THE EFFECT OF ENVIRONMENTAL ENRICHMENT ON THE IMMUNE RESPONSE AND MEASURES OF DISEASE RESILIENCE AND WELFARE IN PIGS

A thesis submitted to the

College of Graduate and Postdoctoral Studies

In partial fulfillment of the requirements

for the degree of Master of Science

In the Department of Large Animal Clinical Sciences

University of Saskatchewan

Saskatoon

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ABSTRACT

Disease is a considerable threat to swine well-being and economic productivity. Further, standard barren rearing environments are associated with chronic stress, which can suppress immune function and growth, and contribute to negative social behaviour. Environmental enrichment may mitigate harmful effects of stress, but more research is needed on types of enrichment that improve swine well-being while being practical to use in commercial facilities. This thesis aimed to identify whether pigs provided a rotation of inedible point-source enrichments (E) differ in immune cell concentrations and behaviour compared to barren-housed controls (C), and to explore relationships between behaviour and immune response within each treatment. Chapter Three compared behaviour, productivity, complete blood counts (CBC), and mortality of C and E pigs in three phases of the experiment: quarantine (Q), polymicrobial natural disease challenge (NDC), and finisher (F). E pigs were more likely to interact with enrichments than C pigs on most observation days, but use declined within each phase. E pigs were also more likely to perform comfort-related postures and less likely to show illness-related postures early in the Q and F phases. Lastly, E pigs demonstrated a greater increase in total white blood cell concentration from pre- to post- challenge. Chapter Four examined relationships between individual pigs' social and exploratory behaviours and their growth rate, CBC values, and disease resilience (classified using mortality, growth, and veterinary treatment rate). E treatment altered Q behaviour: enrichment use was positively correlated with pen rooting and both positive and negative social interactions. Pen rooting was also positively correlated with positive social behaviour in Q and NDC, and with concentrations of white and red blood cells, hemoglobin, and lymphocytes. These results suggest that behavioural influences of enrichment were more likely when enrichment use was highest within a phase. In conclusion, pigs provided with inedible point-source enrichments differed in social and exploratory behaviours and posture frequencies, and provision of enrichment influenced relationships between exploratory behaviour, growth, and cellular immune response. More research is needed on providing enrichment that sustains use and satisfies motivational needs, and therefore may have a greater impact on stress reduction and disease response.

Acknowledgments

First and foremost, I would like to acknowledge the dedication, hard work and long hours put in by my supervisor, Dr. Yolande Seddon. Throughout my studies, she provided a wealth of knowledge, advice, support, and opportunities for personal and professional growth for which I am eternally grateful. Thank you also to Dr. John Harding for his guidance as a committee member.

Thank you also to Dr. Giuliana Miguel-Pacheco for her extensive help with everything from statistical analysis to editing this thesis, to Luis Gonzalez-Gracia and Herman Coceancigh for their assistance with behaviour analysis, and to the members of the swine ethology team at the Western College of Veterinary Medicine for their friendship and advice. As well, many thanks to the dedicated staff at the CDPQ and CRSAD research centres for facilitating and overseeing this experiment.

Thank you to the incredible instructors and mentors who taught me during my undergraduate degree at the University of Alberta and who assisted me in beginning my journey towards this graduate degree. In particular, I want to acknowledge the mentorship provided by Drs. Frank Robinson, Michael Dyck, Graham Plastow, and Clover Bench.

Lastly, I would like to express my gratitude towards my husband, Ian Chenier, for your unwavering positivity and support throughout this long journey.

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LIST OF ABBREVIATIONS

Q: quarantine phase

NDC: polymicrobial natural disease challenge

F: finisher phase

C/CTRL: control (barren-housed) treatment

E/ENR : enrichment treatment

CDPQ : Centre de Développement du Porc du Québec

CRSAD: Centre de Recherche en Sciences Animales de Deschambault

ADG: average daily gain

FCR: feed conversion ratio

TR: veterinary treatment rate

CBC: complete blood count

RBC: red blood cell (concentration)

HGB: hemoglobin

HCT: hematocrit

WBC: white blood cell (concentration)

NEUT: neutrophil (concentration)

LYMPH: lymphocyte (concentration)

N:L: neutrophil to lymphocyte ratio

MONO: monocyte (concentration)

BASO: basophil (concentration)

EOS: eosinophil (concentration)

NK (cells): Natural killer T-cells

CRH: corticotrophin releasing hormone

ACTH: adrenocorticotropic hormone

TH1/ TH2:T helper cell type 1/ type 2

NAAb: natural autoantibody-binding factors

IgG: immunoglobulin- G

IL-: Interleukin

IFN-γ: interferon-γ

TNF-α: tumor necrosis factor-α

HPA axis: hypothalamic-pituitary-adrenal axis

PRRSV: porcine reproductive and respiratory syndrome virus

PCV2/PCVAD: porcine circovirus/ porcine circovirus associated diseases

FAWC: Farm Animal Welfare Committee FAWC

NFACC: National Farm Animal Care Council

OIE: World Organization for Animal Health

EU: European Union

CQA: Canadian Quality Assurance

CDHI: Control of Disease in the Hog Industry

CSHB: Canadian Swine Health Board

GDP: gross domestic product

PVC: polyvinyl chloride

RFID: radio-frequency identification

ELISA: enzyme-linked immunosorbent assay

EDTA: ethylenediaminetetraacetic acid

1. GENERAL INTRODUCTION

Swine production in Canada has evolved significantly over the last century to efficiently produce food that meets the demands of a rapidly growing population (Brisson, 2015), but intensive agriculture faces considerable challenges. Disease is a forefront issue for North American swine producers, negatively impacting economic productivity and animal well-being (VanderWaal and Deen, 2018). Factors such as long-distance transport, continuous flow of large numbers of animals and workers within barns, and an ever-growing number of novel pathogens have rendered conventional biosecurity and vaccine protocols less effective at controlling disease spread (Desrosiers et al., 2011, Zimmerman et al., 2012). Coupled with rising consumer interest in improving animal welfare (Bracke, 2006) and concerns over the impact of farm-level antibiotic use on human health (Van Dixhoorn et al., 2016, VanderWaal and Deen, 2018), investigation into swine management practices that improve health and welfare is increasingly important.

There is a growing interest in controlling disease by producing pigs that are less susceptible to disease (Mulder and Rashidi, 2017). Disease resistance, the ability of an animal to control their pathogen burden without succumbing to illness, has been a long-standing topic of livestock research (Albers et al., 1987, Mulder and Rashidi, 2017). However, disease resistance is typically pathogen-specific, and genetic selection for resistance can unintentionally select against productivity traits (Guy et al., 2012, Wilkie and Mallard, 1999). Instead, the concept of disease resilience is gaining traction in research (Mulder and Rashidi, 2017, Putz et al., 2019). Unlike measuring resistance, the pathogen load does not need to be known to quantify disease resilience- it is instead defined as "the ability to maintain a relatively undepressed production level when infected" (Albers et al., 1987, p.1355). In addition to measures of productivity under disease pressure, there is evidence that resilient and susceptible pigs may differ in immune cell concentrations (Bai et al., 2020), indicative of differences in immune response to infection.

While research on disease resilience in pigs has largely focused on genetic heritability (Bai et al., 2020, Putz et al., 2019), there is also evidence that improvements made to rearing environments may reduce stress and subsequent stress-induced immunosuppression, therefore reducing disease susceptibility (Bolhuis et al., 2003, Luo et al., 2020, Van Dixhoorn et al., 2016).

Barren pens with fully slatted plastic or concrete floors are commonly used on swine farms in North America to improve sanitation and ease of cleaning (Nocella et al., 2010, Tuyttens, 2005). However, this style of housing limits the expression of natural functional behaviours such as rooting and chewing (Petersen et al., 1995) and is associated with behavioural and physiological markers of chronic stress (Beattie et al., 2000, Van de Weerd et al., 2003). In turn, chronic stress can cause immunosuppression (Dhabar, 2009), depressed growth (Barnett et al., 1983, Pearce et al., 1989). Frustration and boredom resulting from restricted behaviour in barren pens increase the likelihood of abnormal behaviours like pen-rooting and sham chewing and negative social behaviours such as oral manipulations and biting of penmates (Beattie et al., 2000, Petersen et al., 1995). Environmental enrichment materials that can be rooted, chewed, and ingested, are of high biological value and can reduce stress, thereby supporting immune function (Ernst et al., 2006, Luo et al., 2017), improving growth performance (Oliveiera et al., 2016), and reducing incidence of negative social behaviours (Petersen et al., 1995, Van de Weerd et al., 2006). However, rooting substrates are often not compatible with slatted flooring and liquid manure slurry systems and require additional labour to clean and maintain (Tuyttens, 2005). Point-source enrichments, objects that are fixed in size and location within a pen such as suspended jute sacks, rubber or plastic toys, and cotton ropes, are a common enrichment alternative that can be used in slatted-floor pens and generally require less labour and cleaning effort than bedded systems (Tuyttens, 2005). Particularly when suspended from the ceiling, point-source objects also can encourage social play and exploration by allowing multiple pigs to interact at once (Van de Weerd et al., 2003). Point-source enrichments have not yet been studied for their influence on disease response, and research in this area would be a valuable addition to the understanding of environmental influence on disease resilience.

The overall objective of this thesis is to determine whether measures of disease response, disease resilience, and behaviour of pigs differ when housed with a rotation of inedible point-source enrichment or in barren pens with suspended metal chain enrichments during a polymicrobial natural disease challenge (NDC). To meet this objective, this thesis begins in Chapter Two with a review of relevant scientific literature on current health and welfare concerns in the North American swine industry, the physiological and psychological impacts of disease and stress, and relationships between rearing environment conditions and measures of health and welfare in swine. The literature reviewed in Chapter Two provides a background that identifies

gaps in the understanding of how to modify or enrich the rearing enrichment in ways that are effective at providing long-term improvements to swine health and/or welfare while also being practical for producers to implement.

Chapter Three introduces the first experiment in this thesis, which aims to determine whether a rotation of inedible point-source enrichments impacts pen-level behavioural and physiological (individual CBC values, pen-level mortality and growth rate) measures of disease response and productivity before, during, and after an NDC. Next, the relationship between individual pig behaviour and immune cell concentrations is a novel area of research that is explored in Chapter Four. Individuals can differ considerably in their stress coping style and in the degree to which rearing environment and social support impact their ability to cope with stress (Reimert et al., 2014), but there is little research on individual-level social behaviour, how point-source enrichment influences this, and how social behaviour corresponds to physiological performance. Further, negative social behaviour can be both a cause and a symptom of stress or disease (Munsterhjelm et al., 2019), while positive social interactions may reduce stress and improve productivity (Camerlink et al., 2012). It is therefore valuable to examine how an individual's social behaviour and immune cell concentrations influence each other, and how those relationships may differ for pigs raised with and without enrichment. Chapter Four of this thesis explores correlations between behaviour, growth, and immune cell concentrations, and categorizes individuals by disease resilience using mortality status, growth rate and veterinary treatment rate. Lastly, Chapter Five provides a general discussion on the experimental results from the previous two chapters, their possible interpretations, and study limitations. Suggestions are then given for areas of future research that could contribute to understanding of the effects of environmental enrichment on population- and individual-level measures of swine well-being disease pressure.

This thesis contributes to the field of understanding of swine welfare by exploring relationships between an individual pig's behaviour, immune cell concentrations, and productivity in response to disease pressure, and how this may be influenced by inedible point-source enrichments. Behavioural expression is a key measurement for understanding of swine health and welfare; as such, examining behaviour as it relates to disease is a useful addition to the development of ways to measure and enhance resilience in swine.

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2. LITERATURE REVIEW

2.1 Introduction

The Canadian swine industry has undergone significant expansion and restructuring over the last century to meet the demands of a rapidly growing population (Brisson, 2015). While the number of hogs in Canada rose from 3.3 million in 1921 to 12.7 million in 2011, the number of hog farms dropped from 452,935 to 7,371 in the same period. Intensification of farming has improved productivity but came with consequences: cross-border movement of animals and feed, coupled with large herds on commercial farms provides an opportunity for rapid disease spread (VanderWaal and Deen, 2018). Disease threatens farmers' livelihoods and increases global food insecurity when outbreaks restrict international food trade. Hog farmers have seen an upsurge in infectious diseases over the last 20 years, including porcine reproductive and respiratory Syndrome virus (PRRSV) and porcine circovirus (PCV2), costing the North American industry hundreds of millions of dollars annually (Brisson, 2015). Additionally, public distrust in intensive agriculture has grown in recent years, with crowded, unnatural housing, mishandling of animals, and painful procedures (tail docking, castration) at the forefront of consumer concerns (Nocella et al., 2010, Spooner et al., 2014a, Vanhonacker et al., 2007).

In addition to economic impacts, disease has serious welfare implications for livestock. Not only can disease cause stress, pain, and suffering (Martínez-Miró et al., 2016), but there is evidence that it also negatively affects social behaviour and affective state (Brown et al., 2018) and depresses growth (Albers et al., 1987). There are a multitude of diseases that can impact swine (Zimmerman et al., 2012), and controlling the direct and indirect spread of many pathogens has proven difficult (Desrosiers, 2011). Methods to mitigate the negative economic and welfare impacts of disease are therefore of interest to the industry. Recently, interest has increased in methods of improving resilience to offset the negative impacts of disease. Disease resilience can be defined as "the productivity of an animal in the face of infection" and may also be indirectly indicated by a reduction in veterinary treatments required while under disease pressure (Bishop, 2012). Not only is animal behaviour a relevant indicator of stress and disease (Munsterhjelm et al., 2019), but there is evidence that providing outlets for natural behaviours can enhance immune function (Ernst et al., 2006, Luo et al., 2017, Luo et al., 2020a) and reduce disease susceptibility, allowing animals to maintain productivity and improved welfare while under disease pressure (Van Dixhoorn et al., 2016). Adapting and expanding the definition from Bishop (2012), a resilient

individual could be described as one that, under increased immune pressure, is able to maintain relatively undepressed productivity, require few or no veterinary treatments, and exhibit few behavioural and physiological indicators of stress, illness, or discomfort.

It is well-documented that effective, biologically relevant environmental enrichment allows for the expression of natural behaviours in pigs; in turn, enriched pigs may benefit from reduced stress (Munsterhjelm et al., 2010, Van de Weerd and Day, 2009), lower aggression (Chou et al., 2019, Telkänranta et al., 2014, Ursinus et al., 2014a,) and improved productivity (Beattie et al., 2000, Van de Weerd and Day, 2009). More recently, research has shown that enrichment can also improve outcomes during infection through improved wound healing (Ernst et al., 2006) and immune response to disease (Luo et al., 2020a, Van Dixhoorn et al., 2016). Enrichment therefore shows promise for improving resilience by modifying the environment to better suit the behavioural, psychological, and/or physical needs of the animals.

This review will outline research on how stress and disease are challenging the swine industry, the impact environment has on swine health and welfare, and how knowledge and application of pig behaviour could be applied to address these concerns using environmental enrichment. Additionally, the physiological effects of the environment will be explored, and opportunities for future research identified.

2.2 Challenges and Regulations in the Canadian Swine Industry

2.2.1 Animal Welfare Concerns

The World Organization for Animal Health (OIE) defines that an animal has good welfare when it is "healthy, comfortable, well nourished, safe, is not suffering from unpleasant states such as pain, fear and distress, and is able to express behaviours that are important for its physical and mental state." (OIE, 2019, p. 1). This definition is a summary of the 'Five Freedoms' that farm animals should have in order to have good welfare, as originally defined in the 1965 Brambell Report (Brambell, 1965). The Five Freedoms (below) are the basis of many modern-day animal welfare assessments and are the criteria upon which many countries' welfare legislations are largely based (OIE, 2019). The following list outlines the Five Freedoms as updated by the Farm Animal Welfare Committee (FAWC) in 2010:

i) Freedom from hunger and thirst - by ready access to water and a diet to maintain health and vigour.

ii) Freedom from discomfort - by providing an appropriate environment including shelter and a comfortable resting area.

iii) Freedom from pain, injury, and disease - by prevention or rapid diagnosis and treatment.

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

v) Freedom from fear and distress - by ensuring conditions and treatment, which avoid mental suffering.

With rapid expansion and intensification of livestock agriculture, beginning in the mid-late 20th century, emphasis on efficiency and productivity rose dramatically (de Jonge and van Trijp, 2013). Swine production shifted to largely indoor housing, where pigs are typically kept at high stocking densities in barren, slatted-floor pens to increase efficiency (Nocella et al., 2010). Many elements of intensive swine facilities that improve efficiency have associated trade-offs with aspects of biological health, natural living conditions, and/or animal welfare (Beattie et al., 2000, Kauppinen et al., 2010). Broadly, many of the welfare issues in modern livestock production stem from a disconnect between the biological needs and drives of the animal and the environment they live in (Beattie et al., 2000). The human-animal relationship is of additional concern (Vanhonacker et al., 2007) as handling can be a significant source of fear and distress (Martínez-Miró et al. 2016).

The Five Freedoms (FAWC, 2010) appear to be violated with many modern swine management practices: for example, comfort and expression of normal behaviours are improved in bedded pens and with greater space allowance (Newberry, 1995) when compared to barren pens with slatted flooring, but the latter is most common in North America (Tuyttens, 2005). Piglets experience pain during procedures such as tail docking and castration (Übel et al., 2015), fear and distress from handling (Martínez-Miró et al. 2016), and a variety of diseases are present and common in commercial facilities (Zimmerman et al., 2012). However, welfare issues are rarely black and white: management practices are typically implemented to manage or improve health and welfare, such as reducing the risk of tail biting by docking tails or improving sanitation with slatted flooring. For this reason, welfare regulations exist for the care and handling of livestock,

and aim to balance the (at times, conflicting) needs of the animals with those of producers and consumers (OIE, 2019).

While profitability and efficiency are crucial elements of modern-day animal production, welfare trade-offs of intensive agriculture have been met with increasing criticism over the years (Bracke, 2006, Grunert et al., 2018, Kauppinen et al. 2010). Surveys of consumers on their perception of livestock welfare have identified natural, uncrowded living conditions, careful handling, and modification or elimination of painful and stressful procedures (castration, tail docking) as some of the most important welfare issues in swine production (Nocella et al., 2010, Spooner et al., 2014a), and the citizens interviewed generally found animal welfare to be problematic at the time of surveying (Nocella et al., 2010, Vanhonacker et al., 2007). In contrast, conventional swine producers are more likely to measure welfare based on physical health and productivity (Bock and van Huik, 2007, Spooner et al., 2014b), place less importance on natural living, space allowance, and reduction in painful procedures (Spooner et al., 2014b, Vanhonacker et al., 2007), and are more likely to believe that farm animals generally have good welfare (Vanhonacker et al., 2007). The surveyed producers generally agreed that good physical health and steady growth are reliable indicators of good welfare (Spooner et al., 2014b).

Despite the perceived importance of welfare-friendly production to consumers, animal welfare attributes are not always highly ranked characteristics for consumers when choosing meat (Grunert et al., 2018). Consumers' willingness to pay for 'welfare-friendly' products appears to differ regionally, and is influenced by factors such as national policy, cultural differences, and consumer awareness of welfare issues associated with animal production (Font-i-Fournols & Guerrero, 2014). This contributes to a disconnect between consumer and producer views on welfare. There is a significant associated cost with improving living conditions and changing management practices, making it less appealing to implement changes if they are unable to receive a price premium on the final products (Bracke et al., 2005). The reluctance of producers to improve welfare presents a need for further research and development of management practices and welfare regulations that are both welfare-friendly and practical to implement.

2.2.2 Regulations on Welfare and Enrichment for Pigs

Welfare regulations exist to provide producers with science-based care requirements that address the physiological and psychological needs of animals (OIE, 2019). The World Organization for Animal Health (OIE) provides guiding principles for the ethical and welfare-friendly use of animals in agriculture and research, and science-based recommendations for maintaining and assessing good welfare at the global level (OIE, 2019). Many countries and geographic regions also have their own set of minimum welfare standards or legislations. For example, the European Union (EU) legislates minimum animal welfare standards through the Council Directive 2008/120/EC (EU, 2009); countries within the EU can then choose to set additional standards above the minimum requirements. In Canada, the National Farm Animal Care Council (NFACC) Code of Practice for the Care and Handling of Pigs (NFACC, 2014) is a set of regulations and 'best practice' recommendations that is not legally mandated but is required to be followed if a producer wishes to sell their pigs using the Canadian Quality Assurance (CQA) program (CQA, 2021). Requirements are updated periodically to reflect up-to-date scientific research on how best to meet the animals' needs, while also considering practicality for producers and expectations of consumers (Veissier, 2008). Welfare requirements such as the CQA program not only ensures that animals are cared for properly, but also to give consumers confidence that industry is addressing welfare concerns and continually improving.

Barren environments are one area of concern in swine production. There is considerable evidence that barren environments are detrimental to the health and well-being of pigs, as they are lacking in stimulation and outlets for performance of natural behaviours (Newberry, 1995). Consequences can include abnormal and aggressive behaviours (Beattie et al., 2000), depressed growth rate (Barnett et al., 1983, Pearce et al., 1989), and immune system suppression (Luo et al., 2017, Van Dixhoorn et al., 2016). The ability to perform natural behaviours is one element of the OIE (2019) definition of good welfare, and one of the 'Five Freedoms' (FWAC, 2010), enrichment as a tool to improve the environment is addressed in both the Code of Practice (NFACC, 2014) and EU Directive 2008/120/EC (EU, 2009). Loose, ingestible rooting substrates are generally regarded as the most consistent influencer on psychological and physical well-being in pigs, due to their species-specific biological relevance (Van de Weerd and Day, 2009). This knowledge has been used to form the EU Directive requirement for provision of multiple forms of enrichment, which must include some manipulable rooting materials, for all pigs in captivity (EU, 2009).

Similarly, the Code of Practice (NFACC, 2014) requirement for enrichment states that producers must provide pigs with "multiple forms of enrichment that aim to improve the welfare of the animals through the enhancement of their physical and social environments" (p. 19). To meet this requirement, the Code states that producers should provide a variety of enrichments from several categories including social, occupational, physical, sensory, and nutritional enrichments. A recommendation is made that pigs should be provided with "continual access to a range of novel suspended toys" (NFACC, 2014, p.19) as well as physical enrichments such as straw, hay, or peat. However, unlike the EU Directive, the Code notes that rooting materials may be substituted for manipulable floor toys in housing systems where substrate is not compatible with the manure management system used. This is a potential downfall of the Code, as it allows broad interpretation on the type(s) and application of enrichment, and pigs may not receive the health and welfare benefits if inappropriate items with little biological relevance are used.

2.2.3 Impact of Disease on the Swine Industry

Production losses and veterinary costs due to disease cost the hog industry hundreds of millions of dollars per year in North America (Brisson, 2015). With agriculture accounting for 11% of Canada's goods GDP and roughly 70% of pork production being exported (Agri-Food Exports, 2019), disease resulting in reduced productivity or trade can have devastating effects on both producers and Canada's agricultural economy. Notably, PRRSV and porcine circovirus (PCV2)/porcine circovirus associated diseases (PCVAD) have challenged the industry, contributing to the 2007 launch of an initiative for the Control of Disease in the Hog Industry (CDHI). The CDHI provided swine producers with funding for detection of and vaccination against PCVAD from 2007 to 2010 and was successful in controlling the outbreak (Agriculture and Agri-Food Canada, 2015). From 2008 to 2015, the CDHI funded 26 research projects on swine diseases and paired with the Canadian Swine Health Board (CSHB) to deliver information on biosecurity and disease risk management to producers. However, they did not find evidence that new biosecurity standards and a developed surveillance network had widespread use in Canada. This indicates that there are significant barriers to adoption of recommended disease prevention practices that need to be evaluated.

Further, differences in transmission modes between common diseases and infections present significant challenge to controlling disease, requiring a multi-pronged approach to herd health management. Since the 1990s, the prevalence of some pathogens requiring direct contact for transmission, such as toxigenic *Pasteurella multocida,* and external parasites like mange mites (*Sarcoptes scabiei*), has decreased considerably in North America (Desrosiers, 2011). Pathogens spread by direct contact can typically be managed with standard biosecurity measures including quarantine of sick animals and maintaining high sanitation standards to protect naïve pigs from contact with infected pigs and contaminated pens (Desrosiers, 2011). Strict biosecurity and selection of genetically superior and disease-negative breeding stock has also allowed breeding facilities to maintain high health standards, thereby providing un-diseased nursery pigs to grow-finish farms. Coupled with an increase in adoption of "all in- all out" systems- as opposed to continuous flow, producers are better equipped to maintain pathogen-free herds, leading to a decrease in prevalence of many direct-contact transmitted pathogens. Meanwhile, other direct-contact transmitted pathogens including *salmonella spp*. and *E. coli* are still prevalent in herds globally, and of concern to human health (VanderWaal and Deen, 2018), indicating gaps in disease management practices that are reducing prevalence of certain diseases while allowing continued spread of others.

In contrast, certain pathogens such as PRRSV and *Mycoplasma hyopneumoniae* can be spread by indirect contact via aerosolized particles, and 'local' spread, where disease is transmitted to unaffected farms from nearby infected facilities, is of concern (Desrosiers, 2011). These diseases are significantly harder to control, as there are many possible modes of infection and standard sanitation, and biosecurity practices often will not suffice to prevent spread. The five infectious swine pathogens identified as highest priority in North America in 2018 were PRRSV, *salmonella* spp., *Escherichia coli* (*E. coli*), PCV2, and *Staphylococcus aureus* (*S. aureus*) (VanderWaal and Deen, 2018). Of these, three are commonly spread by indirect transmission (PRRSV, PCV2 and *S. aureus*), and their prevalence has increased dramatically since the 1960s and 1970s despite stricter biosecurity protocols. While no cases of African Swine Fever have been reported in North America as of June 2022 (US Food and Drug Administration, 2022), it is another pathogen of great economic and welfare concern globally which can be spread directly and indirectly, thus increasing the scope of its potential spread (Government of Canada, 2021).

The ever-growing number of pathogens worldwide presents a significant challenge to on-farm disease control (Zimmerman et al., 2012). Coupled with the high animal density on farms, and frequent travel of both animals and humans between farms, diseases can spread rapidly and across long distances (VanderWaal and Deen, 2018). While improved biosecurity has limited the spread of some pathogens, it has largely been ineffective at preventing the spread of disease spread through indirect means (Desrosiers, 2011). Given the challenges faced in controlling both direct and indirect disease spread, there is increasing interest in methods to reduce the disease susceptibility of the animals in addition to herd health management practices (biosecurity, vaccination, sanitation of facilities). Reducing disease susceptibility through genetic selection and/or environmental modifications that support a robust immune system is one proposed tool to improve disease control where standard health management practices are insufficient (Bai et al., 2020, Mulder and Rashidi, 2017, Plastow, 2016). The concepts of disease resistance, resilience, and susceptibility will be explored in a subsequent section of this review.

2.3 Health, Stress and Disease

Good health for livestock is crucial for productivity and welfare, and begins with a robust, uncompromised immune system that allows animals to respond appropriately to disease challenges and recover with minimal loss to production (Albers et al., 1987). In contrast to disease susceptibility, three broad categories of optimal disease response exist: resistance, tolerance, and resilience. Resistance refers to the "ability to control pathogen burden" without showing signs of infection (Mulder and Rashidi, 2017, p.3346), while a disease tolerant animal can maintain homeostasis and productivity/performance in spite of their pathogen load (Nakov et al., 2019). Mulder and Rashidi (2017) suggest a subtle difference between tolerance and resilience definitions: tolerance is the "ability to maintain production given the pathogen burden" while resilience is the "ability to maintain performance regardless of pathogen burden" (p.3346), allowing resilience to be quantified without knowledge of the pathogen load of an individual.

A 'resilient' animal is one that is better capable of responding to, and coping with, disease pressure than others under the same pressures while maintaining growth performance (Albers et al., 1987, Mulder and Rashidi, 2017, Plastow et al., 2016). Resilient individuals may differ from their susceptible counterparts in their stress coping ability (Reimert et al., 2014), behaviour, cellular immune response, growth rate, morbidity, and mortality (Mulder and Rashidi, 2017).

However, identifying the factors contributing to an animal's resiliency, and using that information to produce and maintain a more resilient herd, is more challenging. Manipulating resilience through altered genetics and/or environmental modifications to support good health may therefore improve productivity and welfare despite disease pressure faced (Plastow et al., 2016). Stress is a key factor that impacts an animal's disease response (Salak-Johnson and Mcglone, 2007), and there is evidence that improving the environment can improve stress coping ability, thereby reducing the immunosuppressive effects of stress, and enhancing disease response and/or resiliency (Reimert et al., 2014). This section outlines the physiology of health, stress, and disease, and reviews previously investigated methods of enhancing resilience in swine.

2.3.1 The Immune System

An animal's immune system exists to protect against threats to their internal homeostasis and works to restore homeostasis when an upset has occurred (Roth, 1992, Chase and Lunney, 2019). Immune system development begins in utero and continues throughout life; development is influenced by a multitude of internal and external factors including genetics, age, sex, and the type, duration, and frequency of stressors to which they are exposed (Moberg, 2000).

Upon recognition of a foreign microorganism in the body, the immune system can mount one of two types of responses: an innate or an adaptive immune response (Chase and Lunney, 2019). The innate immune response is a non-specific response to an unrecognized foreign 'invader', increasing circulation of phagocytic cells (macrophages, neutrophils, lymphocytes), cytokines, chemokines and/or antimicrobial proteins and subsequently recruiting natural killer (NK) T cells (Roth, 1992). Inflammation (localized or systemic) and fever typically result from this response. In addition to inducing inflammation, NK cells also produce cytokines that assist in recognition and memory of the invader so that an adaptive immune response and pathogen-specific antibodies can be produced if the same pathogen is encountered later in life (Gerner et al., 2009). Cytokines are immunomodulating molecules predominantly produced by T-cells and are essential for immune system regulation, and they can be divided into two functional groups: proinflammatory and anti-inflammatory cytokines, which can also produce allergic responses (Berger, 2000). Cytokines produced by helper T-cells (TH) fit into two further sub-categories: TH1- and TH2-type cytokines, of which TH1- type are typically proinflammatory and cell-mediated while TH2-type are largely anti-inflammatory and antibody-mediated, playing a role in

down-regulating the TH1 response to limit self-cell damage (Chase and Lunney, 2019). Intracellular pathogens (e.g., *Salmonella spp.)* typically require a TH1 response while extracellular pathogens (e.g. *Mycoplasma spp.)* require a TH2 response; however, an effective immune response to most pathogens will be balanced, with both cell-mediated (TH) and humoral (TH2) responses mounted. Key cytokines in the porcine immune system include interferon (IFN)-γ, which activates the TH1 proinflammatory response, as well as IL-13, which is an important anti-inflammatory cytokine involved in producing a TH2 response and down-regulating the TH1 response.

In subsequent infections with a recognized pathogen, the innate immune response is activated first and continues to fight infection while the adaptive immune response recognizes and mounts either a humoral (B-cell) or cell-mediated (T-cell) response (Chase and Lunney, 2019). In a cell-mediated response, antigen-specific cytotoxic T-cells, phagocytes, and cytokines are activated to destroy foreign antigens without the activation of antibodies. In the humoral response, B cells are activated to produce antibody-secreting plasma cells after recognition of a known antigen; the antibodies then bind to specific sites on the target antigen cells to neutralize them (Janeway et al., 2001). Immunoglobulin-G (IgG) cells are a primary class of serum antibodies involved in humoral immunity and play a key role in proinflammatory cytokine responses (Chase and Lunney, 2019). Basophils play an important role in humoral immunity as they act as effector cells, binding to antigen-specific immunoglobulins, as well as inducing a shift towards TH2-mediated immunity (Mair et al., 2014). Concentrations of red blood cells (RBC), hemoglobin, and hematocrit may also be measured in a sick animal to assess blood oxygen carrying capacity (George-Gay and Parker, 2003), particularly when infected with pathogens known to cause non-regenerative anemia, such as PRRSV (Halbur et al., 2002). However, previously thought to solely function as blood oxygen carriers, evidence has emerged that RBCs and platelets are also important in immune response modulation (Anderson et al., 2018). Platelets play a role in upregulating immune activation, binding and destroying pathogens, chemokines, and nucleic acids in the blood, and in maintaining inactivity in the absence of foreign antigens (Hottz et al., 2018).

The roles of key immune cells involved in both innate and adaptive immune responses are summarized in Table 2.1.

Table 2.1. Roles of key immune cell types in the innate and adaptive immune responses to disease in swine.

Cell Type	Cell subsets	Function
Mononuclear phagocytes[1]	Monocytes	Phagocytosis of viruses & bacteria
	Myeloid-dendritic cells & macrophages	Antigen presentation
Lymphocytes[1]	Natural Killer T-cells	Non-specific inflammation; produce cytokines
	T-cells	Fight viruses & overactive self-cells
	B-cells	Make specific antibodies (adaptive immunity)
Neutrophils[1]		Fight bacteria and fungi Heal damaged tissues
Basophils[1]		Secrete chemicals that fight allergies & infectious agents
Eosinophils[1]		Anti-parasitic, fight allergies & overactive self-cells
Red blood cells[2]		Immune response modulation Bind & destroy chemokines, pathogens & nucleic acids in the blood

[1]Chase and Lunney, 2019; [2]Anderson et al., 2018

2.3.2 Stress Physiology

Animals in captivity encounter many physical and physiological stressors; events that upset, or threaten to upset, homeostasis (Aich et al., 2009). Main causes of stress in livestock production fit into five key categories: social, environmental, metabolic, immunological, and handling (Martinez-Miro et al., 2016). Acute stressors that pigs encounter include weaning, transportation and mixing with unfamiliar pigs (Martinez-Miro et al., 2016), while continuous or frequent stressors contribute to chronic stress, include antagonistic social interactions (Tuchscherer et al., 2014) and barren environments ill-suited to meet their behavioural needs (Beattie et al., 2000, Van de Weerd et al., 2003). The physiological stress response elicited will vary based on the duration of stressors (acute versus chronic), social status, age, genetics, and individual stress coping style (Reimert et al., 2014, Salak-Johnson and Mcglone, 2007).

At equilibrium, circulating cortisol levels follow a diurnal circadian rhythm, with basal concentrations varying throughout the day, regulated by the hypothalamic-pituitary-adrenal (HPA) axis (Ruis et al., 1997). Stressors trigger the hypothalamic-sympathetic system to release catecholamines (epinephrine and norepinephrine), activating the HPA axis (Swanson and Sawchenko, 1980). In response, heart rate and blood pressure are increased, and vasoconstriction occurs, dropping body temperature (Dallman and Hellhammer, 2011, Ruis et al., 2001). From there, corticotrophin releasing hormone (CRH) stimulates production and release of adrenocorticotropic hormone (ACTH). Finally, ACTH is circulated to the adrenal cortex, where glucocorticoids, commonly known as 'stress hormones,' are released. In swine, cortisol is the primary glucocorticoid released following HPA axis activation. Acute stress causes relatively rapid spikes in blood and salivary cortisol concentrations beyond basal levels and is therefore the most commonly measured stress biomarker in swine (Minton, 1994). In contrast, long-term HPA axis activation due to chronic stress causes continuous 'hypersecretion' of cortisol (Jensen et al., 1996). Counter-regulation by the HPA axis can lead to a blunted cortisol rhythm, reducing diurnal variation (De Jong et al., 2000, Munsterhjelm et al., 2010, Ruis et al., 2001). Subsequently, the animal may be unable to mount an acute stress ('fight or flight') response when needed. Additionally, cortisol dysregulation in humans is known to cause systemic inflammation and pain (Hannibal and Bishop, 2014); while the consequences of a blunted rhythm in pigs are not fully understood, it is likely that a similar relationship exists.

2.3.3 Relationship Between Stress and the Immune System

There is significant feedback between the HPA axis and the immune system, with acute and chronic stressors impacting immunity differently (Dhabhar, 2009). In general, stressful events affect central nervous system processes, which in turn can impact immune system development and activity (Salk-Johnson and McGlone, 2007). Stress in early life, such as that caused by early and abrupt weaning, can impair development of the acquired immune system and of mucosal immunity in the gastrointestinal tract, leaving pigs more susceptible to infection (McLamb et al., 2013, Smith et al., 2010).

At equilibrium, the immune system is in a 'resting surveillance' state, monitoring for foreign antigens and maintaining a baseline leukocyte turnover rate until a foreign antigen is detected

(Dhabar and McEwen, 2001). However, stress can also induce an immune response in the absence of foreign antigens: the stress response triggers release of pro-inflammatory cytokines such as IL-6 which is also released in response to infection (Dobbs et al., 1996). In addition to inflammation, IL-6 induces glucocorticoid production, creating a positive feedback loop that can heighten both the stress response and outward expression of disease symptoms.

A stress response can induce either immunoprotection or immunopathology (Dhabar, 2009). The acute stress response typically induces immunoprotection, triggering re-distribution and maturation of immune cells (neutrophils, lymphocytes, macrophages) in preparation for a 'fight or flight' situation (Viswanathan et al., 2005). Reciprocally, immune activation elicits an acute stress response, inducing the 'fight or flight' physiological response (energy mobilization, vasoconstriction, increased heart rate and blood pressure) that would allow the animal to respond rapidly to danger (Dhabhar and McEwen, 2001). However, induction of immunopathology, in which the body attacks self-cells (e.g., autoimmune disorders) or innocuous antigens (as seen in allergies and asthma), can occur if exposure to self-cells or allergens occurs during acute stress (Dhabhar, 2009). With chronic stress, prolonged HPA axis stimulation and elevated levels of glucocorticoids and pro-inflammatory mediators (e.g., IL-6) decreases the number of circulating leukocytes, inducing immunosuppression (Dhabar, 2009). Importantly, stress can also upset the TH1/TH2 cytokine balance, inhibiting immune system development and function (Salak-Johnson and McGlone, 2007). Prolonged elevation of glucocorticoid levels or blunting of the normal diurnal cortisol cycle seen in chronic stress, can induce immunopathology if glucocorticoid levels result in a dysregulated stress response or a shift from TH1 to TH2 cytokine-driven response (Dhabhar, 2009, Elenkov, 2002). Glucocorticoids are known to both upregulate and downregulate production and release of cytokines, which can disrupt the TH1/TH2 balance required for proper immune function (Salak-Johnson and McGlone, 2007). This disruption is thought to be one mechanism by which stressors such as weaning, mixing, crowding, and thermal stress can cause immune system dysregulation, leaving animals more susceptible to infection if they are unable to mount an appropriate (balanced) cell-mediated or humoral response to pathogens present.

Some individuals are better able to cope with stress than others; poor stress coping not only heightens the negative psychological effects of stress, but also impairs immunity (Ruis et al., 2001). Two stress coping styles (proactive and reactive) have been described (proactive and

reactive), with each style differing in behavioural and physiological response to stressful situations (Koolhaas et al., 1999, Ruis et al., 2001, Reimert et al., 2014). Behaviourally, animals with a proactive coping style are characterized by active avoidance of stressors, propensity for aggression and resistance to handling, low behavioural flexibility, and strong adherence to routines (Koolhaas et al., 1999). In contrast, reactive animals are generally less aggressive, have higher behavioural flexibility, and react more passively to stress; they are also more likely to react with conditioned immobility when fearful. Ruis et al. (2001) found that when subjected to three weeks of social isolation stress, reactive gilts had a greater initial acute stress response (increased cortisol and exploratory behaviour) and a rapid (one day) return to baseline, while proactive gilts were more susceptible to chronic stress, experiencing decreased body temperature and increased noradrenaline excretion in their urine throughout the isolation period. Immune cell concentrations were also different between the two groups- in proactive gilts, lymphocyte concentration decreased while neutrophil concentration increased compared to their starting baseline, but no significant change occurred in reactive gilts. This suggests how failure to compensate for stress-related changes can lead to disease susceptibility and/or increased symptom severity. While stress coping style is at least partially hereditary, there is evidence that improvements to captive environments can enhance stress resiliency and subsequently reduce stress-mediated immune suppression (Reimert et al., 2014). This will be discussed in a later section of this review.

2.4 Swine Behaviour

Animal behaviour is interwoven with nearly every aspect of their well-being, as both a causative/active agent and as a reaction to their physical or emotional state and their surroundings. Each species has a behavioural repertoire: a set of behaviours that is natural, instinctive, species-specific, and serve a physiological function (Mkwanazi et al., 2019). Behavioural expression is impacted by factors such as health status (Escobar et al., 2007), emotional state (Murphy et al., 2014), and environment (Van de Weerd and Day, 2009); monitoring behaviour is therefore a useful tool to measure and compare aspects of health and welfare in both commercial and research settings. Additionally, social behaviour influences, and is influenced by stress and the immune response (Camerlink et al., 2012, Reimert et al., 2014, Tuchscherer et al., 2014), and therefore plays an important role in pig well-being. Observations of behaviours, postures, and time budgets

are a useful metric for evaluating herd health and stress, and recognition of changes facilitate early disease detection (Munsterhjelm et al., 2019).

This section discusses behavioural biology of pigs as a framework to understanding the impact of stress and disease on their behaviour repertoire and social relationships, and the role that environmental enrichment can play in mitigating negative effects.

2.4.1 Health, Stress, and Disease Behaviour

Wild pigs spend a considerable amount of each day searching for food and nesting materials; functional, goal-driven behaviours therefore include rooting, foraging, chewing, and nest-building (Jensen and Pedersen, 2007, Newberry, 1993). Subsequently, much of a wild pig's time is spent awake and active. In contrast, foraging and exploratory behaviours are significantly restricted when captive pigs are housed in non-bedded pens and fed directly from a feed hopper (Van de Weerd and Day, 2009) the biological motivation to perform these behaviours exist even when hunger is sated; pigs therefore are still driven to root and chew even in the absence of natural materials (Lewis, 1999). This leads to boredom, frustration, and re-directed rooting behaviour, characterized by oral manipulation of pen fixtures (Beattie et al., 2000) or other pigs (Fraser et al., 1991, Petersen et al., 1995). Barren-housed pigs also spend significantly more time awake and inactive than those provided with straw (Beattie et al., 2000) Common stereotypies in swine, such as bar-biting and sham-chewing, are also associated with boredom and frustration (Newberry, 1995).

The biological purpose behind specific behaviours should be considered when behavioural changes are observed- for example, huddling or overlaying are commonly attempts at heat conservation due to insufficient thermal regulation of the barn (Hillman et al., 2014), or an indication of underlying illness (Escobar et al., 2007, Grommers et al., 1970). Belly-pressing and hunching are postural indicators of pain or discomfort (Brown et al., 2018), while decreased feeding and drinking activity, isolation from other pigs, and increases in the frequency and duration of sternal lying, sitting, or lying inactive, are commonly seen during infection with diseases such as PRRSV and bacterial pneumonia (Brown et al., 2018, Escobar et al., 2007, Reiner et al., 2009). During an active immune response, these sickness behaviour patterns are cytokine-driven and motivated by energy conservation to allow energy to be re-partitioned away from activity and

towards immune function (Konsman et al., 2002. Rauw et al., 2012). Monitoring the presence or increase in frequency of these behaviours can therefore be used to detect changes in pigs' health status.

2.4.2 Social Behaviour

As gregarious animals, pigs kept in groups provide each other with social support (Tuchscherer et al., 2014, Reimert et al., 2014) and perform a range of affiliative and agonistic social behaviours (Stolba and Wood-Gush, 1989). However, commercial housing of pigs in barren pens with limited space allowance can limit natural functional behaviours and increase social stress, inducing re-directed oral manipulations to penmates and pen fixtures and increase aggression, from which pigs have little space to escape (Beattie et al., 2000, Petersen et al., 1995). Social interaction between grouped pigs can therefore impact the individuals' health, welfare, and growth. In investigating the relationship between social interactions and growth rate in pigs, Camerlink et al. (2012) found that finishing pigs who received oral manipulation by conspecifics for >2% of observations over a 6-hour period had a decreased growth rate compared to those that did not receive oral manipulation. Consequently, recipient pigs are likely experiencing heightened stress as a result. In contrast, pigs that received affiliative social behaviours such as social nosing for >2% of observations had higher growth rates than those that received only neutral or negative social interactions. This reflects the known relationship between oral manipulations and chronic stress (Tuchscherer et al., 2014), of which growth depression is one symptom, mediated by prolonged elevation of blood cortisol (Barnett et al., 1983, Pearce et al., 1989).

The relationship seen by Camerlink et al. (2012) between growth rate improvements and positive social interactions may be linked to the role of social support as a stress mediator. Pigs who undergo a stressful experience may exhibit a lessened behavioural and/or physiological response when social support is provided by conspecifics (Tuchscherer et al., 2014, Reimert et al., 2014). During a social deprivation test, Tuchscherer et al. (2014) found that the presence of another piglet, whether familiar or unfamiliar, reduced the stress-induced cytokine production and proinflammatory activity, and modulated glucocorticoid sensitivity when compared to piglets deprived of all social contact. This indicates that supporting positive social relationships between pigs can reduce the impact of chronic and acute stress and also support immune function. This

reflects similar findings in humans, in which social support decreases disease susceptibility and infection risk from low-level stress (Cobb and Steptoe, 1996).

These studies demonstrate the challenges faced with negative social behaviours and the importance of positive social behaviour in relation to physiological health and well-being of domestic pigs. Given that barren environments are a key stressor leading to abnormal behaviours and aggression (Newberry, 1995, Beattie et al., 2000), environmental improvements that promote positive social interactions can be valuable tools to improve welfare and influence productivity (see Section 2.5.2).

2.5 Environmental Enrichment

Previous sections have outlined swine health and welfare concerns including chronic stress, disease and dysregulated immune function, and abnormal behaviour. Each issue can be caused or influenced by the barren environments in which pigs are typically raised in North America. Environmental enrichment is one management tool that, when applied with the biology of the pig in mind, can mitigate some of the negative effects of standard commercial facilities, potentially improving welfare, productivity, and/or disease resilience.

2.5.1 What is environmental enrichment?

Environmental enrichment is defined as an "improvement in the biological functioning of captive animals resulting from modifications to their environment" (Newberry, 1995, p.230). To implement a successful enrichment protocol, the biological drives of the species in question must therefore be considered. Restriction of functional behaviours can lead to frustration, boredom, chronic stress, development of abnormal behaviours/stereotypies that negatively impact psychological and physical well-being (Newberry, 1995, Lewis, 1999). Therefore, biologically relevant enrichments are those that encourage one or more species-specific functional behaviours, such as straw and other rooting materials (Jensen and Pedersen, 2007, Van de Weerd et al., 2003). These enrichments should be differentiated from items or toys with limited species-specific relevance that do not fulfill behavioural needs or improve well-being (Newberry, 1995). The

following section will review relevant research on various types and applications of enrichment and their influence on measures of health and welfare.

2.5.2 Considerations for enriching the environment

Successful enrichment should increase species-specific behaviour, maintain, or improve measures of health, improve productivity, and be practical to use in the production system (Van de Weerd and Day, 2009). To maximize usage, and subsequent physiological benefits, the best enrichments have properties that make them appealing to the species in question and encourage natural behaviours. Pigs prefer enrichments that are odorous, manipulative, deformable and ingestible (Van de Weerd et al, 2003); cleanliness is also important to pigs, with evidence that use rapidly declines when items become soiled (Bracke, 2007). There should also be sufficient material for multiple pigs to interact with the enrichment(s) at the same time, as pigs show synchronized exploratory behaviour (Docking et al., 2003). Effective enrichment needs to not only be available, but also attractive and rewarding enough to maintain continued, recurrent use (Studnitz et al., 2007). Complexity is valued when the enrichment is responsive to manipulation and rewarding to use- for example, substrates that can be rooted through, chewed, or moved to form a nest- but overly complex enrichments with no reward cause frustration and discontinuation of use (Van de Weerd et al., 2003).

Substrates like straw, peat, or compost are common provisions that allow the animals to perform a variety of natural behaviours such as exploration, foraging, chewing/eating, rooting, and nesting (Van de Weerd and Day, 2009). While bedded systems provide high biological relevance, practicality is a common concern for producers. Bedding is expensive, labour-intensive to clean, presents a significant biosecurity hazard and can be problematic in fully slatted pens that use liquid manure slurry systems (Tuyttens, 2005).

To combat concerns with loose bedding, point-source enrichments are common in pens with slatted flooring. Point-source enrichment, materials restricted to one location of the pen, such as a suspended piece of rope, can add a layer of cognitive complexity to the environment while addressing the previously mentioned downsides to loose substrates (Van de Weerd and Day, 2009). However, a wide variety of materials and presentation styles are available, and not all are effective at improving behavioural expression, health, welfare, and/or productivity of swine. For example,

metal chains are a common provision (Mkwanazi et al., 2019), but have very few properties that are attractive to pigs. In an analysis of 130 different enrichments, Bracke (2008) found that short (nose-height), suspended metal chains, with or without indestructible objects attached, had the lowest interaction rate. Scores representing 'enrichment value' were assigned to enrichments based on 30 criteria including animal interaction frequencies, ability to meet behavioural needs, biological value, and prevalence of aggression and stress in pigs provided different enrichments. Items that scored similarly to single chains (scores of <2.5 out of 10) included rubber mats, rubber/PVC hoses, free toys, and knotted rope; a minimum score of 5.0 was suggested by the authors to represent enrichments that provided sufficient health and/or welfare benefits. Interestingly, compared to these low-scoring enrichments, longer (floor-length) branched chains increased complexity and therefore were explored more, while longer chains placed on the floor were able to be rooted, also increasing interest (Bracke, 2008, Bracke, 2017). While changing the style and presentation of chains can increase use, the material is still unable to be deformed or consumed and provides no 'solution' or reward when manipulated, limiting their usefulness as pig-appropriate enrichment.

Even where fully bedded systems are not practical, enrichments that are deformable and/or consumable can still be provided. Alternatives to bedding can include provision of rooting materials in racks (Fàbrega et al., 2019, Zonderland et al., 2008) or in fixed, smaller amounts that do not interfere with manure systems (Roy et al., 2019). Natural materials such as jute (Ursinus et. al, 2014a), or fresh wood (Telkänranta et al., 2014) are other options for point-source enrichments that can be suspended or loose on the floor, and have properties attractive to pigs (manipulative, edible) that encourage exploration and oral manipulation.

Habituation to point-source enrichments occurs rapidly when they are not destructible and/or renewed regularly (Apple and Craig, 1992). Pigs actively seek out and prefer novelty (Stolba and Wood-Gush, 1989) and destructibility (Bracke, 2007); items that are not destructible or ingestible or are not replaced/rotated often will rapidly decline in use (Trickett et al., 2009, van de Perre et al., 2011, Van de Weerd et al., 2003). Gifford et al. (2007) found frequent rotation of point-source objects to be most effective at maintaining interest, with objects presented for no more than two days at a time with at least five days before re-introduction. Similarly, Van de Perre et al., 2011) saw increased interaction upon introduction of novel toys when presented in a rotation,

but interaction with each object was lower upon re-introduction than during initial presentation. This finding emphasizes the need for enrichments that can continue to maintain relevance and provide novelty over time.

Enrichment placement and presentation is another important consideration. Trickett et al. (2009) found that suspended ropes were preferred over loose wood blocks on the ground. Similarly, Blackshaw et al. (1997) saw preference (increased frequency and duration of interaction) for a plastic-covered metal toy when suspended at pig eye-level when compared to the same toy presented on the floor. A preference for suspended wooden logs, compared to logs on the floor, was also reflected in a study by Giuliotti et al. (2019). Items suspended in a central location also provide a better opportunity for more pigs to interact with the enrichment at once and keeps the toys cleaner as they are off the ground. Trickett et al. (2009) found that interactions with suspended ropes were similar in frequency and duration to those with straw, which is commonly regarded as the most effective biological enrichment for pigs. Given the evidence that suspending enrichments increases interaction, combined with the practicality of use (ease of delivery and cleaning, for example), this practice can increase effectiveness of point-source enrichments for pigs, and corresponds with NFACC's Code of Practice recommendation for provision of a variety of simple suspended enrichments (NFACC, 2014).

The following sections will therefore outline relevant research on various enrichment types and their effect on pig health and welfare.

2.5.3 Influence of Enrichment on Behaviour

The role of enrichment in improving welfare is closely tied to its effect on behaviour by providing opportunities for pigs to perform natural functional behaviours that are otherwise restricted in barren environments (Newberry, 1995). Pigs raised in deep-bedded systems, with ample opportunity to root, chew, nest and otherwise manipulate their environment, are less likely to engage in damaging behaviours than those in minimally enriched environments (Petersen et al., 1995, Zonderland et al., 2008). Meanwhile, there is significant evidence that barren environments increase behavioural manifestations of stress, including stereotypies and aggression towards penmates (Newberry, 1995, Pearce and Paterson, 1993, Petersen et al., 1995). These negative behaviours appear to be re-direction of natural behaviours, such as rooting and chewing, into

damaging behaviours such as tail biting or biting of the pen walls, floors, and fixtures (Beattie et al., 2000). However, provision of effective environmental enrichment can allow expression of innate functional behaviours, thereby reducing the incidence of re-directed biting behaviour (Newberry, 1995, Van de Weerd et al., 2006).

Pearce and Paterson (1993) compared the behaviour of pigs in three environmental conditions: uncrowded, crowded, and crowded with provision of point-source enrichments. Of these groups, the uncrowded pigs showed fewer behavioural signs of stress: less time spent sternally recumbent and more time standing, lying laterally, and engaging in non-aggressive social interactions with penmates. However, the crowded group given enrichments showed significantly less indication of chronic stress than the barren crowded group (less time sitting and standing motionless) and spent more time exploring their environment. The enrichment group were also less reactive to novel objects and humans approaching, and spent more time interacting with these stimuli, showing evidence a greater exploratory drive and reduced fear.

Beattie et al. (2000) found that providing straw and peat significantly reduced the incidence of floor and wall biting, tail biting, and nosing of littermates, compared to control pigs kept in barren pens. The same behavioural changes were seen by Petersen et al. (1995) in pigs given straw, logs, and branches, as well as a decrease in time spent lying inactive. Suspended enrichments appear to also have value in managing aggressive biting behaviours. In pens with straw racks and daily provisions of wood shavings, the lowest incidence in tail and ear biting was found when pigs were also given suspended fresh birch wood, compared to a single suspended metal chain, branched chains, or PVC pipe (Telkänranta et al., 2014). That a reduction in damaging behaviours was seen with addition of fresh wood even in the presence of other rooting materials suggests a preference for suspended, chewable, natural materials. Reductions in frequency and duration of damaging biting behaviours has also been demonstrated with jute sacks (Ursinus et al., 2014a), bamboo sticks and chewable plastic toys (Chou et al., 2019), and a rotation of different suspended toys (Giuliotti et al., 2019, Van de Perre et al., 2011). This evidence supports the idea that oral manipulations and damaging biting behaviours can be at least partially attributed to re-direction of natural foraging behaviours, and that enrichments that can be manipulated, explored, chewed, and rooted provide an alternative outlet for these behaviours (Newberry, 1995, Van de Weerd et al., 2006).

While racks of rooting materials are suggested as an alternative to fully bedded systems, results on the efficacy of straw racks at managing abnormal behaviours are conflicted. In comparing the effects of four point-source enrichment treatments (straw rack, wooden logs, paper, and no enrichment/control) on behaviour of grow-finish pigs, Fàbrega et al. (2019) saw the most environmental exploration and the least tail biting in groups given a straw rack. Holling et al. (2017) noted that a straw dispenser had little effect on tail biting in test herds where prevalence of biting was already low. In contrast, Zonderland et al. (2008) found that a similar straw rack reduced severity of tail wounds but was still not as effective as loose straw.

To identify why some enrichments are more effective than others at altering behaviours, it is necessary to understand the biological drive behind the target behaviour and select enrichments that provide an alternative outlet to express that behaviour. While there is an abundance of evidence that enrichment impacts behavioural expression in pigs, it is of interest to explore the reasons that behavioural changes may occur to understand the importance of enrichment, and how to effectively utilize it. Given that negative social behaviours like tail biting have multi-factorial causes including hunger, unfulfilled behavioural needs, high-stress environments, and illness (Taylor et al., 2012), further understanding on how to target behavioural changes is still needed. Therefore, the following section looks at the role of environment in learning, emotional development, and physiology of pigs.

2.5.4 Effect of Enrichment on Stress

Environment can impact stress in a variety of ways, but results are conflicting and inconclusive in many areas (Salak-Johnson and McGlone, 2007). Stress can have a multitude of different physical manifestations, including immune system depression, negative social behaviours, and decreased growth rate (Camerlink et al., 2012, Ernst et al., 2006). Martínez-Miró et al. (2016) proposed that stressors come from five main categories: social, environmental, metabolic, immunological, and handling (by humans). Biologically relevant enrichment can directly address the environmental stress that comes from barren environments that are lacking in appropriate stimuli and outlets for natural behaviour (Petersen et al., 1995) and may also indirectly address the impact of social and/or immunological stressors (Ernst et al., 2006, Munsterhjelm et al., 2010, Van de Weerd and Day, 2009). Enrichment that provides complexity and some level of

cognitive challenge or puzzle provide stimulation, encourage exploration, and allow freedom of choice over aspects of the pigs' environment and routine (Zebunke et al., 2013). In turn, this can reduce negative emotional states negative emotional states such as boredom and frustration stemming from barren environments (Newberry, 1995) and improve memory and learning (De Jong et al., 2000). Douglas et al. (2012) also demonstrated that environment can impact a pig's affective state and cognitive bias. The researchers observed that pigs housed with enrichment for five pigs reacted more negatively when placed in a barren environment than those raised continuously in a barren pen. This experiment also found that, when raised with enrichment, the pigs are more likely to show optimistic judgment biases, by which they were more likely to respond positively to an ambiguous signal than pigs raised in barren environments. This can partially be attributed to lower stress and more positive affective in enriched pigs.

Abnormal diurnal cortisol rhythms are one physiological indicator of chronic stress; De Jong et al. (2000) found that growing pigs raised in barren environments had a blunted, dysregulated cortisol rhythm compared to those enriched with straw bedding. Additionally, Munsterhjelm et al. (2010) found that timing of enrichment impacted development of a cortisol rhythm demonstrated differences in salivary cortisol rhythms between pigs housed with and without rooting material at three production stages (0-4 weeks, 5-9 weeks and 10-24 weeks of age), and found that early enrichment (0-4 and/or 5-9 weeks) significantly increased the likelihood of a normal cortisol rhythm, while barren housing at 0-4 weeks was correlated with a blunted rhythm at 21 weeks of age, regardless of access to enrichment later in development. A blunted cortisol rhythm is associated with chronic stress, which is consistent with the study's observations of increased tail biting and skin lesions in groups with non-rhythmic pigs from 10-24 weeks.

Serotonin also plays a vital role in stress coping behaviour and physiological stress resilience by modulating the stress response (Puglisi-Allegra and Andolina, 2015), and serotonin regulation is influenced by environment (Ursinus et al., 2014b, Arroyo et al., 2020). Positive experiences including affiliative social interactions, play, and exploration are facilitated by enrichment (Mkwanazi et al., 2019) and enrichment therefore appears to upregulate serotonin release (Arroyo et al., 2020). In humans, serotonin dysregulation and/or depletion is correlated with impulsive behaviour, depression, hostility/aggression and anxiety (Carver and Miller, 2006). This appears to be reflected in pigs; tail biting and fearfulness behaviour in response to novel

objects (avoidance, standing alert, seeking walls of the test arena) were correlated with low blood serotonin and higher platelet uptake velocity in an experiment by Ursinus et al. (2014b), while pigs raised in barren environments were more likely to have dysregulated serotonin uptake and display tail biting and fearful behaviours compared to straw-enriched pigs. Interestingly, fearful (low serotonin) pigs also had lower heart rate variability during the novel object test, which is similar to what is seen in humans with panic disorders, related to parasympathetic nervous system suppression (Carver and Miller, 2006).

Novel situations and handling by humans can be significant sources of fear and acute or chronic stress in livestock (Martínez-Miró et al., 2016). How animals are handled plays a role in the development of cognitive biases and chronic stress, and their environment and exposure to novelty influence development of fear and neophobia. Pearce et al. (1989) demonstrated that pigs who received unpleasant handling by humans during rearing, including abrupt movement in the pen and use of an electric prod, were more fearful of humans, spent more time inactive, and interacted with other pigs less than individuals reared with calm, pleasant handling. However, pigs provided with point-source enrichments (rubber tires, chains, and moveable bars) were less fearful of humans than those in barren environments, even when receiving regular unpleasant handling. Reimert et al. (2014) also found a relationship between fearfulness and environment, with straw-enriched pigs being quicker to approach humans and novel objects in a novelty test and reacting more calmly and with more exploratory drive in a novel environment than barren-housed pigs.

This indicates that enrichment can help reduce behavioural and physiological indicators of chronic stress and fear, possibly mediated by providing an outlet for the animals to perform natural behaviours that they are otherwise deprived of in a barren pen. In turn, reduced stress and fearfulness can improve welfare and measures physical well-being.

2.5.5 Enrichment and Measures of Productivity

Enriched environments are not only important for the welfare and emotional well-being of pigs, but there is also evidence that enrichment can improve measures of growth and productivity by reducing stress, thereby impacting profitability. Oliveira et al. (2016) found that pigs given both wood shavings and suspended toys had higher total and average daily gain (ADG) and improved feed conversion ratio (FCR) compared to those receiving either of the enrichments alone, or none

at all. Similarly, Beattie et al. (2000), found that pigs raised with a greater space allowance and rootable enrichments (peat and straw in a rack) had a higher ADG and lower FCR than barren-raised pigs. This relationship between growth, enrichment held true in an experiment by Luo et al. (2020b) in which pigs housed with rooting substrates from birth appeared to cope better with weaning stress than barren-housed pigs, reflected in a greater weight gain and feed efficiency post-weaning while also displaying fewer oral manipulations. Additionally, barren-housed pigs that were switched to enriched housing post-weaning improved in weight gain and feed intake after the switch when compared to pigs kept continuously in barren pens. These growth rate improvements relationships are likely related to the previously mentioned interaction between environment and stress, as it is known that both acute and chronic stress can decrease feed efficiency (Barnett et al., 1983), growth rate (Pearce et al., 1989) and lead to a compromised immune system, thereby indirectly impacting productivity (Salak-Johnson and McGlone, 2007). Additionally, Peeters et al. (2006) saw no differences in growth rate when pigs were enriched with straw in the finishing phase, two to six weeks before slaughter. This suggests that early life experiences are more critical for improving stress coping, which correlates with the timing of critical development periods for the HPA axis in early postnatal life (Poore and Fowden, 2003). However, Blackshaw et al. (1997) did not find growth rate differences between barren-housed weaner pigs and those housed with an inedible ceiling-suspended toy, loose toy on the floor, or both. Interaction with the point-source enrichments in this experiment was also reported to decline over the three-week trial period, indicating that the type and presentation of enrichment items are likely also significant factors influencing enrichment efficacy at altering productivity.

Evidence on the influence of enrichment on swine carcass traits is somewhat conflicting. Beattie et al. (2000) found that pigs given rootable materials and greater space allowance had a higher growth rate, heavier carcass weight, and increased backfat depth at slaughter than those housed in barren pens with only the minimum space allowance. Additionally, meat from the control (barren) group was less tender with more water loss during cooking. Similarly, Klont et al. (2001) examined carcass traits, meat quality and post-mortem muscle metabolites between pigs raised in either conventional barren housing or housing enriched with straw and space allowances exceeding minimum standards. The study found that carcass traits (weight, backfat depth, and meat percentage) and meat colour did not differ, but meat from the enriched pigs had superior water-holding properties. This was indicated by significantly less post-mortem lactate formation (4- and

24-hours post-mortem) and less drip loss from the longissimus lumborum muscle (2- and 5-days post-mortem). There was a tendency for the same results in the biceps femoris muscles of the enriched pigs as well, but results were not significant. Meanwhile, Peeters et al. (2006) saw no differences in carcass weight, backfat depth, or meat quality (pH, electrical conductivity, colour, water-holding capacity) between barren-housed and straw-bedded pigs. Pre-slaughter handling and transportation stress is correlated with increased post-slaughter lactate formation and increased drip loss, resulting in dry, firm, and dark (Tarrant, 1989) or pale, soft, and exudative pork (Warris, 1998). Prior experience to handling, restraint, and novelty in their environment have been shown to reduce fear and stress during handling and transport (Grandin and Shivley, 2015). If enriched pigs therefore have reduced neophobia, the lower levels of stress and fear in these pigs could explain reductions in drip loss and lactate formation seen by Kont et al. (2001) as described above, but this does not appear to be true for all enriched pigs.

The role of enrichment in productivity could be a key argument supporting its use in commercial pig production. While it is difficult to place a concrete value on behaviour and welfare benefits, productivity measures are quantifiable, making direct comparisons of the costs and benefits of enrichment provision possible. Given that a primary concern for producers is the added cost in supplies and labour (Tuyttens, 2005), further research including a cost-benefit analysis of enrichment provision would be a valuable addition to the discussion. While the variation in results cannot be explained by enrichment type alone, there appears to be a trend towards species-specific enrichment improving productivity, and earlier enrichment provision improving stress coping more profoundly than enrichment given later in life. The effect of environmental enrichment on productivity measures is likely linked to the role of enrichment in changing behavioural expression, stress, and disease, as outlined by the literature summarized within other sections of this review. Therefore, while positive effects of enrichment may not always be outwardly apparent or measurable in a commercial system, the underlying impact can significantly impact the production economics as well as the health and welfare of the animals.

2.5.6 Effect of Enrichment on the Immune Response and Disease Resilience

As discussed earlier in this review, disease is a significant concern in the livestock industry, and there is considerable evidence that chronic stress increases disease susceptibility (see Section

2.3.3). One approach to against disease is through genetic selection for animals that are resistant against common swine pathogens (Mulder and Rashidi, 2017, Nakov et al., 2019); however, there are key issues with this approach that warrant investigation into alternative strategies. Firstly, genetic selection and gene editing for resistance are typically disease-specific, making it an unfeasible goal to produce livestock resistant to a large number of common commercial pathogens (Albers et al., 1987). Additionally, genetic selection for disease resistance can lead to unintended selection for unfavorable productivity or behaviour-related traits (Guy et al., 2012, Wilkie and Mallard, 1999). Altering disease resilience, the ability of an animal to maintain relatively undepressed growth regardless of disease pressure, is an alternative focus (Albers et al., 1987). The definition of resilience does not require a specific immune response or pathogen-resistance to control disease burden, but rather describes a broad biological response of animals that are better equipped maintain homeostasis under challenge than their susceptible counterparts (Mulder and Rashidi, 2017). While the heritability of disease resilience traits has been a primary topic of research (Albers et al., 1987, Mulder and Rashidi, 2017, Putz et al., 2019) there is evidence that environment also plays an important role, partly by modulating stress-induced immunosuppression (Ernst et al., 2006, Luo et al., 2017, Van Dixhoorn et al., 2016).

Enrichment can alter immune cell proliferation (Ernst et al., 2006, Luo et al., 2020a, Reimert et al., 2014) and increase natural autoantibody concentrations (Luo et al., 2017, Luo et al., 2020a), leading to enhanced disease response (Van Dixhoorn et al., 2016) and wound healing (Ernst et al., 2006). Luo et al. (2017) found enhanced immune competence, in pigs enriched with straw and shavings compared to those housed in barren, partially slatted pens, measured by increased concentrations of natural autoantibody-binding factors (NAAb) in response to neural autoantigens produced by regrouping stress. In a later experiment, Luo et al. (2020a) again found higher IgG autoantibody concentrations in pigs enriched from birth with a combination of straw, sawdust, peat, inedible toys, and greater space allowance in response to immune stimulation, as well as increased total lymphocyte and granulocyte (neutrophil, eosinophil, basophil) concentrations and decreased lymphocyte to granulocyte ratio. The opposite effect on immune cell proliferation and ratio was found in pigs raised in barren housing from birth, and those who switched from enrichment to barren housing, suggesting that enrichment can positively influence development of the immune system and antibody production, potentially resulting from stress reduction and better mental state and stress resiliency compared to pigs raised without enrichment

or from whom enrichment has been taken away. Reimert et al. (2014) also observed a lower neutrophil to lymphocyte ratio and haptoglobin concentration in pigs enriched with straw, wood shavings, and point-source enrichments (ball on chain and suspended jute sack) and subjected to regrouping stress. A high granulocyte to lymphocyte ratio is indicative of stress (Davis et al., 2008); lower ratios in enriched pigs seen by Luo et al. (2020a) and Reimer et al. (2014) therefore indicate a protective effect of enrichment against regrouping stress. Lastly, a study conducted by Ernst et al. (2006) providing cognitive enrichment using trained auditory signals leading to a food reward increased IgG and T-cell proliferation while decreasing B cell proliferation, resulting in faster wound healing compared to controls. This response indicates enhanced immune function related to a reduction in stress when compared to standard rearing that is limited in opportunities for reward or goal-driven activity.

To measure the impact of enrichment on disease resilience, Van Dixhoorn et al. (2016) compared the cellular immune response and behaviour of pigs raised in either barren or enriched environments and co-infected with PRRSV and *Actinobacillus pleuropneumoniae.* An intensive enrichment protocol was used in this experiment: barren groups were provided with minimal enrichment in the form of blocks attached to chains, while enriched groups received the same blocks and chains, as well as straw, moist peat, wood shavings, jute, and wooden brooms. Under these conditions, enriched pigs showed significantly less stress-associated behaviour (aggression, mounting oral manipulation of penmates and pen fixtures) and had a markedly improved disease response compared to controls, measured by presence and severity of lung lesions and speed of histopathological clearance of the pathogens. These results are highly significant in understanding the role of effective and complex enrichment on immune response and protection, in comparison to the minimal enrichment (stagnant objects on chains) that is commonly provided in North America (Tuyttens, 2005, Van de Weerd et al., 2003).

2.6 Conclusions

From the outlined research within this review, it is clear that barren environments can be a significant stressor for captive pigs. As a result of barren, unstimulating pens, pigs experience chronic stress and fear, stemming from continuous exposure to environments ill-suited to allow performance of innate functional behaviours, and a lack of novelty and cognitive challenge. In

turn, chronic stress can have a host of deleterious effects on health and welfare, including negative affective state, abnormal and aggressive behaviours, immune suppression, and depressed growth.

Environmental enrichment has a demonstrated positive effect on welfare by reducing the stressful effects of barren housing and enhancing stress coping and disease resilience. However, careful attention must be paid to the biological relevance of the enrichments offered: the maximum benefits are obtained when enrichments are attractive enough to sustain interest and satisfy a behavioural need. Ineffective enrichment will not benefit the health and/or welfare of pigs and can be detrimental if it causes frustration and stress. With successful enrichment that encourages pigs to perform natural species-specific behaviours, the animals (and producers) can see benefits including increased productivity, welfare improvements, and enhanced immune system performance resulting from lower stress. Systems bedded with rooting materials such as straw are largely regarded as the 'gold standard' for enrichment for pigs, but Canadian hog farms most commonly use barren pens with slatted flooring. Point-source enrichments are an alternative to bedding, but effectiveness varies significantly based on factors including presentation style or placement within the pen, cleanliness, physical properties of the enrichments, and novelty or rotation schedule.

2.7 Thesis Objectives & Hypotheses

Based on knowledge gaps identified in this literature review, two experiments were formed and will be outlined in the subsequent chapters of this thesis. The overall research objective of the experiments is to determine how behaviour, productivity, and immune cell concentrations differ in pigs exposed to a polymicrobial natural disease challenge when raised in standard barren fully slatted pens compared to pigs raised in same pens enriched with a rotation of inedible point-source objects. In turn, this will help to identify whether point-source enrichments can influence measures of disease resilience and welfare in growing swine. This is broken down in to four specific objectives:

i) Evaluate the relationship between environment (enriched or barren) and the immune response during a disease challenge through a series of complete blood count panels.

ii) Measure the effect of environment (enriched or barren) on feed intake, growth, morbidity, mortality, and veterinary treatments during a disease challenge.

iii) Determine if behavioural indicators of stress and disease differ between enriched and barren housed pigs.

iv) Examine relationships between the behaviour, growth, and complete blood counts of individuals.

It is hypothesized that pigs raised in enriched environments will be more resilient to a polymicrobial disease challenge than those raised in barren environments, and subsequently have better welfare and productivity outcomes. To determine this, five sub-hypotheses will be explored by comparing enriched to barren-housed pigs:

i) Enriched pigs will perform fewer negative social behaviours and spend more time awake/active and performing positive social behaviours.

ii) Enriched pigs will differ in their immune cell counts during a natural disease challenge.

iii) Enriched pigs will have lower morbidity and mortality during the disease challenge.

iv) Enriched pigs will have improved growth performance during the challenge, indicated by measures such as smaller reduction in average daily gain (ADG), higher feed intake, and a lower feed conversion ratio during the disease challenge.

v) Individuals that perform fewer negative social behaviours, more positive inter- personal behaviours, and interact more frequently with their environment than their penmates will differ in immune cell concentrations and growth rate.

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3. EFFECT OF POINT-SOURCE ENRICHMENT ON PEN-LEVEL SWINE BEHAVIOUR, PRODUCTIVITY, AND IMMUNE CELL COUNTS DURING A POLYMICROBIAL NATURAL DISEASE CHALLENGE

This chapter outlines an experiment exploring the effect of a rotation of point-source enrichments on pen-level pig behaviour, mortality, and growth rate, as well as individual-level immune cell counts in response to a polymicrobial natural disease challenge.

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Drafting of this chapter was completed by Madelena Pedersen-Macnab and Dr. Yolande Seddon with suggestions from Dr. John Harding. Experimental design was completed by Dr. Yolande Seddon with suggestions from Madelena Pedersen-Macnab. Animal handling and data collection was completed by Madelena Pedersen-Macnab and staff at the Centre de développement du porc du Québec and the Centre de recherche en sciences animales de Deschambault. Statistical analysis was completed by Madelena Pedersen-Macnab with suggestions given from Drs. Yolande Seddon and Giuliana Miguel Pacheco.

ABSTRACT

Environmental enrichment is identified as a requirement for good welfare in swine, with benefits including reduced stress and improved productivity. However, research on the effect of point-source enrichments on disease response and resilience is limited. To explore this, seven inedible point-source enrichments were provided in a rotation to growing swine before, during, and after a polymicrobial natural disease challenge (NDC) that included pathogens such as porcine reproductive and respiratory syndrome (PRRSV). Nineteen batches of weaned barrows (n=1220) were randomly assigned to enrichment (E) or control (C) treatments in a quarantine facility (Q), remaining for 19 days (d-19 to d0). Pigs were then transferred to an NDC challenge nursery (N), remaining for four weeks (d0 to d28), before transfer to the onsite finisher unit (F), remaining until slaughter (d28 to d147). Behaviour, productivity, mortality, and complete blood counts (CBC, Blood 1: d-14; Blood 4: d42) were measured throughout the experiment. These indicators were analyzed to determine if differences existed in pen behaviour, productivity, and individual pig CBC measures between enriched and control pigs, as measures of disease resilience.

Rearing with point-source enrichment did not influence mortality, ADG, or FCR in any phase. A greater increase in white blood cell (WBC) concentration from Blood 1 to 4 was seen in E pigs ($x10^9$ cells/L, E: 9.58±1.15, C 8.60±1.14 mean±SEM, $p=0.03$), but no other CBC differences were found. Probability of enrichment use was higher in E than C on all but one of the days measured ($p<0.01$). On d-15 and d70, E pigs were less likely to lie sternally ($p<0.01$) and more likely to lie laterally (d-15: $p<0.01$, d70: $p=0.04$) than C pigs, and more likely to be standing on d-15 ($p<0.01$).

Results show that point-source enrichment use was more likely than chain use, and pen-level frequencies of postural behaviour during the quarantine phase indicate that enrichment provision resulted in higher activity and comfort behaviour. No treatment differences in productivity or most CBC values suggests that a rotation of point-source enrichments did not alter overall cell counts, while the greater increase in WBC may indicate prolonged infection compared to controls.

3.1 INTRODUCTION

Diseases such as porcine reproductive and respiratory syndrome virus (PRRSV) are a leading cause of economic and welfare challenges in the swine industry, contributing hundreds of thousands of dollars in economic losses yearly in North America (Brisson, 2015). Economic impacts of disease include increased mortality, depression of feed intake and feed conversion ratio, cost of veterinary care, and increased labour required for health monitoring, sanitation, and administering veterinary treatments (Escobar et al., 2007, VanderWaal and Deen, 2018). In addition to decreasing profitability, disease negatively impacts animal welfare, causing considerable stress and discomfort (Martínez-Miró et al., 2016, Munsterhjelm et al., 2019), and negative mood (Brown et al., 2018). A multitude of livestock diseases exist, and new pathogens continue to emerge and challenge both human and animal health (Zimmerman et al., 2012). Biosecurity efforts to control indirect spread of pathogens are not always effective (Desrosiers et al., 2011), contributing to a growing interest in ways of supporting livestock health and maintaining productivity, even when faced with a disease challenge (Mulder and Rashidi, 2017, Putz et al., 2019).

Altering disease resilience is one area of interest for improving herd health and reducing the financial cost of disease. Considerable research has been performed studying disease resistance- defined as an animal's "ability to control pathogen burden" without succumbing to infection (Mulder and Rashidi, 2017, p.3346) and disease tolerance, the ability to maintain homeostasis and growth performance despite a known pathogen burden (Nakov et al., 2019). In contrast, the concept of disease resilience encompasses a broader scope of the disease response and does not require the pathogen load of an individual to be known (Mulder and Rashidi, 2017). Resilient individuals are defined as ones who can maintain productivity regardless of disease status or pathogen load (Albers et al., 1987, Mulder and Rashidi, 2017, Nakov et al., 2019).

Resilience is not mutually exclusive with resistance and tolerance; a resilient animal can also fit the definition for disease resistance or tolerance, or all three (Doeschl-Wilson et al., 2012, Mulder and Rashidi, 2012). However, genetic selection for a robust immune response to disease alone may not be the answer; individuals with a highly active immune response may still show depressed growth as energy is partitioned away from growth and towards fighting infection (Doeschl-Wilson et al., 2009, Williams et al., 1997). To further complicate the issue, a high degree of immune responsiveness may also have unintended consequences, such as increased

susceptibility to inflammatory conditions like arthritis (Wilkie and Mallard, 1999). It is therefore crucial to develop a holistic approach to resilience that improves the immunological disease response and productivity while supporting good welfare. To do this, it is of value to further investigate factors impacting stress coping ability, and approaches to mitigate physiological impacts of stress.

There is evidence that standard barren environments impair an animal's ability to cope with and respond to stress and disease (De Jong et al., 2000, Van Dixhoorn et al., 2016). For pigs, barren environments restrict performance of functional behaviours such as rooting, chewing, and nesting, contributing to chronic stress (Newberry, 1995, Van de Weerd et al., 2003). Environmental enrichment- defined as an "improvement in the biological functioning of captive animals resulting from modifications to their environment" (Newberry, 1995, p.230) - is a tool that can allow pigs to perform natural behaviours, thereby reducing the level of stress induced by barren commercial environments (Van de Weerd et al., 2003). Enhancements to the environment that encourage species-specific functional behaviours and provide novelty may provide pigs with a number of health and welfare benefits. Pigs provided with enrichments may demonstrate a higher frequency of positive social interactions (Van de Weerd et al., 2006), reduced aggression and performance of stereotypies (Beattie et al., 2000, Petersen et al., 1995, Ursinus et al., 2014), improved growth (Oliveira et al., 2016), and altered immune response leading to faster wound healing (Ernst et al., 2006) and disease clearance (Luo et al., 2017, Van Dixhoorn et al., 2016). While loose substrates such as straw or shavings are typically considered a 'gold standard' for enrichment demonstrating fairly consistent physiological and behavioural benefits (Tuyttens, 2005, Van de Weerd and Day, 2009), use can be challenging for producers to adopt, especially those operating slatted floor systems; common concerns include cost, labour, biosecurity, and incompatibility with liquid manure handling systems (Tuyttens, 2005).

Point-source enrichments are one alternative to substrate enrichments like straw bedding. Point-source enrichments are objects of a limited size, a set shape, and are fixed in a single location within the pen (Van de Weerd and Day, 2009). To be an effective alternative, point-source enrichments should fulfill a behavioural need, be practical to implement, and improve measures of health and/or productivity. However, many items commonly used in commercial barns have minimal biological relevance and attractiveness to pigs (Bracke, 2017), and their use declines rapidly (Blackshaw et al., 1997, Trickett et al., 2009), thereby reducing potential health, welfare,

and behavioural benefits. Even when rotated often (weekly), items with little biological or behavioural relevance to pigs are interacted with less frequently upon re-introduction than when presented for the first time (Van de Perre et al., 2011). It is not known whether a rotation of enrichments with properties attractive to pigs can influence disease resilience, thereby altering measures of disease response such as mortality, growth, and behaviour. Based on this knowledge gap, this experiment aimed to study whether a point-source enrichment protocol designed to encourage functional behaviour expression is effective at improving measures of disease resilience.

The objective of this study was to determine whether a rotation of inedible point-source enrichments affects the physiological and behavioural responses of pigs to disease. To evaluate this, behaviour, mortality, growth rate, and cellular immune counts were measured before, during, and after a polymicrobial natural disease challenge (NDC) from pens of pigs reared with the provision of a rotation of enrichment (enriched), or a hanging chain (control). It was hypothesized that pigs raised with the provision of a rotation of point-source enrichment will differ in their immune response to those with chains, and subsequently will have better productivity, lower mortality, and perform fewer behavioural indicators of stress and illness.

3.2 MATERIALS AND METHODS

All care, handling and procedures performed on pigs were approved under the Animal Protection Committee of the Centre de Recherche en Sciences Animales de Deschambault (15PO283) and Animal Care and Use Committee at the University of Alberta (AUP00002227). The live pig data collation was overseen by the Centre de Développement du Porc du Québec (Centre for the Development of Pork in Québec) and the herd veterinarian.

3.2.1 Animals and Research Environment

The experiment spanned three stages: 1) quarantine in a high-health nursery for 19 days, 2) exposure of pigs to a polymicrobial natural disease challenge (NDC in a challenge nursery for a four-week period (see Section 3.4.2), and 3) grow-finishing stage in a finisher unit for approximately 15 weeks until slaughter. Two research facilities in Deschambault, Quebec were used in this experiment: the Centre de Recherche en Sciences Animales de Deschambault (CRSAD) for the quarantine phase, and the Centre de Développement du Porc du Quebec (CDPQ)

for the NDC phases (nursery and finisher). Piglets were sourced from 11 farms, with all piglets within a batch sourced from a single breeding farm, with the source farm rotating each batch. Each breeding farm provided pigs for one to three batches during the experiment and sourced genetics from one of seven PigGen Canada members (https://piggencanada.org/). Prior to weaning and entry into the experiment, piglets received standard processing procedures: castration, tail docking, teeth clipping, and iron injections. Pain management was provided during process, as dictated by the Code of Practice (NFACC, 2014). During the quarantine phase piglets were also vaccinated for porcine circovirus 2 (PCV2) with Ingelvac CircoFLEXR® PCV2 vaccination (Boehringer Ingelheim, Ingelheim am Rhein, Germany).

From March 2018 to December 2019, 19 batches of F1 cross (Yorkshire x Landrace) weaned barrows, 19-21 days of age, entered the experiment at 3-week intervals in batches of either 60 or 75 piglets (n=1220 pigs). For the first 10 batches (n=680, Batches 41-50), two facilities were used: the quarantine nursery was in a single room of the CRSAD, and the NDC (challenge nursery and finisher) were separate rooms in the CDPQ test station. Only pigs belonging to this study were housed at either facility during the experiment. These two facilities were located two kilometers apart; pigs travelled a short distance by trailer when they were transferred from the quarantine nursery to the challenge nursery. The finisher unit was located within a separate wing of the CDPQ test station, so pigs were only moved a short distance on foot between the NDC and finisher stages of the experiment.

Upon arrival in the quarantine nursery piglets were randomly assigned to pen groups of four pigs. Pigs remained within their treatments until slaughter, but new pen groups were formed in the subsequent stages. When transferred to the challenge nursery, two pen groups were combined to form new groups of eight pigs. Pigs were re-grouped a final time during transfer to the finisher unit by combining two to three pen groups to form new groups of approximately fifteen pigs. Enrichment treatments continued throughout the finisher stage, which lasted an average of 15 weeks before pigs were sent to slaughter. Pigs were shipped to slaughter in two to three groups per batch; individuals that reached 115 kg live weight or more by d153 (174 days old) were shipped in a first group. The rest of the pigs in a batch would then be shipped on d174 (195 days old), except where illness, antibiotic use, or very low live weight (<80 kg) required individuals to remain in the finisher unit for longer. In these cases, remaining pigs would be shipped in a third group.

The subsequent nine batches consisted of 60 pigs each (n=540 pigs, Batches 54-64), and only the CDPQ test station was used for all three phases. The CDPQ nursery was renovated to create an onsite isolation room (Bloc C) with positive pressure ventilation and enhanced biosecurity to maintain PRRSV freedom, while the remaining nursery pens (Bloc B and D) were operationally unchanged as the challenge nursery phase. With the change in location of the quarantine nursery, group sizes changed from four and eight pigs per pen in the quarantine and challenge nurseries respectively, to 10 pigs per pen in both phases. This change was due to the increased size of pens in Bloc C compared to the CRSAD quarantine used for the first ten batches, but the space allotment per pig remained similar and continued to meet Code of Practice (NFACC, 2014) requirements. As a result, pigs were not re-grouped when they moved from the quarantine nursery to the challenge nursery. The finisher phase location and care protocols remained unchanged throughout the experiment.

Pen dimensions increased with each subsequent phase (Table 3.1). The pens used for each treatment (enrichment or control) were alternated every batch to control for pen location effects that might influence performance, such as pen microclimates resulting from variation in heating/cooling and ventilation in different sections of the room. In the quarantine nurseries, pens had fully slatted plastic flooring, solid walls, an automated waterer, a creep feeder, a heat lamp above a small solid-floor section, and a single suspended metal chain for enrichment. The chain was hung from the ceiling in the center of each pen and reaching pig shoulder-height; the length of the chain was adjusted as needed to match the height of growing pigs. To prevent cross-contamination between batches of pigs, the quarantine room was cleaned, sanitized, and left empty for three days between each batch. Each pen in the NDC was configured with fully slatted plastic Tenderfoot® flooring (Tandem Products Inc., Minneapolis, USA), with a section of solid rubber flooring approximately one-sixth of the width of the pen, placed in front of the feeder in the NDC and finisher units. metal bars between pens allowing nose to nose contact between pigs in adjacent pens, one waterer with two drinking nipples, combination feed hopper/trough, and two suspended metal chains. Pens in the finisher unit had fully slatted concrete floors, solid walls, one waterer, two suspended metal chains and one automated radio-frequency identification (RFID) IVOG® feeding stations (Hokofarm Group, Marknesse, Netherlands) on a small section of solid concrete floor. Prior to finisher room entry, pigs were fitted with unique RFID ear tags that

recorded the weight of feed consumed, the number and duration number of feeding bouts per day. Feed was dispensed into the hopper of the feeding station when a nose paddle was pressed.

Table 3. 1. Pen dimensions, group size, and space allowances for pigs in three experimental phases.

	Quarantine phase		Challenge nursery		Finisher phase
	Batches 41-50, CRSAD nursery	Batches 54-64, CDPQ quarantine	Batches 41-50, CDPQ nursery	Batches 54-64, CDPQ nursery	All batches, CDPQ finisher unit
Pen dimensions, total pen area	0.9 x 1.8 m, 1.6m^2	1.2 x 2.4 m, 3.0m^2	1.2 x 2.4 m, 3.0m^2	1.2 x 2.4 m, 3.0m^2	2.6 x 4.9 m, 12.7m^2
Pigs per pen	4-5	10	7-8	10	9-15
Space allowance per pig	0.3m^2	0.3m^2	0.4m^2	0.3m^2	0.8m^2

3.2.2 Disease challenge

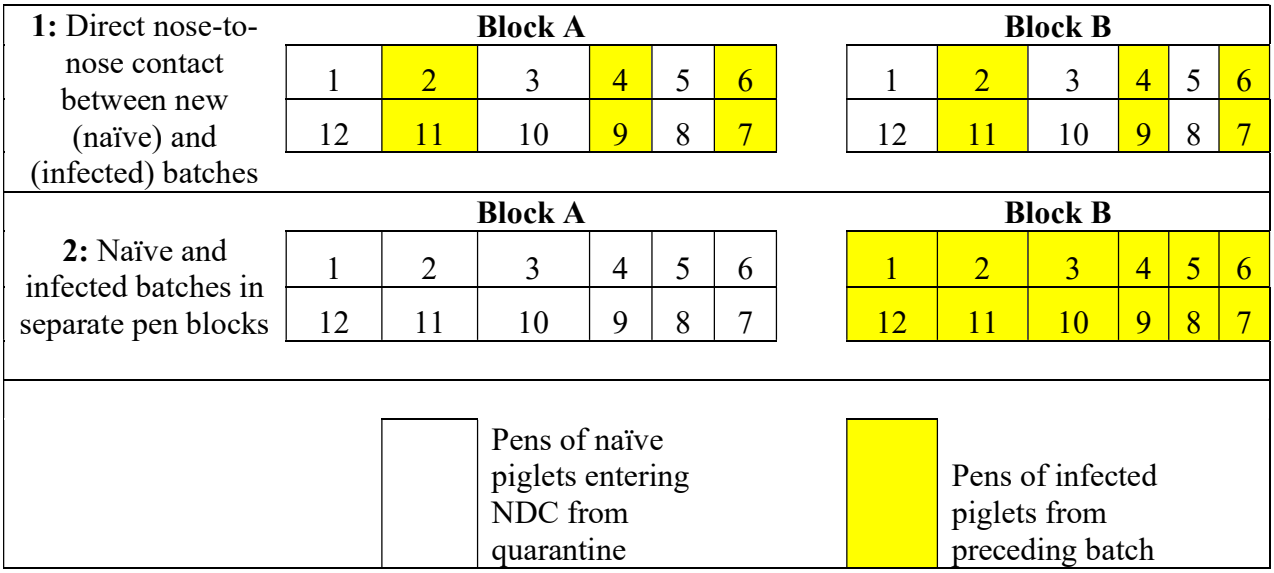
A polymicrobial natural disease challenge (NDC) was used to mimic the multi-pathogen disease pressure that pigs may be subjected to in a commercial facility (Putz et al., 2019). The barn was seeded with three strains of porcine reproductive and respiratory syndrome virus (PRRSV) and multiple bacterial pathogens of significance for swine health were detected in the farm over various batches including but not limited to *Mycoplasma hyopneumoniae*, *Glaesserella parasuis*, *Brachyspira hampsonii*, *Salmonella enterica serovar typhimurium* and *Streptococcus suis*, and two internal parasites (*Cystoisospora suis* and *Ascaris suum*) (Bai et al., 2020, Putz et al., 2019).

A continuous flow model was used in the challenge nursery with entry of new batches every three weeks and pigs remaining for four weeks. This way, purposeful cross-contamination was facilitated by airborne transfer and nose-to-nose contact between naïve and infected pigs during the one-week crossover period to maintain a relatively constant pathogen exposure across

batches of pigs. A sub-group of blood samples, balanced by pen, were collected two weeks and six weeks post-exposure (Bloods 3 and 4, see section 3.3.2for details on blood sampling) were selected and tested for the presence of PRRSV RNA using RT-PCR (Blood 3) and antibodies using enzyme-linked immunosorbent assay (ELISA, Blood 4).

Parenteral treatments were administered on an individual basis as needed and according to an approved veterinary protocol to maintain disease challenge levels that were appropriate for the experimental objectives yet in balance with animal welfare considerations. To control bacterial pathogens, the feed was medicated with tiamulin (21.2 g/tonne) and chlortetracycline (440 g/tonne) for weeks 3 and 4 of the challenge nursery period and with tiamulin (21.2 g/tonne) during week 1 of the finisher period. Pigs deemed unlikely to recover or that were too ill to maintain a reasonable welfare standard were humanely euthanized by the herd veterinarian, according to established Humane Intervention Point guidelines established by the herd and research veterinarians. To lower the pathogen load in the barn following a high-mortality batch, pen assignments were rearranged so that the following batch did not have direct nose-to-nose contact with the previous high-mortality batch (Fig. 3.1).

Fig. 3.1 Arrangement of pens during a natural disease challenge to allow A) direct nose-to-nose contact between naïve pigs entering the experiment and infected pigs from each preceding batch, or B) indirect disease transfer to new batches when disease pressure in the preceding batch increased mortality above a cutoff point of 8%. Adapted from Bai et al., (2020).



3.2.3 Enrichment treatment

Upon entry into the quarantine nursery, pigs were randomly assigned to pens and treatment with approximately 50% of pens receiving the enrichment treatment and 50% left barren (control) except for one suspended metal chain per pen to meet the Code of Practice (NFACC, 2014) requirement for enrichment. Individuals remained within their assigned treatment for the duration of the experiment. Seven enrichment items were used with one type of item presented at a time. The items were rotated three times weekly, with 16 days passing before an item was re-presented to a pen. Each material was selected for having one or more properties that are attractive to pigs: manipulable, malleable, deformable, and odorous (Van de Weerd et al., 2003). The enrichments chosen were a) rooting mat made of rubber with rope woven through it, b) cotton rope, c) 'Porcichew' commercial suspended enrichment (NutraPet, East Yorkshire, UK), d) Easyfix 'Luna' commercial floor enrichment (Easyfix, Ballinasloe, Ireland), e) jute sack, f) Flexible PVC pipe, g) rectangle of tarpaulin material (Fig. 3.2, Table 3.2). The NutraPet Porcichew is available in one size, with a variety of colours and flavour options; the same yellow vanilla-flavoured version was used in all experimental stages. Jute sack, PVC pipe, tarpaulin and cotton rope were replaced between each batch; the rope used on the rooting mat (Fig. 3.2) was also replaced and the rubber mat disinfected. The Porcichew, Lunas, and hanging chains were disinfected but not replaced between each batch unless damaged. The Luna is designed to remain relatively clean while on the floor with multiple rubber spikes on its surface, both for good hygiene and because pigs prefer unsoiled enrichments (Bracke, 2007).

All enrichments were provided at a ratio of one item for every four to seven pigs, to a maximum of two items per pen except for the Easyfix Luna, which was provided as a floor toy (not suspended) at a rate of one item per pen. Each item was attached to a ceiling-suspended chain (two chains per pen) by a metal ring and carabiner clip at a height level with the top of the pigs' backs and adjusted as needed to match the size of the pigs in the pen as they grew. This height was chosen to allow the animals to easily access the items without angling their head upwards. By suspending the items from the ceiling, they remained relatively clean and were accessible at multiple points to allow for more than one individual to investigate the item at one time.

Upon transfer to the finisher unit, the rooting mat and jute sack were removed from the enrichment rotation to prevent ingestion of these less-durable materials by larger pigs. Each phase had a separate set of enrichment items that stayed in the room and were sanitized and/or replaced between each batch. All rubber and plastic materials were cleaned and sanitized between batches, and any enrichments that became too worn and risked being ingested were replaced as needed. Cotton ropes and jute sacks were not reused between batches, so were replaced each batch and if they became frayed or very dirty. The size of the enrichment items increased with each phase to match the size of the growing pigs (Fig. 3.2).

Fig. 3.2. Seven inedible point-source environmental enrichment items provided to grow-to-finish pigs in a three-times-weekly rotation schedule.

Enrichment			
	Porcichew (NutraPet, East Yorkshire, UK)		Tarpaulin
	EasyFix Luna 86 (pictured) Luna 50, and Luna 117 (EasyFix, Ballinasloe, Ireland)		Jute sack
	Three-strand cotton rope		Rooting mat
	Flexible PVC pipe		

Table 3.2. Description of the physical properties and application of point-source swine enrichments used in three experimental phases.

Enrichment	Description	Size	Presentation	Attractive Properties
Porcichew	Vanilla scented plastic disk Antibacterial	One size	Suspended One per 4-7 pigs (2/pen) Washed & reused	Odorous Malleable
EasyFix Luna	Food-grade rubber spiked ball Designed to stay clean[1]	Q: Luna 50 N: Luna 86 F: Luna 117	Floor One per pen (4-15 pigs) Washed & reused	Manipulable Malleable
Cotton rope	Length of cotton rope tied in a knot to make two strands	Q: 13mm diameter, ~30cm length N: 17mm diameter, ~60cm length F: 17mm diameter, ~90cm length	Suspended One per 4-7 pigs (2/pen) Replaced between batches	Deformable Malleable
PVC pipe	Two lengths of pipe crossed in an "X", attached by nut & bolt	Q: 10mm diameter, ~45cm length N: 13mm diameter, ~60cm length F: 20mm diameter, ~90cm length	Suspended One per 4-7 pigs (2/pen) Replaced between batches	Malleable
Tarpaulin	Square of cut tarpaulin	Q: ~30 cm square N: ~45 cm square F: ~60 cm square	Suspended One per 4-7 pigs (2/pen) Replaced between batches	Deformable
Jute sack[2]	Rectangle of jute, folded over a metal ring & gathered with a plastic cable tie	~25x15 cm	Suspended One per 4-7 pigs (2/pen) Replaced between batches	Deformable
Rooting mat	Square of rubber anti-fatigue mat with cotton rope woven through	30x30 cm	Suspended One per 4-7 pigs (2/pen) Mat washed & reused, rope replaced between batches	Malleable Deformable

[1]Q= quarantine, N= natural challenge barn, F= finisher. [2]: EasyFix balls have only three spikes touching the ground at a time to minimize contact with dirt and feces on the floor of the pen. [3]: Jute sack was not used in the finisher phase due to the increased risk of consumption.

3.3 DATA COLLECTION

3.3.1 Productivity Measures

When deaths occurred, the date, batch, pig ID, treatment group, and pen number were recorded in a computer database. Additionally, in the few instances where complications arose during blood sampling (extensive pneumonia, accidental piercing of the phrenic nerve), the herd veterinarian immediately euthanized the individual, and the death was recorded. Due to the low frequency of this occurrence, and that it could not be ruled out that the death was related to the disease challenge, mortalities during blood sampling were included in experimental mortality calculations.

Pigs were weighed individually every three weeks from entry to slaughter beginning on d-19 upon entry into the quarantine nursery (Fig. 3.3). On weeks where blood draws and weighing dates coincided, blood draws were performed at least one day before weighing to minimize risk of handling stress influencing blood parameters and visual health scoring. Deceased pigs were also weighed after death for inclusion in average daily gain (ADG) calculations.

Feed hoppers were filled once a day in the quarantine nursery and NDC by weighing and recording the feed given to each pen every morning. If the feed was not fully consumed by the next morning, progressively less feed would be given each day until a level was reached at which almost all feed was consumed within a 24 -hour period. Conversely, if all feed given to a pen was consumed from one morning to the next, more feed would be added to the ration until a point was reached where not all feed was finished each day and only a small amount of feed was left on the bottom of the hopper the following morning. Therefore, the quantity of feed given on a pen level was either maintained, increased, or decreased to match the relative daily intake level of each pen, but this method was not precise. This method was used to save time and labour as part of the research facility's standard protocol. For feed conversion ratio (FCR) calculations, it was assumed that the feed weighed and given to each NDC pen each day was equal to the feed consumed. In the finisher unit, data from the RFID feeders was automatically exported to a computer program that recorded the feed intake, frequency, and duration of feeding bouts for individuals over a 24-hour period.

Fig. 3.3. Timing of blood sampling, weight recording, and videos recorded for analysis of pen-level behaviour of grow-finish pigs during three experimental phases of a polymicrobial natural disease challenge.

Fig. 3.3a. Quarantine phase (d-19 to d0).

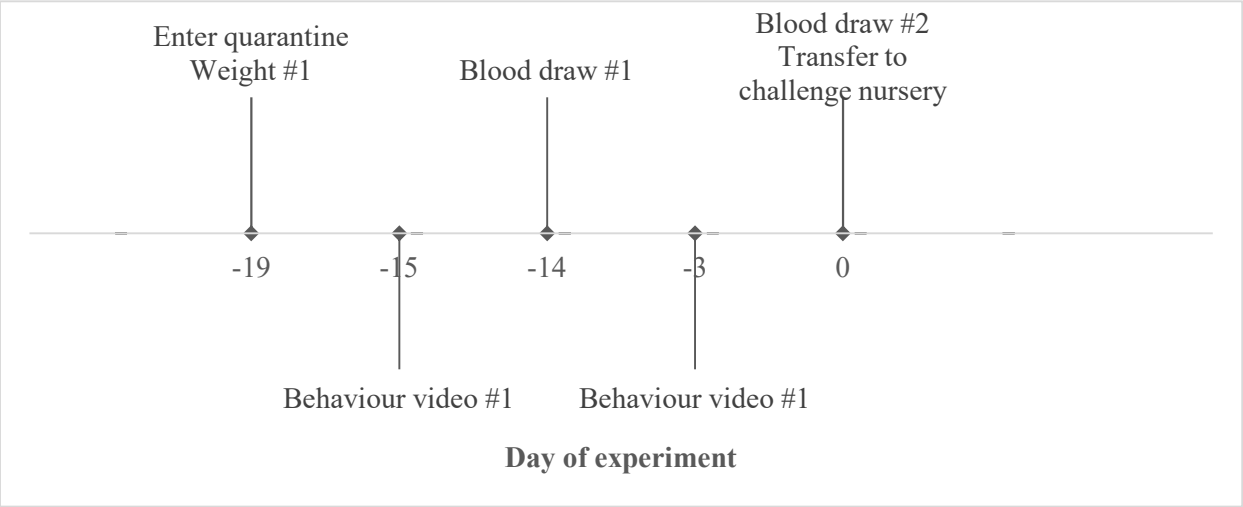


Fig. 3.3b. Challenge nursery phase (d0 to d28).

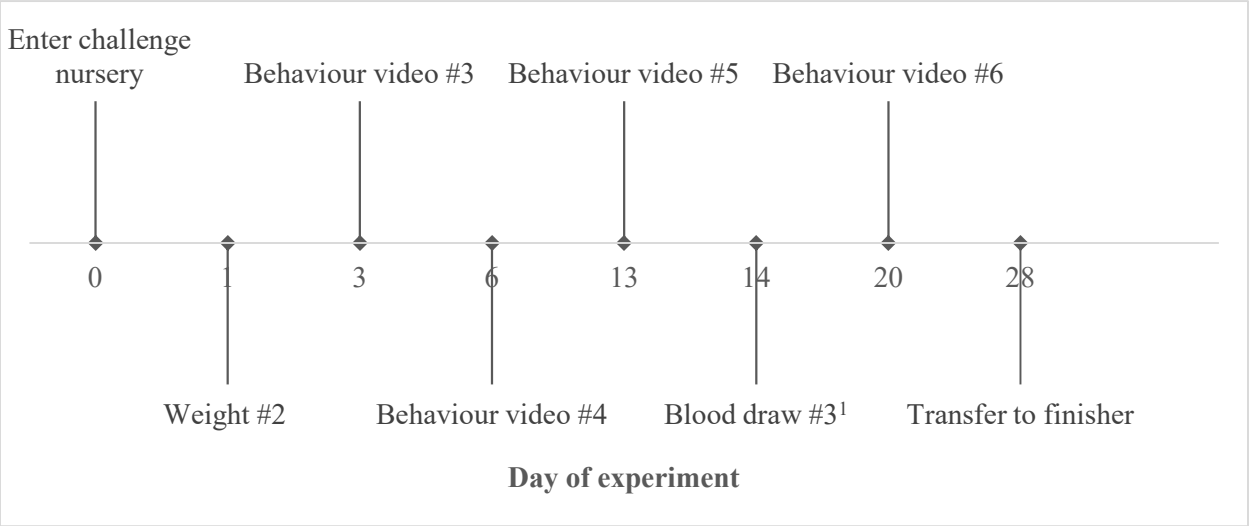
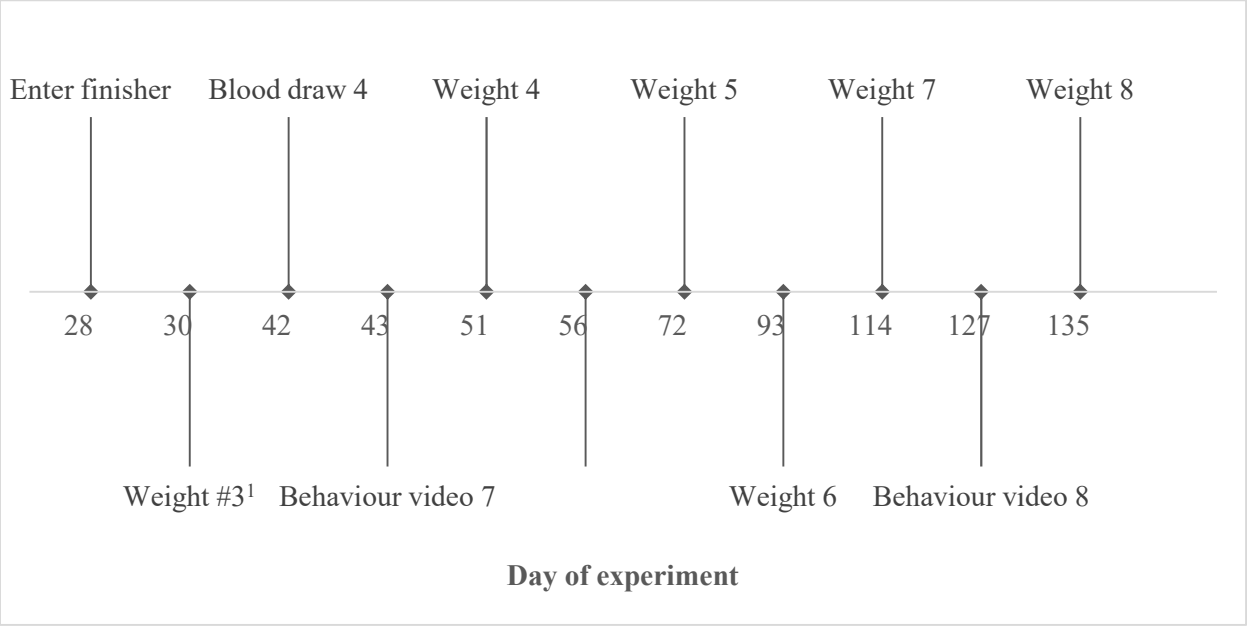


Fig. 3.3c. Finisher phase (d28 to d154).



[1]Blood draws #2 and #5were used only for disease testing and to collect blood transcriptome markers; the data collected is not included in this experiment. Blood draw #3 was only collected for the first eight batches of the experiment.

[2]Weight #3 was collected in the finisher unit but used for NDC growth rate calculations.

3.3.2 Immunology Parameters

Blood samples were drawn a minimum of two times per batch, on which a complete blood count (CBC) was performed to provide a panel of immune cell counts. The samples were taken on d-14 of the experiment (Blood 1) and day 42 (Blood 4) for all batches. Three additional blood samples were collected from pigs in the first 10 batches (Bloods 2, 3, and 5, Fig. 3.3) for use in a separate, overlaid experiment using the same test subjects. These blood samples were not analyzed for the present study.

Blood draws were performed by trained technicians. Several technicians performed sampling in different pens simultaneously: in the quarantine and challenge nurseries (Bloods 1, 2 and 3), one technician would perform the blood draw while a second technician labelled blood vials and recorded any relevant notes on health status. Blood was collected from the external jugular vein into K2 ethylenediaminetetraacetic acid (EDTA) tubes (BD Vacutainer R blood collection tubes, New Jersey, United States), with the piglet restrained in dorsally recumbent in a wooden V-shaped trough. The second technician held the legs and/or snout of the piglet when needed to keep the piglet still. For smaller piglets in the quarantine and challenge nursery, or when the jugular vein was difficult to access, blood was drawn via the cephalic vein in the same manner described above. For Blood 4, blood was drawn from the jugular vein. Pigs that subjectively appeared too ill (severely anemic and/or emaciated) to draw blood from humanely would be excluded from sampling and this information was recorded.

Samples were shipped overnight to a laboratory at the University of Alberta (Edmonton, Alberta, Canada) for analysis using an ADVIA R 2120i Hematology System (Siemens Healthineers, Erlangen, Germany). Complete blood counts (CBC) were performed on each sample, and ten parameters were selected for analysis based on their relevance to disease-specific and generalized cellular immune responses expected from the disease challenge. Six white blood cell traits were evaluated as measures of change in immune cell concentrations from baseline (Blood 1) to post-challenge (Blood 4) based on their roles in innate and adaptive immunity in response to bacteria, viruses, and/or parasites. The cell counts chosen for analysis were: total white blood cell concentration (WBC), neutrophils (NEUT), lymphocytes (LYMPH), monocytes (MONO), basophils (BASO), eosinophils (EOS). These parameters were selected to examine the between-treatment differences in immune cell concentrations when compared to baseline (Blood 1) values by calculating the difference between Bloods 1 and 4 on an individual level (ΔBlood 1-4).

Additionally, neutrophil: lymphocyte ratio (N:L) was calculated as a potential indicator of bacteremia (Jiang et al., 2019), systemic inflammation, and/or acute stress (Zahorec, 2001). Lastly, three red blood cell traits were analyzed: total red blood cell count (RBC), hemoglobin (HGB), and hematocrit (HCT). These measures represent the oxygen carrying capacity of the blood (George-Gay and Parker, 2003), which may be decreased in pigs infected with PRRSV, which can induce non-regenerative anemia (Halbur et al., 2002). Differences in RBC traits between treatments could indicate a difference in disease symptom severity.

3.3.3 Pen-Level Behaviour

A separate set of cameras were used in each of the three growth stages (quarantine, challenge nursery, finisher) to reduce cross-contamination and labour required as there was a constant flow of batches running concurrently in each phase. In the quarantine nursery, four Plotwatcher Pro trail cameras (Day 6 Outdoors, Dover, Florida, United States) were installed above a block of seven pens so that three cameras recorded two pens each and one camera recorded a single pen. In the NDC, four TrendNet View Pro cameras (TrendNet, Torrance, California, United States) were affixed above a block of eight pens so that each camera recorded two pens. The NDC room contained two blocks of 10 pens each, where one block was used per batch and the block used alternated each batch; as such, the cameras were moved from one block to the other in between batches. Arlo Pro wireless security cameras (Arlo Technologies Inc., San Jose, California, United States) were used in the finisher unit: two to three cameras were used to film one to two pens each. Each camera was mounted high on the wall behind the pens to film as much area of the pen(s) as possible.

To reduce labour and potential human error, cameras in the quarantine and challenge nurseries were operated on a timer that turned the cameras on and off during the allocated video recording time periods. The videos were then manually exported from the internal memory of the camera to an external hard drive. The Arlo cameras in the finisher unit were run on battery power (as opposed to a wired connection for Plotwatcher and TrendNet cameras), so to save battery life the cameras were turned on and off manually on the recording days. The Arlo cameras had Wi-Fi connectivity, so videos were uploaded to the Arlo website in real time, then manually downloaded onto an external hard drive at the end of each recording day.

Behaviour data was collected from eight batches were used for analysis in this study (64 pen units in quarantine, 54 in NDC, and 32 in finisher). A minimum of 50% of the pens within each batch were recorded from 8-10 AM and 2-4 PM on each recording day, for a total of four hours of video per day. Video recordings were captured on the same experimental day for each batch, with two recordings in the quarantine nursery, four in the challenge nursery and two in the finisher unit (Fig. 3.3). All pen checks, feeding, and veterinary treatments were performed outside of the hours of video recording to avoid interruptions in the pigs' behaviour.

An ethogram of relevant behaviours was developed (Table 3.3) prior to the behavioural analysis and a single observer was trained to perform the behavioural analysis; the same observer watched and recorded behaviour for all batches in the experiment. The observer was not blind to treatments as the enrichments or chains were visible in each pen. All postures were considered mutually exclusive of each other, while interaction with enrichment was coded separately and could be performed concurrently with other postures. Videos were scan sampled once every three minutes; this method created approximately 80 still frame images for each recording day. A Microsoft® Excel 2016 (Microsoft Corporation, Washington, United States) spreadsheet was used to record the number of pigs performing each behaviour within a single pen in each scan sample. All scan samples for each recording day were viewed for a single pen at a time before moving on to the next pen.

Table 3.3. Ethogram of behaviours and postures observed during pen-level behaviour analysis of pigs during three phases of a polymicrobial natural disease challenge.

Behaviour	Description
Standing	Standing with all four hooves on the ground or in motion; includes walking and standing inactive[1]
Sitting	Upright torso with body weight supported by hindquarters and front hooves[1]
Huddling	Sternal lying with front legs tucked underneath body, weight supported on knees and hind legs[2]
Sternal lying	Lying in a sternal recumbent position (stomach to the floor) with two or more legs extended out from the body[2]

Lateral lying	Lying in a lateral recumbent position with shoulder to the floor and legs extended out from the body[2]
Isolated	Lying at least 1/3 of the width/length of the pen away from any other pigs
Interact with enrichment [a]	Oral or nasal manipulation of the enrichment or chain (control)

[1]Modified from Fabrega et al., 2019, [2]Modified from Barbieri et al., 2012

[a]Not mutually exclusive with other behaviours.

3.4 STATISTICAL ANALYSIS

3.4.1 Common analysis procedures

Statistical analysis was performed using the software SAS 9.4 (SAS Inst. Inc., North Carolina, United States). Microsoft® Excel 2016 (Microsoft Corporation, Washington, United States) spreadsheets were used to store data and perform preliminary calculations. Since groups of pigs were mixed from one phase to the next, each experimental phase (quarantine, challenge nursery, finisher) was analyzed separately. Results were considered significant at $\alpha=0.05$. For all variables, descriptive statistical analysis (proc UNIVARIATE) was first performed on the raw datasets to test for normality of distribution using a Shapiro-Wilk (W) test. Variables with a W statistic $p>0.05$ were determined to follow a normal distribution. If they were not normally distributed, $\log_{10}$ transformations were applied, and the variables were re-analyzed before choosing a model to fit. Where transformations were performed, results in this chapter are presented as raw non-transformed data computed using the final model(s) selected.

3.4.2 Mortality Analysis

Pen-level mortality within each phase was calculated as the number of dead pigs divided by the total number of pigs in the pen, presented as a proportion of the pen that died during that phase. The effect of treatment on pen-level mortality was tested using a generalized linear mixed model (Proc GLIMMIX) with beta distribution, specifying a logistic link function, and batch included as a random effect. Pen location within the barn was not significant within any of the phases, so was not included as a random effect. Mortality in the quarantine nursery was extremely

low, so a constant value of 0.01 was added to each pen mortality calculation to allow zero-mortality pens to be included in the models.

3.4.3 Growth Rate Analysis

Growth rate (ADG) and FCR were calculated on a pen level for each experimental phase. For each three-week period between weightings, the weight gains of all pigs in a pen were summed, including weight gain of any pigs that died during the period by including their weight at death. The total number of days of growth was then calculated by multiplying the number of pigs in the pen by the number of days between weightings that pigs remained in the pen. Where mortalities occurred, the number of days that the pig was alive during the three-week period was added to the total days of growth creating an 'adjusted days of growth' calculation. The ADG was then calculated for each weigh period by dividing the total pen weight gain by the total adjusted days of growth. Lastly, phase-level ADG for each pen was calculated by summing the pen's ADG for each growth period and dividing by the total adjusted days of growth in that phase.

The same calculation procedure was followed for pen-level FCR for each phase, which was calculated for each three-week growth period by summing the total feed intake over the period and dividing by the number of adjusted days of growth within that phase.

The effect of treatment on pen-level ADG and FCR within each phase was tested using a linear mixed model (Proc MIXED) with Tukey adjustment, with batch as a random effect. Where pen location was found to be significant, it was nested within batch as a random effect.

3.4.4 Complete Blood Count Analysis

The CBC traits were analyzed separately for Blood 1, Blood 4, and ΔBlood 1-4, with individual pig as the experimental unit. A conservative approach was taken to remove only extreme outliers most likely to have resulted from error rather than from normal biological variation (Bai et al., 2020). Extreme outliers were identified from studentized residuals as data points three or more standard deviations from the mean of each blood value (Osborne and Overbay, 2004), and were removed for all variables in Blood 1 and Blood 4. ΔBlood 1-4 was calculated using the Blood 1 and Blood 4 datasets with outliers removed so further outliers were not removed.

The fixed effect of treatment on each CBC trait was first analyzed using a linear mixed model (Proc MIXED) with an SMM adjustment for multiple comparisons with unequal sizes, specifying studentized residuals (SAS Institute, 1999). Pen nested in batch was included as a random effect for variables where pen location was found to be significant; for all others, batch alone was included as a random effect. For variables with non-normal residuals, a $\log_{10}$ transformation was applied and the model re-run. For Blood 1 and Blood 4, the following variables were transformed for the final models: WBC, NEUT, LYMPH, N:L, MONO, BASO, and EOS. Transformation did not improve the distribution of the red blood cell traits (RBC, HCT, HGB) in Blood 1 or 4, or any variables in ΔBlood 1-4, so the untransformed data sets were used for those variables. Where transformations were applied, results were checked for accuracy by back-transforming means and comparing to results of the same model run on the untransformed data.

For reference range calculations, average CBC reference values for swine (Iowa State University, 2011) were used to score individual pigs as either '0' (within reference range) or '1' (outside of reference range) for their Blood 1 and Blood 4 CBC values. Reference ranges for pigs 0-42 days of age were used for Blood 1, and values for pigs 42 days- 2 years of age were used for Blood 4. To determine whether the proportion of pigs with cell counts outside of a normal reference range differed by treatment, Proc FREQ was used to produce 2x2 contingency tables, using a Fisher's exact test for independence.

3.4.5 Pen-Level Behaviour Analysis

A generalized linear mixed model (Proc GLIMMIX) with specified logit link function and binomial distribution was fitted to pen-level behaviour data to test the fixed effect of treatment on the probability of each behaviour occurring during a day and time within each pen. The model analyzed the raw count data for the number of pigs observed performing a behaviour of interest within a sampling period (numerator) over the number of pigs that were observed in the pen at that time period (denominator). The effects of batch and pen were tested for each variable and pen nested within batch was included in all final models as a random effect. The effects of treatment, time (AM, PM), and day, and their interaction were explored as fixed effects. A Tukey-Kramer adjustment was applied to the least square means (LSmean). For NDC behaviour models, a 3-way interaction of treatment*day*time was included in the final model, and day was included in a

random intercept statement to account for similarities between days. For quarantine and finisher models, only the 3-way interaction of treatment*day*time was examined; day was not included as a random intercept because each phase had only two days within it. Where day or time were not significant, they were removed from the fixed effects and all other possible interactions (2-way interaction (treatment*day or treatment*time) were explored instead. For quarantine and finisher behaviour models, day could not be included as an intercept as there were only two observation days within each phase.

Two behaviours (huddling and isolated lying) occurred at a very low rate in all phases, as did sitting in the quarantine phase. These behaviours fit the models poorly; descriptive statistics were instead presented for these behaviours.

3.5 RESULTS

3.5.1 Mortality and Growth Rate Results

No significant ($p>0.05$) differences were found between treatments for mortality, ADG, or FCR in any of the three phases (Table 3.4, 3.5).

Table 3.4. Treatment differences in pen-level mortality (LSmean ± SEM) between enriched and control pigs in three experimental phases.

Mortality (proportion of pigs/pen)			
	Treatment		
Experimental Phase	Control[1]	Enrichment[1]	*p*
Quarantine	0.01 ± 0.01	0.01± 0.01	0.79
Challenge nursery	0.18 ± 0.02	0.22 ± 0.02	0.16
Finisher	0.14 ±0.02	0.17 ± 0.02	0.13

[1]Sample sizes: Quarantine phase, N=219 pens (control=124, enrichment=95); challenge nursery phase, N= 128 pens (control=69, enrichment=59); finisher phase, N=65 pens (control=35, enrichment=30).

Table 3.5. Pen-level average daily gain (ADG) and feed conversion ratio (FCR) comparisons between enriched and control pigs in two experimental phases (LSmean ± SEM).

	ADG (kg/day)			FCR (kg gained/kg feed)		
Experimental Phase[a]	Control[1]	Enrichment[1]	*p*	Control[1]	Enrichment[1]	*p*
Challenge nursery	0.28 ± 0.02	0.29 ± 0.02	0.46	0.48 ± 0.02	0.51 ± 0.02	0.14
Finisher	0.87 ± 0.02	0.88 ± 0.02	0.70	0.44 ± 0.01	0.45 ± 0.01	0.50

[1]Sample sizes: Challenge nursery, N=136 pens (control=74, enrichment=62); finisher, N=79 pens (control=42, enrichment=37).

3.5.2 Complete Blood Count Results

Treatment groups did not differ in baseline CBC values (Blood 1, Table 3.6) upon entry into the quarantine phase. The increase in total WBC concentration from Blood 1 to Blood 4 was greater for enriched pigs than for controls (E: 9.58±1.14, C: 8.60±1.14, p=0.03), but none of the individual WBC components (NEUT, LYMPH, MONO, BASO, EOS) or RBC traits differed significantly in Blood 4 or ΔBlood 1-4 (Table 3.6). Additionally, no significant differences were found in the proportion of pigs in each treatment with CBC values within or outside of a normal reference range for Blood 1 and Blood 4 (Table 3.7).

Table 3.6. Comparison of swine complete blood cell (CBC) counts measured at 5 days post entry into the quarantine (Blood 1) and at day 42 post exposure in the finisher (Blood 4) phase, as well as the mean and percent changes from Blood 1 to Blood 4. Results presented as raw (non-transformed) treatment (CTRL= control, ENR= enrichment) LSmean ± SEM.

Trait[1]	Blood 1 n=1129				Blood 4 n=985				ΔBlood 1-4 n=921[3]				
	CTRL n=602	ENR N=527	*p*	Ref. range[2]	CTRL n=534	ENR n=451	*p*	Ref. range[2]	CTRL n=496	CTRL ΔBlood 1-4 (%)	ENR n=425	ENR ΔBlood 1-4 (%)	*p*
WBC[4] ($x10^9$ cells/L)	11.8± 1.06	11.7±1.06	0.91	9.62-25.2	20.1±1.02	20.7±1.02	0.20	11.4-28.9	**8.60±1.14**	**+73%**	**9.58±1.15**	**+82%**	**0.03**
NEUT ($x10^9$ cells/L)	5.13±1.05	4.98±1.05	0.87	2.35-11.9	8.76±1.03	9.13±1.03	0.36	2.0-10.4	3.72±0.89	+73%	4.32±0.90	+87%	0.07
LYMPH ($x10^9$ cells/L)	5.79±1.02	5.82±1.02	0.91	4.02-12.5	8.65±1.02	8.89±1.02	0.11	5.3-17.9	2.99±0.29	+52%	3.23±0.29	+55%	0.19
N:L ratio (no units)	0.89±1.05	0.85±1.05	0.56	N/A	1.02±1.04	1.03±1.04	0.80	N/A	0.15±0.13	+17%	0.19±0.13	+22%	0.48
MONO ($x10^9$ cells/L)	0.36± 1.04	0.36± 1.04	0.70	0.05-2.30	1.17±1.03	1.21±1.03	0.52	0.00-3.70	0.86±0.06	+239%	0.91±0.06	+253%	0.22
BASO ($x10^9$ cells/L)	0.07±1.13	0.08±1.15	0.57	N/A	0.18±1.09	0.19±1.09	0.58	N/A	0.01±0.21	+14%	0.02±0.21	+25%	0.78
EOS ($x10^9$ cells/L)	0.33±1.06	0.33±1.07	0.76	0.00-0.05	0.36±1.05	0.35±1.06	0.71	0.00-1.30	0.04±0.05	+12%	0.04±0.05	+12%	0.91
RBC ($x10^9$ cells/L)	6.01±0.06	6.01±0.06	0.93	4.87-7.88	6.26±0.05	6.23±0.05	0.71	5.88-8.19	0.13±0.15	+2%	0.11±0.15	+2%	0.67
HCT (no units)	0.36±0.01	0.36±0.01	0.90	0.28-0.40	0.35±0.00	0.34±0.00	0.62	0.32-0.43	-0.02±0.01	-6%	-0.02±0.01	-6%	0.35
HGB (g/L)	110±2.84	109±2.85	0.58	80.8-119	104±0.89	103±0.90	0.09	112-147	-4.85±2.56	-4%	-6.35±2.57	-6%	0.11

[1]WBC= white blood cells, RBC= red blood cells, HGB= hemoglobin, HCT= hematocrit, NEUT= neutrophils, LYMPH= lymphocytes, N:L= neutrophil to lymphocyte ratio, MONO= monocytes, BASO= basophils, EOS= eosinophils. [2]Reference range values from Iowa State University (2011). Blood 1 is compared to a reference range for pigs aged 0-6 weeks, and Blood 4 is compared to a reference range for pigs 6 weeks-2 years of age. [3]ΔBlood 1-4 was calculated on raw data with a reduced sample size due to mortalities, missing samples, etc. and is therefore not exactly equal to Blood 4 LSmean – Blood 1 LSmean. [4]Total WBC is equal to the sum of the WBC subsets listed (NEUT, LYMPH, MONO, BASO, EOS) plus concentration of large unstained cells (not analyzed).

Table 3.7. Contingency table comparing the proportion of pigs between treatments with complete blood count (CBC) values outside of a standard reference range (Iowa State University, 2011) in Blood 1 (n=1129) and Blood 4 (n=985). Results expressed as % of pigs in treatment group (number of pigs) that are within or outside of the reference range.

CBC trait[1]		Within Ref. Range, %(N)		Outside Ref. Range, %(N)		*p*
		Control	Enrichment	Control	Enrichment	
WBC	Blood 1	68.7 (388)	66.5 (332)	31.3 (177)	33.5 (167)	0.47
	Blood 4	98.1 (512)	97.5 (428)	1.9 (10)	2.5 (11)	0.66
RBC	Blood 1	91.8 (552)	94.1 (495)	8.2 (49)	5.9 (31)	0.16
	Blood 4	76 (403)	75.7 (337)	24 (127)	24.3 (108)	0.94
HCT	Blood 1	69.2 (364)	73.7 (334)	30.8 (162)	26.3 (119)	0.12
	Blood 4	81.2 (428)	76.8 (341)	18.8 (99)	23.2 (103)	0.10
HGB	Blood 1	67 (402)	67.2 (352)	33 (198)	32.8 (172)	1.00
	Blood 4	18.6 (98)	17.1 (76)	81.4 (428)	92.9 (368)	0.56
NEUT	Blood 1	78 (467)	78.8 (412)	22 (132)	21.2 (111)	0.77
	Blood 4	67.7 (360)	66.5 (298)	32.3 (172)	33.5 (150)	0.73
LYMPH	Blood 1	86.7 (522)	88.1 (464)	13.3 (80)	12 (63)	0.53
	Blood 4	95.4 (501)	96.7 (434)	4.6 (24)	3.3 (15)	0.41
MONO	Blood 1	100 (601)	99.8 (527)	0 (0)	0.2 (1)	0.47
	Blood 4	53.2 (279)	51.1 (527)	46.8 (245)	49 (220)	0.52
EOS	Blood 1	71.1 (427)	75.2 (394)	29 (174)	24.8 (130)	0.12
	Blood 4	99.3 (530)	99.8 (450)	0.8 (4)	0.2 (1)	0.38

[1]WBC= white blood cell concentration, RBC= red blood cell concentration, HGB= hemoglobin concentration, HCT= hematocrit, NEUT= neutrophil concentration, LYMPH= lymphocyte concentration, MONO= monocyte concentration, EOS= eosinophil concentration.

3.5.3 Pen-Level Behaviour Results

In the quarantine phase, the enrichment group had a higher probability of enrichment use than of chain use in control group on d-15, but use declined by d-3 and there were no longer significant differences between treatments (Table 3.8). On d-15, probability was higher for standing and lower for sternal lying in the enrichment group in the AM, but no differences in postures were found on d-3 between treatments. The enrichment group also had a higher probability of lateral lying in the PM across the quarantine phase (no significant day effects) compared to controls.

In the challenge nursery, the probability of enrichment use was greater in the enrichment group than chain use in control groups on d3, d6 (AM only), d13, and d20 (PM only) (three-way interaction of treatment*day*time, Table 3.9). However, there was no difference in the probability for specific postures to occur between treatment groups at any point in the NDC phase.

In the finisher phase, enrichment use was more likely in the enrichment group on d70 and d140, but interaction was low throughout the phase and declined to nearly zero by d140 in both treatments (Table 3.10). Additionally, the probability in the control group for observing lateral lying was lower on d70 (treatment*day interaction), and higher for sternal lying on d70 AM. No other interactions were significant in the finisher phase.

Mean frequencies of low-occurrence behaviours (huddling, isolated lying, sitting) are presented in Table 3.11.

Table 3.8. Mean probability of pigs in the quarantine phase performing a behaviour or posture during a sampling day (Day -15, Day -3) and time (AM, PM). Results presented as LSmean ± SEM.

		Day -15 (n=88 pens)			Day -3 (n=78 pens)			
Behaviour	Time	Enrichment (N=44)	Control (N=44)	Treatment within Day *p*	Enrichment (N=39)	Control (N=39)	Treatment within Day *p*	Treatment * Day * Time *p*
Interact w/ enrichment	**AM**	**0.07±0.01[a]**	**0.01±0.00[b]**	**<0.01**	0.02±0.00[b]	0.01±0.00[b]	0.12	0.003
	PM	**0.05±0.01[a]**	**0.03±0.01[b]**	**<0.01**	0.02±0.01[c]	0.02±0.01[c]	1.00	
Standing	**AM**	**0.30±0.03[a]**	**0.24±0.02[b]**	**<0.01**	0.28±0.03[a]	0.28±0.03[a]	1.00	<0.01
	PM	0.30±0.03[a]	0.29±0.03[a]	1.00	0.30±0.03[a]	0.32±0.03[b]	0.94	
Sternal lying	**AM**	**0.23±0.00[a]**	**0.33±0.01[b]**	**<0.01**	0.29±0.01[b]	0.31±0.01[b]	0.73	<0.001
	PM	0.26±0.01[a]	0.29±0.01[a]	0.25	0.29±0.01[b]	0.32±0.01[b]	0.19	

		Quarantine phase			
Behaviour	Time	Enrichment	Control	Treatment within time *p*	Treatment * Time *p*
Lateral lying*	AM	0.39±0.03[a]	0.37±0.03[a]	0.81	<0.001
	PM	**0.35±0.03[b]**	**0.30±0.03[b]**	**<0.01**	

Within a treatment and time of day (AM/PM), superscripts indicate differences across days ($p<0.05$). Within a day, between treatments, the p value indicated differences between treatments within a day for AM and PM separately. * For lateral lying, superscripts indicate differences ($p<0.05$) within treatment between AM and PM.

Table 3.9. Mean probability of pigs in a natural disease challenge phase performing a behaviour or posture during a sampling day (Day 3, 6, 13, 20) and time (AM, PM). Results presented as LSmean ± SEM for enrichment (E) and control (C) treatment groups.

		Day 3 (n=41 pens)			Day 6 (n=56 pens)			Day 13 (n=58 pens)			Day 20 (n=67 pens)			
Behaviour	Time	E (N=21)	C (N=20)	TRT within Day *p*	E (N=30)	C (N=26)	TRT within Day *p*	E (N=28)	C (N=30)	TRT within Day *p*	E (N=36)	C (N=31)	*p*	TRT* Day* Time
Interact w/ enrichment	**AM**	**0.05±0.01 a**	**0.01±0.00 a**	**<0.001**	**0.03±0.01 a**	**0.01±0.00 a**	**<0.001**	**0.03±0.01 a**	**0.00±0.00 a**	**<0.001**	0.00±0.00 b	0.00±0.00 a	0.12	<0.001
	PM	**0.04±0.01 a**	**0.01±0.00 ac**	**<0.001**	0.02±0.00 a	0.01±0.00 a	0.69	**0.03±0.01 a**	**0.00±0.00 bc**	**<0.001**	**0.01±0.00 b**	**0.00±0.00 b**	**<0.007**	
Standing	**AM**	**0.33±0.02 a**	**0.39±0.03 a**	**0.03**	0.21±0.02 b	0.24±0.02 b	0.22	0.17±0.02 c	0.18±0.02 c	1.00	0.13±0.01 d	0.15±0.01 d	0.63	<0.001
	PM	0.26±0.02 a	0.32±0.02 a	0.06	**0.24±0.02 b**	**0.36±0.03 b**	**<0.001**	0.22±0.02 c	0.24±0.02 c	0.97	**0.14±0.01 d**	**0.20±0.02 d**	**<0.001**	
Lateral lying	**AM**	**0.19±0.03 aT**	**0.12±0.02 a**	**<0.001**	**0.27±0.04 b T**	**0.18±0.03 b**	**<0.001**	0.19±0.03 a	0.19±0.03 b	1.00	**0.31±0.04 b**	**0.22±0.03 b**	**<0.001**	<0.001
	PM	**0.24±0.04 aT**	**0.16±0.03 a**	**<0.001**	**0.25±0.04 a**	**0.13±0.02 a**	**<0.001**	0.16±0.02 bT	0.15±0.02 aT	1.00	**0.31±0.04 a**	**0.24±0.03 bT**	**0.03**	
Sitting	AM	0.02±0.00 a	0.02±0.00 a	1.00	0.02±0.00 a	0.02±0.00 a	1.00	0.02±0.00 a	0.02±0.00 a	1.00	0.02±0.00 a	0.02±0.00 a	1.00	0.005
	PM	0.03±0.00 a	0.02±0.00 a	1.00	0.02±0.00 a	0.03±0.00 a	0.66	0.03±0.00 a	0.03±0.00 a	1.00	0.03±0.00 a	0.02±0.00 a	1.00	
Sternal lying	AM	0.38±0.02 a	0.42±0.02 a	0.18	0.46±0.02 b	0.50±0.02 b	0.12	**0.52±0.02 c**	**0.60±0.02 c**	**<0.001**	**0.49±0.02 d**	**0.57±0.02 d**	**<0.001**	
	PM	0.39±0.02 a	0.44±0.02 a	0.13	0.43±0.02 b	0.43±0.02 a	1.00	**0.48±0.02 c**	**0.56±0.02 b**	**<0.001**	0.47±0.02 c	0.51±0.02 c	0.36	0.003

E= enriched pens, C = control pens, TRT= treatment. Within a treatment and time of day (AM/PM), superscripts indicate differences across days ($p<0.05$). Within a day, between treatments, the p-value indicated differences between treatments within a day for AM and PM separately. T= where appears, significant between two variables at the level of a tendency, $p<0.10$.

Table 3.10. Probability of pigs in the finisher phase performing a behaviour or posture during a sampling day (Day 70, Day 140) and time (AM, PM). Results presented as LSmean ± SEM.

Behaviour	Time	Day 70 (n=48 pens)			Day 140 (n=49 pens)				
		Enrichment (N=23)	Control (N=25)	Treatment within day *p*	Enrichment (N=23)	Control (N=26)	Treatment within Day *p*	Treatment x Day *P*	Treatment x Day x Time *p*
Enrichment interaction	**-**	**0.02±0.00[a]**	**0.00±0.00[b]**	**<0.01**	**0.01±0.00[b]**	**0.00±0.00[b]**	**<0.01**	<0.01	NS
Standing	-	0.35±0.02[a]	0.35±0.01[a]	1.00	0.19±0.01[b]	0.22±0.01[b]	0.22	<0.001	NS
Sitting	AM	0.03±0.00[a]	0.03±0.00[a]	0.99	0.04±0.00[a]	0.03±0.00[a]	0.78	-	0.01
	PM	0.03±0.00[a]	0.03±0.00[a]	0.81	0.04±0.00[a]	0.03±0.00[a]	0.15		
Sternal lying	**AM**	**0.36±0.01[a]**	**0.42±0.01[a]**	**<0.01**	0.31±0.01[b]	0.32±0.01[b]	0.93	-	0.003
	PM	0.36±0.01[a]	0.38±0.01[a]	0.85	0.33±0.01[b]	0.34±0.01[b]	0.99	-	NS
Lateral lying	-	**0.23±0.02[a]**	**0.19±0.01[a]**	**0.04**	0.42±0.02[b]	0.39±0.02[b]	0.51	0.001	NS

E= enriched pens, C = control pens. Within a treatment and time of day (AM/PM), superscripts indicate differences across days ($p<0.05$). Within a day, between treatments, the P value indicated differences between treatments within a day for AM and PM separately.

Table 3.11. Descriptive statistics for average relative frequency of low occurrence pen-level pig behaviours in three experimental phases, separated by treatment, sampling day (Day -15, -3), and time (AM, PM).

Table 3.11a. Quarantine phase.

Behaviour	Time	Day -15 (n=88 pens)				Day -3 (n=78 pens)			
		Control (N=44)		Enrichment (N=44)		Control (N=39)		Enrichment (N=39)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Huddling	AM	0.02	0.00	0.01	0.00	0.02	0.00	0.02	0.00
	PM	0.01	0.00	0.01	0.00	0.03	0.00	0.02	0.00
Isolated lying	AM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	PM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sitting	AM	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00
	PM	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00

Table 3.11b. Natural disease challenge phase.

Behaviour	Time	Day 3 (n=41 pens)				Day 6 (n=56 pens)				Day 13 (n=58 pens)				Day 20 (n=67 pens)			
		Control (N=21)		Enrichment (N=20)		Control (N=30)		Enrichment (N=26)		Control (N=28)		Enrichment (N=30)		Control (N=36)		Enrichment (N=31)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Huddling	AM	0.02	0.00	0.02	0.00	0.02	0.00	0.02	0.00	0.02	0.00	0.01	0.00	0.01	0.00	0.01	0.00
	PM	0.03	0.00	0.02	0.00	0.03	0.00	0.02	0.00	0.02	0.00	0.01	0.00	0.01	0.00	0.01	0.00
Isolated lying	AM	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00
	PM	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00

Table 3.11c. Finisher phase.

Behaviour	Time	Day 70				Day 140			
		Control		Enrichment		Control		Enrichment	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Huddling	AM	0.01	0.00	0.01	0.00	0.02	0.00	0.02	0.00
	PM	0.02	0.00	0.00	0.00	0.02	0.00	0.01	0.00
Isolated lying	AM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	PM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

3.6 DISCUSSION

3.6.1 Pen-Level Behaviour

A decline in use over time is expected with all or most point-source enrichments (Trickett et al., 2009, van de Perre et al., 2011), but factors including presentation style and material choice can reduce habituation (Van de Weerd et al., 2003). This experiment's enrichment protocol was designed with pig preferences in mind: items had properties that are attractive to pigs (odorous, malleable, manipulable; Van de Weerd et al., 2003), suspended in central locations (Trickett et al., 2009) at eye level (Blackshaw et al., 1997), kept clean (Bracke et al., 2007), and rotated three times weekly to reduce habituation (Trickett et al., 2009, Van de Perre et al., 2011). The protocol used in this experiment exceeds the recommendations for enrichment set by the NFACC Code of Practice (2014).

The pattern of enrichment use in both treatments reflected what has been previously reported in the literature: exploration of a rotation of point-source enrichments in treatment pens was higher than with unchanging enrichment (chains) in control pens during most sampling periods (Gifford et al., 2007), and usage level with the chains in control pens was near-zero throughout the study (Bracke, 2017). In an otherwise-barren environment, nearly any kind of enrichment is likely to encourage exploration and manipulation due to the lack of other stimuli. However, items that do not continue to provide novelty, biological relevance and/or those that are too simple or too complex, will lose the animal's interest (Van de Weerd et al., 2003). Even with frequent rotation of seven different items, interest upon introduction and re-introduction of each enrichment declined rapidly within each phase, reaching near-zero usage by the end of the natural challenge phase and into the finisher phase. Findings by Gifford et al. (2007) suggest that short-term (two days) exposure to an object with at least five days before re-presentation of that object is sufficient to maintain novelty, but that was not reflected in the pattern of use in this experiment. In contrast, the downward trend of the probability of enrichment use within each phase reflects an experiment by Van de Perre et al. (2011) in which point-source enrichments were explored more often when rotated, but frequency of contact with enrichments decreased with each re-introduction of an item. The pattern of enrichment use in this experiment supports the concept of intrinsic vs extrinsic reinforcement: inedible point-source items in this study may have provided only intrinsic reinforcement of exploratory behaviour via novelty, which waned over time as the animals became

habituated. While they provided a way to perform functional behaviours like chewing and rooting, performing these behaviours did not result in a tangible 'reward'. In contrast, materials like straw allow pigs to meet a physical or behavioural need while also providing an extrinsic reward (food, nesting materials), and are therefore more likely to sustain use (Tarou and Bashaw, 2007).

Differences in posture probabilities between treatments were apparent on d-15 of the quarantine phase, four days after entering the experiment. Pigs in the enrichment group were more likely to be observed standing and less likely to be lying sternally in the AM, and more likely to be lying laterally in the PM. Standing pigs may have been more active, potentially spending more time exploring their environment, eating, and drinking, or interacting with enrichment, while lateral lying is an indicator of rest and thermal comfort (Brown et al., 2018). Conversely, sternal lying is associated with inactive lying, discomfort, and/or illness, and pigs appear more likely to be lying inactive when in a sternal recumbent position compared to lateral lying (Escobar et al., 2007). Together these results indicate that enriched pigs in the early quarantine phase may have been more comfortable and active. However, these postural patterns were no longer significant by d-3, corresponding with a decline in enrichment use to a level that was not significantly different to the control group, suggesting a possible relationship between probability of enrichment use and comfort-related postures. A similar behaviour pattern was observed in the finisher unit, where enrichment use and lateral lying were more likely, and sternal lying less likely, in the treatment group on d70. By d140, enrichment use was still significantly more likely than in the control group but was less likely than on d70 in both treatment groups.

While enrichment use was more likely in the treatment group than the control group across most days and times in the NDC, it declined throughout the phase, and no other behaviour differences were found. A lack of difference in sickness-related behaviour likely indicates that enrichment use was not high enough to produce stress-mitigating effects that may have influenced immune cell concentrations at the given level of disease pressure that pigs were under in the NDC. Lying behaviour patterns in the finisher unit further support the idea that enrichment did not sufficiently reduce stress: postural differences were significant on d70 when enrichment use was somewhat higher, but disappeared by d140, demonstrating habituation to the enrichment a point where it was no longer sufficient to alter levels of activity.

Perhaps the greatest limitation on the efficacy of the enrichment treatment was the use of inedible point-source enrichments in place of highly valued enrichments such as rooting materials (Van de Weerd et al., 2003). The objects used, and how they are presented, are arguably the most impactful aspects of a successful enrichment protocol. Not all inedible point-source enrichments and enrichment presentation formats are equally attractive to pigs: ideally, the item(s) chosen should encourage and facilitate the performance of at least one species-specific natural behaviour, and improve the health, biological functioning, productivity and/or welfare of the animals (Van de Weerd and Day, 2009). The types of enrichment objects chosen for this experiment, and the consistency of their application are additional factors that likely contributed to usage. If these enrichments were not sufficiently attractive or were difficult and/or uncomfortable to interact with, interaction frequency and duration would likely decrease markedly, reducing their efficacy. Inclusion of a positive control of straw-bedded pens (see Van de Weerd et al., 2006) is one solution that could have helped determine whether the enrichment was too weak, or the disease challenge too strong. Studies using rooting materials as enrichment have demonstrated health and welfare benefits including reductions in abnormal and aggressive behaviours (Beattie et al., 2000, Petersen et al., 1995), improved growth rate (Luo et al., 2020a) and reduction in disease symptom severity (Van Dixhoorn et al., 2016). Edible rooting materials can also positively influence early development of healthy gut microbiota, which is associated with enhanced immune function (Wen et al., 2021). Unfortunately, due to facility restrictions and concurrent use of the test population in other studies, it was not possible to include a positive control of loose, edible rooting materials. However, this limitation is also one of the aspects of the study that provides the most valuable information in relation to commercial application of enrichment. Given that rooting materials have some practicality limitations regarding cost, labour, and interference with manure systems (Tuyttens et al., 2005), this study instead tested materials that are readily available and could be more easily used in commercial operations than bedding or rooting materials.

Finally, pigs were regrouped during each experimental phase, and it was therefore a limitation to the study that it was not possible to perform repeated measures analysis to track changes in behavioural patterns from one phase to the next. Measuring changes in behaviour would have been a valuable addition to more accurately compare changes in pen-level enrichment use over time and to calculate degrees of change in postural frequencies pre- and post- disease challenge and during recovery.

3.6.2 Growth Rate

In the absence of a disease challenge, some researchers have observed an increase in ADG and decrease in FCR with provision of enrichments such as a combination of rooting materials and suspended toys (Luo et al., 2020a, Oliveira et al., 2016), or greater space allowance and racks of rooting materials (Beattie et al., 2000). However, other studies have found no differences in growth rate between barren-housed pigs and those housed with loose straw (Peeters et al., 2006) or with inedible point-source enrichments that were either suspended from the ceiling or free on the floor (Blackshaw et al., 1997). It is hypothesized in the above-mentioned studies that treatment growth rate differences were related to reductions in stress, demonstrated by lower fear reactivity (Beattie et al., 2000, Luo et al., 2020a) and aggression (Oliveiera et al., 2016). Additionally, animals that are more resilient to disease are expected to have a higher growth rate while under disease pressure (Albers et al., 1987, Bai et al., 2020, Bishop , 2012). In this study, enrichment did not influence ADG or FCR during the challenge nursery, or finisher phase of this study where the pigs were under waning disease pressure. If the enrichment protocol had enhanced disease resilience, a difference in productivity between treatments would have been expected. Based on this, it is likely that enrichment use was either too low in frequency or not biologically relevant enough to reduce pen-level stress or alter stress coping ability, the mechanism through which the pigs' productivity may have been improved if the treatment was effective.

One limitation to the reliability of growth data in the quarantine and challenge nursery phases was the feeding method used. Feed was given and recorded only on a pen-level, and the amount given was imprecisely adjusted based on whether a pen's feed allotment was fully consumed over 24 hours. Therefore, it is possible that pens were either without feed for a portion of the night (and were thus unintentionally feed-restricted) or given more feed than they consumed. If these imprecisions occurred regularly, this could have caused increased competition and stress when feed was re-introduced or may have impacted growth rate and/or altered the results of the ADG and FCR calculations for the challenge nursery phase, where treatment differences would have been most likely to be seen if the enrichment protocol had an impact on disease response. Use of automated RFID feeders in the challenge nursery, like those used in the finisher phase, could have improved the reliability of feed consumption data, allowing more accurate tracking of

daily consumption rates on individual and pen-average levels while also helping to ensure that pigs were never without feed during this period.

3.6.3 CBC and Mortality

While there were no significant treatment differences in individual WBC components (NEUT, LYMPH, MONO, BASO, EOS), the greater increase in total WBC concentration from blood 1-4 in the enrichment group could indicate a more robust immune response to infection or result from prolonged or secondary bacterial re-infection (Van Dixhoorn et al., 2016). It is possible that the enrichment objects acted as vectors for re-infection within enriched pens, increasing the length of infection time, but this is somewhat unlikely as this would be expected to be reflected in treatment differences in growth and/or mortality in the finisher unit. However, while statistically significant, the difference in the change in WBC between treatment groups was relatively small and may not have biological relevance when considered alongside the lack of differences in growth rate, mortality, or WBC subsets.

The acute phase of PRRSV infection, which typically lasts around 21-28 days but can span 50 days or more (Sinkora et al., 2014) targets and depletes all white blood cell subsets (Ladinig et al., 2014). However, a large spike in lymphocyte (Sinkora et al., 2014) and neutrophil (Crisci et al., 2019) concentrations is also expected in later-stage infection, with neutrophils expected to peak by d14 post-infection and return to normal by d42. Similarly, infection with *Salmonella spp.* is associated with increases in WBC, NEUT, LYMPH, N:L, MONO, EOS, and BASO in later-stage infection (19 days post-inoculation), with a smaller relative increase in WBC components generally indicating a less-severe infection (Burdick Sanchez et al., 2019). The timing of blood draws was therefore a considerable drawback of this experiment. For the first eight batches, a blood draw was taken on d14 in the challenge nursery, but this blood draw was not taken for the remaining ten batches due to time and labour constraints at the research facility and did not align with the batches for which behaviour sampling was performed. Had results of this blood draw been available for analysis, it could have provided useful data on the disease response of each treatment group during the height of infection. With this, patterns in immune cell concentrations through the disease challenge may have provided additional insight in treatment group differences during the acute stage of the challenge.

Based on these expected relationships, it was anticipated that the total population would have higher LYMPH and NEUT concentrations and lower MONO concentrations in Blood 4 than Blood 1, while control pigs would have a larger increase in NEUT and N:L than enriched pigs from ΔBlood 1-4. In this experiment, N:L ratios were numerically higher in Blood 4 than Blood 1 for both treatment groups as expected and a tendency for a greater increase in neutrophils was found in the enrichment group, but no significant between-treatment group differences were found in lymphocytes or N:L ratios in Blood 4 or ΔBlood 1-4. Both treatment groups experienced a roughly 2.5-fold increase in monocyte concentration, as well as a relatively large increase in neutrophils and lymphocytes from Blood 1 to Blood 4. Given that elevated monocyte concentrations occur in the later stages of acute PRRSV infection (George-Gay and Parker, 2003), and are the major responding WBCs roughly two weeks after infection with swine dysentery (Jonasson et al., 2004), the elevated monocyte concentrations found in Blood 4 would be expected if pigs were still clearing co-infection with secondary pathogens at the time of sampling.

Additionally, elevated cortisol in humans can increase NEUT concentration while decreasing LYMPH count, leading to a higher N:L ratio with onset of acute stress (Davis et al., 2008, Onsrud and Thorsby, 1981), and researchers have observed a larger N:L ratio increase in barren-housed pigs compared to enriched pigs (Luo et al., 2020b, Reimert et al., 2014). While there is limited evidence of enrichment impacting mortality rates in pigs, a high N:L ratio is highly predictive of all-cause mortality in humans (Ye et al., 2020). It would therefore be possible for enrichment to reduce disease challenge mortality if it were sufficient to reduce stress-induced increases in N:L ratio, but this was not the case in this study. Mortality is a very severe disease outcome. Given the low enrichment usage, and the lack of treatment-group differences in less-severe measures of disease response (growth rate, immune cell concentration, behaviour), it is not surprising that treatment-group differences were not observed in any of the phases of the disease challenge.

No significant treatment group differences were found in any of the measured RBC traits. For both treatments RBC concentration increased by just 2% from Bloods 1 to 4, while a small decrease was seen in HGB (C: -4%, E: -6%) and HCT (-6%, both treatments). By Blood 4, less than 20% of pigs in both treatments had HGB values within the standard reference range, with both control and enrichment group means lower than the minimum reference range value. Low

RBC concentration and hemoglobin indicates anemia and represents poor oxygen carrying capacity (George-Gay and Parker, 2003). Infection with *Salmonella spp.* is also associated with changes in RBC traits and may be partially responsible for the values seen in this experiment. Barba-Vidal et al. (2017) saw an increase in HGB and HCT in pigs four days after *Salmonella* inoculation, while Burdick Sanchez et al. (2019) reported a slight decrease from baseline in RBC, HGB and HCT by 19 days post-inoculation. While treatment groups did not differ from each other, these population-level trends could indicate that the effects of infection with PRRSV and/or *Salmonella spp.* on RBC traits may not have cleared by Blood 4. This is consistent with non-regenerative anemia, commonly caused by PRRSV infection (Halbur et al., 2002).

Additionally, dehydration can increase RBC concentration, HCT, and HGB because of a decreased total plasma volume (Bhattarai et al., 2015). Water consumption is sometimes decreased in sick pigs (Escobar et al., 2007), so this could partially explain the increase in RBC concentration, but not the decrease in HCT and HGB. However, the increase in RBC was quite small (2% from Blood 1 to 4 in both treatments) and in the absence of additional measures such as total protein it is not possible to assess dehydration, so this is largely speculative.

Comparison of the proportion of pigs with CBC values within Iowa State University (2011) reference values showed some interesting trends in the total population, despite an absence of treatment differences. Of note, a much greater proportion of pigs had WBC, HCT, LYMPH and EOS concentrations within reference range at Blood 4 than at Blood 1. This is the opposite relationship of what was expected given that Blood 1 provides baseline values for piglets in a high-health nursery prior to the disease challenge, and Blood 4 was taken during disease recovery. Additionally, the proportion of pigs within the expected reference range for WBC, LYMPH and EOS values at Blood 4 was nearly 100% for both treatment groups, despite the timing of the blood draw being only four weeks after entry into the disease challenge. One possible explanation is that the reference range contains an average of both ‘normal’ and ‘abnormal’ values, with pigs included in the reference ranges that may have subclinical infections. Given the high prevalence of disease in commercial swine operations (Desrosiers, 2011), reference interval groups are typically comprised of 95% clinically healthy animals and 5% unwell, which can skew the average values and provide a wider range of accepted ‘healthy’ values than if only clinically healthy animals were included (Bangert et al., 2008). These unexpected results could also be partially attributed to

variation in the age and breed of pigs used to calculate reference values. In the present study, blood 1 was compared to reference values in a narrow age range (0-6 weeks), while Blood 4 was compared to pigs six weeks to two years of age.

The potential for error in blood count data can be sizeable, as there is a high degree of human involvement at multiple stages from blood draws, data recording, labelling, shipping, and handling of samples, and sample processing and analysis (Bai et al., 2020). A relatively high level of individual variability in immune cell counts was expected due to the complex relationship between genetic influence, the environment, and immunological response to a disease challenge (Hill and Mulder, 2010). It was anticipated that individual immune responses and growth rates would vary considerably between individuals, regardless of treatment group. In analyzing the CBC data, it was therefore important to remove data points that were most likely a result of error and had the potential to skew results, while not removing values that were reflecting true immune response variability. Based on the expected occurrence of these two types of outliers, it was decided to remove only extreme outliers three or more standard deviations from the mean, as described by Osborne and Overbay (2004) and Bai et al. (2020). This improved the fit of most CBC models, so was applied across all Blood 1 and Blood 4 variables for consistency.

It is important to note that due to between-pig variability in disease resilience and immune response, least square means comparisons have limitations. It is possible that the results show an averaging effect in which, for example, differences in immune cell values of susceptible pigs compared to average pigs 'cancel out' the differences of resilient individuals. However, this was not a significant concern for this experiment because of the relatively large sample size; this was also a reason that the proportions of each treatment group within reference range was analyzed in addition to least square means.

3.7 CONCLUSIONS

The goal of this experiment was to investigate the impact of a rotation of inedible point-source enrichment items on measures of disease resilience in swine. In this study, enrichment provision did not influence mortality, growth rate, or most concentrations of immune cell subsets before, during, or after a polymicrobial natural disease challenge. On a pen level, frequencies of comfort- and activity-related postures were initially increased in the enrichment group early in the experiment but were largely not affected by treatment afterwards. This study has important

implications in understanding how housing systems in intensive livestock farming affect animals' disease response. This addresses a knowledge gap in the relationship between point-source enrichment use, pig behaviour, productivity, and immune cell concentrationswhile under disease pressure.

Several factors limited the scope of the study but can provide starting points for future research that may expand on the findings outlined in this chapter. In this experiment, only inedible enrichments were used whereas previous research has found that edible, destructible enrichments hold a higher biological value to pigs compared to inedible point-source items, and therefore maintain a higher usage frequency and greater impact on disease response. Low usage that declined over time may be the main factor in the lack of treatment group differences in many of the experimental variables tested, and the level of enrichment use may have been insufficient to alter the average pen-level disease response.

In this experiment, enrichment use declined over time within each phase, suggesting that the rotation schedule and items used were not sufficiently attractive, novel, and/or rewarding to maintain interest long-term within or throughout experimental phases. These results will be valuable for producers when making housing system decisions as they suggest that inedible point-source enrichments may not be sufficient to improve productivity or disease reponse of swine. Enriched pigs initially exhibited more comfort-related behaviours, but enrichment use declined rapidly, and few behavioural differences were observed later in the experiment. This suggests that further research is needed to test alternative enrichment items and protocols that are more practical for producers to implement than straw-bedded pens but will be more effective than the protocol used in this experiment at maintaining interest and providing health and welfare benefits.

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4. EFFECT OF ENVIRONMENTAL ENRICHMENT ON INDIVIDUAL-LEVEL BEHAVIOUR AND MEASURES OF DISEASE RESILIENCE IN SWINE

This chapter explores individual-level behaviour effects of point-source enrichments on a subset of pigs from Chapter Three. The experiment within Chapter four explores interactions between individual-level pig social and exploratory behaviours, as well as the relationships between behaviour and measures of productivity, immune cell concentrations, and resilience in response to a polymicrobial natural disease challenge.

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Drafting of this chapter was completed by Madelena Pedersen-Macnab and Dr. Yolande Seddon with suggestions from Dr. John Harding. Experimental design was completed by Dr. Yolande Seddon with suggestions from Madelena Pedersen-Macnab. Animal handling and data collection was completed by Madelena Pedersen-Macnab and staff at the Centre de développement du porc du Québec and the Centre de recherche en sciences animales de Deschambault. Statistical analysis was completed by Madelena Pedersen-Macnab with suggestions given from Drs. Yolande Seddon and Giuliana Miguel Pacheco.

ABSTRACT

Standard barren pens contribute to chronic stress in pigs, which may suppress immune function and growth and negatively influence social behaviour. Subsequently, illness and negative social behaviour increase stress and depress growth. Environmental enrichment is one tool to aid swine health and welfare by providing outlets for natural functional behaviours, which can decrease stress and modulate social behaviour. This experiment explores relationships between individual-level social and exploratory behaviours and disease response of barren-housed control pigs (C) and those given a rotation of point-source enrichments (E). Two batches of weaned barrows (n=70) were randomly assigned to treatments in a quarantine nursery (Q, D-19 to D0) before entering a polymicrobial natural disease challenge nursery (N, D0-D28), encountering common pathogens such as porcine reproductive and respiratory syndrome virus (PRRSV), then were transferred to a finisher phase (F). Individual behaviour (D-18 and D13), average daily gain (ADG), and post-challenge (D42) complete blood count (CBC) measures were recorded, and relationships explored. Disease resilience was categorized (resilient, average, susceptible, dead) for three batches (n=159) using ADG and veterinary treatment rate.

Enrichment use was higher in E pigs than C pigs on D13 but not on D-18. A positive correlation was found between enrichment use ($p<.001$) and pen-rooting ($rs(df=68)=.52$), positive social ($rs(68)=.47$) and negative social interactions ($r_s(df=68)=.52$) on D-18. Finisher ADG was positively correlated with pen-rooting in both treatments on D13 (C: $rs(df=68)=.38$, E: $rs(df=87)=.41$, $p=.002$), and with positive social behaviour in E pigs on D13 ($r_s(df=77)=.34$, $p=.002$). Pen-rooting was positively associated with positive social behaviour on D-18 ($rs(df=68)=.39$, $p<.001$) and D13 ($rs(df=77)=.31$, $p=.003$) in E pigs, as well as concentrations of white and red blood cells, hemoglobin, and lymphocytes ($r_s(df=82)=.43$, .11, .46, and .44 respectively, $p<.001$). The proportion of pigs in each resilience category did not differ between treatment groups ($p>.05$).

Results demonstrate that treatment groups differed in behaviour and relationships between behaviour and disease response but suggest that the enrichment protocol used may not have been sufficient to enhance disease resilience. Positive relationships between pen-rooting, positive social behaviour, ADG, and CBC measures suggest a beneficial impact of exploratory behaviour on disease performance in enriched pigs.

4.1 INTRODUCTION

Stress and disease are significant concerns in livestock production, impacting animal welfare (Martinez-Miro et al., 2016), economic productivity (Brisson et al., 2015, Escobar et al., 2007) as well as threatening human health (Desrosiers et al., 2011) and consumer perception of the farm animal industry (Nocella et al., 2010, Spooner et al., 2014). Swine producers are under increasing pressure to improve swine health and welfare while maintaining high productivity and minimizing antibiotic use (Van Dixhoorn et al., 2016). Practical and sustainable methods of swine health management that support disease control, enhance herd health, and improve welfare are of high importance to the industry. Disease resilience, the ability of an animal to maintain homeostasis and productivity regardless of its disease status (Albers et al., 1987, Mulder and Rashidi, 2017), is an expanding area of research in sustainable swine health management. Disease response and resiliency can differ significantly between individuals, influenced by the external environment and presence of stressors (Reimert et al., 2014) as well as internal factors such as heritability of resilience-related genetic traits (Bai et al., 2020, Putz et al., 2019), and stress-coping style (Bolhuis et al., 2003, Cobb and Steptoe, 1996). While much of the available research on disease resilience focuses on genetic selection to improve health (Albers et al., 1987, Mulder and Rashidi, 2017), disease response and susceptibility (or resilience), manipulation of genetics alone is not always feasible or cost-effective, and animals raised in environments poorly suited to their biological and behavioural needs may have increased susceptibility. It is therefore of great importance to examine environmental factors contributing to disease resilience (and susceptibility) on an individual level and explore ways in which measures of resilience and welfare may be improved through changes to the environment.

Stress is a key consideration in livestock health and wellness (Salak-Johnson and Mcglone, 2007), with aspects of commercial pig production such as housing conditions and social dynamics contributing to both acute and chronic stress (Chase and Lunney, 2019). Commercial swine facilities commonly raise growing pigs in barren pens with limited opportunity to perform natural behaviours such as rooting and chewing, contributing to chronic stress (Van de Weerd et al., 2003). Environments that restrict expression of innate functional behaviours are a significant source of stress (Munsterhjelm et al., 2010, Newberry, 1995), and can manifest in re-directed behaviours including oral manipulation of penmates (Fraser et al., 1991, Petersen et al., 1995) and rooting at the pen floor and fixtures (Beattie et al., 2000). Stress can be both a cause and a symptom of disease

(Martinez-Miro et al., 2016). Prolonged activation of the neuroendocrine stress response can have an immunosuppressive effect, leaving animals more susceptible to infection (Dhabhar et al., 2009, Tuchscherer et al., 2009). Conversely, immunological stress can cause distressing symptoms including fever, fatigue, and mood changes (Munsterhjelm et al., 2019, Tuchscherer et al., 2014, Reiner et al., 2009), contributing to a cyclical relationship between stress and immune activation.

A pig's behavioural expression is influenced by factors such as health status (Escobar et al., 2007), environment (Van de Weerd and Day, 2009), and their relationship with conspecifics (Reimert et al., 2014). For example, immune system activation may induce a shift away from exploration and activity, and towards energy- and heat- conserving postures such as inactive sternal lying and huddling (Brown et al., 2018, Escobar et al., 2007, Reiner et al., 2009). Additionally, stress and disease can alter social behaviour. Increases in biting behaviour between penmates can be an early indicator of disease (Munsterhjelm et al., 2019) and visibly ill animals are more likely to be recipients of aggression (Bouwman and Hawley, 2010, Riber and Forkman, 2007). Monitoring baseline behaviour and watching for changes over time can be used as an early disease detection tool (Munsterhjelm et al., 2019) and is useful to monitor and quantify the progression of disease, stress, and/or abnormal or aggressive behaviours in both research and commercial applications (Reiner et al., 2009). While social behaviour can be negatively impacted by stress and disease, the opposite can also occur- positive social behaviours such as gentle nose-to-nose contact have been demonstrated to positively influence growth rate (Camerlink et al., 2012), while social support and positive social interactions have shown a protective effect on immune function (Cobb and Steptoe, 1996, Reimert et al., 2014, Tuchscherer et al., 2014). Conversely, growth rate may be lower in recipients of aggression and oral manipulations (Camerlink et al., 2012). This suggests that negative social behaviour in pigs causes stress, negatively impacting their physiological well-being, while positive social behaviours can mitigate some of the negative effects of stress.

Based on the relationships between social behaviour and stress, and between stress and disease, captive environments that mitigate stress may enhance encourage positive social behaviour and enhance immune function and productivity, all of which can be used to measure of disease resilience. Environmental enrichment- modifications made to the environment to improve the behaviour and/or biological functioning of animals (Newberry, 1995)- may be able to mitigate

some of the stress caused by barren pens (De Jong et al., 2000, Van de Weerd et al., 2003). Environments that encourage expression of innate functional behaviours can positively influence swine social behaviour (Beattie et al., 2000, Telkänranta et al., 2014, Van de Weerd and Day, 2009) productivity (Olieveira et al., 2016), and immune response (Ernst et al., 2006, Reimert et al., 2014). Under disease pressure, there is evidence that enrichment protocols that include a variety of edible rooting materials and manipulable point-source items can enhance measures of resilience including immune cell proliferation (Luo et al., 2020, Reimert et al., 2014) and reduce symptom severity (Van Dixhoorn et al., 2016). Biologically relevant enrichment can allow pigs an outlet for natural functional behaviours such as rooting and chewing, reducing frustration and boredom (Newberry, 1995). In doing so, provision of materials that encourage exploration and oral manipulation, such as jute or fresh wood, can decrease the incidence of damaging biting behaviour, which sometimes stems from boredom and unfulfilled behavioural drives (Telkänranta et al., 2014, Ursinus et al., 2014). However, there is little research on the effect of inedible point-source enrichments on social behaviour and disease resilience, despite their common use in pig production (Tuyttens, 2005). Additionally, research suggests that even with frequent rotation between types of point-source enrichments, use declines significantly over time, thereby reducing the potency of stress mitigation (Van de Perre et al., 2011). Therefore, it is of interest to further explore whether these types of enrichments are sufficient to influence social behaviour, productivity, and/or immune function in pigs, and to determine if relationships exist between a pig's behaviour and their physiological response to disease pressure.

This experiment examines the effect of provision of point-source enrichment on individual-level measures of social and exploratory behaviour, and the relationship between individual pig behaviour and growth rate, and immune cell concentrations in weaned nursery pigs undergoing a polymicrobial natural disease challenge (NDC). A series of correlation analyses are performed to tests the hypothesis that pigs who interact more often with point-source enrichment will perform more positive and less negative behaviour and will demonstrate a greater growth rate while under disease pressure. It is also hypothesized that within the enrichment group there will be a positive relationship between positive social behaviour and the concentration of immune cells post- disease challenge. Furthermore, the effect of point-source enrichment on measures of disease resilience is measured to test the hypothesis that a greater proportion of enriched pigs will be classified as resilient compared to barren-housed control pigs, categorized using growth rate and veterinary

treatment rates. This addresses a knowledge gap in the relationship between pig behaviour and their immune function while under disease pressure, as well as the role of enrichment in altering these measures. If pigs provided with point-source enrichments in non-bedded pens differ from barren-housed pigs in their social behaviour and relationships between behaviour and immune function, it will add evidence to the understanding of how rearing environment impacts animals' ability to cope with, and respond to, disease.

4.2 MATERIALS AND METHODS

All care, handling and procedures performed on pigs were approved under the Animal Protection Committee of the Centre de Recherche en Sciences Animales de Deschambault (15PO283) and Animal Care and Use Committee at the University of Alberta (AUP00002227). The project was overseen by the Centre de Développement du Porc du Québec (Centre for the Development of Pork in Québec) and their herd veterinarian.

4.2.1 Animals and Research Environment

A sub-sample of two batches of weaned grow-finish barrows (n=70) were chosen from the total experimental population used in Chapter Three; for additional details on the experimental protocol and facility design, see Chapter Three, Section 3.2. A third batch was included for analysis of disease resilience calculations (see Section 4.3.3) for a total of 159 pigs. The batches selected for this sub-sample followed identical experimental protocols, including the location of experimental facilities for each phase and pen grouping sizes. Videos from the total population of 19 batches were pre-viewed to select 10 batches with the best video quality and pig paint marking visibility to facilitate accurate pig identification for individual behaviour analysis. Unfortunately, of the 19 batches available, 17 batches were unable to be analyzed due to video footage loss or inconsistent/illegible paint markings during one or both of the sampling days selected for this experiment.

This experiment spanned three phases: 1) 19 days in a quarantine phase (d-19 to d0), 2) 28 days in a challenge nursery (d0 to d28), and 3) approximately 18 weeks in a finisher phase (d28 to d135), after which pigs were sent to slaughter. Separate rooms in a research facility at the Centre de Développement du Porc du Quebec (CDPQ, Deschambault, Canada) were used for each experimental phase. Upon entry to the quarantine phase, pigs were randomly assigned to pens in

groups of 10; the pen groupings stayed the same upon transfer to the challenge nursery phase. Pens in the quarantine and challenge nursery phases measured 1.2m by 2.4m (3.0m^2), providing 0.3m^2 of floor space per pig. In the finisher phase, pigs remained in the same treatment groups but were randomly assigned to new groups of 9-15 pigs and were housed in pens that measured 2.6m by 4.9m (12.7m^2), providing a minimum of 0.8m^2 per pig. Pens in all phases had slatted plastic Tenderfoot floors (Tandem Products Inc., Minneapolis, USA) with small (approximately 1/6 of pen width) solid flooring sections, one waterer, one feeder, and two ceiling-suspended metal chains for enrichment. Feed was weighed and hand-delivered into combination feed hopper/troughs in quarantine and the challenge nursery pens once per day, and automated radio frequency identification (RFID) IVOG® feeding stations (Hokofarm Group, Marknesse, Netherlands) were used to deliver feed and record individual consumption in finisher pens. Pigs were individually weighed every three weeks during the entire experiment, beginning on d-19 upon entry into the quarantine phase.

4.2.2 Disease Challenge and Blood Sampling

Following a 19-day quarantine phase, pigs were transferred to a challenge nursery room on d0, where they were exposed to a polymicrobial natural disease challenge (NDC). Individuals remained in the same treatment groups throughout the experiment, and groups of penmates remained the same in the quarantine and challenge nursery phases for this experiment. The NDC was seeded with three strains of porcine reproductive and respiratory syndrome virus (PRRSV), and multiple bacterial pathogens were detected in various batches throughout the experiment including *Mycoplasma hyopneumoniae*, *Haemophilus parasuis*, *Brachyspira hampsonii*, *Salmonella enterica serovar typhimurium* and *Streptococcus suis*), and two internal parasites (*Cystoisospora suis* and *Ascaris suum*) (Bai et al., 2020, Putz et al., 2019). A continuous flow model, with one week of cross-contamination between batches, was used to ensure pathogen transfer. Exposure to PRRSV was confirmed by random blood sampling within each batch 4 weeks and 6 weeks post-entry (Bai et al., 2020).

Blood samples were collected into EDTA tubes all pigs in the quarantine phase (d-14) and the finisher phase (d42); only finisher-phase blood values were analyzed for this experiment. Samples were shipped overnight to the University of Alberta (Edmonton, Alberta, Canada) for

complete blood count (CBC) using an ADVIA R 2120i Hematology System (Siemens Healthineers, Erlangen, Germany). The CBC included counts of 6 white cell types (total white blood cell count (WBC), lymphocytes (LYMPH), neutrophils (NEUT), monocytes (MONO), basophils (BASO), and eosinophils (EOS), and three hemogram measures (total red blood cell count (RBC), hematocrit (HCT), hemoglobin (HGB)). Additionally, a neutrophil: lymphocyte ratio (N:L) was calculated. These cell subsets were selected for their roles in the adaptive and innate immune responses to the pathogens involved in the NDC (George-Gay and Parker, 2003, Halbur et al., 2002, Sinkora et al., 2014) and were used to explore relationships between immune cell concentrations post-disease challenge and individual pig behaviour.

4.2.2 Enrichment

Fifty percent of the pens were assigned as controls, receiving two suspended metal chains per pen, and the other half assigned an enrichment treatment. Pigs were randomly assigned to pen upon entry and remained in the same treatment group throughout the experiment. For enrichment treatment pens, seven different inedible point-source enrichments were provided to pigs and rotated three times weekly, with only enrichment object presented at a time. Six of the seven enrichment objects[1] were attached to ceiling by suspended chains hung at pig shoulder-height and provided at a ratio of one item to every five to seven pigs (two items per pen). The enrichments provided were a) rooting mat, b) cotton rope, c) 'Porcichew' commercial suspended enrichment (NutraPet, East Yorkshire, UK), d) Easyfix 'Luna' commercial floor enrichment (Easyfix, Ballinasloe, Ireland), e) jute sack, f) Flexible PVC pipe, g) rectangle of tarpaulin material. The sizes of the enrichments and the height of the suspended chains increased in each phase as the pigs grew. For more detailed information on the enrichment items and protocol used, see Chapter 3, Section 3.2.3.

[1]The EasyFix Luna ball was given as a floor toy, and only one ball was given to each pen (10-15 pigs).

4.2.3 Behaviour Data Collection

Behaviour data was collected for 90% of pens in two batches, with 10% of pens not visible within the available camera footage (n=70 pigs). For each batch, videos were recorded from 8 to

10 AM and 2 to 4 PM on two days, once in the quarantine barn pre-challenge (d-18, n=6 pens) and once in the NDC during the disease challenge (d13, n=7 pens). In the quarantine phase, videos were recorded using two Plotwatcher Pro trail cameras (Day 6 Outdoors, Dover, Florida, United States) installed overhead above a block of four pens so that two pens were recorded by each camera, recording still images at a rate of 40 frames per second. Three to four TrendNet View Pro cameras (TrendNet, Torrance, California, United States) were used in the challenge nursery, placed above a block of six pens so that each camera recorded one to two pens, producing continuous video recordings over each two-hour sampling period.

Immediately prior to each scheduled video recording for behaviour data collection, pigs were individually marked with a stock marker paint. A different mark was used for each pig within a pen, following a chart corresponding to each pig's unique ear tag ID number. Scan samples were taken every two minutes (Camerlink et al., 2012), recording the behaviour(s) engaged in by each pig based on the ethogram shown in Table 4.1. If a pig was not engaged in any social behaviours during a scan sample, or the marking on their back was unclear or obstructed, it was coded as 'no behaviour observed'. To accurately categorize the behaviours within context, 15 seconds before each scan sample were watched. Observations were performed by a single observer who was not blinded to treatments as the enrichment objects or chains were visible in the videos.

Table 4.1. Ethogram of social and exploratory behaviours observed during individual pig behaviour analysis.

Category	Behaviour	Description
Positive Social Behaviour	Social nosing[1]	Gentle sniffing or nose-touching another pig's face or body
Negative Social Behaviour	Tail biting	Oral or nasal manipulation directed towards a pig's tail
	Ear biting	Oral or nasal manipulation directed towards a pig's ear
	Belly nosing[1]	Using their nose/mouth to push or bite at another pig's stomach
	Other bite	Oral or nasal manipulation of any other body part of a pig

	Head-butt	Using their head to hit or push another pig's head or body
	Mounting[1]	Climbing on the back or head of another pig that is standing or sitting
Exploratory Behaviour	Enrichment use	Oral or nasal contact with the enrichment or suspended metal chain
	Rooting (pen-directed)[2]	Oral or nasal contact with the pen floor, walls, or fixtures

Where either the behaviour or unique marking placed on the pig was not clear, observations were not included in analysis.

[1]Adapted from Camerlink et al., 2012; [2]Adapted from Van de Weerd et al., 2006

4.3 CALCULATIONS AND STATISTICAL ANALYSIS

4.3.1 Common procedures amongst all variables

Microsoft Excel (2016) spreadsheets were used to store data and perform preliminary calculations. Next, all statistical analysis was performed using the software SAS 9.4 (SAS Inst. Inc., North Carolina, United States).

4.3.2 Growth Rate Calculations

The average daily gain (ADG) of individual pigs was calculated for each phase (quarantine, challenge nursery, finisher) by dividing the weight gained by an individual during each phase by the total number of days the pig remained in that phase. For pigs that died during a phase, their weight at death was used, divided by the number of days that they were alive during that phase.

4.3.3 Resiliency Calculations and Analysis

Pigs were assigned to one of four disease resilience classification groups: dead, susceptible, average, or resilient. Classification cut-off points were calculated using whole-population quartiles for finisher-phase ADG and number of veterinary treatments given (treatment rate, TR) in the challenge nursery (Bai et al., 2020, Bishop, 2012). Pigs that died during the experiment were classed as 'dead'; those in the 25% quartile of ADG and 75% quartile for TR were classed as

'susceptible', and pigs in the 75% quartile for ADG and 25% quartile for TR were classed as 'resilient'; all others were classed as 'average'. Using these classifications, frequency tables were produced (Proc FREQ) and a Fisher's Exact Test performed to examine the distribution of resiliency classifications (dead, susceptible, average, resilient) within each treatment.

4.3.4 Individual Behaviour Calculations and Analysis

Several behaviours occurred at a very low frequency in the scan samples collected, thus, all negative social behaviours (Table 4.1) were combined within each sampling day (d-18 and d13). By doing so, four behavioural categories were used in analysis: rooting at pen fixtures, interacting with enrichment, gentle social nosing (positive social), and sum of negative behaviours. The number of observed occurrences of each behaviour by an individual pig were summed for each four-hour sampling day. The relative frequency of the behaviours observed per pig was then calculated by dividing the number of observations of each behaviour by the number of scan samples in which that pig was observed. Calculated frequencies of social and exploratory behaviours were then compared between treatment groups using a Kruskall-Wallis test (Proc NPAR1WAY) and used in correlation analysis within treatment groups (see Section 4.3.5).

4.3.5 Correlation Analysis

Correlation analysis was performed to examine relationships between the relative frequencies of social and exploratory behaviours within a behaviour sampling day and between treatment groups. Behaviours in each sampling day were also tested for relationships with phase-level ADG (quarantine, challenge nursery, and finisher phases), and with ten CBC parameters (WBC, NEUT, LYMPH, N:L, MONO, BASO, EOS, RBC, HCT, HGB) from Blood 4 (taken on Day 42). The CBC parameters used in correlation analysis had previously been analyzed in Chapter Three, where studentized residuals were used to remove extreme outliers three or more standard deviations from the mean of each blood value (See Chapter Three, Section 3.4.4 for further explanation). Within each behaviour video day, a matrix of Spearman rank correlations was created using Proc CORR to determine if relationships existed between behaviour frequencies, growth rate in each phase, and Blood 4 CBC values. Scatterplots were produced to visually examine the relationship between variables. To reduce the likelihood of Type I error when

producing a multi-variable correlation matrix, a Bonferroni correction was applied to adjust the alpha significance level to p=.003 (Chen et al., 2017).

4.4 RESULTS

4.4.1 Social & Exploratory Behaviour

On d-18, mean behaviour frequencies (Fig. 4.1) were higher in the enrichment (E) group than the control group (C) for pen rooting (C: 0.7%, E: 1.2%, p=.04), positive social behaviour (C: 0.0%, E: 0.3%, p=.01), and negative social behaviour (C: 0.8%, E: 2.8%, p=.03), but mean frequency of enrichment use was not different between treatments (p=.69). Enrichment use frequency was significantly higher in the enrichment group on d13 (C: 0.1%, E: 4.1%, p<.01; Fig. 4.2), as was negative social behaviour (C: 1.4%, E: 2.5%, p=.04), while frequencies of pen rooting and positive social behaviour were not significantly different (p=.17 and .86 respectively).

Overall stronger and more significant correlations were found between behaviour frequencies in the enrichment group than in the control group on d-18 and d13 (Fig. 4.3). On d-18 in the enrichment group (Fig. 4.3b), there were moderate positive correlations between enrichment use and pen-directed rooting ($r_s(df=68)=.52$, p<.001), positive social nosing ($r_s(df=68)=.47$, p<.001), and negative social behaviours ($r_s(df=68)=.52$, p<.001). Similarly, pen rooting was positively correlated with social nosing ($r_s(df=68)=.39$, p<.001). No significant correlations were found on d-18 in the control group (Fig. 4.3a); a low positive correlation was found between chain use and negative social behaviour ($r_s(df=52)=.36$, p=.007), but was not significant after a Bonferroni correction was applied.

On d13 in the challenge nursery, the only significant correlation found in the control group (Fig. 4.3c) was between positive social nosing and negative social behaviour ($r_s(df=68)=.35$, p<.001). In the enrichment group (Fig. 4.3d), enrichment use and negative social behaviour were positively correlated ($r_s(df=87)=.38$, p<.001), as were positive social nosing and pen rooting ($r_s(df=87)=.31$, p<.001). No other behaviours were significantly correlated.

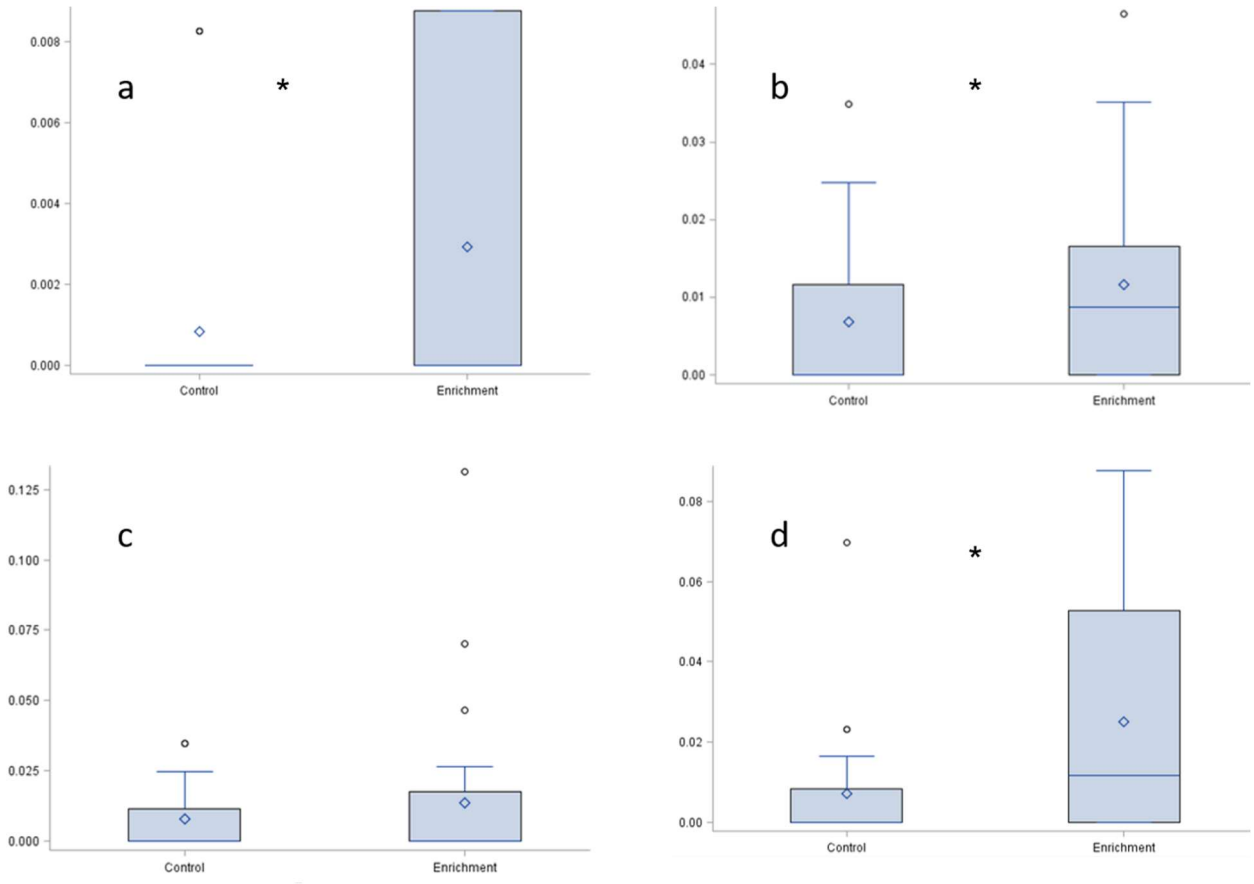

Fig. 4.1 Frequency of behaviour performed by pigs reared in control (n = 30 individuals) and enriched (n=30 individuals) groups on Day -18 in the quarantine phase: a: Social nosing; b: rooting; c: interaction with enrichment; d: negative social behaviour. Within each plot and treatment, mean represented by ◊, median by horizontal lines within the box, upper and lower quartile represented by edges of the box, maximum/minimum by the box tails, outliers by the open circles. Where asterisk appear, significant difference between treatment groups, $p<0.05$.

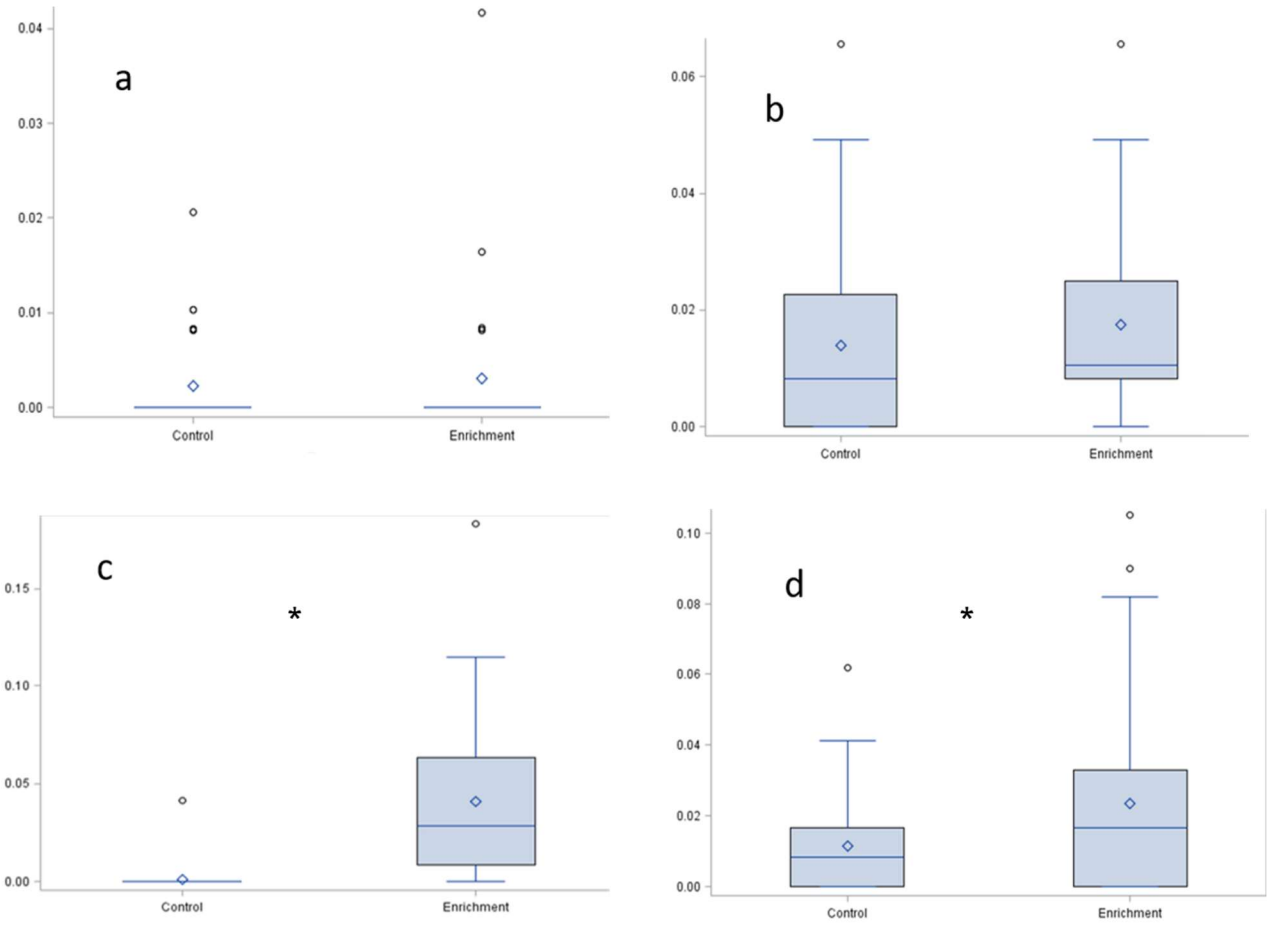

Fig. 4.2 Frequency of behaviour performed by pigs reared in control (n = 40 individuals) and enriched (n= 30 individuals) groups on Day 13 in the NCB phase: a: Social nosing; b: rooting; c: interaction with enrichment; d: negative social behaviour. Within each plot and treatment, mean represented by ◊, median by horizontal lines within the box, upper and lower quartile represented by edges of the box, maximum/minimum by the box tails, outliers by the open circles. Where asterisk appear, significant difference between treatment groups, $p<0.05$.

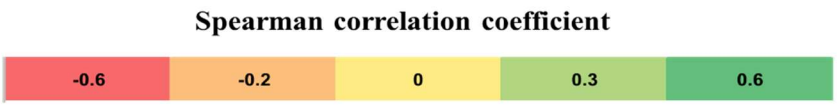

Fig. 4.3a: Day -18, c ontrol (n=30).

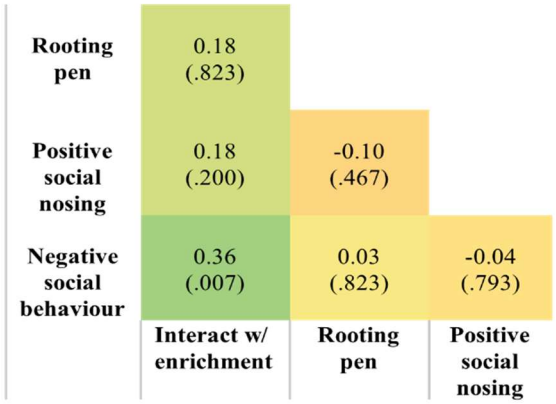

Fig. 4.3b: Day -18, enrichment (n=30)

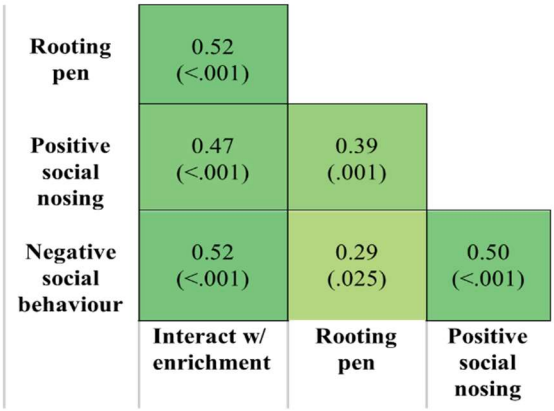

Fig. 4.3c: Day 13, control (n=30).

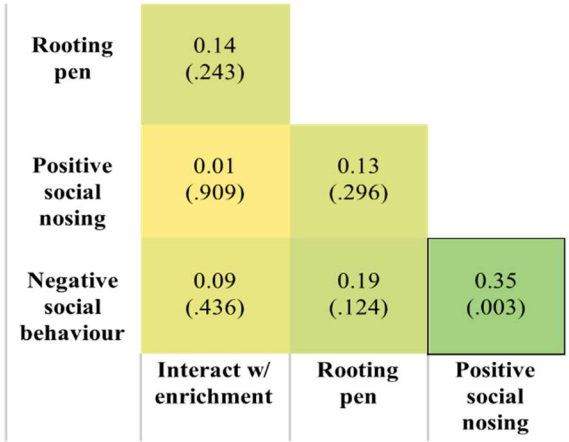

Fig. 4.3d: Day 13, enrichment (n=40).

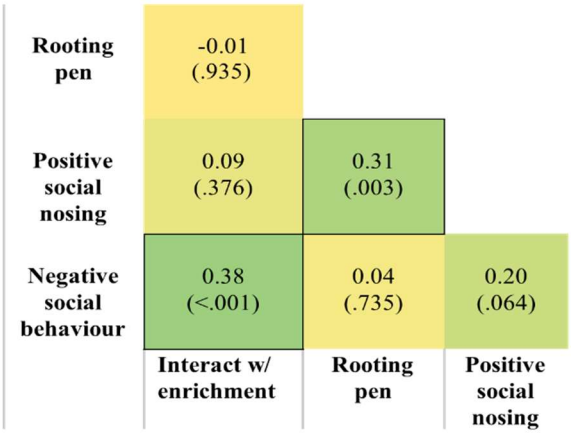

Fig. 4.3. Heat map representation of a Spearman correlation matrix of behaviour frequencies of pig social and exploratory behaviour within enrichment and control treatment groups during a quarantine phase (Day -18, Fig. 4.3a, b), and challenge nursery phase (Day 13, Fig. 4.3c, d). P-values of the correlations are in parentheses. Significance is established as $p \leq .003$.

4.4.2 Growth Rate & Resiliency Correlations

A low negative correlation was found between negative social behaviour on d-18 and quarantine ADG in the control group (rs(df=52)=-.32, p=.017), but after a Bonferroni correction was applied no correlations between quarantine behaviour and growth rates in either treatment group were significant (Fig. 4.4a and b). On d13, low positive associations were found between pen rooting and finisher ADG in both the control (rs(df=63)=.38, p=.002 Fig. 4.4c) and enrichment groups (rs(df=77)=.41, p<.001, Fig. 4.4d), and between positive social nosing and finisher ADG in the enrichment group only (rs(df=77)=.34, p<.001, Fig. 4.4d).

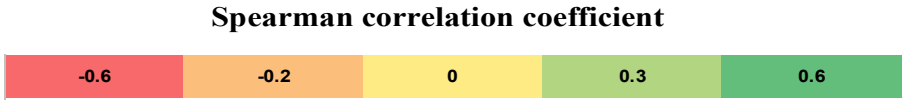

Fig. 4.4a: Day -18 behaviour, control treatment (n=30).

Interact w/ enrichment	-0.07 (.599)	-0.11 (.441)	-0.20 (.161)
Rooting pen	0.25 (.073)	0.06 (.669)	0.28 (.051)
Positive social nosing	0.06 (.648)	-0.13 (.366)	-0.20 (.165)
Negative social behaviour	-0.32 (.017)	-0.15 (.271)	0.16 (.280)

Fig. 4.4b: Day -18 behaviour, enrichment treatment (n=30).

Interact w/ enrichment	-0.07 (.548)	-0.13 (.302)	0.19 (.132)
Rooting pen	0.04 (.756)	-0.18 (.152)	0.18 (.172)
Positive social nosing	-0.08 (.503)	-0.18 (.142)	0.22 (.081)
Negative social behaviour	-0.13 (.296)	-0.26 (.036)	0.02 (.867)
	Quarantine ADG	**NCB ADG**	**Finisher ADG**

Fig. 4.4c: Day 13 behaviour, control treatment (n=40).

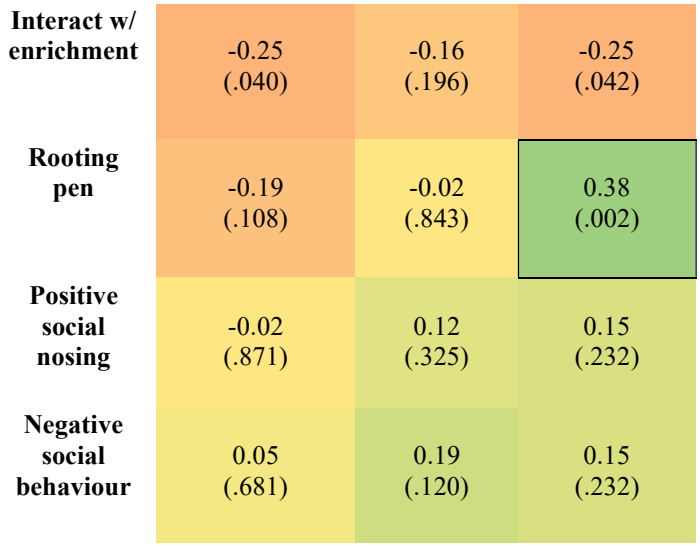

Fig. 4.4d: Day 13 behaviour, enrichment treatment (n=30).

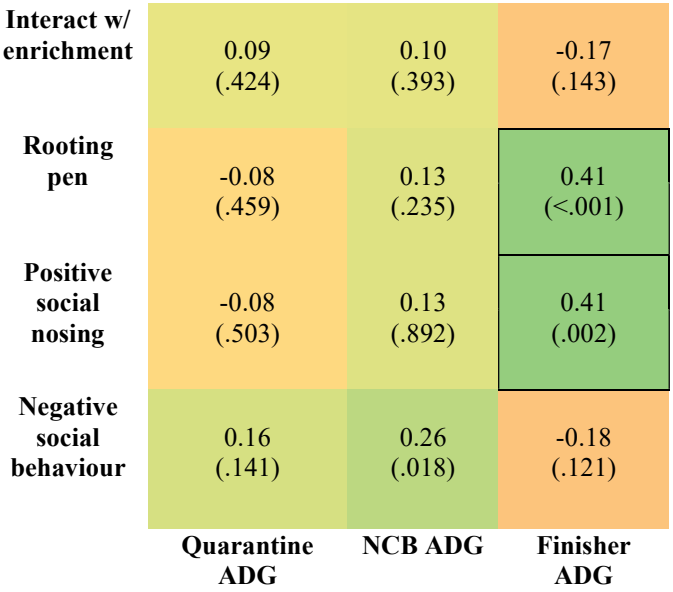

Fig. 4.4. A heat map representation of a matrix of Spearman correlation coefficients between frequencies of individual pig behaviour in a quarantine phase (Day -18, Fig. 4.4a, b), and the challenge nursery phase (Day 13, Fig. 4.4c, d) and the average daily gain (ADG) in the quarantine, challenge nursery, and finisher phases, within two treatment groups (enrichment and control). P-values of the correlations are in parentheses, significance is established as $p \leq .003$.

4.4.3 Complete Blood Count Correlations

No significant relationships were found between any quarantine phase (d-18) behaviours and CBC measures in either treatment group ($p>.003$, Fig. 4.5a, b). On d13, significant positive correlations were found in the enrichment group (Fig. 4.5d) between pen rooting and concentrations of WBC ($rs(df=82)= .43$, $p<.001$), RBC ($rs(df=82)= .44$, $p<.001$), HGB ($rs(df=82)=.46$, $p<.001$), and LYMPH ($rs(df=82)= .44$, $p<.001$) from a blood sample collected on d42. Positive social nosing was also positively correlated with HGB in the enrichment group only ($rs(df=82)=.39$, $p<.001$). No significant relationships were found between d13 behaviour and CBC measures in the control group (Fig. 4.5c).

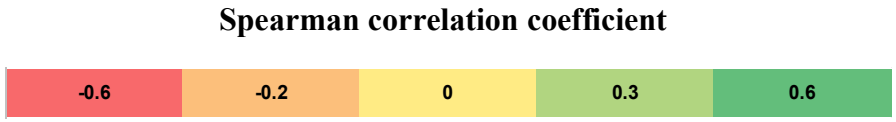

Fig. 4.5a: Day -18, control treatment (n=30).

	Total WBC	Total RBC	Hematocrit	Hemoglobin	Neutrophil	Lymphocyte	N:L ratio	Monocyte	Basophil	Eosinophil
Interact w/ enrichment	-0.09 (.513)	-0.39 (.005)	0.00 (.986)	-0.29 (.034)	0.11 (.418)	-0.16 (.264)	0.03 (.836)	0.00 (.987)	0.14 (.318)	-0.18 (.209)
Rooting pen	0.11 (.428)	-0.06 (.649)	-0.15 (.281)	-0.17 (.225)	0.12 (.403)	0.08 (.585)	0.07 (.635)	-0.07 (.609)	0.22 (.111)	0.08 (.581)
Positive social nosing	0.04 (.803)	0.09 (.510)	-0.10 (.490)	0.03 (.807)	-0.07 (.635)	0.07 (.613)	-0.02 (.915)	0.29 (.040)	0.21 (.141)	0.08 (.560)
Negative social behaviour	-0.02 (.865)	-0.05 (.700)	0.16 (.271)	0.05 (.723)	0.16 (.270)	-0.12 (.407)	0.27 (.056)	0.00 (.988)	0.16 (.248)	-0.03 (.816)

Fig. 4.5b: Day -18, enrichment treatment (N=30).

Interact w/ enrichment	0.33 (.007)	0.31 (.012)	-0.23 (.062)	0.30 (.016)	0.16 (.201)	0.32 (.009)	0.07 (.563)	0.04 (.776)	0.15 (.237)	-0.11 (.374)

	Total WBC	Total RBC	Hematocrit	Hemoglobin	Neutrophil	Lymphocyte	N:L ratio	Monocyte	Basophil	Eosinophil
Rooting pen	0.29 (.017)	0.11 (.378)	-0.12 (.359)	0.07 (.581)	0.22 (.079)	0.28 (.026)	-0.05 (.695)	-0.10 (.457)	0.15 (.243)	0.00 (.974)
Positive social nosing	0.16 (.217)	0.12 (.332)	-0.15 (.227)	0.05 (.722)	-0.06 (.623)	0.19 (.125)	-0.13 (.299)	-0.12 (.365)	-0.03 (.827)	-0.01 (.926)
Negative social behaviour	0.08 (.527)	0.04 (.758)	0.16 (.210)	0.05 (.676)	0.17 (.181)	-0.06 (.630)	0.19 (.137)	0.31 (.013)	0.13 (.299)	0.05 (.697)

Fig. 4.5c: Day 13, control treatment (N=40).

Interact w/ enrichment	0.05 (.665)	-0.07 (.565)	-0.13 (.294)	0.01 (.941)	-0.06 (.646)	0.02 (.887)	-0.02 (.876)	0.08 (.511)	0.10 (.436)	-0.06 (.639)
Rooting pen	0.01 (.930)	0.00 (.980)	0.08 (502)	0.10 (.444)	-0.06 (.646)	0.00 (.976)	-0.14 (.277)	0.03 (.789)	0.17 (.166)	-0.20 (.112)
Positive social nosing	0.08 (.540)	0.08 (.532)	-0.11 (.357)	0.13 (.303)	0.08 (.519)	0.11 (.382)	0.07 (.564)	0.08 (.548)	-0.04 (.758)	0.06 (.635)
Negative social behaviour	-0.13 (.298)	-0.13 (.291)	0.09 (.481)	-0.03 (.779)	-0.07 (.553)	-0.14 (.261)	0.01 (.912)	0.17 (.175)	0.25 (.043)	-0.10 (.413)

	Total WBC	Total RBC	Hematocrit	Hemoglobin	Neutrophil	Lymphocyte	N:L ratio	Monocyte	Basophil	Eosinophil

Fig. 4.5d: Day 13, enrichment treatment (N=30).

	Total WBC	Total RBC	Hematocrit	Hemoglobin	Neutrophil	Lymphocyte	N:L ratio	Monocyte	Basophil	Eosinophil
Interact w/ enrichment	-0.01 (.926)	-0.06 (.586)	-0.19 (.082)	-0.01 (.896)	-0.07 (.554)	0.10 (.352)	-0.13 (.239)	-0.18 (.095)	0.06 (.581)	-0.15 (.180)
Rooting pen	0.43 (<.001)	0.44 (<.001)	-0.29 (.008)	0.46 (<.001)	0.11 (.326)	0.44 (<.001)	0.11 (.320)	0.21 (.052)	0.09 (.390)	0.00 (.984)
Positive social nosing	0.29 (.009)	0.24 (.029)	-0.25 (.023)	0.39 (<.001)	0.21 (.055)	0.28 (.010)	0.21 (.063)	0.00 (.969)	-0.04 (.699)	-0.01 (.934)
Negative social behaviour	-0.03 (.779)	-0.02 (.843)	-0.15 (.166)	0.00 (.974)	-0.11 (.320)	0.09 (.404)	-0.13 (.252)	-0.20 (.071)	0.06 (.582)	-0.03 (.771)

Fig. 4.5 A heat map matrix of Spearman correlation coefficients between frequencies of individual pig behaviour and complete blood count values measured on Day 42 during a natural disease challenge. Correlations are presented for behaviour observed during a quarantine phase (Day -18, Fig. 4.5a, b), and challenge nursery phase (Day 13, Fig. 4.5c, d) within two treatment groups (enrichment and control). P-values of the correlations are in parentheses, significance set at p<.003.

4.4.3 Disease Resilience Classification

The proportion of pigs within each treatment group classified as dead, susceptible, average, or resilient did not differ statistically ($p>.05$, Table 4.2).

Table 4.2. Results of a Fisher's Exact Test analysis on the distribution of individuals classified as resilient, susceptible, average, or dead. Presented as the number of pigs in the classification (% of treatment group). n=159 pigs, p=0.61.

Treatment	Resiliency Classification (N (%))			
	Dead	Susceptible	Average	Resilient
Enrichment (N=89)	10 (11.2%)	8 (9.0%)	56 (62.9%)	15 (16.9%)
Control (N=70)	5 (7.1%)	7 (10.0%)	50 (71.4%)	8 (11.4%)

4.5 DISCUSSION

4.5.1 Individual behaviour

Aggression and oral manipulations of conspecifics are often more prevalent in barren-housed pigs than pigs raised with species-specific enrichment (Van de Weerd and Day, 2009) and can have several influencing factors such as boredom, frustration, feed restriction, and re-direction of natural rooting behaviour due to a lack of a suitable outlet for the behaviour (Fraser et al., 1991, Lewis, 1999, Petersen et al., 1995). Rooting behaviours may also be re-directed towards pen floors, walls, and fixtures in the absence of appropriate rooting materials (Beattie et al., 2000). The enrichment treatment group in this study performed more pen rooting, positive social, and negative social behaviours than control pigs on d-18 of the quarantine phase but did not interact significantly more with the point-source enrichments than the control pigs did with bare chains. During this phase, enrichment use was positively correlated with pen- rooting, positive social nosing, and negative social behaviours in the enrichment group, but interestingly these relationships were not found in the control group. As pigs entered the experiment just one day prior (d-19), it is reasonable to expect that there would be a variety of correlated social behaviours on d-18 due to an overall increase of activity, and that many individuals would engage in both positive and negative interactions as pigs were transported and mixed in unfamiliar groups the day prior (d-19),

contributing to an unstable social hierarchy (Camerlink et al., 2021). That these relationships were found only in the enrichment group, and that more social and exploratory behaviours were performed overall suggests that these behaviours may not have been entirely distinct from each other and may have been performed sequentially. Rather, that provision of enrichments was related to more activity and more overall oral- nasal exploratory behaviour within the group compared to the control pens.

Positive social nosing was positively correlated with negative social behaviours in the enrichment group in the quarantine phase, and in the control group in the challenge nursery phase. Similar findings were observed by O'Malley et al. (2022), who found a positive correlation between agonistic interactions and non-agonistic social nosing in pigs at three-, six-, and nine-weeks post-mixing, but not immediately after mixing. As described above, a combination of positive and negative social behaviours is reasonable to expect shortly after mixing, as seen in the enrichment group, but social instability and agonistic behaviour typically lessen by two weeks post-mixing (Stookey and Gonyou, 1994). It is possible that in the absence of manipulable enrichments, control pigs performed re-directed exploratory oral-nasal behaviours towards penmates sequentially with positive social behaviours, while enriched pigs had access to items for exploration and biting/chewing behaviour (Van de Weerd and Day, 2009) and did not directly transition between positive and negative social behaviours as often despite performing more negative social behaviours than control pigs on d13.

A low positive correlation existed between negative social behaviour and metal chain enrichment use in the control group in both phases but was not significant after a Bonferroni correction was applied. Chain interaction in the control group was near-zero on d13, which is likely why no significant relationships were found between enrichment use and any other behaviours. In contrast, the enrichment group interacted more often with the point-source items and performed more negative social behaviours than control pigs on d13, and a positive correlation between enrichment use and negative social behaviours was found in both the quarantine and challenge nursery phases in the enrichment group only. As previously discussed, relationships within the enrichment group on d-18 appear to correspond with greater overall activity, but the relationship between enrichment use and negative social behaviours on d13 could indicate that interaction with point-source enrichments did not sufficiently satisfy the pigs' behavioural needs for rooting,

chewing, and exploration, while pen-rooting may have been more satisfying than enrichment use. A lack of outlets for species-specific behaviours is thought to cause frustration and/or boredom, which can lead to re-directed oral manipulations from object to penmate or pen fixtures in the absence of species-appropriate outlets for these behaviours (Beattie et al., 2000). When enrichments provide an effective outlet for functional behaviours that are otherwise restricted in barren pens, a reduction in oral manipulation of penmates is expected (Telkänranta et al., 2014), which was not reflected in this study.

In a meta-analysis of common enrichment items, Bracke (2017) found that suspended metal chains were similar in enrichment value to several point-source enrichments used in this study (rubber rooting mat, PVC hose, free toy, cotton rope). Enrichment value was scored out of 10 (RICHPIG score) based on assessment criteria including interaction rate, prevalence of negative behaviours, and growth performance of the pigs. A score of 5 was suggested as the minimum for enrichments to provide significant health and/or welfare benefits, and each of the aforementioned items scored <2.5 out of 10; in contrast, rooting materials such as straw, soil, and silage scored ≥ 7.0 out of 10. This agrees with the results of this study, suggesting that while a rotation of inedible point-source enrichments influenced behavioural differences compared to barren-housed control pigs, the enrichments likely did not provide sufficient stress mitigation to reduce negative social behaviour and did not appear to encourage positive social behaviour in the NDC.

Several study limitations may have impacted reliability of results and should be considered in the discussion of these results. Firstly, the size of the population sub-sample was limited by data loss during the experiment. A portion of video data was lost due to equipment failure, and additional footage was unusable for individual behaviour scoring as the markings on pigs were not correctly applied prior to video recording. As a result, there were more pigs in the enrichment treatment group than the controls, and more pigs sampled on d13 than d-18. Further, due to a combination of unclear paint markings and limited camera quality, not all behaviours performed during scan samples were able to be accurately identified. Where a behaviour or pig's marking was unclear, behaviours were not able to be included in the dataset. This limited the frequency of all behaviours recorded, therefore limiting the conclusions that can be drawn. Additionally, performance of social or exploratory behaviours by an individual can be subtle and short-lived, and therefore scan sampling only captures a representative portion of the activities in a pen. To

better capture the full range of behaviours performed, future studies looking to include individual behaviour data could consider continuous sampling instead. To combat issues with readability of paint markings, smaller pen groups would be ideal to aid in identifying individuals by process of elimination when only part of a marking is visible. This could dramatically decrease the number of 'unreadable' behaviours that are excluded from analysis. In combining these changes to the methodology, it could also be possible to categorize whether pigs were performing or receiving social behaviours. This would allow for a more sensitive comparison of social behaviour and allow more detailed comparisons against measures of disease resilience.

4.5.2 Growth and immune parameters

Investigation into relationships between social behaviour and immune function in pigs is a fairly novel area, and one that provides additional insight into factors that can impact disease resilience on an individual level. Given the high degree of individual variability in resilience and stress coping style and ability (Koolhaas et al., 1999, Reimert et al., 2014), recording individual social behaviour adds valuable information on the impact of point-source enrichment on measures of immune function and disease resilience. In this experiment, the NDC pressure was quite intense, meaning that environmental influences would likely have to be similarly strong to produce differences in disease response on a population-average level. Because of this, individual-level relationships within treatments were of great interest to quantify the direction and intensity of relationships between behaviour and measures of disease resilience.

A polymicrobial natural disease challenge model was used in a study by Van Dixhoorn et al. (2016) to compare the disease response of minimally enriched and highly enriched pigs, in which enriched pigs showed less frequent stress- and disease-related behaviour, cleared PRRSV faster in sera, were less likely to have lung lesions, and had less severe lesions when they were present. While the present experiment did not measure virus levels in sera or lung lesions, the concept of enhanced disease resilience mediated by reduced stress can be applied to explore the measured immune parameters in this experiment.

On d13 of the NDC, a low positive correlation was found between pen-rooting and finisher phase ADG in both the enrichment and control groups, and between pen rooting and positive social behaviour in the enriched pigs only. Individuals performing a higher frequency of pen-rooting on

d13 could have been more resilient than their counterparts, and thus able to partition more energy towards exploratory activity (pen rooting) in the NDC and compensatory weight gain once they reached the finisher phase (Rauw, 2012). Positive social behaviour on d13 was also positively correlated with finisher ADG in the enrichment group, agreeing with findings by Camerlink et al. (2012), who found a relationship between positive social behaviour and higher growth rate. This indicates that the individuals performing positive social behaviours while under disease pressure may have been experiencing a more positive affective state, and that provision of point-source enrichments may have influenced this affective state, which was reflected in their growth rate during recovery in the finisher unit.

Quarantine (d-18) behaviour was not related to ADG in any of the experimental phases for either treatment, indicating that d-18 behaviour was not predictive or influential of differences in immune function that may have resulted in altered growth rate during the challenge. As well, no relationships found between behaviour (d-18 or d13) and challenge nursery phase ADG in either treatment. This supports the idea that interaction with the point-source enrichments or metal chains was not sufficient to influence stress (and subsequent growth rate) during the acute phase of the disease challenge.

Pen-rooting in the enrichment group was positively correlated with post-challenge (d42) concentrations of WBC, LYMPH, RBC, and HGB, while positive social nosing was positively correlated with finisher ADG and post-challenge HGB. Increases in RBCs and HGB can indicate compensation from low blood oxygen levels (George-Gay and Parker, 2003), which would make sense during recovery from respiratory disease after the NDC. Conversely, low RBC and HGB concentration might have been expected during acute infection as anemia is a possible symptom of PRRSV (Cuartero et al., 2002) and of bacterial pathogens such as *Mycoplasma suis* (Stadler et al., 2021) and *Salmonella spp.* (Burdick Sanchez et al., 2019). However, we know from Chapter Three that the majority of individuals in both treatments had RBC, HGB, and HCT values within the normal range for healthy pigs.

Coupled with higher WBC and LYMPH, indicative of an active immune response to infection (Li et al., 2016), these cellular responses could mean that enriched pigs that performed more pen-directed exploration and had positive social contact experienced heightened immune responsiveness during the disease challenge, while the higher finisher ADG could then be

reflective of compensatory re-partitioning of energy towards growth in the pigs with more-active cellular immune responses (Rauw, 2012). Moreover, stress is associated with a decrease in LYMPH (Mcglone et al., 1993, Ruis et al., 2001), while challenge nursery phase pen-rooting in the enrichment group was correlated with an increase in LYMPH in this study, possibly indicating that pen-rooting was not related to high stress. In this case, rather than a reflection of frustration or boredom, pen-rooting may be a marker of increased activity in pigs experiencing stress-reducing effects of enrichment, or perhaps of those that were more resilient and therefore less affected by the disease pressure.

Several relationships between behaviour, growth rate, and CBC measures were significant at the 95% confidence level, but not at the adjusted significance level ($p<.003$). Based on previous work demonstrating links between social behaviour and growth rate (Camerlink et al., 2012), and the positive association between positive social nosing and finisher ADG demonstrated in the enrichment group of this experiment, it was expected to see a negative correlation between negative social behaviour and ADG within the quarantine and challenge nursery phases. This relationship was significant for both treatment groups at the 95% confidence level but was no longer statistically significant after a Bonferroni correction was applied. It is possible that the null hypothesis was incorrectly accepted for these relationships, and there may have been a mild negative influence of aggression on growth rate within both treatments. Lastly, an additional blood draw taken mid-NDC would have contributed additional understanding of the relationships between individual behaviour and cellular immune response and may have provided clarity on some relationships found in this experiment. Concentrations of immune cell subsets typically vary during different stages of disease (George-Gay and Parker, 2003), so future research could examine how social behaviour and immune responses may change and influence each other during both early- and late-stage infection.

4.5.3 Disease resilience classifications

A lack of significant difference in the proportion of resilient and susceptible pigs within each treatment reflects the main findings from Chapter 3. Based on this result, it can be inferred that the enrichment protocol did not provide sufficient stress mitigation effects to influence average growth rate, mortality, or disease severity (estimated by the number of veterinary treatments

required). Genetics are a strong influencing factor for disease resilience (Wilkie and Mallard, 1999), so environmental impacts would have to outweigh an animal's genetic pre-disposition to influence disease outcome. It is also possible that enrichments were beneficial, but that use of enrichment items, particularly porous items such as the cotton rope, acted as a vector for re-infection within a pen, thereby adding disease pressure to enrichment pens and negating stress-mitigation benefits. If future studies include the use of point-source enrichments during a disease challenge, it could therefore be beneficial to routinely swab enrichment items to test for presence of pathogens that may be spread through oral contact with the enrichments.

Chronic stress caused by barren environments and social stress can induce immune system suppression (Dhabar, 2009), while there is evidence that enrichments that encourage expression of natural functional behaviours can mitigate environmental stress and reduce its immunosuppressive effects (Luo et al., 2017, Van Dixhoorn et al., 2017). Previously discussed results indicated that behaviour of pigs was influenced by the presence of enrichment, and the behaviours influenced some CBC values, but the relationships found do not clearly point to a reduction in stress sufficient to influence disease resilience. To explore whether the enrichment group and control group were similarly affected by chronic stress from relatively barren environments that lacked biologically relevant outlets for natural behaviours, a positive control of a highly enriched group could have been included as a comparison.

4.6 CONCLUSIONS

The main goal of this experiment was to examine relationships between individual pig behaviour and corresponding measures of growth rate and immune cell concentrations following a natural disease challenge, and to determine if enriched and barren-housed pigs differ in disease resilience. While enriched and barren-housed pigs in this experiment did not differ in the proportion of pigs classified as resilient or susceptible, the results show interesting relationships between disease response and behaviour within treatments. Preliminary data on the behaviours of individual pigs showed that pigs raised with a rotation of point-source enrichment performed a greater level of oral-nasal-facial behaviours, suggesting that the provision of enrichment increased activity, but may not have satisfied behavioural needs, resulting in redirected rooting and oral manipulations towards penmates and pen fixtures. Pigs performing pen rooting during disease

challenge was associated with increased average daily gain in the finisher and higher counts of several immune cells post-disease challenge, which may suggest pigs that are more active showing functional behaviours may be associated with a good recovery from disease, despite the behaviours being directed towards the pen rather than the enrichments provided.

From the results of this experiment, there appears to have been more variability in behaviour and disease response within the enrichment group than within the control group, which could mean that the enrichment treatment impacted behaviour and disease response for some individuals, while control pigs had a more uniform response. Further work should be explored to confirm this, which can lead to developments in phenotypic measures for disease resilience. Several associations were found between behaviour and immune function in enriched pigs, including unexpected positive relationships between pen-rooting and positive social nosing, post-challenge growth rate, and several immune cell subsets. While pen-rooting in the enrichment treatment group may have resulted from an exploratory drive that was unfulfilled by the enrichments provided, the relationships found may indicate a positive impact of pen-directed exploration on immune function. From a resource allocation perspective, pigs under intense disease pressure need to shift energy expenditure towards immune function and away from growth and exploratory behaviours to successfully manage disease and return to homeostasis. To extend this perspective, pigs engaging in more activity, demonstrated by greater frequency of exploratory and social behaviours while under disease pressure, are likely more resilient, and differences in immune cell concentrations associated with increases in these behaviours could be indicative of a more robust and balanced immune response.

Future studies examining the impact of different types of enrichment on individual behaviour, productivity, and disease response would provide valuable information to producers. Environmental enrichment is a Code of Practice requirement, and provides demonstrated health and welfare benefits, but not all enrichment is equal. The enrichment schedule tested in this experiment did not produce a clear difference in disease resilience when compared to pigs kept in standard barren pens. Therefore, it would be beneficial for further research to expand on the findings of this study by comparing a positive control, such as a straw-bedded system, to various inedible point-source enrichments in a rotation schedule and with stagnant presentation. More

work is needed to make recommendations to producers on enrichment protocols that effectively improve health and welfare of pigs while being practical to implement on-farm.

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5. GENERAL DISCUSSION AND CONCLUSIONS

The objectives of this thesis were to examine the effect of inedible point-source enrichments on pig disease response, and to explore relationships between pig behaviour and immune function when under the pressure of a polymicrobial natural disease challenge. In Chapter Three, the influence of inedible point-source enrichments on disease response was explored through a series of pen-level behaviour, growth, mortality, and immune cell counts before, during, and after a polymicrobial natural disease challenge (NDC). In Chapter Four, relationships were explored between individual-level pig social and exploratory behaviours and measures of disease response (growth rate, immune cell counts) within enriched and barren-housed control treatment groups. Additionally, the effect of housing type (enriched or barren) on disease resilience was tested by categorizing pigs based on their performance (growth rate, veterinary treatments) in the disease challenge.

Barren pens with slatted flooring are common in North American swine production for their ease of cleaning and maintenance (Tuyttens, 2005), but can cause stress, frustration, and boredom by limiting the expression of natural functional behaviours (Beattie et al., 2000). In turn, barren environments are associated with a higher likelihood of abnormal, aggressive, or stereotypic behaviour (Mkwanazi et al., 2017), immunosuppression (Luo et al., 2017, Van Dixhoorn et al., 2016), and depressed growth (Barnett et al., 1983, Pearce et al., 1989). There is considerable evidence supporting the provision of loose, ingestible substrates as enrichment to reduce environmental stress (Van de Weerd and Day, 2009). Inedible point-source enrichments are commonly used in commercial swine facilities as an alternative to loose substrates (Mkwanazi et al., 2017), but evidence of their benefit to health and/or welfare is conflicting or absent in several areas of research (Bracke, 2006). Therefore, a knowledge gap exists in the understanding of the role of inedible point-source enrichments on the swine stress response, and how they may influence immune function, behaviour, and growth performance of pigs under disease pressure.

To address this knowledge gap, experiments in Chapters Three and Four of this thesis explored the provision of inedible point-source enrichments, rotated three times weekly, as a possible tool to improve swine health and/or welfare outcomes before, during, and after a polymicrobial natural disease challenge (NDC). In Chapter Three, results showed that point-source enrichment usage

was higher in the enriched treatment group than with metal chains in control pens during most sampling days but declined over time from the beginning to end of the experiment and within each phase. In Chapter Four, mean enrichment use was higher in the enrichment group on Day 13 (NDC phase), but not on Day -18 (quarantine phase), but was low (1-5% of observations) overall. Despite frequent rotation and more than two weeks between re-presentations of the same items (Trickett et al., 2009, van de Perre et al., 2011), it appears that habituation to the enrichments occurred. The observed decline in use within each phase reflects previous findings on point-source enrichments (Van de Perre et al., 2011), and suggests that the items used likely did not provide sufficient novelty, complexity, and/or intrinsic reward to maintain a high level of interest despite the introduction of two new items (rooting mat and jute sack) during the NDC to support continued engagement during the acute disease challenge. As discussed by Stolba and Woodgush (1989) and Trickett et al. (2009), novelty is a valued component of enrichment, particularly when objects do not provide a tangible reward such as food, and habituation to indestructible items occurs rapidly (Apple and Craig, 1992). Gifford et al. (2007) found that rotation of enrichments every two days with at least five days between re-presentation was sufficient to retain novelty, but this was not directly reflected in the results of either chapter in this thesis. Even with rotation and provision of novel objects, enrichments need to fulfill biologically driven behavioural needs in order to reduce stress and provide physiological benefits (Bracke, 2008, Van de Weerd and Day, 2009), and it appears that the enrichments in this experiment likely did not meet these criteria.

In Chapter Three, enriched pigs were more likely than control pigs to interact with the provided enrichments near the beginning of the quarantine phase (d-15) and early in the finisher phase (d70), and the provision of enrichments was associated with a higher probability of standing during the morning of d-15 of the quarantine phase. As well, enriched pigs were more likely to be lying laterally and less likely to lie sternally on d-15 (PM) and on d70 in the finisher phase. The differentiation between sternal and lateral lying is important for assessing stress and discomfort, particularly in the context of the NDC. Sternal lying is most seen in pigs that are lying awake and inactive and is associated with illness and thermal discomfort or heat conservation (Escobar et al., 2007), while standing is indicative of activity and exploration, and lateral lying is most likely when pigs are asleep or resting and maintaining a comfortable body temperature (Brown et al., 2018). Increased lateral lying (and sleeping) in enriched pigs in the quarantine and finisher phases could have resulted from increased activity, facilitated by the higher probability of enrichment use

compared to control pigs. While probability of enrichment use remained higher in the treatment group than the control group throughout most of the NDC phase and near the end of the finisher phase (d140), postural differences between treatments were not found. This indicates that while the rotation of point-source enrichments was more attractive than the unchanging metal chains, the enrichment use was insufficient in either frequency/duration or in biological relevance (or both) to alter probabilities of health- or sickness-related postures.

Compared to interactions with point-source objects, studies have demonstrated up to 20% higher interaction frequency with straw (Scott et al., 2007, Petersen et al., 1995) compared to <2% (Scott et al., 2007) for pens with suspended manipulable toys; this reported interaction level is similar to the frequency observed across multiple time points in both chapters of this experiment. This context is important when discussing the results of the experiments in this thesis. It is possible that frequent and sustained interaction with enrichments is necessary to effectively mitigate stress, and it appears that materials provided on the ground that can be rooted (even if inedible) sustain interaction and interest better than suspended point-source materials (Bracke, 2017), and therefore infrequent interaction with point-source enrichments may not result in significant impacts on immune function and growth performance. This is supported by the lack of treatment differences found in average growth rate, mortality, or post- disease challenge concentrations of immune cells in Chapter Three.

To the best of our knowledge, no studies have found a link between enrichment use and mortality in pigs, which concurs with the absence of difference in mortality rates in this study. In agreement with Blackshaw et al. (1997), results of Chapter Three showed no significant differences in growth rate (ADG, FCR) between barren-housed pigs and those reared with suspended inedible enrichments in any experimental phase. In contrast, experiments in which weaned pigs were provided edible rooting substrates (Luo et al., 2020) or a combination of rooting substrates and suspended toys (Oliveira et al., 2016) saw higher ADG and lower FCR in enriched pigs compared to those raised in barren pens. The afore-mentioned studies, together with the findings of Chapter Three of this thesis, suggest that inedible point-source enrichments may be less likely to influence growth rate in pigs than environments enriched with rooting substrates.

It is not definitively known what an ‘ideal’ response to the NDC would represent based on CBC values alone, and thus the comparisons between treatments should be considered exploratory

in combination with known measures of disease response such as morbidity, mortality, growth rate, and behaviour. In the absence of differences in average between-treatment differences in mortality and growth rate in Chapter Three and disease resilience classifications using veterinary treatment rate and growth in Chapter Four, it can be inferred that the provision of inedible point-source enrichment did not alter immune function as a whole, but several interesting relationships existed between individual behaviour frequencies, growth, and CBC values.

Significant positive correlations were found between challenge nursery phase pen rooting (Day 13) and post-challenge (Day 42) concentrations of total WBC, RBC, hemoglobin, and lymphocytes within the enrichment group. Pen rooting was also positively correlated with positive social behaviour (and not correlated with negative social behaviour) in both phases (Day -18 and Day 13). In the absence of effective enrichment such as straw or other loose substrates (Bracke, 2008), rooting and oral manipulation behaviours may be directed towards pen floors, walls, and fixtures, or penmates (Petersen et al., 1995); these re-directed rooting behaviours are typically thought to reflect negative emotional states such as boredom and frustration (Beattie et al., 2000). While increased concentrations of WBCs and subsets can indicate active infection or inflammation (Chase and Lunney, 2019), pen rooting in the enrichment group was also positively correlated with finisher ADG, and mortality was not different between treatments in either Chapter Three or Chapter Four. Therefore, it is more likely that correlations between pen rooting and above-listed immune cell concentrations point to a portion of enriched pigs with a more active and robust WBC response that were able to partition additional energy towards growth and exploratory behaviour in the finisher unit, performing less sickness behaviour (Rauw, 2012) and potentially demonstrating greater re-population of hemoglobin and RBCs that could have been depleted during active infection (George-Gay and Parker, 2012). In a separate experiment using the same population of pigs as the present study, Bai et al. (2020) found a relationship between resilient pigs (classified as high growth rate and low veterinary treatment rate) and higher RBC concentration in Blood 4 and a smaller decrease in RBC from Blood 1 to Blood 4 compared to susceptible, dead, and average pigs. The unexpected correlations between cell counts, growth rate, and pen rooting may therefore warrant further exploration of pen rooting as a phenotypic expression of disease resilience in pigs enriched with inedible point-source objects.

Differences in average posture frequencies and change in WBC concentrations (Chapter Three), and within-treatment relationships between individual behaviour and immune responses (Chapter Four) could indicate subtle differences in behavioural and immunological measures of disease response, even in the absence of overall treatment differences in the proportion of pigs classified as disease resilient based on growth rate and veterinary treatments. This is further supported by Chapter Three results, in which the enrichment group demonstrated a greater increase in total WBC concentration from pre- to post- disease challenge than the control group. The results of Chapter Four suggest that pen rooting in this experiment is more likely to represent positive exploratory behaviour than a negative state of frustration or boredom, indicating that some enriched individuals either had a more active immune response to infection and subsequently were more active during the NDC or were performing more frustration-related behaviour during late-stage infection. Use of cell counts alone to assess immune function is a significant limitation of this study. Future investigation on this subject could include antibody titers (Zhao et al., 2020) and/or inflammatory cytokine markers (interleukins, tumor necrosis factor-α, interferon-γ) in early- and late-stage infection to assess whether pigs with high leukocyte concentrations in Blood 4 (D42) were recovering from the disease challenge or fighting an ongoing infection (Gómez-Laguna et al., 2010, Ladinig et al., 2014a). Additionally, Previous research suggests that pigs may return to pre-infection concentrations of leukocytes by day 19 post-infection (Ladinig et al., 2014b), so inclusion of a blood draw during early-stage infection in the challenge nursery would have provided valuable insight into the disease challenge progression and degree of cellular immune responses experienced within treatments.

Results of Chapter Three are mostly in agreement with Ernst et al. (2006), who found no significant differences in white blood cell (WBC) and subset concentrations (total WBC, neutrophils, lymphocytes, monocytes, basophils, eosinophils, neutrophil to lymphocyte ratio) between pigs with and without cognitive enrichment. However, enriched pigs in the Ernst et al. (2006) experiment demonstrated faster wound healing and higher immunoglobulin G (IgG) concentrations than the non-enriched control group. On the contrary, Luo et al. (2020) found higher concentrations of total WBC, lymphocytes, and greater neutrophil to lymphocyte (N:L) ratio in addition to higher IgG in pigs enriched with a combination of rooting and point-source materials. A decrease in lymphocytes and increase in neutrophils is associated with acute stress (Davis et al., 2008); had the enrichment treatment reduced stress and stress-induced immunosuppression, it

would be expected that the enrichment group would differ in concentrations of neutrophils, lymphocytes, and/or N:L ratio in Chapter Three, and that enrichment use would be negatively correlated with neutrophils and N:L ratio and positively correlated with lymphocyte concentration, but none of these relationships were found. Similarly, if pen-rooting were indicative of stress, the opposite relationship would be expected: instead, pen-rooting was positively correlated with both total WBC and lymphocyte concentration. Based on this information, it cannot be determined if the point-source enrichments affected immunological markers of stress, but pen-rooting behaviour in the enrichment group reflects similar relationships with immune cells to those measured in pigs provided with rooting substrates in the Luo et al. (2020) experiment. Measuring IgG for the experiments in this thesis could have added additional value to determine if a similar relationship existed and is an area of future research that could be explored.

The relationship between environment and physiological well-being is complicated and multi-faceted, with the stress response located at the epicentre of the issue. Compared to a different disease challenge model examined by Van Dixhoorn et al. (2016), in which point-source enrichments were provided alongside straw bedding, the results of Chapter Three suggest that a protocol of inedible point-source enrichments alone may not have sufficient stress-mitigating effects to influence disease response on a population level. As discussed in Chapter Three, a lack of a positive control of straw is a limitation to this study and could have provided a useful point against which the present study's results could be compared to determine if a more biologically relevant enrichment protocol could provide sufficient benefit to influence measures of disease resilience in pigs.

While treatment differences in immune cell concentrations and growth rate indicate physiological influence of enrichment, physical indicators of stress and illness are arguably more important measures of welfare and well-being. Experiments in both chapters saw treatment-level behavioural differences, even when significant physiological differences were absent. This is a primary reason that Chapter Four delved into exploration of behavioural and physiological measures of disease response on an individual level- a novel approach to the understanding of disease resilience and its relationship with environment and behaviour. In doing so, unexpected relationships were found between pen rooting, typically considered a negative behaviour, and immune function, suggesting that pen activity and exploration may be useful measures for

examining disease resilience. Behaviour, immune response, and disease resilience can vary considerably between individuals, and based on this, Chapter Four provides preliminary understanding to potential behavioural measurements of disease resilience.

In conclusion, provision of a rotation of inedible point-source enrichments before, during, and after a polymicrobial natural disease challenge was associated with differences in pen-level postures and individual-level social and exploratory behaviour, but no clear association was found between enrichment and immune function or disease resilience. The results of this thesis do not mirror those of studies providing biologically relevant rooting materials as enrichment, suggesting that the enrichments provided were not sufficiently rewarding, and thus did not appear to mitigate stress and improve immune function or productivity during the disease challenge. Preliminary investigation on disease performance and individual behaviour relationships indicates that exploratory behaviours such as pen rooting may be a useful indicator of individual disease resilience, with greater activity corresponding to compensatory growth and a more active immune response. Further research is needed on the efficacy of suspended inedible point-source enrichments for improving pig well-being: investigation into alternative enrichment materials and provision methods for use in fully slatted rearing pens could provide additional value, finding an effective ‘middle ground’ between barren and fully bedded systems that satisfies the needs of animals and producers alike.

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