Australian Government
Department of Agriculture

AUSTRALIAN Pork™

Review of Innate Immunity in Pigs

Final Report
APL Project 2010/1029

December 2011

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i. Acknowledgements

This project is supported by funding from Australian Pork Limited and the Department of Agriculture.

Appreciation is expressed to Dr Jo Pluske (SciEcons Consulting) for her input into this project.

ii. Executive Summary

Disease and enhanced microbial load are considered to be major factors limiting performance and efficiency of feed use by pigs in Australian piggeries. Broad spectrum antibiotics also have been added to diets of pigs to reduce microbial load and enhance performance. However, use of these antibiotics has been implicated in the development of antibiotic resistance in bacteria with serious ramifications for human antibiotic use. Copper sulphate and zinc oxide at concentrations well in excess of requirements for pigs have been used as alternatives to antibiotics particularly during the post-weaning period. However, microbial resistance to copper has been observed and the potential for contamination of the environment with these elements and their toxic effects on ruminants and other animals has resulted in concerns by the community about use of these compounds as in-feed additives.

This review was commissioned by Australian Pork Limited to obtain a detailed understanding of the innate immune system in animals and how it can be manipulated. In particular, the review identified nutritional, environmental, animal selection and other factors that may either reduce or enhance the capacity of the immune system of a pig to resist the impact of high microbial loads found within many piggeries.

The review encompasses an overview of the immune system including physical barriers, the innate immune system and adaptive immune system, which also needs to be discussed in order to gain a more complete understanding of the innate system. Details regarding development and maturation of the immune system as well as inhibitors of the immune system and stimulation of microbial growth are also discussed in this review. In addition, breeding for enhancement of the immune system; the impact of the immune system on animal performance; and possible ways for manipulating the immune system in pigs, are explored.

The review has identified many factors that regulate the innate and adaptive immune system responses in pigs and the consequences of their manipulation on health and performance. The most important outcomes from the review are listed. Practical strategies for optimising the immune response and decreasing the adverse effects from disease are suggested. Finally, areas needing adoption or further research are identified.

A. Major Outcomes from the Review

1. Pigs exposed to conventional housing systems with high microbial loads grow round 20% more slowly than gnotobiotic pigs or pigs in 'clean' environments.
2. Mounting an immune response is expensive in terms of energy and protein/amino acids. The enhanced rate of protein turnover associated with the production of immune cells, antibodies and acute phase proteins increases energy expenditure by 10-15% of maintenance needs and protein requirements by 7-10%. The requirements for tryptophan, sulphur containing amino acids and threonine are increased by a further 10%.

3. There are negative outcomes for pig health and productivity from both under- and over-responsiveness of the immune system. Maximising the immune response is not the desired outcome, but the response should be appropriate for specific circumstances.
4. An inadequate immune response caused by selective breeding for low immunity, inadequate/excess availability of nutrients or stress increases susceptibility to disease and inability to produce immunoglobulins following vaccination.
5. Over stimulation of the immune response with excess production of pro-inflammatory cytokines causes excessive production of the prostaglandin PGE_2, which is primarily responsible for anorexia, fever, increased proteolysis and reduced pig performance. Negating the negative effects of PGE_2 appears not to adversely affect the ability of the immune system to combat pathogens, but improves pig performance.
6. Breeding pigs for fast-lean growth, without consideration of immunity, reduces their capacity to mount an immune response and reduces performance in an environment with high microbial load.
7. Heritability of most immune variables is moderate to very high (~10-90%) and these variables are amenable to selection.
8. There appears to be little opportunity for single gene selection except perhaps for the AA allele of the FUT1 M307 adhesion gene for *E. coli* F18. There may be future opportunities to identify other potential single gene alleles responsible for specific diseases from an understanding of the mechanisms of pathogen invasion of the host or its ability to evade the immune system.
9. Both immunity and productivity should be improved by breeding pigs using selection indexes that include productive and immune response traits. Several different aspects of the immune system need to be included in a selection index to cover responses from the innate and adaptive immune systems.
10. Selective breeding of pigs under environments with high microbial loads inadvertently leads to improvements in animal health and productivity when animals are reared in these environments.
11. The immune response is extremely sensitive to under- and over- supply of nutrients including tryptophan, methionine, valine, threonine, β-carotene, folic acid, vitamin B_6, vitamin B_{12}, vitamin C, vitamin E, riboflavin, iron, zinc, sodium, copper, manganese and selenium.
12. n-6 polyunsaturated fatty acids stimulate pro-inflammatory cytokine production and are associated with autoimmune diseases, anorexia, reduced growth rates and protein accretion, whereas n-3 polyunsaturated fatty acids increase resistance to pathogenic diseases. The ratio of n-6:n-3 fatty acids in the diet is critical for controlling the adverse effects of n-6 fatty acids and an optimal ratio of 4:1 (n-6:n-3) or less.
13. Certain dietary components including high protein, undigested nutrients in the distal intestines and iron promote microbial growth and gastrointestinal tract (GIT) diseases.
14. Temperatures below thermoneutrality, diurnally fluctuating temperatures and draughts increase pig morbidity and mortality, increase pro-inflammatory cytokine production, reduce leukocyte numbers and reduce the production of immunoglobulins.
15. Atmospheric ammonia, endotoxins and viable bacteria greatly exacerbate the severity of pathogenic organisms including parasites. Concentrations above 5 ppm for ammonia, 1 $\mu g/m^3$ for endotoxins and 50,000 CFU/m^3 for viable bacteria appear to be detrimental to pig growth and health.
16. Social and psychological stress reduce immune cell numbers increase pro-inflammatory cytokine production and cortisol release with resulting reductions in feed intake, protein deposition and performance.

17. Pigs at weaning, particularly early-weaned pigs, are most vulnerable to disease because of their low nutrient intake, an innate immune system that matures after exposure to microbes and an adaptive immune system that develops between 4 and 7 weeks of age. Reducing microbial load and assisting the immune system of weaned pigs has high priority.
18. Pigs, like other animals, suffer from endotoxin tolerance where continual stimulation of the innate immune system with a specific antigen and its Toll-like receptor leads to down-regulation of the immune response after 4-5 days. Approximately 3 weeks without exposure to the antigen is required to fully restore the innate immune response.

B. Four Possible Strategy Areas for Improving Immune System Outcomes for Pigs

1. Decrease the presence of agents that elicit an immune response

Reduce atmospheric load of viable bacteria, ammonia and endotoxins: The primary drivers of poor air quality are pen and shed cleanliness. Procedures to maintain shed ammonia below 5 ppm, endotoxins below 1µg/m^3 and viable bacteria below 50,000 CFU/m^3 are outlined in the review, and include batch rearing, thorough cleaning between batches, spraying water above floors and pens prior to people working in pig buildings and setting a maximum of 300 pigs/air space with an air volume (m^3/pig) of at least 0.0118*Live weight (kg) + 1.82.

Add antimicrobial agents to the diet or drinking water: Alternatives to in-feed antibiotics include Zn oxide at doses exceeding 2000 ppm; organic and inorganic acids; plant derived compounds (carvacol, cinnamaldehyde, oleoresin, allicin, thymol, rosemarinic acid, tochopherols and phenols); antimicrobial peptides (lactoferrin, lactoferricin, lactoferrampin, lysozome, indolicin, purothionin); bacteriophages; clays (kaolin, bentonites, zeolites). The value of zinc and acids is well established. Many of the other compounds have small positive effects on growth of young pigs, but results are inconsistent. The medium chain fatty acids lauric ($C_{10:0}$) and myristic acid ($C_{12:0}$) are toxic to most gram positive and gram negative bacteria and to viruses and their potency is markedly increased when fed as 1-monoglycerides. A combination of monolaurin and monomyristin in the ratio of 2:1 was found to be most potent. Further research into specific antimicrobial peptides and bacteriocins may be worth conducting.

Limit microbial growth stimulants in the diet: Reducing undigested nutrients in the distal intestines can be achieved by replacing traditional grains with cooked rice or groats; eliminating grains particles >1.0 mm; adding glucanase, xylanase and phytase enzymes to the diets; replacing plant proteins with readily digestible animal plasma or milk proteins; reducing the content of amino acid balanced protein to 17.5% for weaner pigs; and avoiding excess iron in diets.

Feed probiotics: Probiotics are fed to young pigs to reduce the number of pathogenic organisms by specific bacteriocin targeting and blocking adhesion sites, competing for nutrients, or increasing GIT acidity. Many experiments with probiotics over 50 years have shown variable and inconsistent results in terms of disease control and animal productivity, however and for example, work by Beale *et al.* (2011) using an avirulent strain of *E. coli* shows promise in reducing post-weaning diarrhoea.

2. Breed pigs for enhanced immunity and high productivity

Select pigs for a single gene allele: Selecting pigs for a single gene allele is relatively straight forward. The AA allele for the *E. coli* F18 adhesion gene is a possibility, but others genes need to be identified. F18 represents approximately 60% of *E. coli* infections.

Use a selection index that includes immune traits: The high heritability of most immune traits means that they can be readily incorporated into selection indexes that also include desired productivity

traits. Selecting pigs, particularly in a 'clean' environment without including immune traits in the index, is likely to produce progeny that are less capable of dealing with 'dirty' environments. Best results are likely to occur when pigs are selected in the same environment that the progeny will be reared and immune traits are included in the selection index.

3. Regulate the immune response for specific situations

The immune system must be capable of mounting a sufficient immune response for a specific situation and the negative effects of over-stimulation of the immune response (anorexia, fever, proteolysis) should be minimised.

Ensure the diet is balanced for optimal immune response**:** Formulate diets that closely meet the requirements of pigs without deficiencies of excesses of nutrients.

Eliminate stressors: Ensure pigs are kept in thermoneutral temperatures, with cold, fluctuating temperatures and draughts avoided. Avoid social stress by unnecessary mixing of pigs.

Modulate immune system with n-3 and n-6 fatty acids: Maintain a ratio of 4:1 (n-6:n-3) or less in diets. This criterion should be included in all feed formulation software.

Provide compounds resembling microbial PAMPS that stimulate Toll-like receptors: The innate immune system of young, newly weaned pigs can be stimulated by feeding PAMPS (pathogen-associated molecular patterns) and stimulating specific Toll-like receptors. When given to 1-day old chickens, toll-like receptor agonists including unmethylated cytosine-guanosine oligodeoxynucleotide, cationic peptides (BT/TAMUS 2032) produced by gram negative bacteria, serum-opsonized salmonella and fungal β-glucan have reduced the effects of salmonella infections by priming the innate immune system. Less research on Toll-like agonists has been conducted with pigs. Mannan oligosaccharide, which binds to Toll-like receptors, when assessed over many experiments improved growth of pigs by 8.5% over the first 7 days after weaning. The response dropped dramatically over subsequent weeks presumably because of endotoxin tolerance. Toll-receptor agonists need to be identified for specific weaner pig diseases and administered for only 4-5 days prior to be pigs coming into contact with the pathogen to avoid endotoxin tolerance.

Provide pre-formed antibodies: Preformed antibodies against specific pathogens are useful for pigs up to about 7 weeks of age. The most common source of preformed antibodies is porcine plasma. At least 10% is required in the diet of weaned pigs during the first 1-2 weeks after weaning. The effectiveness of feeding plasma declines with age and cleanliness of the environment.

Vaccinate against pathogens: Vaccines are effective against many pig pathogens. Frequently autogenous vaccines developed from organisms derived from diseased pigs at a specific location are required. Vaccines need to be administered 2-3 weeks before onset of a disease and have low efficacy when maternal antibodies are present in young pigs. Vaccination of pigs less than 6 weeks of age is generally ineffective because of the immaturity of the adaptive immune system.

4. Negate the effects of pro-inflammatory cytokines

Reduce the production and activity of pro-inflammatory cytokines: Conjugated linoleic acid when added to diets of chickens and mice is extremely effective for reducing the production of pro-inflammatory cytokines and eliminating the adverse anorexic and weight loss symptoms associated with excess pro-inflammatory cytokines. Anti-inflammatory cytokines also reduce pro-inflammatory cytokine production. An experiment with administration of IL-5 has increased performance of

weaned pigs in a commercial environment, but remains unpractical until a non-labour intensive method for administration can be developed. Similarly, experiments using the receptor antagonist for IL-1 (IL-1ra) improve the performance of pigs challenged with the PRRS virus or reared in commercial conditions, but are unpractical due to difficulty in administration.

Use NSAIDs: Non steroid anti-inflammatory drugs are known to reduce inflammatory responses in animals by depressing pro-inflammatory cytokine production and the production of the COX-2 enzyme. A few experiments with pigs show small positive effects on intake, performance and health from the use of aspirin, indomethacin and ketoprofen.

Nullify the production of PGE_2: The prostaglandin PGE_2 has been shown to be responsible for many of the adverse effects of anorexia, fever, reduced activity and increased proteolysis associated with excess pro-inflammatory cytokines. PGE_2 is produced from dietary and cell membrane phospholipids via secretory phospholipidase A_2 ($sPLA_2$) to produce arachidonic acid, which is catalysed by the COX-2 enzyme. Strategies have been used to nullify the production of PGE_2 by using COX-2 inhibitors and antibodies against $sPLA_2$ produced in hen eggs. These antibodies have been successful in increasing performance of young chicks raised in conventional environments. One experiment with pigs showed a small positive effect.

C. Recommendations

1. Develop an adoption process to apply on farms which includes the many known practices that ensure the immune system of commercially raised pigs is not excessively challenged by and is primed to respond appropriately to pathogenic organisms. These practices include:
    a. Maintaining the desired air quality considering the methods outlined in the review.
    b. Ensuring pigs are not held in environments below their lower critical temperatures.
    c. Formulate and feed diets that do not limit an immune response because of either deficiencies or excesses of nutrients.
    d. Formulate diets with a ratio of n-6:n-3 polyunsaturated fatty acids of 4:1 or less.
    e. Provide ingredients and feed processing practices that enable the majority of nutrients to be digested in the proximal small intestine.
    f. Develop and implement an appropriate vaccination schedule for sows and their progeny.

2. Undertake research to improve the resistance of weaned pigs to pathogens and to negate over-stimulation of the immune system. Specific factors that warrant investigation either alone or in combination in commercial piggery environments include:
    a. Feeding 1-monoglyceride lauric acid and 1-monogluceride myristic acid singly and in combination in the diet of pigs for several weeks post-weaning.
    b. Feeding of both cis-9, trans-11 and trans-9, cis-12 CLA molecules to pigs for several weeks post-weaning.
    c. Provide either in the diet or by other means, selected NSAIDs for 1-2 weeks post weaning.
    d. Feed porcine plasma for 1-2 weeks post weaning.
    e. Provide COX-2 inhibitors, specifically celecoxib, for several weeks post-weaning.
    f. Develop $sPLA_2$ antibodies in either hen eggs or cow milk and feed for several weeks post weaning. If antibodies to $sPLA_2$ are successfully developed, they would have wider application for treatment whenever a febrile disease occurs or is likely to occur.

3. Investigate the feasibility for other possible areas of future research including:
    a. Identification of toll-receptor agonists associated for diseases specific to weaner pigs in Australia and for which there is no successful non-antibiotic treatment. These toll-like agonists would be fed or administered for 4 days prior to exposure to the pathogen to avoid endotoxin tolerance but prime the innate immune system.
    b. Identify possible single gene alleles that could block the virulence of pathogens specific to pigs in Australia and for which there is no successful non-antibiotic treatment. Once identified, the feasibility of including them in a pig breeding program would need to be assessed.
    c. Identify potential antimicrobial peptides such as those listed above, bacteriocins and/or bacteriophages that could be included in weaner diets to reduce microbial load and/or specific pathogens.
    d. Investigate possible existing methods for administering anti-inflammatory cytokines or IL-1ra to pigs peri-weaning that are feasible for use on commercial farms.

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1. Background to Research

Disease and enhanced microbial load are considered to be major factors limiting performance and efficiency of feed use by pigs in Australian piggeries. Over many years, the inclusion in diets of copper sulphate (Braude, 1967) and zinc oxide (Poulsen, 1995) at concentrations well in excess of requirements for pigs has been used to enhanced growth and efficiency of feed use, particularly during the post-weaning period. These elements are known to reduce microbial populations within the digestive system (Shurson *et al.* 1990; Dunning and Marquis, 1998), which is considered to be, at least partially, responsible for their growth promoting effects (Zhou *et al.*, 1994). However, microbial resistance to copper has been observed (Aarestrup *et al.*, 2002) and the potential for contamination of the environment with these elements and their toxic effects on ruminants (Ópezalonso *et al.*, 2000) and other animals has resulted in concerns by the community about use of these compounds as in-feed additives.

Broad spectrum antibiotics also have been added to diets of pigs to reduce microbial load and enhance performance. In particular, the use of antibiotics in diets at growth promoting levels ("antibiotic growth promotants"; AGPs) has been implicated in the development of antibiotic resistance in bacteria with serious ramifications for human antibiotic use (Witte, 2000; Collignon, 2003). Consequently, many European countries have banned the use of in-feed antibiotics for use as growth promotants and other countries, including Australia, are making efforts to reduce their use.
This review was commissioned by Australian Pork Limited to obtain a detailed understanding of the innate immune system in animals and how it can be manipulated. In particular, the review is to identify nutritional, environmental, animal selection and other factors that may either reduce or enhance the capacity of the immune system of a pig to resist the impact of high microbial loads found within many piggeries. The outcome from the review is to recommend further research and/or adoption of practices that may stimulate the immune system of pigs and reduce the use of antibiotics within the Australian pig industry.

2. Objectives of the Research Project

a. Undertake a comprehensive review of factors affecting the innate immune system in animals and ways it can be manipulated in intensively housed pigs.
b. Recommend practices that may stimulate the innate immune system and reduce the impact of disease in intensively housed pigs.
c. Recommend novel research, development and/or extension activities that may reduce antibiotic use and enhance performance of intensively housed pigs.

3. Research Methodology

This desk-top study will comprise of a literature review beginning with an overview of the immune system. Physical barriers, the innate immune system and adaptive immune system will be discussed in detail. Development and maturation of the immune system will be the focus of the next section followed by of inhibitors of the immune system and stimulation of microbial growth. Breeding for enhancement of the immune system will then be discussed before the impact of the immune system on animal performance is explored in the proceeding section. Possible ways for manipulating the immune system in pigs will be the subject of the final section of this review.

4. Literature Review

4.1. Overview of the Immune System in Animals

Although the review was specified to cover the innate immune system in pigs, a detailed understanding of the immune system and how it may be manipulated cannot be obtained without dual consideration of the innate and adaptive immune systems. The two systems are interdependent. An overview of how animals protect themselves from potentially pathogenic microbiota and parasites is presented.

4.1.1. Physical Barriers

The primary causes of disease in pigs that originate from external sources are through invasion of the body by viruses, bacteria, fungi or parasites. The first defence the animal has to these invasions is the physical barriers of the surfaces exposed to the exterior of the animal; the skin, gut lining, lung epithelium and the urinogenital tract linings. The skin area is approximately 1.5 m^2 for a pig weighing 100 kg (Kelley *et al.*, 1973). However, the total surface area of all the exterior organs of the animal is approximately 400 m^2. This is an enormous area of the animal that must be defended against possible invaders. Although the number of organisms on the skin can reach 3 million/cm^2, animals have evolved an array of strategies for minimising the chances these organisms will invade the tissues. For example, squamous cells grow and migrate towards the surface where they die to form a protective layer against microbial infection. In addition, secretions from sebaceous glands, which produce sebum, are rich in triglycerides, waxes, paraffins and cholesterol which act to waterproof the skin, but also have antimicrobial activity, particularly through the secretion of weak acids. Many of the organisms on the skin are known as commensal bacteria that have adapted to living in this relatively harsh environment. Approximately one in five commensal bacteria on the human skin inhibit the growth of wound infecting bacteria *Staphylococcus aureas* and so also help protect individuals from an infective invasion following trauma of the skin.

Interleukin-1 (IL-1) also has an important role in maintaining the integrity of the skin barrier function. The IL-1α precursor is synthesised by epithelial cells and appears to be evenly distributed between epidermal cells and the stratum corneum (Hauser *et al*., 1986). The *N*-terminal amino acids of the precursor are removed by specific proteases, particularly calpain, to form the mature IL-1α molecule. Within the epidermis, IL-1α stimulates the proliferation of fibroblasts and keratinocytes to increase the depth of the skin and improve the effectiveness of the barrier to microbial entry (Barland *et al*., 2004).

The mouth and buccal cavity are lined with a tough mucous membrane and are continually washed with saliva that drains towards the throat to prevent microbiota infecting saliva glands and increasing the opportunity they will be swallowed. Saliva contains the enzyme lysozyme, which is antibacterial, and the secreted mucus contains IgA immunoglobulins. Some of the *Streptococcus* species that reside in the mouth produce hydrogen peroxide, which is also an antimicrobial agent.

The stomach is lined with cells that produce hydrochloric acid and the pH within the stomach of pigs is sufficiently low to kill many microbiota entering the digestive tract in feed of water. However, several important organisms including *Escherichia coli* and *Salmonella* strains as well as eggs of parasitic worms are resistant to the strong acid conditions of the stomach.

The intestines are large organs designed to facilitate digestion and absorption of nutrients from ingested feed. The small intestine in particular has an extremely large surface area with a multitude of villi to increase the absorptive area. The intestines rely greatly on the antimicrobial activity of the

stomach to protect against pathogenic microbiota. Consequently, there has been much research over recent decades examining the effectiveness of acidifiers adder to the diets of young pigs (Jacela *et al*., 2009). There are many commensal microbiota in the intestines that are known to promote epithelial barrier function and increase mucosal tolerance to bacteria within the lumen (Mirpuri *et al*., 2010). Non-pathogenic *E. coli* colonise intestinal cells and appear to enhance the integrity of the epithelial barrier through toll-like receptor-9 (TLR-9) activation of interferon-αA (IFN-αA), which induces the expression of guanylate binding protein-1 (GBP-1). This GBP-1 protein has been shown to prevent epithelial cell apoptosis and thus enhance the number of protective cells in the intestine.
Mucus secreted into the lumen of the intestines also has a major role protecting against pathogenic microbes, while stimulating commensal organisms. Mucus contains glycoproteins, antimicrobial molecules and immunoglobulins. The glycoproteins are secreted by goblet cells and act as a physical barrier through high viscosity. The mucus has an inner and an outer layer. The inner layer has a high concentration of antimicrobial compounds secreted by Paneth cells of the intestinal epithelial. The antimicrobial compounds are comprised primarily of α-defensins, which interact with the negatively-charged phospholipids in bacterial cell membranes to form pores that disrupt membrane function and result in cell lysis. The Paneth cells also secrete lysozyme and phospholipase A2, which have strong antibacterial and antifungal action. The inner mucus also contains specific IgA antibodies secreted by B cells in the lamina propria. These IgA antibodies act against previously recognised antigens. The thicker outer layer of muscus contains lower concentrations of antimicrobial compounds to allow commensal organisms to survive in the intestinal lumen. Pathogens can penetrate the mucus barrier through motility and enzymic degradation, thereby reducing its viscosity and effectiveness as a physical barrier.

The epithelium of the upper respiratory tract is thin and relatively prone to infection. However, lysozyme is also present in nasal secretions and has an antimicrobial action. Large particles are prevented from entering the lungs by the presence of hairs and vibrissae in the nose. Smaller particles are frequently trapped in mucus secreted from nasal mucosal cells which have cilia to direct the mucus away from the trachea and towards the oesophagus. Sneezing is also a protective reflex action to expel irritants in the upper respiratory tract.

The trachea and bronchi are also lined with a ciliated mucous membrane that traps microbiota and small particles that have escaped the protective mechanisms of the upper respiratory tract. The cilia in this region of the respiratory tract sweep upwards to carry the mucous away from the lungs and to the pharynx where it is swallowed. Microbiota that reach the alveoli have little barrier resistance and rely on the innate and adaptive immune systems for control.

4.1.2. The Innate Immune System

The innate immune system is the next defence against pathogenic microbes that manage to penetrate the physical barrier and enter body tissues. The innate immune system needs to be extremely fast acting because a single bacterium can double in approximately 30 minutes and, if uncontrolled, will produce around 100 trillion bacteria in 24 hours (Sompayrac, 2008). The major feature that distinguishes the innate immune system from the adaptive immune system is that the components of the innate immune system already possess the properties that allow an immediate and substantial attack on invading pathogens, whereas the adaptive immune system needs time to increase the numbers of specific cells that will be effective against an individual pathogen. A second feature that separates the two systems is that the innate immune system does not change with repeated exposure to a specific pathogen, while the adaptive system has a 'memory' for past pathogens and can initiate a significantly more rapid response on repeat exposures.

The innate immune system have evolved over millions of years to produce genes that code for receptors that recognise molecular structures shared by groups of microbes that are essential for their survival, but are rarely associated with mammalian tissues. These unique microbial molecules are called pathogen-associated molecular patterns (PAMPS). They are non-specific antigens because they do not identify properties of an individual microorganism, but rather of groups of organisms that share the same chemical structures. These PAMPS include lipopolysaccharides (LPS) found in the outer membrane of gram-negative bacteria cell walls; peptidoglycan and lipotechoic acid (LTA) from gram-positive bacteria cell walls; mannose, which is a sugar found at the terminal of microbial glycolipids and glycoproteins and is rare in mammalian tissues; bacterial, viral and fungal unmethylated cytosine-phosphate-guanine (CgP) which is uncommon in mammals; bacterial flagellin; the amino acid *N*-formylmethionine found in bacterial proteins, double-stranded and single-stranded RNA from viruses; and unique molecules displayed on stressed, injured and infected cells. The innate immune system recognises about 10,000 different molecular patterns from pathogens.

The innate immune system primarily comprises the complement protein system; the phagocytes, macrophages and neutrophils; cells that release inflammatory mediators, basophils, mast cells and oesinophils; and natural killer (NK) cells. Acute phase proteins and cytokines are released, or their release is stimulated by innate immune cell activity. These proteins and cytokines have an important role in controlling the response to a pathogenic invasion. In addition, dendritic cells, which reside below epithelial cells, have been classed as being part of the innate immune system. These dendritic cells do not directly destroy invading pathogens, but present the antigen characteristics of the pathogens to cells of the adaptive immune system so specific cells within this system can be multiplied and deployed against the invader.

4.1.2.1. The Complement Protein System

The complement system assists the ability of phagocytes and antibodies to remove pathogens form an organism. The complement system consists of approximately 25 proteins, glycoproteins or protein fragments and accounts for about 5% of the globulin fraction of blood. These components of the complement system are primarily synthesised by liver hepatocytes, but some are produced by activated macrophages, blood monocytes and epithelial cells of the gastrointestinal tract (GIT). Like most components of the immune system, the complement system must be activated before it can function against invading organisms.

The most abundant complement protein is complement component 3 (C3). There are three activation pathways (classical, alternative and lectin activation) which all generate homologous variants of the protease, C3-convertase. In the alternative pathway, C3 molecules within the blood spontaneously cleave into two smaller particles, C3a and C3b. The latter portion, C3b, is extremely reactive and binds to either amino or hydroxyl groups and will attach to proteins and carbohydrates on the surface of microbial invaders. If the C3b does not attach to one of these chemical groups within about 60 microseconds, it is neutralised by binding with water. However, in the relatively rare event that C3b does attach to a foreign particle, it sets off a chain reaction. The bound C3b protein attaches with another complement protein B, while complement factor D cleaves part of B to form C3bBb. This combined molecule is a potent convertase and cleaves other C3 protein molecules in the blood into C3a and C3b fragments. The C3b fragment attaches to the invader with further factor B and D involvement to create a positive feedback loop. In addition, the C3bBb convertase is also active against complement component C5 to produce C5b, which then combines with other complement proteins C6, C7, C8 and C9 to build the 'membrane attack complex" (MAC). Once the MAC is formed a hole is opened in the bacterium and it dies.

There is a system within the body of animals to ensure that the C3b proteins do not bind to host tissue cells. There are proteins within the blood that cleave C3b molecules to make them inactive. In addition, host cells have a surface protein, decay accelerating factor, which accelerates the destruction of convertase, C3bBb, by other blood proteins. Further, the cell surface protein, protectin (CD59), will dismantle the MAC structure before it can damage the host cells. These cell surface protecting proteins are individual and species specific, and are the primary reason for rejection of tissue transplants between species and individuals.

In the classical activation pathway, C1, a protein complex composed of C1q, C1r and C1s sub-units, binds to the Fc region of IgG and IgM antibodies that have bound with an antigen. The C1 complex binds to the antibody via C1q, but it must be cross linked to at least two antibodies before it is activated. The C1 complex does not bind to antibodies without an attached antigen. The C1 complex is normally bound to an inhibitory molecule, C1-INH, but this is removed if two C1 complexes bind in close affinity to each other. The binding of C1q to several antibodies activates C1r, which activates C1s to form an activated C1qrs, an enzyme that cleaves C2 into C2a and C2b and also C4 into C4a and C4b. The C4b and C2a fragments combine on the cell membrane to produce a C4bC2a complex, which is a C3 convertase and cleaves C3 into C3a and C3b.

The lectin activation pathway for complement proteins functions through a protein, mannose-binding lectin, which is synthesised mainly in the liver. This protein can bind to mannose which is found on the surfaces of many yeasts, fungi, viruses, bacteria and protozoa. The protein does not bind to mammalian cells. The mannose binding lectin (MBL) must be activated before it can convert C3 to C3b to start the chain reaction described above. There is another protein in the blood, mannose-binding lectin-associated serine protease, which, when bound to MBL functions like a convertase and cleaves C3 into C3a and C3b. The C3b then binds to the surface of the invader and the process of MAC formation occurs rapidly.

The complement system has another important function in addition to the creation of MACs. When C3b is attached to the surface of an invading pathogen, it can be cleaved to an inactive form, iC3b, by complement factor I in the presence of C4b and factor H. This complex is incapable of forming MACs (Rother and Till, 1988). However, it remains adhered to the invading cell. Phagocytes, particularly macrophages, have receptors that bind to the iC3b molecule, thus opsonising the invading organism to facilitate phagocytosis.

There is one further activity of the complementary system. The C3b and C5b fragments released from the cleavage of C3 and C5 do not attach to the surface of invading pathogens, but attach to macrophages and neutrophils to activate them into more effective killers of the invading cells. These C3a and C5a fragments are also called anaphylatoxins because they contribute to analphylactic shock.

4.1.2.2. Phagocytes

There are two phagocytes in the innate immune system, macrophages and neutrophils. Both cell types are produced in bone marrow from stem cells along with many other white (dendritic cells, oesinophils, basophils, mast cells, B lymphocytes, T lymphocytes, natural killer cells) and red (erythrocytes) blood cells. Macrophages live for several months and have different functions including phagocytosis of cell debris and invading organisms, antigen presentation to helper T cells and, in the hyperactive state, produce tumor neucrosis factor (TNF) which has the capacity to destroy tumor and viral infected cells. In contrast, neutrophils have a life span of only a few days and their function is to eliminate invading organisms through phagocytosis, degranulation with the release

of toxic compounds and enzymes into the tissues and formation of extracellular traps composed of fibres of chromatin and serine proteases, which trap and digest invaders.

Macrophages

Macrophages are released from bone marrow as monocytes that remain in the blood for about 3 days (Sompayrac, 2008). There are approximately 2 billion monocytes in the blood of a human at any time. Monocytes travel though capillaries where some enter tissues spontaneously and mature into macrophages that reside beneath the epithelium of all external tissues. Here the macrophages act as sentinel cells ready to ingest external invaders that have entered the tissues. Macrophages can destroy any organic particle by first engulfing it in an external pouch or vesicle, which is then interiorised into the cell to form a phagosome. The phagosome is fused with an internal lysosome, which contains an array of acid hydrolase enzymes including lipases, amylase, proteases, nucleases and phosphoric acid monoesters. These compounds within the lysosome destroy the organic material within the phagosome.

Macrophage cells have three stages of activation (Zhang and Mosser, 2008). In the resting state, they act as scavengers removing debris from dead cells and any organisms that invade the tissue. Macrophages become activated by TNF-γ released from NK cells and helper T cells as these cells respond to the presence of an invading organism. A hyperactivated state occurs when the toll-like receptors on the surface of the macrophage are stimulated by PAMPS, particularly LPS from gram-negative bacteria and LTA from gram-positive bacteria. When activated, the macrophages increase phagocyte activity and also use their major histocompatability complex proteins, class II (II MHC) to present antigens from the phagocytosed particles to its surface to further stimulate activity of helper T cells.

Furthermore, activated macrophages release the proinflammatory cytokines IL-1, IL-6, IL-8 and TNF-α, as well as nitric oxide, into the blood stream. The release of these compounds stimulate blood flow to the infected area, cause cells lining the blood vessels in the area to contract, which increases fluid penetration, and attract other killer cells, particularly neutrophils, to the site requiring defence. The chemicals also stimulate nerve endings at the site of infection. All these reactions lead to swelling, inflammation and soreness in area of infection. The degree of activation of the phagocytes influences the intensity of these responses.

In addition, the cytokines released by activated phagocytes stimulate the production from the liver of a large number of acute-phase proteins, while depressing the production of other proteins. Some of the acute-phase proteins such as C-reactive protein, mannose-binding protein, ferritin, ceruloplasmin, serum amyloid A and haptoglobin act to destroy or inhibit growth of microbes. Several of these proteins act as opsonins on microbes to mark their presence for other killer cells, while others either bind to iron or inhibit iron uptake by the microbes. Another group of acute-phase proteins, α 2-macroglobulin and coagulation factors, stimulate coagulation and limit spread of infection by trapping pathogens in local blood clots. Concomitant with the increase in acute-phase protein production in the liver is a decrease in the production of proteins including albumin, transferring, transthyretin, retinol-binding protein and transcortin (Ritchie *et al*., 1999). It is believed that the reduced synthesis of these proteins provides amino acids needed for the production of the acute-phase proteins when needed to fight an infection.

Neutrophils

The human body contains approximately 20 billion neutrophils, but individual cells have a lifespan of only around 5 days (Pillay *et al*., 2010). These cells are programmed to die through apoptosis.

Neutrophils are attracted to sites of inflammation through the release of IL-1, IL-8 and TNF-α from macrophages and other cells and the complement protein, leukotriene B4, and complement fragment C5a. Neutrophils in blood take around 30 minutes to exit the blood and become fully active at the site of inflammation. Neutrophils are inactive while in the blood.

There is a rather intricate process that enables the neutrophil to exit the blood at the precise point where it is needed in the body. When cells within blood vessels close to the site of inflammation bind to IL-1 and TNF-α released by active macrophages, they synthesise a protein, selectin, that is expressed on the surface of the blood vessel epithelium. This process takes approximately 6 hours. Neutrophils within the blood have an adhesion molecule, selectin ligand, which is attracted to selectin and greatly reduces the speed of the neutrophil. However, the slowing neutrophil will only stop if there is clear evidence of microbial invasion. If the slowing neutrophil binds to C5a or LPS in the region, it immediately releases a presynthesised and stored protein, integrin, onto its surface. Integrin binds tightly to another protein, intercellular adhesion molecule, which is located at intervals along the epithelium of blood vessels. This binding stops movement of the neutrophil, where it breaks through the blood vessel epithelium into the tissues. The neutrophil locates the site of infection partially by binding to formyl methionine released by macrophages. Formyl methionine is an amino acid common in bacteria, but not found in mammalian cells.

Once activated, neutrophils are extremely efficient phagocytes. These activated cells release myeloperoxidase to produce oxygen radicals, antimicrobial peptides, particularly α-defensins, and cathelicidin, as well as lysosome granules that are toxic to microbes. However, these substances also damage nearby healthy cells of the host (van Wetering *et al*., 2004; Nicholls and Hazen, 2005). Neutrophils are the predominant cells in pus formed around microbial infected tissue. The death of neutrophils and host tissue results in the local release of a range of heat shock proteins and alarmins. These proteins bind to toll-like receptors on macrophages and stimulate the release of nitrous oxide and TNF-α to further stimulate the inflammatory response and recruit more neutrophils and other cells to fight the invading organisms.

4.1.2.3. Basophils, Mast Cells and Oesinophils

Basophils, mast cells and oesinophils form part of the innate immune system that has a primary role combating invading parasites and intestinal worm infections. The three cell types are formed from myeloid cells in bone marrow and their synthesis is stimulated in response to IL-3, IL-5 and granulocyte macrophage colony-stimulating factor (GM-CSF) released primarily by helper T type 2 cells and macrophages (Shearer *et al*., 2003). They are distinguished by their capacity to produce the cytokines, IL-4 and IL-13. Each of the cell types forms internal granules that contain toxic substances and are release when activated to combat an invasion by parasites. These cells are associated with type 2 immunity and also play a major role in the development of allergies, asthma and other immunological disorders (Spellberg and Edwards, 2001).

Basophils and mast cells are structurally and functionally similar and in total constitute around 2% of leukocytes, but are dominated by mast cells. These cells have granules that contain histamine, anti-blood coagulant proteoglycans (heparin and chondroitin), serine proteases, serotonin and other eicosanoid compounds. Mast cells migrate to the epithelial layer of all barrier tissues of the host and can have an extremely long life of up to years if not activated.

Oesinophils represent from 1-6% of leukocytes. Oesinophils synthesise and store granular proteins before exiting the bone marrow. They migrate to the thymus, spleen, lymph nodes as well as the epithelial cells of the lower GIT, ovary and uterus. They do not migrate to the lung, skin, upper

intestinal tract and other internal organs under normal circumstances. Oesinophils are attracted to the site of infection by IL-3, IL-5 and GM-CSF released from helper T cells, where they degranulate and release cationic proteins, proteases and a neurotoxin to destroy the tissues of the invading parasite. The cationic proteins and neurotoxin are riboneucleases which also have anti-viral activity. The cationic proteins create pores in the membranes of tissues in invading parasites that enable the entry of cytotoxic molecules to destroy the invader. The peroxidise form reactive oxygen and reactive nitrogen compounds that promote oxidative stress in cells of the invading organism leading to cell death by apoptosis and necrosis.

4.1.2.4. Natural Killer Cells

Natural killer (NK) cells are the first line of defence against viral and tumour infected cells. NK cells are regarded as being part of the innate immune system, but are formed from the same progenitor bone marrow cells as the B and T cells of the adaptive immune system. There is some evidence that, like components of the adaptive immune system, NK cells can also undergo clonal expansion and generate memory cells (Sun and Lanier, 2011).

IL-15, produced by stromal cells in bone marrow, stimulates the development of NK cells, which mature within the bone marrow. The mature NK cells flow with blood from the bone and are largely stored in the blood, liver and spleen until attracted to a site of inflammation, in a similar manner to neutrophils, where they proliferate rapidly. NK cells have a half-life of around 7 days. NK cells are frequently attracted to sites of infection by cytokines (Il-2, IL-12, IL-15, IL-18) released from viral or tumour infected cells and the chemokine C ligand 5 (CCL5).

Activated NK cells themselves release a range of cytokines and chemokines including IFN-γ, TNF-α, granulocyte-macrophage colony-stimulating factor, macrophage inflammatory protein (MIP-1α), MIP1β and CCL5, thereby acting as a regulator of the adaptive immune system. A major function of NK cells is to destroy viral and tumour infected cells, bacteria, parasites and fungi. There are two mechanisms used by NK cells locate the target cell for destruction; i) receptors that bind directly to the Fas (antigen-binding fragment) protein on the surface of target cells, and ii) receptors that bind to the Fc region of antibodies (IgG) that have bound to the target cell in a process called antibody-dependent cellular cytotoxicity (ADCC). Once the NK cell has bound to the target cell directly or the antibody with the target cell, it releases perforin proteins to form pores in the membrane of target cells creating aqueous channels through which released proteases called granzymes entre and lead to cell apoptosis. The granzyme proteases degrade structural cytoskeleton proteins and chromosomes, with the cell fragments being removed by phagocytes. The direct binding to Fac proteins on the surface of cells is important for containing infected cells prior to induction on the adaptive immune system. The effectiveness of the ADCC process has been shown to depend on the oligosaccharide composition of the Fc region of the IgG antibody (Shinkawa, *et al*., 2003). Oligosaccharides low in fucose resulted in a 50-fold increase in cellular cytotoxicity.

NK cells are potent destroyers of targeted cells. Thus, an intricate system has evolved to ensure that they destroy only viral or tumour infected cells in the body and not normal healthy cells. The system relies on the net balance between the activation of stimulatory and inhibitory receptors on the NK cells (Backström *et al*., 2004). The inhibitory receptors react with MHC class I molecules presented on the surface of normal healthy cells. Many viruses have evolved the ability to prevent the MHC class I system presenting fragments of normal cellular protein to the surface of body cells, so when these molecules are not present there is reduced inhibitory receptor binding. Alternatively, viral and tumour infected cells release unusual carbohydrates and proteins, particularly MHC Class I polypeptide related sequence A (MICA) and sequence B (MICB) on their surface that are recognised

by the G2D type II receptor of NK cells. Other stress molecules released by infected cells and recognised by NK cells include TFN-α and TFN-β.

4.1.3. The Adaptive Immune System

Unlike the innate immune system that has evolved receptors to recognise about 10,000 common molecular structures from invading organisms and can act immediately against them, the adaptive immune system has evolved receptors to recognise almost any conceivable invading organism (approximately 100 million possibilities), but requires time to increase the number of component cells and antibodies available to attack an identified specific organism. In addition, the adaptive immune system has evolved to respond rapidly to a repeat exposure from an individual organism, whereas the innate immune system has no memory of a previous exposure.

There is a close interaction between the innate and adaptive immune systems. Frequently an invading organism can be controlled by the innate immune system alone. However, when this does not occur, the components of the innate immune system activate and direct the adaptive immune system to assist with destruction of the invader.

The major components of the adaptive immune system are B cells and the antibodies they produce, T cells that destroy virus infected cells and produce many cytokines that regulate parts of the immune response, a cellular antigen presentation system, and the lymphoid system to integrate immune responses. Both B cells and T cells use an intricate system involving rearrangement of four distinct DNA segments to create an enormous number of different receptors, with B cells capable of recognising any organic molecule including proteins, lipids and carbohydrates and T cells capable of recognising only protein fragments. Each B cell and T cell has a unique receptor pattern, with many copies of this receptor being displayed on the cell surface. B and T cells initially require a dual stimulus to be activated. One stimulus is the B or T cell receptors binding in a cluster to the unique cognate antigens. However, these antigens could be from invading organisms or from the animals own tissues. The second stimulus is needed to prevent the host tissues from being attacked. For B cells, the co-stimulation can be from either a T helper cell or IFN-γ released from NK cells or killer T cells. For T cells, the co-stimulation is from proteins, particularly B7 proteins, expressed on the surface of antigen presenting cells.

Within an animal there are initially only around thirty B or T cells that have a receptor capable of responding to a specific antigen. Hence, once a B or T cell has been activated, it proliferates by doubling in size and forming two daughter cells each with the unique receptors. Each cell division takes about 12 hours and the proliferation continues for around seven to fourteen days to produce a clone of from 1,000 to 20,000 identical cells. Thus, the adaptive immune system is extremely versatile in the organisms it can identify, but takes time to respond to an initial threat. However, if the same antigen is identified at a later stage, the response is more immediate. First, the B and T cells do not require co-stimulation the next time there is a clustering of bound receptors. Second, a larger number of the unique cloned cells remain following activation than were initially present before the specific antigen was recognised by the immune system. These remaining cells include long-lived B cells that reside in bone marrow and continue to produce the specific antibodies as well as B 'memory' cells which proliferate slowly to maintain the number of long-lived B cells. Similarly, with T cells, some remain in the tissues ready for a subsequent attack by the specific invader, whereas others remain in the lymphoid organs and bone marrow and proliferate rapidly on subsequent occasions when the specific antigen is present.

4.1.3.1. B Cells

B cells are produced from stem cells in bone marrow. Approximately one billion are produced each day and 3 billion are circulating in the blood of a human at any time. The primary role of B cells is to produce antibodies that bind a specific antigen. The basic structure of an antibody is two pairs of two different proteins, the longer heavy-chain and the shorter light-chain. Each antibody molecule has two identical antigen binding (fragment antigen binding, Fab) regions consisting of the short chain and the corresponding section of the long chain protein. The remaining section of the long chain protein is called the Fc (fragment crystallisation) region and is important for binding to immune cells or other antibodies to develop antibody classes.

Antibody structures are first presented on the surface of maturing B cells, with a short section of a few amino acids from the heavy-chain protein Fc region inserted through the cell surface to act as an anchor. The protruding portion of the antibody structure is called the B cell receptor and there are many identical receptors protruding from the surface of each B cell, but the receptors differ between B cells. When these B cell receptors bind in a cluster to antigens and there is co-stimulation to activate the B cell into proliferation, the mature, cloned B cells secrete the B cell receptor, without the amino acids of the heavy chain used to attach it to the cell, into the blood as an antibody.

The signal for activation of the B cell once the receptor has bound with the epitope of the cognate antigen is via two additional proteins, Igα and Igβ, which are associated with the heavy-chain protein and protrude a few amino acid sequences beyond the cell surface, but have long sequences deep inside the cell. Stimulation of the enzymes within the B cell that result in cell proliferation requires the signal to be sent from many Igα and Igβ proteins concentrated within a region of the B cell surface. Most invading bacteria, viruses and parasites have many closely located copies of the same antigen on their surface. Thus, B cell receptors for the specific antigen become 'clustered' together when a pathogen is engaged. This clustering or 'cross-linking' of B cell receptors can provide a sufficiently concentrated response through the Igα and Igβ proteins to stimulation B cell proliferation and production of antibodies. However, the signal is greatly increased if another receptor on the B cell surface binds to the complement fragment for an antigen that has been opsonised by cells of the innate immune system.

Antibodies evolved so they could bind to any conceivable antigen contained in an invading organism and these invaders are continually mutating. Hence, two different processes (DNA segment rearrangement and somatic hypermutation) evolved to allow B cells to compete with the ever-changing invaders. The former occurs as the initial bone marrow stem cells mature into B cells and the second occurs only in proliferating B cells that have been activated following binding to a specific antigen.

DNA Segment Rearrangement

The genes responsible for B cell receptor heavy-chain proteins are located on chromosome 14 in humans. The heavy-chain genes consist of separate gene segments known as variable (V, ~40), diversity (D, ~25), joining (J, 6) and constant (C, 9). During initial differentiation of stem cells into B cells, one of the twenty five D segments first joins with one of the six J segments by the process of gene deletion. One of the V gene segments is then joined to the DJ segment by the same gene deletion process. Finally the selected VDJ segment joins to the C segment. The class of immunoglobulin produced (μ, δ, γ, ε or α) depends on which ones of the nine constant gene segments is incorporated with the VDJ segment fractions. Diversity in the amino acid sequence of the heavy-chain proteins in the region for antigen binding come from which V, D and J segments

were selected and by the enzyme, terminal deoxynucleotidyl transferase, which incorporates a variable number of DNA bases into the D region of the new gene sequence. The DNA code is then transcribed to mRNA and translated into an amino acid chain. There is only about one chance in nine that the translation will produce a full-length, viable, heavy-chain protein. Gene rearrangement proceeds simultaneously on the two chromosomes 14 of the maturing B cell and, if one produces a successful heavy-chain protein, it transported to the B cell surface and the process ceases on the other chromosome. If a viable heavy-chain protein is not produced by either chromosome the progenitor B cell dies through apoptosis.

Once the heavy chain protein has been transported to the cell surface, the light-chain gene segments, located on either chromosome 2 (κ) or 22 (λ) in humans, undergo a similar process of gene segment rearrangement by gene deletion. However, for the light-chain, only gene segments V, J and C are rearranged. The rearranged light-chain amino acid sequence produced must be of the right length and have an acceptable bonding structure with the existing heavy-chain protein. If this does not occur, the cell dies through apoptosis. The combination of the variable heavy-chain protein with the variable light-chain protein both produced through gene deletions, results in an enormous number of potential antigen receptors. Each receptor is unique and one is likely to bind to any biological antigen.

Somatic Hypermutation

Continuing improvement in the affinity of an antibody produced by the proliferating B cell for its cognate antigen comes from somatic hypermutation in the V, D and J regions of the chromosome responsible for antigen binding. Somatic hypermutation occurs within B cells only after the antibody class produced by the cell has been switched from IgM to the most appropriate class for the cognate antigen through rearrangement of gene segments in the C region of the chromosome (Ziqiang *et al*., 2004).

The mutation rate in the VJD segment of the proliferating B cell is from one per 1,000 to one per 100,000 bases per generation compared with a normal mutation rate in mammalian DNA of about one per 100 million bases per generation. The B cell hypermutations are primarily single base substitutions, with occasional insertions or deletions. The actual mutations that occur in any single B cell may increase, have no change to or decrease the affinity of the antigen binding region to the specific cognate antigen. B cells must be continually restimulated by binding to the antigen for them to continue to proliferate. Thus, the B cells with receptors that have mutated to a higher antigen affinity are more rapidly stimulated than those with lower affinity and proliferate more frequently. The process of somatic hypermutation within the maturing B cell population ensures that antibodies produced are continually developing higher average affinity to the cognate antigen. There are gene repair mechanisms within B cells to control the outcome of somatic hypermutation (Liu *et al*., 2007), but sometimes these are not effective and result in B cell lymphoma diseases.

4.1.3.2. Antibodies

An activated B cell can release about 10,000 antibodies per second. There are five classes of antibodies produced by B cells: IgM, IgD, IgG, IgA and IgE. The class of antibody produced depends on the region of the C segment of the genes that is incorporated with the VJD segment during gene rearrangement of the heavy-chain proteins. When B cells are first activated, the C_μ region of the constant gene segment is transcribed to produce mainly IgM antibodies, but also a small number of IgD molecules are produced during the protein translation process. The predominant IgM antibody is composed of five of the basic double, heavy-chain and light-chain antibody components, joined at the base of the Fc region. Once the IgM binds to an antigen, the classical complement system is

activated. IgM is particularly effective in activating the complement system because the Fc segments of five antibody units are always within close proximity from each other. IgM antibodies are thought to be the first antibodies that evolved and show a clear interaction between the innate and adaptive immune systems. Because the B cell antibodies can recognise and bind to any conceivable antigen, they greatly extend the capacity of the innate immune complement system to destroy invading organisms, including viruses.

Antibody Class Switching

Once a B cell has bound to an antigen and starts to proliferate it will frequently change the antibodies produced from IgM to one of the other classes that is more suitable for the specific invading pathogenic organism. This process is called antibody class switching and is achieved by a gene deletion process that brings the Cγ, Cε or Cα segments of the Fc region of the heavy-chain genes adjacent to the VJD segment for the production of IgG, IgE and IgA antibodies, respectively. The different antibody classes have a range of specific sites in the Fc region that bind receptors from different immune system cell types that will provide the most appropriate response for the specific pathogen antigen. The particular switching that takes place is determined by cytokines released by primarily T helper cells. The predominant type of T helper cell, Th1 or Th2, has a major influence on which antibody class switch occurs. For example, Th1 cells secrete IL-2, IFN-γ, TFN and lymphotoxin-α (LT-α) which stimulate type I immunity and favour the switching from IgM to IgG. Alternatively, Th2 cells secrete Il-4, Il-5 Il-9, Il-10 and Il-13, which stimulates type II immunity favouring switching to IgE. Environments with high concentrations of TGF-β and IL-5 will result in IgA antibodies. There are differences between animal species in the actual cytokines that stimulate specific class switching.

IgG antibodies are the most common antibodies in blood and are composed of a single structure with two heavy-chain and two light-chain regions. There are four different IgG classes: IgG1, IgG2, IgG3 and IgG4, in order of prominence in blood. The IgG classes differ in the number of disulphide bonds linking regions of the protein chains and thus provide different binding efficiencies for specific molecules and cells. IgG1 is specifically efficient at binding antigens of invading organisms to opsonise them for phagocytes, particularly macrophages and neutrophils. Alternately, NK cells bind best to IgG3 antibodies which facilitate the destruction of virus infected cells through antibody-dependent cell-mediated cytotoxicity. IgG molecules are the only antibodies that pass across the foetal placenta to protect the unborn and peri-natal young. Furthermore, IgG antibodies have a relatively long half-life of about 3 weeks compared with about one day for IgM antibodies.

IgA antibodies consist of two double, heavy-chain and light-chain antibody components (like IgG) joined at the base of the Fc region. This structure provides several unique properties to the IgA antibodies including an ability to cross the intestinal epithelium and epithelium of other tissues such as the respiratory tract and mammary gland. The structure also makes the IgA antibodies that have passed into the intestines resistant to intestinal acids and enzymes. Approximately 80% of B cells are located in mucosal tissue of animals and produce IgA antibodies. Each IgA antibody has four binding sites for any cognate antigen. Thus, with the many IgA antibodies binding to the repeat antigens on invading organisms, IgA can complex across numerous organisms preventing their attachment to the intestinal epithelium and facilitating their removal from the digestive tract in mucus and faeces. This process is particularly important for protesting young animals while consuming milk from the many microbial loaded materials ingested from the environment. The structure of IgA also makes it incapable of activating the complement system and thereby protecting the epithelial tissues from an activated complement.

IgE antibodies have a single molecular structure similar to IgG antibodies, but the Fc region binds specifically to mast cells, basophils and oesinophils and facilitates degranulation of these cells when the antibodies are bound to their cognate antigen. Although these three immune system cells bind to the same Fc receptor on IgE antibodies, the substances released by mast cells and basophils upon degranulation are most effective against small parasites, whereas those released by oesinophils are more effective against larger parasitic helminths.

When the cognate antigen for an IgE antibody is from an allergen and there is a sufficient cluster of antigen-attached antibodies to stimulate degranulation of mast cells, the compounds released attack tissue cells of the host rather than a non-existent parasite. Because B cells have memory of previous antigen encounters, the second and subsequent exposures to an allergen antigen can result in a substantial allergic reaction and in extreme forms, anaphylactic shock. In particular, the histamines released by the mast cells increase permeability of blood capillaries and fluid moves from blood to the tissues. If the allergen spreads throughout to body, as for example with bee sting proteins, the massive loss of fluid from blood can result in such a reduction in blood volume to initiate cardiac arrest. The released histamines also cause smooth muscle contraction which, in severe cases, can lead to sufficient constriction of bronchi and bronchioles in the lung to cause suffocation.

Allergic reactions occur when the antigen from an allergen is bound to IgE rather than IgG antibodies, because mast cells, basophils and oesinophils cannot bind to the IgG antibodies. People who suffer from allergies have been reported to have from 1,000 to 10,000 times more IgE antibodies than those who do not have allergies (Sompayrac, 2008).

Most T helper cells at birth are Th2 type cells, because Th1 cells release TNF-α, which activates NK cells, and IL-2, which results in proliferation of NK and killer T cells that would result in destruction of the foetal placenta because it is not 'self' for the mother. The predominance of Th2 cells in the foetus leads to a predominance in IgE antibodies. However, when the young are exposed to microbes soon after birth, they elicit a Th1 type response, which results in the production of IgG antibodies. If allergens are encountered at the same time, these too will be bound to IgG antibodies and the B cells will have an IgG memory. Thus, subsequent exposure to the allergen will not result in the degranulation of mast cells and oesinophils. However, as occurs in many modern societies, young people are not as exposed to microbes as during evolution and the switching from Th2 to Th1 does not occur with the result that allergens become bound to IgE antibodies which remains the memory for subsequent exposures.

4.1.3.3. T Cells

T cells, like B cells are produced by stem cells in bone marrow, but mature in the thymus rather than in bones. There are about one trillion T cells in the blood of humans. T cells also have receptors composed of two glycoprotein chains, either α and β or γ and δ. The former are regarded as traditional T cells and comprise 95% of all T cells. Each α or γ protein chain is comprised of a V, J and C gene segments, whereas each β or δ protein chain has a V, D, J and C segments. Maturing T cells in the thymus undergo gene recombination in the V, D, J segments through gene deletion in the β chain proteins. If these rearrangements produce a viable protein, the V and J segments of the α chain are rearranged. Large variation in the amino acid sequence of these T cell receptors is produced by a similar process used for B cells. The $\alpha\beta$ T cells also express either a CD4 or a CD8 co-receptor that separates helper T cells from killer T cells, respectively. The helper T cells that express the CD4 protein bind to the class II MHC complex on antigen presenting immune cells, whereas the killer T cells that express the CD8 proteins bind to class I MHC molecules on any cell.

The α and β chains are the antigen binding components of the T cell receptors, with most of the structure outside the cell and a few amino acids holing them to the cell surface. These αβ chains are held in close proximity to a group of six proteins (δ, γ, 2ε and 2ζ) called CD3 protein complex, each of which has several amino acids external to the cell membrane, but most of the structure within the cell. Similar to B cells, a clustering of T cell receptors by binding to numerous cognate antigens causes activation of the T cell via the CD3 proteins. Depending on the affinity of the T cell receptors for MHC-self antigens, the T cells may undergo apoptosis, be neutralised or activated. Only those with weakly positive affinity are chosen.

When killer T cells bind to the class I MHC antigen complex indicating a virus, other pathogen or tumour infected cell and the B7-CD28 co-stimulation occurs, the cell releases perforin, granzymes and granulysin. The perforin forms aqueous channels in the cell membrane allowing the granzyme serine proteases and cysteine proteases to initiate the caspase cascade which leads to cell apoptosis. The γδ T cells normally do not have either CD4 or CD8 receptors for binding to MHC proteins and have less diversity in gene segments than αβ T cells. The γδ T cells are primarily located in epithelial tissues, particularly in gut mucosa. There is evidence γδ T cells have receptors that recognise antigens that are common to organisms that may invade specific epithelial zones and can react quickly like the innate immune system. Unlike the other T cells which bind only to peptides, the γδ T cells can bind to lipid antigens.

4.1.3.4. Antigen Presentation

Antigen presentation is a crucial component of the adaptive immune system, where cells present on their surface fragments of proteins synthesised within the cell and captured by phagacytosis from outside to cell. The protein fragments are presented to the cell surface via two MHC type molecules, class I and class II. Both classes of MHC molecules have special regions where they bind short peptides and present them for recognition by T cells. By presenting short fragments from proteins, the MHC system greatly increases the potential for T cells to recognise specific epitopes from invading organisms that may not be available to them directly from the organism due to the intricate folding of proteins.

Major Histocompatability Complex Proteins: Class I

Class I MHC molecules present to the cell surface only peptides that have been synthesised within a cell. This is particularly important for viruses and other pathogens that replicate within the cell. The class I MHC system functions in all cells of the body including the antigen presenting cells, but bind only to killer T cells, which can then destroy an infected cell.

Proteasomes are protein complexes within cells that produce proteases and degrade defectively synthesised and damaged proteins into smaller peptides and eventually amino acids that can be used in further protein synthesis. Some proteasome produced peptides with 8-11 amino acids are transported into the endoplasmic reticulum by two associated ATP-binding-transporter proteins (transported associated with antigen processing proteins; TAP1, TAP2). The class I MHC molecules initially reside within the endoplasmic reticulum. The molecules are composed of a protein produced from one of three genes, which is bound to β2-microglobulin. The class I MHC genes are called human leukocyte antigen (HLA-A, HLA-B and HLA-C) and have a large polymorphism, with 400-700 variations each in humans. An individual animal has the potential to have six different HLA produced proteins, three from each chromosome. Each class I MHC molecule binds the ends of a peptide with specific amino acids, but the intermediate amino acids can vary widely. Hence, there is a large variation of possible antigens attached to the class I MHC molecules for any individual, but a far larger number for a population. Both the TAP transporter proteins and class I MHC molecules

have a higher affinity for binding to peptides with terminal hydrophobic and basis amino acids. When the protein segment is bound to the class I MHC molecule, the complex is transported to the surface of the cell and the antigen displayed.

Specialised antigen presenting cells such as macrophages have evolved a mechanism to ensure that they produce more appropriately sized peptides with hydrophobic or basic terminal amino acids than normal body cells. Binding to receptors on the macrophage of IFN-γ, which is released by activated NK cells, neutrophils and macrophagees, up-regulates the synthesis three proteins within the proteasome that produce more peptides of appropriate length and terminal amino acids for the class I MHC molecules.

Major Histocompatability Complex Proteins: Class II

Class II MHC molecules are confined only to activated immune antigen presenting cells, the phagocytes, dendritic cells and macrophages, and B cells. They display to the surface of these cells only antigens that are derived from organisms external to the cell. The class II MHC molecules are composed of two proteins, α and β chains, synthesised in the cytoplasm and transported to the endoplasmic reticulum where they combine with the variant chain protein. The gene coding the class II MHC proteins (HLA-D) is also highly polymorphic to bind to a wide range of peptide structures. Class II MHC molecules display peptides with from 13 to 25 amino acids, with the binding sites for the peptide in the middle rather than the ends as for class I MHC molecules.

The variant chain protein protects the binding sites for antigens while the class II MHC molecule is transported from the endoplasmic reticulum to an endosome within the cytoplasm that has merged with a phagosome containing material from outside the cell. Proteases within the endosome degrade these exogenous proteins from the phagosome into peptides. Another protein called HLA-DM catalyses the release of the variant chain protein from the class II MHC molecule, where the exogenous peptides are attached and the complex presented to the cell surface for binding to helper T cells.

T cells bound to MHC molecules on the surface of cells require co-stimulation before being activated. The co-stimulation occurs when a membrane protein, cluster of definition 28 (CD28) on the T cell surface bind with a membrane protein, B7, on an activated antigen presenting cell. The three antigen presenting cell types have different functions and mechanisms.

Dendritic cells and macrophages are phagocytes. Dendritic cells are formed in bone marrow and migrate to all tissues of the body, but only express large numbers of MHC molecules and B7 membrane proteins when activated. They can be activated by cytokines, particularly TNF-α, released from other activated immune cells, such as neutrophils and macrophages, or by PAMPS from bacteria and viruses, like LPS, that stimulate Toll-like receptors within the cell or on the cell surface. The latter mechanism provides a strong link between the innate and adaptive immune systems.

Activated dendritic cells cease being phagocytes and migrate to lymph nodes where they encounter and activate appropriate T cells which recognise the cognate antigen presented. The number of activated dendritic cells depends on the strength of their activation signals and thereby activates an appropriate number of T cells for the situation. Activated dendritic cells survive in the lymph nodes for about seven days so they continually provide an indication of the number and type of T cell that need to be activated. Furthermore, dendritic cells ensure their replacement by the release of IL-4

and granulocyte-macrophage colony stimulating factor (GM-CSF) to stimulate the correct number of monocyte cell differentiation into dendritic cells.

Similar to dendritic cells, macrophages only present sufficient MHC and B7 molecules to activate T cells after they have been activated by TNF-α or PAMPS. However, macrophages remain located in the tissues and do not migrate to lymph nodes. T cells, activated by dendritic cells in lymph nodes, migrate to areas of inflammation within the body but require continual restimulation whenever there is an organism requiring elimination.

B cells are particularly important for antigen presentation late in an infection and following subsequent exposure to the same organism. When activated or memory B cells bind to the cognate antigen, the B cell receptor and antigen complex moves into the cell where the antigen binds to class II MHC molecules and is transported back to the cell surface. The high affinity of B cells for the antigen, results in many MHC molecules presenting the antigen to T cells to rapidly exceed the threshold cross-linking for T cell activation. B cell activation of T cells has been estimated to be 100 to 10,000 fold more than by the other antigen presenting cells, particularly when there are low concentrations of antigen.

4.2. Development and Maturation of the Immune System

There are many excellent and comprehensive reviews pertaining to the development and maturation of the immune system in the pig and as such, only an overview will be provided as part of this review. As noted by Burkey *et al.* (2009), a major function of the immune system is to identify and eliminate pathogens. In vertebrates, the immune system is subdivided into the innate and adaptive arms of immunity that has collaborated to form a sophisticated mucosal immune system. The collaborative, mucosal immune effort offers protection from harmful pathogens while also being tolerant of dietary antigens and normal microbial flora (Burkey *et al*., 2009).

Knowledge regarding the intricacies of mucosal immunity as it applies to the inductive and effector sites is particularly important in pigs because of the development of these sites as the pig matures (Burkey *et al*., 2009). The period from birth through weaning represents a critical time for pigs because they are immunologically incompetent and so are exposed to and must mount appropriate immune responses toward or be tolerant of dietary and environmental antigens. Mucosal immunity, including the inductive and effector components of the gut-associated lymphoid system (GALT), is extremely important in guiding the immune response toward an appropriate and effective immune response that strives to maintain intestinal homeostasis. Not only is the maintenance of intestinal homeostasis important for the development of the neonatal pig, but it also has important ramifications for health and performance throughout the productive lifetime of the animal. Knowledge with respect to porcine mucosal immunity is important in the understanding of the interrelationships between GIT physiology, immunology, and the resident microbiota.

Early-life exposure to appropriate microbial flora drives expansion and development of an efficient immune system (Inman *et al*., 2010). Aberrant development causes increased likelihood of allergic disease or increased susceptibility to infection and hence factors affecting microbial colonization may also affect the direction of immune responses in later life. Inman *et al.* (2010) suggested that there is a need for a manipulable animal model of environmental influences on the development of microbiota and the immune system during early life. In this regard, they assessed the effects of rearing under low- (farm, sow) and high-(isolator, milk formula) hygiene conditions on the intestinal microbiota and immune development in neonatal piglets, because they can be removed from the mother in the first 24 h for rearing under controlled conditions and, due to placental structure,

neither antibody nor antigen is transferred *in utero*. They found that microbiota in both groups was similar between 2 and 5 days. However, by 12–28 days, piglets reared on the mother had more diverse flora than siblings reared in isolators. Dendritic cells accumulated in the intestinal mucosa in both groups but more rapidly in isolator piglets with the minority of 2 to 5-day-old farm piglets, whose microbiota resembled that of an older (12 to 28-day-old) pig, also accumulating dendritic cells earlier than the other farm-reared piglets (Inman *et al*., 2010). Consistent with dendritic cell control of T-cell function, the effects on T cells occurred at later time-points, and mucosal T cells from high-hygiene, isolator pigs made less IL-4 while systemic T cells made more IL-2.

Inman *et al*. (2010) also suggested a correlation between microbiota and dendritic cell recruitment, linked to a variable not directly controlled in this study. The observed differences in immunological development in the low- and high-hygiene groups do not give an indication of the propensity to develop allergic disease. However, given the central role of the dendritic cells in the generation and direction of immune responses the difference in MHCII + dendritic cell numbers in the low- and high hygiene groups is an important observation. These workers noted that the functional significance of the increased dendritic cells in the high-hygiene piglets is not clear: in particular, the extent to which this early increase in dendritic cells was linked to the later decrease in the ability of mucosal T cells to make IL-4, and increased ability of systemic cells to secrete IL-2. It may be, for example, that IL-4 production coincided with increased immunoglobulin class-switching within the gut in response to the more dramatic changes in the flora in farm-reared piglets (Inman *et al*., 2010).

In an Australian context, where there are piglets born in outdoor-based production units, a greater exploration between the rearing environment and its effects on intestinal microbiota and neonatal immunological development would serve as an excellent experimental model for delineation of such effects in indoor-born piglets. In this regard, work by Mulder *et al*. (2009) demonstrated clear effects of the rearing environment on the development of immune function (inflammation) and the microbiota composition. Such a study under Australian conditions could yield potentially valuable experimental data for such relationships.

Emery and Collins (2011) concluded that during the first four weeks after the birth of a piglet there is an amazing interaction between developmental changes in the porcine gut and the gut associated lymphoid system, establishment of the microbiome and gut ecosystem, and functional maturation of the digestive process and immune competence. While cells of the innate immune system such as mononuclear phagocytes and leucocytes are present at birth at reduced numbers, their populations and migratory and functional activity gradually matures to adult levels over the next four weeks. To protect the growing piglet from the insult of pathogens in the immediate period after birth while the whole interactive system is maturing and vulnerable, colostral bioactive molecules and specific antibodies which should be most relevant to cover endemic pathogen challenge will integrate with the innate immune system to reduce pathogen load (by direct killing or uptake, or interference with attachment or establishment) or ameliorate pathogenic effects (by neutralisation of toxins) and hold the host-pathogen balance in favour of the developing pig (Emery and Collins, 2011). Concurrently the microbiome is also enabled to the exclusion of introduced pathogens.

In addition to antibodies, colostrum and milk contain a variety of bioactive/trophic peptides that have been shown to enhance gut development and exhibit anti-microbial activity. As acknowledged by Emery and Collins (2011), these peptides include insulin-like growth factors (IGFs) and epidermal growth factor (EGF) and bear resemblance to many moieties found in serum and inducible secretions that constitute the innate immune system. Most of the antimicrobial compounds documented in the innate immune system are present in sow colostrum or have been produced by

the foetal liver and are present at reduced levels in serum and secretions at birth. These neutralise invasive pathogens prior to ingestion by phagocytic cells. The exact interplay between GIT epithelium and gastrointestinal microflora and the developing immune system under the influences of bioactive peptides (in milk) is complex and dynamic and much is required of the operation to protect the health and growth of the piglet through optimal digestion, nutrient partitioning and a functional gut ecosystem.

Furthermore, the adaptive immune system is poorly developed in piglets at birth and lacks the capacity to develop antibodies and T cell presentation (Stokes *et al*., 2004). The development of the adaptive immune system commences from shortly after birth, but the first IgA antibodies do not appear in the gut of piglets until around four weeks of age (Thompson *et al*., 2008). Full competence for antibody production and T cell activity is not gained in pigs until they are about seven weeks of age (Stokes *et al*., 2004).

4.3. Inhibitors of the Immune System and Stimulation of Microbial Growth

4.3.1. Nutrient Deficiencies or Excesses

The immune system is influenced greatly by the nutrition of an animal with impairment of the immune response being affected by both deficiencies of energy and individual nutrients and by excesses of specific nutrients. There are many reports of energy and protein malnutrition depressing immune function (Scrimshaw and SanGiovanni, 1997; Fock *et al*., 2008; Rodriguez *et al*., 2011). Furthermore, immune function is known to be affected by deficiencies of specific amino acids, fatty acids, vitamins, macro-minerals and trace elements. These nutrients appear to be required in amounts that exceed the minimum needed to prevent classical deficiency symptoms. Suchner *et al.* (2000) termed them *immunonutrients* that are required in amounts needed to cover both traditional metabolic and immunological requirements.

Specific nutrients known to affect immune function include arginine, tryptophan, methionine, valine, threonine, vitamin A, β-carotene, folic acid, vitamin B_6, vitamin B_{12}, vitamin C, vitamin E, riboflavin, iron, zinc, sodium, copper, manganese and selenium (Daly, *et al*., 1990; Butcher and Miles, 2002; Chandra, 2002, Marcos, *et al*., 2003). Antioxidants are important for reducing oxidative stress in immune cells and reducing immune cell apoptosis (Victor and De la Fuente, 2002). Dietary lipids and particularly the balance of n-3 to n-6 fatty acids are also known to affect the composition of immune cells, their immune function and the inflammatory response (De Pablo and Alvarez de Cienfuegos, 2000; Innis and Jacobson, 2007).

Excess amounts of many nutrients are known either to have negative effects directly on components of the immune system or to stimulate microbial growth and thereby enhance the challenge for the immune system. Excess intake of vitamin E, vitamin A, polyunsaturated fatty acids, methionine and zinc have been shown to reduce the effectiveness of the immune system (Kumari and Chandra, 1993; Friedman *et al*., 1998; Butcher and Miles, 2002; Faber *et al*., 2004). Alternately, high intakes of undigested or slowly digested nutrients are known to increase substrates for the growth of intestinal microbes and increase morbidity and mortality from pathogenic organisms (Pluske *et al*., 1996; Hampson *et al*., 2000; Pluske *et al*., 2002). Similarly, excess of dietary iron (Bullen *et al*., 2006) and dietary protein (Wellock *et al*., 2006; Houdijk, *et al*., 2007; Heo *et al*., 2008, 2009; Opapeju, *et al*., 2009) have been shown to stimulate microbial growth and susceptibility to enteric microbial diseases.

4.3.2. General Nutritional Status

Numerous experiments involving many animal species provide results to show that general under-nutrition reduces the capacity of the immune system to respond to vaccines or specific antigens, and to decrease their resistance to diseases. However, there are other reports that suggest severe under-nutrition does not depress some components of the immune system.

Zilstra *et al*. (1999) examined the effects of nutrition on the response of young piglets to a rotavirus infection. There were three treatments from two days of age; i) non-infected fully nourished; ii) infected fully nourished; and iii) infected, malnourished. The first two treatments were offered a liquid diet formulated to meet nutritional requirements of the young pigs. The non-infected fully nourished group was pair fed to the infected fully nourished group. The malnourished group received the same feed that had been diluted 50% with water and electrolytes. The infected pigs were challenged with rotavirus on day two of age. Intestinal damage and diarrhoea was observed within two days of infection in both fully nourished and malnourished groups. Symptoms subsided by day 9 in the fully nourished group but not until day 16 in the malnourished group. Malnutrition intensified MHC class I gene expression and reduced MHC II gene expression relative to the fully nourished piglets. Expression of jejunal $CD4^+$ and $CD8^+$ T-lymphocyte numbers were increased in the fully nourished infected group, but there was no change in T cell numbers in the malnourished group compared to the control pigs. The immediate post-weaning decline in intake will have substantial negative consequences on the immune capacity of the pig.

Fock *et al*. (2008) examined the effect of a low protein diet on the response of mice to and LPS challenge. The mice were fed either a 4% or a 20% protein diet until the low protein group lost approximately 20% of their body weight. Both groups were then given 1.25 µg of LPS intravenously. The malnourished mice had significantly less immune cells in the blood, bone marrow and spleen. The malnourished mice also showed significant differences in the IL-10 response to LPS, which suggested an impaired immune response to LPS. Similarly, there are numerous experiments with sheep that show the immune response to intestinal parasites is depressed in poorly nourished animals compared with well-fed counterparts (Wagland *et al*., 1984; Liu *et al*., 2003).

Severe malnourishment has been shown to reduce the effectiveness of vaccines. Malafaia *et al*. (2009) fed mice either a nutritionally balanced diet containing 14% casein or a diet containing 3% casein and deficient in iron and zinc. Mice from both groups were vaccinated with *Leishmania chagasi* antigen vaccine after the malnourishment had been established. Both groups were then given nutritionally adequate diets and challenged with live *Leishmania chagasi* organisms. The vaccine was shown to cause a significant reduction in the number of parasites in the liver and spleen of fully nourished mice, but the number of parasites in the spleen of the malnourished mice increased. There was a marked reduction in IFN-γ production in response to the vaccine in the malnourished mice. In another experiment with mice examining the effects of malnourishment on response to vaccines, Naraynan, *et al*. (1978) obtained results which suggested undernutrition depressed cell-mediated immunity, but not humoral responses.

There is also evidence that immune memory is depressed in malnourished animals (Martin *et al*., 2006). Deer mice were inoculated with the KLH antigen and developed a full antibody and T-cell response. The mice were then split into two groups. One group was fed *ad libitum*, while the second group was fed 70% of the amount eaten by the first group. Two weeks after the primary inoculation, the mice were given a second inoculation of KLH. The nutrient restricted mice produced 95% less IgG against KLH after the second antigen challenge than the well fed mice, suggesting that there were severe nutritional constraints on immunological B cell memory.

However, contrary to the examples of impaired immunological response resulting from general malnutrition, Crenshaw *et al.* (1986) found that young pigs offered a nutritionally inadequate diet did not have a depressed antibody production against human red blood cells. Pigs offered the inadequate diet grew at 0.17 kg/d compared with 0.35 kg/d for those fed the adequate diet, but did not show any difference in antibody production in response to the red blood cell challenge.

4.3.3. Proteins and Amino Acids

4.3.3.1. Increased Requirements for Amino Acids

Stimulation of the immune system and particularly the release of pro-inflammatory cytokines, IL-1β, IL-6 and TNF-α, have major effects on protein and amino acid metabolism in animals (Klasing, 2007). The pro-inflammatory cytokines stimulate the uptake of amino acids by the liver primarily for production of acute phase proteins, while reducing amino acid uptake by skeletal muscles through an increase in the rate of protein degradation and a decrease in the rate of protein synthesis (Lang *et al*., 2007). Immune stimulation also enhances the production of reactive oxygen and nitrogen free radicals, which contain unpaired electrons that have strong antimicrobial properties (Colditz, 2002). These free radicals can also damage cells of the animal. However, a peroxidative protection system has been developed in animals to inactivate the free radicals. In particular, the production of glutathione, which contains a free and readily oxidisable sulfhydryl group, is increased markedly during stimulation of the immune system (Brosnan and Brosnan, 2009).

The major acute phase proteins produced by pigs include haptoglobin, fibrinogen, α-antitrypsin, lipopolysaccharide binding protein, C-reactive protein, serum amyloid A, kallikrein-related pig acute phase protein, albumin, transferring, retinol binding protein and cortisol binding protein (Rakhshandeh and de Lange, 2011). The production of acute phase proteins increases many fold during immune stimulation (Jahoor *et* al., 1999). The amino acid composition of these acute phase proteins differs markedly from that of skeletal muscle (Rakhshandeh and de Lange, 2011). Thus, the synthesis one gram of a specific acute phase proteins, such as albumin, requires the breakdown of six grams of muscle protein to provide the cysteine needed. Rakhshandeh and de Lange (2011) suggest that because of the differences in amino acid composition of acute phase proteins relative to skeletal muscle, the supply of glutamine, arginine, tryptophan, threonine, methionine and cysteine may become critical to an effective immune response in growing pigs. However, after reviewing the literature, Rakhshandeh and de Lange (2011) concluded that most of the additional amino acids needed for the synthesis of acute phase proteins can be provided from endogenous sources, except for tryptophan and the sulphur containing amino acids, methionine and cysteine. Although the increase in amount of dietary tryptophan and sulphur containing amino acids needed depends on the magnitude of the immune response, results from Rakhshandeh and de Lange (2011) suggest that tryptophan requirements increase by more than 10% and sulphur amino acids by approximately 20%. Furthermore, these authors suggest that there is a greater need for methionine than cysteine with the ratio of methionine:cysteine increasing from 0.57 to 0.62.

The GIT of pigs is coated with a continuous layer of mucus produced by goblet cells. Mucus protects the gut tissues from mechanical damage associated with the consumption of dietary fibre and also acts as a barrier to pathogenic organisms. Mucus contains glycoproteins which are particularly rich in threonine (10-13%) compared with muscle tissue (5-6%). Several GIT infections, particularly helminth infections (Khan, 2008) and trinitrobenzene sulfonic acid-induced ileitis (Rémond *et al*., 2009), increase substantially mucin synthesis and the number of goblet cells. The increase in mucin production would be expected to increase the requirements for threonine. Indeed, Rémond *et al*. (2009) showed that experimentally-induced ileitis increased the flux of

threonine across the portal drained viscera in mini-pigs by almost 7-fold. Furthermore, Law *et al.* (2007) showed that mucin production and the number of goblet cells were significantly reduced in neonatal pigs fed threonine deficient diets. Similarly, Faure *et al.* (2005) showed that threonine deficiency in rats significantly reduced mucin fractional synthesis rates in the duodenum, ileum and colon.

Other gut diseases including inflammatory bowel diseases, ulcerative colitis, Crohn's disease (Faure *et al.*, 2006) and dextran sulphate sodium-induced colitis (Faure *et al.*, 2003) have been shown to significantly reduce mucin production. These diseases may be expected to reduce the requirements for dietary threonine. However, Faure *et al.* (2006) showed that providing a mixture of amino acids containing threonine, serine, proline and cysteine to rats with dextran sulphate sodium-induced colitis significantly increased the number of goblet cells in the ulcerated area and increased colonic mucin production by 95%.

The experiments outlined above suggest that requirements for threonine are likely to be increased in pigs with GIT infections. However, the magnitude of this increase is unclear. There appear to be no experiments investigating the effects of increasing the dietary concentration of threonine above that needed for optimal growth on the performance of pigs with GIT infections. Experiments with broiler chickens (Mroufyan *et al.*, 2010) and laying hens (Azzam *et al.*, 2011) suggest that threonine requirements recommended for maximum growth or egg production are insufficient for optimal immune responses. Azzam *et al.* (2011) recommend an increase of approximately 30% in threonine requirement for optimal immune response in laying hens.

4.3.3.2. Excess Dietary Protein and Amino Acids

Numerous studies have shown that reducing the protein content of diets not containing antibiotics and fed pigs immediately after weaning reduces their susceptibility to GIT infections and diarrhoea (e.g., Göransson, 1997; Callesen and Johansen, 2006; Wellock *et al.*, 2006; Heo *et al.*, 2008, 2009; Yue and Qiao, 2008). For example, Callesen and Johansen (2006) showed that reducing the crude protein content of the diet from 21% to 18% for pigs weaned in a commercial facility reduced the incidence of diarrhoea by 25%.

Lowering the protein content of the diet reduces the amount of undigested protein available to microbes within the GIT. Pigs immediately after weaning produce relatively small quantities of hydrochloric acid in the stomach (Kil and Stein, 2010). Since dietary proteins generally have a high buffering capacity, the relatively high pH within the stomach of newly weaned pigs reduces pepsin activity and protein digestion. An increase in undigested protein stimulates the growth of pathogenic microbes and protein fermentation products including ammonia and amines such a putrescine, which can damage the intestinal epithelium and increase the incidence of diarrhoea (Potter and Kentworthy, 1969; Ball and Aherne, 1987; Dong *et al.*, 1996).

Reducing the protein content of the diet below recommended concentrations has been shown to reduce growth rate of pigs unless individual amino acids are added to the diets to improve the balance of amino acids available to the animals (Wellock *et al.*, 2006; Htoo *et al.*, 2007; Heo *et al.*, 2008; Yue and Qiao, 2008). Evidence suggests that the protein content of diets offered to weaned pigs from around 6 kg can be reduced to 17.5% without a detrimental effect on growth rate provided it has an ideal amino acid pattern (Heo *et al.*, 2009). The ideal amino acid pattern can be determined by the computer simulation model, AUSPIG (Black *et al.*, 1986), or in relation to lysine as estimated by (Rademacher *et al.*, 1999). Diets will generally need to be supplemented with lysine,

threonine, methionine, tryptophan, isoleucine and valine for the most commonly used protein sources in Australian pig diets.

Although gastrointestinal diseases are likely to result in increased requirements for tryptophan, methionine and threonine, there is evidence showing too much of these amino acids can be toxic to animals. For example, excess methionine has been shown to reduce feed intake and growth rate in several animal species including pigs (Edmonds *et al*., 1987), rats (Rotruck and Boggs, 1977), cats (Fau *et al*., 1987) and chickens (Acar *et al*., 2001). A 4% excess of DL-methionine compared with NRC (1979) recommended requirements, when added to a 20% protein diet offered to pigs weighing 8.6 kg, was found to reduce growth rate by 52% relative to the control diet (Edmonds *et al*., 1987). Excess methionine has been related to toxicity of the liver through an increase in the generation of mitochondrial reactive oxygen species and oxidative damage to liver mitochondrial DNA (Gomez *et al*., 2009). Edmonds *et al*. (1987) showed also that diets with 4% excess tryptophan or threonine above the recommended NRC (1979) requirements reduced growth rate by 28% and 5%, respectively.

4.3.4. Fatty Acids

The lipids in feed commonly fed to pigs consist mainly of neutral fats (specifically triglycerides), esters of fatty acids and glycerol (Rossi *et al*. 2010). The degree of unsaturation, the length of the carbon chains and the isomeric form of the fatty acids greatly influence both the physical and the chemical characteristics of fat. Grundy (1994) noted that saturated fatty acids are divided into two groups: long chain and medium chain with saturates having 8 and 10 carbon atoms belonging to the medium-chain group. The medium-chain fatty acids (MCFA) are absorbed directly into the portal circulation as free fatty acids. Fatty acids with a chain of less than six carbon atoms are called short-chain fatty acids (SCFA), and are the major end products of bacterial fermentative reactions in the colon and are the principal anions in the hindgut of mammals (Pluske *et al*., 1999). Essential fatty acids (EFA) cannot be made endogenously by humans and other animals and hence must be extracted exogenously from dietary sources (Rossi *et al*. 2010).

4.3.4.1. Short and Medium Chain Fatty Acids

Rossi *et al*. (2010) surmised that SCFA and MCFA may be useful in diets for post-weaning piglets because weaning is considered a stressful event that is associated with a sudden change of diet, reduced feed intake, growth performance and elevated incidence of enteric disease. Pluske *et al*. (1997) explained that the overall mass of the small intestine decreases along with the mucosal component and the intestinal villi and hence digestive and absorptive functions of the gut are impaired so contributing to poor performance after weaning. Rossi *et al*. (2010) reported that in attempts to reduce in-feed antibiotics, much research has been directed towards increasing intestinal SCFA and MCFA content through different nutritional strategies, with an improvement in growth performance of pigs fed sodium butyrate.

Rossi *et al*. (2010) concluded that the anti-inflammatory mechanism of butyrate is related to its ability to differentially regulate cytokine expression and secretion by porcine peripheral blood mononuclear cells. Hamer *et al*. (2008) described several studies that suggested bacterial metabolites such as butyrate might affect the host immune response. Butyrate is a major end-product of intestinal microbial fermentation of dietary fibre and is important as an energy source for intestinal epithelial cells and maintaining colonic homeostasis. In an extensive literature review, these authors noted that apart from promoting satiety, butyrate produces beneficial effects by influencing colonic mucosal functions including inhibition of inflammation and carcinogenesis, reinforcing various components of the colonic defence barrier leading to enhanced protection

against luminal antigens, and decreasing oxidative stress. One important component of this barrier is the mucous layer covering the epithelial lining consisting of mainly mucin glycoproteins and trefoil factors (TFFs) (Hamer *et al*. 2008).

Mucin glycoproteins are classified into neutral and acidic subtypes with sulfomucins and sialomucins categorised as acidic and sulfomucins generally having greater resistance to bacterial degradation. The effects of a number of fermentable dietary fibres on the mucous layer have been studied with varying results. For example, resistant starch increased the number of acidic mucins, but did not affect the number of goblet cells in rats (Hamer *et al*. 2008). In contrast, fructo-oligosaccharides (FOS) increased the number of goblet cells in piglets. Alterations in goblet cell function, composition and thickness of the intestinal mucous layer have been found in several intestinal disorders (Hamer *et al*. 2008). It should, however, be noted also that some equivocal results have been reported, which can partly be explained by the different butyrate concentrations and models used. In addition, a few animal and *in vitro* studies demonstrated negative effects at higher butyrate concentrations on permeability and visceral sensitivity of the large intestine (Hamer *et al*. 2008).

With respect to conjugated linoleic acid (CLA), O'Shea *et al*. (2004) noted that aspects of both the innate and adaptive immune responses are affected by dietary CLA supplementation with evidence suggesting that the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers exert distinct effects on immune function. In particular, studies have indicated that these isomers have differential effects on specific T-cell populations and immunoglobulin subclasses. O'Shea *et al*. (2004) reported that with regard to a pig model, CLA could influence critical pathways of thymocyte differentiation in that phenotypic analysis of T-cell subsets in the thymus and peripheral blood revealed that CLA acted first on immature thymocytes and then on modulated mature peripheral blood T cells. These authors also reported that (i) CLA rectified tissue inflammation and weight loss associated with the treatment of as inflammatory bowel disease (IBD); (ii) with longer-term feeding of CLA, the effects of CLA persisted beyond the period of dietary supplementation; (iii) interstitial pneumonia tended to be more severe in infected pigs fed a control diet than those fed CLA.

4.3.4.2. Omega-3 and Omega-6 Fatty Acids

There are two families of EFA, omega-3 (e.g., linseed) and omega-6 fatty acids (e.g., vegetable oils, such as corn, sunflower and soybean). Rossi *et al*. (2010) suggested that the omega-3 PUFA activity in modulating mediators of humoral and cellular immunity may present possible benefits for livestock suffering health problems related to acute respiratory or gastrointestinal diseases. A study by Kelley (2001) revealed that a reduction in the amount of fat intake enhanced several indices of the immune response, including lymphocyte proliferation, natural-killer–cell activity, cytokine production, and delayed-type hypersensitivity. In addition, supplementation of human diets with omega-3 fatty acids reduced several aspects of neutrophil, monocyte, and lymphocyte functions, including the production of inflammatory mediators. Despite a few studies reporting conflicting evidence, Kelley (2001) suggested that fish oils have been used successfully in the management of several inflammatory and autoimmune diseases even though there is a need to determine safe and adequate intake levels of omega-3 fatty acids. Kelley (2001) explained that omega-3 PUFA are anti-inflammatory and omega-6 PUFA are pro-inflammatory and as a result, they produce different types of eicosanoids that have different effects on immune cells. Changing the ratios between omega-3 and omega-6 PUFA therefore alters the type and concentration of eicosanoids produced with, in general, eicosanoids derived from omega-3 PUFA being less potent mediators of inflammation than those derived from arachidonic acid metabolites with the effects of eicosanoids being dose dependent such that the same cells can be simulated or inhibited depending on the dose (Kelley 2001). Furthermore, this review suggested that increasing PUFA intake increases oxidative stress that can cause lipid peroxidation if not counterbalanced by antioxidant nutrients. Safe and adequate supplemental levels of fish oils and vitamin E need to be established with dose–response studies comparing the effects of dietary omega-3 fatty acids, linolenic acid, eicosapentanoic acid and docosahexaenoic acid from foods rather than supplements (Kelley 2001).

Miles and Calder (1998) explained that all mammals can synhesize fatty acids *de novo* from acetyl-CoA with the end product of the fatty acid synthetase enzyme being palmitic acid, which can then be elongated to stearic acid. However, cell membranes require unsaturated fatty acids to maintain their structure, fluidity and function and, as animal tissues are unable to synthesize linoleic and *a*-linolenic acids, these fatty acids must be consumed in the diet (essential fatty acids). Unlike marine plants, in mammals the omega-3 and omega-6 PUFA are not metabolically interconvertible. It is the formation of these long-chain omega-3 PUFA by marine algae and their transfer through the food chain to fish that accounts for their abundance in some marine fish oils (Miles and Calder 1998). Miles and Calder (1998) provided a review of studies that have investigated the effects of the amount and type of fat in the diet on immune cell functions and the effects of omega-3 PUFA. They showed how omega-3 PUFA might affect immune cell function partly by regulating the expression of genes encoding for proteins involved in cellular responses and in communication between cells. One mechanism by which omega-3 PUFA could affect gene expression is through changes in the signal transduction pathways which link cell surface receptors to the activation of nuclear transcription factors (NF) (Miles and Calder 1998). Alternatively they suggested that omega-3 PUFA or their derivatives might bind directly to NF thereby altering their activity.

Polyunsaturated fatty acids have specific metabolic and nutritional effects because they affect gene expression involved in intestinal inflammation and give rise to lipid mediated products. Knoch *et al*. (2009) explained that there is evidence to suggest that there are beneficial effects of long-chain PUFA, particularly omega-3 PUFA, and for a variety of inflammatory diseases omega-3 PUFA have been shown to modulate the expression of genes that code for proteins involved in inflammation, lipid metabolism and energy utilization. Knoch *et al*. (2009) reported, in a pig study, that omega-3 PUFA failed to protect from IBD but accelerated colon regeneration and clinical remission by

activating peroxisome proliferator activated receptors. Knoch *et al*. (2009) noted that many studies have focused on the anti-inflammatory effects of omega-3 PUFA as indicated by reports of histological assessments, circulating PUFA concentrations or ex vivo production of inflammatory mediators. A range of animal models of acute chemically-induced colitis show primarily anti-inflammatory effects of fish oil rich in omega-3 PUFA (Knoch *et al*. 2009).

Knoch *et al*. (2009) also noted that most of the animal studies they reviewed used a mixture of various fatty acids in the diets and therefore any observed anti-inflammatory or immunomodulatory effects have been assigned to a fatty acid mix rather than pure PUFA. Genome-wide studies use modern technologies and knowledge derived from the human genome project to provide a more comprehensive understanding of the multiple pathways by which PUFA can regulate transcriptional and metabolic processes (Knoch *et al*. 2009). A large part of the evidence regarding anti-inflammatory and immunomodulatory effects of PUFA has arisen from cell culture studies where single fatty acids have been added (Knoch *et al*. 2009). In order to define effects of dietary PUFA on IBD, for example, animal model systems with diets of well-defined fatty acid composition and purity would be advantageous and should incorporate the "omics" tools (transcriptomics, proteomics, metabolomics) available to nutrition research (Knoch *et al*. 2009). Once hub genes that are differentially expressed in response to PUFA have been identified by transcriptome analyses, other techniques (including chromatin immunoprecipitation and transactivation assays) can be applied in order to define direct targets of the PUFA-responding central genes (Knoch *et al*. 2009). Such an approach can also be used in the pig.

Hwang (1989) explained that EFA can affect immune responses based on the observation that EFA deficiency can accentuate or improve symptoms of certain autoimmune diseases in animals, that supplementation of linoleic acid to animals reversed such effects, and that the treatment of animals with cyclooxygenase inhibitors abrogated the effect of linoleic acid. Derived from EFA, PUFA, with 20 carbons, are precursors of prostaglandins and other enzymatic metabolites and are involved in the immune response. These compounds - eicosanoids - possess diverse biological actions in endocrine, metabolic, and nervous systems. Hwang (1989) suggested that because biosynthesis of eicosanoids in tissues can be modulated by the composition of dietary fatty acids, there is a possibility that manipulating dietary EFA can be used not only as a preventative measure but also as a supplemental means to manage certain chronic diseases that result from immunological disturbance.
Low n-6 PUFA intakes enhance whereas high intakes decrease certain immune functions (Harbige 2003). Low intakes of long-chain n-3 fatty acids (fish oils) enhance certain immune functions, whereas high intakes are inhibitory on a wide range of functions, e.g., antigen presentation, adhesion molecule expression, Th1 and Th2 responses, pro-inflammatory cytokine and eicosanoid production, and they induce lymphocyte apoptosis (Harbige 2003). In this regard, vitamin E has a critical role in long-chain n-3 PUFA interactions with immune functions, often reversing the effects of fish oil (Harbige 2003). Further investigations are warranted examining dose-response relationships with dietary vitamin E levels in diets for weanling pigs given the importance of vitamin E to aspects of immune function.

4.3.4.3. Effects of Dietary Fatty Acids on Disease

The effects of dietary fatty acids on animal autoimmune disease models depend on both the autoimmune model and the amount and type of fatty acids fed (Harbige 2003). Diets low in fat, essential fatty acid deficient (EFAD), or high in long-chain n-3 PUFA from fish oils, increase survival and reduce disease severity in spontaneous autoantibody-mediated disease, whereas high-fat, LA-rich diets increase disease severity (Harbige 2003). In experimentally induced T-cell-mediated autoimmune disease, EFAD diets or diets supplemented with long-chain n-3 PUFA augmented

disease, whereas n-6 PUFA prevented or reduced the severity (Harbige 2003). In contrast, in both T cell- and antibody-mediated autoimmune disease, the desaturated/elongated metabolites of LA are protective. PUFA of both the n-6 and n-3 families are clinically useful in human autoimmune-inflammatory disorders, but the precise mechanisms by which these fatty acids exert their clinical effects are not well understood (Harbige 2003).

It is clear however that many of the observations made on the effects of n-3 PUFA fatty acids on immune functions in health are relevant to the effects of these fatty acids observed in infection and autoimmunity (Harbige 2003). Much evidence exists, consistent with the generalised immunosuppressive effects of high fish oil intakes, demonstrating impaired resistance to certain experimental infections (Harbige 2003). However, there is also a good deal of evidence for a protective effect of fish oils in other experimental infections (Harbige 2003). The immunological mechanisms behind this apparent paradox are unclear, but vitamin E in some cases is important in this interaction. Consistent with their immunosuppressive and anti-inflammatory effects, long-chain n-3 PUFA from fish oils increase the survival and reduce the severity of spontaneous autoantibody-mediated disease in animal models (Harbige 2003).

For example, rats were sensitised (immunised) with neuroantigen (spinal cord homogenate) on day 0 and treated 7 d later (until day 21) with EPA-rich fish oil (MaxEPA) at 500 mg/kg EPA, or γ-linolenic (GLA)-rich fungal oil (FGO) at 500 mg/kg GLA. There were significant differences in the course of the disease compared with control experimental autoimmune encephalomyelitis (EAE) values at day 28 ($P < 0.05$) and day 36 ($P < 0.025$) in the fish-oil-treated group, i.e., fish oil prolonged the disease course. Fungal oil markedly reduced the incidence of EAE compared with controls, i.e., full protection against clinical disease. Linoleic acid-rich safflower oil at 500 mg linoleic acid/kg body weight was without effect and importantly does not contain GLA. At higher doses, safflower oil reduced the clinical severity of EAE but not the incidence. There was also no difference between controls and linseed oil at 500 mg α-linolenic acid/kg (Harbige 2003).

Zentek *et al.* (2011) reported that MCFAs are found at higher levels in milk lipids of many animal species and in the oil fraction of several plants, including coconuts, palm kernels and certain *Cuphea* species. Medium-chain triglycerides (MCTs) and fatty acids are efficiently absorbed and metabolized and are therefore used more commonly in piglet nutrition given they provide instant energy and also have physiological benefits beyond their energetic value contributing to several findings of improved performance in piglet-feeding trials. In their review, Zentek *et al.* (2011) reported that MCFAs affect the composition of the intestinal microbiota and have inhibitory effects on bacterial concentrations in the digesta, mainly on *Salmonella* and coliforms. They noted that some feed specifications had up to 8% of a non-esterified MCFA mixture in feed, but due to the sensory properties this can have a negative impact on feed intake that could be overcome by using up to 15% MCTs. Zentek *et al.* (2011) reported that feeding sows with diets containing 15% MCTs resulted in a lower mortality of newborns and better development, particularly of underweight piglets. They concluded that MCFAs and MCTs offer advantages for the improvement of energy supply and performance of piglets and may stabilize the intestinal microbiota, expanding the spectrum of feed additives supporting piglet health in the post-weaning period.

Miura *et al.* (1993) suggested that results obtained with animal models show that both the amount and type of dietary fat modulate aspects of the immune system. They observed that in the rat, absorption of octanoic acid (C8), most of which appeared to be transported to portal blood, did not produce a significant elevation of lymphocyte flux or increased proliferative response of lymphocyte in intestinal lymph. Odle *et al.* (1989) concluded that MCT may be better used than long-chain

length fatty acids and that there may be differences between the utilisation of even-MCT and odd-MCT, depending on the age of the neonate pig. They surmised that this could be related to chain length effects on digestion and absorption because plasma decanoate concentration changed little, even though it composed 25% of the even-MCT supplement.

Messens *et al*. (2010) reported that *Salmonella typhimurium* was responsible for more than half of the reported cases of human salmonellosis in Belgium in 2007 and was the predominant serovar isolated from slaughter pig carcasses. However, octanoate or caprylic acid is reported to have an antibacterial effect. For example, to lower the *Salmonella* contamination of pork meat, measures can be taken at the primary production level, e.g. by reducing the shedding of *Salmonella* through the use of feed additives such as MCFAs (Messens *et al*. 2010). To investigate the effect of MCFAs (sodium caproate, sodium caprylate and sodium caprinate) on the pig intestinal microbial community, Messens *et al*. (2010) developed an *in vitro* continuous culture system, simulating the porcine caecum. The system was monitored by plating on selective media, PCR-DGGE and HPLC analysis of fermentation products. By the addition of 15mM caprylate, significant reductions of coliforms and *Salmonella* counts (by 4.69 $\log_{10}$ units; 95% confidence interval: 4.19–5.18) could be achieved, while other bacterial populations were clearly less affected. Messens *et al*. (2010) reasoned that this concentration seems economically feasible in pig feed, provided that the substance can reach the caecum without being absorbed. Hence they concluded that caprylate, for example in the form of encapsulated beads or as triacylglycerol oil, might have potential as a *Salmonella*-reducing additive in pig feed.

Van Immerseel *et al*. (2004) conducted a study to evaluate the MCFA caproic, caprylic, and capric acid, for the control of *Salmonella* serovar Enteritidis in chickens. Results suggested that all MCFA were growth inhibiting at low concentrations *in vitro*, with caproic acid being the most potent. They concluded that MCFA have a synergistic ability to suppress the expression of the genes required for invasion and to reduce the numbers of bacteria in vivo.

Dierick *et al*. (2002) examined four selected MCFAs (C6:0–C12:0) containing a variety of fat sources and six lipases, under gastric-simulated conditions (pH 3 to 6, 3 h incubation, 37.8C, with complex substrate), to find if appropriate amounts of MCFAs could be liberated that could provide a valuable alternative for nutritional antibiotics used in piglet nutrition. They found that depending on the conditions applied, up to 20% of the MCFAs could be enzymatically released into the medium.

Finally, Han *et al*. (2011) compared the effects of eucalyptus-derived medium chain fatty acids (E-MCFAs), zinc oxide (ZnO), and antibiotics on performance, nutrient digestibility, and serum chemistry parameters of nursery pigs. They found that growth performance of pigs supplemented with microencapsulated E-MCFAs did not differ from that of pigs supplemented with antibiotics or pharmacological levels of ZnO. Although there is evidence to suggest that eucalyptus oil stimulates the immune system, they found that there were no changes in serum parameters such as IgG, TNF-α, or cortisol, suggesting that mechanisms other than immune-system stimulation are responsible for the performance enhancement observed in pigs fed eucalyptus. Hence they concluded that eucalyptus-MCFAs can be used as a growth promoter in diets fed to nursery pigs.

4.3.5. Pig Housing Environment

The effects of various aerosol pollutants found in pig buildings on the severity of several diseases have been investigated. Hamilton *et al*. (1996) examined the effect of atmospheric ammonia concentration on the severity of atrophic rhinitis. Pigs were exposed to ammonia concentrations of 0, 5, 10, 15, 25, 35 and 50 ppm and one group of pigs at each concentration was inoculated with

pathogenic *Pasteurella multocida* type D. The severity of rhinitis increased curvilinearly with the concentration of ammonia. Pigs exposed to only 5 ppm ammonia had significantly more severe rhinitis than those not exposed to ammonia. The greatest effect occurred with an ammonia concentration of 10 ppm and the response gradually declined as the ammonia concentration increased to 50 ppm. The authors speculate that the decline in severity may have been caused by a toxic effect of the higher ammonia concentrations on the pathogenic organism. Subsequent studies (Hamilton *et al*., 1998a) revealed that gaseous ammonia had a marked effect on the colonisation by *P. multocida* of the nasal cavity, but not the tonsil. Colonisation by the pathogenic organism resulted in almost complete exclusion of commensal flora from the region and colonisation regressed rapidly following the removal of ammonia from the air.

Drummond *et al*. (1981a) conducted a similar experiment to Hamilton *et al*. (1996) in which weaned pigs were inoculated with *Bordetella brochiseptica* and exposed to aerial ammonia concentrations of 0, 50 or 100 ppm. Inoculation with *B. brochiseptica* was reported to significantly depress growth rate over 4 weeks by 26 % and feed intake by 12 %, but there was no significant effect of ammonia concentration. The numbers of pigs used in the experiments were small and results of the effects of individual treatments were not presented in the paper, but were given as fitted regression models. Thus, an independent assessment of the synergistic effect of the disease and airborne ammonia concentrations is not possible. Nevertheless, the authors reported that the clinical symptoms of nasal discharge and turbinate bone shrinkage were progressively more severe in inoculated pigs as the concentration of atmospheric ammonia increased.

Drummond *et al* (1981b) investigated the impact of 100 ppm atmospheric ammonia on the performance and clinical responses over 4 weeks of 9-week old pigs experimentally infected with the roundworm *Ascaris suum*. The presence of atmospheric ammonia exacerbated the effects of the roundworm infection. Mean growth rates of the pigs compared with control animals were depressed by 32%, 28%, and 61% for the ammonia exposed, ascarid infected and combined ammonia plus ascarid infection. The observed depression in feed disappearance compared to the control pigs was 18%, 18% and 35% for the respective treatments. Despite the effects on pig performance, the clinical symptoms and histopathology of the ascarid infection were not exacerbated by the high concentration of atmospheric ammonia.

Murphy T. (unpublished) collected viable airborne bacteria from within a pig building and increased their numbers under laboratory conditions. Groups of pigs received increasing doses of the cultured viable bacteria through intranasal inoculation and were subjected to concentrations of 0, 25 or 50 ppm ammonia in the air during 20 minute feeding bouts over several weeks. Growth rate of the pigs was related negatively to increasing ammonia exposure only in pigs inoculated with viable bacteria cultured from air collected in pig buildings. The growth rate depression was related closely to the severity of non-specific bacterial infections within the lungs of the inoculated pigs. This experiment is important because it has demonstrated that pig performance can be depressed through lung infections caused by general airborne bacteria, which were predominantly *Streptococcus*, found in pig buildings without the need to be associated with traditional respiratory disease causing organisms.

There is evidence also that the severity of diseases can be exacerbated by the presence of dust and particularly dust high in antigenic or endotoxin materials. Hamilton *et al*. (1998b) exposed pigs to a fine dust made from dried ovalbumin at concentrations from 0 to 16.6 mg/m^3, with and without inoculation with *P. multocida*. Exposure to the ovalbumin dust increased significantly colonisation of both the nasal cavity and tonsil by the pathogenic organism and increased the severity of atrophic

rhinitis. Exposure to the ovalbumin without the disease agent was found to increase significantly the concentrations of serum anti-ovalbumin IgG and IgA antibodies. However, when infected with the pathogen, the capacity of the animals to produce antibodies to ovalbumin was significantly reduced. These results suggest that exposure of pigs to an antigenic dust at the concentrations found within many pig buildings, increases greatly the susceptibility of the animals to *P. multocida* and the toxins produced by the organism depress antibody production, thereby enhancing the virulence of the disease. Further studies by Hamilton *et al*. (1999) have shown that the combined effects of ammonia and antigenic dust are synergistic, with the greatest colonisation of the upper respiratory tract occurring when 5 mg/m^3 of respirable dust was combined with 50 ppm ammonia. In this study, the presence of both dust and ammonia without a *P. multocida* challenge was found to cause mild turbinate atrophy in the pigs.

A similar synergistic effect of airborne bacterial lipopolysaccharide endotoxin on the severity of symptoms induced by the porcine reproductive and respiratory syndrome (PRRS) virus has been observed by Van Reeth *et al*. (2000) using gnotobiotic pigs and Labarque *et al*. (2002) using pigs from a PRRS negative herd. Intratracheal inoculation of the pigs with the PRRS virus alone resulted in fever for a period of 1 to 5 days, but little clinical change to respiratory function. Exposure to LPS alone resulted in fever and lethargy, but few signs of respiratory change. However, the concurrent exposure of pigs to the PRRS virus and LPS resulted in severe fever and lethargy as well as major changes to respiratory function. Development of the disease was considered by Van Reeth *et al*. (2000) to be exacerbated by the production of pro-inflammatory cytokines in the lungs, particularly TNF-α. Previous studies have shown that humans exposed to dust from swine building containing 22.4 mg/m^3 of inhalable dust and 1.2 μg/m^3 endotoxins resulted in a 4-fold increase in the concentration of TNF-α and a 14-fold increase in another pro-inflammatory cytokine, IL-6 (Wang *et al*., 1996). The increase in these cytokine concentrations is believed by Wang *et al*. (1996) to be partially responsible for decline in lung function associated with work in pig buildings.

Cargill *et al*. (1999) compared a batch production system based on age segregated rearing with a continuous flow production system and found that improvements in air quality were associated with a reduction in the prevalence of pneumonia and pleurisy and in the number of lung lesions at slaughter. The study showed that the prevalence of respiratory disease was related strongly to the concentrations of airborne viable bacteria. Growth rate of the pigs was negatively related to both the severity of respiratory disease and the concentration of airborne viable bacteria, but not to the concentrations of either total or respirable dust. These findings suggest that there may be an interaction between the concentration of viable bacteria and the presence of pathogenic organisms in determining the severity of respiratory disease and growth performance of pigs.

In summary, the experiments suggest there are significant interactions between atmospheric ammonia, endotoxins and viable bacteria concentrations and the capacity of a pig to resist pathogenic microorganisms. Murphy T. (unpublished) conducted a controlled study to investigate the impact of a range of concentrations of airborne viable, non-pathogenic bacteria, on pig performance. This study showed that bacteria cultured from air samples collect within pig buildings can cause non-specific lung infections and depress performance when inoculated into the nasal cavity of pigs exposed simultaneously to 25 ppm or more of gaseous ammonia. Atmospheric ammonia has been shown to significantly reduce the production of IgG, IgA and IgM antibodies to Newcastle disease in broiler chickens (Wang *et* al., 2010). The concentration of viable, but non-pathogenic, bacteria within pig buildings appears to be a major factor determining whether the immune system of pigs can cope with microbial challenges occurring in commercial pig buildings. The report from Murphy *et al*. (2000) suggests that viable bacterial numbers should be kept below 50,000 colony

forming units (CFU)/m^3. The other experiments cited suggest that to maintain optimal health and integrity of the immune system the concentration of atmospheric ammonia should be maintained below 5 ppm and the concentration of endotoxins below 1µg/m^3. Black (2003) suggested several management strategies that may maintain these low concentrations of viable bacteria, ammonia and endotoxins:

- Maintaining an air volume (m^3/pig) of at least 0.01176*Pig weight (kg) + 1.823
- A maximum of 300 pigs/air space
- Less than 10% of the floor wet and dung covered
- A batch rearing system with thorough cleaning between batches, high pressure hose, with degreasing agent and final disinfectant
- Allow floors to dry before introducing a new batch of pigs
- Spray water above floors and pens prior to people working with pigs in buildings
- Less than 30% of the floor with slats

The concentrations of airborne viable bacteria are considerably higher in the deep litter sheds than conventional sheds measured in Australia (Banhazi *et al.* 2000; Pattison *et al.*, 2002). However, feed intake and performance of pigs reared in deep litter sheds has been reported to be greater than for pigs reared in conventional buildings (Payne *et al.* 2003). There is evidence that the increased performance of pigs raised in the deep litter structures could be due to other factors including improved temperature regulation (Hayne *et al.*, 2000) and a reduction in stress through more species typical behaviours such as play (Lay *et al.*, 2000).

4.3.6. Climate Fluctuations

There is strong evidence that pigs exposed to cold conditions have greater clinical disease than pigs housed under favourable climatic conditions. Cargill and Byrt (1983) showed that the incidence of scouring increased in neonatal pigs and death rate increased from 0.1 to 1.3 per litter when conditions were changed from a constant temperature of 29°C to a daily fluctuating temperature from 21°C to 29°C. Similarly, Le Dividich (1981) found that a fluctuation in temperature of 4°C daily during the first week after weaning decreased the growth rate of piglets by 10% and significantly increased post-weaning diarrhoea. Wathes *et al.* (1989) have demonstrated an interrelationship between cold stress and feed intake in young pigs exposed to *E. coli.* The incidence and severity of diarrhoea in piglets challenged with an enterotoxigenic strain of *E. coli* was greater in those housed at 15°C and suffering from cold stress than for those housed at 30°C, when feed was offered *ad libitum*. However, when piglets were offered a limited amount of feed and housed at 15°C, they did not become infected by *E. coli* following inoculation and grew at the same rate as control pigs housed at the same temperature.

Fluctuations in airspeed within a piggery environment also increase the susceptibility of young pigs to enteric diseases. Scheepens *et al.* (1991) observed marked increases in diarrhoea, coughing, sneezing and haemorrhagic ear lesions in pigs exposed to draughts and low temperatures compared with pigs housed under thermoneutral conditions. Hessing and Tielen (1994) confirmed these observations and showed also that the health of young pigs was adversely affected by mixing in unfamiliar groups.
Cold stress in young pigs has been shown to significantly increase the production of pro-inflammatory cytokines compared with pigs housed in thermoneutral environments. Frank *et al.* (2003) measured a highly significant increase in serum IL-1β and IL-6 concentrations and higher liver and spleen mRNA concentrations for these cytokines in pigs weaned at 17 days of age and held at a temperature 10°C below pigs in a thermoneutral environment. The antibody titre response of mice to an inoculation with sheep red blood cells has been found to be depressed by cold stress (Cichon *et al.*, 2002). Mice held at 5°C for 16 days produced significantly less antibodies than mice held at

23°C for 16 days, with antibody production being strongly related to the mass of the thymus and spleen. On the contrary, Hangalapura *et al.* (2004) did not observe a decline in antibody production in chickens exposed to cold stress following inoculation with keyhole limpet hemocyanin, but they did observe a significant increase in lymphocyte proliferation. In another study, (Hangalalura *et al.* (2003) confirmed the lack of effect of cold stress in chickens on antibody production, but observed a significant increase in the production of toxic reactive oxygen intermediates.

The strong negative effect on performance and health of pigs being housed in temperatures below their lower critical temperature may be one of the reasons why pigs reared in deep-litter sheds grow faster than pigs in conventional sheds (Trezona *et al*., 2007). Pigs housed in deep-litter sheds also are less susceptible to draughts because they can partially bury themselves in the litter.

4.3.7. Endotoxin Tolerance

Continual stimulation of the innate immune system is known to down-regulate the immune system through a phenomenon called endotoxin tolerance (Van Epps, 2006; Romani *et al*., 2008; Biswas and Lopez-Collazo, 2009; Bourquin *et al*., 2011). Initial exposure of the innate immune system to foreign organism PAMPS such as LPS, lipoproteins and fungal antigens recognised by specific Toll-like receptors on immune cells results in an inflammatory response from pro-inflammatory cytokines released by macrophages, neutrophils and dendritic cells. However, repeated or continual exposures to the same or related antigens, which bind to the same toll-like receptors, result in the inflammatory response being markedly reduced. Similarly, continual exposure to some fungal disease causing organisms (*Candida and Aspergillus* spp.) has been shown to cause failure to eradicate the organism which is removed by the innate immune system following a single exposure (Romani *et* al., 2008).

Initial experiments with LPS showed that larger doses were continually required to induce an inflammatory response in rabbits when the PLS was administered daily (Beeson, 1947). However, the full inflammatory response was regained after a three week interval from the last LPS administration. An inflammatory response within animals is known to go through an initiation (pro-inflammatory) phase, an adaptive (anti-inflammatory) phase and a resolution (restoration of homeostasis) phase (McCall *et al*., 2011). Endotoxin tolerance appears to be active, particularly during the initiation phase of an infection. Although the mechanisms for endotoxin tolerance are still being elucidated, they are likely to be related to those responsible for the phase changes in the inflammatory cycle. Two distinct signalling pathways initiated by LPS binding to TLR-4 (myeloid differentiation primary response [MyD88] and Toll IL-1 receptor domain-containing adaptor-inducing IFN-β [TRIF]), changes in cellular NF-κB (nuclear factor kappa-light-chain enhancer of B cells) and changes in chromatin that affect the transcription of microRNAs and disrupt the translation of inflammatory genes are thought to be largely responsible for the endotoxin tolerance phenomenon (Sato *et al*., 2002; Shen *et al*., 2008; Biswas and Lopez-Collazo, 2009; McCall *et al*., 2011).

The implications of endotoxin tolerance for the pig industry are i) that continual stimulation of the immune system by exposure to large microbial loads is likely to result in a down regulation of the innate immune response, and ii) if Toll-like receptor agonists are to be used to prime the innate immune system, particularly in the neonate, to improve protection against specific antigens (Kogut *et al*., 2010), repeat antigen delivery would need to be given at intervals identified in relation to the inflammatory cycle (Bourquin *et al*., 2011).

4.4. Breeding for Enhancement of the Immune System

4.4.1. Single Gene Trait Selection

More than 2000 genes are thought to regulate the immune response in pigs (Mallard and Wilkie, 2003). However, only a small number of pig diseases are controlled by one or a few genes and are amenable to single gene selection. Some of these single gene diseases are more correctly considered to be metabolic disorders such as rickets caused by vitamin D deficiency due to lack of a gene for the synthesis of renal 25 hydroxy-cholecalciferol-1-hydroxase (Winkler *et al*., 1986). Similarly, porcine atherosclerosis and hypercholesterolemia have been associated with a defect in the Apo lipoprotein B100 gene (Nichols *et al.* 1992). Selective breeding to remove such diseases is relatively simple (Mallard and Wilkie, 2003).

Other diseases such as post-weaning diarrhoea and oedema disease, caused by variants of *E. coli* F18, depend on expression of F18 receptors on the enterocytes of the small intestine of pigs that bind to the fimbriae of the organism and allow colonisation of the tissue. The F18 receptors are linked to a single gene, FUT1, which codes for synthesise of fucosyltransferase 1 (Nagy *et al*., 1992). A specific region of this gene responsible for the adhesion, FUT1 M307, is polymorphic in pigs and has three main forms GG, GA and AA (Luo *et al*., 2010). Approximately 95% of 447 pigs from Large White, Landrace and Songliao Black breeds examined by Luo *et al*. (2010) were GG and GA genotypes and formed adhesions with the *E. coli* organism. There were no differences in the percentage of each polymorphic class between the three breeds examined by Luo *et al.* (2010). Only 5% of the pigs were AA genotype. All of these AA genotype animals did not form adhesions to either variant of the F18 fimbriae and are considered to be resistant to the diseases. Thus, several groups (Meijerink *et al*., 1997; Frydendahl *et al*., 2003; Luo *et al*., 2010) have recommended selecting for the AA variant of the FIT1 M307 gene as a means for reducing the impact of diseases caused by *E. coli* F18 in young pigs. Approximately 60% of enteropathogenic *E. coli* strains appear to be of the F18 serotype (Blanco, *et al*., 2006).

Luo *et* al. (2010) used a relatively simple PCR amplification technique on a small ear biopsy to determine the genotype in the FUT1 M307 region of the genome. Such a technique would be amenable to practical pig breeding. Nevertheless, the protein encoded by the FUT1 gene is a Golgi stack membrane protein and changing to the AA genotype may have other consequences for the pig. Horak *et al.* (2005) showed that sows selected for the AA genotype produced a significantly lower number of live piglets than the other genotypes. On the contrary, Jaing *et al.* (2005) observed that AA genotype finisher pigs had less back-fat and superior meat quality traits compared with the other genotypes. More recently, Kadarmindeen (2008) examined on-farm and research station results in pigs selected for the AA allele and observed significant positive effects on growth rate and backfat thickness compared pigs with possessing other alleles.

4.4.2. Multi-Gene Trait Selection

The response to most infectious diseases in pigs involves many components of the innate and adaptive immune systems, which are controlled by numerous genes and are not amenable to single gene manipulations. However, there have been several experiments with pigs (Mallard *et al*., 1992; Wilkie and Mallard, 1999; Bates *et al*., 2009) and poultry (van der Zijpp and Leenstra, 1980; Siegel *et al*., 1982; Martin *et al*., 1990; Pinard *et al*., 1992; Mashaly *et al*., 2000; Kuehn *et al*., 2006) using general stimulants of the immune system or specific disease causing organisms to select animals for either high or low immune responses. These experiments show clearly that immune response genes are heritable and can be readily manipulated through selection.

The most comprehensive experiment in pigs was undertaken by Wilkie and his colleagues in Canada. These researchers used four antigens to stimulate antibody, cell mediated immunity and monocyte phagocytotic responses in Yorkshire pigs (Mallard *et al*., 1992). The antigens were hen egg white lysozyme for measuring antibody response; a purified protein derived from Bacillus Calmette Guerin to stimulate IgG and T cell mediated delayed-type hypersensitivity; concanavalin-A from jack beans for a lymphocyte blastogenic response; and irradiated *Salmonella typhimurium* to measure macrophage phagocytosis. Estimated breeding values (EBVs) were calculated for each pig based on the responses to the individual antigens. The pigs were selected for high, low or control breeding groups based on the combined EBVs obtained. Selection for macrophage phagocytosis was removed from the index after one generation because of low heritability estimates. The pigs were selectively bred over 9 generations and various responses measured over latter generations.

Antibody responses to a range of antigens including carbohydrates 1 and 5, and LPS 1 from *Actinobacillus pleuropneumonia*, the bacterium *Mycoplassma hyorhinis*, several commercial vaccines and an inactivated influenza vaccine all showed higher antibody titres and antibody avidity in the high immune response line than in the control or low response lines (Appleyard *et al*., 1992; Magnusson *et al*., 1999; Wilkie and Mallard, 2000). There were no animals in the high immune response line that did not have a major antibody response to normal porcine vaccines, compare with many non-responders in the low immune selection line. The high immune line also showed less peritonitis and pleuritis following infection with *Mycoplassma hyorhinis* than the low response line. Furthermore, when the pigs were challenged naturally with parvovirus, the high line had significantly less mummified foetuses than the low response line. The high line selected pigs also reached 100 kg at least 10 days earlier than the low line animals. There were similar differences between the lines in performance when pigs were reared in low-disease status research facilities or in commercial environments with high prevalence of microbial challenge. Mallard and Wilkie (2003) suggest that the improved performance of the high line pigs related to better health, less subclinical disease and higher feed intake than in the low line animals. There were no significant differences between the selection groups in carcass measurements, except subcutaneous fat depth was slightly less in the high immune response line.

Despite the clear superior performance of the high immune response line pigs, there was one indication of a negative impact from greater immune stimulation. At post-mortem following infection with *Mycoplassma hyorhinis*, pigs from the high line were found to have a significantly higher score for arthritis than the low line. The arthritis scores were highly correlated with inflammatory cytokine concentrations (Magnusson *et al*., 1999). Analysis of blood collected from the eighth generation of pigs following injection of hen egg white lysozyme revealed that the high immune line had an IgG_1:IgG_2 ratio close to 1, whereas the ratio for the low line was significantly less (Crawley *et al*., 2005). This results suggests that selection for the high immune response increased type 2 immunity with the T2 helper cells producing more IL-4, IL-5, IL-9, IL-10 and IL-13 that stimulate antibody production, rather than type 1 immunity where T1 helper cells predominantly produce IL-2, IFN-γ and LT-α, which promote phagocytic activity (Spellberg and Edwards, 2001).

Selective breeding of poultry for enhanced immune response have also been successful (van der Zijpp and Leenstra, 1980; Siegel *et al*., 1982; Martin *et al*., 1990; Pinard *et al*., 1992; Mashaly *et al*., 2000; Kuehn *et al*., 2006). After 30 generations of selection for antibody response to sheep red blood cells, a high proportion of birds from the low response line did not have detectable antibody titres 10-14 days after inoculation (Kuehn *et al*., 2006). There appear to be a major difference in the poultry and pig selection experiments. The positive relationship between effectiveness of the immune response and performance characteristics observed by Wilkie and his colleagues in pigs has

not been observed in broiler chickens selected for antibody response to sheep red blood cells. Under these circumstances there have been strong negative correlations between antibody production and body weight of chickens, with increases in maintenance energy requirements for the high immune response selected lines (van der Zijpp and Leenstra, 1980; Mashaly *et al*., 2000). Negative correlations were also observed between antibody response to sheep red blood cells and productive traits including age to first egg, percentage hen day egg production, percentage fertility and duration of fertility in laying White Leghorn birds (Siegel *et al*., 1982).

4.4.3. Heritability of Immune Traits

Numerous estimates have been made of the heritability of immune response variables in pigs. Wilkie and Mallard (1999) provided heritability estimates after eight generations of selection of 0.27 for antibody production to hen egg white lysozyme, 0.16 for delayed-type hypersensitivity to Bacillus protein, 0.16 for lymphocyte blastogenesis to concanavalin-A and 0.07 for serum IgG concentrations. These heritabilities are regarded as low to moderate. Edfors-Lilja *et al*. (1994) also measured heritability of several immune traits from the progeny from 19 sires and 55 dams of the Yorkshire breed. Some measures were taken on the pigs without treatment, whereas others were made following treatment with concanavalin-A. An extremely high heritability of 0.87 was obtained for the number of polymorphoneuclar leukocytes, whereas medium heritabilities of 0.3-0.4 were found for phagocytic capacity of leukocytes, concanavalin-A induced cell proliferation, IL-2 production and total white blood cell count. Low heritability values of <0.08 were found for total number of lymphocytes, serum Ig concentrations and IFN-α production. Large differences between litters from individual dams were found for many of the immune response variables measured.

More recently, Flori *et al*. (2011) examined a wide range of immune response variables in French Large White pigs three weeks after administration of the *Mycoplasma hyopneumoniae* vaccine. A total of 54 variables were measured including; i) total white blood cell numbers, lymphocyte counts and proportions of various leucocyte subsets; ii) innate immune response variables of phagacytosis and *in vitro* production of IL-1B, IL-6, IL-8, TNF, IL-12 and IFN-α following stimulation ; iii) adaptive immune response variables of lymphocyte proliferation, total IgG, IgA, IgM, specific IgG sub-types and *in vitro* production of IL-2, IL-3, IL-10 and IFN-γ following stimulation; iv) acute phase protein concentrations of C-reactive protein and haploglobin. The highest heritabilities approached 0.9 for *in vitro* stimulated IL-2 and IL-10 production. Forty two of the 54 variables had heritability values greater than 0.2.

Clapperton *et al*. (2009) have also estimated the heritability for 23 innate and adaptive immune traits in pigs from seven farms with either specific pathogen-free or non-specific pathogen-free environments. Direct heritability estimates ranged from 0.09 for $CD16^+$ cell count to 0.93 for $CD8a^+$ cells. Although there was some variation in heritability estimates for the different environments, the authors conclude that heritabilities are generally unaffected the health status of the herd.

4.4.4. Selection of Pigs for Disease Resistance

There is strong evidence of wide variation between pig genotypes and strains in many immune trait variables (Nguyen *et al*., 1998; Mallard and Wilkie 2003; Lowenstein *et al*., 2004; Lunney, 2010). In addition, polymorphism has been observed in many of the immune response genes (Cai *et al*., 2011; Liu *et al*., 2011). These observations of moderate to high heritabilities, wide variation between pig genotypes and substantial gene polymorphism confirm that many of the innate and adaptive immune response variables are under genetic control and could be readily altered by selection. However, significant heterosis effects on immune responses from cross-breeding have not been observed (Meeker *et al*., 1987). The desirability of selecting for specific diseases has been discussed by Stear *et al*. (2001) and Bates *et al*. (2010). The general conclusion is that direct selection of pigs resistant to a disease may lead to slow progress and potential negative correlations with performance, whereas index selection techniques including several measures of immunity response and performance variables should lead to animals with disease resistant animals with good performance. There is evidence that pigs bred for resistance to a single organism, may be more susceptible to other organisms (Stear *et al*., 2001).

4.5. Impact of the Immune System on Animal Performance

Stimulating the immune system results in a considerable cost to an animal through the use of energy, amino acids and other nutrients and can lead to a substantial depression in animal performance. Several methods have been used to assess the cost of the immune system including i) consideration of the mechanisms involved in the development, maintenance and use of the immune system; ii) studies of differences between gnotobiotic (germ-free) and conventional animals, iii) measuring the impact of antibiotic treatments, iv) measuring the effects of stimulation of the immune system with non-pathogenic antigens and v) breeding animals for enhanced immunity.

There is clear evidence of trade-offs in resource use between the immune system and productive responses in animals including growth, pregnancy, lactation and overall reproductive success (Lochmiller *et al*., 2000). These trade-offs are particularly exacerbated during periods of nutrient deficiencies (Norris and Evans, 2000; French *et al*., 2011) and climatic stress (Freeman, 2007; Martin *et al*., 2008). Many of the studies quantifying the impact of the immune system on animal performance have been conducted with chickens, other birds, rodents and humans, with relatively few involving pigs. Although there are differences in details of the immune system between animal types, the principles are similar across animal species and apply generally to pigs.

Many of the biochemical and biophysical mechanisms responsible for the impact of the immune system on animal performance are understood and further refinement of this knowledge continues. There are both direct costs to the animal through the use of nutrients for production and maintenance of components of the immune system, and indirect impacts on metabolic systems through the release of cytokines, chemokines and other products from the immune cells.

4.5.1. Cost of the Immune System: Estimates Based on Mechanisms

Klasing (2004, 2007) has made the most comprehensive attempt to quantify the cost of the immune system based on an understanding of the mechanisms involved in chickens. The immune system was divided into three phases for the analysis. First, development of the immune system including initial production of monocytes in bone marrow, proliferation of the B and T cells and their presence in epithelial and lymphoid tissues. Second, maintenance of the immune system function, such as production of complement proteins, replacement of immune cells, particularly neutrophils, relatively small output of antibodies from B memory cells and routine phagocytosis of apoptotic cells resulting from cell turnover. Third, use of the immune system when responding to foreign organisms and

antigens, including production of acute phase proteins, proliferation of antigen specific B and T cells, antibody production and cell-mediated toxicity. The analyses by Klasing and colleagues also concentrated on estimating the costs for lysine because it is predominantly used for protein synthesis in animals. However, results from Sirimongkolkasem (2007) indicate that the immune system has a higher requirement than lysine for several amino acids, particularly cysteine. In addition, Klasing has made some estimates of energy use based on protein turnover assumptions, but has not considered all energy costs of the immune system.

4.5.1.1. Protein Costs

Klasing (2004) estimated that development of the innate immune system in chickens represents only 0.18% of total lysine requirements of the bird. However, development of the acquired immune system was estimated to take up to 2.2% of the total lysine required by chickens and 3 % of total energy expenditure. The adaptive immune system is more expensive in terms of nutrient requirements than the innate immune system because the process of gene segregation and development of B and T cells with effective receptors is an inefficient process. Approximately one in nine B and T cells form effective receptors, so many cells are synthesised and destroyed by apoptosis in order to cover the majority of antigens that the animal will likely encounter.

Considering the lysine used for the routine production of antibodies, particularly intestinal IgA, and the turnover of leukocytes, Klasing (2004) estimates that maintenance of the immune system may use up to 3% of the lysine requirements of a chicken. Other costs, such as the production of complement proteins were not considered, but their contribution to lysine use will be small. Subsequently, Klasing (2007) suggested that the cost for maintenance of the immune system in a pathogen unchallenged bird may be as low as 0.5-2% of total lysine requirements.

The greatest cost of the immune system to an animal comes on the first encounter with a pathogenic organism or cognate antigen. Both the innate and adaptive immune systems are greatly up-regulated. Klasing (2007) estimated that the total cost of all the changes associated with up-regulation of both immune systems was from 7-10% of total lysine requirements. The major contributors to the increase in lysine requirements were hypertrophy of the liver and production of acute phase proteins. Klasing (2004) predicted that acute phase protein production was the biggest cause of increased lysine requirement and alone accounted for 4.6% of all lysine needs. Acute phase proteins have a higher ratio of cysteine to lysine than muscle protein (1.49:1; Sirimongkolkasem, 2007). Thus, the requirement for cysteine can be estimated to increase by approximately 7% due to acute phase protein release during the period of acute response to a pathogen.

4.5.1.2. Energy Costs

A comprehensive evaluation of the energy needs of the immune system has been made by Buttgereit *et al*. (2000). ATP required for the major components of an immune response are considered including migration of immune cells, phagocytosis, cytokinesis, antigen processing and presentation by MHC molecules, lymphocyte activation, changes in the function of ionic pumps, increases in synthesis of protein and RNA/DNA in cell proliferation, antibody synthesis and killer T cell cytotoxicity. Although the assessment of ATP needs of the immune system were largely qualitative, Buttgereit *et al*. (2000) used *in vitro* lymphocyte cultures exposed to the antigen, concanavalin A, from jack beans (*Canavalia ensiformis*) to quantify changes in ATP uptake for some of the immune processes. There was a 70% increase in ATP used for protein synthesis and a many fold increase in ATP used for RNA/DNA synthesis. In addition, there were major increases in the oxygen used by Na^+K^+ATPase associated with the sodium pump and Ca^{2+}-ATPase used for calcium channelling.

Overall, the lymphocytes exposed to concanavalin A increased oxygen consumption by approximately 15% compared with control cultures.

Acute infection commonly induces fever and increases body temperature. Although the amount of energy needed to raise body temperature will vary depending on the relative proportions of lean and fat tissue, approximately 3.5 kJ/kg of energy is required to raise the temperature of a pig by one degree centigrade (Black, AUSPIG model) and approximately 6.5 kJ/kg is needed to maintain the temperature of an adult human one degree higher (Segerstrom, 2007). Thus, approximately 10 kJ/kg of energy is required to increase and maintain an elevated temperature of one degree centigrade during fever. For a pig weighing 80 kg with an increase in temperature of 2°C, this represents 1.6 MJ/day and is around 15% of basal metabolic rate. Several empirical measurements of oxygen consumption in humans with sickle cell disease (Borel *et al*., 1998) or other forms of trauma induced fever (Roe and Kinney, 1965) suggest that fever increases metabolic rate by around 10-15%. The experiments of Borel *et al*. (1998) suggest that the majority of the increase in energy expenditure is due to increases in the rate of protein turnover associated with infection.

Experiments with Pigs

No experiments appear to have been conducted where the energy cost of a non-pathogenic immune response has been measured in pigs. However, the administration of a non-pathogenic endotoxin to sheep, which resulted in a 1.5°C increase in body temperature, was shown to be associated with an increase of 33% in heat production (Baracos *et al*., 1987). Nevertheless, there are measurements of the effects of pathogenic organisms on energy production in pigs.

Bray (1996) measured oxygen consumption in individual pigs before and after the inoculation of one lung with either saline or the pathogenic bacterium, *Actinobacillus pleuropneumoniae* serotype 1. The severity of the infection in each animal was assessed *post mortem* from measurement of the percentage of the lung that was damaged by the organism. Body temperature of all infected pigs rose by at least 2°C within 12 hours of inoculation and remained elevated for 2 days or more when lung damage exceeded 10%. Voluntary feed intake declined to at least 20% of the pre-inoculation amount by day 2 post-inoculation in all infected pigs. Despite the large fall in feed intake, oxygen consumption on day 2 post-inoculation was at least 20% higher than during the pre-inoculation period (Bray *et al*., 1993). Black *et al*. (1998) compared oxygen consumption between the saline treated pigs, heat stressed pigs and pleuropneumonia affected pigs at equivalent relative intakes and concluded that the pathogen increased energy requirements by as much as 70% on day 2 and 50% on day 5 post-inoculation depending on the severity of the disease.

4.5.2. Studies with Gnotobiotic Animals

There have been numerous studies with pigs, chickens and other animals comparing responses in germ-free environments with those when the animals are reared either conventionally or exposed to specific organisms. These studies can be used to assess the cost of stimulating an immune response. In most cases, the conditions relating to infection of the conventional animals were uncontrolled and there would have been a wide range in the extent and type of microbial infection. However, in other studies, specific non-pathogenic bacteria were introduced into germ-free animals.

Shurson *et al*. (1990) found that feed intake was 60% higher in germ-free than conventionally reared pigs offered the same diet and that the growth rate of the germ-free pigs was almost double that of conventionally reared pigs. However, the initial weights of the pigs were not given in the paper. On the contrary, Miller *et al*. (1982) reported that conventionally reared neonatal piglets grew 80% faster than germ-free piglets fed diets adequate in iron. However, in this experiment, the

conventionally reared piglets were over twice as heavy at the start of the experiment than the germ-free animals, so a true comparison of the effects on microbes on performance cannot be made. A fairer comparison of the impact of initiation of the immune response on pig performance comes from the study of Loynachan *et al.* (2005) where germ-free piglets were compared with germ-free piglets of similar weight that had been inoculated with a non-pathogenic bacteria *Lactobacillus paracasei*. The germ-free piglets grew 20% faster and had a 21% better feed conversion efficiency than the piglets exposed to the bacterium. Siggers *et al.* (2007) showed more recently that the absence of microbes in germ-free neonatal pigs resulted in a more robust intestinal mucosa and heavier small intestines and pancreas than conventionally reared pigs. However, another study with chickens showed that the weight of intestinal segments is greater in conventionally reared birds than germ-free birds (Muramatsu *et al.*, 1988), despite the germ-free chickens having an 18% faster growth and an 11% better feed conversion efficiency.

More experiments with germ-free chickens have been conducted than with germ-free pigs. Many of these experiments have been summarised by Lochmiller and Deerenberg (2000) who conclude that germ-free chickens fed nutritionally adequate diets have similar or slightly lower metabolisable energy intakes, greater rates of protein and energy retention, lower maintenance energy requirements and greater growth rates (5-30%) compared with conventionally reared chickens. Several of the experiments summarised by Lochmiller and Deerenberg (2000) included comparisons between germ-free chicks and germ-free chicks inoculated with *Streptococcus faecalis* that normally inhabits the gut.

Lochmiller and Deerenberg (2000) also summarised six papers reporting experiments with germ-free rodents compared with conventionally reared or streptococcus inoculated animals. These results appeared more variable than for the germ-free chickens, with several studies showing little difference between the germ-free and conventionally reared animals. However, in other experiments metabolic rate and rectal temperature were lower in the germ-free animals (Levenson *et al.*, 1969; Sewell *et al.*, 1975). In one experiment (Wostmann *et al.*, 1983), energy absorption was greater in the conventionally reared rats compared with pair-fed, germ-free rats. Lochmiller and Deerenberg (2000) argue there may be advantages to an animal from specific populations of microbiota in the gut that influence the comparisons between germ-free and conventionally reared animals and make assessing the impact of an immune response using this technique less reliable. The same criticism is less likely to be true when germ-free animals are compared with germ-free counterparts inoculated with a single non-pathogenic organism. In such experiments, the germ-free animals had growth rates between 5% and 15% greater than the single organism infected animals (Lochmiller and Deerenberg, 2000).

Marked differences in the activation of the immune system have been observed between germ-free animals and those reared conventionally. A consistent finding in those experiments where it has been measured is a many fold (up to 100-times) larger number of IgA, IgG and IgM secreting lymphocytes in the gut and immune associated organs of conventionally reared animals compared with germ-free counterparts (Hooijkass *et al.* 1984; Pereira *et al.*, 1986; Bakker *et al.*, 1995, Pabst and Rothkötter, 1999).

4.5.3. Impact of Antibiotic Treatment

Knowledge of protective responses enables identification of the microbial components that induce protection as well as new means to enhance formulation of and delivery of vaccines to mucosae in young animals (Emery and Collins 2011). There is an urgent need to replace antibiotics in feed but this can only be achieved if proven alternative means are available and more effective vaccines are an

option with a proven cost-effective track record (Emery and Collins 2011). Alternatively prebiotics may enhance gut function or 'chemically control pathogens' (Emery and Collins 2011).

Antimicrobial compounds added to feed have been investigated as growth promotants in pigs since the early 1950's (Luecke *et al.*, 1951) and, until recent years, have been used widely around the world in pig production facilities. Results from many experiments investigation the impact of in-feed antibiotics on performance of pigs has been summarised over time by Hays (1979), Zimmerman (1986) and Cromwell (2002). The average improvement in growth rate for young pigs up to about 26 kg live weight was 15%-16% and the improvement in feed conversion efficiency from 6.5% to 7% (Hays, 1979; Zimmerman, 1986). Responses in individual experiments ranged from a small percentage improvement (4%) to well over a doubling in growth rate (Zimmerman, 1986). The response to antibiotics in young pigs has been far greater than observed in older pigs, presumably because of a lower stimulation of the immune system through the influence of B and T memory cells and their ability to produce antibodies and killer T cells rapidly after subsequent exposures to a cognate antigen. Corresponding average increases for grower-finisher pigs from about 27 to 92 kg to in-feed antibiotics were 3.6%-4.0% for growth rate and 2.1%-2.4% for efficiency of feed use (Hays, 1979; Zimmerman, 1986).

All analyses show that the magnitude of the response to antibiotics depends on the microbial load experienced by the pigs and the variety of pathogenic organisms present (Melliere *et al.,* 1973; Cromwell, 2002, Holt *et al*., 2011). Consequently, there is a strong negative correlation between the growth rate of the control animals and the size of the response to in-feed antibiotic treatment (Melliere *et al*., 1973). Thus, the improvements in pig performance resulting from the use of in-feed antibiotics are generally greater on commercial pig enterprises than on experimental research facilities. Cromwell (2002) showed under the conditions prevailing on one farm that mortality was reduced from 15.6% to 3.1% when antibiotics were added to feed.

Similar observations on the effect of in-feed antibiotics have been made with broiler chickens. Fuller *et al.* (1979) examined the effects of inoculating gnotobiotic chicks with a range of bacteria isolated from the crop and caecum of birds with and without a faecal filtrate. Growth rate was depressed by aerobic bacteria, particularly streptococcus species, but not by anaerobic organisms. The effects of the organisms were particularly marked when given in combination with faecal filtrate. Growth rate in the germ-free chicks was depressed by 5-15% following the inoculations, but could be fully restored following treatment with antibiotics.

Roura *et al*. (1992) investigated the effects of in-feed antibiotics on the performance of chicks reared in clean or dirty environments. Chicks in the clean environment grew 8% faster and used feed 22% more efficiently than chicks in the dirty environment. The inclusion of in-feed antibiotics significantly improved the growth and efficiency of feed use by chicks in the dirty environment, but did not affect the performance of chicks in the clean environment.

There is evidence antibiotics have a direct effect on the immune system as well as an indirect effect through reducing microbial challenge. Roura *et al.* (1992) investigated the effects of antibiotics for conventionally reared chicks injected with LPS to stimulate an immune response. The antibiotics were shown to change some of the effects of LPS by reducing the changes in several immune responses such as size of the spleen, liver concentration of the acute phase protein, metallothionine, and liver zinc concentration. Gillissen (1988) reviewed the direct and indirect effects of antibiotics on animals and concluded that antibiotics could directly reduce many responses of the stimulated immune system including phagocytosis, killer T cell activity, antibody production, lymphocyte

proliferation, production of interleukins and expression of immune cell receptors. Thus, the cost of the immune system assessed by measuring the influence of in-feed antibiotics may underestimate the true cost of stimulating the immune system to animals. Antibiotics appear to act directly to down-regulate the immune system and reduce the apparent impact of immune stimulation.

4.5.4. Impact of Treatment with Specific Antigens and Vaccination

Several experiments have been conducted to investigate the effect of administration of non-pathogenic antigens and vaccination on the performance of pigs. Schinckel (1995) investigated the impact of providing *E.* coli-derived LPS and multiple vaccines on the performance of pigs reared in high-health status, research facilities. The antigenic challenge was either moderate or intense. Pigs in the former group received five mixed-antigen injections between the age of 12 and 84 days, while pigs in the intense group received eight injections over the same period. There was little difference between the moderate or intense groups in the variables measured. At an age of 107 days, antigen treated pigs were approximately 20% lighter, consumed about 27% less feed and had significantly poorer feed conversion efficiency between the age of 45 and 54 days than the control animals. However, after day 107, the antigen treated pigs grew 11% faster through compensatory gain. Over the period from 12 days of age to 109 kg, the antigen treated pigs had a growth rate only 13% less than the control pigs. The antigen treated pigs also has less back-fat and smaller loin eye-muscle area than the control pigs at the end of the experiment.

Similarly, Potter *et al*. (2009) showed that pigs vaccinated with commercial porcine circovirus type 2 and *Mycoplasma hyopneumoniae* vaccines had a reduced growth rate and feed intake compared to control animals over the period from 21 to 56 days of age. However, after that time and when the animals were transferred to commercial conditions, the growth rate of the vaccinated pigs was greater than the control animals (Bergstrom *et al*., 2009). There were differences between the commercial vaccines, with one vaccine not changing performance of the young pigs. Fangman *et al*. (1998) also showed that vaccination of early weaned piglets with a vaccine against infectious bovine rhinotracheitis virus significantly reduced their performance relative to control piglets. Similarly, Meeker *et al*. (1987) found significant negative correlations between the antibody response to pseudorabies and atrophic rhinitis vaccines and weaning weight and days to 100 kg, but positive correlations with back-fat thickness.

There are numerous examples of the effect of antigens with other animals and birds. Demas *et al*. (1997) injected adult and aged mice with either saline of keyhole limpet hemocyanin (KLH) and measured the responses in the immune system and on metabolism. The KLH injected mice mounted a significant antibody response, increased body temperature by 1.5°C and increased oxygen consumption by around 15%. The adult mice maintained the increase in oxygen consumption for a longer time than the aged mice. However, there was no significant effect of injection with KLH on the weight of the mice fifteen days after the injection.

Pilorz *et al*. (2005) also used KLH as a non-pathogenic immune stimulant in young guinea pigs and found a significant increase in IgG production compared with the saline injected control animals. However, in this experiment, there was no significant effect of the immune stimulation on either metabolic rate or body weight gain.

Several research groups have used sheep red blood cells (SRBC) as a non-pathogenic antigen to investigate the metabolic impact of the immune system in several animal species. Ots *et al*. (2001) injected wintering, free-living, male great tits (*Parus major*) with SRBC and observed a 9% increase in basal oxygen consumption, an 8% lower plasma albumin concentration and a 3% decline in body

weight over the six to ten days between the inoculation and re-capture of the birds compared with the sham-injected controls. There was a significant relationship between the magnitude of immune response and the impact on the birds. Those birds that mounted the greatest immune response, as measured by SRBC antibody titre, lost the most weight and had the highest oxygen consumption.

Fair *et al.* (1999) injected you growing Japanese quail (*Coturnix coturnix japonica*) with SRBC and found an increase in total white blood cells and particularly lymphocytes compared with control birds. The SRBC challenge resulted in a significant decline of 15% in the rate of weight gain, a decrease in wing length and an increase in fluctuating asymmetry in the masses of primary feathers.

There are other examples of a negative impact of vaccination on animal performance. For example, Wagland *et al.* (1984) found that lambs vaccinated with the irradiated intestinal parasite larvae of *Trichostrongylus colubriformis* showed a strong negative correlation between weight gain and serum antibody titre during the vaccination period.

4.5.5. Effects of Breeding for Fast Growth on the Capacity of the Immune System

There is strong evidence from experiments with pigs, broiler chickens and turkeys that breeding for enhanced growth rate and performance is associated with reduced effectiveness of the immune system. Rauw *et al.* (1998) reviewed many of the negative effects resulting from selection of animals for high rates of production efficiency, including impacts on the immune system. The association between enhanced performance and immune competency has been examined in pigs. Several experiments investigated correlations between growth rate and immune variables in groups of pigs varying in performance, but not selected for improved performance (Clapperton *et al.*, 2005; Clapperton *et al.*, 2008; Clapperton *et al.*, 2009), whereas others examined the impact of selecting for increased performance on immune variables and resistance to disease (Frank *et al.*, 1997; Clapperton *et al.*, 2006). In pigs not selected for performance, but with differing growth rates, there were negative correlations between growth rate and immune variables such as $CD11R1^+$ positive cells, number of monocytes, number of B cells $\gamma\delta^+$ T cells, $CD8a^+$, monocytes and acute phase proteins (Clapperton *et al.*, 2005b; Clapperton *et al.*, 2008). These authors suggested that the slower growth rate of pigs with high immune response variables could have been due to the impact of sub-clinical infections. However, a subsequent experiment (Clapperton *et al.*, 2009) confirmed the negative correlations between performance and immune variables for pigs grown under both high and low health status conditions. Clapperton *et al.* (2008) examined pigs with known genetic backgrounds and showed that the negative correlations between immune response variables and performance were both phenotypic and genetic.

The immune response in pigs selected over seven generations for high or low lean content under either *ad libitum* or a restricted intake conditions, and for feed intake were examined by Clapperton *et al.* (2006). From the eighth generation animals were randomly selected to maintain the lines. Subsequently, immune variables and growth rates were measured at 14 and 24 weeks of age for all animals when fed *ad libitum*. Male pigs had significantly higher concentration of white blood cells, neutrophils, lymphocytes and oesinophils than the female pigs. There were differences in the immune response variables for those pigs selected under restricted compared with *ad libitum* feeding conditions. The pigs selected for high lean growth with restricted feeding had higher white blood cell counts, higher lymphocyte numbers with increased numbers of $CD8a^+$, $CD11R^+$ cells and monocytes. In a previous experiment with the same pigs, Clapperton *et al.* (2005a) showed higher concentrations of the acute phase protein, α_1-acid glycoprotein, in the line selected for high lean under restricted feeding conditions. However, there were no consistent immune cell differences for

the high and low lean lines selected under *ad libitum* conditions or in the high and low feed intake lines in either reported experiment.

These results from Clapperton *et al*. (2005a, 2006), which suggest that selection for increased performance enhances immune function variables, is contrary to the negative genetic correlations between performance and immune response variables subsequently observed by Clapperton *et* al. (2008), but concur with the observations of Wilkie and colleagues (Raymond *et al*., 1998; Mallard and Wilkie, 2003) where the line of pigs selected for high immunity also had higher growth rates. These observations suggest that selection for high growth rate under conditions of moderate disease challenge results in the selection of animals with higher capacity to reduce the effects of mild or subclinical disease. However, the positive correlation was observed only when pigs were selected under a restricted feeding regime and, perhaps indicates that priority of nutrients for the immune system is higher when pigs are selected under circumstances where there is competition for nutrients. None of the pigs were challenged with disease causing organisms in the experiment, so the impact on survival and performance characteristics of the differences in immune variables between the high and low lean lines could not be determined.

Frank *et al*. (1997) placed young pigs from high and low lean selected lines in either a segregated early-weaning environment with disinfected grower facilities or conventional weaning facility with continuous-flow grower pig management. The microbial load from the two environments would be expected to be different. There was little difference between the selected lines in mortality recorded under the 'clean' environmental conditions with 3.6% and 2.8% of pigs, respectively, from the high lean and low lean groups dying during the trial. However, under the 'dirty' conditions, mortalities were 18.5% and 5.6%, respectively, for the high and low lean groups. This study provides clear evidence that selection of pigs for high performance reduces their capacity to survive under disease challenging conditions.

Contrary to the observation of Clapperton *et al*. (2006) that pigs selected for high lean content displayed higher blood leukocyte counts than those selected for the low lean line, Miller *et al*. (1992) found that chickens selected for high weight gain had lower antibody titres when challenged with sheep red blood cells than those selected for low weight gain. In addition, Nestor *et al*. (2011) showed that turkeys selected for increased body weight at 16 weeks of age had lower phagocytic activity following vaccination with inactivated Newcastle disease virus and *Pasteurella multocida* than control birds, although there were no differences in antibody production between the strains. However, when these strains of turkeys were challenged with live *Pasteurella* and Newcastle disease organisms, mortality was significantly higher in the turkeys selected for high 16 week weight, than for the control birds or another commercial turkey strain (Nestor *et al*., 1996a,b; 1999).

4.5.6. Disease Susceptibility in Elite Athletes

The effect of exercise on immune function and susceptibility to disease appears to depend on the severity of excursion. Moreira *et al*. (2009) reviewed 162 publications and concluded that moderate exercise generally enhances immune function, whereas intense exercise temporarily impairs immune competence and results in a higher incidence of upper respiratory tract infections. Moderate physical activity in non-athletes decreases susceptibility to respiratory tract infections (Murphy *et al*., 2008). These observations have led to the 'J' curve hypothesis, with improvements in immune function as exercise rate increased, but then depression in function with heavy exercise (Nieman, 1994). However, another analyses of published results suggest that an 'S' curve would be a better description of the effects of exercise on immune function (Malm, 2006). This analysis suggested there are substantial improvements in immune function resulting from moderate exercise, then a

decline with high exercise, but an improvement again with excessive long-term exercise. This analysis suggested that the elite athletes undertaking excessive exercise have an immune function similar to the non-exercise group.

There have been several experiments with rodents undergoing different exercise routines and then challenged with specific diseases. Cannon and Kluger (1984) challenged mice that were either sedentary or had been trained on exercise wheels for 16-18 days with an approximate LD50 dose of *Salmonella typhimurium*. The mice undertaking exercise had significantly greater survival than the unexercised mice. Chao *et al*. (1992) examined the effect of exercise through swimming on the recovery of mice infected with *Toxoplasmosis gondii*. One group of mice was made to swim 45 minutes per day from the day of inoculation, while the other group remained sedentary. Swimming had little effect on the acute phase of the infection, but significantly reduced the time for recovery. Swimming was shown to significantly reduce the elevation in the serum concentrations of TNF-α. The timing of the exercise appears to be important in affecting the immune response. Ilbäck *et al*. (1991) exercised mice by swimming either before or after challenge with *Streptococcus pneumonia* and *Francisella tularenis* bacteria. Strenuous exercise immediately before the bacterial challenge substantially reduced susceptibility of the mice to the diseases, whereas exercise after the challenge significantly increased mortality from both diseases.

The improvement in immune response with moderate exercise is believed to be due to a reduction in pro-inflammatory cytokines, particularly TNF-α and its soluble receptor, and an increase in anti-inflammatory cytokines, particularly the anti-inflammatory form of IL-6 (Petersen and Pedersen, 2005). During exercise, IL-6 is produced by muscle fibres and stimulates the production of other anti-inflammatory cytokines, IL1 receptor antagonist (IL-1ra) and IL-10, while inhibiting the production of TNF-α. In addition, IL-6 stimulates lipolysis and fatty acid oxidation, thus reducing body fat content. Adipose tissue contributes significantly to the production of TNF-α and its receptor. It is believed that the reduction in pro-inflammatory cytokine production resulting from moderate exercise provides protection against chronic diseases associated with inflammation. Serum TNF-α concentration was shown to be reduced in exercised mice compared with sedentary counterparts and resulted in more rapid recovery from toxoplasmosis (Chao *et al*., 1992).

Although lymphocyte cell numbers increase during intense exercise, their numbers and function significantly decline after the exercise has been completed (Shinkai *et al*., 1992; Kakanis *et al*., 2010). In addition, neutrophil phagocytic function declines along with NK cell activity following intense activity, while pro-inflammatory cytokines and oesinophils numbers increase (Lin *et al*., 2010; Kakanis *et al*., 2010). Furthermore, IgA and IgM concentrations have been found to decline after intense exercise (Gleeson and Pyne, 2000). These immunoglobulin concentrations generally return to normal within 24 hours of cessation of the exercise. However, training at an intense level over many years can result in a chronic suppression in immunoglobulin concentrations. The low concentration of immunoglobulins, particularly IgA_1 subclass, has been associated with increased risk of respiratory illness in athletes (Gleeson and Pyne, 2000, Gleeson 2006). There is evidence that increased cortisol production during intense exercise may contribute to the post-exercise depression in helper and killer T cell counts and other immune functions (Shinkai *et al*., 1992).

Moderate exercise appears to be beneficial to animals in terms of increased production of anti-inflammatory cytokines and reduced production of pro-inflammatory cytokines with reduced anorexia and increased protein synthesis compared with sedentary behaviour. These changes to immune function may be one explanation for the superior performance of pigs reared in deep-litter

sheds where they undertake moderate exercise compared with pigs reared in small pens (Trezona *et al*., 2007), despite a higher load of airborne viable bacteria (Banhazi *et al*., 2002).

4.6. How the Immune Response Reduces Animal Performance

Substantial evidence indicates that high responsiveness of the innate immune system and the release of pro-inflammatory cytokines is responsible for various types of autoimmunity including arthritis, neurodegenerative diseases, inflammatory bowel disease and cachexia associated with AIDs infection in humans (Blach-Olszewska and Leszek, 2007) as well as increased proteolysis, increased energy expenditure and depressed growth rates in animals (Spurlock, 1997; Allen, 2000; Romanovsky *et al*., 2005). The cytokines, IL-1β, IL-6 (acting as a pro-inflammatory cytokine), TNF-α and IFN, particularly have been implicated as major causes of the negative responses to the immune system in animals and humans. Each of these cytokines has been linked directly to the induction of fever, anorexia, depressed activity and increased sleep (Kapás *et al*., 1992; Harden *et al*., 2008; Nilsberth *et al*., 2009; Reyes-Vasquez and Dafny, 2011). IL-1β, IL-6 and TNF-α have also been linked to increased muscle proteolysis (Spurlock, 1997; Lang *et al*., 2007).

4.6.1. Adverse Effects of Cytokine Administration

The early experiments of Klasing *et al*. (1987) demonstrated that the immune response to SRBC, the immune stimulant sephadex, LPS derived from *E. coli* or *S. typhimurium* or *S. aureus* significantly reduced feed intake, growth rate and the efficiency of feed use in chickens. Feeding control chicks the same amount of feed eaten by the immune stimulated chicks did not reduce weight gain by the same amount as observed with immune stimulation. However, intraperitoneal injection of crude IL-1 to a pair-fed group resulted in a reduction in weight gain similar to the immune stimulated chicks. Further, *in vitro* studies with isolated chick skeletal muscle cultures showed that IL-1 significantly increased the rate of protein degradation, but did not alter the rate of protein synthesis. These studies showed clearly that there was an adverse effect of IL-1 administration on net protein synthesis greater than caused by the reduction in feed intake.

Similar increases in the rate of protein degradation in cultured rat muscle cells were observed by Goldberg *et al*. (1984) following addition of a crude IL-1 preparation. Goldberg *et al*. (1984) measured a 5-fold increase in the release of prostaglandin E_2 (PEG2) in the cultures. Further studies showed that addition of PGE_2 to the cultures had a similar effect on protein degradation rate to the addition of IL-1 and that the adverse effects of IL-1 could be reversed with addition of aspirin or indomethacin, which prevent the synthesis of PEG_2.

Subsequently, numerous studies have shown that intraperitoneal administration or direct administration into the brain of the cytokines IL-1β, IL-6, TNF-α, TNF-β and IFN-α cause fever, anorexia, increased proteolysis and other symptoms of disease such as reduced activity and increased sleep in many different animal species including rats, mice, cats, chickens and rabbits (Kapás *et al*., 1992; Lenczowski *et al*, 1999; Sachot *et al*., 2004; Harden *et al*., 2008; Nilsberth *et al*., 2009; Reyes-Vasquez and Dafny, 2011). For example, rats receiving increasing intracerebroventricular doses of either IL-1β or IL-6 showed dose dependent increases in body temperature and decreases in voluntary wheel-running activity (Harden *et al*., 2008). Only rats receiving the highest dose of IL-1β (100 ng) showed a reduction in feed intake. However, when IL-1β and IL-6 were administered in combination at the lowest dose rates, feed intake and body mass were significantly reduced, indicating strong synergism between the cytokines. Other studies suggest that IFN-α can induce fever in rats without the involvement of IL-1 (Dinarello, *et al*., 1984).

Although individual cytokines are known to have more than one physiological action, Kapás *et al.* (1992) showed that, for TNF-α, amino acids from positions 31-36 were responsible for induction of fever and changes in non-rapid eye-movement sleep, whereas amino acids from 69-100 were responsible for anorexia. This observation suggests that cytokines have the potential to bind to numerous cell receptors to produce an array of physiological effects. Inflammatory cytokines are released within the first hour of an inflammatory immune response (Yang and Cook, 2003). The cytokines are released through activation of the NF-κB pathway and transcription/translation of microRNAs derived from the inflammatory cytokine genes.

4.6.2. Modes of Action of Pro-Inflammatory Cytokines

There is strong evidence that PGE_2 and other prostaglandins, like $PGF_{2\alpha}$, are the principal mediators in the induction of fever and other adverse responses in animals to cytokines (Nilsberth *et al*., 2009; Markworth and Cameron-Smith, 2011). The prostaglandins are synthesised from dietary linoleic acid and body cell membrane phospholipids via a family of phospholipase A2 (PLA_2) enzymes to produce arachidonic acid, which requires the activity of the cyclooxygenases (COX)-1 and -2 and various prostaglandin synthases to produce the range in forms of prostaglandins. The pro-inflammatory cytokines, particularly acting synergistically, stimulate PLA_2 and the COX-2 enzyme synthesis, which greatly increases prostaglandin production (Akiba *et al*., 2001).

There is a diverse family of over 25 mammalian PLA_2 enzymes that hydrolyse the sn-2 position of membrane glycerophospholipids (Boilard *et al*., 2010). One class, the cytosolic ($cPLA_2$), act on the inner leaflets of cell membranes, whereas another class, the secretory ($sPLA_2$), act on the outer leaflets of the membranes. The pro-inflammatory cytokines stimulate the synthesis of the $sPLA_2$, but not the synthesis of $cPLA_2$ (Akiba *et al*. 2001). The $sPLA_2$ are secreted particularly from macrophages and mast cells (Granata *et al*., 2005; Triggiani *et al*., 2009), but are also found in bee sting and snake venom. During the initial phases of an antigenic challenge and PAMP recognition by the Toll receptors, these innate immune cells within the epithelium and lumen of the GIT and respiratory tract increase greatly the release of $sPLA_2$ (Granata *et al*., 2005). Immune stimulation within the lumen is needed for marked increases in $sPLA_2$ production, such that the concentration of $sPLA_2$ in the lumen of the small intestine of mice was approximately 2.5-fold lower with parenteral nutrition compared with normal feeding (Pierre, 2011).

Bidgood *et al*. (2000) demonstrated that addition of $sPLA_2$ to cultured fibroblast cells increased the production of PGE_2 in a dose dependent relationship, with a concomitant increase in COX-2 expression. Inhibitors of $sPLA_2$, such as LY311727, prevent the cytokine stimulated increase in both COX-2 expression and in PGE_2 synthesis (Akiba, *et al*., 2001; Bidgood *et al*., 2000). Inhibitors of COX-2, such as NS-398, prevent both the cytokine and $sPLA_2$ stimulated increase in PGE_2 synthesis (Bidgood *et al*., 2000). Furthermore, inhibitors of either $sPLA_2$ or COX-2 have been shown also to have clinical effects in reducing pro-inflammatory disease symptoms in animals. For example, Coulthard *et al*. (2011) showed that an inhibitor to $sPLA_2$-IIA significantly decreased joint swelling, gait disturbances and histopathology scores in rats with antigen-induced arthritis. Similarly, the administration of COX-2 inhibitors (including various non-steroid anti-inflammatory drugs and SC-236), but not the COX-1 inhibitor ((SC-560), has been shown to reduce the extent of fever in several animal species (Zhang *et al.,* 2003; Romanovsky *et al*., 2005).

Pro-inflammatory cytokines are known also to stimulate the release of various hormones and other metabolism mediating peptides including adrenocorticotropic hormone (ACTH), leptin, melanocyte-stimulating hormone (MSH) and others (Blalock, 1989; Sachot *et al*., 2004; Reyes-Vasquez and Dafny, 2011). It is likely that changes in endocrine status of animals are responsible for the increase in

protein degradation rate and reduction in synthesis rate associated with an acute immune response. Endotoxin challenge or administration of IL-1β or TNF-α to rats significantly reduces the concentrations of growth hormone and insulin like growth factor (IGF)-1, while increasing the release of ACTH and subsequent concentrations of corticosteroids (Spurlock, 1997). These changes in endocrine status are sufficient to reduce the net rate of muscle protein synthesis. There is evidence that the pro-inflammatory cytokines reduce the translational efficiency of mRNA during protein synthesis by suppressing the activity of the serine kinase, the mammalian target of rapamycin (mTOR), either directly or more probably through the stimulated glucocorticoid concentrations (Lang *et al*., 2007; Kuehn *et al*., 2010; Frost and Lang, 2011). There is also some evidence that increased concentrations of PGE_2 resulting from the actions of pro-inflammatory cytokines increases the rate of muscle protein degradation (Barcos *et al*., 1983; Smith and Tisdale, 1993; Lorite *et al*., 1997). However, there are other experiments that have showed PGE_2 inhibitors to have no effect on the rate of protein breakdown (Barnett and Ellis, 1987; Hasselgren *et al*., 1987).

Anorexia is another major physiological change associated with an acute immune response. Sachot *et al*. (2004) showed that the intake depression and fever associated with intraperitoneal injection of LPS to rats was partially reversed by administration of a leptin antiserum. In addition, the LPS stimulated increase in IL-1β mRNA in the hypothalamus of the animals, which was also attenuated by the presence of the leptin antiserum. Leptin is known to stimulate the concentrations of mTOR in the hypothalamus of animals, which results in stimulation of anorexogenic peptides in the hypothalamus, particularly α-MSH (Black *et al*., 2009). Several inflammatory diseases and cachexia have been reported to result in marked increases in plasma α-MSH (Catania *et al*. 1991). Intracerebroventricular administration of α-MSH has been found to significantly enhance the negative response in feed intake and activity associated with LPS administration to rats (Huang *et al*. 1999). However, administration of antagonists, either natural agouti-related peptide (AgRP) or synthetic (SHU-9119), to the α-MSH receptors, melanocortin receptor 3 and 4 (MC3-R/MC4-R), reversed the symptoms caused by LPS (Huang *et al*. 1999, Marks *et al*. 2001). Similarly, the symptoms did not appear in MC4-R knockout mice following administration of LPS. These results show a central role for the hypothalamic melanocortin system in fever and cachexia and that a blockade of MC4-R signalling normalises feed intake and activity without an increase in morbidity or mortality (Marks *et al*. 2001, Catania 2007).

4.7. An Optimal Immune Response

A major outcome from this review is that there are negative outcomes for animal health and productivity through both under- and over- responsiveness of the immune system. For example, pigs (Mallard and Wilkie, 2003) or chickens (Kuehn *et al*., 2006) selected over many generations for low immune response fail to produce antibodies following vaccination and are more susceptible to specific pathogens than animals selected for high immune response. On the contrary, high responsiveness of the immune system, and particularly release of the pro-inflammatory cytokines IL-1β, IL-6, TNF-α and IFN-α, results in anorexia, fever and increased muscle proteolysis with a reduction in animal performance. The negative effects of over-responsiveness of the immune system appear to be in excess of the need to combat invading pathogens and can be large. Schinckel (1995) showed that young pigs challenged with LPS were 20% lighter and consumed 27% less feed than the unchallenged group. Furthermore, environmental and genetic conditions that result in the production of high a proportion of IgE within the antibodies produced by an animal can increase the risk of development of many autoimmune diseases and allergies through over stimulation of oesinophils and mast cells and their subsequent degranulation damaging host cell tissues.

There is also clear evidence of trade-offs between nutrient use by the immune system and productive responses in animals, particularly during periods of nutrient restriction. This competition for nutrients is particularly important during the period immediately after weaning when the intestine of the piglet is being invaded with microbes, there is a substantial increase in the number of leukocytes and other immune cells in the intestinal epithelium and nutrient intake is limited. Requirements for the immune system change dramatically as a pig grows through the first weeks of life. Initially the piglet is protected through passive immunity by antibodies produced in colostrums and milk from the sow. Then, as the GIT is invaded by environmental organism and feed antigens, immune cells invade the GIT epithelium and the innate immune system develops and matures. Finally, from around four weeks until seven weeks of age the adaptive immune system undergoes development, the piglet gains capacity to produce an array of antibodies and the immune system is fully developed.

In addition, animals selected for fast growth have a reduced capacity to mount an immune response and have lower performance and increased mortality when placed in an environment with high microbial challenge (Frank et al., 1997). Another important finding is that continual stimulation of the immune system with the same antigen leads to immune tolerance and a less effective on-going immune response.

The results presented indicate that there is not one optimal immune response that should be targeted for all animals, but the immune response must be appropriate for specific circumstances. An animal must have the capacity to mount a substantial immune response against invading pathogenic organisms, but the negative effects of pro-inflammatory cytokines should be minimised. Possible ways to achieve these objectives are explored in the following section.

4.8. Manipulating the Immune Response to Increase Pig Performance

The literature reviewed suggests that a range of strategies could be used or explored to improve the health and productivity of commercially raised pigs. These strategies fall into four main areas as follows: i) decrease the presence of agents that elicit an immune response or are pathogenic; ii) breed pigs with enhanced immunity, but maintain high rates of productivity; iii) regulate the immune response either up or down to suit specific circumstances; iv) negate the adverse effects of pro-inflammatory cytokines, while maintaining disease control.

4.8.1. Decrease the Presence of Agents that Illicit an Immune Response

Comparisons between pigs reared in conventional sheds with gnotobiotic pigs (Loynachan *et al*., 2005), pigs reared in isolation (Jolie, 1999), pigs reared in 'clean' environments (Williams, 1998; Cargill *et al*., 1999; Lee *et al*., 2005; Renaudeau, 2009) or pigs receiving antibiotics (Zimmernam, 1986) shows clearly that presence of the microbial load in conventional sheds depresses feed intake and growth rate by around 20%. The strong negative correlation observed between growth rate of pigs and the concentration of viable bacteria in the atmosphere suggests that more than 50,000 CFU/ m^3 will depress performance (Murphy *et al*., 2000). Furthermore, pathogenic diseases in pigs are exacerbated by the presence of atmospheric ammonia at more than 5 ppm and airborne endotoxins at more than 1 $\mu g/m^3$ (Black, 2003). These observations suggest that strategies to microbial load in the atmosphere and airborne pollutants should substantially increase pig performance. Possible strategies are considered briefly.

4.8.1.1. Reduce Atmospheric Microbial Loads and Air Pollutants

The air quality factors affecting pig performance and practical strategies for improving air quality in commercial piggeries have been reviewed by Cargill *et al*. (1999); Black (2003), Holyoak (2007) and Banhazi *et al*. (2009). The primary drivers of air quality, including ammonia, viable bacteria and respirable particle concentration, are pen and shed cleanliness (Banhazi *et al*., 2009). The principal strategies for improve pen and shed cleanliness include batch rearing, thorough cleaning between batches and spraying water above floors and pens prior to people working in pig buildings. The number of pigs reared within one air space also appears to have a substantial effect on viable bacteria in the atmosphere and pig performance. Recommended minimal stocking densities and methods for improving air quality within piggeries are given in section 4.3.5.

4.8.1.2. Add Antimicrobial Agents to the Feed or Water

Several recent reviews have assessed the effectiveness of antimicrobial agents as alternatives to commonly used in-feed antibiotics (e.g., Anon., 2005; Thaler and Sulabo, 2009; Edwards and Edwards, 2011). Although many compounds and commensal organisms have been evaluated, none appear to be as effective as traditional antibiotics. However, several are being used widely as antibiotic replacements within the pig industry including Zn oxide at doses exceeding 2000 ppm, and various organic and inorganic acids. There are both microbial resistance and environmental contamination concerns with continuing use of Zn oxide, whereas acids have wide acceptability by pig producers and environmentalists.

The list of compounds either suggested or evaluated as potential antimicrobial compounds is large and includes MCFA and SCFA; compounds found in plants (carvacrol an oil from oregano, cinnamaldelyde from the cinnamon tree, capsicum oleresin from chilli peppers, allicin from garlic, thymol from thyme, rosmarinic acid from sage, and tochopherols and phenols from a range of plants); antimicrobial peptides such as lactoferrin, lactoferricin, lactoferrampin, lysozyme, neutrophil peptides like indolicin and purothionin from wheat; bacteriocins (peptides produced by bacteria to eliminate other similar types of bacteria), bacteriophages (viruses that cause lysis of specific bacteria) and clays such as kaolin, bentonites and zeolites which appear to reduce microbial numbers and absorb microbial endotoxins.

Many of these compounds have been shown to have small positive benefits on either growth rate or reduction of diarrhoea in early-weaned pigs. For example, plant extracts (Manzanilla *et al*., 2004), essential plant oils (Windisch *et al*., 2008), eucalyptus oil (Han *et al*., 2010), antimicrobial peptides (Tang *et al*., 2009) and clays (Trckova *et al*., 2009; Song *et al*., 2012) have been shown to have significant benefits. Research is continuing to evaluate many of these natural antimicrobial compounds.

Several of the most promising new approaches that require further understanding and quantification of their value in pig diets are antimicrobial peptides, bacteriocins and bacteriophages. The evidence to date indicates that both bacteriophages and bacteriocins have high specificity for particular serotypes, which may limit their widespread application as bactericides (Smith and Huggins, 1983). However, further evaluation of different bacteriophage and bacteriocin types and their combinations could provide a broad-spectrum bactericide.

There also may be considerable value in further research into the use of antimicrobial peptides. Levy *et al*. (2000) noted that the bactericidal permeability-increasing protein (BPI), found in the primary granules of adult neutrophils, has a high affinity for LPS and other endotoxins and exerts selective cytotoxic, antiendotoxic and opsonic activity against gram-negative bacteria. Addition of

recombinant 21-kDa N-terminal BPI fragment (rBPI21) to neonatal blood reduced the growth and tumor necrosis factor (TNF)-inducing activity of representative gram-negative clinical isolates. Use of rBPI21 was found to potentially be of clinical benefit to neonates suffering from gram-negative bacterial infection and/or endotoxemia. Similarly, Tang *et al.* (2009) showed that a peptide generated by the fusion of lactoferricin and lactoferrampin in *Photorhadbus lmninescens* and fed to young piglets challenged with enterotoxigenic *E. coli* resulted in 21% faster growth than in control piglets. These peptide treated pigs also recovered more rapidly from diarrhea, had enhanced serum glutathione, IgA, IgG and IgM concentrations and a decreased concentrations of *E. coli* in the gut.

More research into MCFA as bactericides would also be of value. It has long been known that consumption of bovine whole-milk reduces GIT infections in children compared with low-fat milk (Koopman *et al*., 1984). Subsequent research has shown that milk fats, their digestion products and a group of lipids with an aliphatic-amino-alcohol backbone called sphingolipids have significant antimicrobial activities (Sprong, *et al*., 2001). The bactericidal activity has been shown to depend on the chain length and degree of saturation of the fatty acids. The fatty acids $C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{16:0}$, and $C_{18:0}$ were found to have little bactericidal activity, whereas $C_{14:0}$, $C_{18:1}$ and $C_{18:2}$ had moderate activity but only against gram-positive bacteria. However, $C_{10:0}$ (lauric acid) and $C_{12:0}$ (myristic acid) were shown to be toxic to all microbes tested including gram-positive bacteria, gram-negative bacteria and viruses. Lauric acid was found to have a slightly greater antimicrobial activity than myristic acid. In addition, a breakdown product of sphingolipids, lysosphingolipid, was also shown to have high antimicrobial activity (Sprong *et al*., 2001). Recent research (Batovska *et* al., 2009) has shown that the bactericidal activity of lauric acid can be increased 3-fold or more for most bacterial species tested when presented as a 1-monotriglycerids (monolaurin) rather than as the free fatty acid. Furthermore, Batavska *et al*. (2009) observed that the minimal inhibition concentration against the bacterial strains tested was approximately 4-fold less when monolaurin was mixed in the ratio of 2:1 with monomyristin (monotriglyeride of myristic acid) or in a ratio of 1:2 with monocaprin (monotriglyceride of $C_{8:0}$, capric acid).

In summary, there is an opportunity to improve the antimicrobial activity of diets, particularly for young pigs during the early post weaning period, by investigating the use of monolaurin, monomyristin, monocaprin, selected antimicrobial peptides, bacteriocins and bacteriophages and the synergies between them. The use of these compounds in combination with organic and inorganic acids should also be investigated.

4.8.1.3. Limit Microbial Growth Stimulants in the Diet

Undigested nutrients in the distal small intestine and colon of pigs increase the growth of intestinal microbes and increase morbidity and mortality from pathogenic organisms (Pluske *et al*., 2002; Wellock *et al*., 2006). Furthermore, newly weaned pigs fed fermentable carbohydrates including laculose, inulin, sugarbeet and wheat starch increased the production of pro-inflammatory cytokines IL-1β and IL-6 compared with pigs on normal diets (Pie *et al*., 2007). Young pigs immediately after weaning are particularly susceptible to GIT pathogenic infection (Emery and Collins, 2011). The digestive system of weaned pigs is still adapting to the consumption of solid feed and has a lower digestive capacity than older pigs. Compared with pigs that have been consuming solid feed for several weeks, recently weaned pigs have higher gastric pH lowering pepsin activity, lower overall production and efficacy of digestive enzymes and lower mucin secretion with reduced intestinal barrier protection. Consequently, strategies that either increase digestion in the proximal small intestine of fermentable dietary ingredients or reduce the amounts of dietary nutrients that exceed the requirements of the pig could reduce the intestinal microbial load and growth of pathogenic organisms.

Strategies that have been successful in increasing the digestion of nutrients in the proximal small intestine of pigs include: replacing wheat, barley, triticale, sorghum or maize in the diet with grains that have more digestible starch such as cooked rice (Pluske *et al*., 2002; Vicente *et al*., 2008) or groats; removing grain particles greater than 1.00 mm through sieving and regrinding or some other method (Henman *et al*., 2011); adding glucanase, xylanase, phytase and other enzymes to the diet (Omogbenigun *et al*., 2004); replacing plant derived protein ingredients with more readily digestible spray dried plasma or milk proteins (Bosi *et al*., 2004); and reducing the protein content of the diet (Wellock *et al*., 2006). However the impact on pig performance of several of these strategies has been shown to be variable. For example, there is a wide range in outcomes from adding glucanase and xylanase enzymes to the diets of pigs (Bedford and Shulze, 1998). Furthermore, reduction in dietary protein concentrations to around 17.5% for newly weaned pigs requires supplementation with crystalline amino acids to ensure an optimum amino acid balance (Heo *et al*., 2009) that adds considerable cost to the diet.

A high concentration of iron stimulates microbial growth and normal functioning of the immune phagocyte cells depends on low concentrations of free ionic iron in body tissues (Bullen, *et al*., 1968, 2006). Low tissue iron concentrations in animals are maintained by the binding of free iron to lactoferrin and transferring. The increase in the gut microbial population in conventionally reared compared with gnotobiotic pigs increases iron requirements with all conventionally reared pigs not given iron supplements dying (Miller *et al*., 1982). The full role of dietary iron in influencing the growth of pathogenic microbes while ensuring sufficient iron for metabolic purposes and the potential role of antibacterial peptides such as lactoferrin appears to require further clarification.

4.8.1.4. Probiotics

The application of probiotics is a much researched topic in the pig industry. The science of probiotics attempts to alter the microbial species in the GIT to be more favourable for animal health. There have been many recent reviews covering aspects of the theory, benefits and disadvantages of probiotics (e.g., Thaler and Sulabo, 2009; Cho, *et al*., 2011; Emery and Collins, 2011). The bacteria used in probiotic therapy are generally lactic acid producing *Lactobacillus*, *Streptococcus* and *Bifidobacteria* species isolated from the intestinal flora of animals. However, specific strains of avirulent *E. coli* also have been selected to compete with enterotoxigenic strains of the same species (Beale *et al*., 2011). The proposed functions of probiotics are to compete with pathogenic bacteria for nutrients and intestinal binding sites, release compounds that are toxic to the pathogenic organisms such as bacteriocins and stimulate the immune system to enhance its ability to negate pathogenic organisms.

Although, several studies that have shown positive effects on pig health and growth rates (Davis *et* al., 2008; Beale *et al*., 2011), the magnitude of the differences from untreated animals is generally small. De Lange *et al*. (2010) have suggested that the positive effects of manipulating the gut microbiota on gut health may be counterbalanced by the increased energy and nutrient costs needed to support these microbes. Thaler and Sulabo (2009) concluded that, although probiotics have been used for over 50 years, the variable and small improvements resulting from current microbial strains and application techniques suggest probiotics are unlikely to be of major value as a replacement of in-feed antibiotics.

4.8.2. Breeding Pigs for Enhanced Immunity and High Productivity

Pigs can be bred for improved resistance to disease. This will rarely involve single gene selection and more commonly be based on broad selection criteria because more than 2000 genes regulate the immune response (Mallard and Wilkie, 2003). Selecting pigs for single gene allele type is relatively

straightforward. The most promising single gene identified to date is the AA allele of FUT1 M307 for adhesion of *E. coli* variant F18. Although the allele represents only 5% of the pigs populations so far examined, pigs with the allele do not form an epithelial adhesion with the F18 strain and are resistant to the disease. A small ear biopsy and PCR assay has been used to identify the FUT1 M307 alleles in large groups of pigs (Luo *et al*., 2010) and could be used in pig breeding programs. There is contradictory evidence whether selection for the AA allele has positive or negative effects on pig productivity (Horak *et al*., 2005; Jaing *et al*., 2005). Before embarking on a breeding program for the AA allele, the prevalence of F18 *E. coli* strain relative to other enterotoxigenic forms of the organism in the target environments for the pigs should be considered.

The heritability of most immune traits examined is high ranging from <0.1 to ~ 0.9 and there strong polymorphism in the immune response genes within pig populations. Hence, rapid progress can be made selecting pigs for high immune response. However, a high immune response without major pathogenic challenge will result in reduced performance through competition by the immune system for nutrients and the negative effects of pro-inflammatory cytokines on intake and protein deposition. Furthermore, there are several different components of the innate and adaptive immune systems that need to be enhanced to confer widespread disease resistance (Mallard and Wilkie, 2003). Thus, the benefits obtained from breeding pigs for enhanced immune systems will depend on the environments to which future generations of the pigs are to be exposed. A relatively low immune response would be needed for pigs exposed to environments with low microbial and pathogen loads, whereas a greater immune response would be needed for pigs destined for high environmental loads.

The best result in terms of both resistance to disease and productivity will come from index selection techniques that include several measures of immune response along with economically important production traits. In addition, the greatest progress and success should come when the pigs are selected within the same environment the progeny will be raised.

4.8.3. Regulate the Immune System for Specific Situations

Pigs must have the capacity to mount an immune response when required, but the negative impacts of over stimulation of the immune system should be minimized. Thus, dietary or environmental factors that limit the capacity of the immune system should be removed, while methods for enhancing the immune system when subjected to specific challenges should be explored. Enhancing immunity is particularly important during the first 7 weeks of life before the adaptive immune system becomes fully developed, but it will also be beneficial for older pigs when exposed to specific pathogens.

4.8.3.1. Sufficient Dietary Nutrients for Optimal Immune Response

The efficiency of the immune response is extremely sensitive to both nutrient deficiencies and nutrient excesses. The immune response is depressed when amino acids, total energy, essential minerals and vitamins are lacking in the diet (sections 4.3.1-4.3.4; Beisel *et al*., 1981; Savino and Dardenne, 2010). The requirements for tryptophan, sulphur containing amino acids and threonine need to be increased by approximately 20% above needs to maximum growth for the uncompromised response of the immune system (section 4.3.3.1). The immune system is also particularly sensitive to deficiencies in zinc (Haase and Rink, 2009). In addition, excess of many nutrients, including zinc (Faber *et al*., 2004), amino acids (section 4.3.3.2), vitamins E, A and C (Cook, 2011). Thus, careful consideration of the needs of the immune system should be given when formulating diets for pigs and particularly early post weaning diets.

4.8.3.2. Eliminate Stressors

Physical and psychological stressors stimulate the release cortisol and catecholamines, which in turn stimulate the release of cytokines from immune cells within the body. Catecholamines, particularly, stimulate the production of the pro-inflammatory cytokines IL-6 from peripheral tissues including the liver, spleen and pancreas (De Simoni *et al*., 1990), and TNF-α, IL-1 and IL-8 from lung mononuclear and epithelial cells via the action of the α2-adrenergic receptors (Linden, 1996; Le Tulzo *et al*., 1997). Thus, direct activation of the immune system through stressors stimulates the release of pro-inflammatory cytokines and also reduces the magnitude of the immune response (Salak-Johnson and McGlone, 2007).

Management strategies should be employed that keep pigs within their zone of thermal comfort, because temperatures below the lower critical temperature, fluctuating low temperatures, draughts and temperatures above the zone of thermal comfort increase stress on pigs and reduce their response to disease challenge (Cargill and Byrt, 1983; Scheepens *et al*., 1991; Morrow-Tesch *et al*., 1994). Cold stress has been shown to significantly increase the production of the pro-inflammatory cytokines IL-1β and IL-6 in young pigs (Frank *et al*., 2003).

The social environment also affects stress and immune response. Rudine *et al*. (2007) mooted that pig performance, immunity, and behaviour may be influenced by production system and social status and hence compared a conventional indoor housing system was an outdoor system. They found that body weight and average daily gain were not influenced by the production system but immune and blood measures were affected by the production system. The percentage of phagocytosis was greater ($P \leq 0.05$) and antibody titres to SRBC challenge tended to be greater ($P=0.066$) among outdoor-reared pigs compared with indoor-reared pigs. Outdoor-reared pigs had higher haemoglobin concentrations ($P<0.005$), percentage of haematocrit ($P<0.005$), mean corpuscular volume ($P<0.005$), and mean corpuscular haemoglobin ($P<0.005$) compared with indoor-reared pigs. They also found that dominance order influenced the immune system with dominant pigs having greater phytohaemagglutinin stimulated lymphocyte proliferation ($P<0.01$) compared with submissive pigs. Furthermore, Ernst *et al.* (2006) presented pigs (7–20 weeks of age and housed in groups of 8) with environmental enrichment by providing equipment provoking attention and cognitive activity, which was rewarded by feed. They found that the experimental animals had a significantly higher concentration of IgG as well as an increased *in vitro* T-cell proliferation to ConA but a reduced LPS-induced proliferation of B-cells, while basal salivary cortisol concentrations were similar. Whilst Turner *et al.* (2006) acknowledged that research has indicated that large group size, the mixing of unfamiliar pigs and social defeat have all been shown to compromise immune function, they found that this was unlikely to be the case with finisher pigs.

4.8.3.3. Modulate the Immune System with Saturated, n-3 and n-6 Fatty Acid

Dietary fats modulate the immune response and can have substantial effects on health and growth of animals (Harbige, 2003; Yaqoob, 2004). Wilkinson and Newman (in press, 2012) showed that feeding pregnant gilts and their progeny diets containing sunflower oil (high in n-6 polyunsaturated fatty acids) caused significant reductions in birth weight, growth rate, feed intake and increased mortality of their progeny to slaughter weights compared with diets containing fish oil (high in n-3 polyunsaturated fatty acids) or tallow (high in saturated fatty acids). Many other studies with animals and humans have shown negative effects of inclusion in diets of n-6 polyunsaturated fatty acids compared with n-3 polyunsaturated fatty acids on a range of autoimmune diseases, growth rate and protein accretion (Lorite *et al*., 1997; Calder, 2001; Simopoulos, 2002a; Gottrand, 2008). There is also evidence that n-3 fatty acids in the diet increase resistance to pathogenic disease, but the results are equivocal (Anderson and Fritsche, 2002).

The predominant n-6 fatty acids are linoleic acid (18:2), γ-linoleic acid (18:3) and arachidonic acid (20:4). Linoleic acid is readily converted to arachidonic acid, which is the precursor for PGE_2 and leukotrine B_4 synthesis and leads to the release of pro-inflammatory cytokines, anorexia, fever and protein degradation. Feeding diets high in fish oil with high concentrations of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) partially replaces linoleic and arachidonic acids in cell membranes which decreases the production of the pro-inflammatory cytokines, PGE_2 and leukotrine B_4 and their adverse effects (Simopoulos, 2002a).

Recent research (Abbott, *et al*., 2009) suggests that the ratio of n-3:n-6 fatty acids in the diet is more critical for determining the balance of fatty acids in animal cell membranes than the total quantity provided in the diet. The research by Abbott *et al*. (2009) and Simopoulos (2002b) suggests that the ratio of n-6:n-3 in diets fed to animals for optimal immune response and minimal adverse health consequences is 4:1 or less. Thus, consideration of the n-6:n-3 ratio should be included in diets formulated for pigs and should not exceed 4:1 for optimal health response.

4.8.3.4. Enhance the Immune System through Stimulation of Toll-Like Receptors

Immune competence in pigs is low at birth. Development of the innate immune system is stimulated greatly by microbial invasion of the GIT and airways and is generally fully functional by four weeks of age in conventionally reared pigs, while the adaptive immune system produces few antibodies before four weeks of age and is fully functioning by around seven weeks (Stokes *et al*., 2004). Pigs are particularly susceptible to diseases like coccidiosis (Worliczek *et al*., 2009) and rotavirus (Tzipori and Williams, 1978) only during their first few weeks of life before the innate immune system has fully developed (Worliczek *et al*., 2009). Consequently, methods to stimulate earlier development of the innate immune system, particularly against specific types of pathogenic organisms, have been suggested (Kogut, 2009). The principle involves providing to the pig with compounds in the diet or by other routes that resemble microbial PAMPS and stimulate specific Toll-like receptors to enhance development and effectiveness of the innate immune cells and their interaction with the adaptive immune system.

Toll-like receptor agonists such as unmethylated cytosine-guanosine oligodeoxynucleotide have been shown in chicks to increase innate immune cell numbers, degranulation of immune cells and generation of reactive oxygen species (He *et al*., 2005). When day-old chicks were given the synthetic nucleotide prior to a challenge with live *Salmonella enteritidis* bacteria, there was a significant reduction in Salmonella invasion of organs and in chick mortality. Similarly, the gram-negative bacterium produced cationic protein (BT/TAMUS 2032) when provided at 12, 24 and 48 ppm in the diets for 4 days to post-hatch chicks challenged with *Salmonella enteritidis*, significantly decreased *Salmonella* invasion of organs in a dose dependent manner. The functional efficiency of the heterophils in the BT-treated chicks was also dose dependently up-regulated with increased phagocytosis, oxidative bursts and degranulation compared with control birds (Kogut *et al*., 2007). Another Toll-like receptor agonist, serum-opsonized *Salmonella enteritidis*, was shown to increase degranulation and oxidative bursts by chicken heterophils when added to the feed of young chickens (Kogut, 2009). Addition of β-glucan to the diet stimulated Toll-like receptors and leukocyte oxidative bursts and there was a major synergistic effect of providing the opsonized organism and β-glucan in the diet.

There appear to be few experiments where Toll-like receptors have been deliberately stimulated in young pigs. Feeding 2% sugar beet oligosaccharides was shown to increase the expression of the TRL2 receptor and TNFα mRNAs in young pigs given different oral doses of *Bifidobacterium animalis*, but effects on pig performance were not measured (Trevisi *et al*., 2008). Li *et al*. (2006) showed that

feeding 50 ppm of a yeast derived β-glucan in the diet of young pigs increased plasma IL-6, IL-10 and TNF-α concentrations after a challenge with LPS compared with pigs not receiving the supplement. In one 28 day experiment, but not in another, a small positive effect was observed in growth rate and feed intake when 50 ppm β-glucan was added to the diet (Li *et* al., 2006). Earlier studies by Dritz *et al*. (1995) did not show a production response to the addition of β-glucan when offered for 28 days to pigs from weaning.

Fungal-derived mannan oligosaccharide (MOS) is also recognized as a PAMP and stimulates Toll-like receptors in animals (Shashidhara and Devegowda, 2003). Many experiments have been conducted investigating the effects of MOS fed to young pigs and chickens. A review of 54 experiments in which MOS was fed to pigs immediately after weaning showed wide variation in results with only 11 reports showing a significant improvement in growth rate (Miguel *et al*., 2004). An analysis of 41 experiments that included growth measurements showed that growth rate was increased compared with control pigs by an average of 8.5% during the first 15 days and by only 2.1% for the remainder of the experiments. Corresponding values for average feed intake were 3.5% and 1.2% for the first and second phases of growth. In a recent experiment in which 2% MOS was fed to 3-week-old pigs challenged with the porcine reproductive and respiratory syndrome (PRRS) virus, MOS significantly improved the efficiency of feed use during days 7-14 following the challenge (Che *et al*., 2011). MOS increased the serum concentrations of IL-10, white blood cell count and lymphocyte numbers, and increased the concentration of TNF-α in the PRRS challenged pigs. MOS also reduced the increase in body temperature associated with the PRRS challenge. These results are typical of a Toll-like receptor stimulated increase in the efficiency of the innate immune cells and an increase in anti-inflammatory cytokines, while depressing the pro-inflammatory cytokine, TNF-α.

Compounds that stimulate Toll-like receptors appear to have been less effective in pigs than in young chickens. The differences between species may relate to the compounds used for chickens being more directly related to known PAMPS of specific targeted pathogens than those used in pig experiments. It may also relate to the length of time the compounds were fed, being for a much shorter time in the chicken experiments. Endotoxin tolerance is known to rapidly down-regulate the innate immune response within 4-5 days of exposure to a particular antigen and then take around three weeks without the antigen to return to a full response (Beeson, 1947). This phenomenon of endotoxin tolerance may explain the reduced effect of diet additives like MOS during the latter times of the experiments.

Further work investigating the value of target Toll-like receptors may be warranted. There is an opportunity to identify PAMPS from specific pathogenic organisms that infect pigs within the first 4-5 weeks of life such as coccidiosis, rotavirus, *E. coli* and feed them to pigs for no more than 5 days prior to the expected time of infection.

4.8.3.5. Provide Pre-Formed Immunoglobulins for General or Specific Pathogens

The adaptive immune system produces few antibodies before four weeks of age and is not fully functioning until around seven weeks of age. Antibodies are provided to young pigs in colostrum, but provision of preformed antibodies in the diet immediately after weaning can improve growth rate and feed intake. One way to provide preformed antibodies is from porcine or other animal plasma or whole blood. Ferreira *et al*. (2009) reviewed the results from 25 scientific papers investigating the effects of spray-dried porcine plasma added to the diets of young pigs. This review and other published information indicate that feed intake and growth rate can be substantially improved during the first week after weaning when about 10% dried plasma is included in the diet. The benefits of dietary plasma decreases as weaning age increases from 14 to 28 days and as the

amount in the diet falls below 8%. The response to dietary plasma also depends on the cleanliness of the environment. There appears to be no benefit feeding plasma to young pigs reared in clean environments (Coffey and Cromwell, 1995; Zhoa *et al*., 2007).

Although feeding spray-dried plasma was found to reduce the population of *E. coli* in the small intestine of pigs in some experiments (Ferreira *et al*., 2009), this was not observed in an experiment in which pigs were challenged with pathogenic *E. coli* (Van Dijk *et al.* 2002). Nevertheless, in this challenge experiment, pigs given 8% spray-dried plasma in their diets consumed more feed, grew faster and had less diarrhoea than pigs not receiving the plasma. In this experiment, mortality was similar in the pigs receiving or not receiving plasma. However, in an experiment with broilers where the birds were naturally infected with pathogenic *E. coli* and *Salmonella*, the inclusion of spray-dried avian plasma reduced mortality from 50% to less than 10% (Campbell *et al*., 2006).

The IgG-rich component rather than the albumin-rich component has been shown to be responsible for the improvements in growth rate and feed intake of pigs offered porcine plasma (Pierce *et al*., 2005). Bovine spray-dried plasma was found to be less effective that porcine plasma for improving performance of young pigs (Pierce *et al*., 2005). However, separated and dried bovine plasma IgG-rich fraction appeared to be as effective as porcine plasma. The research from Kats *et al*. (1994) suggests that there is a synergistic effect of feeding spray-dried porcine plasma with spray-dried porcine blood in the ratio of 7.5:1.63% in the diet, but the reasons for the interaction is not known.
The major effect of dietary plasma on pig performance is through the action of preformed antibodies on pathogenic and other organisms within the GIT. However, dietary plasma also appears to have an effect on the cytokine and endocrine status of pigs. Pigs fed plasma have a lower mRNA expression for IL-1β, IL-6 and TNF-α than pigs not receiving the plasma (Touchette *et al*., 2002). However, following an interperitoneal injection of LPS, pigs fed plasma showed a significantly greater response in serum TNF-α and IFN-γ than pigs not receiving the plasma. Similarly, Carroll *et al.* (2002) found plasma fed pigs had a greater ACTH and cortisone response following LSP administration than pigs not given plasma.

In summary, feeding porcine plasma or the immunoglobulin rich fraction at concentrations of at least 8% in the diet for the first 1 to 2 weeks after weaning is likely to be beneficial in pigs weaned from 14 to 28 days and in conventional 'dirty' environments. The effects are likely to be greater for pigs that have received little or no colostrum (Serge and Kaeberle, 1962). The benefits also are likely to be better when the plasma is derived from pigs reared in the same environment as the piglets to which is will be fed to ensure antibodies for the specific organisms in the environment are present in the plasma. Low temperature drying of the plasma is also important to ensure the immunoglobulins are not denatured.

4.8.3.6. Vaccinate against Specific Pathogens

Vaccination is one of the most important strategies for manipulating the immune system of the pig. Vaccines contain either live, attenuated organisms with reduced virulence that multiply within the pig or inactivate whole organisms, antigenic parts of organisms, synthesized antigens or detoxified toxins (toxoids) that cannot multiply in the pig. The advantage of live vaccines is that they provide a larger antigenic stimulus to the immune system and do not require repeat vaccination. The major disadvantage is that live vaccines are extremely sensitive to storage prior to vaccination. Examples of live vaccines are for PRRS, pseudorabies and classical swine fever.

Components of vaccines act as antigens and stimulate the production of antibodies by B cells. Frequently, antigens of pathogenic organisms are specific to the particular serovars present at a

location and general vaccines are ineffective. In these circumstances, autogenous vaccines are manufactured from the specific organisms isolated from diseased pigs at the locality where the vaccines are to be used. Vaccines have been developed for most pig viruses (pseudorabies, foot-and-mouth, parvovirus, PRRS, swine fever, swine influenza, transmissible gastroenteritis) and bacteria (*Actinobacillus pleuropneumonia*, atrophic rhinitis, Clostridial diseases, *E. coli*, *Mycoplasma*, erysipelas, Glassers disease, Leptospirosis, Pasteurellosis, Streptococcal meningitis).

B cell activation to a new antigen and production of sufficient quantity of antibodies to protect an animal from a disease takes 10 to 21 days and for most non-living antigens a second vaccination is required for complete antibody protection. Vaccines given by intramuscular injection are not as effective for respiratory and GIT pathogens as for systemic diseases because mucosal immunity and IgA antibodies are required. Nasal vaccines can be useful for respiratory diseases in stimulating the production of IgA. Vaccines are not efficacious if the pigs are already developing a disease when the vaccination occurs. Vaccines have low efficacy if maternal antibodies are still present (Suradhat *et al.*, 2007). In addition, vaccines are generally ineffective for pigs less than 4 weeks of age (Suradhat *et al.*, 2007). This inability to form antibodies is partly due to the lack of sufficient B cells to develop full antibody protection and partly due to the presence of maternal antibodies derived from colostrums. Nevertheless, vaccinations from 6 weeks of age are generally successful. Vaccination of the sow against diseases the piglet is likely to encounter may help protect the young piglet, but frequently the sow will have sufficient antibodies against the organisms in the environment.

In summary, vaccines are extremely important for stimulating the immune system of pigs and preventing many common diseases. However, vaccinations are best given 2 to 3 weeks before the expected onset of the disease and not after pigs have been exposed to the disease. Vaccinations before pigs are about six weeks of age have low efficacy.

4.8.4. Negate the Adverse Effects of Pro-Inflammatory Cytokines

The adverse effects of pro-inflammatory cytokines can be negated by i) reducing the amount of cytokines produced, ii) using antagonists to the cytokines to reduce their activity or iii) nullifying the production PGE_2, which is primarily responsible for their adverse effects.

4.8.4.1. Reduce the Production and Activity of Pro-Inflammatory Cytokines

Use of Conjugated Linoleic Acid

Early experiments in which structural derivatives of linoleic acid (cis-9, trans-11; trans-9, cis-12), CLA, were added to the diets of chickens (Cook *et al*., 1993) and mice (Miller *et al*., 1994) showed that CLA protected the animals against the immune-stimulated weight loss, while enhancing other measures of immune function. Dietary CLA protected the animals against damage from cytokines associated with an over-responsive immune system and had positive outcomes for disorders such as type I immune hypersensitivity (Whigham *et al*., 2001), cachexia (Graves *et al*., 2005) and arthritis (Huebner *et al*., 2010).

Subsequent experiments demonstrated that CLA influenced the production of cytokines by interrupting the inflammatory signalling pathway prior to activation of NF-κB and transcription/translation of microRNAs (Li *et al*., 2005). The effect on cytokine production was found to be as soon as 90 s following immune stimulation (Whigham *et* al., 2001). The experiments with CLA suggest it may be possible to reduce the production of pro-inflammatory cytokines without diminishing the effectiveness of the immune response for disease control.

Use of Anti-Inflammatory Cytokines or Pro-Inflammatory Cytokine Antagonists

Anti-inflammatory cytokines (Il-4, IL-5, IL-8, IL-10, Il-11, IL-13) can reduce the production of pro-inflammatory cytokines and their manipulation may improve the performance of animals with a pro-inflammatory cytokine response (Opal and DePalo, 2000). Interleukin-4 (IL-4) and interleukin-5 (IL-5) have been shown to reduce the production of pro-inflammatory cytokines both *in vitro* (Zhou *et al*. 1994) and *in vivo* (Lubberts *et al*. 1998). Lubberts *et al*. (1998) showed that administration of recombinant IL-4 and IL-10 to arthritic mice caused significant decreases in IL-1 and TNFα concentrations, thereby inhibiting the progression of the disease. Recombinant IL-4 was shown to suppress the transcription of pro-inflammatory cytokine genes, including IL-1, IL-8 and TNFα in porcine macrophages *in vitro* (Zhou *et al*. 1994).

In addition to anti-inflammatory cytokines, the action of pro-inflammatory cytokines may be subverted by using cytokine receptor antagonists. Cytokine receptor antagonists are able to block the action of pro-inflammatory cytokines. The IL-1 receptor antagonist (IL-1ra) binds specifically to the IL-1 receptor, but unlike IL-1, does not initiate signalling processes within the cell. The effect of IL-1ra is to effectively block available IL-1 receptors, thereby preventing the cascading effects of IL-1 which include the stimulation of IL-8 and IL-6 production (Dinarello, 1996) and the production of PGE_2 with its anorexic, fever and proteolytic effects.

IL-1ra has received attention as a therapeutic for various diseases associated with detrimental inflammatory responses. IL-1ra was found to reduce IgE production in guinea pigs either sensitised to cow milk and prevent intestinal anaphylaxis (Theodorou *et al*., 1993) or sensitised with ozone and prevent airway hyperactivity (Verhein *et al*., 2008). The success of IL-1ra in reducing inflammation *in vitro* and in pre-clinical studies *in vivo* has resulted in phase I clinical trials using IL-1ra genes in a retrovirus vector to reduce the production of pro-inflammatory cytokines in human patients (Evans *et al*. 1998). Of particular significance is the protective effect that IL-1ra has provided against LPS, *E.*

coli, *Staphylococcus*, *Klebsiella*, gram negative sepsis, IL-1-induced sleep, food and water suppression, hemorrhagic shock, in various species including humans, baboons, guinea pigs, rats, mice and rabbits. Two experiments have been reported showing a small positive effect of recombinant IL-1ra administration to pigs experimentally challenged with *Mycoplasma hyopneumoniae* and PRRS-modified live virus (Dionissopoulos *et al*., 2006) or reared in a commercial environment (Black *et al*., 2001). In the experiment of Dionissopoulos *et al*. (2006), the pigs were around 30 kg at the start of the experiment and the IL-1ra treated animals grew significantly faster over 28 days at 270 g/d compared with 200 g/d for their disease challenged counterparts not receiving IL-1ra. There was no effect of IL-1ra on feed intake. In the experiment conducted under commercial conditions (Black, 2001), pigs were weaned at 28 days and nine treatments were applied for the following 6 weeks. There were ten treatments: three 'cytokine' treatments; IL-5 (an anti-inflammatory cytokine), IL-1ra and saline; and three in-feed antibiotic treatments, normal commercial rate of antibiotic, half normal and no antibiotics. The cytokines of saline were administered by intramuscular injection twice weekly at the rate of 100 µg/dose for IL-5 and 200 µg/dose for IL-1ra. The tenth treatment was IL-5 delivered as a plasmid to pigs receiving normal antibiotics. The pigs were reared in replicate groups of 16 animals, 8 males and 8 females.

Figure 1 shows the changes in the average pig weight over six weeks for each treatment. Both IL-5 and IL-1ra treatments resulted in significantly heavier animals after 6 weeks compared with the saline controls when there were no in-feed antibiotics. However, the 'cytokine' treatments were similar to the saline treatment when in-feed antibiotics were used at either normal or half normal rates. The number of deaths during 6 weeks was also reduced in the 'cytokine' treatments, with 6, 3 and 1 dying for the saline, IL-5 and IL-1ra treatments not receiving antibiotics. There results shows that anti-inflammatory cytokines and IL-1ra were able to replace antibiotics in the experiment conducted under commercial rearing conditions. However, the frequent intramuscular injection makes the procedure non-practical until a method for continuous delivery of the cytokines following one single intervention is developed.

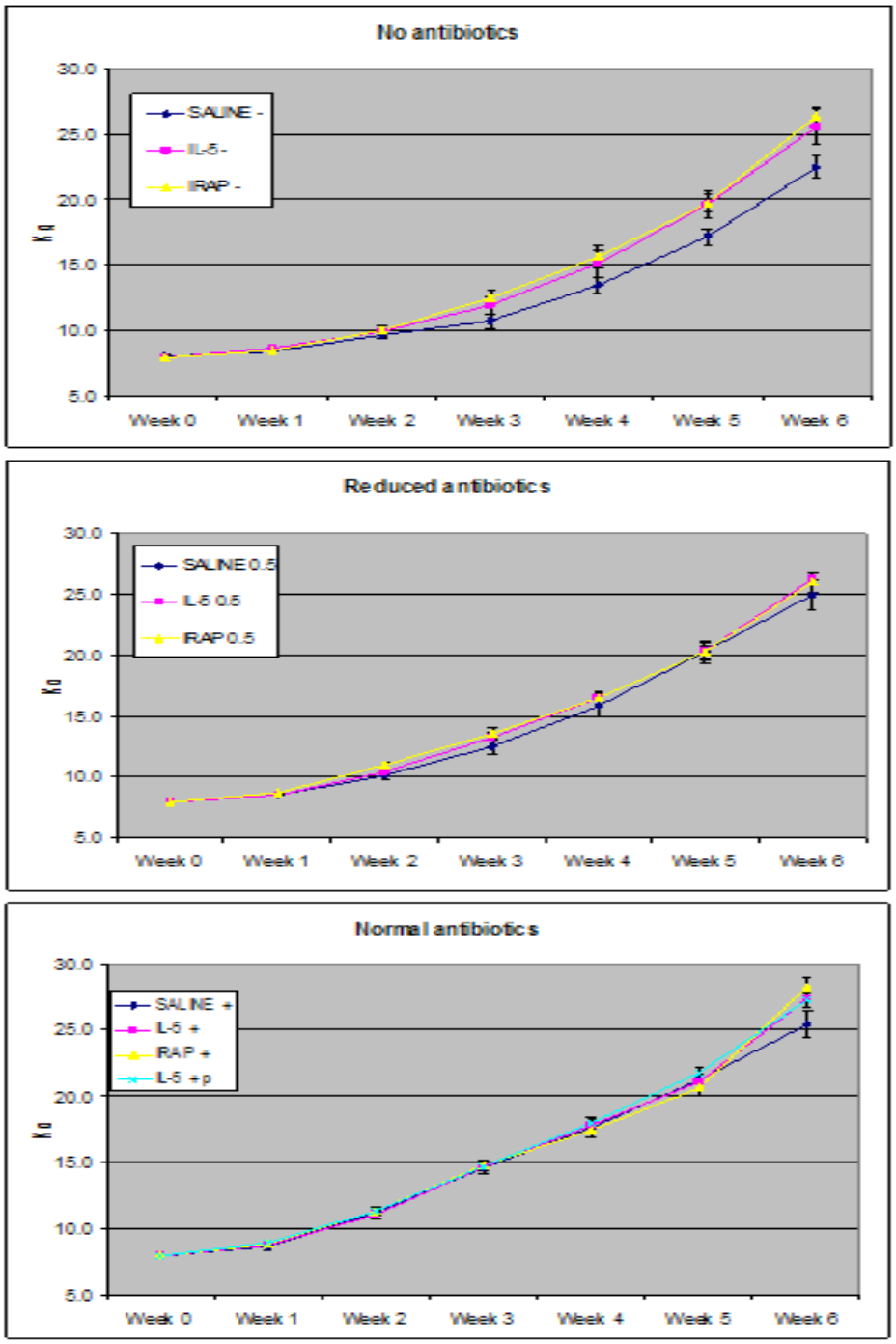

Figure 1: Effect of saline, IL-5 or Il-1ra injection or IL-5 palsmid on the mean weight of piglets receiving no in-feed antibiotic, half normal rate of in-feed antibiotic or normal in-feed antibiotic.

Use of NSAIDs

Non steroidal anti-inflammatory drugs have been known for many years to reduce the inflammatory response in animals. The current available NSAIDs include aspirin, dicloferac, obuprofen, indomethacin, ketoprofen, ketorolac, naproxen, piroxicam, sulindac, tiaprofenic acid, celecoxib and meloxicam. The early experiments of Goldberg *et al.* (1984) showed clearly that aspirin and indomethacin prevented the production of PGE_2 when added to cultured muscle cells that had been treated with IL-1. Subsequently, NSAIDs have been shown to reduce the inflammatory response by depressing the production of both pro-inflammatory cytokines and COX-2. NSAIDs reduce the synthesis of IL-1β, TNF-α and INF-γ by inhibiting the activity of NF-κB and the transcription of the pro-inflammatory genes in NK and $\gamma\delta$T cells (Inaoka, *et al*., 2006; Vaish and Sanyal, 2011). Most of the NSAIDs inhibit the synthesis of both COX-1 and COX-2 enzymes, whereas celecoxib and meloxicam reduce the synthesis of COX-2 only (Mastbergen *et al*., 2002). Inhibition of the COX-2 enzyme prevent the synthesis of PGE_2 which is the primary factor causing fever, anorexia and reduced net protein synthesis in animals with a stimulated immune response.

Early experiments showed that the addition of 125 or 250 ppm of aspirin to the diet of weaned pigs increased growth rate and reduced scouring in pigs after weaning without apparent detrimental effects on the immune response (Xu *et al*., 1990). Similarly, Johnson and Borell (1994) found that treatment of pigs with indomethacin before administration of LPS from *E. coli* prevented anorexia and inactivity caused by LPS. Ketoprofen, but not flunixin, when injected intramuscularly into pigs at a rate of 3 mg/kg eight and 32 hours after endobronchial challenge with *Actinobacillus pleuropneumoniae* (APP) significantly improved feed intake and reduced the rise in body temperature compared with the untreated controls (Swinkels *et al*., 1994). Flunixin was also found in the APL Growth Gap program not to improve the performance of APP challenged pigs (Black *et al*., 2001). Bures *et al.* (2011) recently found that treatment of pigs with indomethacin and a probiotic, *E. coli Nissle*, increased the number of methane producing bacteria and excretion of methane in breath. The apparent limited number of experiments examining the impact of NSAIDs on pig performance when their impacts on pro-inflammatory cytokine and PGE_2 production are so well documented, suggest that further investigation of their impact immediately after weaning may be warranted.

4.8.4.2. Nullify the Production of PGE_2

Cook (2004) and his colleagues have conducted much research into nullifying the production of PGE_2. He argued that, as newly born animals move from a virtually germ-free uterine environment to conventional environments loaded with microbes, there is a large increase in movement of immune cells into the GIT. These immune cells release cytokines which stimulate the production of PGE_2, the prime agent that suppresses feed intake and growth rate by as much as 20%. Cook (2004) suggested that if prostaglandins both stimulate the release of pro-inflammatory cytokine and are also primarily responsible for the adverse effects of the cytokines, regulation of prostaglandin production may be a means for stimulating growth of conventionally reared animals.

Prostaglandins are produced predominantly from cell membrane phospholipids via secretary phospholipid A2 to produce arachodonic acid which is catalysed by the COX-2 enzyme and finally via various prostaglandin synthases. Stachowska *et al.* (2007) found that CLA down-regulated the activity of $sPLA_2$ from the pancreas of pigs, while earlier studies had shown $sPLA_2$ to stimulate the synthesis of prostaglandins in intestinal epithelial cells following antigen stimulation (Grossman *et al*., 2000). Furthermore, severe systemic endotoxemia in rats was found to increase the release of $sPLA_2$ into the GIT (Zayat *et al*., 2008), indicating that both GIT and systemic infections increase the release of $sPLA_2$ into the intestines, which leads to increased concentrations of PGE_2 and immediate release of pro-inflammatory cytokines.

Cook (2004) suggested that neutralisation of the effect of intestinal $sPLA_2$ may be an effective way to reduce the negative impact of cytokine induced reduction in feed intake, net protein synthesis and performance of conventionally reared animals. He proposed the production of antibodies to $sPLA_2$ that could be included in diets. Cook and Trott (2010) have used laying hens to produce a large quantity of antibodies against $sPLA_2$ in eggs. Earlier, Cook (2004) reported an increase in growth of conventionally reared chickens of 5.4 % following the inclusion of egg produced $sPLA_2$ antibody in their diets. Similarly, Corrigan *et al*. (2007) reported a small increase in growth rate of young piglets fed diets containing egg produced $sPLA_2$ antibody. Further evaluation of the $sPLA_2$ antibody in weaner diets is warranted.

5. Conclusions

This review has provided a detailed analysis of the innate immune system with not only a description of this specific system but also how it relates to the immune system as a whole, the animal and the environment. By exploring factors such as inhibitors of the immune system and stimulation of microbial growth, the associated impacts on the animal, from a practical perspective, can be better understood. How the immune system relates to breeding and management decisions also indicates the importance of this system on production. In particular there appears to be considerable propensity to modify/hasten the innate (and possibly adaptive) immune system(s) of the pig, particularly in the early stages of growth and development. Specific methods for manipulating the immune system are discussed. Findings from this review form the basis for recommendations regarding practices that may stimulate the innate immune system and reduce the impact of disease in intensively housed pigs as well as options that may reduce antibiotic use and enhance performance of intensively housed pigs. Several specific areas for research are indicated.

6. Technical Summary

There are three components to the pig's immune system: natural, innate and acquired immunity. The ability of a pig to resist disease necessitates a well-developed immune system and an adequate level of energy. From a production perspective, an immune system that is functioning well will reduce disease outbreaks and hence reduce mortality levels, as well as increase feed efficiency and average daily gain. Management decisions associated with space and stress are extremely important because both will negatively affect the immune system if pigs are housed in an overcrowded, stressful environment. A functioning immune system is also contingent upon good diet formulation and in particular adequate vitamin and minerals. In addition the immune system can only protect against pathogens if the flow of pigs, cleaning and maintenance is of a high standard.

To better understand the immune system, this review was commissioned by Australian Pork Limited. In particular, the review sought to identify nutritional, environmental, animal selection and other factors that may either reduce or enhance the capacity of the immune system of a pig to resist the impact of high microbial loads found within many piggeries.

Host defense against bacterial invasion requires an innate immune system with the ability to respond to infection independent of prior exposure to the pathogen. It is recommended that further understanding is needed regarding the interplay between the microbiome, for example in the GIT, and disease states. The use of modern molecular-based tools aligned to bioinformatics can advance research questions in this area, however, the work is (still) relatively costly and needs to be specifically targeted.

There are already a number of existing feed additives/products on the commercial feed market that impart some effects on innate immune function, especially for younger pigs (e.g., the peri-weaning period), however these effects tend to be of a small magnitude, variable, and studies providing convincing evidence of the long-term benefits are limited. Nevertheless this is an area of research that will continue to attract attention because of increasing pressure to reduce antimicrobials' use. The mechanism exists within Program 2 of the HIAP CRC to explore such projects further.

Technologies being used to stimulate innate immunity in other animals, including man, should be evaluated further to assess their potential translation for use in the Australian pig industry. Many such technologies would require a (very) long lead-in time with likely registration issues, nevertheless, identification of the most promising technologies could occur, for example via the formation of a high-level specialist working group set up by APL/HIAP CRC and charged with exploration of such initiatives.

An opportunity exists to review current research and then conduct further targeted research in the translation of mechanisms responsible for regulation/maturation of innate immune function in outdoor-bred/outdoor-reared pigs to indoor pigs.

7. Implications and Recommendations

The review has identified many factors that regulate the innate and adaptive immune system responses in pigs and the consequences of their manipulation on health and performance. The most important outcomes from the review are listed. Practical strategies for optimising the immune response and decreasing the adverse effects from disease are suggested. Finally areas needing adoption or further research are identified.

7.1. Major Outcomes from the Review

1. Pigs exposed to conventional housing systems with high microbial loads grow round 20% more slowly than gnotobiotic pigs or pigs in 'clean' environments.
2. Mounting an immune response is expensive in terms of energy and protein/amino acids. The enhanced rate of protein turnover associated with the production of immune cells, antibodies and acute phase proteins increases energy expenditure by 10-15% of maintenance needs and protein requirements by 7-10%. The requirements for tryptophan, sulphur containing amino acids and threonine are increased by a further 10%.
3. There are negative outcomes for pig health and productivity from both under- and over-responsiveness of the immune system. Maximising the immune response is not the desired outcome, but the response should be appropriate for specific circumstances.
4. An inadequate immune response caused by selective breeding for low immunity, inadequate/excess availability of nutrients or stress increases susceptibility to disease and inability to produce immunoglobulins following vaccination.
5. Over stimulation of the immune response with excess production of pro-inflammatory cytokines causes excessive production of the prostaglandin PGE_2 which is primarily responsible for anorexia, fever, increased proteolysis and reduced pig performance. Negating the negative effects of PGE_2 appears not to adversely affect the ability of the immune system to combat pathogens, but improves pig performance.
6. Breeding pigs for fast-lean growth, without consideration of immunity, reduces their capacity to mount an immune response and reduces performance in an environment with high microbial load.

7. Heritability of most immune variables is moderate to very high (~10-90%) and these variables are amenable to selection.
8. There appears to be little opportunity for single gene selection except perhaps for the AA allele of the FUT1 M307 adhesion gene for *E. coli* F18. There may be future opportunities to identify other potential single gene alleles responsible for specific diseases from an understanding of the mechanisms of pathogen invasion of the host or its ability to evade the immune system.
9. Both immunity and productivity should be improved by breeding pigs using selection indexes that include productive and immune response traits. Several different aspects of the immune system need to be included in a selection index to cover responses from the innate and adaptive immune systems.
10. Selective breeding of pigs under environments with high microbial loads inadvertently leads to improvements in animal health and productivity when animals are reared in these environments.
11. The immune response is extremely sensitive to under- and over- supply of nutrients including tryptophan, methionine, valine, threonine, β-carotene, folic acid, vitamin B_6, vitamin B_{12}, vitamin C, vitamin E, riboflavin, iron, zinc, sodium, copper, manganese and selenium.
12. n-6 polyunsaturated fatty acids stimulate pro-inflammatory cytokine production and are associated with autoimmune diseases, anorexia, reduced growth rates and protein accretion, whereas n-3 polyunsaturated fatty acids increase resistance to pathogenic diseases. The ratio of n-6:n-3 fatty acids in the diet is critical for controlling the adverse effects of n-6 fatty acids and an optimal ratio of 4:1 (n-6:n-3) or less.
13. Certain dietary components including high protein, undigested nutrients in the distal intestines and iron promote microbial growth and GIT diseases.
14. Temperatures below thermoneutrality, diurnally fluctuating temperatures and draughts increase pig morbidity and mortality, increase pro-inflammatory cytokine production, reduce leukocyte numbers and reduce the production of immunoglobulins.
15. Atmospheric ammonia, endotoxins and viable bacteria greatly exacerbate the severity of pathogenic organisms including parasites. Concentrations above 5 ppm for ammonia, 1 μg/m^3 for endotoxins and 50,000 CFU/m^3 for viable bacteria appear to be detrimental to pig growth and health.
16. Social and psychological stress reduce immune cell numbers increase pro-inflammatory cytokine production and cortisol release with resulting reductions in feed intake, protein deposition and performance.
17. Pigs at weaning, particularly early-weaned pigs, are most vulnerable to disease because of their low nutrient intake, an innate immune system that matures after exposure to microbes and an adaptive immune system that develops between 4 and 7 weeks of age. Reducing microbial load and assisting the immune system of weaned pigs has high priority.
18. Pigs, like other animals, suffer from endotoxin tolerance where continual stimulation of the innate immune system with a specific antigen and its Toll-like receptor leads to down-regulation of the immune response after 4-5 days. Approximately 3 weeks without exposure to the antigen is required to fully restore the innate immune response.

7.2. Four Possible Strategy Areas for Improving Immune System Outcomes for Pigs

1. Decrease the Presence of Agents that Elicit an Immune Response

Reduce atmospheric load of viable bacteria, ammonia and endotoxins: The primary drivers of poor air quality are pen and shed cleanliness. Procedures to maintain shed ammonia below 5 ppm, endotoxins below 1 μg/m^3 and viable bacteria below 50,000 CFU/m^3 are outlined in the review, and include batch rearing, thorough cleaning between batches, spraying water above floors and pens

prior to people working in pig buildings and setting a maximum of 300 pigs/air space with an air volume (m^3/pig) of at least 0.0118*Live weight (kg) + 1.82.

Add antimicrobial agents to the diet or drinking water: Alternatives to in-feed antibiotics include Zn oxide at doses exceeding 2000 ppm; organic and inorganic acids; plant derived compounds (carvacol, cinnamaldehyde, oleoresin, allicin, thymol, rosemarinic acid, tochopherols and phenols); antimicrobial peptides (lactoferrin, lactoferricin, lactoferrampin, lysozome, indolicin, purothionin); bacteriophages; clays (kaolin, bentonites, zeolites). The value of zinc and acids is well established. Many of the other compounds have small positive effects on growth of young pigs, but results are inconsistent. The medium chain fatty acids lauric ($C_{10:0}$) and myristic acid ($C_{12:0}$) are toxic to most gram positive and gram negative bacteria and to viruses and their potency is markedly increased when fed as 1-monoglycerides. A combination of monolaurin and monomyristin in the ratio of 2:1 was found to be most potent. Further research into specific antimicrobial peptides and bacteriocins may be worth conducting.

Limit microbial growth stimulants in the diet: Reducing undigested nutrients in the distal intestines can be achieved by replacing traditional grains with cooked rice or groats; eliminating grains particles >1.0 mm; adding glucanase, xylanase and phytase enzymes to the diets; replacing plant proteins with readily digestible animal plasma or milk proteins; reducing the content of amino acid balanced protein to 17.5% for weaner pigs; and avoiding excess iron in diets.

Feed probiotics: Probiotics are fed to young pigs to reduce the number of pathogenic organisms by specific bacteriocin targeting and blocking adhesion sites, competing for nutrients, or increasing GIT acidity. Many experiments with probiotics over 50 years have shown variable and inconsistent results in terms of disease control and animal productivity, however and for example, work by Beale *et al.* (2011) using an avirulent strain of *E. coli* shows promise in reducing post-weaning diarrhoea.

2. Breed Pigs for Enhanced Immunity and High Productivity

Select pigs for a single gene allele: Selecting pigs for a single gene allele is relatively straight forward. The AA allele for the *E. coli* F18 adhesion gene is a possibility, but others genes need to be identified. F18 represents approximately 60% of *E. coli* infections.

Use a selection index that includes immune traits: The high heritability of most immune traits means that they can be readily incorporated into selection indexes that also include desired productivity traits. Selecting pigs, particularly in a 'clean' environment without including immune traits in the index, is likely to produce progeny that are less capable of dealing with 'dirty' environments. Best results are likely to occur when pigs are selected in the same environment that the progeny will be reared and immune traits are included in the selection index.

3. Regulate the Immune Response for Specific Situations

The immune system must be capable of mounting a sufficient immune response for a specific situation and the negative effects of over-stimulation of the immune response (anorexia, fever, proteolysis) should be minimised.

Ensure the diet is balanced for optimal immune response: Formulate diets that closely meet the requirements of pigs without deficiencies of excesses of nutrients.

Eliminate stressors: Ensure pigs are kept in thermoneutral temperatures, with cold, fluctuating temperatures and draughts avoided. Avoid social stress by unnecessary mixing of pigs.

Modulate immune system with n-3 and n-6 fatty acids: Maintain a ratio of 4:1 (n-6:n-3) or less in diets. This criterion should be included in all feed formulation software.

Provide compounds resembling microbial PAMPS that stimulate Toll-like receptors: The innate immune system of young, newly weaned pigs can be stimulated by feeding PAMPS (pathogen-associated molecular patterns) and stimulating specific Toll-like receptors. When given to 1-day old chickens, toll-like receptor agonists including unmethylated cytosine-guanosine oligodeoxynucleotide, cationic peptides (BT/TAMUS 2032) produced by gram-negative bacteria, serum-opsonized salmonella and fungal β-glucan have reduced the effects of salmonella infections by priming the innate immune system. Less research on Toll-like agonists has been conducted with pigs. Mannan oligosaccharide, which binds to Toll-like receptors, when assessed over many experiments improved growth of pigs by 8.5% over the first 7 days after weaning. The response dropped dramatically over subsequent weeks presumably because of endotoxin tolerance. Toll-receptor agonists need to be identified for specific weaner pig diseases and administered for only 4-5 days prior to be pigs coming into contact with the pathogen to avoid endotoxin tolerance.

Provide pre-formed antibodies: Preformed antibodies against specific pathogens are useful for pigs up to about 7 weeks of age. The most common source of preformed antibodies is porcine plasma. At least 10% is required in the diet of weaned pigs during the first 1-2 weeks after weaning. The effectiveness of feeding plasma declines with age and cleanliness of the environment.

Vaccinate against pathogens: Vaccines are effective against many pig pathogens. Frequently autogenous vaccines developed from organisms derived from diseased pigs at a specific location are required. Vaccines need to be administered 2-3 weeks before onset of a disease and have low efficacy when maternal antibodies are present in young pigs. Vaccination of pigs less than 6 weeks of age is generally ineffective because of the immaturity of the adaptive immune system.

4. Negate the Effects of Pro-Inflammatory Cytokines

Reduce the production and activity of pro-inflammatory cytokines: Conjugated linoleic acid when added to diets of chickens and mice is extremely effective for reducing the production of pro-inflammatory cytokines and eliminating the adverse anorexic and weight loss symptoms associated with excess pro-inflammatory cytokines. Anti-inflammatory cytokines also reduce pro-inflammatory cytokine production. An experiment with administration of IL-5 has increased performance of weaned pigs in a commercial environment, but remains unpractical until a non-labour intensive method for administration can be developed. Similarly, experiments using the receptor antagonist for IL-1 (IL-1ra) improve the performance of pigs challenged with the PRRS virus or reared in commercial conditions, but are unpractical due to difficulty in administration.

Use NSAIDs: Non steroid anti-inflammatory drugs are known to reduce inflammatory responses in animals by depressing pro-inflammatory cytokine production and the production of the COX-2 enzyme. A few experiments with pigs show small positive effects on intake, performance and health from the use of aspirin, indomethacin and ketoprofen.

Nullify the production of PGE_2: The prostaglandin PGE_2 has been shown to be responsible for many of the adverse effects of anorexia, fever, reduced activity and increased proteolysis associated with excess pro-inflammatory cytokines. PGE_2 is produced from dietary and cell membrane phospholipids via secretory phospholipidase A_2 ($sPLA_2$) to produce arachidonic acid, which is catalysed by the COX-2 enzyme. Strategies have been used to nullify the production of PGE_2 by

using COX-2 inhibitors and antibodies against $sPLA_2$ produced in hen eggs. These antibodies have been successful in increasing performance of young chicks raised in conventional environments. One experiment with pigs showed a small positive effect.

7.3. Recommendations

1. Develop an adoption process to apply on farms which includes the many known practices that ensure the immune system of commercially raised pigs is not excessively challenged by and is primed to respond appropriately to pathogenic organisms. These practices include:
    a. Maintaining the desired air quality considering the methods outlined in the review.
    b. Ensuring pigs are not held in environments below their lower critical temperatures.
    c. Formulate and feed diets that do not limit an immune response because of either deficiencies or excesses of nutrients.
    d. Formulate diets with a ratio of n-6:n-3 polyunsaturated fatty acids of 4:1 or less.
    e. Provide ingredients and feed processing practices that enable the majority of nutrients to be digested in the proximal small intestine.
    f. Develop and implement an appropriate vaccination schedule for sows and their progeny.

2. Undertake research to improve the resistance of weaned pigs to pathogens and to negate over-stimulation of the immune system. Specific factors that warrant investigation either alone or in combination in commercial piggery environments include:
    a. Feeding 1-monoglyceride lauric acid and 1-monogluceride myristic acid singly and in combination in the diet of pigs for several weeks post-weaning.
    b. Feeding of both cis-9, trans-11 and trans-9, cis-12 CLA molecules to pigs for several weeks post-weaning.
    c. Provide either in the diet or by other means, selected NSAIDs for 1-2 weeks post weaning.
    d. Feed porcine plasma for 1-2 weeks post weaning.
    e. Provide COX-2 inhibitors, specifically celecoxib, for several weeks post-weaning.
    f. Develop $sPLA_2$ antibodies in either hen eggs or cow milk and feed for several weeks post weaning. If antibodies to $sPLA_2$ are successfully developed, they would have wider application for treatment whenever a febrile disease occurs or is likely to occur.

3. Investigate the feasibility for other possible areas of future research including:
    a. Identification of toll-receptor agonists associated for diseases specific to weaner pigs in Australia and for which there is no successful non-antibiotic treatment. These toll-like agonists would be fed or administered for 4 days prior to exposure to the pathogen to avoid endotoxin tolerance but prime the innate immune system.
    b. Identify possible single gene alleles that could block the virulence of pathogens specific to pigs in Australia and for which there is no successful non-antibiotic treatment. Once identified, the feasibility of including them in a pig breeding program would need to be assessed.
    c. Identify potential antimicrobial peptides such as those listed above, bacteriocins and/or bacteriophages that could be included in weaner diets to reduce microbial load and/or specific pathogens.
    d. Investigate possible existing methods for administering anti-inflammatory cytokines or IL-1ra to pigs peri-weaning that are feasible for use on commercial farms.

8. Intellectual Property

Not Applicable.

9. References

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10. Publications Arising

Not Applicable.