt, 1988). This connection demonstrates how the water intake data can be utilised to highlight an inadequacy of the building. In addition, measuring water intake continuously is often an easier and more cost effective measure to gauge how much feed is being consumed than recording the feed intake (Bird, 2005).

Of the analysis involving only data from the second replicate, the negative relationship between the FCR and the consumption of water at pen and individual pig level could be indicative of feed wastage within the pens, or of different body tissue deposition in the pigs. The negative relationship between water consumption and FCR suggests the pigs could be depositing a greater proportion of fat and by so doing the pigs would be

expected to have an increased FCR, and also utilising less dietary water than those depositing more lean tissue. A higher FCR is associated with disease challenge, and pigs with chronic immune stimulation have been found to have a greater proportion of adipose tissue laid down (Williams, 1998). Therefore, this relationship between FCR and water intake may reflect the ongoing immune stimulation in the building as demonstrated in the APPs of other pigs (Chapter 5).

Although not significant, the data from this study found a strong tendency for a reduction in water consumption in relation to the severity of scour. Of relevance to disease monitoring, this tendency for a reduction in water consumption was seen one week prior to the change in clinical signs. This relationship between water consumption and the scour score is in agreement with data on the consumption of water in relation to enteric disease outbreaks in weaner pigs, in that a decrease in consumption occurs prior to disease onset (Madsen and Kristensen, 2005), and from retrospective analysis of water consumption data from a number of case studies in which a decrease in consumption is seen prior to disease outbreak (Crabtree et al., 2008, Bird, 2007). A reduction in drinking behaviour has been reported in sick pigs, triggered by the action of cytokines (Reiner et al., 2009). Cytokines are produced soon after pathogen recognition (Borghetti et al., 2011). Secreted from macrophages and helper T-cells, the cytokine tumor necrosis factor – alpha (TNF – α) and Interleukin – 1 (IL – 1) are responsible for inducing the behavioural and metabolic changes seen within animals and humans during infection (Bluthe et al., 1991). However, the number of days prior to a disease outbreak that a change in the water consumption occurs has been found to differ. Madsen and Kristensen (2005) reporting three days, and Bird (2007) citing three days prior to swine influenza, and Crabtree et al., (2008) citing 1 week, although reference to the specific disease in question is not given. It is plausible that the number of days prior to a disease outbreak that water consumption change occurs will differ according to the infection itself and the severity of disease. If this is the case, these differing patterns in water reduction could assist in the detection of different diseases.

In view of the statistical tendencies and numerical trends shown in this study, an increased number of replicates may be required to determine whether the use of the daily water consumption, at this level, is able to provide accurate information on impending disease.

The lack of significant difference between the majority of the pen health scores and the water consumption could also require further replicates to asses if there are significant relationships. However, with the health score being broad and covering a range of heath conditions, this could also indicate that the water consumption differs in relation to specific conditions and levels of severity, and this is worthy of further investigation.

7.6 Conclusion

The usage of water by pens of pigs was related to a number of animal and environmental factors such as the weight and number of pigs within the pen, and environmental temperature. Modelling of the relationships between water usage and these factors under different conditions could provide a predictive tool enabling deviation from normality to be reliably established as an early warning system for health and performance problems. A tendency for a differences in the quantity of water consumed one week before scour symptoms are alter, suggests that water consumption could be utilised to help detect the occurrence of sub-clinical disease in pigs and this should be investigated further.

Chapter 8: General discussion

This thesis has investigated a selection of health management strategies aimed at improving the detection and limiting the spread of disease in finishing pigs. The effect of a disinfection routine applied in all-in/all-out (AIAO) and CF housing systems on pig health through mitigating environmental challenge and improving air quality was explored (Chapter 3). In addition, the extent of reduced performance in finishing pigs suffering from clinical disease was documented in a commercial production setting (Chapter 3). To extend this over a longer timescale the lifetime growth performance of > 700 pigs under commercial management was documented and relationships between birth weight, growth profile and disease examined (Chapter 4). Following relationships observed between plasma APPs at slaughter, and pre-slaughter disease state and growth rate (Chapter 3), the use of OF for the measurement of APPs in individual and pooled OF samples was explore to assess its usefulness in understanding the development of sub-clinical disease within growing pigs (Chapter 5). A procedure for optimising pooled OF collection in terms of utility, and the sample representation of OF obtained from groups of ≤25 pigs was then determined (Chapter 6). Finally, the use of automated recording of the water intake of pigs was explored for its ability to provide information on pig health and wellbeing and in particular to act as an early warning indicator of disease (Chapter 7). The results of this thesis provide a contribution to the growing pool of knowledge to stimulate developments that can be taken forward for the benefit of a sustainable pig industry by improving the welfare and performance of the pig, and the profitability of producers.

New health challenges will continue to present themselves to the pig industry, which in response needs to have a robust set of control measures in place. Past history (BPEX, 2011c) has shown that the ever fluctuating, and at times extremely volatile, nature of the global pig meat market can result in a producer's profit quickly turning into a loss. These challenges place a greater emphasis on the need to improve the management and the productivity of finisher pigs, and to address health challenges in a cost-effective and sustainable manner. Finisher pigs are the end product of on-farm production, and are at the point where profit should be consolidated. Failing to monitor pigs carefully results in

lack of knowledge of how the herd is actually performing and consequently failure to address any existing problems. The result is suboptimal performance and revenue.

8.1 Realising the extent of lost production

Losses from disease arise from a reduced growth rate and feed efficiency, veterinary treatment costs of sick animals and, at worst, mortality. Whilst producers can visually identify lost production from poor growing individuals, especially when a disease outbreak occurs, there still remains the question as to how much production potential is being lost in the group as a whole. This can be deceptive, as demonstrated by Wood and Lysons (1988) who found a deterioration in FCR of 0.58 over a four year period when dysentery was endemic within a study herd, equating to an additional cost of £7.31 per pig at the time of publishing. They commented that the greatest losses to production were from sub-clinical, rather than clinical cases. This demonstrates that the extent and cost of production lost per farm as a result of disease cannot be truly known unless measured, and commonly it is not. Attempts to measure the cost of the most common diseases have been made at various levels: for example for pneumonia from data collected in experiments (Straw et al., 1989), swine dysentery from production data of a farm before and after eradication (Wood and Lysons, 1988), and an estimation of the economic cost of PRRSv to the United States industry through comparing data from infected and uninfected herds (Neumann et al., 2005).

Whilst no specific veterinary diagnosis of diseases was made in this thesis, (apart from initial diagnostics on the study farm as detailed in chapter 3, section 3.2.1) reductions in the ADG of pigs were linked to clinical respiratory and enteric disease symptoms (coughing and severity of scour appearance in faeces) (Chapter 3), just as a producer could observe. The growth reductions in pigs suffering from clinical respiratory and enteric problems, but which recovered without hospitalisation, was of the order of 80-90g/day. This, when calculated at 4p/g cost of production, accounting for input costs, sale value, FCR and interest rates (Richardson, 2011), equated to a loss of £3.20 - £3.60 per pig through lost gain alone. Additional to this loss would be the costs of drugs and labour for treatment. However, when comparing the average daily gain of all pigs who suffered respiratory symptoms and enteric problems (and thus also those that did not recover and remain on experiment), the reductions in average daily gain were substantial at -0.37kg

and -0.17kg in comparison with daily gain of pigs suffering no health problems. When calculated as the average cost of production, this equates to £14.80 per pig suffering from respiratory problems, and £6.80 per pig suffering from enteric problems (assuming 4p/g gain, Richardson, 2011). Again these data show the loss based on reduced growth alone; treatment costs would be additional and, depending on the severity of infections, the extent of effect on FCR will alter to a greater extent than that calculated by Richardson (2011). It was not possible to measure FCR on an individual pig basis in this study.

The average lung score across all replicates in the study reported in Chapter 3, was low at 4.5, which compares to a mild lung score as described by Burch (2007a). However, the growth reductions found in this study were similar to that expected from severe scores by Burch (2007a). Taking this into account, the estimated loss could be far greater than that estimated by Burch (2007a). The greater reduction in daily gain in pigs found in this thesis (Chapter 3), could be related to concurrent infections, and could also serve as an example for how growth reductions as a result of disease could differ when in combination with a variety of farm housing and management factors.

The pigs on trial in Chapter 3 were under conditions of experimental management and prompt veterinary treatment was given to clinical cases. Data from the longitudinal study of pigs (Chapter 4) revealed that under commercial farm management treatment was prioritised to the younger pigs and pigs later in the finisher stages received very little veterinary treatment, even though the most adverse health conditions occurred at these stages. Slow growing pigs and sick pigs were often left within the normal production flow with little intervention. Maintaining poorly growing pigs in such a situation is likely to result in a significant financial loss.

This study provides a concerning insight into what might be occurring on a number of farms, if not all. Other work recording the administration of animal veterinary treatments on individual farms to form an animal treatment index (ATI) by which farms could be benchmarked for pig health, revealed a number of farms displaying an ATI of 0.0 on pigs sent to slaughter indicating no veterinary treatments were administered (Blaha et al., 2006). The farm under study (Chapter 4) suffered from persistent disease problems in the finisher stage and lack of intervention at this stage may reflect an act of acceptance by the producer. Where disease persists endemically on a farm there is, therefore, a danger that

the impaired performance of the pigs can become accepted as the normal situation. On many farms more attention is devoted to the breeding and weaning pigs than to the finishers, placing importance on the fertility and health of weaners, which are more clearly apparent. However, the data from these trials should reiterate that the finishers are the end product, and that attention to detail should remain until the point of slaughter.

To encourage producers to take an active role in recording how disease could be affecting their herd, Burch (2007a) estimated the cost of pneumonia from severity of lung scores. This provides a means by which producers using the British Pig Health Scheme surveillance at slaughter can estimate the amount of lost production per pig based on the severity of lesions. In 2007, Intervet launched the ResPig® model (Intervet/Schering-Plough Animal Health, 2001), allowing a service by which to calculate economic losses associated with a range of porcine respiratory pathogens and, in addition, to calculate the cost-benefit of applying a vaccination strategy. Included in the package for the ResPig® model was the opportunity for producers to have disease diagnostics conducted to provide an up to date picture of the state of disease on the unit. However, despite this offer, the uptake of the model in the UK was poor (J. Richardson, personal communication, 19th August, 2011). However, Intervet NL provide the ResPig model as a charged service to Dutch pig producers, and sell over €500,000 worth per year (J. Richardson, personal communication, 19th August, 2011). This clearly displays the difference in attitude and approach to health management between some Dutch and UK pig producers.

The figures calculated in this thesis demonstrate that, when clinical signs are present, significant compromise to the performance of pigs has occurred and those pigs that have suffered from disease will thereafter lag the growth rate of others (Chapter 3 and Chapter 4). Sick and slow growing pigs may remain in the system a long time and cause significant financial loss per pig. With so little monitoring on the majority of farms, the net negative effect is likely to be more costly than a producer`s expectation or estimation. Little uptake of tools on offer from industry (e.g. ResPig®) to quantify lost production could indicate that a change in attitude is required.

8.2 The use of health scores for objective observation

In beginning to recognise the extent of health problems on a unit, direct observation of the pigs is the simplest approach available to producers (Friendship, 2005) and can be employed immediately at no extra cost. However, at this simple level, shortfalls easily arise in the standard and consistency of observation on farms due to differences in the perception and attitude of individuals, the approach to observation taken, and varying opinions as to which symptoms require action. In addition, the increasing size of farms and the adoption of systems with larger group sizes make direct observation more difficult.

Disease scoring of organs at slaughter has been developed and used successfully for many years; see Goodwin et al. (1969), whose lung scoring system for pneumonia accurately quantifies the severity of pneumonia infection in individual pigs and is now used in the British Pig Health Scheme's national abattoir surveillance system (BPHS, 2011). This incorporates measurement and reporting of other lesions including papular dermatitis, milk liver spot, pyaemia, tail-biting and pericarditis (BPHS, 2011). Abattoir based systems are easier to employ and give good coverage of the population in that cohort, but are not informative about all diseases, as for example enteric disease or disease occurring early in life where lesions may have resolved by slaughter. The application of health scoring to live pigs during on-farm observation is intended to introduce methods through which the severity of health conditions can be measured longitudinally in groups of pigs with a degree of consistency and accuracy, using simple methodology that all producers can employ. The benefit is realised where an observation technique can be informative on the extent of disease and/or lost production arising from certain clinical measurements. Previous studies have examined cough scores as a measure of pneumonia and live weight gain in pigs (Morris et al., 1995), coughing and sneezing to estimate pneumonia and atrophic rhinitis (Morés et al., 2001), faecal scores, cleanliness scores and overall health scores as a measure of enteric health and general health (Wellock et al., 2006).

Studies have found significant associations between health scores and disease level, however, the relationships have not always been strong. Morris et al., (1995) concluded there was no relationship beyond chance between weekly cough scores per pig and lung score recorded at slaughter in their study. Cough scores of individual pigs were only weakly related to the severity of pneumonia (r = 0.32), as determined by thoracic

radiograph on a weekly basis and estimation of the percentage of lung tissue consolidated by pneumonia lesions at slaughter, leading Straw et al., (1990) to conclude the cough scores were not a useful measure. From the health scores tested throughout this thesis, no associations were found between cough, sneeze and scour scores and APPs in live pigs (Chapter 5). This could indicate that the triggers for the APPs were unrelated to the clinical signs observed in the health scores, which were very low overall. However, relationships were found between cough scores and incidence of pneumonia in live pigs, cough scores and lung scores at slaughter, and a high pen health score related to a low pen daily live weight gain (Chapter 3). In addition, whilst not significant, an anticipated relationship was seen, when a reduction in water consumption was observed in relation to severity of scour score (Chapter 7). There was a tendency for a reduction in water consumption to occur a week prior to symptoms, suggesting that the scour score was representative of the severity of the ongoing enteric problems within the pig.

The strength of association between cough score and lung score at slaughter in this study ($r = 0.36$) was similar to that found by Straw et al., (1990), with a stronger relationship found between coughs and the prevalence of pneumonia in live pigs ($r = 0.55$). However, whilst both Straw et al., (1990) and Morris et al., (1995) assessed pigs on an individual level, the work in this thesis used a simpler level of cough scores (number of coughs in 5 minutes heard from the group of pigs) to assess the group as a whole. Therefore, despite the weak correlation, its presence suggests this is something that can be used and could be built upon. It is important to note which scoring systems do and do not work, and to the test the robustness of those that do in practice.

New projects involving farm surveys are now adopting health scores developed in the EU Welfare Quality project as a means to benchmark the welfare status of farms against those of others (see Welfare Quality, 2009), and incidentally are utilising the method of cough scoring used in this study to assess prevalence of respiratory disease. Amongst methods by which pig herd health can be benchmarked, the ATI developed by Blaha et al., (2006) is only effective if producers are actually administering veterinary treatments, as does not always appear to be the case (Chapter 4), and actually recording them. On the other hand, observational health scores, if robust enough, could be conducted by vets or trained auditors. When the pig is displaying the clinical sign, there can be no confusion as to what is occurring on the farm.

Considering the ease with which health scores can be conducted this approach could justify more research. If reliability can be confirmed, a very cheap and quick tool will be developed through which producers can monitor the progression of disease on their farms. To be worthwhile health scores should be able to reflect the severity of the disease, and if this could be linked to reductions in performance the added benefit would be even more valuable.

8.3 The importance of housing and environment

The management of housing and environment is pivotal to successful health management of pigs. Part of this thesis attempted to look at the interactive affects of housing and environment on pig health and performance (Chapter 3), and of key interest in this study was to ascertain whether washing within a CF system was able to reduce the total immunological load placed on the pigs from the surrounding environment. The work conducted in this thesis (Chapter 3) found no difference in the health and productivity of pigs between AIAO and CF housing. Whilst there were differences in the level of ammonia and airborne bacteria between the AIAO and CF systems, the levels were all lower than those found to cause disease problems. A lack of difference between treatments in the serum Hp and CRP levels from a sub-sample of pigs provides further evidence to confirm the lack of differences in health and productivity between the systems. It also suggests that the study`s attempts to create unhygienic conditions were not sufficient to create chronic immune stimulation, as has been found in pigs housed in less hygienic conditions (Petersen et al., 2005).

A failing of this study could be the attempts to simulate the AIAO and CF systems in a controlled way by simulating the systems in a controlled building. The design of this study is open to criticism, as small scale simulation of commercial environments will potentially not produce the same magnitude of response in the pig as is found in the commercial setting, in addition to results being non-representative of larger farms. However, conducting this type of labour-intensive research on a variety of commercial farms operating AIAO and CF can also bring problems due to introducing a large amount of variability in housing systems and their management. Only through creating controlled and comparable systems can this be avoided. Others have recreated controlled AIAO and

CF systems with success (Ice et al., 1997, Scheidt et al., 1995), which gave a basis to attempt it in this thesis.

Despite the absence of differences in the current study, there is no doubt that the environment in which we house pigs will greatly influence the extent to which pigs will reach their genetic growth potential (Robertson, 1997). On-farm pathogen presence, combined with the aerial and surface hygiene of housing systems, contributes to the overall immune challenge facing the pigs. Farm hygiene has been linked to the level of immune activation as measured by APPs (Petersen et al., 2005), pigs housed in unwashed pens have been found to have a lower average daily live weight gain than pigs placed in cleaned and disinfected pens (Cargill and Banhazi, 1996), and the synergistic effect of aerial contaminants has been found to play a key role in severity of respiratory disease (Robertson, 1997, Robertson et al., 1990). In summary, research demonstrates that in the presence of pathogens additional factors may act as immunosuppressants (Robertson, 1997), or potentially overload the immune systems of the pig. As a result the effect of reducing one element of the total challenge could mean the difference between pigs succumbing to disease or not. This approach has been well demonstrated in the effective Madec principles for the control of PMWS (Madec et al., 2000, American Association of Swine Veterinarians, 2007), a disease in which environment is a key factor for determining the outcome (Madec et al., 2000). The Madec principles demonstrate what can be achieved through directly improved management rather than reliance on conventional control measures such as antimicrobials and vaccinations.

This thesis (Chapter 3) did show that a cleaning and disinfection routine did significantly reduce the quantity of bacteria on the pen surfaces. Whilst no specific pathogens were enumerated, the measure of a total viable count provides a simple method to evaluate the total bacterial load remaining on the surface of the pens. Cleaning and disinfection routines are a vital step in the control of disease, and regularly advised in control measures (Done et al., 2001). With muck and mud reducing the efficacy of disinfectants against pathogens (Thompson et al., 2007), the importance of following a specific disinfection procedure involving a prewash is recognised. Due to time constraints and differing opinions, it is not surprising that surveys have revealed that there are some failings in the process of cleaning and disinfection within the industry (Mannion et al., 2007). The availability of cleaning robots a such as the Multiclean (Nilfisk-ALTO,

Denmark) offers a solution to producers who fail to achieve sufficient cleaning and disinfection through lack of time, loathing of the job. or health concerns. The robots should ensure high quality cleaning of a consistent standard. However, as the robots are programmed to clean by the operator, studies have shown that if utilised incorrectly the post cleaning cleanliness can be poor (Pedersen and Kai, 1998, cited in Zhang et al., 2006), and the robots can use up to 40% more water than manual cleaning (Zhang et al., 2006). Further development of the technique is required in order to optimise use of robots, and ongoing work includes the development of an automated optical scanner to assess efficiency of cleaning (Zhang et al., 2006). If programming and efficiency of robots can be optimised it should bring benefits to pig health management through ensuring high quality washing and disinfection. Benefits to the respiratory health of farmers have also been realised as farmers are not present to inhale bioaerosols generated during the cleaning activity (Hiel et al., 2009).

This work found the use of disinfection did not give any benefit to the performance of the pigs in the CF system. Cleaning in a room with pigs still in occupation would not normally be recommended due to the generation of bioaerosols from the cleaning and disinfection process. An increase in bioaerosols was not found in this study as a result of washing, but there was an association with an increase in ammonia. Instead, it became apparent that there were far better areas for producers to focus on when seeking to improve the environment of the pig; principally the main factors influencing air quality. These include pig stocking density, pen hygiene and ventilation. The overall message drawn from this is that, in terms of pig health, a continued holistic approach needs to be taken in providing and supporting the correct environment for the pig.

8.4 The application of APPs in the pig industry

APPs have regularly been cited as having potential as a biomarker for health surveillance in veterinary medicine, providing information on the extent of ongoing inflammatory processes to assist diagnosis and prognosis of disease states (Petersen et al., 2004, Murata et al., 2004). One reason for the interest in APPs for pig health management is as a measure by which individual herd health can be benchmarked against other herds. In addition, the APPs can provide additional diagnostic information on the extent of disease on a unit and whether, and where within the production system, sub-clinical disease is

occurring. Sub-clinically infected growing pigs will often have no overt clinical signs of disease, but will have poor growth performance as the body mounts an immune response and increases catabolism of protein, as has been demonstrated in piglets injected with LPS (Daiwen et al., 2008). Where growth rate has been measured, a relationship between APPs and daily live weight gain is commonly reported (see Eurell et al., 1992, Clapperton et al., 2005, Franek and Bilkei, 2004). The work in this thesis also found relationships between the growth rate of pigs and the APP concentrations in plasma and OF. The association between APPs and growth is a valuable one and can link APPs to areas of sub-clinical disease activity. This information can be utilised by the producer to determine the extent of lost productivity occurring on a particular unit as a result of immune activation. When used in association with current knowledge of the circulating pathogens on the farm, and an assessment of hygiene status which has also been found to relate to APP immune activation (Petersen et al., 2005), the measurement of APPs could assist in determining where management changes should be made, and measure the success of any changes.

If APPs were to be adopted as a biomarker, a regular sampling regime may be required, or be of greater benefit. Recent work on selecting infection biomarkers for the automated continuous screening of pig health suggests that frequent observations are required to allow a continuous stream of data for monitoring of individual pigs/the herd (Tambuyzer et al., 2011). In such a scenario, only non-invasive sampling techniques would be suitable for industry application. The development of a time resolved immunoflouretic assay that can successfully detect levels of CRP and Hp in OF (Gutiérrez et al., 2009a, Gutiérrez et al., 2009b) is a significant development and now really opens possibilities for the application of APPs in pig health. In due course, the possibilities will expand with the refinement of assays to detect additional APPs. The measurement of APPs in OF examined in this thesis builds upon emerging work (Gutiérrez et al., 2009c, Gutiérrez et al., 2011), and helps test the robustness of the technique in a variety of settings and on farms of differing health status. The values obtained in this study can contribute to knowledge that can be eventually used in establishing benchmarks for pig herd health.

There has been much discussion surrounding the potential benefit of APPs to benchmark farm health status. However, the highly variable nature of APPs and the wide variety of different farming systems, management types and health and hygiene statuses mean that a

lot more work is required before farms can be confidently benchmarked by APP level. Of the large amount of research conducted on APPs in pigs, the vast majority to date has focused on comparing levels of APPs in clinically healthy and clinically sick pigs (Petersen et al., 2002), most often from experimentally induced states through which the course of infection can be mapped (Heegaard et al., 1998, Barbé et al., 2011, Martín de la Fuente et al., 2010). This work has been important to determine which APPs react to which disease conditions. However, studying the APP responses of pigs with one specific disease is unrepresentative of the true situation, of pigs on farms. They are often subject to multiple concurrent infections and factors related to management and hygiene status may interplay and possibly have immunosuppressive effects. In such a scenario it is possible that the typical APP response observed with one experimentally induced infection may not occur, especially as the strength of infections may differ. Any APP monitoring to be done for benchmarking purposes has to be robust enough to take this into account.

One of the much discussed benefits of APP measurement, the detection of sub-clinical disease, is also an area in which little research has actually been done. A few papers have approached the subject, and the response of APPs in sub-clinical states has been found to be either as great as found in clinical states (Sorensen et al., 2006), show no response at all (Grau-Roma et al., 2009), or show lower levels in the days before clinical symptoms developed (Harding et al., 1997). Characterisation of levels of APPs during sub-clinical disease is required because it is highly likely that the response may differ from when a clinical disease state is seen. It was a key interest to explore the use of APPs for the detection of sub-clinical disease in this thesis (Chapter 5). The poor growth rate and relatively few clinical symptoms observed suggest that the pigs could have been experiencing chronic sub-clinical disease. Figure 8.1 shows a range of Hp levels from a variety of studies and compares values from clinically healthy pigs, pigs with known diseases and pigs with unknown and thus potentially subclinical states, to the results found in this study in which a sub-clinical disease state is postulated (source Seddon). By separating the results in this thesis into groups based on the ages of the pigs (in order to generate more comparisons), it demonstrates that a number of the groups had values comparable to pigs regarded as clinically healthy, and the remaining had values within the range of pigs seen to have known disease conditions including diarrhoea, respiratory

disease and lameness. This middle of the range value may be indicative of disease presence.

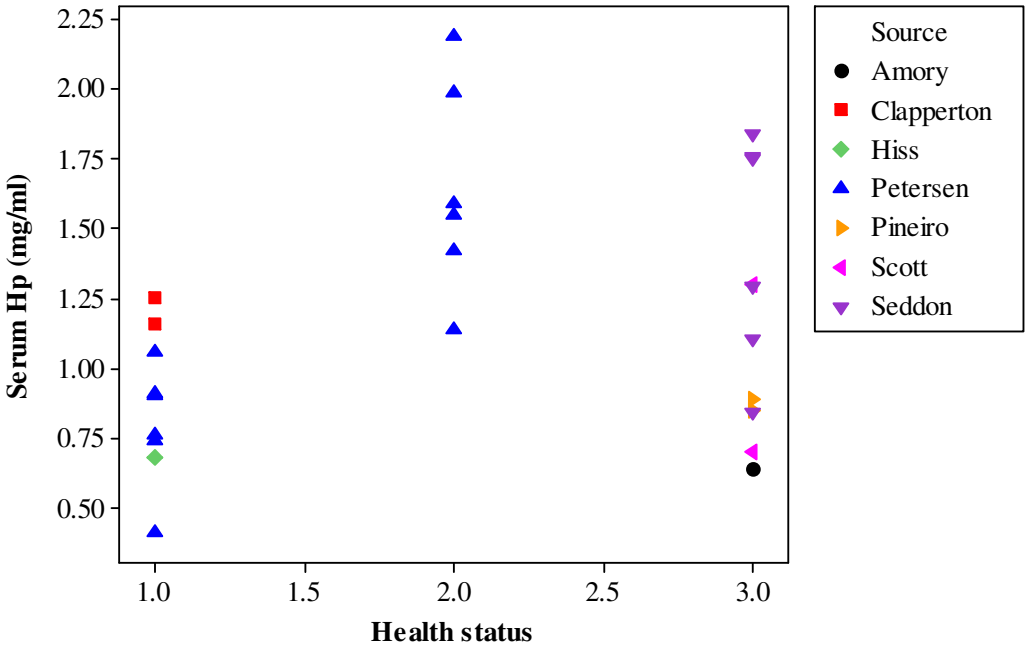

Figure 8. 1 Concentration of serum Hp of pigs clinically healthy (1), diseased (2) and of unknown health status (3). Source of reference: (Amory et al., 2007, Clapperton et al., 2005, Hiss et al., 2003, Petersen et al., 2002, Piñeiro et al., 2009c, Scott et al., 2005). (This graph contains the results of multiple pigs from the trial in Chapter 5, displaying different ages for a greater number of comparisons for the graph. Using only the mean, it would be at the position of 1.51mg/ml).

In the few papers that have looked at sub-clinical disease (Sorensen et al., 2006, Harding et al., 1997), it was not possible to compare the levels directly as the authors do not provide direct reference values.

This study is one of the first investigations to assess sub-clinical disease through APPs in OF under commercial conditions, and one that could be used to contribute to benchmark values. Few papers are currently available displaying levels of APPs in OF, due to no commercial analysis kit being currently available. It currently appears that only one research group (Murcia Veterinary University) is running the analysis. Figures 8.2, 8.3, 8.4 and 8.5 display the concentrations of Hp and CRP in serum and OF found in this study, compared to the work by Gutiérrez et al., (2009c), split over age groups. The values of serum Hp, which was related to finisher and lifetime ADG (Chapter 5) are

higher than values found for healthy pigs, but lower than values found for pigs with clinical PRRSv, and therefore could again be relating to a lesser level of disease burden than those monitored by Gutiérrez et al., (2009c) but still showing immune activation.

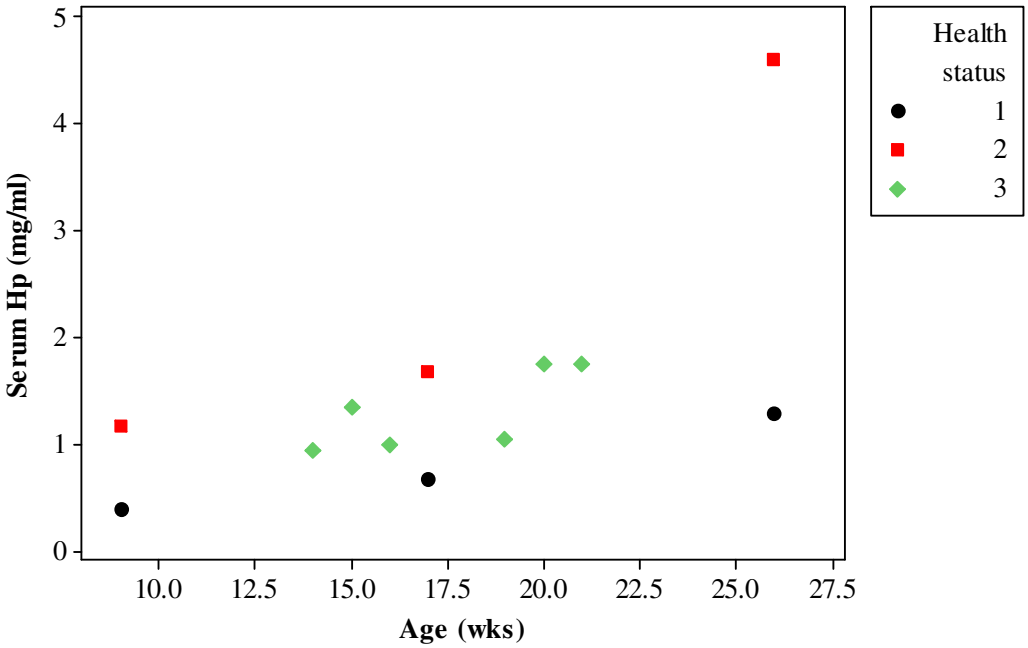

Figure 8. 2 Concentration of Hp in serum from pigs clinically healthy (1), diseased (2, Gutiérrez et al., 2009c) and of unknown healthy status (3, Chapter 5) at differing ages.

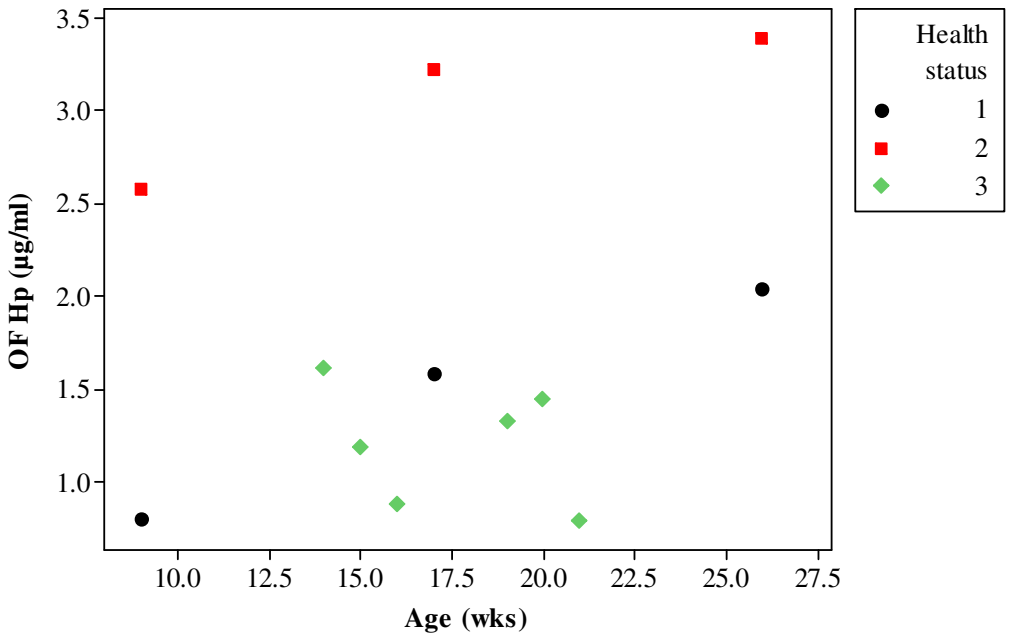

Figure 8. 3 Concentration of Hp in OF from pigs clinically healthy (1), diseased (2 Gutiérrez et al., 2009c) and of unknown healthy status (3, Chapter 5) at differing ages.

The concentrations of Hp in OF found in this study are lower than those found by Gutiérrez et al., (2009c), however, associations between the lifetime ADG were found, and may indicate the lower levels are linked better to more chronic conditions.

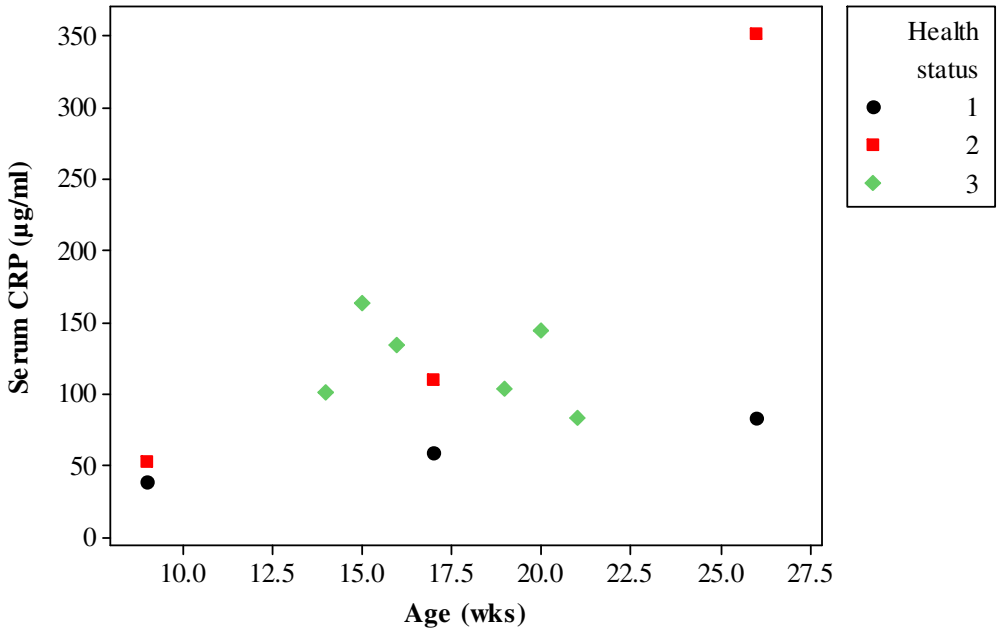

Figure 8. 4 Concentrations of CRP in serum from pigs clinically healthy (1), diseased (2 Gutiérrez et al., 2009c) and of unknown healthy status (3, Chapter 5) at differing ages.

The concentration of CRP in serum was not found to relate to any production parameters in the pigs (Chapter 5). However, fig 8.4 demonstrates that the concentrations of CRP in serum are higher than those found by Gutiérrez et al., (2009c). Fig. 8.5 displays that the concentration of CRP in OF from this thesis was similar to that found for healthy pigs by Gutiérrez et al., (2009c). Yet it was concentrations of CRP in OF that had significant relationships to the productivity of the pigs in the finisher stage and the length of time the pigs were in the finisher building. Whilst this is only a comparison with one study, this is an interesting observation. For APPs to be significantly representative of disease, in the case of CRP, a significant increase in concentrations is frequently thought to be expected. The data from the OF suggests that this is not necessarily the case and lower values can provide meaningful information.

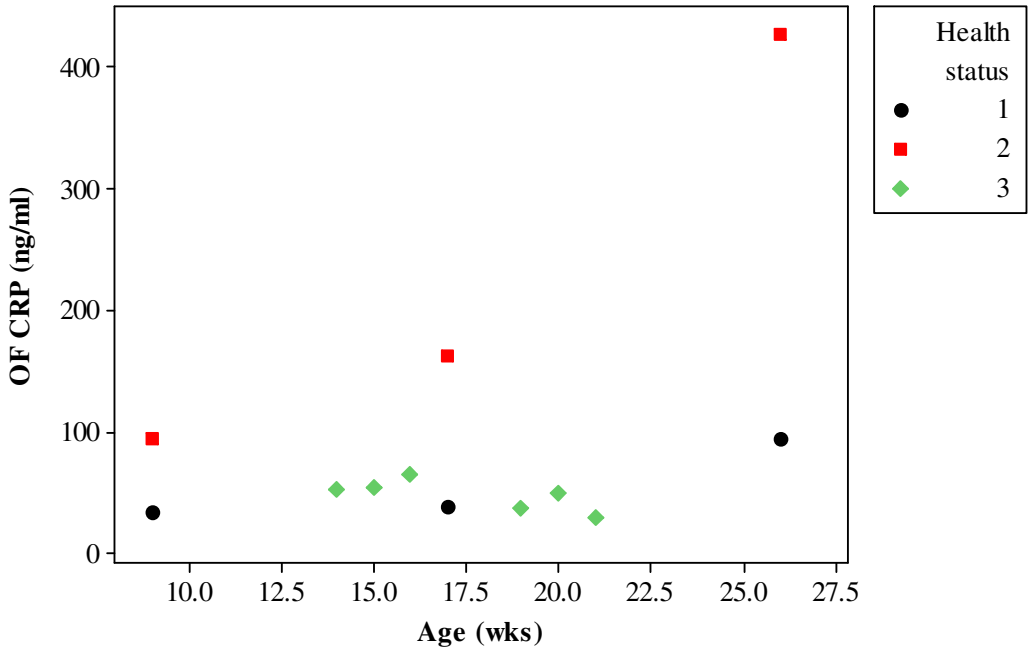

Figure 8. 5 Concentrations of CRP in OF from pigs clinically healthy (1), diseased (2 Gutiérrez et al., 2009c) and of unknown healthy status (3, Chapter 5) at differing ages.

In the sub-clinical state, there may be varying APP levels present depending on the severity of the sub-clinical case and the duration of sub-clinical activity. Knowing what the level of APP activation may mean in terms of losses to production would be very useful for their practical application to industry. The pig industry already provides benchmark herd performance within (BPEX, 2010b) and across countries (BPEX, 2010a). However, studies of APPs need to record the growth rate (and ideally FCR) of the pigs in order to relate to this, and the fact that few have done so is a real failure in APP research.

For OF to be an easy diagnostic medium to monitor APPs, ideally a quick test is needed that can provide levels of the APPs quickly and simply as a penside diagnostic. The Pig-MAP stick® available through PigCHAMP Pro Europa S.A., Segovia, Spain is one example on the market, but currently only works for detecting APPs in serum and meat juice.

Where farmers are in an area of constantly circulating disease challenge, it has been suggested that changes in diet formulation can be made to assist the pigs in coping with the immune challenge, and to reduce the extent of lost production through modified feed

utilisation efficiency until a long term solution to the health problem can be realised. This gives some potential for alterations to diet formation appropriate to meet the changed nutrient requirements of pigs with high immune stimulation. In such cases appetite and lean tissue growth rate are reduced, and a different balance of amino acids is needed to reflect their utilisation for immunological rather than growth processes. This use of APPs can also prove useful in the experimental setting, where changes arising from experimental procedures can be closely monitored.

8.5 The use of OF pooled samples – cutting costs in diagnostics

The ability to detect pathogens in OF, and the increasing sensitivity of diagnostic tests, is now making it possible to utilise a pooled OF sample for screening of disease pathogens. Use of a pooled sample offers significant cost savings in health monitoring, allowing a large number of individuals to be screened via analysis of one sample, or at most a few samples. This is a technique already being utilised in the dairy industry, which analyses bulk milk samples for the detection of disease (Thobokwe et al., 2004).

The collection of a pooled OF sample by absorbent cotton rope was proposed by Prickett et al., (2008a). Work in this thesis analysed the behavioural activity of the pigs in response to rope provision. This is an important step to determine what level of chewing activity, and thus OF sample deposition, can be expected in different scenarios. Important sampling questions include decisions on the number of ropes and the ideal pig:rope ratio to optimise group representation, and how this is affected by housing system – with fully slatted and straw bedded systems both being common growing pig housing systems within the UK and wider Europe. The work was able to confirm that provision of 1-2 ropes to groups of ≤25 pigs for 45 minutes optimised the number of pigs to chew the rope and, in addition, demonstrated that the uptake of rope was poorer in straw bedded housing systems. This could have implications for how representative a pooled OF sample collected from enriched environments might be, since the provision of additional ropes did not improve uptake in straw systems. These systems also operate with larger group sizes, for which the best strategies are currently unknown.

No analytical work was conducted on the OF collected in Chapter 6 due to financial constraints. To determine the robustness of this collection technique a logical next step

would be to link analytical values from OF samples collected from individual pigs known to have chewed the rope (as verified by the video footage) to analytical values from the pooled OF sample. This would also help to determine how long individual pigs need to chew the rope to be represented in the OF sample. However, studies continue to demonstrate that use of a pooled sample has the possibility detect the presence of circulating pathogens within pen groups. The sampling technique now being used for PCV-2 virus allows its detection from day two post infection (Prickett et al., 2011), also PRRSv (Prickett et al., 2008b) and swine influenza (Detmer et al., 2011). To add to the growing array of evidence, the negative relationship between the concentration of CRP in a pooled OF sample and the average daily live weight gain of the pen of growing pigs (Chapter 5) is the first evidence to suggest the possibility of measuring the APPs of a greater number of pigs usefully with one sample. However, as this relationship was not replicated for Hp, future work is required to determine if the relationship in CRP is by chance or if there are factors which could enable Hp to be used in a pooled sample.

The use of pooled samples drastically reduces analysis costs, although some sceptics of OF have argued that a pooled serum sample would achieve the same cost reductions (G. Wade West, personal communication, February 2010). However, major benefits arise from the sampling possibilities for OF as a non-invasive sampling technique which utilises the pigs' desire to chew since sample collection requires minimal interaction with the stockperson, and no requirement for a vet on site. This brings multiple benefits in terms of reduced stress for the pigs, less veterinary costs for the farmer, and improved biosecurity resulting from less vet circulation between diseased farms. This type of sampling would be particularly useful for routine disease surveillance, and would be especially effective for surveillance required in the regional pig health improvement schemes which require health status testing of all participating farms. In the long term, reduced costs of sampling could lead to increased disease surveillance operations that are required for the quick and timely discovery of herds becoming re-infected with disease.

Further research will reveal whether there are limitations to the number of pathogens that can be detected in the pooled OF, and the time frame in which they actually become detectable in OF. However, if a non-invasive diagnostic fluid is required, OF offers many possibilities by being easy to collect. Work on pigs in the USA shows the great potential for this technique, but it is still hard to find work carried out in Europe documented in

literature. This is necessary as some pathogen strains, such as for PRRSv, differ between Europe and the USA. The experiment in this thesis (Chapter 5) is one of the first to document attempts to measure the European strain of PRRSv in OF. However, it is not a very good example of practical application, as it transpired that the majority of the pigs had seroconverted to PRRSv.

8.6 Water consumption monitoring – a component of precision livestock monitoring

The development of systems that can provide an early warning indicator of disease is of increasing interest and importance to the livestock industry. The monitoring of water consumption holds real potential in this respect, as the consumption closely follows the metabolic state of the individuals, and therefore the analysis of changes can provide information on the productivity and health of the animals. The specific interest in water consumption in this study related to its potential as an early indicator of disease. In farms operating AIAO production for weaner pigs by building, deviations within daily water consumption patterns have been found to alter one day prior to the clinical signs of postweaning scour (Madsen and Kristensen, 2005), and one week prior to observation of (unnamed) clinical symptoms in pigs (Crabtree et al., 2008).

The work in this thesis analysed the water consumption of pens of pigs in CF housing and used a less refined level of analysis by looking at the total daily water intake. The results were not significant, but demonstrated that when pigs were showing immediate signs of scour a reduction in water consumption was observed in relation to the severity of scour. In addition, in one week, there was a relationship which approached significance despite limited replication between a reduction in the total daily water consumption of pens of pigs one week prior to a change in the clinical severity of scour (chapter 7). This encouraging finding should stimulate further study to develop more sophisticated pattern analyses and algorithms for automated disease alerts.

Apart from the benefits arising from water intake as a lead indicator of health changes, the results in this thesis found relationships with body weight, live weight gain, feed intake, temperature and group size. The presence of these relationships suggests that the modelling of water consumption could be taken to a level that provides a fully automated commercial package delivering information not only on current and predictive

information on pig health, but also of the rate of feed intake and growth performance. This would make the use of water consumption a far more powerful monitoring tool, and would enable the capture and storage of growth and performance data automatically, something producers are constantly encouraged to achieve. With automated monitoring, the process of keeping pig heath and productivity records could be carried out with low labour costs and yet be highly accurate. This would offer producers a better awareness of shortfalls in production on their farm. The benefits of this to the industry have been repeatedly highlighted, for example by Bird and Crabtree (2000).

The tools enabling the automated measurement of water consumption are a simple addition to piggery building environmental control systems, as offered for example by Farmex Ltd (Reading, UK). However, unlike the poultry industry, it is proving apparent within the pig industry that there is unlikely to be uptake unless there is a fully automated system analysing the data continuously and providing simple output interpretation. Currently there is no infrastructure for this in place and such a development is a future challenge for the industry.

8.7 Final conclusion and findings from the thesis

The findings of this thesis demonstrate that chronic underperformance of growing pigs results in severe economic loss. When clinical signs are present there is a large associated deficit in performance and pigs that experience set-backs will continue to lag behind. Simultaneously, slow growing pigs, potentially sub-clinically affected, can affect profits equally severely and remain untreated within the system. This emphasises the need to target disease as early as possible and take prompt action.

The measurements examined in this thesis should be regarded as disease indicator tools, which should prompt further veterinary investigation, not a diagnosis in itself. Simple health scores provide a method to increase a producer`s awareness of health problems occurring on the farm, and can be linked to performance measures. Whilst these can only measure clinical symptoms other tools to help identify disease early, and in the sub-clinical stage, are now on their way to development and future reality. Using pooled OF samples, the future of disease detection could be one of low diagnostic costs and increased sampling. In time, automated systems of water monitoring could provide

producers with a method to oversee many aspects of production and have greater control of health and productivity monitoring. However, further work is required to make all of this possible for the industry.

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Appendices A: Determination of Acute phase proteins by Time Resolved Immunofluoretic assay

1. Determination of CRP in serum and OF

CRP concentrations were measured in serum and OF as previously described by Martinez-Subiela et al., (2007). Serum and OF samples were diluted 1:2000 and 1:4 respectively. Biotinylated anti-CRP antibodies, produced by Murcia Veterinary University in accordance to the technique described by Martinez-Subiela et al., (2007), were pipette into streptavidin microtitration strips (Perkin-Elmer Life and Analytical Sciences), (300ng/well) and incubated at room temperature for 45 minutes, following which the plate was washed four times with DELFIA wash buffer (Perkin-Elmer Life Sciences, Finland). Samples and standards also diluted in DELFIA assay buffer were added to wells at 200µl/well, and wells incubated for a further 45 minutes at room temperature. The standard curve was performed using purified CRP with concentrations in range of 0-250ng/ml. Following incubation a second wash was performed and europium-labelled anti-CRP antibody solution (200 µL/well) were added to each well. The plate was incubated for a further 45 minutes and then washed. Finally, 200 µl/well of DELFIA enhancement solution (Perkin-Elmer Life Science) were added to the plates and incubated for 20 minutes. The fluorescence was measured in a Victor 1420 multilable counter (Perkin-Elmer Life Sciences).

2. Determination of Hp in serum and OF

Haptoglobin concentrations were measured in line with the procedure described by Gutiérrez et al., (2009a). In brief, serum and OF samples were diluted with DELFIA assay buffer by 1:10,000 and 1:10 respectively. In one step, 75 µL of biotinylated antibody (50 ng/well), 50 µL of diluted sample and known standards also diluted with DELFIA assay buffer, and 75 µL of Eu chelate - labeled antibody (20 ng/well), (DELFIA, Finland), were added to streptavidin microtitration strips (Perkin-Elmer Life and Analytical Sciences). The strips were incubated for 15 minutes at room temperature with continuous shaking. Following this strips were washed four times with DELFIA wash concentrate, and 200 µL of enhancement solution (DELFIA enhancement solution) was added. The strips were shaken for a further five minutes, following which the fluorescent signals were measured in a Victor 1420 multilable counter (Perkin-Elmer Life Sciences).

Appendices B: Real-Time PCR for detection of PRRSv

1. Real-time PCR for detection of PRRSv in OF

The RT-PCR was performed as described by Kleiboeker et al., (2005), except for the following alterations:

1) Mastermix consisted of Quantitect Probe RT-PCR mastermix (cat no 204443, Qiagen)
2) The primers and probes which Kleiboeker et al., (2005) directed to North American PRRSV strains were not changed except for the first 5' base of the NA For2 primer was changed (A to G).
3) The real-time RT- PCR was conducted on a Stratagene MX3000P PCR instrument using the touchdown cycling conditions as described in point four.
4) The cycling conditions were 50°C for 30 min (1 cycle), followed by 95°C for 15 min (1 cycle). The touchdown stage which was 17 cycles of 94°C for 20 seconds, 72°C for 45 seconds, where a decrease of 1°C in annealing temperature is made for each of the 17 cycles. Finally 38 cycles of 94°C for 20 seconds and 55°C of 45 seconds.
5) Double the standard recommended amount of Reverse Transcriptase enzyme (a total of 0.5µl, supplied in the Quantitect probe kit) and Qiagen Hotstart Taq enzyme was added to the PCR. An additional 0.125ul of hotstar taq (Qiagen cat no 203203 supplied at 5 units per µl) was added to each PCR reaction also.
6) PCR reactions were run simultaneously with another 2µl of the DNA extract from OF in a 25µl PCR reaction, with no reverse transcriptase (RT) enzyme added. This was to check for the false amplification of contaminating DNA.
OF samples were tested in triplicate, plus one control PCR with no reverse transcriptase added.

With only a limited number of European strains examined by Kleiboeker et al., 2005, an alignment was made of ORF7 nucleotide sequences from European PRRSV strains isolated by the AHVLA Virology Department between 2003 and 2005, and also 47 NCBI sequences (including isolates provided by researchers in Poland that demonstrated more variability than other European strains, including the Lelystadt strain). The alignment revealed that the regions to which Kleiboeker et al., (2005) had directed the European

strain forward primer and probe contained a degree of variability. As previous work had revealed that the virus can undergo a high degree of mutation (Drew et al., 1997), it was judged prudent to re-design the European primers and probes to more conserved regions of the ORF7 gene. This would increase the probability that the RT-PCR assay would be able to detect newly emerging strains (C. Fearnley, personal communication, 18th July 2011).

Upon arrival at the AHVLA, OF samples were immediately stored at -80°C, until processed. RNA was extracted from OF using the following method:-

- 1ml of OF was centrifuged at 12,000 rpm for 10 minutes to remove the larger fragments.
- RNA was extracted from 140µl of OF using the QIAmp Viral RNA kit (cat no 52906) and eluted in 35 µL of elution buffer from the kit.
- The extracted RNA (2 µL) was tested using the PRRSv PCR (in a total PCR reaction volume of 25 µL).

2. Real-time TaqMan® PCR for the detection of PRRSv in serum

The Roche MagNApure automated nucleic acid extraction robot was run to extract the RNA from 200 µL of serum, using the extraction kit (MagNA Pure LC RNA isolation kit-high performance (cat no 03 542 394 001). The RNA was eluted in 50 µL of elution buffer (supplied in the kit). 2 µL of the RNA was used in a 25 µL total volume RT-PCR. The recommended quantity of reverse transcriptase and Qiagen Hotstart Taq enzyme (supplied/included in the Qiagen Quantitect Probe RT-PCR mastermix (cat no 204443), were added to the PCR. The serum RNA was tested in triplicate (C. Fearnley, personal communication, 18th July, 2011).