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Investigating the use of dietary magnesium on pig performance, welfare and stress during key life events

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The candidate confirms that the work submitted is her own, except where work which has formed part of jointly-authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

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I conducted the search. Myself, Louise Dye, and Lisa Collins wrote and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Abstract

Some processes and practices commonly used in pig production may be experienced by the animals as stressful, negatively impacting pig performance and welfare, as well as farm productivity and profit. Research in rodents and humans has demonstrated that dietary magnesium is effective at reducing stress. However, research in other species is limited. This thesis investigated the impact of dietary magnesium supplementation on stress, performance, and welfare during key life events in farmed pigs. A systematic review indicated that magnesium supplementation can positively impact welfare, stress, and behaviour, although the literature was limited. A survey of farmers reflected this finding, but highlighted gaps between scientific research and commercial application in the timing of supplementation. To examine the effect of supplementary magnesium phosphate, with or without phytase, on physiological and behavioural measures of stress during regrouping, a study was conducted with 240 pigs over five weeks. In a separate study, two types (phosphate and sulphate) and levels (0.2% and 0.3%) of magnesium were supplemented in the diet of 240 pigs pre- and post-weaning. Magnesium phosphate improved performance and reduced body lesions scores post-weaning, but not in grower pigs. However, magnesium phosphate positively impacted pig behaviour in grower pigs despite no difference in cortisol measures. Despite an increase in dietary magnesium level, there were no further benefits of supplementing magnesium phosphate with phytase. Post-weaning, magnesium sulphate resulted in poorer performance and faecal scores. Taken together, the results of these novel studies demonstrate that dietary magnesium phosphate may improve or maintain performance post-weaning, and can improve pig behaviour and welfare during stressful events. The effects of magnesium phosphate on the stress response during these events, and potential interactive effects with other nutrients, needs further exploration. This thesis elucidates the benefits and advances understanding of the impact of magnesium supplementation on pig welfare, stress, and performance.

Table of Contents

List of Tables

Table of Figures

Abbreviations

UK	United Kingdom
%	Percentage
N/A	Not applicable
ID	Identification
Kg	Kilograms
g	Grams
Vs.	Versus
°C	Degrees centigrade
nm	Nanometre
ml	Millilitre
rpm	Revolutions per minute
MgP	Magnesium phosphate
MgS	Magnesium sulphate
FTU	Phytase units
HPA	Hypothalamic–pituitary–adrenal
ATP	Adenosine triphosphate
NMDA	N-methyl-D-aspartate
RFID	Radio frequency identification
PSE	Pale, soft and exudative
ADG	Average daily gain
ADFI	Average daily feed intake
FCR	Feed conversion ratio
ELISA	Enzyme-linked immunosorbent assay
ICP-OES	Inductively coupled plasma - optical emission spectrometry

SE	Standard Error
AIC	Akaike Information Criterion
lm	Linear model
glm	General linear model
glmm	General linear mixed model
clmm	Cumulative link mixed model (ordinal regression)
glmnb	Negative binomial model
glmmnb	Negative binomial mixed model

Chapter 1. Introduction

Modern intensive farming systems are under increasing pressure to produce meat and animal products for a growing global human population. As a consequence, it has been predicted that by the year 2030 global meat supply will reach 374 million metric tonnes, while pig meat output is projected to be 127 million metric tonnes (1). To meet this increasing demand, production systems are required to be more sustainable, efficient, and productive than ever (2,3), a challenge which is made all the more difficult with turbulent world events (4,5). Ensuring good animal welfare remains a priority while increasing outputs seemingly poses an additional demand (6,7). However, there is evidence that good livestock welfare is not only good for the animal but often goes hand-in-hand with farm efficiency and profit (8,9). Therefore, it is important to assess and improve animal welfare within these farming systems at the same time as ensuring production performance is maintained or enhanced. This is particularly important during common stressful events, such as weaning and regrouping, which have been shown to negatively impact animal welfare and farm productivity (10,11).

1.1 Stress

Stress challenges the homeostasis of living organisms, and the consequent stress response may be defined as a biological response aimed at restoring this homeostasis (12–14). In general, stress can be caused by physical or psychological events (stressors) which in turn initiate the stress response, resulting in the activation of the hypothalamic-pituitary-adrenal (HPA) axis which stimulates release of glucocorticoids from the adrenal cortex (14–16). The stress response can be both acute, lasting minutes or hours, or chronic, lasting days or weeks (17). Although stressors can be both positive and negative, for example arousal due to fighting or excitement (18–20), for the purpose of this thesis, stress and the stress response refers to the response to a negative stressor.

1.2 Stress and Magnesium

Magnesium is an essential mineral involved in hundreds of enzymatic processes and physiological functions in the mammalian body, including adenosine triphosphate (ATP) production and immune function (21). Magnesium has been shown to be closely linked with stress and mood via a number of biological mechanisms. Magnesium is a N-methyl-D-aspartate (NMDA) receptor antagonist, and this inhibitory effect on NMDA results in its antidepressant-like effects on mood (22,23). It has also been proposed that the anti-depressive effect of magnesium is due to interactions with the serotonergic and dopaminergic systems (21,24). Magnesium has been shown to interact with corticotropin-releasing factor (CRF), a key factor in HPA axis activation (25). Similarly, magnesium can prevent over activation of the HPA axis by reducing the release of adrenocorticotrophic hormone (ACTH) and control adrenocortical sensitivity to ACTH and release of glucocorticoids (21,26). Conversely, magnesium deficiency has been shown to be cause HPA axis dysregulation, depression and anxiety in both humans (27–29) and animals (23,30,31), once again demonstrating the role and potential of magnesium in regulating the stress response (32,33).

1.3 Stress within Pig Production

Within pig production, acute stressors and chronic stressors, such as transport, rough handling, and heat stress (34–36), impact on pig health, welfare, behaviour, and productivity (17). Poor welfare can occur when the animal struggles to adapt to a stressor, and if repeated or enduring, the stressor can have a significant effect on the animal's wellbeing. Furthermore, when the body is under stress, nutritional energy is redirected away from growth and the immune system to facilitate the stress response resulting in reduced productivity (37). Stress can also result in a change in behaviour, such as an increase in fighting and tail biting (38–40). These stress-related, reactive behaviours not only require energy but can result in injury and illness, further impacting upon the animals' health and welfare, and farm production costs and profit (8,39).

In the UK, a wide range of different pig production systems are in operation. All of these systems present different challenges for the pigs living and growing within them, for instance pigs reared solely indoors may have a carefully controlled climate but may lack space and/or varied enrichment, with the opposite for outdoor bred or reared pigs (41). Yet despite these differences, most pigs will experience the same key life events, including weaning, regrouping, and transport. Therefore reducing stress during these key life events should represent an improvement in welfare in multiple pig production systems.

1.4 Pig Nutrition

Nutrition is an important factor in pig production. At the most basic level, ensuring the animal receives the right type and amount of nutrients is essential to ensure optimal growth and health. In pig production, precision nutrition is becoming more common as this way of feeding allows the farm to specifically tailor the diets to the needs of each group of pigs (42,43). This type of feeding has been shown to be a successful way to lower production costs, increase nutrient efficiency, improve pig welfare, and reduce the environmental impact of the production system (42). Precision nutrition can also involve the inclusion of specific supplementary components that may provide further benefits for productivity, health and welfare (44–47), including magnesium (48). Typically, due to the high amount of cereal components, the magnesium content of pig feed is more than sufficient to meet their requirements (0.04% per Kg of feed) and no supplementation is needed (49). In terms of nutritional physiology (50,51) and stress (52), pigs are similar to humans. This similarity between the two species suggests that additional magnesium in the diet should have a similar effect on stress as has been observed in humans (50,53).

1.5 Conclusion

As consumer demand for high welfare pork products at an affordable price increases, optimising welfare within intensive systems is crucial. Many key life events in pig production are stressful for the pig and consequently can negatively impact pig performance and welfare. The capacity for magnesium to regulate the stress response may be beneficial in terms of pig production by reducing stress during these key life events.

1.6 Thesis objectives, hypotheses and structure

1.6.1 Thesis Aim

The main aim of this thesis is to investigate the impact of supplementary magnesium in the diet on stress, welfare, and performance during key life events in pigs.

1.6.2 Thesis Objectives

Five main objectives will be addressed in this thesis:

(i) Conduct a systematic review of the current literature to determine whether this supports the use of supplementary dietary magnesium as an intervention to reduce stress in pigs.

(ii) Conduct a survey of farmers to (i) explore current practice of pig farmers regarding the use of magnesium in pig nutrition, and (ii) explore current opinions of pig farmers on the potential use of magnesium in pig nutrition.

(iii) Investigate how supplementary dietary magnesium, with or without phytase, may affect (i) pig performance; (ii) pen faecal cortisol; (iii) focal pig salivary cortisol; and (iv) focal pig hair cortisol, during regrouping in grower pigs.

(iv) Investigate how supplementary dietary magnesium, with or without phytase may affect (i) focal pig behaviour; and (ii) focal pig skin lesion scores during regrouping in grower pigs.

(v) Explore how supplementary dietary magnesium may affect (i) performance; and (ii) focal pig lesion scores, in pigs post-weaning.

1.6.3 Hypotheses

I expect that stress and the secondary effects of stress during regrouping and weaning will be reduced by supplementing farmed pigs' feed with magnesium. Therefore, I hypothesise that pigs that have had supplementary dietary magnesium during a key stressful event will have reduced physiological measures of stress, lower lesion scores, fewer instances of aggressive and harmful behaviour, and improved performance in comparison with pigs on the same diet without a magnesium supplement. Physiological measures of stress will be assessed by focal pig salivary and hair cortisol, and pooled pen faecal cortisol measures. Pig performance will be measured by average daily gain, average daily feed intake, and feed conversion ratio.

1.6.4 Thesis Structure

In this thesis a background in the format of a systematic review shows the current scientific evidence for the influence of supplementary magnesium on stress and behaviour in pigs (Chapter Two). Following this, the results of a survey of farmers explores their views and experience with supplementary magnesium in practice (Chapter Three). I then investigate how adding supplementary magnesium to pig feed before, during and after a regrouping stressor can influence pig behaviour and skin lesion scores (Chapter Four), as well as pig performance and cortisol levels (Chapter Five). In Chapter Six, two different types and levels of magnesium are supplemented in the feed of piglets before, during and after weaning, and its impact on performance and skin lesion scores assessed (Chapter Six). The findings and their implication for magnesium as a nutritional intervention to reduce stress during key life events in farmed pigs is discussed as a whole (Chapter Seven).

Chapter 2. Is Magnesium Supplementation An Effective Nutritional Method To Reduce Stress In Domestic Pigs? A Systematic Review

2.1 Introduction

It is not uncommon for commercially farmed domestic pigs (*Sus scrofa domesticus*) to experience negative stress during their lifecycle. Acute stress (such as transportation or regrouping) and chronic stress (such as excessive heat or over-stocking for an extended period of time) can both be detrimental to the animal's health and welfare, and have economic impacts due to increased susceptibility to disease, increased mortality, poor meat quality and poor performance (54–56). To understand how an environment, situation or event is affecting an animal, stress can be assessed by measuring physiological, physical, and behavioural changes. Physiological measures of stress, such as heart rate or cortisol, have typically been the most common method of measuring a stress response in animals. For example, hair cortisol has been shown to be a good marker for chronic stress (57), whereas blood and salivary cortisol changes much faster in response to acute stressors (58). However, whilst these measures assess the level of arousal of the individual, they do not indicate valence — the physiological changes observed can be the result of positive (excitement) or negative stress, making interpretation difficult. These physiological measures are more easily interpreted and more useful when used in conjunction with behavioural measures, allowing for the valence of the animal to be assessed (59–61). Physical changes like skin lesion scores can also be used. For example in pigs, lesions on the main body are likely the result of fighting and aggressive interactions (62), whereas tail lesions often signs of non-aggressive harmful behaviours (38).

Harmful social behaviours, such as tail and ear biting resulting in ear and tail lesions, are often multifactorial with factors such as genetics, access to enrichment and stocking density influencing the frequency and severity; however, they can also be exacerbated by stress (63). Acutely stressful events, such as transport or regrouping, can also lead to an increase in aggressive behaviours such as fighting, due to the disruption and subsequent re-establishment of the dominance hierarchy (39). Not only are these types of harmful and aggressive behaviours detrimental to the pigs' welfare but they can have a huge economic impact for the farmer or producer. Performance measures, including growth rate and reproduction (64,65), are all negatively impacted by a high level of stress, as well as resulting damage and skin lesions increasing the risk of disease and mortality. Later, aggression before slaughter can cause carcass damage resulting in a penalty for the producer (66,67), and higher stress levels have also been shown to negatively affect meat quality causing, for example, pale, soft and exudative (PSE) meat that is unattractive to the consumer (68,69).

Often acutely stressful events are unavoidable in current commercial farming systems, such as key events that involve a change of environment or social structure, including weaning, regrouping (also known as mixing), or transportation, Therefore, research which focuses on improving the welfare of commercially farmed pigs, especially during these periods, is crucial for the animals and producer.

The five freedoms (70,71) describe the basic needs of an animal to guard against poor welfare. The five freedoms are the freedom from hunger and thirst; freedom from discomfort; freedom from pain, injury or disease; the freedom to express normal behaviour and freedom from fear and distress. These basic requirements should be met before other areas can be addressed to ensure a good, or even positive (72) welfare state is met. Providing a nutritionally balanced diet with access to water meets the most basic requirement. However, nutrition can also improve welfare beyond simply meeting the animals' basic needs. For example, providing a varied diet in terms of texture and taste, allowing a choice of diet, or providing the diet in an enriching and stimulating way will allow for the animal to express more of its natural behaviour (73–75). Adding additional nutrients above the required level to maintain bodily function and growth, such as increased tryptophan (76) or fibre content (77), has also been shown to improve behaviour, welfare and performance. In farmed animal species, supplementary magnesium has been seen to improve productivity, including increased eggshell strength in aged laying hens (78), reduced weight loss in heat-stressed hens (79), improved growth rate in sheep (80), and reduced time between weaning and next oestrous cycle in pigs and dairy cattle (81).

As a vital mineral for mammalian function, magnesium acts as a co-factor for over 300 different enzymes and plays key roles in processes including ATP production and immune function (82–84). A large body of research also suggests that magnesium may play a role in reducing stress, anxiety and depression in humans via multiple mechanisms including the serotoninergic, glutamatergic and adrenergic systems (21). Multiple reviews have concluded that there is evidence for beneficial effects of magnesium despite the poor quality of some experimental research (for reviews see: Stress and anxiety: (27,85); Depression: (86,87)). In commercial pig production, magnesium may be added to pig feed during a stressful event in an attempt to alleviate this (88,89). Swine diets typically contain sufficient magnesium to maintain growth and normal bodily function due to the level of magnesium in the cereal components of the feed; however, supplementation can be implemented with a range of different magnesium compounds or products. Although magnesium is generally thought to be beneficial in reducing stress, there remains a lack of substantive evidence to support its effectiveness in pigs.

Our aim was to conduct a systematic review to evaluate the available scientific evidence and determine whether this supports the use of supplementary dietary magnesium as an intervention to reduce stress in pigs. Included papers could focus on chronic or acute stress but must include a dietary magnesium treatment and at least one measure of stress, for example physiological measures such as cortisol, adrenaline and heart rate; skin lesions or observed behaviour.

2.2 Method

2.2.1 Search

A systematic review was conducted in April 2020 using the search engine Web of Science due to its wide range of source databases (90). The Web of Science default time span of 1900 – 2020 was applied. The search terms ‘magnesium’, ‘pig’, ‘swine’, ‘livestock’, ‘behaviour’, ‘aggression’ and ‘stress’ were used in combination using Boolean operators. The search term string used was “(magnesium OR mg) AND (behaviour OR behavior OR stress OR aggression OR aggressive OR cortisol) AND (pig OR pigs OR swine OR porcine OR livestock)”.

The references of the final corpus were checked to ensure no literature was missed. Five further studies were found; however, one was a conference abstract (91) and three were not accessible (92–94) and, therefore, are not included in this review. The final paper found in the reference check was included in the final corpus (95).

2.2.2 Inclusion and Exclusion Criteria

Duplicates were removed and the remaining papers were filtered in four stages: (1) title; (2) abstract; (3) methods; and (4) full paper. Papers were included if: (1) pigs were the main study species, with a focus on the whole live animal; and (2) the study included dietary magnesium and at least one measure of stress. Papers were excluded if they were: (1) review papers; (2) conference abstracts; (3) in vitro; or (4) research not including a magnesium supplement or a measure of stress. Papers were also excluded if the abstract or full text could not be accessed or was not in English (Figure 1).

2.2.3 Information Extraction

The following information was extracted from the final remaining papers: (1) aim of study; (2) sample size, sex and age of individuals or stage of production; (3) genotype; (4) experimental treatment(s); (5) dietary treatments (type of magnesium supplement, dose, administration method); (6) measured outcomes of stress; and (7) results.

2.3 Results

2.3.1 Characteristics of Included Studies

The initial search identified 2,379 studies that were filtered according to the inclusion and exclusion criteria (as defined in 2.2.2), resulting in a final corpus of sixteen papers (Table 1; Figure 1).

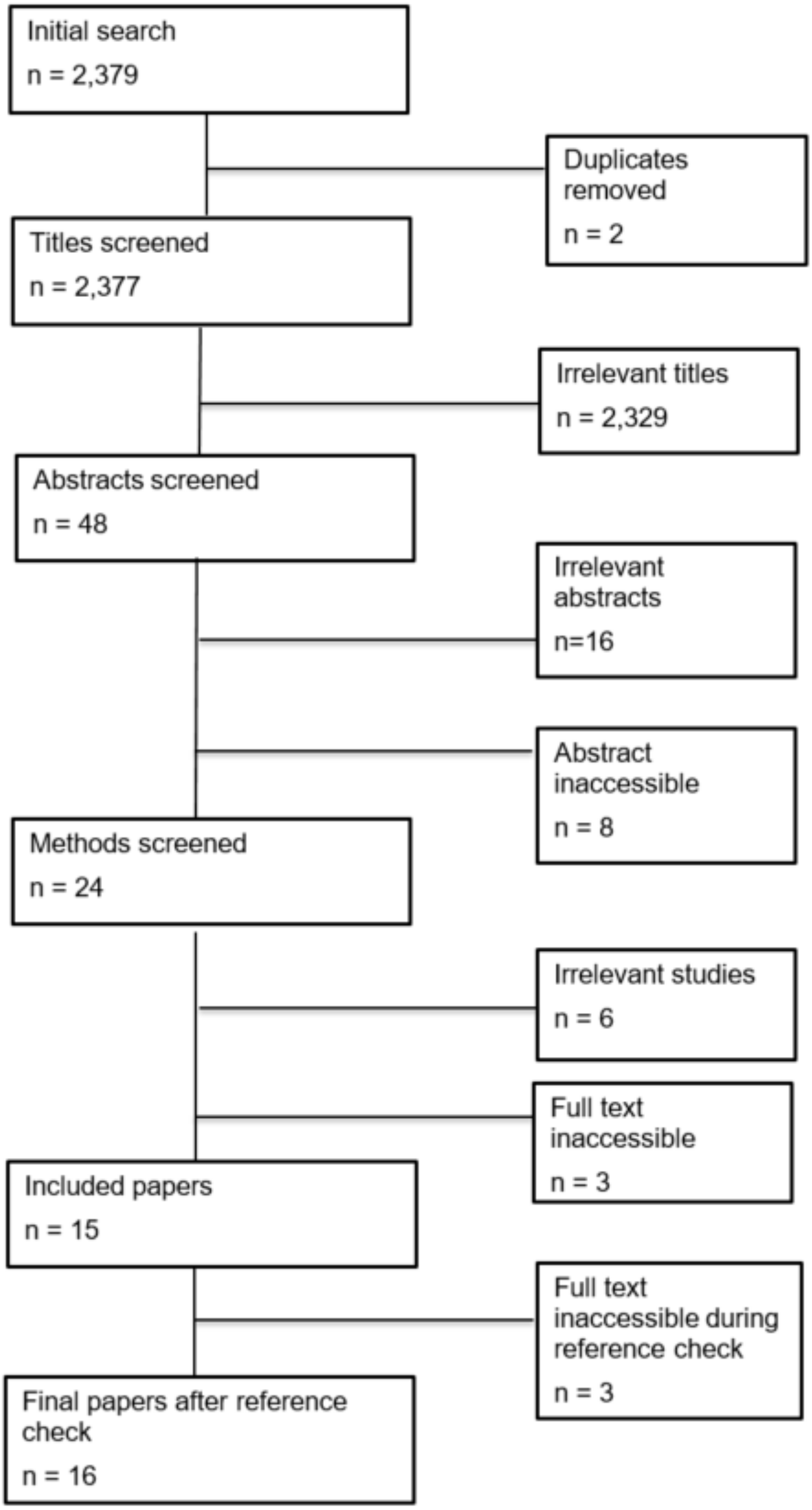

Figure 1. Flowchart to show the study selection process. Irrelevant studies included those that did not have pigs as their study species or include a measures of stress

Five studies included male and female pigs, seven only used male animals and four did not report the sex of the animals used (96–99). Sample sizes (including all treatments and controls) across the studies were highly variable, ranging from 10 to 448 pigs in total (average sample size of 124 with a standard deviation of 150). Thirteen of the sixteen studies focussed on the effect of magnesium in the finishing phase (approximately 50kg to slaughter) and two in the grower phase (approximately 20kg to 50kg live weight); one study did not specify the stage of production or age of the pigs used (96).

Six studies used Large White x Landrace pigs, three used a combination of Landrace, Large White and Pietrain breeds, two used Pietrain x Hypor animals and one used only Landrace and one a Duroc x Large White x Yorkshire. Two studies did not specify breed, only that the animals were halothane gene positive or negative (100,101).

Seven studies chose to include pigs that expressed or carried the halothane gene (96,98–100,102–104). This genotype results in the pigs being more susceptible to porcine stress syndrome, a genetic condition characterised by stress induced hypothermia (105). Three of these studies compared groups of pigs positive for the halothane gene with animals either negative (102,103) or carriers (100), whereas both studies by Peeters et al., (98,99) used only carriers of the gene and one study did not state the genetic profile of the animals used (96).

2.3.2. Treatments

2.3.2.1 Dietary Treatment

A total of ten different magnesium supplements were used across the sixteen studies. Four supplements were used in multiple studies; magnesium acetate was used by both Peeters, et al., (2005) and (2006) (98,99). Two studies used magnesium-rich marine algae extract with a magnesium level of 59,520mg/Kg (88,89) and two used magnesium sulphate (95,103). Magnesium aspartate, also known as magnesium aspartate hydrochloride, was another popular choice with six studies choosing to use this supplement (95–97,100,106–108). Other magnesium supplements were magnesium mica (109), magnesium fumarate (104), magnesium carbonate (102), magnesium oxide (110) and magnesium chloride (95). The dose varied greatly between studies with twenty different doses administered. The majority of studies included magnesium at a level of <1g (31.25% of the studies) or between 1 and 5g (50.00% of the studies). Only one used a dose between 5-10g and three >10g (Table 1). Six studies compared two or more different amounts of the specific magnesium supplement (97,100,104,106,108,110). There were ten different durations of supplementation ranging from 2 to 115 days (average of all durations in each study was 22.24 days with standard deviation of 33.65 days). One study supplemented during a live weight range (30-100kg) rather than days (104), and two studies compared long and short-term supplementation (97,100). Two different supplementation methods were used. Thirteen studies opted to add the supplement to the pigs standard feed, a further two added it to drinking water (98,99) and one supplemented both feed and water depending on the length of application (97).

2.3.2.2 Methods of Inducing and Measuring Stress

Stress was often induced by slaughter (100,102–104,110), and measured in terms of behaviour and skin lesions in or following the lairage period (100), handling and stunning procedures (95,102,103) or blood parameters following slaughter (97,104,110). Transport, an acute stressor, was included in multiple studies (97–99,101,107,108) during which some were transported within their original groups (101); some were mixed and then transported (99) and some experienced a transport simulation (98). Others used common stressors experienced on a commercial farm, such as regrouping (88,89), withdrawal of feed (89), handling technique (95,106) or exercise (96).

A total of thirteen studies used physiological measures to quantify stress and six used behavioural measures with four studies employing both techniques (Table 1; Table A 1). Stress was typically assessed by measuring cortisol, with seven studies using plasma or serum (97,104,107,108,110) and three using salivary cortisol (88,89,98). Other physiological measures used to quantify stress included norepinephrine levels in two studies (104,106), adrenaline and noradrenaline (95) and one study measured tachycardia and hyperventilation (96). The level of aggression or harmful behaviours was assessed using behavioural observations in six studies (88,89,98,100,102,103). Lesion scores were used in a further four studies (88,89,99,103).

2.3.3. Outcomes of Included Studies

Of the final corpus of studies (Table 1), ten found that supplementary magnesium significantly reduced at least one measure of stress. A further two studies found supplementary magnesium reduced serum cortisol levels, although not significantly (107,108). Two studies found supplementary magnesium resulted in a statistically significant increase in stress (100,103) suggesting that it may be harmful in some instances. Two studies found no difference in measures of stress between dietary treatments. Apple, et al., (101) showed that 25g/Kg magnesium mica had no effect on stress and similarly, D'Souza, et al., (95) found no significant difference between a control diet and three different magnesium-supplemented diets on adrenaline and noradrenaline.

Sample size or power calculations were not reported and the total number of animals used in the sixteen studies ranged from 10 to 448 with eight studies using between 1 and 50 pigs, two using 51 to 100 and five having a total sample size of over 100 animals (Table 1). Six of the 15 studies appear to have less than ten animals per treatment group (including dietary, genotype and stressor treatments) (96,101,102,104,107,108). Thus, the results from studies with a low sample size should be interpreted with caution.

2.3.3.1 Cortisol and Physiological Measures

Salivary cortisol was reduced in two studies (88,89) and plasma or serum cortisol in three (97,104,110). A further two studies found magnesium aspartate reduced serum cortisol concentrations; however, these were non-significant trends (107,108). Porta, et al. (97) found mixed results depending on the length of time and application method. They observed that serum cortisol was decreased in pigs receiving 5mg/Kg of magnesium aspartate hydrochloride in feed for 115 days; however, if magnesium was administered at a higher level (40mg/Kg) in water for 5 days before slaughter serum cortisol was increased in comparison to the control. Peeters, et al., (98) also added magnesium to water and found pigs receiving magnesium acetate at 3g/L for 2 days before a transport stressor resulted in salivary cortisol level not returning to baseline as quickly as in control pigs, suggesting that magnesium did not positively influence stress.

O'Driscoll, et al., (89) showed that during the regrouping stressor, supplemented females had lower cortisol levels than control females; however, during a 21 hour feed withdrawal, there was no significant difference in salivary cortisol between dietary treatments. In a second study (88) magnesium also significantly lowered salivary cortisol levels.

Other physiological measures were also used to measure stress. D'Souza, et al., (106) showed that overall boars fed supplementary magnesium aspartate had significantly lower plasma norepinephrine than pigs that received the control diet. Ehrenbergt and colleagues (96) found supplementary magnesium reduced hyperventilation and tachycardia over a 24h period after stress.

2.3.3.2 Behaviour

Magnesium was found to have a beneficial influence on aggressive or harmful behaviours in three studies including reduced duration (but not frequency) of aggressive behaviours (88), and pigs being slower to perform the first retreat attempt in the abattoir stunning unit (102). Two found no effect of magnesium in the diet on behaviour (89,103). Caine et al., (100) found supplementing feed with 40mg/Kg of magnesium aspartate hydrochloride for 7 days resulted in an increase in aggressive behaviours, although a long-term low-level of magnesium in the diet (magnesium aspartate hydrochloride 5mg/Kg in feed for 43 days before slaughter) had no effect. In another study, when pigs were placed in a vibration crate designed to simulate transport the magnesium-supplemented pigs were visibly calmer and spent more time lying down (98).

2.3.3.3 Skin Lesion Scores

All but one of the studies measuring lesion scores found reduced lesions in supplemented pigs in comparison to the control (88,89,99). Panella-Riera, et al. (103) on the other hand found the opposite effect. Panella-Riera, et al. (103) found pigs had more severe skin lesions (typically due to biting during an aggressive encounter) when they received a diet containing elemental magnesium (1.2g/Kg) in combination with L-tryptophan (8g/Kg). Peeters, et al. (99) found skin lesions in the loin area were reduced.

2.3.3.4 Halothane Genotype

Although now bred out of commercial pig herds, many studies in this review focus on problematic halothane-genotype pigs. Two studies found halothane-genotype pigs responded positively to increased dietary magnesium, evidenced by pigs taking longer to show the first retreat attempt in the abattoir stunning unit (103) or reduced hyperventilation and tachycardia following transport stress (96). One study showed no difference between genotypes (102); however, others found that halothane-genotyped pigs had higher plasma norepinephrine (104) and aggressive behaviours were more frequent in pigs carrying the halothane gene in comparison to control or non-halothane-genotype individuals (100). The final two studies involved only pigs that carried the halothane genes and so no comparison could be made between these and individuals with a different genotype (98,99).

Table 1. Summary of extracted information for the final review corpus.

	Weaner	Grower	Finisher	Halothane-Genotype	Physiological Measures	Behavioural Measures	Supplement Dose <1g	Supplement Dose 1-5g	Supplement Dose 5-10g	Supplement Dose >10g	Reduction in stress measure?
Apple, et al., (2005)			x	x	x					x	
Caine, et al., (2000)			x	x		x	x				
D'Souza, et al., (1999)			x		x					x	
D'Souza, et al., (1998)			x		x		x				x
Ehrenbergt, et al., (1991)	Not reported				x		x				x
O'Driscoll, et al., (2013a)		x			x	x				x	x
O'Driscoll, et		x			x	x	x				x

	Weaner	Grower	Finisher	Halothane-Genotype	Physiological Measures	Behavioural Measures	Supplement Dose <1g	Supplement Dose 1-5g	Supplement Dose 5-10g	Supplement Dose >10g	Reduction in stress measure?
al., (2013b)											
Otten, et al., (1995)			x	x	x		x				x
Panella-Riera, et al., (2008)			x	x		x		x			x
Panella-Riera, et al., (2009)			x	x		x		x			
Peeters, et al., (2005)			x	x	x	x		x			x
Peeters, et al., (2006)			x	x	x	x		x			x
Porta, et al., (1995)			x		x			x			x

	Weaner	Grower	Finisher	Halothane-Genotype	Physiological Measures	Behavioural Measures	Supplement Dose <1g	Supplement Dose 1-5g	Supplement Dose 5-10g	Supplement Dose >10g	Reduction in stress measure?
Tang, et al., (2009)			x		x			x			
Tang et al., (2008)			x		x			x			
Tarsitano, et al., (2013)			x		x			x	x		x
Total %	0	12.50	81.25	43.75	81.25	43.75	31.25	50.00	6.25	18.75	62.50

2.4 Discussion

The aim of this systematic review was to examine the current scientific literature exploring the use of magnesium to reduce stress in pigs. Sixteen studies, published between the years 1991 and 2013, met the inclusion criteria. Ten of these reported at least one positive significant effect of supplementary magnesium on physiological measures of stress and/or measures of harmful or aggressive behaviour (Table 1). Not all studies found supplementary magnesium to be beneficial; including Caine, et al., (100) who found that short-term, high doses of magnesium (40mg/kg for 7 days) increased the frequency of aggressive, and Panella-Riera, et al., (103) who reported that the carcases of pigs fed for 5 days before slaughter on a diet supplemented with 1.2g of elemental magnesium and 8g of L-tryptophan had an increased number of skin lesions, suggesting they were more active or fought more during the transport or slaughter period. In both studies, supplementary magnesium was only given for a very short period of time, five and seven days before slaughter respectively.

A common theme throughout this literature was porcine stress syndrome, a genetic condition characterised by hypothermia induced by stress (105) which can often result in sudden death and poor meat quality. In the UK, the halothane gene has now been removed from commercial pig production through genetic selection, rendering the results from these studies less relevant to current UK commercial pig production, although they may remain relevant to pig production in other countries. Overall, the results of the seven studies focusing on porcine stress syndrome susceptible pigs, suggests that magnesium supplementation in some cases may have a positive impact on animals that are genetically susceptible to stress (Table A 1). Alternatively, if focusing on the nine studies that did not include halothane-genotype pigs, five studies found at least one measure of stress was improved when the pigs received magnesium. One of these five studies however, also showed that magnesium increased serum cortisol levels when given at a low-level for a longer period of time (97). A further three found no significant effect. This suggests that more research to determine appropriate dose regimens is required.

There is also large amount of literature examining how magnesium may improve meat quality, although not all studies include measures of stress (111–113). Thirteen of the sixteen studies retrieved in this review were concerned with the effects of magnesium on meat and thus discussed measurements of stress from the perspective of improving pork quality. These studies also tended to focus only on the end stage of the commercial pig's life; for example, both Apple, et al. (101) and Porta, et al. (97) focused on transport and slaughter stress. Although the later stages of the pigs' lifetime may seem like the most obviously stressful period, stress is likely to occur at various points throughout the whole life and may have a cumulative impact on welfare and performance. Therefore, it would be worthwhile to explore further, the effect of including magnesium during earlier life stages.

Throughout the literature, cortisol was the most common measurement taken to indirectly assess levels of stress. Cortisol was measured either in the plasma, serum or saliva with concurrent recording of behavioural measures including the frequency and duration of aggressive behaviour (Table 1). Cortisol is an easy to obtain measure of arousal or stress and so it is unsurprising that so many of the studies used cortisol measures. However, cortisol is highly variable even within an individual, and can be elevated due to both positive and negative arousal; as such, cortisol measures may be more interpretable when contextualised with behavioural responses that can help to infer the valence of the response (59).

Although measures of cortisol and behaviour were common across the studies, in terms of the nutritional treatment there was a lack of consistency between methodologies with often no clear reasoning for the doses, durations or types of magnesium used. As shown by the number of studies extracted in this review, this is a relatively new nutritional method that is yet to achieve scientific consensus on when and how it may be most beneficial, or even harmful. Cost will be key in terms of farmers' willingness to implement a new strategy. Investing in additional magnesium will need to be cost effective and worthwhile for the producer, either because the magnesium is a cheap strategy to implement, or stress is reduced in a large enough proportion of the livestock (with clear benefits, such as improved performance) to make the treatment a worthwhile investment. Based on the studies in this review, there appears to be no clear conclusion regarding the best method to administer supplementary magnesium in order to reduce stress and further research should strive to validate appropriate dosage, duration and application of magnesium.

Despite inconsistency between methodologies rendering valid comparisons between studies difficult, it is clear from the results that supplementary magnesium can have beneficial effects on reducing measures of stress, aggression, and improve meat quality in pigs of varying genotypes. A large amount of research was focused on the end of the commercial pig's life and although this is a key time in terms of pork quality, it would also be beneficial to investigate further how introducing magnesium into the diet earlier on in life may improve welfare, performance and other key measures. Overall, there is a limited amount of scientific evidence to support the use of magnesium to reduce aggression and stress on commercial pig farms; however, the weight of the evidence for magnesium supplementation in pigs is positive and more thorough investigation of the impact of magnesium on stress in pigs is merited.

Chapter 3 Farmer's Views And Experiences With Supplementary Dietary Magnesium In Pig Production

3.1 Introduction

Agricultural science strives to optimise farming in a world that currently demands more food at a lower price and higher standard than ever before (114,115). New and alternative farming methods are frequently researched and scientifically tested to improve our agricultural systems in terms of their efficiency and sustainability, whilst also aiming to improve conditions for workers and the welfare of livestock (116–119). In recent years new farming technologies have allowed farms to maximise outputs by, for example, collecting livestock health and performance data automatically and employing innovative arable farming techniques (120,121).

Pig nutrition is one aspect of farming where innovation is crucial. The basic requirement of livestock feed is to meet the nutritional requirements of the animal, while remaining cost effective (43). As nutrition influences pig performance, it is a key factor in pig production (122). Beyond meeting the animals' nutritional requirements, new methods and feed formulations are continually being developed that not only improve performance and economic outputs (123,124), but can also increase the sustainability of our livestock production systems (3,42). One example of this is the use of alternative protein sources which are more environmentally friendly, such as insect proteins in livestock feed (118,125). Likewise, precision feeding techniques are becoming increasingly common and are able to tailor the feed to the nutrient requirements of the animal at each particular age or stage, while reducing nutrient loss in faeces and environmental pollution (42,126).

Diet formulations may include supplementary nutrients or components that aim to maximise key areas, such as nutrient efficiency (127) or health (128). For example, phytase is an enzyme often included in pig feed in order to release and utilise the natural bound phosphorous in the cereal elements of the diet, reducing the need for supplementation with phosphorous itself, which can be expensive and damaging for the environment (44,129). Similarly, including copper in pig feed can improve growth and health status due to its bactericidal and bacteriostatic properties (130,131). Likewise, whilst including supplementary magnesium in the diet is not necessary for the animal's maintenance requirements (49), it may improve the pig's ability to cope with stress (48). The body's response to acute and chronic stressors requires energy as the animal's physiological systems become activated and behaviour may change as a coping mechanism (17,37). This response can negatively impact other processes, especially in cases of chronic stress, such as growth and the immune system, and therefore by reducing stress, magnesium may have a positive impact on pig performance, health and welfare (81,89) (see also Chapter 2).

Previous research indicates that including supplementary magnesium in commercial pig diets can improve meat quality and some stress and welfare measures, such as skin lesion scores (48) (see also Chapter 2). It is important to ensure that research findings are applicable in real-life commercial farm settings. Pig production systems worldwide vary greatly in multiple aspects, for example the type of system and genotype of the animals (41,132), and as such not all new feed formulations will have the same effect on each farm. The implementation and impact of scientific research to support evidence-based decision making on farm relies heavily on communication between researchers and industry stakeholders, including key parties, such as vets and nutritionists. This is especially true when scientifically recommended components (such as supplementary magnesium) can come at an extra cost for the farmer or producer in comparison with standard feed. Investigation of the inclusion of supplementary magnesium in pig diets requires insight into the current understanding and application of this method within the commercial industry setting.

3.1.1 Study Aims and Objectives

The aim of this survey was to explore the current thinking and practices among pig farmers on the use of supplementary magnesium in pig nutrition. A secondary aim was to inform and aid current and future research into the use and acceptance of magnesium in pig nutrition which is commercially applicable. The objectives of this survey study were to:

(i) Explore current practices of pig farmers regarding the use of magnesium in pig nutrition.

(ii) Explore current opinions of pig farmers on the potential use of magnesium in pig nutrition.

3.2 Method

3.2.1 Ethical Approval

This study was approved by the University of Leeds School of Psychology Research Ethics Committee on the 19th November 2020 (REF: PSYC-143). Minor amendments to the survey questions were approved on 25th May 2021 and 13th June 2021.

3.2.2 Survey

The survey was designed and made available on the platform “Qualtrics” (133) and was structured with both open and closed questions to elicit the respondents’ opinions and experiences of using supplementary dietary magnesium, or supplements containing magnesium. The survey consisted of three sections, comprising questions regarding (i) the participant’s farm and herd, (ii) the participant’s knowledge about the use of magnesium in pig nutrition, and (iii) the participant’s experience of and willingness to use supplementary magnesium (see Appendix B for the full survey). The main target population was farm managers or staff with an understanding of the feeding regime on the farm. Farmers from any type of farming system were eligible to participate including commercial farms, independent farms and smallholders. Originally, the survey was aimed at the UK only however, on the 13th June 2021 due to a low response rate, it was opened to participants worldwide. Participation was completely voluntary, and no incentives were offered.

Before beginning the survey, consent was required from all participants. Information about the survey, data protection, anonymisation, sharing of anonymous research data and consent was provided to the participants in written form at the beginning of the survey (Appendix B). All responses were anonymous and so once the survey responses had been submitted, it was not possible to identify or withdraw a respondent from the dataset. No personal or identifying information was collected in the survey.

3.2.3 Recruitment

Survey participants were recruited via a combination of email, social media and a paid advertisement in Pig World Magazine's e-mail newsletter advert (see Appendix B for the full advert). Relevant companies and individuals (such as Pig Discussion Groups, Agriculture and Horticulture Development Board, and the National Federation of Young Farmers Clubs) were contacted via e-mail or social media message inviting them to participate in the survey and to distribute the survey to pig farmers. In some cases, this resulted in distribution by other means, such as posts on the National Pig Association forum, the Animal Welfare Research Network, and Garth Pig Practice newsletter. The survey was also sent out by Morrison's supermarket to their network of farmers via e-mail. Responses were collected from 26th January 2021 until 31st August 2021.

3.2.4 Inclusion and Exclusion Criteria

It was made clear in the opening survey statement that participants should only take part if they were (i) employed or engaged with farming pigs, and (ii) over the age of 18; it was made clear that they should not participate if they were (i) not working with pigs in a farming capacity, or (ii) under the age of 18. The relevant section of the opening survey statement was as follows: "*By completing this survey, you are agreeing that you are a pig farmer or keeper based in the UK, over the age of 18 and to the anonymous information you provide being used in my PhD project and stored in accordance with the University of Leeds Privacy Policies"* to which a link was provided.

3.2.5 Data Analysis

All data analysis was performed in Microsoft Excel. Firstly, all test responses were removed from the data set. One respondent was removed due to repeated incoherent responses. The total number of respondents after exclusions was twenty-five. Due to the small number of responses, a descriptive analytical approach was taken and no formal statistical tests were performed. After exploring the responses, it became clear that two of the respondents who stated they had previously used supplementary magnesium were still using it and therefore all respondents who had experience using supplementary magnesium were grouped for analysis to enable a comparison of those respondents with and without experience of using magnesium (11 responses in total).

3.3 Results

A full breakdown of the number and percentage of respondents to each question and answer is available in Appendix B.

3.3.1 Respondent Background

Of the 25 respondents, 24 were located in the United Kingdom and one in Ireland. The majority of respondents said their pig herd was *"Closed"* (n=17, 68%) and described their farm as "*Farrow to finish*" (n=18, 72%). Most participants reported the total size of their pig herd was approximately "*1000-5000 pigs*" (n=11; 44%) with a smaller number reporting a larger herd size of "*5000-10,000 pigs*" (n=7; 28%). The most commonly reported dam line genotype was Large White cross Landrace (n=18, 72%) and the most common sire line was Duroc (n=14, 56%). Although respondents reported using a range of pig systems, 11 (44%) reported using an indoor slatted pig system and 6 (25%) had indoor straw systems. A further two reported their pigs were outdoor bred and reared on straw indoors and only one person selected outdoor bred and reared indoors on slats.

3.3.2 Respondent Views on Additional Magnesium Supplementation

All participants, regardless of their experience of supplementary magnesium, were asked their views on whether magnesium supplementation in pigs may be effective at (i) reducing stress, (ii) reducing aggressive or harmful behaviours, (iii) improving performance, and (iv) improving meat quality (Table B 1).

Of the 25 respondents, 15 (60%) endorsed the statement "*I am unsure whether additional dietary magnesium reduces stress in pigs*", and seven selected "*Yes, additional dietary magnesium reduces stress in pigs in some circumstances*". When asked about aggressive or harmful behaviours, 15 respondents (60%) selected "*I am unsure whether additional dietary magnesium alters aggressive and/or harmful behaviours in pigs*". Similarly, in terms of the effect of additional magnesium on pig performance, the majority (n=17; 68%) selected that they were unsure whether magnesium would affect this outcome. The remaining eight respondents selected that "*yes they believe additional dietary magnesium may improve pig performance in some circumstances*". Finally, when asked about magnesium supplementation and meat quality, 16 respondents (64%) endorsed the response "*I am unsure whether additional dietary magnesium improves meat quality*" (Figure 3).

3.3.3 Experience with Additional Magnesium Supplementation

Of the 25 respondents, 14 had never used supplementary magnesium (56%), 9 (36%) had previously used supplementary magnesium and two were currently using supplementary magnesium (8%). When asked how they became aware of using additional magnesium three respondents answered "*word of mouth*", two answered "*advice from nutritionist*", two answered "*own research*" and four respondents answered that they heard through other means (not stated). Of those that had used supplementary magnesium, the majority reported that the main reason they started using it was because of “*aggressive or harmful behaviours*” (n=6; 54.55%); two answered that it was because of stress within the herd or group, and one answered that it was for health reasons. Of the two participants that reported “*other*”, one stated they decided to use additional magnesium following “*vet advice to help with a health issue*”, and the other was to “*reduce constipation in pre-farrowing sows*”.

3.3.4 Method of Magnesium Supplementation

Seven participants (63.64%) added the supplementary magnesium to feed, two added it to water and the final two participants selected *“other”,* stating that they used magnesium feed blocks. Five reported that the additional magnesium was given or would be given for *“2-4 weeks”*, three reported *“<1 week”* and a further three reported *“3-6 months”.* In terms of the stage of production magnesium supplements were or would be used at, one participant reported giving magnesium to *“Weaners (4-7 weeks of age)”*, four (36.36%) to *“Growers (8-12 weeks of age)*”, three to *“Finishers (13 weeks – slaughter weight)*”, two to *“Breeding sows*” and one to *“other*” (reporting using additional magnesium in “growers and finishers”).

The reported types of magnesium used in the supplementary magnesium varied considerably. Two respondents reported using magnesium oxide, two magnesium sulphate, one reported using magnesium phosphate, two selected *“other*”, stating that “*Calcium Magnesite*” and “*Emgevet in water*” (containing Magnesium Aspartate Hydrochloride) were used, and four

(36.36%) respondents selected *"don't know*". The level of supplementary magnesium used varied across respondents with one selection each for "*0.01-0.1%*" and "*0.3 -0.4%*", and a further three respondents (27.27%) selected "*0.1 - 0.2%*". The final four (54.55%) participants selected "*other*", 3 of which said they don't know, one said, "*By advise from the vet*" (sic) and one stated "*different for age groups*" (sic).

3.3.5 Reported Effects of Supplementary Magnesium

Most respondents (54.55%, n=6/11) reported observing a small decrease in stress within the herd or group (Figure 2. Number of responses for each multiple choice answer for the questions: "Since giving additional magnesium to your pigs, have you noticed any change in stress in the herd or group of pigs?" and "Since giving additional magnesium to your pigs, have you noticed any change in harmful or aggressive behaviours?"). The majority (n=7/11; 63.64%) reported observing a small decrease in aggressive or harmful behaviour which they attributed to the supplementary magnesium. However, some also reported a large increase in stress (n=2/11; 18.18%) or aggressive/harmful behaviours (n=1/11; 9.09%). Others reported no change in stress (n=2/11; 18.18%) or behaviour (n=2/11; 18.18%). In terms of pig performance measures, most participants (n=9/11; 81.82%) observed no change. Eight participants (n=8/11, 72.72%) reported that they do not receive feedback on meat quality and three reported no observed impact of supplementary magnesium on meat quality (Table B 1).

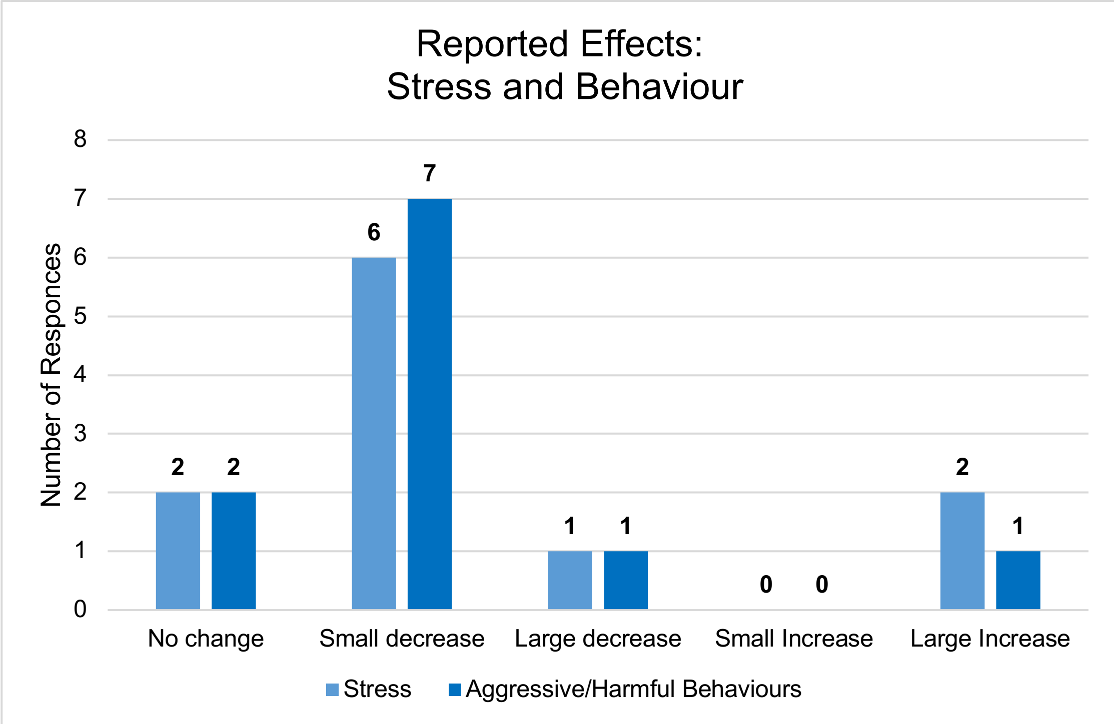

Figure 2. Number of responses for each multiple choice answer for the questions: "Since giving additional magnesium to your pigs, have you noticed any change in stress in the herd or group of pigs?" and "Since giving additional magnesium to your pigs, have you noticed any change in harmful or aggressive behaviours?"

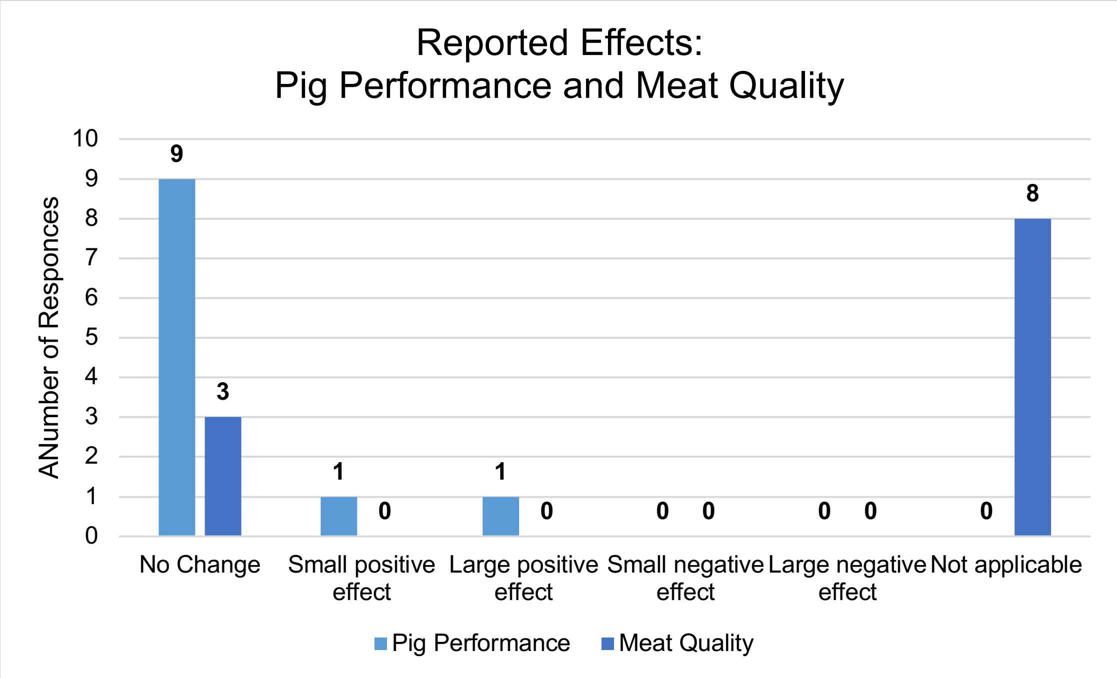

Figure 3. Number of responses for each multiple choice answer for the questions: "Since giving additional magnesium to your pigs, have you noticed any change in performance?" and "Since giving additional magnesium to your pigs, have you noticed any change in meat quality?"

All respondents who reported a decrease in stress within the group or herd also reported observing a decrease in aggressive or harmful behaviours (n=7, two magnesium oxide, one Emgevet in water, others unknown). Of the two respondents who reported observing a positive effect of magnesium on pig performance, one found supplementary magnesium also reduced stress and aggressive and/or harmful behaviours (unknown type of magnesium, at 0.1 – 0.2 %) whereas the other did not see any change in stress and aggressive and/or harmful behaviours (this participant reported using calcium magnesite at an unknown level).

Only two types of magnesium, magnesium sulphate and oxide, were reported as being used by more than one respondent. Of the two respondents that supplemented with magnesium sulphate, one supplemented (0.01 – 0.1 %) during the grower and finisher stages and reported an increase in stress and aggressive behaviours, and no change in performance. In contrast, the other respondent applied supplementary magnesium (approximate level of magnesium not known) during the grower phase and reported no change in stress, behaviour, or performance. Two respondents reported supplementing with magnesium oxide, one at an approximate level of 0.1 – 0.2 % for 2-4 weeks during the weaner stage and the other for less than one week in breeding sows (approximate level not known). Both found that the magnesium oxide resulted in a small decrease in stress and aggressive/harmful behaviours but no change in performance. Although the type of magnesium is unknown, a further two participants used a supplementary feed block containing magnesium (the brand or formulation was not specified) during the finisher phase and again reported that it resulted in a small decrease in stress and aggressive/harmful behaviours but not performance.

Of the 11 survey participants who reported currently using, or having previously used supplementary magnesium, eight (72.73%) reported they would recommend it to other producers and three reported they would not.

Table 2. Number of responses and percentage of responses answering each of the options relating to questions about stress, behaviour, meat quality, and performance outcomes during or after using supplementary magnesium in the pig herd (n=11).

Question	Answer	Number of responses	%
Q20 & 34: Since / While giving additional magnesium to your pigs, did you noticed any change in stress within the herd or group?(Stress can be defined as the animal making physiological or behavioural changes to cope with its environment, e.g. being more alert)	No, I saw no change in stress within the herd or group	2	18.18
	Yes, I saw a small decrease in stress within the herd or group	6	54.55
	Yes, I have seen a large decrease in stress within the herd/group	1	9.09
	Yes, I saw a large increase in stress within the herd or group	2	18.18
Q21 & 35: Since / While giving additional magnesium to your pigs, have you noticed any change in aggressive or harmful behaviours?	No, I saw no change in aggressive and/or harmful behaviours	2	18.18
	Yes, I have seen a large decrease in aggressive and/or harmful behaviours	1	9.09
	Yes, I have seen a small decrease in aggressive and/or harmful behaviours	7	63.64
	Yes, I saw a large increase in aggressive and/or harmful behaviours	1	9.09
Q22 & 36: Since / While giving additional magnesium to your pigs, have you seen any change in performance?	No, I saw no change in pig performance measures	9	81.82
	Yes, I have seen a small positive effect on pig	1	9.09

Question	Answer	Number of responses	%
	performance measures		
	Yes, I saw a large positive effect on pig performance measures	1	9.09
Q23: Since / While giving additional magnesium to your pigs, have you seen any change in meat quality?	Not applicable - I do not receive feedback on meat quality.	8	72.73
	No, I saw no change in meat quality	3	27.27

3.3.6 Additional Comments from Respondents

Seven (28%) of the 25 survey participants left an additional comment at the end of the survey. These comments included requests or interest in knowing more about magnesium, that genetics can play a large role, and that as with all products they work on some farms but not others. One respondent suggested that the ratio of the ingredients is important not just the addition of one mineral. These comments can be found in full in Appendix B.

3.4 Discussion

This survey aimed to gain a better insight into current opinion and degree of consensus among farmers on the use of supplementary magnesium in pig production. In total 25 farmers participated in the survey, 11 of whom stated that they are currently, or have previously, supplemented with additional magnesium. Many reported a positive effect of this supplementation on stress and/or behaviour but not pig performance measures or meat quality (Figure 3). There was a wide range of farming backgrounds including genotype and type of farming system. Despite the small sample size, this variation in farming background allowed for a broad view of applications of magnesium in pig farming.

When asked whether magnesium may influence stress, behaviour, performance, or meat quality in pigs most respondents were unsure (Table B

1). This uncertainty may be due to a lack of assessment or observation of these qualities on farm, or it may be that some areas, such as ‘stress’, are less easily observed on farm. Typically, in livestock science research, specific tools and assessments are employed to assess pig welfare and performance, such as behavioural observations, physiological measures (e.g. cortisol) and feed intake in combination with pig weights. However, these tools often require time or equipment that many farmers do not have or cannot afford. Based on the reported outcomes of magnesium supplementation in this survey, it could be suggested that the majority of the respondents agreed that additional supplementary magnesium can be effective at reducing stress and aggressive/harmful behaviours (Figure 2). However, as this conclusion is based on firsthand reports it should be remembered that placebo or confirmation bias may have influenced adopting farmers’ views. Despite this, the consensus of this survey is in agreement with much of the previous scientific literature which has shown that skin lesion scores (88,89,99), harmful behaviours (88,89,103), cortisol (88,104,110), and catecholamine hormones (95) can be reduced with magnesium supplementation.

Most participants who had experience supplementing with magnesium said they would recommend it to others; again reflecting the view that the overall outcome of supplementing with magnesium appears to be positive. This is key, as personal recommendation within the farming community often plays a crucial role in wider adoption of novel techniques or approaches in agriculture. Despite the overall positive consensus, one respondent reported a large increase in stress alongside a large decrease in aggressive and/or harmful behaviours (unknown magnesium compound supplemented at 0.1 - 0.2%), and one a large increase in stress and a large increase in aggressive or harmful behaviours (magnesium sulphate supplemented at different levels for different age groups). This negative impact of magnesium supplementation has previously been reported in some studies that showed a negative impact on behaviour (100), cortisol levels (97) and skin lesion scores (103). Despite the mostly positive effect on stress and behaviour, this did not appear to translate into reported pig performance (Figure 3). It may be predicted that a reduction in stress response and/or stress related behaviours would counter any negative effects of the stress on performance, such as average daily gain and feed conversion ratios (17). However, performance can be impacted by multiple factors, and typically, in research studies, feed consumption and pig weights are monitored closely using regular measurements made by hand or using specialist equipment. It is possible, and likely, that these careful regular measures are not made on some farms and therefore, more subtle, less obvious, changes in pig performance are unlikely to be observed. However, this hypothesis cannot be confirmed via this survey.

When exploring the reasoning for employing magnesium supplementation, it is interesting to note that no respondents reported that they began using supplementary magnesium to improve meat quality. This suggests that despite a large amount of the scientific literature focusing on the use of magnesium to improve pork quality, this is not typically a reason for the farmer to investigate and consider its use. Magnesium supplementation has been shown to improve meat quality by reducing stress (e.g. during transport, lairage, or slaughter itself) and counteracting the effects of stress hormones, including catecholamines (110,134,135). Reducing the occurrence of pale, soft and exudative (PSE) meat by supplementing with magnesium before slaughter is beneficial in terms of consumer preference. Despite this, farmers don’t typically see the end product on the shelves, especially those operating large commercial units, as was reflected by the report from eight participants that they don’t receive feedback on meat quality.

There is a lack of research and consensus on the method by which magnesium should be supplemented in pig production and in previous studies the type and level of magnesium has varied greatly (100–102,110). In this survey, respondents reported using a wide range of methods to apply supplementary magnesium and many did not know the type or level of magnesium used. Where the type of magnesium used was known, inorganic forms, such as magnesium oxide and magnesium sulphate, were most frequently reported. Literature in both humans and rodents has demonstrated that the efficacy of magnesium varies depending on the type magnesium, duration and method of application, with inorganic compounds less easily absorbed than organic magnesium compounds (136–138).

3.4.1 Limitations

The main limitation of this survey is the low response rate, which means that the survey is unlikely to be representative of the population of pig farmers in the UK. Thus, the conclusions that can be drawn are limited and interpreted with caution. E-mail and social media platforms including Twitter, Facebook and Linkedin were used as the main way to target pig farmers. Recruitment for surveys through social media provides many advantages including the potential to recruit respondents worldwide and rapid, low-maintenance dissemination of the survey (139–141). However, this method does limit the pool of respondents to people that use these social media platforms. Recruiting participants via other means as well as social media and email, such as postal surveys (142), was not within the scope of this study and a sampling frame of all pig farmers in the UK to whom postal surveys could be sent was also not available. Similarly, there was no incentive offered to complete the survey, but had an incentive been offered, a greater number of responses might have been obtained.

The survey was reliant on the respondent's subjective experience. The questions about stress and behaviour all rely on the respondent's individual interpretation of the question and their observations on farm which might be influenced by their assumptions and beliefs about pig farming practices and magnesium or feed supplements in general. Typically, in scientific research, stress would be measured in a number of different ways including physiological measures and behavioural observations (57,58,60,143,144). Similarly, pig performance is measured differently on different farms, with some farms monitoring it regularly while others less so. This subjectivity is clear from a few responses which are inconsistent with answers to other questions by the same participant. For example, one respondent stated they saw a large increase in stress within the herd as well as a reduction in aggressive/harmful behaviours in response to magnesium supplementation.

Furthermore, their reason for not continuing with the supplementation was that the aggressive behaviour had stopped, which appears contradictory to the earlier stated increase in stress. Therein lies the problem of inferring from responses in a survey, without further input from the respondent. Such inconsistent answers have to be taken at face value or discounted

3.4.2 Conclusion

In conclusion, although the overall perception of magnesium is largely positive, the variation in application and response to magnesium supplementation highlights the need for further research to explore the most appropriate level and type of magnesium to supplement with and to examine objectively the effects on health, behaviour and performance. Despite this small limited survey, the results reflect the view of the majority of pig farmers surveyed that supplementary magnesium has the potential to reduce stress and undesirable behaviours. This outcome is consistent with the current scientific literature.

Chapter 4. Supplementary Magnesium To Reduce Behavioural Measures Of Stress during Regrouping In Growing Pigs

4.1. Introduction

Regrouping or mixing, where stockpersons create new social groups of pigs with unfamiliar conspecifics, is a common pig management practice, that often results in aggression - a major welfare issue in pig production (10,39,145–147). Regrouping can occur multiple times throughout a pig's lifecycle for many reasons, including moving animals to a new location, or grouping pigs of similar weight in order to reduce within-pen weight variation (148,149). Regrouping occurs multiple times throughout a commercial pig's lifespan and can result in an increase in aggression for two main reasons. Firstly, the established social hierarchy is disrupted, and secondly, unfamiliar conspecifics are introduced creating competition (39,145,150,151). Alongside these two main social stressors, there are many other factors at play that may influence the occurrence of harmful behaviours and aggressive events, for example environmental enrichment (152) and individual temperament (66) which might mediate the experience of and response to stress. Although it is not uncommon for unfamiliar individuals to be aggressive when re-establishing a dominance hierarchy, conditions within a pig production system can exacerbate the animals' behavioural responses. Intensive pig production systems involve repeated regrouping, limited space to escape conflict (153), and pens of pigs homogenous in terms of weight (154) and therefore competitive ability, increasing the likelihood of intense fighting.

4.1.1 Aggression and Harmful Behaviours

Aggressive and harmful behaviours between pigs poses a significant and persistent animal welfare and economic issue. These behaviours, such as fighting and tail biting, increase the risk of injury, disease, and mortality (38,62,155–157). Simultaneously, the energy to engage in negative behaviours, and to cope with any subsequent health challenges, such as injury, can result in reduced growth and pig performance (158). Consequently, this can increase production costs due to the decrease in health status and productivity of the group (8,10,159,160). Although both harmful and aggressive behaviours can result in the same health, performance, and economic consequences, they are the result of different motivations. Aggressive behaviour, such as fighting, is typically the result of a specific stressful event such as regrouping. On the other hand, harmful behaviours such as tail and ear biting, are non-aggressive abnormal behaviours that are the result of multiple factors, including nutrition, temperature, and environmental enrichment (161,162). The multifactorial nature of harmful behaviours makes them less predictable than aggressive behaviours however, they can also be exacerbated by stress or stressful events (163). Consequently, reducing stress is important for the management and reduction of aggression and harmful behaviours, which in turn is critical for both animal welfare and farm productivity.

Reducing the frequency of exposure to stressful events that can result in aggression or exacerbate harmful behaviours— such as regrouping — is the obvious way to reduce these behaviours however, this is often not possible. Strategies to mitigate the negative behavioural changes associated with stressful events have been widely researched (39,147,164,165) with examples of mitigations including: increasing the use of environmental enrichment (166), altering the size (167) or weight distribution (154) of the group, and increasing the amount of fibre in the diet (168). There are also promising findings that including supplementary magnesium in the diets of commercial pigs may reduce aggression and harmful social behaviours (Chapter Two; (48)). For example, Peeters, et al. reported that pigs who received supplementary magnesium were visibly calmer after transport simulation (98), and O'Driscoll, et al. reported that supplements including magnesium reduced the duration of aggressive behaviours in growing pigs (88). Furthermore, several studies have reported that including supplementary magnesium can reduce skin lesion scores (88,89,99).

4.1.1. Magnesium and Phytase

Supplementary magnesium has been shown to reduce cortisol during acute stress including transportation (101) and slaughter (104,110). Typically, there is adequate magnesium content in swine diets due to the large number of cereal components (49). Increasing the level of magnesium in pig diets may be a nutritional method to reduce stress, without requiring major changes to production or housing. Research to date has been limited and generally focuses on the end of a pigs' life (Chapter Two; (48)). There is therefore a need to understand the impact of supplementary magnesium on the physiological measures of stress in pigs during stressful events, such as regrouping.

Furthermore, combining supplementary magnesium with additional nutritional components may enhance any positive effects. Phytase is a phytate degrading enzyme commonly added to pig feed in order to release natural bound phosphorous in cereal elements of the diet (169,170). Phytase reduces the need for additional phosphorus to be added to the diet, and has been shown to improve the availability and digestibility of trace minerals, including magnesium (127,171). Therefore, including phytase in the diet with supplementary magnesium may result in a synergistic effect by further increasing the total magnesium content of the diet. The possible synergistic effects of magnesium and phytase supplementation has not been researched previously.

4.1.1 Aim and Hypotheses

The aim of this study was to assess how supplementary magnesium phosphate, with or without additional phytase, may influence skin lesion scores and the duration of aggressive and harmful social behaviours during regrouping in grower pigs.

H1: A diet supplemented with magnesium will reduce the duration of aggressive behaviour and result in lower skin lesion scores after regrouping compared with the same diet without magnesium supplementation.

H2: A diet supplemented with magnesium will reduce the duration of harmful behaviours and result in lower skin lesion scores after regrouping compared with the same diet without magnesium supplementation.

H3: A diet supplemented with magnesium and phytase will further reduce the duration of aggressive behaviours and result in lower skin lesion scores than a diet supplemented with magnesium alone.

H4: A diet supplemented with magnesium and phytase will further reduce the duration harmful behaviours and result in lower skin lesion scores than a diet supplemented with magnesium alone.

4.2. Method

4.2.1 Ethical Approval

This study was carried out between October and December 2018 under the project licence number PPL 70/7895 (expiry date 18/12/18).

4.2.2 Sample Size

A sample size estimate of 24 focal pigs per dietary treatment was calculated based on an effect size of 14 (the difference in least squared means of bouts of aggression in treatment and control pigs reported in the literature (88,89)).

A standard deviation of 3.2 (based on the standard deviation in the number of fights per pig reported by Andersen et al. (167)), at power of 0.9 and a significance level of 0.05.

4.2.3 Animals and Housing

Two-hundred and forty Large White cross Landrace piglets were available for inclusion in this study. The piglets remained with the sow until four weeks old and then were housed in groups of five pigs per pen (pen size: 1.5m x 1.5m) until nine weeks of age when the pens were regrouped to pens of ten pigs (pen size: 1.5m x 2.5m). All piglets were housed in traditional farrowing crates with the sow until they were weaned. At weaning, the piglets were vaccinated (Porcilis PCV and Porcilis M Hyo ID Once) and weighed to determine the weaning weight before being allocated to the pens of five. Any pigs weighing less than 5kg, or with any obvious injury/illness, or with intact tails were not included in the study. Each initial pen of five pigs was balanced by sex, weight, and origin litter, with the pre- and post-regrouping pen in mind to ensure both were balanced. Each pen of five either contained two or three female pigs to ensure an equal sex ratio (1:1) when regrouped in the larger pen size of ten pigs. Where possible, only one pig per origin litter was included in each pen of five and ten. Furthermore the within-pen weight variation was kept to 3.5kg or less. Each pair of five pig pens received the same dietary treatment so no treatments were mixed during regrouping. The pigs remained in the same pens throughout the study. On the day of regrouping a dividing wall was removed between each pair of pens to create the larger, balanced pen of ten pigs. In total there were 24 pens of 10 pigs which were spread across three rooms. In each room there were two pens of ten pigs per diet with random distribution of diets across the rooms.

4.2.3.1 Treatment groups and focal individuals

Four treatment groups were included in this study: (i) control group, (ii) supplementary magnesium phosphate (MgP) diet, (iii) phytase diet, and (iv) combined supplementary magnesium phosphate and phytase diet. The number of focal pigs required per treatment group was calculated using sample size calculation (section 2.2). Two focal pigs in each pen of five were identified at weaning by selecting the heaviest and lightest weight pig in each pen. This resulted in 44 female and 52 male focal pigs in total (Control: 12 females, 12 males; MgP: 12 females, 12 males; Phytase: 7 females, 18 males; MgP and Phytase: 15 females, 8 males). These were marked with a pattern of marker spray on the back between the shoulders avoiding the hair sampling area (rump). The spray was reapplied every 1-2 days as required.

4.2.4 Diet

For the first 20 days post-weaning (from 4 to 7 weeks of age, i.e. the pre-treatment period), all pigs received the same standard three-diet regime (Primary Diets) in a five-spaced feeder. This feeding regime consisted of 2kg/pig of ‘Elite’ followed by 4kg/pig of ‘Ultra Wean’ and finally approximately 8kg/pig of ‘Ultra Sprint’. These diets contain 0.12%, 0.13% and 0.12% of magnesium respectively.

All pigs were fed one of the four study diets (Table 3) from 7 to 12 weeks of age (i.e. the treatment period), in two, one spaced feeders. Due to a lack of consensus in the current scientific literature (48), the level and type of magnesium compound used in this study was based on the level and type used by a large UK (United Kingdom) based pig feed manufacturer. All diets were formulated specifically for this study (Table 3) and at the time of manufacture, a sample of each diet was retained and sent for analysis at Sciantec and Primary Diets (Table 6). A feed sample was collected for each diet each week throughout the study and stored in a freezer. To ensure consistency in dietary components, a composite sample was created and analysed by Sciantec for magnesium and Primary Diets for phytase content (Table 7).

Table 3. Formulation of each study diet. Formulated and manufactured by Primary Diets.

	Diet			
Ingredient (%)	**Control**	**Mg**	**Phytase**	**Mg + Phytase**
Barley	30.00	30.00	30.00	30.00
Wheat	39.66	39.46	39.60	39.40
Soya	22.04	22.07	22.05	22.08
Full Fat Soya Bean	3.50	3.52	3.51	3.53
Premix	0.25	0.25	0.25	0.25
L-Lycine HCL	0.48	0.48	0.48	0.48
DL-Methionine	0.11	0.11	0.11	0.11
L-Tryptophan	0.18	0.18	0.18	0.18
L-Valine	0.01	0.01	0.01	0.01
Vitamin E	0.09	0.09	0.09	0.09
Phytase	0.00	0.00	0.03	0.03

Ingredient (%)	Diet			
	Control	Mg	Phytase	Mg + Phytase
Feed enzyme (xylanase)	0.01	0.01	0.01	0.01
Limestone Flour	0.67	0.75	0.67	0.75
Dicalcium Phosphate	0.49	0.36	0.49	0.36
Magnesium phosphate	0.00	0.15	0.00	0.15
Salt-PDV	0.44	0.44	0.44	0.44
Sodium Bicarbonate	0.06	0.06	0.06	0.06
Pellet binder	0.80	0.80	0.80	0.80
Soya Oil	1.21	1.26	1.23	1.28

4.2.5 Skin Lesion Scoring

Focal pigs were lesion scored using the same scoring system as outlined in Stevens, et al. (172) (Table 4). Skin lesions were scored once a week from weaning to the end of the trial by the same observer (Emily Bushby). An additional score was taken the day after regrouping resulting in two lesion scores for each pig in that week. Focal pigs were scored on the back, tail, left and right ears, shoulders, flank and hindquarters. Scores were recorded manually on a paper scoring sheet and then transferred onto a master Excel spread sheet each week.

Table 4. Lesion scoring system as outlined by Stevens, et al. (172)

Score	Scoring system
0	No injuries.
1	One small superficial lesion.

2	More than one small superficial lesion or one deeper superficial lesion.
3	One or several big and deep lesions. If deep only one single lesion, if not so deep several red lesions.
4	One very big, deep and red lesion. Or many deep red lesions.
5	Many very big, deep and red lesions covering the area.

4.2.6 Focal Pig Behaviour

Behaviour was recorded using 2MP Sony Exmor IP Cameras with one camera placed above each pen of ten pigs. Video recordings were made between the hours of 11am-12pm and 2pm-3pm, after feeding and other sampling had finished. Videos were collected on six days across the whole experimental period: on each of two consecutive days: (i) one week before regrouping; (ii) on the day of and day after regrouping; and (iii) one week after regrouping.

Before regrouping the dividing panel separating the pens of ten into pens of five meant that the main cameras were unable to capture the whole of the front pens. Therefore these pens were filmed using Canon Legria handheld camcorders on tripods. Two camcorders on tripods were placed in each room and, after an hour of filming, were moved along the corridor to film the next two front pens. This meant that each pen was not always filmed at the same time and there was only one hour of footage per front pen per day before regrouping.

The videos were analysed to determine each focal pig's duration of active, inactive, and aggressive and harmful behaviours (Table 5). These behaviours were coded for the first and last 15 minutes of each hour (11:00-11:15am; 11:45am-12:00pm; 2:00-2:15pm; 2:45-3:00pm) on each of the six recording days. BORIS software (173) and an ethogram adapted from O'Driscoll, et al., (88,89) (Table 5) were used. The videos were analysed blind to diet (i.e. the diets provided in each pen were unknown during video analyses).

Due to some of the footage running at half speed, to ensure the same amount of time was analysed for each pen, the sample of time analysed was as close to the intended time as possible. Four pens were excluded from before and after (20 pens were able to be analysed), and three pens from during (21 pens were analysed) the regrouping time points, due to missing data.

Table 5. Ethogram adapted from O'Driscoll, et al. (88,89)

Behaviour	Description
Active	Actively performing a behaviour (e.g. exploring enrichment, walking, biting, playing)
Inactive	Lying, standing or sitting while not performing any behaviour
Pig out of view	Pig's head is out of view and behaviour is not able to be categorised
Fighting	Mutual pushing parallel or perpendicular, ramming or pushing of the opponent with the head, with or without biting in rapid succession and/or head thrusting. Lifting the opponent by pushing the snout under its body.
Body Biting	Biting (mouth open) any part of another pig, but not as part of head thrust, or fight (often repeated in rapid succession).

Tail Biting	Biting (mouth open) another pig's tail, but not as part of head thrust, or fight (often repeated in rapid succession).
Ear Biting	Biting (mouth open) another pig's ears, but not as part of head thrust, or fight (often repeated in rapid succession).
Head thrust	Ramming or pushing another pig with the head (with or without biting), but not as part of a fight.
Other aggressive behaviour	Any other harmful or aggressive behaviours.

4.2.7 Statistical Analysis

Data was stored, and the skin lesion scores calculated, in Microsoft Excel. All statistical analysis was carried out using RStudio software (174). The data were analysed as a two-by-two factorial dietary treatment design. Before running the models, collinearity was examined using the *vif* function from the ***car*** package, where values over five were determined to be collinear (175).

4.2.7.1 Skin Lesion Scores

Lesion scores were separated into tail score, ear score (sum of both ears) and body score (sum of all main body areas: left and right flank, hindquarters, shoulders and back). In order to include the weight of each pig in the analysis, the weight of each pig at the closest time point to the lesion score recording was added to the data and model for that time point. The dates that each pig was lesion scored were separated into during the standard diet (4-7 weeks of age), and before (7-9 weeks of age), and after regrouping (9-12 weeks of age).

An ordinal regression model using the *clmm* function from the **ordinal** package (176) was used to analyse the skin lesion score data. Lesion score (body, ear, or tail) was included as the response variable and test time period, weight, sex, magnesium and phytase dietary treatments as the fixed predictor variables. The mixed pen location, pig ID nested with mixed pen location were included as random effects within the models. For the body and ear lesion score analyses, all two-way interactions between the fixed variables were included. Due to low variation and convergence issues with the tail lesion score model, two alternative models were compared: (i) a simplified model with only fixed and random effects; and (ii) the full model with no random effects. Based on the AIC values, the simplified model fitted best and therefore was employed. All three models were fitted using backwards stepwise deletion based on the AIC values using the *drop1()* function (177).

4.2.7.2 Behavioural observations

Out of sight or time not coded for a specific behaviour accounted for 6% of the total data. These were treated as missing data and were not included in the analyses. Each behaviour (active, inactive, fighting, tail biting, ear biting, body biting) was analysed separately as well as the grouped duration of harmful (tail biting, ear biting, body biting), referred to henceforth as grouped harmful behaviours.

Due to the variation within pig ID and pen location (random effects) being very small leading to overfitting and convergence issues, three models were compared using the AIC values using the *mod.sel* function from the package

MuMIn (178). These were models including pig ID within pen location as a random effect, including pig ID as a random effect, and no random effects included. To analyse the behaviour data, a negative binomial model was employed as this type of model is able to analyse non-normal count data, such as duration of time (179). The duration of each behaviour (active, inactive, fighting, tail biting, ear biting, body biting) using the *glm.nb* function from the **MASS** package (180). The *stepAIC* or *drop1* functions were used to fit the models using backwards stepwise deletion based on the AIC values. The rounded duration of each behaviour in seconds was included as the outcome variable with the time point, dietary treatments, and the focal pig's sex and weight (day 33 weight as it was the closest weight to the observation time points) included as fixed effects. Interaction effects included were Diet.mg*Diet.p, Sex*Diet.mg, Diet.mg*Weight, Diet.mg*Time.Point, Sex*Diet.p, Diet.p*Weight, Diet.p*Time.Point, Time.Point*Weight, Sex*Time.Point, and Weight*Sex.

4.3 Results

4.3.1 Model Comparison for Behavioural Data

There was no collinearity in the predictor variables, as all values were less than two (176). The within pig ID and pen location (random effects) variation was extremely small (p<0.001) and models including random effects indicated overfitting and convergence issues. Three models were compared using the AIC values using the *mod.sel* function from the package **MuMIn** (178). These were models including all fixed and interactive effects (as described in section

5.2.3) and either including pig within pen as a random effect, including pig as a random effect or included no random effects. The final models for the ear biting, tail biting, fighting and time spent inactive fit best with no random effects included. The final model for the grouped harmful behaviours and the body biting data, included only pig ID as a random effect. The final model for the time each pig spent active fit best with both mixed pen location and pig ID nested within mixed pen location included as random effects.

4.3.2 Diet

The diets supplemented with magnesium were shown to have a higher level of total magnesium at the point of manufacture (Table 6) and throughout the study (Table 7).

Table 6. Mineral analysis of the feed sample taken from each study diet at the point of manufacture.

	Diet			
	Control	**Mg**	**Phytase**	**Mg + Phytase**
Ash (%)	4.0	4.1	4.1	4.4
Crude Fibre (%)	2.7	2.9	3.2	3.0
Crude Protein (%)	19.3	19.9	18.9	19.3
Moisture (%)	11.4	11.3	11.4	11.6
Total Oil (%)	4.13	4.08	3.75	4.11
Magnesium (%)	0.15	0.20	0.17	0.21
Phytase activity (FTU/Kg)	640	518	2020	1420

Table 7. Mineral analysis of the composite feed sample at the end of the study (day 55).

	Diet			
	Control	Mg	Phytase	Mg + Phytase
Magnesium (%)	0.16	0.20	0.16	0.23
Phytase activity (FTU/Kg)	877	625	2090	2000

4.3.3 Skin Lesion Scores

As predicted, there were significantly higher body scores after regrouping than during the standard diet period or before regrouping (p<0.001). This was consistent for both the magnesium and phytase treatment groups (p<0.001; Table 8). Likewise, there were significantly higher ear scores after regrouping than during the standard diet or before regrouping time periods (p<0.001). This was also the case for the magnesium diet (p=0.028; Table 8). Tail lesion scores increased post-regrouping in comparison with before regrouping (p=0.01) and tail lesion scores increased with increasing weight (p=0.005). There was no significant difference in focal pig tail lesion scores between dietary treatments (p>0.001; Table 8).

Table 8. Odds ratio, 95% confidence interval, p-values, and raw means with standard errors for the body score ordinal cumulative link mixed model. P-values in italics indicate significance in comparison with the control. The final minimal model as determined by backward stepwise deletion. Due to conversion issues with the model the additive model with mg diet – phytase diet interaction is reported.

	Odds Ratio	95% Confidence Interval	P-Value	Raw Mean & SE
Body Lesion Scores				
Control	-	-	-	8.967 ± 0.374
Mg	0.598	-1.174 - 0.147	0.127	8.320 ± 0.259
Phytase	0.854	-0.728 - 0.413	0.588	9.120 ± 0.281
Mg * Phytase	-	-	-	8.394 ± 0.376

	Odds Ratio	95% Confidence Interval	P-Value	Raw Mean & SE
Standard diet (0-20 days)	-	-	-	2.687 ± 0.212
Before regrouping (21-33 days)	5.441	0.156 - 3.231	*0.030*	5.892 ± 0.235
After regrouping (34-55 days)	157.481	3.517 - 6.600	*<0.001*	12.765 ± 0.167
Sex (male)	-	-	-	9.242 ± 0.264
Sex (female)	-	-	-	8.457 ± 0.277
Weight	1.096	-0.003 - 0.187	0.059	-
Before regrouping * Phytase	1.228	-0.317 - 0.728	0.440	6.000 ± 0.327
After regrouping * Phytase	1.684	0.015 - 1.026	*0.043*	13.255 ± 0.245
Before regrouping * Mg	0.733	-0.837 - 0.216	0.248	5.579 ± 0.311
After regrouping * Mg	0.437	-1.441 - -0.213	*0.008*	11.920 ± 0.252
Mg * Weight	1.022	-0.0006 - 0.044	0.057	-
Before regrouping * Weight	0.970	-0.126 - 0.066	0.543	-
After regrouping * Weight	0.920	-0.177 - 0.011	0.086	-
Ear Lesion Scores				
Control	-	-	-	2.818 ± 0.123
Mg	1.497	-0.238 - 1.046	0.217	2.692 ± 0.121
Phytase	-	-	-	2.937 ± 0.137
Mg * Phytase	-	-	-	2.500 ± 0.119
Standard diet (0-20 days)				1.135 ± 0.120
Before regrouping (21-33 days)	2.700	0.525 - 1.461	*<0.001*	1.946 ± 0.090
After regrouping (34-55 days)	10.457	1.878 - 2.815	*<0.001*	3.770 ± 0.068
Sex (male)	1.192	-0.051 - 0.402	0.129	2.855 ± 0.089
Sex (female)	-	-	-	
Before regrouping * Mg	0.607	-1.163 - 0.166	0.141	1.811 ± 0.127
After regrouping * Mg	0.490	-1.348 - -0.075	*0.028*	3.570 ± 0.083
Tail Lesion Scores				
Control	-	-	-	0.600 ± 0.062
Mg	0.769	-0.721 - 0.196	0.262	0.517 ± 0.057
Phytase	1.057	-0.376 - 0.488	0.799	0.718 ± 0.062
Mg * Phytase	1.043	-0.597 - 0.682	0.895	0.612 ± 0.059
Standard diet (0-20 days)	-	-	-	0.166 ± 0.038
Before regrouping (21-33 days)	294.004	381.843 - 393.210	0.977	0.222 ± 0.028
After regrouping (34-55 days)	589.699	381.147 - 393.906	0.974	1.033 ± 0.047
Sex (male)	0.928	-0.407 - 0.259	0.662	0.621 ± 0.042

	Odds Ratio	95% Confidence Interval	P-Value	Raw Mean & SE
Sex (female)	-	-	-	0.604 ± 0.043
Weight	1.023	0.006 - 0.039	*0.005*	-

4.3.4 Behavioural Observations

4.3.4.1 Time spent active (non-aggressive)

Focal pigs were significantly more active after regrouping than before ($p<0.001$) and during regrouping ($p<0.001$). Similarly, focal pigs receiving supplementary magnesium phosphate were more active after regrouping than during ($p=0.010$), whereas focal pigs receiving phytase were less active during regrouping than the control diet ($p=0.006$). However, overall the magnesium diet ($p<0.001$) and magnesium and phytase combined diet ($p=0.014$) were significantly less active than control focal pigs. There was no effect of focal pig sex or weight on non-harmful/aggressive activity level.

4.3.4.2 Time spent inactive

Focal pigs receiving the supplementary magnesium diet were significantly more inactive than focal individuals on the control ($p<0.001$) diet. Likewise, there was a trend for focal pigs receiving magnesium to spend more time inactive than focal pigs on the phytase diet ($p=0.05$). Males were spent more time inactive during regrouping than females whereas in contrast, male focal pigs on the magnesium dietary treatment spent less time being inactive than females ($p=0.001$).

4.3.4.3 Combined harmful and fighting behaviour

There was no significant impact of dietary treatment, time point, focal pig weight or sex on the duration of time spent engaging in fighting and harmful behaviours ($p>0.05$).

4.3.4.4 Harmful behaviours

During regrouping, the duration of time spent engaging in harmful behaviours increased with increasing weight ($p=0.042$). However, during regrouping the duration of harmful behaviours performed by focal pigs was significantly lower than before regrouping ($p=0.013$). Dietary treatment and sex did not impact the duration of harmful behaviours.

4.3.4.5 Fighting

There was significantly more fighting during regrouping than before ($p=0.002$) or after ($p<0.001$). Furthermore, focal pigs spent significantly less time fighting after regrouping than before the event ($p=0.010$). These time point differences were consistent across all dietary treatments (Table 9). Focal pigs receiving magnesium supplementation alone ($p=0.03$) or in combination with phytase ($p=0.009$) spent significantly less time fighting than those focal individuals on the phytase only treatment diet. Male focal pigs fought significantly more than females overall ($p<0.001$) and when on the phytase dietary treatment ($p=0.045$).

4.3.4.6 Body biting

Focal pigs receiving the phytase dietary treatment spend significantly longer body biting then focal pigs on the control diet ($p=0.038$). There was no effect of time point or focal pig sex or weight.

4.3.4.7 Ear biting

There was significantly less time spent ear biting during regrouping than before ($p<0.001$) or after ($p=0.01$). Focal pigs receiving supplementary magnesium phosphate spent less time ear biting after regrouping than the control diet focal pigs ($p=0.046$). Male focal pigs spent significantly longer ear biting than female focal pigs overall ($p=0.025$) and males receiving supplementary magnesium phosphate also spent significantly longer ear biting than female focal pigs on the same diet ($p=0.004$).

4.3.4.8 Tail biting

The duration of tail biting was significantly less after regrouping than before regrouping ($p=0.009$). Male focal pigs spent more time tail biting than females ($p=0.038$). Overall, the duration a focal pig spent tail biting decreased with decreasing weight showing that the lightest weight pigs spent the most time tail biting ($p=0.044$). There was no effect of diet on tail biting duration.

Table 9. Estimates, z-values, p-values, and raw means with standard errors for each behavioural negative binomial model. P-values in italics indicate significance in comparison with the control. The final minimal model as determined by stepwise backwards deletion.

	Estimate	**z-value**	**P-Value**	**Raw Mean & SE**
Active (non-aggressive) (*r^2ML: 0.197*)				
Intercept	5.732	33.519	*<0.001*	-
Control	-	-	-	281.860 ± 11.336
Mg	-0.471	-3.615	*<0.001*	209.173 ± 13.339
Phytase	0.00006	0.001	0.999	243.241 ± 12.770
Mg * Phytase	0.352	2.439	*0.014*	250.205 ± 12.459
Before regrouping	-	-	-	225.373 ± 11.867
During regrouping	0.080	0.790	0.429	230.666 ± 9.431
After regrouping	0.338	3.089	*0.002*	286.506 ± 11.118
Sex (male)	-	-	-	243.426 ± 8.684
Sex (female)	-	-	-	250.594 ± 9.678
Weight (day 33)	-0.010	0.006	0.126	-
Mg * Before regrouping	-	-	-	200.916 ± 19.451
Mg * During regrouping	0.301	2.558	*0.010*	231.950 ± 11.543
Mg * After regrouping	0.086	0.727	0.467	266.447 ± 15.948
Phytase * Before regrouping	-	-	-	236.795 ± 16.082
Phytase * During regrouping	-0.325	-2.716	*0.006*	214.309 ± 12.502
Phytase * After regrouping	-0.178	-1.466	0.142	288.068 ± 15.309
Inactive (non-aggressive) (*r^2ML:0.114*)				
Intercept	5.913	24.418	*<0.001*	-
Control	-	-	-	393.220 ± 11.561
Mg	0.225	3.892	*<0.001*	428.565 ± 16.113
Phytase	0.301	1.343	0.179	382.047 ± 15.604
Mg * Phytase	-	-	-	417.367 ± 10.640
Before regrouping	-	-	-	409.200 ± 13.026
During regrouping	-0.076	-1.168	0.242	417.987 ± 11.708
After regrouping	-0.119	-1.809	0.070	385.123 ± 10.485
Sex (male)	-0.442	-1.835	0.066	401.521 ± 10.022
Sex (female)	-	-	-	407.589 ± 9.358
Weight (day 33)	0.003	0.283	0.776	-
Weight (day 33) * Sex (male)	0.017	1.690	0.090	-
Mg * Sex (male)	-0.252	-3.132	*0.001*	400.348 ± 17.605

	Estimate	z-value	P-Value	Raw Mean & SE
Mg * Sex (female)	-	-	-	434.929 ± 9.672
Phytase * Sex (male)	0.141	1.679	0.093	401.028 ± 12.717
Phytase * Sex (female)	-	-	-	399.661 ± 14.069
Phytase * Weight (day 33)	-0.018	-1.793	0.073	-
Before regrouping * Sex (male)	-	-	-	386.189 ± 21.008
Before regrouping * Sex (female)	-	-	-	431.605 ± 14.971
During regrouping * Sex (male)	0.190	2.068	*0.038*	431.121 ± 14.362
During regrouping * Sex (female)	-	-	-	403.815 ± 18.702
After regrouping * Sex (male)	0.123	1.314	0.188	384.054 ± 15.855
After regrouping * Sex (female)	-	-	-	386.222 ± 13.886
Harmful behaviours & fighting (*r^2ML:0.052*)				
Intercept	2.742	16.154	*<0.001*	-
Control	-	-	-	16.139 ± 1.893
Mg	-0.035	-0.195	0.845	13.796 ± 2.008
Phytase	-	-	-	18.316 ± 1.784
Mg * Phytase	-	-	-	16.552 ± 1.604
Before regrouping	-	-	-	19.169 ± 1.644
During regrouping	-0.359	-1.560	0.118	16.172 ± 1.594
After regrouping	0.069	0.315	0.752	13.899 ± 1.407
Sex (male)	0.306	1.681	0.092	17.769 ± 1.343
Sex (female)	-	-	-	15.090 ± 1.176
Mg * Before regrouping	-	-	-	18.098 ± 2.385
Mg * During regrouping	0.311	1.271	0.203	17.558 ± 2.374
Mg * After regrouping	-0.417	-1.706	0.088	10.636 ± 1.361
Before regrouping * Sex (male)	-	-	-	21.455 ± 2.433
Before regrouping * Sex (female)	-	-	-	16.301 ± 2.068
During regrouping * Sex (male)	0.006	0.025	0.980	17.986 ± 2.469
During regrouping * Sex (female)	-	-	-	14.032 ± 1.880
After regrouping * Sex (male)	-0.433	-1.774	0.076	13.121 ± 1.834
After regrouping * Sex (female)	-	-	-	14.867 ± 2.196
Harmful behaviours (*r^2ML: 0.054*)				
Intercept	3.564	5.621	*<0.001*	-

	Estimate	z-value	P-Value	Raw Mean & SE
Control	-	-	-	14.800 ± 1.913
Mg	-0.033	-0.183	0.854	11.346 ± 1.470
Phytase	-	-	-	17.021 ± 1.794
Mg * Phytase	-	-	-	15.181 ± 1.520
Before regrouping	-	-	-	17.596 ± 1.635
During regrouping	-1.968	-2.468	*0.013*	12.613 ± 1.309
After regrouping	-1.334	-1.619	0.105	14.620 ± 1.530
Sex (male)	-	-	-	15.274 ± 1.243
Sex (female)	-	-	-	14.791 ± 1.237
Weight (day 33)	-0.033	-1.235	0.216	-
Mg * Before regrouping	-	-	-	17.563 ± 2.363
Mg * During regrouping	0.198	0.780	0.435	13.055 ± 1.892
Mg * After regrouping	-0.466	-1.882	0.059	11.100 ± 1.468
Weight (day 33) * During regrouping	0.068	2.034	*0.042*	-
Weight (day 33) * After regrouping	0.059	1.660	0.097	-
Fighting (*r^2ML: 0.472*)				
Intercept	1.528	1.586	0.112	-
Control	-	-	-	24.384 ± 6.453
Mg	0.379	0.283	0.776	25.800 ± 8.811
Phytase	1.363	2.298	*0.021*	23.608 ± 5.306
Mg * Phytase	1.085	1.732	0.083	26.600 ± 7.065
Before regrouping	-	-	-	27.304 ± 5.401
During regrouping	1.213	2.275	*0.022*	30.148 ± 5.192
After regrouping	-1.651	-2.287	*0.022*	6.818 ± 1.134
Sex (male)	1.910	4.448	*<0.001*	28.166 ± 4.301
Sex (female)	-	-	-	17.578 ± 3.817
Weight (day 33)	-0.004	-0.105	0.916	-
Mg * Before regrouping	-	-	-	23.000 ± 11.661
Mg * During regrouping	1.450	2.640	*0.008*	34.928 ± 7.458
Mg * After regrouping	0.833	1.164	0.244	6.000 ± 2.258
Phytase * Before regrouping	-	-	-	29.642 ± 7.708
Phytase * During regrouping	-1.667	-2.619	*0.008*	27.588 ± 6.523
Phytase * After regrouping	0.147	0.188	0.850	8.285 ± 1.148
Phytase * Sex (male)	-1.087	-2.003	*0.045*	26.777 ± 5.482
Phytase * Sex (female)	-	-	-	19.909 ± 5.511

	Estimate	z-value	P-Value	Raw Mean & SE
Mg * Weight (day 33)	-0.087	-1.586	0.112	-
Body biting (*r^2ML: 0.075*)				
Intercept	2.411	12.493	*<0.001*	-
Control	-	-	-	12.394 ± 2.218
Mg	-0.307	-1.660	0.096	8.750 ± 1.884
Phytase	0.391	2.069	*0.038*	17.675 ± 3.069
Mg * Phytase	-	-	-	13.155 ± 2.228
Before regrouping	-	-	-	14.528 ± 2.153
During regrouping	0.041	0.227	0.820	14.812 ± 2.360
After regrouping	-0.332	-1.746	0.080	11.047 ± 2.190
Sex (male)	-	-	-	14.714 ± 1.981
Sex (female)	-	-	-	12.303 ± 1.587
Ear biting (*r^2ML: 0.169*)				
Intercept	3.376	15.684	*<0.001*	-
Control	-	-	-	21.366 ± 3.959
Mg	-0.199	-0.701	0.483	16.600 ± 2.638
Phytase	-	-	-	19.710 ± 2.741
Mg * Phytase	-	-	-	19.833 ± 2.581
Before regrouping	-	-	-	24.888 ± 3.083
During regrouping	-0.729	-2.792	*0.005*	12.707 ± 1.795
After regrouping	0.105	0.457	0.647	20.660 ± 2.456
Sex (male)	-0.460	-2.239	*0.025*	18.871 ± 2.024
Sex (female)	-	-	-	20.500 ± 2.278
Mg * Sex (male)	0.822	2.825	*0.004*	22.000 ± 3.621
Mg * Sex (female)	-	-	-	16.700 ± 2.184
Mg * Before regrouping	-	-	-	27.333 ± 4.728
Mg * During regrouping	0.006	0.018	0.985	14.086 ± 2.254
Mg * After regrouping	-0.673	-1.987	*0.046*	16.083 ± 2.569
Tail biting (*r^2ML: 0.217*)				
Intercept	4.211	3.921	*<0.001*	-
Control	-	-	-	21.366 ± 3.959
Mg	-1.926	-1.499	0.133	16.600 ± 2.638
Phytase	-	-	-	19.710 ± 2.741
Mg * Phytase	-	-	-	19.833 ± 2.581
Before regrouping	-	-	-	24.888 ± 3.083
During regrouping	-0.339	-1.505	0.132	12.707 ± 1.795
After regrouping	-0.704	-2.937	*0.003*	20.660 ± 2.456

	Estimate	z-value	P-Value	Raw Mean & SE
Sex (male)	0.645	2.074	*0.038*	18.871 ± 2.024
Sex (female)	-	-	-	20.500 ± 2.278
Weight (day 33)	-0.100	-2.007	*0.044*	-
Mg * Sex (male)	-0.734	-1.771	0.076	22.000 ± 3.621
Mg * Sex (female)	-	-	-	16.700 ± 2.184
Mg * Weight (day 33)	0.097	1.646	0.099	-

4.4 Discussion

Aggression during and after regrouping is common and can have negative consequences for pig welfare, productivity, and farm profit (39,160). The aim of this study was to assess how supplementary magnesium phosphate, with or without additional phytase, may influence skin lesion scores and the duration of aggression and harmful behaviours during regrouping in grower pigs. It was hypothesised that magnesium supplemented diets would reduce the duration of aggressive and harmful behaviours and result in lower skin lesion scores post-regrouping. Furthermore, it was hypothesised that an increased level of phytase consumed with the magnesium would improve the bioavailability of magnesium within the diet and have a positive synergistic effect. Due to a lack of consensus in the current literature (see Chapter Two), the type and level of magnesium used in this study was selected based on the level added to feed by one UK based pig feed manufacturer. However, analysis of the feed in this study showed the maximum difference in total magnesium contents of the diets in this study was 0.05%. This small difference between the diets was partly due to the high magnesium content in the control diet, and the additional magnesium not notably increasing the level in the magnesium enhanced diets. As predicted, the magnesium and phytase diet had the highest total magnesium content of all the diets with 0.21 - 0.23% in comparison to 0.20% in the magnesium phosphate supplemented diet. This shows that phytase did increase the availability of magnesium in the diet by releasing further magnesium from the feed that was previously bound by phytate.

As expected, regrouping was a stressful event for the pigs involved, highlighted by the increase in fighting during the regrouping period, as well as an increase in ear and body lesion scores after regrouping in comparison with the previous time points. Moreover, at the during regrouping time point significantly less time was spent by the focal pigs engaging in harmful behaviours, such as ear biting, than before or after regrouping. This reduction in harmful behaviours and increase in fighting behaviours on the day of and after the stressor is likely due to the stress caused by regrouping. This highlights that fighting and aggression increases during stressful events whereas harmful behaviours are caused by other factors (161,162). Furthermore, focal pigs were most active after regrouping than before or during. This could suggest that after the initial aggression, the time taken for the newly formed social structure and dominance hierarchy to settle took longer for the pigs in this study.

Overall including supplementary magnesium phosphate in the diet reduced the duration of time the pigs spent fighting, ear biting and in general activity. Focal pigs consuming a diet containing supplementary magnesium phosphate spent significantly less time fighting during the study period than pigs receiving phytase. Furthermore, they spent significantly less time ear biting after regrouping than pigs receiving a control diet during the same time period, a finding which reflects results from existing literature (89,103). It was also found that focal pigs receiving a diet containing magnesium phosphate or magnesium phosphate in combination with phytase spent significantly less time in general activity (and were more inactive) than focal pigs on a diet containing phytase only. However, magnesium supplemented pigs were more active during regrouping while phytase diet pigs were less active. These results in combination suggest that the supplementary magnesium resulted in a calming or stress reducing effect, and is in agreement with existing literature that has suggested that magnesium supplementation in commercially farmed pigs can have a positive impact on behaviour and welfare (48,98).

It was further hypothesised that including phytase within the diet alongside magnesium would have a positive synergetic effect due to the increase in total dietary magnesium. However, in this study including phytase with the supplementary magnesium did not appear to enhance the effects of magnesium and, in contrast to the magnesium phosphate treatment, phytase supplemented without magnesium had a significantly negative impact on the behaviour and skin lesion scores. It was found that focal pigs receiving phytase spent more time fighting than those on the control or magnesium phosphate diets, and engaged in more body biting behaviour than the control treatment. The increase in these undesirable behaviours suggests that phytase may have a negative impact on pig behaviour and/or levels of stress. Including phytase in pig feed is common (127,181), and therefore understanding any negative behavioural changes caused by the addition of phytase to the diet should be investigated further in future research.

Skin lesion scoring is often employed instead of, or in complement to, behavioural observations (62,145,155). Recording skin lesion scores based on the number and severity of the lesions, is a useful welfare indicator and is often used as a proxy for aggressive behaviour (62,182). Skin lesions in different areas may be the result of different behaviours. For example, body lesions can be the result of increased activity within the pen, and may reflect increased time spent in play or aggression, whereas tail and ear lesions are more likely to be the result of harmful ear and tail biting behaviours (150,172). In this study, there was no effect of dietary treatment on skin lesion scores, a finding that conflicts with much of the current scientific literature (88,89,99). Furthermore, considering that focal pigs receiving supplementary magnesium were less active and spent less time fighting it would be expected to see this reflected in the skin lesion scores. Why this wasn't the case in this study warrants further investigation, in particular whether this may be due to the type and level of magnesium used.

Ear and body lesion scores were higher after regrouping in comparison with before or during regrouping, and tail lesion scores were significantly higher after regrouping in comparison with before. This increase in skin lesion scores post-regrouping is in agreement with previous research (62) and shows the impact stressors such as regrouping can have. However, it should be noted that the increase in skin lesion scores could also be due to the decreasing pen space per pig as the pigs increase in size. Moreover, skin lesions can take time to heal and due to the short time between lesions scoring days, the increase in score could also be due to the cumulative effect of new and old lesions.

Individual differences between pigs can result in variation in behaviour (66,166,183), for example pig weight. Despite an overall reduction in harmful behaviours during regrouping, at this time point there was a significant increase in the time spent performing harmful behaviours and the pigs' weight, as such that heavier individuals were more likely to spend longer engaging in these behaviours. In contrast, the duration of time spent tail biting increased with decreasing weight, showing that the lightest weight pigs spent the most time tail biting. This links with the lesion score result showing that tail lesion scores increased with increasing pig weight, highlighting that the lighter pigs were doing the tail biting while heavier pigs were being bitten, resulting in higher tail lesion scores. Typically this is thought to be due to the smaller pigs competing with heavier individuals for resources (38,184,185). Furthermore, sex differences in behaviour is also a common occurrence (88,183,186). In this study, male focal pigs spent more time fighting, and spent more time ear and tail biting than female focal pigs. Moreover, males spent significantly more time inactive during regrouping than females, however, within the magnesium dietary treatment group this result was reversed. This suggests that supplementary magnesium may have differing effects depending on the sex of the animal, although further investigation is needed to explore this. In terms of sex, differences in behaviour and stress response to a magnesium supplement have been shown in two previous studies (88,89) but has otherwise, to the authors knowledge, not been investigated. This sex difference is a key finding that could have implications for commercial application of the nutritional supplement and therefore should be researched further in future.

4.4.1 Conclusion

Overall, magnesium phosphate included in the diet formulation at 0.15% reduced overall activity level, time spent fighting and ear biting behaviours at some time points highlighting a calming effect of the supplement. This positive effect may impact males and female pigs differently, a result which merits further research. However, supplementing the diet with magnesium phosphate and phytase resulted in a higher level of total magnesium in the diet but did not show the same benefits welfare. In conclusion, supplementary magnesium phosphate had a positive impact on the behaviour and welfare of growing pigs.

Chapter 5. Supplementary Magnesium To Reduce Physiological Measures Of Stress During Regrouping In Growing Pigs

5.1 Introduction

In commercial pig production, management strategies and husbandry practices can cause stress for the animals involved (11,17,187). Regrouping, the splitting and mixing of established groups (38,150), is typically carried out for management purposes, such as to reduce within-group variation in weight (39,188). Regrouping disrupts the established dominance hierarchies within a group of pigs, which must be re-established, resulting in social stress and an increase in harmful and aggressive behaviours (39,189,190). Whilst the re-establishment of dominance hierarchies after regrouping may be considered a normal process, the conditions on modern pig farms can exacerbate the negative effects of regrouping on pig health and welfare. For example, limited pen space means there is less room available for pigs to escape social conflict, and the often intense aggressive bouts of fighting can result in an increase in the release of stress related hormones, such as catecholamines and glucocorticoids, among the individuals (20,191). Catecholamines, such as adrenaline and noradrenaline, and glucocorticoids are stress hormones that allow the body to respond to stressful circumstances by altering physiological systems, for example cardiac and immune responses (192,193). Cortisol is the main glucocorticoid released by the adrenal glands as the hypothalamic pituitary adrenal (HPA) axis is stimulated during stress (17,194). Although the release of cortisol is designed to help and protect the animal while navigating stressful situations, due to the HPA axis being closely linked with endocrine systems such as the immune system (195,196), it can also negatively affect animals' health and performance. For example, an increase in cortisol can result in lower levels of immune cells, such as immunoglobulins and lymphocytes, which are key biological components when fighting infection (197–199). The negative impact of regrouping stress on the pigs' health and welfare can adversely affect performance and the economic efficacy of the farm (160).

5.1.1 Reducing Stress

Since, in most commercial systems, it is not possible to avoid regrouping completely, reducing the stress of regrouping should be a priority for welfare and productivity. Previous research has shown that the negative impact of stressful events can be mitigated in several ways. For example, pig welfare can be improved in the longer-term by ensuring sufficient enrichment is available and that group stocking densities are adequate or optimal (152,191). Whereas gentle handling of pigs', when required, can reduce or limit the immediate stress of a situation (35,200). Typically these mitigations require a change in either the farmers' behaviour, farm management strategies, or physical environment. Often implementing these changes can be unrealistic (e.g. straw bedding in slatted systems) and more costly than any negative effects of stressful events (10,149), highlighting the need for commercially viable solutions. Alternatively, nutrition plays a key role in pig welfare and productivity, and adding additional nutrients may reduce the impact of stressful events without needing to make large changes to the animals' environment.

5.1.2 Cortisol as a Measure of Stress

The HPA axis is the interaction the hypothalamus, the pituitary gland, and the adrenal glands (15). Endocrine pathways between these three organs regulate normal physiological functioning as well as the stress response. The way in which the HPA axis responds to stressful stimuli is underpinned by a number of different factors including early life experience, age (196,201), sex (202,203), or type of stressor (15). Involved in the stress response are a number of different hormones including catecholamines, such as adrenaline and noradrenaline, and glucocorticoids, such as cortisol. These stress hormones allow the body to respond to stressful circumstances by altering physiological systems, for example cardiac and immune responses (192,193). For humans, pigs, and many other mammals, cortisol is the main glucocorticoid released by the adrenal glands as the hypothalamic pituitary adrenal (HPA) axis is stimulated during stress (17,194). Cortisol level is often recorded as a measure of arousal in relation to a negative stressor however, it is important to note that other typically non-stressful stimuli can also result in HPA axis stimulation and consequently an increase in cortisol level (e.g. exercise or excitement) (18,19,204,205). Therefore, measuring cortisol, or other glucocorticoids, is an effective way to understand the animals' state of arousal but this does not always directly translate into a measure of negative stress and other measures, such as behavioural observations, should be considered simultaneously.

Cortisol is secreted and deposited throughout the body, including saliva (206), urine (207), faeces (191), milk (208), hair (57,209), and blood (32), all of which can be collected and used to measure cortisol, and, consequently, arousal levels in pigs (143,210). However, not all measures of cortisol are equal. Some require more invasive techniques (e.g. blood) than others (e.g. saliva), and some are better measures of acute or chronic stress. Cortisol levels in hair can be an indication of long-term stress levels as cortisol is deposited and stored in the hair over time (57,209). Saliva and plasma cortisol concentrations change rapidly within a matter of minutes giving an indication of short-term or acute stress (143,206,211). In addition, blood sampling procedures can induce stress and lead to elevated and unreliable measures. It is therefore necessary to select the type of cortisol measurement carefully based on what type of stress is to be assessed and how the sampling procedure may influence these results (Table 10).

Table 10. A summary of cortisol measures used to assess arousal in pigs.

Cortisol measure	Acute/chronic stress measure	Invasive/Non-invasive	Sampling impact	Reference
Saliva	Acute (minutes)	Non-invasive	Potential	(206,212)
Urine	Acute (hours)	Non-invasive	None	(207)
Faeces	Chronic (days)	Non-invasive	None	(191,213)

Milk	Acute (hours)	Non-invasive	Potential	(208)
Hair	Chronic (weeks)	Non-invasive	None	(57,209)
Blood	Acute (minutes)	Invasive	Potential	(214)

5.1.3 Aim and hypotheses

The aim of this study was to assess how supplementary magnesium, with or without additional phytase, may influence physiological measures stress. Salivary, faecal and hair cortisol measures were chosen to allow for the measurement of short-, medium- and long-term stress during regrouping. The study involved pigs between 7-12 weeks of age (grower pigs) as it allowed for the avoidance of other stressful events, such as weaning.

Three hypotheses were tested:

H1: Focal pigs that have had supplementary dietary magnesium will have lower salivary and hair cortisol post-mixing compared to pigs on the same diet without a magnesium supplement.

H2: Pens of pigs that have had supplementary dietary magnesium will have lower pooled faecal cortisol post-mixing in comparison with pens of pigs on the same diet without a magnesium supplement.

H3: Pigs that have had a diet with supplementary magnesium and additional phytase will have lower salivary, hair and pooled faecal cortisol compared with pigs supplemented with only dietary magnesium.

5.2 Method

The study methodology, including ethical approval, animals and housing, pig performance, and power calculation is described in Chapter Four.

5.2.1 Health data

All pigs were checked by a member of the farm staff each day and the number of pigs in the pen, the faecal consistency (Table 11), the number of off colour or sick individuals, any medication given, and any dead/removed pigs were recorded.

Table 11. Faecal consistency scoring system as used routinely by the technical staff at Leeds University Farm (National Pig Centre). Assessed at pen level. Maximum score 4.0; minimum score 1.0.

Score	Faecal scoring system
1	Faeces in the pen are firm.
2	Faeces in the pen are soft and spread slightly.
3	Faeces in the pen are very soft and spread readily.
4	Faeces in the pen are a watery, liquid consistency.

5.2.2 Performance data

5.2.2.1 Pen Feed Intake

During the pre-treatment period, when all pigs consumed the standard diet, trough weights were recorded every weekday and the weight of feed consumed was recorded every day. During the pre-treatment period, the weekend feed intake was recorded as an average taken from the amount given on the Friday and the trough weights on the following Monday. To measure pig feed intake during the treatment period, all feed added to the troughs were weighed, and the feed remaining in the troughs on day 33 (the day before regrouping) and day 55 (at the end of the study) were weighed, allowing calculation of average daily feed intake.

5.2.2.2 Pig Weights

All pigs were weighed (i) individually at weaning, (ii) at the start of the treatment period (day 20), (iii) before mixing (day 33), and (iv) at the end of the study diet (day 55). Feed conversion ratio (FCR), average daily gain (ADG), and average daily feed intake (ADFI) throughout the pre-treatment and treatment periods were calculated using pig weights and daily feed intake.

5.2.3 Cortisol

5.2.3.1 Saliva

To keep sampling time to a minimum and reduce any influence of the animals circadian rhythm (206,212), saliva was collected from one pig per pen for each diet and room (half of the total focal pigs, n=48). Saliva was collected at six different sampling times. To obtain baseline salivary cortisol levels, saliva was collected in the week prior to commencement of the dietary treatment (pre-treatment period). During this week, saliva was collected on three consecutive days to allow for one habituation day and sampling on the two days following. During the treatment period, saliva was collected (i) during the week before mixing, (ii) the day after mixing, and (iii) a week after regrouping. Samples were always taken between 8am and 11am. Saliva was collected using a synthetic Salivette swab (Sarstedt) attached to a large cable tie which was placed into the focal pig's mouth for 60-90 seconds or until thoroughly moistened. The swab was removed from the cable tie and placed into the corresponding container labelled with the date, room, pen and pig ID.

As per manufacturer's guidelines, immediately following collection each sample was centrifuged at 1000xg at room temperature for two minutes to remove the saliva from the swab. The swab was removed from the tube and samples were frozen at -20°C. Salivary cortisol was analysed using an enzyme-linked immunosorbent assay (ELISA) kit (Salimetrics, State College, PA, USA). Samples were prepared for analysis by thawing overnight at 5°C before centrifuging at 1500xg for 15 minutes. Clear samples were then pipetted into clean Eppendorf tubes to reduce the risk of recontamination with the pellet before being analysed according to the kit protocol. The ELISA plates were read using a plate reader at 450nm. Following this, the optical densities were used to calculate the concentration of cortisol in each sample. This was done in RStudio (174) using the ***nplr*** package (215) to create a 4-parameter logistic regression curve.

5.2.3.2 Faeces

Pen level pooled faecal samples were collected from the pen floor between 8am and 4pm. A minimum of 20-30g of faecal matter was collected into a plastic sealable bag, labelled with the date, room, and pen number. Faeces were collected from two locations around the pens of five animals and from a minimum of three locations for pens of ten animals. Samples were mixed thoroughly to ensure a pooled sample representative of the pen. Faecal samples were frozen immediately after collection.

Before analysis, samples were thawed overnight at 5°C and prepared as per manufacturer's guidelines (DetextX, DRG Diagnostics, Marburg, Germany). To analyse the samples, approximately 5g of sample was weighed out into glass dishes, before being dried in an oven at 55^{o}C for 24 hours. The dried sample was ground down into a fine powder using a pestle and mortar. 0.5g of powdered sample was placed into a falcon tube with 5ml of ethanol (1ml of ethanol for each 0.1g of sample) and shaken for 30 minutes at 200rpm. This mixture was centrifuged at 3000xg for 20 minutes and 1ml of the supernatant was transferred into an Eppendorf. The sample was then dried using a speedvac and dissolved using 150 microliters of ethanol, before diluting in 3ml of assay buffer. The sample was analysed using the pan-specific cortisol ELISA kit (DRG Diagnostics, Marburg, Germany). The ELISA plates were read using a plate reader at 450nm. Following this, the optical densities were used to calculate the concentration of cortisol in each sample. This was carried out in RStudio (174) using the ***nplr*** package (215) to create a 4-parameter logistic regression curve.

5.2.3.3 Hair

A hair sample was taken from each focal pig at the end of the study (day 55; n=92). Hair was collected from the left and right side of the focal pig's rump using small pet clippers before being transferred into a tin foil pouch and sealed in a plastic bag labelled with the date, room, pen, and pig ID. The clippers were applied gently to avoid any possibility of cutting the skin. Between samples, the pet clippers were cleaned, and any remaining hair removed using a toothbrush. Samples were stored in the fridge at 5°C until analysis.

Hair cortisol samples were prepared and analysed based on the published methods of Carroll et al. (2018) and Davenport et al. (2006) (57,216). Each hair sample was washed in isopropanol twice followed by one wash using water and left to air dry for 48 hours. 80mg of each hair sample was weighed out (to account for 20mg loss during the cutting, weighing and grinding process) and cut into small sections. The hair was dampened slightly using water and then ground down using a pestle and mortar. The ground hair was transferred into a 15ml falcon tube and 1.5ml of methanol was added. The samples were left for 16 hours overnight at room temperature. Following extraction, 1ml of the methanol was transferred to a clean Eppendorf tube and dried in a speed vacuum centrifuge machine. Before analysis, each sample was reconstituted with 0.4ml of assay buffer from the ELISA kit (Salimetrics, State College, PA, USA). The samples were then analysed according to the kit protocol. The ELISA plates were read using a plate reader at 450nm. Following this, the optical densities were used to calculate the concentration of cortisol in each sample. This was done in RStudio (174) using the ***nplr*** package (215) to create a 4-parameter logistic regression curve.

5.2.4 Plasma

At the end of the experimental period (12 weeks of age), blood was collected from all focal pigs (n=92) by a trained member of the farm staff in accordance with the Home Office licence (70/7895; expiry: 18/12/2018). Blood was collected from the jugular into a 6ml heparinised vacutainer and immediately placed on ice before being centrifuged at 2000xg, 4°C for 15 minutes. Plasma was removed using a pipette and stored in an Eppendorf tube at -20°C until analysis.

Plasma samples were thawed overnight at 5°C before being de-proteinised using nitric acid. One part plasma was mixed with 4 parts acid before being left on ice for 10 minutes. The sample was then centrifuged at 16000xg for 10 minutes and diluted in deionised water to make 10ml. This sample was sent to the School of Earth and Environment at the University of Leeds for mineral analysis using inductively coupled plasma - optical emission spectrometry (ICP-OES). All samples were analysed for levels of calcium, copper, iron, magnesium, phosphorus, potassium, sodium and zinc.

5.2.5 Statistical analysis

All data was stored in Microsoft Excel. The health score averages per week and the final lesion scores were all calculated in Microsoft Excel. All statistical analyses were performed in RStudio (174) as a two-by-two factorial design by dietary treatment. Combining or sub-setting of datasets was completed using the *merge* and *subset* R functions respectively. Then, with the exception of the faecal cortisol, faecal scores, and post-weaning skin lesion scores, all data were analysed as follows. Firstly, the distributions of the relevant data were checked for normality using a histogram, qq plot and Shapiro-Wilk test. Then a gamma distribution was confirmed by testing the data using the *gamma_test* function. Any non-normal or non-gamma distributed data were transformed using an appropriate transformation (see sections 5.2.5.1 – 5.2.5.6). A generalized linear mixed model with a gamma distribution using the *glmer()* function including all relevant fixed variables and two-way interactions (see sections 5.2.5.1 – 5.2.5.6). nAGQ was set to equal 0 due to convergence issues and the *drop1* function was then used to fit the model using backwards stepwise deletion based on the Akaike Information Criterion (AIC) values. If interactions were not significant, these were removed from the model and the additive only model results reported (with the exception of the magnesium diet * phytase diet interaction to ensure analysis of all treatments). The **pscl** package was used to calculate the pseudo R^2 value based on the final model with the random effects removed (179,217). Post-hoc analysis was performed using the *emmeans* function from the **emmeans** package (218).

An ordinal regression model using the *clmm* function from the **ordinal** package (176) was used to analyse the faecal scores data. Before running the models, collinearity was examined using the *vif* function from the **car** package (175), where values over five were determined to be collinear. This was done using a general linear mixed model version of the model with only additive fixed effects and no interactions. The ordinal model included all relevant fixed effects and two-way interactions. The *drop1* function was used to fit the model using backwards stepwise deletion based on the AIC values.

5.2.5.1 Health data

To assess the impact of dietary treatments on pig health, an ordinal regression model was fitted with the average faecal score (rounded to two decimal places) as the outcome variable, and dietary treatment, and the time point included as fixed effects. The regrouped pen location ID was included as a random variable. The final minimal model included the magnesium dietary treatment, time point and an interaction between the two variables as fixed effects.

5.2.5.2 Performance data

To analyse the ADG, ADFI and FCR a generalised linear model with a gamma distribution was fitted. Due to the presence of zero values, ADG was transformed by adding 1 to all values. The ADG, ADFI or FCR were included as the outcome variable with dietary treatments, time point, and all two-way interactions included as fixed effects. For the ADG analysis the regrouped pen location ID, and pig ID nested within the regrouped pen location, were included as random predictor variables. For the ADFI and FCR analysis only the regrouped pen location ID was included as a random effect. The ADFI final minimal model included only time point as a fixed effect. The final minimal models for ADG and FCR included the magnesium dietary treatment, time point and an interaction between the two variables as fixed effects.

5.2.5.3 Salivary Cortisol

A general linear mixed model was then fitted with square root salivary cortisol as the outcome variable, and the sample time point, sex of the focal pig, dietary treatments, and the weight of the focal pig (on the closest weigh day to the sample time) included as fixed effects. All two-way interactions between the fixed effects were included. The regrouped pen location ID, and the pig ID nested within the regrouped pen location, were included as random predictor variables. The model was fitted using the s*tep()* function. This resulted in a minimal model with sample time point and weight as fixed effects.

5.2.5.4 Faecal Cortisol

A generalized linear mixed model with a gamma distribution using the *glmer* function was employed to analyse the faecal cortisol data. nAGQ was set to equal 0 due to convergence issues. The time point at which the sample was taken, dietary treatments, and two-way interactions between the variables were included as fixed effects in the generalised linear model. Faecal cortisol was the outcome variable and the regrouped pen location ID was included as a random variable. The final minimal model only included time point as a fixed effect.

5.2.5.5 Hair Cortisol

The hair cortisol data was log transformed and then analysed using a general linear mixed model using the *lmer* function and fitted using AIC values and the *Step* function. Logged hair cortisol was the outcome variable, and dietary treatments, the focal pigs' sex and weight on the day of hair collection (day 55) included as fixed effects as well as all two-way interactions between the variables. Pen location ID was included as a random effect. As all fixed effects could be removed via backwards stepwise deletion, the final model reported included sex, weight, both phytase and magnesium dietary treatments and an interaction between the two dietary treatments.

5.2.5.6 Plasma Minerals

All plasma mineral data was analysed using a generalized linear model with a gamma distribution using the *glmer()* function. nAGQ was set to equal 0 due to convergence issues. For all models, the mineral level was the outcome variable with magnesium and phytase dietary treatments, sex, and weight of the focal pig, plus their interactions included as fixed variables. Pen location ID and pig ID nested within pen location ID were included as random effects. To achieve a gamma distribution, before analysis potassium and copper were log transformed using the *log()* function, and sodium was square root transformed using the *sqrt()* function.

5.3 Results

5.3.1 Pig Health

Overall, of the two-hundred and forty pigs, forty-one received antibiotics (twenty-two focal pigs), five pigs died (three focal pigs) and five were removed (one focal pig) during the course of the study. Before regrouping faecal scores were slightly higher overall. Before regrouping, pigs receiving magnesium supplemented diets had higher faecal scores than the control dietary treatment (Table 12).

Table 12. Odds ratio, 95% confidence intervals and p-values from the model output for the faecal score analysis. Minimal model as determined by backwards stepwise deletion. Descriptive means and standard errors of the raw data for all diets and time points.

	Odds Ratio	**95% CI**	**p-Value**	**Raw Mean & SE**
Control	-	-	-	2.130 ± 0.018
Mg	0.898	-0.716 – 0.503	0.731	2.176 ± 0.029
Phytase	-	-	-	2.113 ± 0.019
Mg * Phytase	-	-	-	2.143 ± 0.023
Standard diet (0-20 days)	-	-	-	2.143 ± 0.012
Before regrouping (21-33 days)	0.511	-1.285 – -0.056	*0.032*	2.147 ± 0.024
After regrouping (34-55 days)	0.671	-1.139 – 0.342	0.291	2.120 ± 0.019
Before regrouping (21-33 days) * Mg	2.521	0.060 – 1.788	*0.035*	2.200 ± 0.040
After regrouping (34-55 days) * Mg	1.252	-0.819 – 1.268	0.673	2.125 ± 0.027

5.3.2 Pig Performance

As expected, average daily feed intake, average daily gain, and feed conversion ratio increased over time ($p<0.001$) however, dietary treatment did not impact on feed intake or feed conversion ratio (Table 13). The magnesium diet resulted in a slightly lower ADG before regrouping than the control at this time point (p=0.018).

Table 13. Estimate, t-value and p-values from the model output for the ADFI, ADG, and FCR analyses. Minimal models as determined by backwards stepwise deletion. ADG was transformed by +1 to all values. FCR was transformed by +30 to all values. Descriptive means and standard errors of the raw data for all diets and time points.

	Estimate	**t-value**	**p-Value**	**Raw Mean & SE**
ADFI *(r^2ML: 0.932)*				
Intercept	2.361	64.48	*<0.001*	-
Control	-	-	-	0.756 ± 0.063
Mg	-	-	-	0.786 ± 0.062
Phytase	-	-	-	0.828 ± 0.071
Mg * Phytase	-	-	-	0.751 ± 0.062
Standard diet (0-20 days)	-	-	-	0.423 ± 0.005
Before regrouping (21-33 days)	-1.204	-32.48	*<0.001*	0.867 ± 0.019
After regrouping (34-55 days)	-1.598	-43.69	*<0.001*	1.321 ± 0.031
ADG *(r^2ML: 0.560)*				
Intercept	0.737	119.923	*<0.001*	-
Control	-	-	-	0.542 ± 0.016
Mg	-0.006	-0.716	0.474	0.559 ± 0.015
Phytase	-	-	-	0.579 ± 0.015
Mg * Phytase	-	-	-	0.531 ± 0.014
Standard diet (0-20 days)	-	-	-	0.363 ± 0.006
Before regrouping (21-33 days)	-0.102	-15.731	*<0.001*	0.559 ± 0.010
After regrouping (34-55 days)	-0.163	-26.152	*<0.001*	0.740 ± 0.009
Sex (male)	-	-	-	0.552 ± 0.011
Sex (female)	-	-	-	0.553 ± 0.011
Before regrouping (21-33 days) * Mg	0.021	2.352	*0.018*	0.540 ± 0.015

	Estimate	t-value	p-Value	Raw Mean & SE
After regrouping (34-55 days) * Mg	0.010	1.229	0.219	0.733 ± 0.013
FCR *(r^2ML: 0.758)*				
Intercept	0.841	48.801	*<0.001*	-
Control	-	-	-	1.457 ± 0.051
Mg	0.022	0.908	0.364	1.445 ± 0.050
Phytase	-	-	-	1.458 ± 0.057
Mg * Phytase	-	-	-	1.459 ± 0.055
Standard diet (0-20 days)	-	-	-	1.173 ± 0.014
Before regrouping (21-33 days)	-0.190	-9.572	*<0.001*	1.566 ± 0.025
After regrouping (34-55 days)	-0.295	-13.798	*<0.001*	1.795 ± 0.042
Before regrouping (21-33 days) * Mg	-0.045	-1.609	0.108	1.594 ± 0.041
After regrouping (34-55 days) * Mg	0.001	0.035	0.972	1.757 ± 0.035

5.3.3 Cortisol

5.3.3.1 Salivary Cortisol

Salivary cortisol was significantly higher one day after regrouping (p=0.015) and lower eight days after regrouping (p=0.044) compared with the baseline. Salivary cortisol level significantly decreased with increasing weight (p=0.001) but was not influenced by dietary treatment (Table 14).

Table 14. Estimate, t-value and p-values from the model output for salivary cortisol analysis. Minimal model as determined by backwards stepwise deletion. Descriptive means and standard errors of the raw data for all diets and time points.

R^2C: 0.220	Estimate	t-value	p-Value	Raw Mean & SE
Intercept	0.161	11.258	*<0.001*	-

R^2C: 0.220	Estimate	t-value	p-Value	Raw Mean & SE
Control	-	-	-	0.115 ± 0.007
Mg	-	-	-	0.123 ± 0.006
Phytase	-	-	-	0.105 ± 0.007
Mg * Phytase	-	-	-	0.124 ± 0.006
Baseline/Standard diet	-	-	-	0.122 ± 0.005
Before regrouping (4 days)	0.014	0.935	0.347	0.113 ± 0.005
After regrouping (1 day)	0.036	2.583	*0.015*	0.128 ± 0.008
After regrouping (8 days)	0.063	1.972	*0.044*	0.103 ± 0.006
Weight	-0.004	-3.104	*0.001*	-

5.3.3.2 Faecal Cortisol

Faecal cortisol level was significantly higher one and eight days after regrouping in comparison with the baseline (p<0.001) and four days before regrouping (p<0.001). Pooled pen faecal cortisol level was also significantly lower than on day eight than day one after regrouping (p=0.006; Table 15).

Table 15. Estimate, t-value and p-values from the model output for pooled pen faecal cortisol analysis. Minimal model as determined by backwards stepwise deletion. Descriptive means and standard errors of the raw data for all diets and time points.

r^2ML: 0.403	Estimate	t-value	p-Value	Raw Mean & SE
Intercept	0.002	28.487	*<0.001*	-
Control	-	-	-	576.663 ± 29.664
Mg	-	-	-	616.819 ± 36.729
Phytase	-	-	-	605.116 ± 38.729
Mg * Phytase	-	-	-	551.311 ± 32.921
Baseline/Standard diet	-	-	-	490.396 ± 12.079
Before regrouping (4 days)	-0.00006	-0.599	0.549	505.373 ± 18.437

r^2ML: 0.403	**Estimate**	**t-value**	**p-Value**	**Raw Mean & SE**
After regrouping (1 day)	-0.0008	-9.318	*<0.001*	843.610 ± 45.412
After regrouping (8 days)	-0.0005	-5.386	*<0.001*	672.283 ± 46.433

5.3.3.3 Hair Cortisol

There was no significant effect of weight (p=0.867), sex (p=0.924) or dietary treatment (p=0.215) on the level of cortisol in the hair at the end of the study period (Table 16).

Table 16. Estimate, t-value and p-values from the model output for log transformed hair cortisol level at the end of the study period. Minimal model as determined by backwards stepwise deletion. Descriptive means and standard errors of the raw data for all diets and sexes.

R^2C: 0.076	**Estimate**	**t-value**	**p-Value**	**Raw Mean & SE**
Intercept	-1.760	-7.963	*<0.001*	-
Control	-	-	-	0.186 ± 0.017
Mg	-0.052	-0.516	0.612	0.177 ± 0.012
Phytase	-0.131	-1.285	0.215	0.161 ± 0.007
Mg * Phytase	0.140	0.961	0.348	0.176 ± 0.011
Sex (male)	0.006	0.096	0.924	0.175 ± 0.009
Sex (female)	-	-	-	0.173 ± 0.006
Weight (day 55)	0.0009	0.168	0.867	-

5.3.4 Plasma Minerals

Focal pigs on the magnesium diet had significantly lower levels of plasma zinc than pigs on the control diet. Male focal pigs had showed higher plasma phosphorous levels than females (p=0.043). Furthermore, male focal pigs' plasma calcium (p=0.045), phosphorus (p=0.044) and sodium (p=0.05) increased with increasing weight. Sex and weight did not impact plasma zinc levels. There was no significant influence of diet, sex and weight on plasma iron, copper, potassium or magnesium level at the end of the study (Table 17).

Table 17. Estimate, t-value and p-values from the minimal model output (as determined by backwards stepwise deletion) for each plasma mineral level at the end of the study period. Potassium and copper are log transformed. Sodium is square root transformed. Descriptive means and standard errors of the raw data for all diets and sexes

	Estimate	**t-value**	**p-Value**	**Raw Mean & SE**
Magnesium *(r^2ML: 0.054)*				
Intercept	3.429	10.533	<0.001	-
Control	-	-	-	0.311 ± 0.006
Mg	-0.042	-0.276	0.783	0.317 ± 0.010
Phytase	-0.214	-1.307	0.191	0.337 ± 0.013
Mg * Phytase	0.206	0.888	0.375	0.311 ± 0.011
Sex (male)	-0.074	-0.690	0.490	0.326 ± 0.008
Sex (female)	-	-	-	0.310 ± 0.006
Weight (day 55)	-0.004	-0.540	0.589	-
Phosphorus *(r^2ML: 0.055)*				
Intercept	0.348	4.161	*<0.001*	-
Control	-	-	-	2.303 ± 0.057
Mg	-	-	-	2.361 ± 0.096
Phytase	-	-	-	2.541 ± 0.124
Mg * Phytase	-	-	-	2.250 ± 0.088
Sex (male)	0.212	2.015	*0.043*	2.375 ± 0.077
Sex (female)	-	-	-	2.334 ± 0.052
Weight (day 55)	0.002	0.933	0.350	-
Sex (male) * Weight	-0.005	-2.008	*0.044*	-
Potassium *(r^2ML: 0.078)*				
Intercept	0.649	12.142	*<0.001*	-
Control	-	-	-	4.266 ± 0.165
Mg	-0.026	-1.027	0.304	4.468 ± 0.147
Phytase	-0.029	-1.096	0.273	4.552 ± 0.206

	Estimate	t-value	p-Value	Raw Mean & SE
Mg * Phytase	0.035	0.925	0.354	4.315 ± 0.151
Sex (male)	-0.031	-1.788	0.073	4.563 ± 0.139
Sex (female)	-	-	-	4.229 ± 0.088
Weight (day 55)	0.001	1.264	0.206	-
Sodium *(r^2ML: 0.097)*				
Intercept	0.108	11.328	*<0.001*	-
Control	-	-	-	70.726 ± 1.429
Mg	-	-	-	71.639 ± 2.476
Phytase	-	-	-	76.465 ± 3.076
Mg * Phytase	-	-	-	68.386 ± 2.301
Sex (male)	0.020	1.690	0.091	74.162 ± 1.969
Sex (female)	-	-	-	69.100 ± 1.255
Weight (day 55)	0.0002	1.185	0.236	-
Sex (male) * Weight (day 55)	-0.0006	-1.960	*0.050*	-
Calcium (*r^2ML: 0.088*)				
Intercept	0.413	5.606	<0.001	-
Control	-	-	-	2.072 ± 0.045
Mg	-	-	-	2.135 ± 0.068
Phytase	-	-	-	2.241 ± 0.084
Mg * Phytase	-	-	-	2.049 ± 0.055
Sex (male)	0.166	1.791	0.073	2.168 ± 0.050
Sex (female)	-	-	-	2.070 ± 0.038
Weight (day 55)	0.001	0.949	0.342	-
Sex (male) * Weight (day 55)	-0.004	-1.999	*0.045*	-
Copper (*r2ML: 0.104*)				
Intercept	0.298	27.426	*<0.001*	-
Control	-	-	-	33.206 ± 1.155
Mg	-0.001	-0.197	0.844	34.199 ± 1.343
Phytase	-0.009	-1.523	0.128	37.795 ± 2.132
Mg * Phytase	0.010	1.248	0.212	32.668 ± 1.433
Sex (male)	-0.002	-0.810	0.418	35.827 ± 1.256
Sex (female)	-	-	-	32.845 ± 0.862

	Estimate	t-value	p-Value	Raw Mean & SE
Weight (day 55)	-0.0002	-0.996	0.319	-
Zinc (*r2ML: 0.106*)				
Intercept	0.061	5.442	*<0.001*	-
Control	-	-	-	18.106 ± 0.479
Mg	0.032	2.111	*0.034*	17.131 ± 0.790
Phytase	-	-	-	17.817 ± 1.151
Mg * Phytase	-	-	-	16.220 ± 0.688
Sex (male)	0.003	1.472	0.141	17.257 ± 0.661
Sex (female)	-	-	-	17.330 ± 0.433
Weight (day 55)	-0.0001	-0.576	0.564	-
Mg * Weight (day 55)	-0.0007	-1.839	0.065	-
Iron (*r2ML: 0.038*)				
Intercept	0.042	3.661	*<0.001*	-
Control	-	-	-	23.685 ± 1.746
Mg	-0.003	-0.796	0.425	25.628 ± 2.272
Phytase	-0.007	-1.501	0.133	27.738 ± 2.040
Mg * Phytase	0.010	1.414	0.157	23.626 ± 1.685
Sex (male)	-0.00004	-0.011	0.991	25.444 ± 1.416
Sex (female)	-	-	-	24.608 ± 1.333
Weight (day 55)	0.00007	0.235	0.813	-

5.4 Discussion

The aim of this study was to investigate whether supplementary magnesium in pig feed could reduce physiological measures of arousal (cortisol) and improve performance during a common stressful event – regrouping. It was hypothesised that a diet supplemented with magnesium phosphate would result in a reduction in stress, or an increased ability to cope with stress, and therefore a reduction in the level of cortisol in pen level pooled faecal, individual hair, and individual salivary cortisol levels.

To measure stress pre- and post-regrouping, three different measures of cortisol were assessed. Pen level faecal samples showed a higher level of cortisol one and eight days after regrouping in comparison with the baseline and four days before regrouping levels. Furthermore, focal pig salivary cortisol was increased one day post-regrouping in comparison with baseline cortisol levels. These increases in salivary and faecal cortisol confirm that this was a stressful event for the pigs involved, and may also show that regrouping stress, or arousal due to regrouping, can lasts days rather than hours, as highlighted by the lower focal pig salivary cortisol level but still increased faecal cortisol level on day eight after regrouping compared with the baseline levels. In contrast to the hypotheses, there was no significant effect of the increased dietary magnesium content, or dietary phytase, on faecal, or salivary cortisol levels. This was unexpected due to the behavioural differences between the diets reported in chapter four and physiological changes demonstrated in response to magnesium supplementation in previous research (88,89,104,110). However, existing literature has shown that cortisol and other physiological measures can vary in response to magnesium and factors including the level or type of magnesium supplement can have differing results (48,97). Similarly, there was no difference between the dietary treatments in hair cortisol level at the end of the study period. This may be due to the time needed for the cortisol to deposit in the hair; as the hair sample was taken only two to three weeks post-stressor, this may not have been long enough to show an effect (219). Likewise, the level of magnesium supplementation may not have been enough to result in a change, reflected by the lack of difference in the diets in terms of faecal and salivary cortisol. Salivary cortisol can change within minutes and is influenced by factors such as weight, sex, age, and time of day (206). In this study, the pig's individual weight significantly influenced their salivary cortisol level with heavier pigs having a lower level than lighter pigs. This contrasts with previous research showing that salivary cortisol increased with increasing weight (206,220). Why the opposite was found in this study is unknown and requires further investigation.

It was expected that if magnesium supplementation reduced stress, pig performance measures would also be improved due to less energy being required by the stress response (221). In this study, there was no statistically significant difference between the diets in terms of average daily feed intake or feed conversion ratio however, the magnesium diet result in slightly lower average daily gain during the before regrouping period only. As there was no improvement in cortisol levels, the lack of positive change in pig performance is understandable, as again it is possible that the supplementation was not sufficient to result in any measurable changes in performance data. This is further highlighted in the plasma data, as the magnesium supplemented diets did not result in an increase in focal pig plasma magnesium levels. This suggests the level of supplementation was not enough to increase circulating levels of magnesium.

Despite this, focal pigs consuming a diet containing magnesium phosphate diet a lower level of zinc plasma at the end of the study. Zinc and magnesium are closely linked, with magnesium often regulating zinc levels. In humans high dietary zinc has been shown to inhibit magnesium absorption (222,223), yet a study by Molina-López, et al. demonstrated that erythrocyte magnesium level was positively correlated with erythrocyte zinc level (224). More research is needed to understand why plasma zinc was lower in focal pigs consuming magnesium phosphate in comparison with the control. A lower level of plasma zinc was not observed in the combined magnesium and phytase dietary treatment group which is unexpected as this diet had the highest magnesium content of the four diets. Furthermore, in humans and pigs phytate can have an inhibitory effect on zinc bioavailability and absorption (44,225), and therefore it would be expected that diets containing phytase (where phytate in the diet is broken down) would have resulted in the increase in focal pig zinc status.

Although there were no further effects of diet on plasma mineral status, there were significant differences between sexes. Male focal pigs had higher levels of plasma phosphorous compared with their female counterparts and furthermore, in the case of sodium, phosphorous, and calcium, there was a significant interaction between sex and weight. The level of these minerals in the plasma increased with weight for males, but decreased with weight for females. Although sex (226–228) and weight (229–231) have been shown to influence pig production outcomes previously, why the effect of weight and sex is influencing plasma levels of sodium, phosphorous, and calcium in this way requires further investigation.

Overall, supplementary dietary magnesium and magnesium in combination with phytase, did not result in a reduction in cortisol levels or improvement in pig performance during and after regrouping. As standard pig feed has a relatively high level of magnesium already, a higher level of supplementation may be needed to show further positive or negative effects. It also may explain why there was a lack of difference between the dietary treatments for almost all aspects of this study. Increasing the level of magnesium in the feed may be most easily achieved with an increased dose, as absorption has been shown to increase with increased supplementation (136). However, there are also differences between compounds, for example it has been shown that organic compound may be more bioavailable than inorganic compounds (136,138). Using a different type of magnesium to supplement the diet may have a better efficacy than magnesium phosphate, but this would need to be investigated further to identify the most appropriate compound. Furthermore, some magnesium compounds, such as magnesium L-threonate (232), have been shown to increase magnesium levels in the brain in rodents, which may provide further benefits for stress reduction. However, research is currently limited and focuses on cognitive function (232). This type of magnesium is also much more expensive than other common compounds like magnesium oxide or phosphorus. Therefore, this type of magnesium is unlikely to be used in pig production unless further research shows it is cost-effective.

5.4.1 Conclusion

In conclusion, supplementary dietary magnesium phosphate added at 0.15% with or without phytase, did not improve cortisol levels or pig performance during and after regrouping. Future research should ensure there is sufficient magnesium in the diet to increase the level beyond the control. Moreover, exploring supplementation with other types of magnesium, and how phytase may impact the effects of magnesium supplementation, requires further investigation.

Chapter 6. The Impact Of Magnesium Supplementation During Weaning On Pig Performance And Welfare

6.1 Introduction

Weaning is the first and most significant life event experienced by commercially farmed pigs. For the young piglet, weaning poses multiple physiological, environmental, and social challenges which can result in an increase in stress and stress-related neuroendocrine responses (233). Separation from the sow, re-grouping with unfamiliar individuals in a novel environment, and an abrupt change in diet are all significant stressors that occur during the weaning process (64). Post-weaning aggression is not uncommon as piglets are often grouped into larger pens of conspecifics; in some systems these will include a mix of familiar littermates and unfamiliar pigs from other litters. This results in the re-establishment of a dominance hierarchy (234) and commonly fighting between pen-mates results in injury, poor health, and poor performance which can impact the animal long after the weaning period (39). The associated stress can increase the likelihood of harmful behaviours such as tail biting (38), a common and significant welfare issue in modern pig production. Beyond unfamiliar conspecifics and re-establishing hierarchies, the abrupt transition from an easily digested milk to an often novel, less digestible, solid, cereal-based diet is difficult for the piglets' immature gut and can result in a period of reduced feed intake immediately post-weaning (235). Methods such as feeding small amounts of the post-wean diet as creep feed have been shown to aid this dietary transition, improve feed intake and encourage gut maturity (236). However, piglet gut health during- and post-weaning is still one of the largest physiological challenges for the pig and producer, often having a huge economic and welfare cost (for review see: (237)). As well as the gut, the piglet's immune system is also relatively immature leaving them vulnerable to health challenges. Post-weaning diarrhoea is a particularly significant issue at weaning, resulting in reduced feed intake, poor growth, and poor health during the first week post-weaning (64).

The weaning period has the potential to be financially costly for the farmer (64) due to the possible negative impact on the piglets' health and immune system (238), behavioural responses and injury rates (62), and growth and performance (239). Although these issues are not unique to the weaning period, the compounded nature of these stressors — presented during what is a key developmental life stage — mean that there is significant potential for weaning to have a lasting impact throughout later life stages (229). Many current mitigations for weaning focus on reducing the effects of the stressor, and not the stress itself, for example dietary-related mitigations are implemented in order to improve performance or tackle an issue like post-weaning diarrhoea (237). The possibility of improving pig behaviour and welfare through this period and beyond with specific dietary support should not be overlooked, as including a dietary supplement that may prepare the pig to cope with the weaning transition by reducing stress could potentially prevent issues, such as post-weaning diarrhoea (237).

In humans, magnesium has been shown to be closely linked to stress (33). Supplementary dietary magnesium has been shown to have beneficial effects on subjective anxiety in humans (27), and some studies have shown that increased magnesium in the diet can reduce cortisol and improve performance and welfare measures in pigs (48). There are many types of magnesium compounds that can be used to supplement the diet, all of which contain different amounts of elemental magnesium, with some being more easily absorbed and utilised by the body than others (136). In livestock production, it is likely that cheaper and more available compounds are typically favoured in an effort to keep feed costs low.

Currently there is very little knowledge and research on whether and how supplementary dietary magnesium affects the pig stress response, as well as a lack of consensus on the most effective type and method of application (for review, see (48)). Despite the limited evidence, magnesium supplementation is a method used by some farmers to improve the health and/or behaviour of pigs on their farm. Magnesium supplementation in weaner piglet diets has been carried out previously but this has not been with the specific aim of reducing weaning stress (240–242). To our knowledge, this is the first study to specifically explore the impact of supplementary dietary magnesium phosphate on pig performance and welfare post-weaning.

6.1.1 Aim and hypotheses

The aim of this study was to investigate whether supplementary dietary magnesium, before and during weaning, can reduce weaning stress resulting in improved pig welfare and performance.

H1: Pigs that consume supplementary dietary magnesium will have improved performance and lower skin lesion scores overall post-weaning compared to pigs on a standard diet, in line with the hypothesis that magnesium reduces the levels of stress.

H2: The impact of magnesium supplementation would be dose dependent, with pigs that consume higher levels of supplementary magnesium expected to have lower skin lesion scores and improved performance post-weaning compared to pigs supplemented with a lower level of dietary magnesium.

H3: It was expected that different types of supplementary magnesium will have different effects on performance and skin lesion scores.

6.2 Material & Methods

6.2.1 Ethical Approval

This study was approved by the University of Leeds Animal Welfare Ethical Review Board on the 11th February 2021 (211102EB/LC).

6.2.2 Sample Size

Power calculations were conducted to determine sample size using the RStudio (174) using the function *pwr.f2.test* from the package **pwr** (243). A sample size estimate of 24 focal pigs per dietary treatment was calculated based on the following: u (degrees of freedom for numerator, number of dietary treatments minus one) = 3, f2 (standard medium effect size (244)) = 0.5, significance level = 0.05, power = 0.8.

6.2.3 Animals and housing

Twenty-seven litters of piglets (sow line - JSR 9T; sire line – TenderShire Rattlerow) were housed alongside the sows in a free-farrowing crate (overall pen size: 2.4m x 2.4m). Forty-eight hours after farrowing, the crates were opened to allow the sow free movement. As part of the standard commercial procedures at the National Pig Centre all piglets were weighed, sexed, tail docked (on veterinary advice), and tagged with a unique RFID tag. In order to balance litter sizes, the farm staff cross-fostered piglets during the first two weeks post-farrowing. This included creating a “smalls” litter containing low weight piglets from other litters and using a nurse sow kept on from the previous farrowing batch. All cross-fostering occurred before the commencement of the trial, in the first two weeks of age. All piglets had access to a creep area which was inaccessible to the sow. During the first two weeks post-birth each litter participated in a study investigating the effect of creep area lighting colour on time spent in the creep feed area. All lights were removed before the present study commenced.

Piglets remained in their litters (birth or fostered) until four weeks of age, when they were weaned as per standard procedures at the National Pig Centre. During weaning, farm staff vaccinated and weighed all pigs individually, recording any injuries or illness. Pigs were then allocated to a post-weaning pen determined by litter, previous light treatment, creep feed dietary treatment, sex and weaning weight. Pigs weighing less than 5kg, with any obvious injury/illness or with intact tails (in order to minimise behavioural changes due to variation in tail length) were excluded from the study at this stage. This resulted in twenty-four pens of ten pigs (two-hundred and forty pigs in total; average weaning weight = 9.18kg, standard deviation = 1.64kg), with six pens (2.5m x 1.75m) for each of the four dietary treatments (sixty pigs per treatment). Following weaning, all pigs remained in the same pen for three weeks post-weaning except if removed from the study due to illness or injury.

Each pen had standard slatted flooring with two five-space feeders with continuous access to feed and water. At weaning, the heaviest and lightest male and female pigs in each pen were selected as focal pigs, resulting in four focal pigs per pen. Focal pigs were marked with a pattern (cross, dot, two dots or stripe) for identification using non-toxic agricultural marker spray on the back. The marker spray was reapplied once or twice a week as required.

6.2.4 Dietary treatments

All diets were formulated by a pig nutritionist at a specialist pig feed manufacturer (Primary Diets) and, as standard, tested at the point of manufacture to ensure the diets were nutritionally complete. A two-stage diet regime was used, with both diets tailored to the specific nutritional needs of the animals during each stage. Stage one fed as creep feed and from weaning to day 13 post-weaning, and the stage two diet fed from day 13 to day 20 post-weaning. Four different dietary treatments were investigated: Control (standard diet with no supplementary magnesium, 0.15% total magnesium content); Magnesium phosphate (standard diet with supplementary magnesium phosphate resulting in ~0.2% total magnesium content; MgP (0.2%)); High magnesium phosphate (standard diet with supplementary magnesium phosphate resulting in ~0.3% total magnesium content; MgP (0.3%)); Magnesium sulphate (standard diet with supplementary magnesium sulphate resulting in ~0.2% total magnesium content; MgS (0.2%)). Feed samples were collected weekly throughout the study.

6.2.4.1 Pre-Weaning Diet

All piglets had access to the farm's standard creep feed diet (Initiator; Primary Diets) until two weeks pre-weaning. After this point, the litters received one of the first stage treatment diets as creep feed (Table 18). Each litter was allocated a diet balanced by previous light treatment (see section 2.2), litter size, room, and sow parity. The piglets had *ad libitum* access to feed in creep feeder troughs mounted on the wall in the creep area.

6.2.4.2 Post-Weaning Diet

At weaning, pigs were allocated to pens to receive the same treatment diet as their allocated creep feed. This ensured all pigs were in a consistent treatment group throughout the study. The pigs had *ad libitum* access to feed. The stage one diet was fed until 13 days post-weaning (Table 18) and stage two diet from day 13 to 20 post-weaning (Table 19).

Table 18. Diet formulation for the first stage weaner starter feed, also used as creep feed for two weeks pre-weaning. Formulated and manufactured by Primary Diets.

	Diet			
Ingredient (%)	**Control**	**MgP (0.2%)**	**MgP (0.3%)**	**MgS (0.2%)**
Magnesium Phosphate	0.00	0.30	0.72	0.00
Magnesium Sulphate (7H2O) Epsom Salts	0.00	0.00	0.00	0.73
Micronised Barley	10.00	10.00	10.00	10.00
Wheat Raw Whole Meal	19.68	19.36	18.90	18.50
Micronised Wheat Meal	10.00	10.00	10.00	10.00
Micronised Oats	10.00	10.00	10.00	10.00
Fishmeal	7.25	7.25	7.25	7.25
Soya Hypro	16.95	16.95	16.95	16.95
Full Fat Soyabean	2.50	2.50	2.50	2.50
Vitamin / Mineral Premix	0.50	0.50	0.50	0.50
Dried Skim Milk	4.00	4.00	4.00	4.00
Whey Powder	11.41	11.41	11.41	11.41
L-Lysine HCL	0.32	0.32	0.32	0.32
L-Methionine	0.19	0.19	0.19	0.19
L-Threonine	0.19	0.20	0.20	0.20
L-Tryptophan	0.03	0.03	0.03	0.03
L-Valine	0.07	0.07	0.07	0.07
Vitamin E	0.02	0.02	0.02	0.02
Flavour	0.02	0.02	0.02	0.02
Sweetener	0.01	0.01	0.01	0.01

Ingredient (%)	Diet			
	Control	MgP (0.2%)	MgP (0.3%)	MgS (0.2%)
Benzoic acid	0.50	0.50	0.50	0.50
Limestone Flour	0.00	0.15	0.36	0.00
DCP	0.92	0.67	0.33	0.93
Salt-PDV	0.01	0.01	0.01	0.01
Soya Oil	5.44	5.55	5.72	5.87

Table 19. Diet formulation for the second stage weaner starter feed. Formulated and manufactured by Primary Diets.

Ingredient (%)	Diet			
	Control	MgP (0.2%)	MgP (0.3%)	MgS (0.2%)
Magnesium Phosphate	0.00	0.27	0.69	0.00
Magnesium Sulphate (7H2O) Epsom Salts	0.00	0.00	0.00	0.66
Micronised Barley	15.00	15.00	15.00	15.00
Wheat Raw Whole Meal	36.60	36.23	35.66	35.34
Micronised Wheat Meal	5.00	5.00	5.00	5.00
Fishmeal	5.50	5.50	5.50	5.53
Soya Hypro	22.72	22.80	22.93	22.95
Full Fat Soyabean	2.50	2.50	2.50	2.50
Vitamin / Mineral Premix	0.50	0.50	0.50	0.50
Whey Powder	7.25	7.25	7.25	7.25
L-Lysine HCL	0.24	0.24	0.24	0.23
L-Methionine	0.13	0.13	0.13	0.13
L-Threonine	0.12	0.12	0.12	0.12
Vitamin E	0.01	0.01	0.01	0.01
Flavour	0.02	0.02	0.02	0.02

Ingredient (%)	Diet			
	Control	MgP (0.2%)	MgP (0.3%)	MgS (0.2%)
Sweetener	0.01	0.01	0.01	0.01
Benzoic acid	0.50	0.50	0.50	0.50
Limestone Flour	0.02	0.17	0.39	0.00
DCP	1.05	0.82	0.47	1.05
Salt-PDV	0.18	0.18	0.18	0.18
Soya Oil	2.65	2.76	2.92	3.02

6.2.5 Data collection

6.2.5.1 Health Data

Pre-weaning, daily health checks were conducted on all litters, recording all medications administered and deaths. As standard at the National Pig Centre, post-weaning, daily health checks for each pen were conducted and recorded on an electronic handheld device including: (i) the number of pigs in the pen; (ii) faecal consistency (Table 11); (iii) pig cleanliness (1 – 4); and (iv) the number of pigs that visually looked off-colour or ill; (v) any medication administered; and (vi) any deaths.

6.2.5.2 Performance

The performance measures assessed for the two weeks pre-weaning were total creep feed intake per pen and average daily gain per pig. Post-weaning, average daily gain, average daily feed intake, and feed conversion ratio was recorded per week. To do this, the amounts of creep feed: given, discarded if spoilt, and remaining in the trough, were weighed and recorded daily for the two weeks pre-weaning. Post-weaning, the trough weights and amount of feed provided was recorded during the working week and during the first weekend. As routine procedure on the farm, during the second and third weekend, only the amount of feed added was recorded and then the Friday and Monday trough weights were averaged across the weekend days.

In addition to being weighed at weaning, all pigs were weighed on the day of weaning and then 6, 13, and 20 days post-weaning.

6.2.5.3 Skin Lesion Scoring

Focal pigs were visually scored for skin lesions using the same scoring system as (172) Stevens, et al., (Table 20). Pigs were lesion scored on the back, tail, face, left and right ears, shoulders, flank and hindquarters. Skin lesion scores were recorded the day after weaning and then on each weighing day (1, 6, 13, and 20 days post-weaning). Scores were recorded manually onto a paper scoring sheet at the time of scoring, and later transferred onto an Excel spreadsheet to calculate the final scores. Tail lesion scores were recorded as a single value per observation, with a minimum score of 0 and maximum score of 5. Ear lesion score was the summed total of the scores from the right and left ears (sum of two parts with a minimum score: 0; maximum score: 10). Body lesion scores were calculated as the sum of the scores for the back, the left and right shoulders, flanks and hindquarters (sum of seven parts with a minimum score: 0; maximum score: 35).

Table 20. Skin lesion scoring system as outlined by Stevens, et al. (172). Faecal scoring of faecal consistency as used routinely by the technical staff at Leeds University Farm (National Pig Centre) assessed at pen level.

Score	Lesion scoring system	Faecal scoring system
0	No injuries	N/A
1	One small superficial lesion	Faeces in the pen are firm
2	More than one small superficial lesion or one deeper superficial lesion	Faeces in the pen are soft and spread slightly
3	One or several big and deep lesions. If deep only one single lesion, if not so deep several red lesions	Faeces in the pen are very soft and spread readily
4	One very big, deep and red lesion. Or many deep red lesions	Faeces in the pen are a watery, liquid consistency
5	Many very big, deep and red lesions covering the area.	N/A

6.2.6 Statistical Analysis

All statistical analyses were completed in RStudio (174). The pre- and post-weaning performance data was analysed as follows. Firstly, the distribution of the data was checked for normality using a histogram, qq plot, and Shapiro-

Wilk test. Any non-normal data was transformed using a natural log transformation using the *log* function or by adding a constant value. Linear mixed models were employed using the *lmer* or *lm* function from the ***lme4*** (245) and ***lmerTest*** (Kuznetsova et al., 2017) packages. The *step* or *drop1* function was used to fit the most parsimonious model using backwards stepwise deletion based on the Akaike Information Criterion (AIC) (177). Where all fixed effects can be removed the original model with the interactions removed is reported. The residuals of the final model were checked for normality using a combination of a histogram, qqplot and, fitted vs residual values plots. The ***emmeans*** function and package was used for post-hoc pairwise comparisons (247). The *r.squaredGLMM* function from the package ***MuMIn*** was used to calculate r^2 values where possible.

6.2.6.1 Pre-Weaning Performance

The total creep feed intake was log transformed using the *log()* function and analysed using a general linear model using the *lm()* function. The dietary treatment, previous light treatment, the number of pigs in the litter/pen and an interaction between diet and light were included as fixed variables in the model. Pre-weaning average daily gain was analysed using the lmer() function with dietary treatment, sex, previous light treatment, total creep feed intake for the litter, and their interactions were included in the model analysing the pre-weaning average daily gain. Milk sow and birth sow were included as random factors.

6.2.6.2 Post-Weaning Performance

As the post-weaning average daily gain data was not normally distributed when combined or transformed, a separate analysis was done for each week post-weaning (0-6 days, 7-13 days and 14-20 days). For these models, sex, diet, and their interaction were included as fixed variables with location ID (pen) included as a fixed effect. The feed conversion ratio data was transformed by adding 30 to all FCR values and then a gamma generalised linear mixed model was employed to analyse the FCR and average daily feed intake data. Dietary treatment and week were included as fixed effects and location ID (pen) as a random effect.

6.2.6.3 Post-Weaning Skin Lesion Scores

An ordinal regression model using the *clmm* function from the ***ordinal*** package (176) was used to analyse the skin lesion and faecal scores. Before running the models, collinearity was examined using the *vif* function from the ***car*** package (175), where values over five were determined to be collinear. This was done using a general linear mixed model version of the model with only additive fixed effects and no interactions. The full ordinal model including all relevant fixed variables and two-way interactions was run before the *drop1* function was used to fit the model using backwards stepwise deletion based on the AIC values. For all skin lesion score analyses, testing day, dietary treatment, sex, weight, and their interactions were included as fixed predictor variables. The location ID (pen) and pig ID (tag) nested within location ID (pen), were included as random effects. Due to convergence issues tail lesion score model, a model containing the magnesium and phytase dietary treatments, an interaction between dietary treatments, sex and weight as fixed effects and only Pig ID included as a random effect is reported. The average faecal score for the pen each week was rounded to one decimal place and used as the outcome variable to analyse faecal scores. The dietary treatment, week number and an interaction between diet and week were included as fixed predictor variables and location ID (pen) included as a random effect.

6.3 Results

During the study, one pig died and four were removed from the study due to poor condition and/or illness.

6.3.1 Diet

The diets were tested after manufacture and the magnesium content of each first stage diet was as follows: control: 0.14%; MgP (0.2%): 0.20%; MgP (0.3%): 0.26%; MgS (0.2%): 0.22%. The magnesium content of the second stage diets were as follows: control: 0.15%; MgP (0.2%): 0.19%; MgP (0.3%): 0.24%; MgS (0.2%): 0.22%.

6.3.2 Pre-Weaning Performance

The total creep feed intake for all twenty-seven litters during the two weeks pre-weaning was not affected by dietary or light treatment (Table 21). Furthermore, pre-weaning average daily gain was not affected by dietary or light treatment however, there was an interaction between sex and creep feed intake (p=0.012; Table 21) showing that in male piglets only pre-weaning average daily gain increased as the total creep feed intake increased.

Table 21. Estimate, t-value and p-values from the model output for the pre-weaning log transformed total creep feed intake. Minimal model as determined by backwards stepwise deletion. Descriptive means and standard errors of the raw data for all diets and time points. Significant p-values are in italics.

	Estimate	**t-value**	**p-value**	**Raw mean and SE**
Total Creep Feed Intake (r^2ML: 0.389)				
Intercept	-0.259	-0.820	0.422	-
Control (diet)	-	-	-	1.013 ± 0.070
MgP (0.2%)	-0.153	-0.437	0.666	0.863 ± 0.056
MgP (0.3%)	0.466	1.342	0.195	1.589 ± 0.118
MgS (0.2%)	-0.477	-1.329	0.199	0.687 ± 0.030
Control (light)	-	-	-	1.168 ± 0.105
No Light	-0.559	-1.057	0.303	0.595 ± 0.069
White Light	0.679	1.893	0.073	1.504 ± 0.112
Blue Light	0.040	0.114	0.910	0.893 ± 0.042
Green Light	-0.032	-0.091	0.928	0.727 ± 0.024
ADG (r^2M: 0.025)				
Intercept				-
Control (diet)	-	-	-	0.303 ± 0.007
MgP (0.2%)	-	-	-	0.318 ± 0.009
MgP (0.3%)	-	-	-	0.313 ± 0.008
MgS (0.2%)	-	-	-	0.294 ± 0.012
Control (light)	-	-	-	0.286 ± 0.009
No Light	-	-	-	0.264 ± 0.014
White Light	-	-	-	0.319 ± 0.012
Blue Light	-	-	-	0.313 ± 0.009
Green Light	-	-	-	0.326 ± 0.008

	Estimate	t-value	p-value	Raw mean and SE
Sex (male)	-0.010	-0.757	0.449	0.315 ± 0.007
Sex (female)	-	-	-	0.300 ± 0.006
Total Creep Feed Intake	-0.007	-0.506	0.616	-
Sex (male) * Total Creep Feed Intake	0.026	2.501	*0.012*	-

6.3.3 Post-Weaning Performance

Overall pigs receiving supplementary magnesium sulphate had a significantly lower average daily feed intake than all other dietary treatments (control: p<0.0001; MgP 0.2%: p=0.001; MgP 0.3%: p<0.0001). Furthermore, supplementing with magnesium phosphate to result in a diet with 0.3% magnesium content resulted in significantly higher average daily feed intake than 0.2% (p=0.014). As expected, average daily feed intake increased over time for all dietary treatments (p<0.001; Table 22).

The magnesium sulphate (0.2%) dietary treatment resulting in significantly lower average daily gain than the control and 0.3% magnesium phosphate diet during the first week post weaning (Control: p= 0.043; MgP 0.3%: p=0.016), lower than both magnesium phosphate diets during the second week post-weaning (MgP 0.2%: p=0.015; MgP 0.3%: p=0.037), and significantly lower ADG than the 0.2% magnesium phosphate diet during the third week post weaning (MgP 0.2%: p=0.009). In terms of the magnesium phosphate diets, during the first week post-weaning there was no statistical difference in ADG between the magnesium phosphate diets or control. During the second week post-weaning, both diets supplemented with magnesium phosphate resulted in significantly higher ADG than the control diet (MgP 0.2%: p=0.020; MgP 0.3%: p=0.048). No difference was observed during the third week post weaning (Table 22). Sex did not influence ADG during the post-weaning period.

Overall, magnesium sulphate (0.2%) dietary treatment resulted in a significantly higher FCR than the control diet. There was no statistical difference between the magnesium phosphate diets and the control, or between the three weeks post-weaning (Table 22).

Table 22. Estimate, t-value and p-values from the model output for post-weaning average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR). FCR data was transformed by +30. Minimal model as determined by backwards stepwise deletion. Descriptive means and standard errors of the raw data for all diets and time points. Significant p-values are in italics.

	Estimate	**t-value**	**p-value**	**Raw mean and SE**
ADFI				
Intercept	8.572	14.603	<0.001	-
Control	-	-	-	0.363 ± 0.050
MgP (0.2%)	1.597	1.754	0.079	0.397 ± 0.059
MgP (0.3%)	-1.164	-1.500	0.133	0.403 ± 0.055
MgS (0.2%)	5.064	4.594	*<0.001*	0.319 ± 0.049
Week 1	-	-	-	0.105 ± 0.007
Week 2	-5.780	-9.376	*<0.001*	0.370 ± 0.010
Week 3	-6.948	-11.647	*<0.001*	0.636 ± 0.016
Control * Week 1	-	-	-	0.116 ± 0.009
MgP (0.2%) * Week 1	-	-	-	0.098 ± 0.011
MgP (0.3%) * Week 1	-	-	-	0.135 ± 0.013

	Estimate	t-value	p-value	Raw mean and SE
MgS (0.2%) * Week 1	-	-	-	0.073 ± 0.012
Control * Week 2	-	-	-	0.358 ± 0.017
MgP (0.2%) * Week 2	-6.948	-1.987	*0.047*	0.398 ± 0.012
MgP (0.3%) * Week 2	0.853	1.046	0.295	0.403 ± 0.022
MgS (0.2%) * Week 2	-4.762	-4.186	*<0.001*	0.323 ± 0.016
Control * Week 3	-	-	-	0.616 ± 0.022
MgP (0.2%) * Week 3	-1.780	-1.932	0.053	0.695 ± 0.018
MgP (0.3%) * Week 3	1.028	1.303	0.192	0.673 ± 0.036
MgS (0.2%) * Week 3	-4.905	-4.405	*<0.001*	0.561 ± 0.022
ADG				
Week 1 (r^2ML: 0.108)				
Intercept (Control)	0.034	1.613	0.122	0.034 ± 0.013
MgP (0.2%)	-0.036	-1.179	0.252	-0.001 ± 0.013
MgP (0.3%)	0.036	1.179	0.252	0.070 ± 0.016
MgS (0.2%)	-0.065	-2.156	*0.043*	-0.031 ± 0.011
Sex (male)	-	-	-	0.015 ± 0.011
Sex (female)	-	-	-	0.020 ± 0.009
Week 2 (r^2M: 0.091)				
Intercept (Control)	0.370	17.681	*<0.001*	0.370 ± 0.014
MgP (0.2%)	0.074	2.517	*0.020*	0.444 ± 0.016
MgP (0.3%)	0.062	2.100	*0.048*	0.432 ± 0.018
MgS (0.2%)	-0.024	-0.829	0.417	0.345 ± 0.017
Sex (male)	-	-	-	0.401 ± 0.013
Sex (female)	-	-	-	0.396 ± 0.011
Week 3 (r^2ML: 0.095)				
Intercept (Control)	0.513	19.650	*<0.001*	0.514 ± 0.019
MgP (0.2%)	0.066	1.813	0.084	0.580 ± 0.017
MgP (0.3%)	0.004	0.130	0.897	0.518 ± 0.019
MgS (0.2%)	-0.064	-1.748	0.095	0.449 ± 0.018
Sex (male)	-	-	-	0.527 ± 0.014
Sex (female)	-	-	-	0.506 ± 0.013
FCR				
Intercept	0.031	15.587	*<0.001*	-
Control	-	-	-	1.506 ± 0.428
MgP (0.2%)	0.003	1.295	0.195	-1.406 ± 1.789

	Estimate	t-value	p-value	Raw mean and SE
MgP (0.3%)	-0.0003	-0.156	0.876	1.882 ± 0.511
MgS (0.2%)	-0.001	-0.786	*<0.001*	3.526 ± 2.877
Week 1	-	-	-	1.955 ± 2.645
Week 2	0.001	1.612	0.581	0.935 ± 0.012
Week 3	0.0007	0.385	0.700	1.240 ± 0.016
Control * Week 1	-	-	-	2.351 ± 1.284
MgP (0.2%) * Week 1	-	-	-	-6.323 ± 5.038
MgP (0.3%) * Week 1	-	-	-	3.415 ± 1.394
MgS (0.2%) * Week 1	-	-	-	8.376 ± 8.797
Control * Week 2	-	-	-	0.966 ± 0.021
MgP (0.2%) * Week 2	-	-	-	0.898 ± 0.015
MgP (0.3%) * Week 2	-	-	-	0.931 ± 0.020
MgS (0.2%) * Week 2	-	-	-	0.946 ± 0.032
Control * Week 3	-	-	-	1.201 ± 0.028
MgP (0.2%) * Week 3	-	-	-	1.205 ± 0.034
MgP (0.3%) * Week 3	-	-	-	1.301 ± 0.029
MgS (0.2%) * Week 3	-	-	-	1.255 ± 0.033

6.3.4 Post-Weaning Skin Lesion Scores

All diets containing supplementary magnesium resulted in significantly lower body lesion scores in comparison with the control diet (MgP 0.2%: p=0.027; MgP 0.3%: p<0.001; MgS 0.2%: p<0.001). There was no significant difference between the magnesium dietary treatments. Female focal pigs had higher body scores overall (p=0.027) however, male pigs receiving the magnesium phosphate 0.3% diet had higher body lesion scores than females (p=0.002). Overall, body score increased with increasing weight (p<0.001) which was particularly evident on day 20 post-weaning (p=0.013). Furthermore, body lesion scores were significantly lower on day 13 than day 1 post-weaning.

The magnesium sulphate (0.2%) diet resulted in lower ear lesions scores than pigs receiving the control and both magnesium phosphate dietary treatment (Control: p=0.031; MgP 0.2%: p=0.045; MgP 0.3%: p=0.021). Male focal pigs had higher ear scores than females overall (p=0.111), and when receiving both magnesium phosphate diets (0.2%: p=0.003; 0.3%: p=0.017). Overall, heavier focal pigs had significantly lower ear lesion scores than lighter weight focal pigs (p=0.005), which was particularly evident on day 13 post-weaning (p<0.001).

Overall, male focal pigs had significantly lower tail lesion scores than female focal pigs (p=0.021). Furthermore, in male focal pigs' tail score significantly increased with increasing weight (p=0.029). Tail lesion scores were significantly higher on day 6 than day 1 and 13 (Day 1: p=0.014; Day 13: p=0.029). Tail lesion scores were significantly lower on day 13 (p=0.045) post-weaning in comparison than tail scores on day 1 post-weaning. Dietary treatment did not influence tail lesion scores.

Table 23. Odds ratio, 95% confidence interval, p-values, and raw means with standard errors for the body, ear and tail lesion score ordinal cumulative link mixed models (minimal model as determined by backward stepwise deletion). P-values in italics indicate significance.

	Odds Ratio	95% Confidence Interval	p-Value	Raw Mean & SE
Body Score				
Control	-	-	-	5.913 ± 0.380
MgP (0.2%)	0.480	-1.384 – -0.082	*0.027*	4.989 ± 0.427
MgP (0.3%)	0.303	-1.853 – -0.531	*<0.001*	4.764 ± 0.403

	Odds Ratio	95% Confidence Interval	p-Value	Raw Mean & SE
MgS (0.2%)	0.330	-1.760 – -0.451	*<0.001*	3.612 ± 0.371
Weight	1.331	0.153 – 0.419	*<0.001*	-
Sex (male)	0.475	-1.403 – -0.083	*0.027*	4.726 ± 0.293
Sex (female)	-	-	-	4.908 ± 0.278
Day 1	-	-	-	7.010 ± 0.447
Day 6	0.316	-2.774 – 0.473	0.165	3.934 ± 0.328
Day 13	0.185	-3.268 – -0.097	*0.037*	2.402 ± 0.253
Day 20	1.679	-1.031 – 2.069	0.512	5.903 ± 0.382
Sex (male) * Control	-	-	-	5.333 ± 0.526
Sex (male) * MgP (0.2%)	1.910	-0.276 – 1.571	0.169	4.958 ± 0.629
Sex (male) * MgP (0.3%)	4.251	0.512 – 2.382	*0.002*	5.666 ± 0.630
Sex (male) * MgS (0.2%)	1.463	-0.555 – 1.317	0.425	2.933 ± 0.468
Weight * Day 6	0.999	-0.172 – 0.171	0.997	-
Weight * Day 13	0.927	-0.224 – 0.073	0.321	-
Weight * Day 20	0.841	-0.310 – -0.035	*0.013*	-
Ear Score				
Control	-	-	-	3.684 ± 0.139
MgP (0.2%)	0.596	-1.152 – 0.120	0.112	3.638 ± 0.171
MgP (0.3%)	0.683	-1.012 – 0.251	0.237	3.752 ± 0.159
MgS (0.2%)	0.498	-1.332 – -0.060	*0.031*	2.924 ± 0.181
Weight	1.202	0.055 – 0.312	*0.005*	-
Sex (male)	0.659	-0.930 – 0.096	*0.111*	3.606 ± 0.124
Sex (female)	-	-	-	3.389 ± 0.111
Day 1	-	-	-	4.641 ± 0.156
Day 6	0.442	-2.480 – 0.847	0.336	3.747 ± 0.132
Day 13	1.709	-1.218 – 2.290	0.549	2.076 ± 0.151
Day 20	0.772	-1.880 – 1.363	0.754	3.526 ± 0.102
Sex (male) * Control	-	-	-	3.600 ± 0.196
Sex (male) * MgP (0.2%)	2.969	0.362 – 1.815	*0.003*	3.979 ± 0.239
Sex (male) * MgP (0.3%)	2.401	0.151 – 1.601	*0.017*	4.000 ± 0.246
Sex (male) * MgS (0.2%)	1.070	-0.668 – 0.804	0.855	2.822 ± 0.272
Weight * Day 6	0.986	-0.190 – 0.162	0.879	-
Weight * Day 13	0.758	-0.438 – -0.115	*<0.001*	-
Weight * Day 20	0.876	-0.272 – 0.009	0.068	-
Tail Score				

	Odds Ratio	95% Confidence Interval	p-Value	Raw Mean & SE
Control	-	-	-	0.423 ± 0.064
MgP (0.2%)	-	-	-	0.489 ± 0.083
MgP (0.3%)	-	-	-	0.280 ± 0.061
MgS (0.2%)	-	-	-	0.290 ± 0.065
Weight	1.013	-0.071 – 0.098	0.758	-
Sex (male)	0.287	-2.309 – -0.181	*0.021*	0.349 ± 0.048
Sex (female)	-	-	-	0.394 ± 0.050
Day 1	-	-	-	0.293 ± 0.056
Day 6	1.627	0.096 – 0.877	*0.014*	0.560 ± 0.095
Day 13	0.608	-0.981 – -0.010	*0.045*	0.184 ± 0.048
Day 20	0.973	-0.650 – 0.595	0.931	0.451 ± 0.065
Weight * Sex (male)	1.098	0.009 – 0.179	*0.029*	-

6.3.5 Post-Weaning Faecal Scores

As expected during the post-weaning period, faecal scores during the first week post-weaning were significantly higher than the following two weeks ($p<0.0001$; Table 24). Pens of pigs on the magnesium sulphate diet had significantly higher faecal scores (indicating looser faeces) than pens receiving the control ($p=0.001$) and magnesium phosphate 0.3% ($p=0.01$) diets.

Table 24. Odds ratio, 95% confidence interval, p-values, and raw means with standard errors calculated from the final faecal score ordinal cumulative link mixed model. P-values in italics indicate significance.

	Odds Ratio	95% Confidence Interval	p-Value	Raw Mean & SE
Control	-	-	-	2.177 ± 0.055
MgP (0.2%)	2.100	-0.0002 – 1.484	0.050	2.322 ± 0.087

	Odds Ratio	95% Confidence Interval	p-Value	Raw Mean & SE
MgP (0.3%)	1.329	-0.459 – 1.028	0.453	2.216 ± 0.067
MgS (0.2%)	4.199	0.682 – 2.187	*<0.001*	2.488 ± 0.088
Week 1	-	-	-	2.575 ± 0.072
Week 2	0.220	-2.160 – -0.867	*<0.001*	2.195 ± 0.046
Week 3	0.145	-2.613 – -1.239	*<0.001*	2.133 ± 0.048

6.4 Discussion

This study aimed to investigate whether diets supplemented with magnesium, before, during, and post-weaning, can result in improved pig welfare and performance post-weaning. All piglets had access to one of four dietary treatments as creep feed for two weeks before weaning. The results from this period showed no difference in the amount of creep feed consumed by each litter or in the average daily gain of each piglet during the two week pre-weaning period across the four treatment groups. Therefore, including supplementary magnesium in the creep feed did not provide any advantage or disadvantage to the piglets in the pre-weaning period.

Pig performance post-weaning varied significantly depending on the level and type of supplementary magnesium. This was expected as previous research has shown variation in results when different types and levels of magnesium are added to the diet (100). In this study, both magnesium phosphate diets resulted in a significantly higher average daily gain during the second week post-weaning. This may be due to less energy being required by the stress response and active behaviours, such as fighting, which is also indicated by the significantly lower body scores recorded for pigs consuming the magnesium supplemented diets. In future, it would be beneficial to include a further control group with no weaning stressor to explore whether supplementary magnesium can improve performance when no stressor is present.

These results are also a clear indication that magnesium phosphate at either 0.2% or 0.3% in post-weaning diets can improve pig performance during the second week post-weaning. However, this improved performance did not appear to continue into the third week. Unlike the first and second weeks post-weaning, by week three is likely that the weaning stress is greatly reduced and the pigs have settled into their new environment and diet. Therefore, the lack of difference in performance may show that the magnesium supplementation provided no advantage, in terms of growth, during this third week. It should be noted that overall ADFI for pigs receiving the magnesium phosphate (0.3%) diet was higher than pigs on the magnesium phosphate (0.2%) diet, suggesting they may be less feed efficient. Despite the improvement in ADG and differences in ADFI between the magnesium phosphate diets, in this study there was no statistical difference in FCR between the dietary treatments which suggests there was no differences in terms of overall feed efficiency.

Magnesium sulphate is a common inorganic magnesium compound which in a previous studies, when supplemented in the sow diet pre-farrowing was shown to improve piglet viability (81,248). However, in this study pigs receiving supplementary magnesium sulphate at 0.2% not only had significantly poorer faecal scores throughout the three-week post-weaning period, but also had lower average daily gain and average daily feed intake during the first week compared with the control and both magnesium phosphate diets. This poor performance is most likely due poor faecal scores which indicate a higher level of loose faeces. Loose faeces are common during the first week post-weaning in piglets (64,237) and can be seen generally across all the diets in this study. However, the consistently poorer faecal scores and performance in this dietary group shows this type of magnesium was not well tolerated by the weaner piglets in this study and therefore is not ideal for supplementation at this age.

All supplementary magnesium diets significantly reduced the frequency and severity of body skin lesions throughout the post-weaning period compared with the non-supplemented control diet. Furthermore, there was no statistically significant difference between the magnesium diets, suggesting either dietary treatment has the same welfare benefit in terms of body lesion scores. Magnesium sulphate supplemented diet resulted in lower ear lesion scores, and there was no difference between dietary treatments in the severity and frequency of tail lesions in this study. Recording the frequency and severity of skin lesions on different areas of the pigs' bodies can be indicative of activity levels within the pen and can be used as a proxy indicator of aggression or harmful behaviours (62). An increase in skin lesions can result in an increased chance of disease, (e.g. infections in the spine) and poor welfare (249). Here, all levels and types of supplementary magnesium appeared to reduce the amount of activity or harmful behaviours occurring during weaning and the post-weaning period, possibly due to a reduction in stress or increased ability to cope with the stress. This is in agreement with previous research carried out on older pigs experiencing different stressors, which showed that an increased level of magnesium, or supplement containing magnesium, can reduce skin lesion scores (89,99). Dietary treatment did not influence tail scores, suggesting that there was no effect of the magnesium treatments on tail-biting related behaviours.

Similarly, to Chapter Four, in this study there were also individual differences between sexes and weight in skin lesion scores. Skin lesion scores varied between sex for body, ear and tail lesion scores, with females having higher body and tail lesion scores overall, and males having higher ear lesion scores overall and higher body lesion scores when receiving the magnesium phosphate (0.3%) dietary treatment. Sex differences in skin lesion scores are not uncommon (66,250) and have been found in previous research using the same welfare assessment tool (89,250). Weight was also a significant factor in lesion score variation, with body lesion scores increasing with weight, and tail lesion scores also increasing with weight but for male pigs only. This is in agreement with previous literature that has also shown that skin lesion scores typically increase with pig liveweight (62,251). However, why the opposite was found for ear lesions in this study requires further exploration but may be related to the differing behavioural motivations (62,162,172). The differences between sexes and weight in this study again highlights the importance of accounting for individual variation (252).

Overall including supplementary magnesium phosphate at either 0.2% or 0.3% total magnesium content of the diet reduced the frequency and severity of skin lesions during the post-weaning period, and improved pig performance during the second week post-weaning. In contrast, including magnesium sulphate at 0.2% total magnesium in the diet was not well tolerated by the newly-weaned piglets and exacerbated issues, such as post-weaning diarrhoea, resulting in poor performance. Although there was some variation in pig performance, there was no clear dose-dependent benefit of including magnesium phosphate at 0.3% total magnesium content instead of 0.2%. A cost benefit analysis was not conducted as part of this study, but it can be assumed that if similar performance and welfare outputs can be achieved with a lower supplementation, it is likely that this will cost less to implement and therefore provide the largest economic gain for the farmer. It should also be noted that in order to keep the study non-invasive and minimally intrusive, cortisol and other physiological indicators of stress were not collected. However, in future investigating such measures would be beneficial to further understand the impact of magnesium supplementation on the physiological aspects of weaning stress.

6.4.1 Conclusion

This study demonstrates that supplementary magnesium phosphate in the creep and post-weaning diet has potential to improve pig welfare and performance post-weaning. Further work is needed to confirm the possible benefits of magnesium supplementation at weaning, as well as its impact on stress by including physiological measures. However, this small dietary change has the potential to greatly improve outcomes during a challenging life-stage for commercially farmed pigs.

Chapter 7. Discussion And Conclusions

7.1 General discussion

Pig production is facing an uncertain future due to unprecedented challenges including African swine fever, labour shortages, poor pig prices, and increasing costs of grain (253,254). Improving and optimising pig performance and welfare has never been more important. This thesis aimed to investigate the impact of supplementary dietary magnesium on pig welfare, performance, and stress during key life events. These stressful events can have immediate and long term effects on pig welfare and productivity (229,255,256). Four studies which address these aims were presented in this thesis. Firstly, a systematic review of the current scientific literature on the effects of magnesium in reducing stress and aggression in pigs was undertaken (presented in Chapter Two; (48)). This review highlighted that there is limited research on the effects of supplementary dietary magnesium on reducing stress and improving pig performance and welfare during key life events throughout the pigs' lifespan. In order to understand how supplementary magnesium is currently used, farmers' views and experiences with magnesium supplementation were explored using an online survey in Chapter Three. This showed, despite the small sample size, that farmers had limited experience of the use of magnesium and that when it is used, it is typically applied to reduce undesirable behaviours, including aggression. This underpinned the need for experimental evidence to evaluate the potential for magnesium to improve performance and welfare in commercially farmed pigs. Finally, the impact of supplementary magnesium on pig performance, welfare, and stress was empirically tested during exposure to two key stressors which occur during the lifetimes of the vast majority of commercial pigs: regrouping and weaning.

Taken together, the findings of the studies presented in this thesis highlight a gap between the existing scientific evidence for magnesium supplementation and current rationale for usage by farmers. Diets including supplementary magnesium phosphate were shown to be effective at improving pig behaviour around a regrouping stressor, as well as improving pig welfare and performance measures during the post-weaning period. This represents a strong and important contribution to understanding the potential for the application of magnesium in pig production. The research presented in this thesis also highlights important questions to be addressed in future research.

7.1.1 The impact of magnesium supplementation on stress reduction in pigs: current scientific and anecdotal evidence

Chapter Two systematically reviewed and synthesised the existing scientific literature on the impact of supplementary dietary magnesium on stress and aggression in pigs. Nearly a third of the research was published before the year 2000 (5 of 16 studies), with only three studies published within the past decade (88,89,110), showing a lack of progression or direction in this research area. Of the three most recent studies, one did not include behavioural or welfare observations (110), and the other two did not directly supplement with a magnesium compound but instead used a magnesium-rich algae supplement (88,89). This existing research, and that conducted in the 2000’s and 1990's, provides some important contributions to our understanding of magnesium in pig health and wellbeing, but much more research is needed to determine exactly which types of magnesium supplementation are effective and how magnesium can contribute to pig production. Despite the dearth of high quality existing research, the overarching consensus was that magnesium supplementation has potential to reduce stress and improve pig welfare as demonstrated by lower skin lesion scores (88,99), lower salivary and plasma cortisol levels (88,89,97,104,107,110), and fewer incidences of aggressive behaviour (88,98).

When researching a technique or supplement which is commercially applicable, it is important to consider how it is currently viewed or used. Chapter Three aimed to understand how magnesium supplementation is currently being used in pig production, and farmers' views of this. The results of this survey were consistent with the findings of the systematic review, specifically, that supplementing commercial pigs' diet with magnesium can be beneficial in reducing stress and aggression. Despite the focus of the scientific literature on meat quality and slaughter, anecdotal evidence collected in the survey (Chapter Three), showed that farmers would include supplementary magnesium in the diets of pigs of all ages, with a minority (4/11) of respondents supplementing during the finisher stage, which might be most effective in terms of influencing meat quality. This is a clear contrast with the existing scientific literature which, with the exception of a few studies (88,89,96,98), primarily focused on improving meat quality by reducing stress towards the end of life. This difference in application is particularly evident since the majority of the studies in the review (11/16; Chapter Two) investigated the effect of magnesium on meat quality, whereas eight of eleven respondents in the survey (Chapter Three) stated that they do not receive feedback on meat quality. Hence this was not their motivation to use magnesium, and they were supplementing for other reasons. This important difference between the science and farm-level application demonstrates the lack of translation of research findings and highlights the need for more scientific research into magnesium supplementation during earlier life-stages, such as the weaner and grower phases, to bridge the gap between scientific research and commercial application. The survey revealed that farmers were unaware of any clear recommended method of magnesium supplementation and consequently there was a large variation in the type of magnesium offered, the method of delivery, and duration of supplementation reported. This variation was also seen in the scientific research where multiple levels, types, and durations of magnesium supplementation were employed, with no clear rationale or evidence provided by the investigators for the selection of one method over another.

Based on the reported scientific evidence, and first-hand views and experiences, it is apparent that magnesium supplementation can be beneficial within pig production, in terms of reducing stress and undesirable behaviours, such as aggression. Since the beginning of this PhD project in October 2017, to the author’s knowledge, there has minimal research focusing on magnesium supplementation and pig performance (81), and no new research which included stress or behavioural outcomes. Despite limited up-to date research, it is clear that magnesium is being used commercially to improve behavioural parameters. This highlights the demand for specific, evidence-based research to drive this area forward and accurately inform nutritionists, vets, and producers and farmers of the best practice across different life stages and contexts.

7.1.2 The impact of magnesium supplementation on pig stress during regrouping

Underpinning the positive impact of magnesium on pig welfare and performance is its ability to interact with multiple biological mechanisms, including the stress response. Magnesium influences the stress response via multiple mechanisms (21,33), and magnesium supplementation has been demonstrated to reduce measures of stress in rodents and humans (23,27). In this thesis, stress was assessed using a combination of physiological and behavioural measures. Including both types of measures allowed the assessment of arousal and valence of the arousal, which is important when attempting to understand an animal's experience of a stressor (59). To the author's knowledge, the studies presented in Chapters Four and Five are the first studies to determine the impact of supplementary dietary magnesium phosphate on the physiological and behavioural effects of stress in pigs during regrouping. Due to a lack of consensus in the literature, the type and level of magnesium supplementation in this study was chosen based on recommendations from a UK pig feed manufacturer.

The increase in pen level faecal cortisol, individual salivary cortisol, as well as an increase in duration of fighting behaviour and higher ear and body lesion scores, shows that regrouping was a significant stressor for the pigs in this study and highlights the negative physiological, behavioural and welfare impact stressors such as regrouping can have. Magnesium phosphate supplementation had a positive impact on behaviour and resulting in “calmer” focal pigs, that spent shorter durations of time fighting and active during the overall study period, as well as less time ear biting after regrouping. It could therefore be interpreted that these animals were less stressed than those on other dietary treatments and consequently expect to see this reflected in cortisol levels. However, magnesium supplementation did not influence salivary, faecal or hair cortisol levels. This lack of change in salivary cortisol contrasts with previous research which showed that a supplement containing magnesium can reduce salivary cortisol in pigs (88,89). Although it should be noted that these studies used a “magnesium-rich” supplement and whilst the results could be a consequence of the increase in magnesium, they could equally be attributable to other minerals in the supplement, the combination of minerals, or the specific supplement. In addition, differences in methodology make it difficult to draw comparisons between the research presented in this thesis and existing literature (48). However, with the exception of two studies (97,98), the majority of existing research, despite all supplementing with different types of magnesium, have shown a decrease in plasma and serum cortisol or norepinephrine in comparison to control diets (97,104,106–108,110), and thus have shown a positive effect of magnesium on the stress response. This is not what was shown in Chapter Five and why this was the case in this study could be due to a number of factors, including but not limited to, the possibility that magnesium may have reduced stress around regrouping but not overall arousal hence the lack of change in behaviour but not cortisol. Alternatively, a higher level of magnesium phosphate may be required in pigs between 7-12 weeks of age, to produce measurable effects of the supplementation on the cortisol post-regrouping.

Individual variation is important to investigate and include in livestock behaviour studies (252). In Chapters four and five of this thesis there was a clear pattern between weight, cortisol and tail biting. Throughout the study period salivary cortisol level and tail biting behaviour increased with decreasing weight, while tail lesion scores increased with increasing weight. This paints a picture of lighter weight focal pigs biting the tails of heavier individuals, possibly due to a factor that is also resulting in their higher level of arousal, as shown by the increase in salivary cortisol level. Higher levels of cortisol in lower weight individuals in comparison with heavier pigs was not expected based on previous research (206,220) and this, in combination with the tail biting outcomes, may reflect that the smaller pigs were more greatly impacted by environmental or social factors than heavier pigs. Previously lighter weight pigs have been shown to be more likely to bite the tails of heavier pigs in an attempt to compete for resources, such as feed (38,184,185). However, to fully understand the links between these outcomes further research is needed.

Despite the lack of difference in cortisol levels between the diets supplementary magnesium phosphate was shown to have a statistically significant positive impact on the duration of aggressive and harmful behaviours. This in agreement with the results of Chapters Two and Three which found that dietary magnesium supplementation can have benefits in terms of behaviour and welfare in commercially farmed pigs during stressful key life events.

7.1.2.1 Magnesium and phytase supplementation effects during regrouping

Phytase is commonly used in pig feed to facilitate the release of bound phosphorous (44,127) but has also been shown to increase the absorption and retention of magnesium (127,169,171). In Chapters Four and Five, a combined magnesium and phytase diet was included to explore the impact of these supplements applied in combination on pig performance and measures of stress and welfare. As expected, the combined phytase and magnesium diet had a higher total magnesium content than the diet supplemented with magnesium alone. This result shows that by including phytase the level of magnesium in the diet can be increased, reducing the need for higher levels of magnesium inclusion during formulation. This is beneficial in terms of the cost effectiveness of the supplementation which does not require high levels of magnesium (the more costly ingredient). In addition, including phytase is common practice and this study found that phytase may be included alongside supplementary magnesium (181) with no adverse effects and a positive synergistic increase in available magnesium resulted.

The results of the studies presented in Chapter Four and Five report that, similarly to supplementing with magnesium alone, including phytase with magnesium did not result in any change in performance parameters, nor salivary, faecal or hair cortisol level. This shows that despite the increased level of magnesium in the diet this did not result in an increase in effects of the magnesium. There also was no difference between the magnesium only and combined treatment diet when it came to the behavioural changes. This may be due to there not being a large enough difference in magnesium content between the two dietary treatments to show a statistically significant change however, more research would be needed to examine the reason for this result.

Somewhat unexpectedly, it was observed that phytase when supplemented without magnesium had a significantly negative impact on pig behaviour, with those focal individuals having a higher duration of time fighting and body biting than other treatment groups. A result that warrants further investigation considering the prevalence of phytase supplemented diets within the pig industry. As the main focus of this thesis was on the effects of magnesium, phytase was not investigated further but future studies should consider investigating the behavioural effects of phytase as well as the impact of magnesium combined with other supplements. These findings would have important implications for pig nutrition and production, and would highlight the importance of considering potential positive and negative nutrient interaction effects.

7.1.3 The impact of magnesium supplementation on pig performance during regrouping and weaning

Pig performance, farm productivity and profit are all directly linked and are typically the main factors considered in the uptake of a new technique or method in pig production (149,160). During the regrouping study presented in Chapter Four, dietary treatment did not impact performance measures. During a period of high stress nutritional energy is directed away from growth to fuel the stress response and related behaviours, consequently negatively impacting pig performance (36,37). Therefore, a positive or mitigating impact of magnesium supplementation would be expected as behavioural measures in Chapter Four indicated that the pigs were experiencing a reduced level of stress compared with the control diet. However, there was also no statistically significant change in cortisol measures reported in Chapter Five, suggesting that any behavioural or stress related change may not have been large enough to observe physiologically and consequently, in terms of pig performance.

Chapter Six is, to the author's knowledge, the first study to investigate the impact of magnesium phosphate supplementation on pig welfare and performance post-weaning. This study showed that weaner pigs fed a diet with the same total magnesium content (0.2%) with the same type of magnesium (magnesium phosphate) as was administered to grower pigs in Chapters Four and Five, had greater average daily gain (ADG) compared to pigs on the control diet. Although this could be due to differences in age and stressor, we cannot rule out a genetic effect as the regrouping study involved pigs of a different genotype to the weaning study. Research has shown that genetics can significantly impact pig behaviour (144,165,257), and therefore, this difference should be kept in mind when comparing the two studies presented here. These results have important implications for pig production, as they suggest that supplementary dietary magnesium phosphate has the potential to be an effective method of improving or maintaining pig productivity during weaning, a life event which typically results in a poor performance during the first one to two weeks post-weaning (11,64,258). Although magnesium phosphate supplementation resulted in an improvement in pig performance, including magnesium sulphate at the same level led to consistently poorer performance throughout the three weeks post-weaning. This poor performance is not surprising considering the poor faecal scores also observed in this treatment group, indicating that magnesium sulphate is not suitable for piglet of this age.

As demonstrated in Chapter Six, there was no significant difference in pig performance post-weaning between the diets supplemented with magnesium phosphate to a level of 0.2% or 0.3% total magnesium. Yet, a total magnesium content of 0.2% did not impact performance post-regrouping in grower pigs in Chapters Four and Five, and so it would be appropriate to investigate an increased level, for example 0.3%, of magnesium phosphate in the diet. These results suggest that a total magnesium content of 0.2% might be sufficient for weaner pigs (two to seven weeks old), and consequently one might expect that older, grower stage pigs (seven to twelve weeks old), would require an increased level of supplementation. Furthermore, this finding underlines the economic importance of establishing the correct level of supplementation, since including more magnesium to reach a dietary level of 0.3% during weaning, at further cost to the producer, does not provide further performance benefits. Conversely, the cost of including magnesium phosphate at the lower level during the grower period does not pay off in terms of pig performance.

7.1.4 The impact of magnesium supplementation on pig welfare during regrouping and weaning

Improving the welfare of commercial farmed pigs is not only beneficial for the animal but also positively affects pig performance and farm productivity (8,9). Both Chapter Four and Six presented in this thesis demonstrated a positive impact of magnesium supplementation on pig welfare during stressful life events. A 'calming' effect of magnesium supplementation has previously been reported (98,259) which was also found in Chapter Four. Despite a calming effect being observed in terms of behaviour in Chapter Four, a reduction in skin lesion scores as a result of magnesium supplementation was not observed during this age and stressful event. This suggests that magnesium phosphate supplementation at this level, age, or type of stressful event may not be sufficient alone to tackle these issues. It may also be the case that magnesium supplementation may be more beneficial when combined simultaneously with other strategies, such as increasing environmental enrichment (260). In contrast, in Chapter Six, magnesium phosphate supplementation significantly reduced body lesion scores after weaning, and magnesium sulphate supplementation also reduced ear lesions scores post-weaning. The results of these studies build upon existing research (88,98,99,259) and highlight the valuable effect magnesium supplementation can have on pig welfare during stressful key life events.

Magnesium sulphate supplementation during weaning resulted in poor faecal scores (and consequently performance, see section 7.1.3) throughout the post-weaning period, an effect not seen in magnesium phosphate supplementation. Post-weaning diarrhoea is common during the post weaning period and can have a detrimental impact on the performance, health, and welfare of the pig (237,261,262). Therefore, despite the positive effects of magnesium sulphate on body and ear lesion scores, overall magnesium sulphate negatively impacted pig health and welfare. In contrast, previous research has shown magnesium sulphate supplemented in sows does not negatively impact their health (81). This again highlights the importance of establishing the most appropriate type and level of magnesium supplementation for each age and stage. Typically cheaper compounds are used to supplement pig diets, such as magnesium sulphate or oxide (110,263), but including a more expensive supplement may be cost-effective in the long term. Studies in rodents have shown cognitive benefit of diets supplemented with magnesium L-threonate, a compound that can cross the blood brain barrier and elevate brain magnesium (232,264,265). Magnesium has been shown to reduce stress by interacting with mechanisms within the brain (21,23). Therefore, it may be expected to see further benefits in terms of the stress response by directly elevating the magnesium level within the brain. This supposition requires further research, to understand how supplementing pig feed with magnesium L-threonate may impact welfare and productivity, and to compare its efficacy with cheaper compounds.

Overall, the results of the studies presented in this thesis show that addition of magnesium phosphate in pig feed can improve pig welfare during key life events in commercial pig production systems. This is likely to have a further positive impact on pig health, farm productivity and profit during these potentially stressful events (9).

7.2 Study limitations

The studies presented in this thesis have limitations, for example two key limitations are time constraints to the experimental work presented, and low response rates to the survey of farmers. Each of these limitations are addressed below.

The low response rate in the survey is a main limitation of the work presented in Chapter Three. Although the survey provides a brief insight into farmer views and experiences, the low number of responses limits the potential to extrapolate to the wider agricultural population. The low response rate may be due to several reasons, for example the use of social media to recruit respondents since this limits the pool of respondents to those within the target group that use social media. There was also no incentive to complete the survey; it is likely that the response rate may have been higher if one had been used. Furthermore, despite publicising the survey through various online platforms, potential eligible participants may have viewed it as irrelevant to their current practice and therefore not participated. As of 2019 the total number of pig holdings in the UK was 10,539 (266), therefore the 24 participants in survey that were from the UK would only represent 0.24% of the total UK population. In order to fully understand the prevalence of magnesium supplementation in current farming practices, a substantially larger study would need to be carried out, including a wider pool of farmers with varied experience —or lack thereof of— magnesium supplementation. Offering an incentive, and advertising or distributing the survey through other well-known outlets and organisations, such as Pig Progress or the National Farmers Union, may also have increased the sample size. Attending events, such as the Pig and Poultry Fair, with paper surveys and directly targeting participants would be a further way to boost the response rate. Unfortunately, due to the ongoing pandemic many of these in person events were online and therefore this was not possible for this survey. Despite this limitation, the responses collected in Chapter Three include a range of farming systems, types of magnesium, pig genotypes, and stages of magnesium supplementation, and so gives a relatively broad, if shallow, overview of farmer views and experiences with magnesium supplementation.

In Chapter Four, a behavioural analysis was conducted in order to directly measure the duration of activity, inactivity, aggressive and harmful behaviours. However, due to the time-consuming nature of continual behavioural analyses, only short sections of time were assessed (two hours per pig at three separate time points). This provides a small limited insight into any changes in behaviour and ideally, much larger time samples would have been analysed. Furthermore, only the duration of behavioural bouts was analysed, and not the frequency of behaviours. Including the frequency would have given further insight into the details of the focal pigs' behaviours, such as the number of bouts of fighting. Similarly, due to time limitations, behavioural observations were not included in Chapter Six, where skin lesion scoring was used as a proxy to assess focal pig welfare. Although this is relatively common in livestock welfare research (62,249), including behavioural observations would have allowed for a more specific and in-depth understanding of how the magnesium supplementation was affecting the animals' behaviour. Likewise, due to the cumulative and ethical implications of collecting multiple samples for scientific research, physiological measures such as salivary or faecal cortisol, which would have given further insight into the pigs' stress response and underlying physiological mechanisms, were not included in the study. Although these additional measures would provide further insight, the positive effect of magnesium on pig performance and welfare post-weaning indicates that the diet did reduce stress during this time period.

In this thesis, it was only possible to compare two different types and levels of magnesium supplementation due to the sample sizes needed and the time associated with conducting such large animal-based studies. If time had permitted, it would have been beneficial to examine the dose response by increasing level of magnesium phosphate in growing pigs with the regrouping stressor to establish whether a higher total magnesium content in the diet would provide any additional benefit to that seen with the dose that resulted in ~0.2% total magnesium content. Likewise, there is wide range of magnesium compounds with different levels of bioavailability (137,267) and it would have been beneficial to examine the potency of different forms of magnesium on these outcomes. For example, does including a more expensive magnesium supplement, such as magnesium L-threonate, confer additional or greater benefits (232,264). As only magnesium phosphate and sulphate were investigated in this thesis, the effects of magnesium supplementation observed are limited to these magnesium compounds.

Although the conditions in both empirical studies presented here are similar to commercial conditions, the maximum pen and group size was ten pigs which is not representative of commercial pig production. Group sizes are typically much larger under commercial conditions and research has shown that the effect of regrouping, aggression, and behaviour can vary depending on the size of the group (39,167,268). To understand fully how dietary magnesium phosphate or other magnesium compounds may impact pig behaviour, welfare, and performance under commercial conditions, further research is needed with larger group sizes.

Despite these limitations, the evidence provided by the four studies presented in this thesis advances our understanding of magnesium supplementation in pig production and identifies knowledge gaps. Furthermore, the data collected provides further information about the impact of magnesium supplementation on the stress response in non-human mammals, where previously this has mainly focused on rodents. Pigs are good models for humans both in terms of nutrition and stress (50–52) and so the results in this thesis may also be relevant to understanding efforts to combat stress in humans (27).

7.3 Future work

This thesis contributes to the current scientific literature and advances our current knowledge about magnesium supplementation and stress in pig production, and in non-human mammals.

7.3.1 Establishing the most effective type and level of magnesium supplementation for each age and stage of pig production

The presented work provides important insights, alongside highlighting the need for establishing the best method and type of magnesium supplementation and application. It is imperative that future work to identify the most appropriate types of magnesium, while establishing the optimum magnesium content of the diet for each age or stage of pig production is conducted. Expanding on the types and levels of magnesium used in this thesis, or other magnesium supplementation methods from industry, should be the starting point for this. The effects of magnesium toxicity are unknown in pigs however the National Research Council states the maximum tolerable level it 0.3% (49), and so this level should not be exceeded. It would be beneficial to compare less commonly used compounds with magnesium L-threonate – a magnesium compound that has been demonstrated to elevate brain magnesium (232,265). Furthermore, the effects of supplementing magnesium alongside other co-nutrients should be considered, including whether any other supplements may boost its efficacy without the need for increased concentration, or indeed exert anti-nutrient effects thus negating any benefits. When deciding on the type and level of magnesium to include in the diet, cost is a vital consideration, especially as the recommendations made here are intended to be implemented in the commercial farming community. Therefore, including a cost-benefit analysis would be a useful inclusion for any future work.

This is a large area to explore with many possible hypotheses. However, the research presented in this thesis suggests that future work should focus on addressing three main questions:

(i) *What level of dietary magnesium supplementation is appropriate for grower (seven to twelve weeks old), and finisher (twelve weeks old to slaughter) stage pigs?*

(ii) *Can dietary magnesium L-threonate supplementation during key life events further reduce stress and positively affect welfare and productivity, when compared with common forms of magnesium?*

(iii) *In terms of production and welfare, which magnesium compound is most suitable and cost effective for dietary supplementation in farmed pigs?*

7.3.2 Investigating the effect of magnesium supplementation on behaviour and physiological measures post-weaning

Cortisol and behaviour should be measured directly in future studies. Although skin lesion scoring can be used as a proxy for activity and aggressive behaviour within the pen, it leaves us unable to further unpick how magnesium impacts on the pig during the post-weaning period. In future, it would be beneficial to include measures of arousal, such as faecal or salivary cortisol, post-weaning to address the question: *How does magnesium phosphate supplementation affect the stress response in piglets post-weaning?*

7.3.3 The effect of magnesium supplementation on behavioural responses

To further understand the possible impact of magnesium supplementation on pig behaviour, sampling and analysing a longer period of time would be beneficial. Behavioural observations can be carried out using multiple methods including continuous sampling, such as was attempted in Chapter Four. However, due to the time-consuming nature of analysing animal behaviour from video footage, there often has to be a compromise between the ideal amount of time assessed and the reality of carrying this out. Very recently, scientists have begun to utilise technology to make behavioural monitoring more accessible and less time consuming. Technology has been developed to automatically detect individual pigs and changes in behaviour which can give an indication of the health and welfare status of the group and/or animal (269–272). This also enables a much larger sample of time to be analysed, without any additional cost in time for the producer or researcher.

Utilising this technology when possible may be beneficial when aiming to assess the behavioural response of a nutritional supplement like magnesium.

7.3.4 The effect of magnesium supplementation on welfare and performance throughout the pigs' lifespan

In Chapters Four, Five, and Six the impact of supplementary magnesium in the diets was explored for short periods of time, a total of five weeks in each study. Therefore, it was not possible to explore the long-term impact of magnesium in the feed, or the long-term impact of improving performance and welfare during these stressful events. Previous research has found differences in the efficacy of magnesium supplementation when applied for different lengths of time and thus investigating these differences further would be a useful direction for future research. Moreover, it would be interesting to address how reducing stress and improving pig welfare and performance during key life events may impact the pig over its lifespan. For example, does this improved performance and welfare post-weaning result in improved performance later in life, or result in a shorter time to slaughter weight. Future research should aim to collect performance and welfare data throughout the pigs' lifespan to address the question: *How can supplementary magnesium during key life events impact on pig performance and welfare throughout the animals' lifespan?*

7.3.5 The impact of supplementary dietary magnesium in different pig production systems

The data collected during the studies in Chapters Four, Five and Six, were carried out on an indoor pig unit. In the UK alone there are many variations in pig production systems, including outdoor, partially outdoor, indoor straw based, and small holdings, all with different challenges (11). The impact of stressful life events on the pigs in these systems is likely to vary and consequently the impact of dietary magnesium is likely to vary across these systems. Likewise, as in this thesis the maximum group size was ten pigs which is not representative of most groups sizes on commercial farms, future studies should ensure that research is conducted using larger groups of pigs. Overall, future research should aim to address the question: *Does variation in production system (e.g., indoor vs. outdoor) impact the efficacy of supplementary dietary magnesium on pig performance and welfare?*

7.3.6 The impact of supplementary dietary magnesium in combination with other mitigations.

There are a wide range of other methods to reduce stress or improve welfare and performance during stressful life events (39,147,164,165). Although supplementary magnesium in the diet has been proven to improve pig behaviour and welfare (Chapters Four and Six; (48,88,89)), combining this dietary change with other commercially feasible mitigations, for example including more enrichment, may result in a synergistic effect on the stress response during these key events, and further impact pig welfare and performance. Therefore, future research should aim to answer the question:

Does supplementary magnesium work synergistically with other mitigations to improve pig welfare and performance during stressful life events?

7.3.9 The effect of magnesium phosphate supplementation, with or without phytase, on mineral homeostasis.

An area outside of the scope of this thesis and therefore not explored was the impact of magnesium phosphate supplementation, alone or in combination with phytase, on other mineral absorption and metabolism. In particular, calcium, phosphorus, and magnesium homeostasis are closely linked (171,273,274). For example, the inclusion of phytase in the diet not only increases the level and digestibility of phosphorus in the diet but also calcium and magnesium (171,275). Moreover, a decrease phosphorous absorption can occur if the calcium to phosphorus ratio is too wide (276). In the study presented in Chapters Four and Five, both magnesium phosphate, a compound containing phosphorus, and phytase, an enzyme used to release phosphorus from phytate, were supplemented together one of the dietary treatment groups. It would be expected that by including both of phytase and magnesium phosphate that there will be higher levels of both magnesium and phosphorus absorption, which will likely impact calcium homeostasis (274). Furthermore, the higher level of magnesium and phosphorus in the diet could have wider effects, such as increased excretion of phosphorous and environmental impacts (277). The implications of including both magnesium phosphate singularly and in combination with phytase should be explored in terms of mineral homeostasis, absorption and metabolism. How supplements impact other nutrients is an important factor to consider and therefore future work should aim to answer the question: *How does magnesium phosphate supplementation, with or without phytase, effect magnesium, calcium, and phosphorus absorption, metabolism, and homeostasis?*

7.4 Conclusions

Taken together the studies presented in this thesis advance our understanding of the impact of magnesium supplementation within pig production and in non-human mammals. The results of the novel studies presented show that some of the negative behaviour, welfare and performance effects that occur during weaning and regrouping can be mitigated with magnesium phosphate supplementation. Furthermore, this thesis identifies, and begins to close, the gaps in existing scientific evidence. It highlights the differences between previous research aims and current on-farm application, while pinpointing specific questions to be addressed by future research in order to address these aims. Despite the promising results found previously and the use of supplementary magnesium commercially, to the author’s knowledge, this is the first piece of scientific research investigating the effect of magnesium on stress, and consequently performance and welfare, in pigs for eight years. This once again highlights the need for up to date research to allow evidence-based application of this beneficial dietary supplement in pig production.

In conclusion, supplementary magnesium phosphate in commercial pig diets is a promising but under researched technique which offers the potential to optimise pig performance and welfare during stressful key life events.

Appendix A

Supporting material for Chapter Two

Table A 1. Extracted information from included studies including aim, animal information, dietary treatments, outcomes measured, and results summary. (+ positive result; - negative result; o no effect)

Author	Aim of study	Genotype, Sex & Age/Stage of Production	Experimental Treatment (s)	Dietary Treatment (s)	Measured outcomes	Results	Size of Effect	Results summary
Apple, et al., (2005)	Effect of magnesium supplementation on performance, transportation stress and meat quality effects	(1) Halothane gene carriers (2) Mixed sex (3) Finisher stage (n=36; Magnesium supplemented = 18)	*Control* – pigs remain in pen. *Stress* – 3 hours of transport.	*Control* *Magnesium mica* - 2.5% magnesium mica 71 days in feed	*Cortisol* (blood taken every 30 minutes for 3 hours)	Transport stress treatment significantly increased cortisol levels but there was no significant diet – treatment interaction.	Cortisol decreased by 20.85% in magnesium diet pigs exposed to transport stress in comparison to control diet.	o

Author	Aim of study	Genotype, Sex & Age/Stage of Production	Experimental Treatment (s)	Dietary Treatment (s)	Measured outcomes	Results	Size of Effect	Results summary
Caine, et al., (2000)	Effect of magnesium supplementation at a high or low doses for long or short periods on performance, behaviour, meat quality and carcass composition in pigs with different halothane genotypes	(1) 50% positive for halothane gene, 50% carrier (2) Mixed sex (3) Finisher stage (n=142; magnesium supplemented = 48)	*Lairage and slaughter*	*Control* *Long term low level* - Magnesium aspartate hydrochloride 5mg/Kg in feed for 43 days before slaughter *Short term high level* - Magnesium aspartate hydrochloride 40mg/Kg for 7 days in feed before slaughter	*Behaviour* (assessed for 2h, 3 days prior to slaughter and during the 1h lairage period in the abattoir)	Increased aggression in short-term high-dose pigs. Aggression was twice as high in carrier genotype pigs. Long term low level magnesium had no effect.	Aggression increased by 113.04% and 68.97% in short term high dose pigs in comparison to long term low dose and control diets respectively (3 days prior to slaughter).	-

Author	Aim of study	Genotype, Sex & Age/Stage of Production	Experimental Treatment (s)	Dietary Treatment (s)	Measured outcomes	Results	Size of Effect	Results summary
D'Souza, et al. (1998)	Investigate whether dietary magnesium can improve meat quality.	(1) Large white X Landrace (2) Male (3) Finisher stage (n=48; magnesium supplemented = 24)	*Minimal handling* at the abattoir *Heavy handling* at the abattoir	*Control* *Magnesium aspartate 100mg* supplemented in feed 5 days prior to slaughter *Magnesium aspartate 230mg* supplemented in feed 5 days prior to slaughter	*Plasma epinephrine and norepinephrine* - blood collected at time of exsanguination.	Pigs receiving supplementary magnesium had significantly lower plasma norepinephrine than control pigs but there was no difference in epinephrine. There was no difference between handling or supplement doses.	A magnesium diet decreased plasma norepinephrine by 50.00% when pigs were exposed to minimal handling at the abattoir.	+

Author	Aim of study	Genotype, Sex & Age/Stage of Production	Experimental Treatment (s)	Dietary Treatment (s)	Measured outcomes	Results	Size of Effect	Results summary
D'Souza, et al., (1999)	Compare the effect of three different magnesium supplements on the pork quality of pigs that are stressed before slaughter.	(1) Large White X Landrace (2) Male (3) 90Kg – Finisher (n=48)	*Negative handling in abattoir* - All pigs experienced 15 electric shocks 5 minutes before slaughter.	*Control* *Magnesium aspartate 40g* *Magnesium sulphate 31.6g* *Magnesium chloride 38.3g* Supplements fed for 5 days prior to slaughter. All magnesium diets are equal to 3.2g elemental magnesium per magnesium diet.	*Plasma noradrenaline and adrenaline* - blood collected at time of exsanguination.	No significant difference found in plasma adrenaline and noradrenaline between diets.	Adrenaline SED – 0.896 Noradrenaline SED – 0.522	o

Author	Aim of study	Genotype, Sex & Age/Stage of Production	Experimental Treatment (s)	Dietary Treatment (s)	Measured outcomes	Results	Size of Effect	Results summary
Ehrenbergt, et al, (1991)	Can dietary magnesium reduce the effects of porcine stress syndrome?	(1) Landrace (2) Sex not reported (3) Age not reported (n=10; magnesium supplemented = 10)	*Stress* - run on an ergometer at 1.3m/s for 10 minutes.	*Magnesium aspartate hydrochloride 40mg* per Kg in feed for 2 days	*Respiratory rate* *Rectal temperature* *Heart rate* *Blood* - sampled before and over 24h after stressors	Magnesium reduced hyperventilation and tachycardia after stress over a 24h period. Glucose and lactate were significantly reduced during and after stress.	Not reported	+

Author	Aim of study	Genotype, Sex & Age/Stage of Production	Experimental Treatment (s)	Dietary Treatment (s)	Measured outcomes	Results	Size of Effect	Results summary
O'Driscoll, et al. (2013a)	Does magnesium from an organic source (marine algae) improve the welfare of undocked pigs?	(1) Large white X Landrace (2) Mixed sex (3) 4 - 20 weeks (n=448; magnesium supplemented = 224)	*Mixing* - 56 days *Out of feed event* - at day 112, where all pigs had no access to food for 21 hours.	*Control* *Marine algae extract (Mg – 59,520ppm)* 5% in feed throughout study (92 days). (Separate male and female control and supplement groups)	*Salivary cortisol* - collected from focal pigs 1 day before and 2 days after the mixing and out of feed events *Skin and tail lesions* - recorded for focal pigs 1 day before and 2 days after the mixing and out of feed events *Behaviour* - recorded after mixing and during out of feed event	*Mixing*: Salivary cortisol was lower in supplemented females than control females. No effect of supplement on aggressive and harmful behaviours or number of skin or tail lesions *Out of feed event*: No effect of diet on behaviour. No effect of diet on salivary cortisol. Supplemented pigs had fewer body lesions overall	Female supplemented pigs on average had salivary cortisol levels that were 20.94% lower than control pigs during mixing. Control animals had 24.24% higher lesion scores than control pigs during the out-of-feed event.	+

Author	Aim of study	Genotype, Sex & Age/Stage of Production	Experimental Treatment (s)	Dietary Treatment (s)	Measured outcomes	Results	Size of Effect	Results summary
O'Driscoll, et al. (2013b)	Does magnesium from an organic (marine algae) source improve welfare of growing pigs?	(1) Large white X Landrace (2) Mixed sex (3) 4 – 21 weeks (focus on grower /finisher period) (n=448; magnesium supplemented = 224)	*Mixing* - on day 56	*Control* *Marine algae extract (Mg – 59,520ppm) 0.05%* in feed throughout study (63 days) (Separate male and female control and supplement groups)	*Salivary cortisol* - collected from focal pigs between days 46-56 and on the same days as the lesion scores were recorded *Tail and skin lesions* – recorded for focal pigs on multiple days *Behaviour* – of focal pigs continually observed for 5 minutes each morning and afternoon	No change in frequency of aggressive/harmful behaviours but the duration was significantly less for supplemented pigs. No effect of diet on tail lesions. Female supplemented pigs had significantly lower skin lesions than other pigs. Supplemented pigs had lower shoulder and ear lesion scores and salivary cortisol.	Duration of aggressive/harmful behaviours was 8.33% less for the supplemented group in comparison to the control. Salivary cortisol was 11.56% lower in supplemented pigs.	+

Author	Aim of study	Genotype, Sex & Age/Stage of Production	Experimental Treatment (s)	Dietary Treatment (s)	Measured outcomes	Results	Size of Effect	Results summary
Otten, et al. (1995)	Investigate the effects of dietary magnesium on stress, blood metabolites and meat quality in different genotype pigs.	(1) 18 landrace, 18 pietrain. (18 were halothane gene positive) (2) 24 males & 12 females (3) Finisher stage (n= 36; magnesium supplemented = ~24)	*Slaughter*	*Control* *Magnesium fumarate 10g/kg* in feed from 30kg – 100kg live weight *Magnesium fumarate 20g/kg* in feed from 30kg – 100kg live weight	*Plasma cortisol, epinephrine and norepinephrine* - blood samples at 35Kg, 57Kg and 87Kg.	Both 10g/kg and 20g/kg of supplementary magnesium reduced plasma cortisol and norepinephrine Magnesium supplementation had no significant impact on plasma epinephrine concentration	Norepinephine was decreased by 31.94% and 18.85% when dietary magnesium as added at 10g and 20g respectively in comparison to the control. Cortisol was decreased by 30.67% and 32.91% dietary magnesium as added at 10g and 20g respectively in comparison to the control.	+

Author	Aim of study	Genotype, Sex & Age/Stage of Production	Experimental Treatment (s)	Dietary Treatment (s)	Measured outcomes	Results	Size of Effect	Results summary
Panella-Riera, et al., (2008)	Do natural tranquilisers (magnesium) have different effects depending on the pigs' genotype.	(1) Landrace, large white and pietrain. 34 halothane gene positive and 27 negative (2) Male (3) Finisher stage (n=61; magnesium supplemented = ~20)	*CO_2 stunning and slaughter*	*Control* *Magnesium carbonate 1.28g/kg* in feed 5 days prior to slaughter	*Behaviour* - on the raceway before entering the CO_2 stunning unit and in the decent to the pit.	In halothane gene negative pigs, magnesium supplemented pigs took longer to attempt the first retreat in the stunning unit. The opposite occurred in halothane gene positive pigs. All other behavioural results were non-significant	Retreat attempts in the CO_2 stunning unit were 2.51% lower when pigs were halothane gene negative in comparison to halothane gene positive.	+

Author	Aim of study	Genotype, Sex & Age/Stage of Production	Experimental Treatment (s)	Dietary Treatment (s)	Measured outcomes	Results	Size of Effect	Results summary
Panella-Riera, et al., (2009)	Investigate the effects of supplementing with magnesium with tryptophan on meat quality, feed intake, mortality and behaviour in pigs with different genotypes.	(1) 33 halothane positive (large white and landrace). 33 halothane negative (pietrain) (2) Males (3) Finisher stage (n=69; magnesium supplemented = ~23)	*CO_2 stunning and slaughter*	*Control* *Elemental magnesium 1.2g/kg and 8g L-tryptophan 8g/kg* in feed 5 days prior to slaughter	*Behaviour* - recorded in the corridor of the abattoir before stunning and during exposure to CO_2.	No difference in feed intake or in behaviour in the abattoir. Magnesium supplemented pigs had more severe skin lesions.	Magnesium and tryptophan diet resulted in a 494.64% increase in “severe skin damage” and a 125.23% increase in “skin damage effecting quality” in comparison to a control diet. “Slight skin damage” and “no skin damage” decreased by 69.96% and 100% respectively.	-

Author	Aim of study	Genotype, Sex & Age/Stage of Production	Experimental Treatment (s)	Dietary Treatment (s)	Measured outcomes	Results	Size of Effect	Results summary
Peeters, et al., (2005)	Effect of magnesium supplementation on stress responses of pigs during transportation.	(1) Peitrain x Hypor (halothane carriers) (2) Sex not reported (3) Finisher stage (n=126; Mg supplemented= 21)	*Transport simulation* - groups of 3, pigs were subjected to vibration for 2 hours in a vibration crate designed to simulate transport.	*Control* *Magnesium acetate 3g/L* in water for 2 days.	*Behaviour* - level of restlessness were observed by camera above the vibration station. *Salivary cortisol* – collected the day before and after treatment and after the recovery period	Magnesium treated pigs spent more time lying down during the second half an hour of vibrations. Supplemented pigs were visibly calmer than controls. Salivary cortisol levels of magnesium pigs did not return to the level recorded before the stressor as quickly in comparison to other dietary treatments.	Salivary cortisol was 30.36% higher in magnesium diet pigs than the negative control after stress.	+/-

Author	Aim of study	Genotype, Sex & Age/Stage of Production	Experimental Treatment (s)	Dietary Treatment (s)	Measured outcomes	Results	Size of Effect	Results summary
Peeters, et al., (2006)	Effect of magnesium supplementation on stress responses, skin lesion and meat quality.	(1) Peitrain x Hypor (halothane carriers) (2) Sex not reported (3) Finisher stage (n=352; Mg supplemented = 22)	*Transport and slaughter*	*Control* *Magnesium acetate 3g/L* for 2 days in drinking water	*Plasma cortisol* - 10 control and 10 magnesium supplemented pigs selected for blood sampling *Skin lesions* – recorded after slaughter	No effect of magnesium on cortisol measurements at slaughter. Magnesium supplemented pigs had fewer loin lesions.	A magnesium diet resulted in 43.06% fewer loin lesions than the control diet.	+

Author	Aim of study	Genotype, Sex & Age/Stage of Production	Experimental Treatment (s)	Dietary Treatment (s)	Measured outcomes	Results	Size of Effect	Results summary
Porta, et al., (1995)	Can magnesium supplementation reduce stress and improve meat quality.	(1) Landrace (2) Sex not reported (3) Finisher stage (n=45; magnesium supplemented = 15)	*Transport and slaughter*	*Control* *Magnesium aspartate hydrochloride 40mg/Kg* in water 5 days before transport & slaughter. *Magnesium aspartate hydrochloride 5mg/Kg* in feed for 115 days.	*Serum cortisol, epinephrine and norepinephrine* - blood collected at slaughter	Serum cortisol was lower in high-level short-term magnesium supplementation in comparison to control pigs. Cortisol was higher in low-level long-term magnesium supplemented pigs. Serum epinephrine was significantly lower in both magnesium groups.	Serum cortisol was reduced by 19.51% when magnesium was given at 40mg for 5 days. Serum cortisol was increased by 53.66% when magnesium was given at 5mg for 115 days.	+/-

Author	Aim of study	Genotype, Sex & Age/Stage of Production	Experimental Treatment (s)	Dietary Treatment (s)	Measured outcomes	Results	Size of Effect	Results summary
Tang et al. (2008)	Effect of magnesium supplementation on blood parameters and meat quality in relation to transport stress.	(1) Duroc x Large White x Yorkshire (2) Male; (3) Finisher stage (n = 36; magnesium supplemented = 12)	*Control* *Stress*- 2 hours of transportation.	*Control* *Magnesium aspartate 1000mg/Kg* 5 days before slaughter *Magnesium aspartate 2000mg/Kg* 5 days before slaughter	*Serum cortisol* - blood was collected during slaughter.	Magnesium decreased serum cortisol levels but not significantly.	In the transport before slaughter treatment group there was a 14.98% and 17.90% decrease in serum cortisol when supplemented with 1000mg/Kg and 2000mg/Kg respectively	o
Tang, et al., (2009)	Effect of magnesium supplementation on blood parameters and meat quality in relation to transport stress.	(1) Large White x Landrace (2) Male; (3) Finisher stage (n = 24; magnesium supplemented = 12)	*Control* – no transport *Stress* - 1.5h of transportation.	*Control* *Magnesium aspartate 1000mg/Kg* in feed 9 days before slaughter	*Serum cortisol* - blood was collected immediately after stressor or non-stressor	Non-significant trend for magnesium supplemented pigs to have lower serum cortisol	When exposed to the transportation treatment, the magnesium diet showed a 15.48% decrease in serum cortisol in comparison to the control.	o

Author	Aim of study	Genotype, Sex & Age/Stage of Production	Experimental Treatment (s)	Dietary Treatment (s)	Measured outcomes	Results	Size of Effect	Results summary
Tarsitano, et al. (2013)	Evaluate the effects of magnesium supplementation 7 days before slaughter on meat quality and performance.	(1) Landrace x Large White (2) Male (3) Finisher stage (n = 48; magnesium supplemented = 36)	*Transport and slaughter*	*Control* *Magnesium oxide 0.2%* for 7 days in feed. *Magnesium oxide 0.4%* for 7 days in feed. *Magnesium oxide 0.6%* for 7 days in feed.	*Plasma cortisol* - blood was collected immediately after slaughter	Supplementary magnesium decreased the concentration of plasma cortisol concentration.	Serum cortisol was decreased by 23.68% in comparison to the control when magnesium was included at 0.4% or 0.6%. Serum cortisol was increased by 13.58% when magnesium was included at 0.2%	+

Appendix B

Supporting material for Chapter Three.

Survey

The use of additional magnesium to reduce stress within UK pig herds.

Diet has a major impact on your pig herd's welfare and productivity. My PhD project, which is supervised by Professors Lisa Collins and Louise Dye at the University of Leeds, is testing whether magnesium improves performance, health and welfare. I would like to understand what UK pig producers think about using additional magnesium, and your experiences and thoughts on this practice.

This survey should take less than 10 minutes to complete. All answers will be anonymised and no identifying information will be collected. Please do not leave any information that may identify you or your farm in answer to any of the questions. By completing this survey, you are agreeing that you are a pig farmer or keeper based in the UK, over the age of 18 and to the anonymous information you provide being used in my PhD project and stored in accordance with the University of Leeds Privacy Policies (see here: https://dataprotection.leeds.ac.uk/wp-content/uploads/sites/48/2019/02/Research-Privacy-Notice.pdf)

This survey is voluntary and you can opt out at any point during the survey by closing down the survey. However, due to the anonymous nature of the survey, once the survey has been submitted it will not be possible to withdraw your answers.

If you have any comments or questions about the survey or the project, please email me: Emily Bushby (bsevbu@leeds.ac.uk) or my supervisors (l.collins@leeds.ac.uk or l.dye@leeds.ac.uk).

Thank You!

1. In what country is the farm located?
   [DROPDOWN SELECTION OF COUNTRIES AUTOMATICALLY CREATED BY QUALTRICS]

   Afghanistan
   Albania
   Algeria
   Andorra
   Angola
   Antigua and Barbuda
   Argentina
   Armenia
   Australia
   Austria
   Azerbaijan
   Bahamas
   Bahrain
   Bangladesh
   Barbados
   Belarus
   Belgium
   Belize
   Benin
   Bhutan

Bolivia

Bosnia and Herzegovina

Botswana

Brazil

Brunei Darussalam

Bulgaria

Burkina Faso

Burundi

Cambodia

Cameroon

Canada

Cape Verde

Central African Republic

Chad

Chile

China

Colombia

Comoros

Congo, Republic of the...

Costa Rica

Côte d'Ivoire

Croatia

Cuba

Cyprus

Czech Republic

Democratic Republic of the Congo

Denmark

Djibouti

Dominica

Dominican Republic

Ecuador

Egypt

El Salvador

Equatorial Guinea

Eritrea

Estonia

Ethiopia

Fiji

Finland

France

Gabon

Gambia

Georgia

Germany

Ghana

Greece

Grenada

Guatemala

Guinea

Guinea-Bissau

Guyana

Haiti

Honduras

Hong Kong (S.A.R.)

Hungary

Iceland

India

Indonesia

Iran

Iraq

Ireland

Israel

Italy

Jamaica

Japan

Jordan

Kazakhstan

Kenya

Kiribati

Kuwait

Kyrgyzstan

Lao People's Democratic Republic

Latvia

Lebanon

Lesotho

Liberia

Libyan Arab Jamahiriya

Liechtenstein

Lithuania

Luxembourg

Madagascar

Malawi

Malaysia

Maldives

Mali

Malta

Marshall Islands

Mauritania

Mauritius

Mexico

Micronesia, Federated States of...

Monaco

Mongolia

Montenegro

Morocco

Mozambique

Myanmar

Namibia

Nauru

Nepal

Netherlands

New Zealand

Nicaragua
Niger
Nigeria
North Korea
Norway
Oman
Pakistan
Palau
Panama
Papua New Guinea
Paraguay
Peru
Philippines
Poland
Portugal
Qatar
Republic of Moldova
Romania
Russian Federation
Rwanda
Saint Kitts and Nevis
Saint Lucia
Saint Vincent and the Grenadines
Samoa
San Marino
Sao Tome and Principe
Saudi Arabia
Senegal
Serbia
Seychelles
Sierra Leone
Singapore
Slovakia
Slovenia

Solomon Islands
Somalia
South Africa
South Korea
Spain
Sri Lanka
Sudan
Suriname
Swaziland
Sweden
Switzerland
Syrian Arab Republic
Tajikistan
Thailand
The former Yugoslav Republic of Macedonia
Timor-Leste
Togo
Tonga
Trinidad and Tobago
Tunisia
Turkey
Turkmenistan
Tuvalu
Uganda
Ukraine
United Arab Emirates
United Kingdom of Great Britain and Northern Ireland
United Republic of Tanzania
United States of America
Uruguay
Uzbekistan
Vanuatu
Venezuela, Bolivarian Republic of...
Viet Nam

Yemen

Zambia

Zimbabwe

2. How would you describe your farm?

- Farrow to finish
- Wean to finish
- Grower to finish
- Breeding herd
- Other (please state)

3. Is your herd open or closed?

- Open
- Closed

4. Approximately, what is the total size of your pig herd?

- 0 – 500
- 500 – 1000
- 1000 – 5000
- 5000 – 10,000
- 10,000 – 15,000
- More than 15,000 (please state)

5. Is your farm an indoor or outdoor pig system?

- Indoor (slatted)
- Indoor (straw)
- Outdoor
- Outdoor bred and reared indoors (slatted)
- Outdoor bred and reared indoors (straw)
- Indoor bred (slatted) and reared outdoors
- Indoor bred (straw) and reared outdoors
- Other (please state)

6. Which genotype/breed is the dam line? Please select all that apply.

- Large white
- Landrace
- Large White X Landrace
- Duroc
- Welsh
- Hampshire
- Berkshire
- Gloucestershire old spot
- Tamworth
- Saddleback
- Lop
- Oxford Sandy and Black
- Other (please state)

7. Which genotype/breed is the sire line? Please select all that apply.

- Large white
- Landrace
- Large White X Landrace
- Duroc
- Welsh
- Hampshire
- Berkshire
- Gloucestershire old spot
- Tamworth
- Saddleback
- Lop
- Oxford Sandy and Black
- Other (please state)

8. Please indicate how much you agree or disagree with each statement below by selecting the answer which most reflects your views.

8(a) Additional magnesium (in addition to the standard amount in feed) may reduce stress in pigs. (Stress can be defined as the animal making physiological or behavioural changes to cope with its environment, e.g. being more alert)

- Yes, additional dietary magnesium reduces stress in pigs
- Yes, additional dietary magnesium reduces stress in pigs in some circumstances
- No additional dietary magnesium increases stress in pigs
- No, additional dietary magnesium increases stress in pigs in some circumstances
- No, additional magnesium does not influence stress in pigs
- I am unsure whether additional dietary magnesium reduces stress in pigs

8(b) Additional magnesium (in addition to the standard amount in feed) may reduce aggressive or harmful behaviours in pigs.

- Yes, additional dietary magnesium reduces aggressive and/or harmful behaviours in pigs
- Yes, additional dietary magnesium reduces aggressive and/or harmful behaviours in pigs in some circumstances
- No, additional dietary magnesium increases aggressive and/or harmful behaviours in pigs
- No, additional dietary magnesium increases aggressive and/or harmful behaviours in pigs in some circumstances
- No, additional magnesium does not influence aggressive and/or harmful behaviours in pigs
- I am unsure whether additional dietary magnesium alters aggressive and/or harmful behaviours in pigs

8(c) Additional magnesium (in addition to the standard amount in feed) may improve pig performance measures (e.g. average daily gain).

- Yes, additional dietary magnesium improves pig performance
- Yes, additional dietary magnesium improves pig performance in some circumstances

- No, additional dietary magnesium negatively affects pig performance
- No, additional dietary magnesium negatively affects pig performance in some circumstances
- No, additional magnesium does not alter pig performance
- I am unsure whether additional dietary magnesium alters pig performance

8(d) Additional magnesium (in addition to the standard amount in feed) may improve meat quality.

- Yes, additional dietary magnesium improves meat quality
- Yes, additional dietary magnesium improves meat quality in some circumstances
- No, additional dietary magnesium negatively affects meat quality
- No, additional dietary magnesium negatively affects meat quality in some circumstances
- No, additional magnesium does not alter meat quality
- I am unsure whether additional dietary magnesium improves meat quality.

9. Do you or have you previously used magnesium in pig feed or water in addition to standard amounts in the mineral pack or feed?

- Yes, I currently use magnesium (skip to question 8)
- Yes, I have previously used magnesium (skip to question 9)
- No, I have never used magnesium (skip to end of survey)

10. *Currently using additional magnesium*

10(a). When adding additional magnesium, do you add it to:

- Feed
- Water
- Both
- Other (please state)

10(b). At which age or stage of production do you typically use additional magnesium? Please select all that apply:

- Piglets (approximately 0-4 weeks of age)
- Weaners (approximately 4-7 weeks of age)
- Growers (approximately 8-12 weeks of age)
- Finishers (approximately 13 weeks – slaughter weight)
- Breeding sows
- Boars
- Other (please state)

10(c). Approximately, how long do you expect each pig will receive additional magnesium?

- < 1 week
- 1-2 weeks
- 2-4 weeks
- 1 - 3 months
- 3 - 6 months
- 6 - 12 months
- 12 – 24 months
- Permanently / indefinitely
- Other (please state)

10(d). Which type of additional magnesium do you currently use?

- Magnesium Phosphate
- Magnesium Oxide
- Magnesium sulphate
- Magnesium Mica
- Magnesium Aspartate
- Magnesium Chloride
- Magnesium Fumarate
- Magnesium Carbonate
- Magnesium Acetate
- Other (please state)

- I do not know the type of magnesium

10(e). If you have changed type of magnesium, please state which type of additional magnesium you previously used.

- Magnesium Phosphate
- Magnesium Oxide
- Magnesium sulphate
- Magnesium Mica
- Magnesium Aspartate
- Magnesium Chloride
- Magnesium Fumarate
- Magnesium Carbonate
- Magnesium Acetate
- Other (please state)
- I do not know the type of magnesium
- Not applicable

10(f). If possible, can you indicate approximately, how much additional magnesium you currently use?

- 0.01 – 0.1 %
- 0.1 – 0.2 %
- 0.2 – 0.3 %
- 0.3 – 0.4 %
- Other (please state)

10(g). Where did you first hear about using additional magnesium?

- Advice from nutritionist
- Word of mouth
- Own research
- Magazine or news article
- Other (please state)

10(h). What are the main reasons you decided to use additional magnesium? Please select all that apply.

- Stress within the herd or group
- Aggressive or harmful behaviours
- Meat quality
- Performance
- Health
- Advised by a nutritionist
- Other (please state)

10(i). Since giving additional magnesium to your pigs, have you noticed any change in stress in the herd or group of pigs? (Stress can be defined as the animal making physiological or behavioural changes to cope with its environment, e.g. being more alert)

- Yes, I have seen a large decrease in stress within the herd/group
- Yes, I have seen a small decrease in stress within the herd/group
- Yes, I have seen a large increase in stress within the herd/group
- Yes, I have seen a small increase in stress within the herd/group
- No, I have seen no change in stress within the herd/group

10(j). Since giving additional magnesium to your pigs, have you noticed any change in aggressive or harmful behaviours?

- Yes, I have seen a large decrease in aggressive and/or harmful behaviours
- Yes, I have seen a small decrease in aggressive and/or harmful behaviours
- Yes, I have seen a large increase in aggressive and/or harmful behaviours
- Yes, I have seen a small increase in aggressive and/or harmful behaviours

- No, I have seen no change in aggressive and/or harmful behaviours

10(k). Since giving additional magnesium to your pigs, have you seen any change in performance?

- Yes, I have seen a large positive effect on pig performance measures
- Yes, I have seen a small positive effect on pig performance measures
- Yes, I have seen a large negative effect on pig performance measures
- Yes, I have seen a small negative effect on pig performance measures
- No, I have seen no change in pig performance measures

10(l). Since giving additional magnesium to your pigs, have you seen any change in meat quality?

- Yes, I have seen a large positive effect on meat quality
- Yes, I have seen a small positive effect on meat quality
- Yes, I have seen a large negative effect on meat quality
- Yes, I have seen a small negative effect on meat quality
- No, I have seen no change in meat quality
- Not applicable - I do not rear pigs to slaughter age
- Not applicable - I do not receive feedback on meat quality.

10(m). Based on your experience, would you recommend using additional magnesium to other producers?

- Yes
- No

11. *Previously used additional magnesium*

11(a). When adding additional magnesium, did you add it to:

- Feed
- Water
- Both
- Other (please state)

11(b). At which age or stage of production did you use additional magnesium? Please select all that apply:

- Piglets (0-4 weeks of age)
- Weaners (4-7 weeks of age)
- Growers (8-12 weeks of age)
- Finishers (13 weeks – slaughter weight)
- Breeding sows
- Boars
- Other (please state)

11(c). Approximately, how long did each pig receive additional magnesium?

- < 1 week
- 1-2 weeks
- 2-4 weeks
- 1 - 3 months
- 3 - 6 months
- 6 - 12 months
- 12 – 24 months
- Permanently / indefinitely
- Other (please state)

11(d). Which type of additional magnesium did you previously use?

- Magnesium Phosphate
- Magnesium Oxide
- Magnesium Sulphate
- Magnesium Mica
- Magnesium Aspartate

- Magnesium Chloride
- Magnesium Fumarate
- Magnesium Carbonate
- Magnesium Acetate
- Other (please state)
- I do not know the type of magnesium

11(e). If you have changed type of magnesium, please state which type of magnesium you previously used.

- Magnesium Phosphate
- Magnesium Oxide
- Magnesium Sulphate
- Magnesium Mica
- Magnesium Aspartate
- Magnesium Chloride
- Magnesium Fumarate
- Magnesium Carbonate
- Magnesium Acetate
- Other (please state)
- I do not know the type of magnesium
- Not applicable

11(f). If possible, can you indicate approximately, how much additional magnesium you previously used?

- 0.01 – 0.1 %
- 0.1 – 0.2 %
- 0.2 – 0.3 %
- 0.3 – 0.4 %
- Other (please state)

11(g). Where did you first hear about using additional magnesium?

- Advice from nutritionist

- Word of mouth
- Own research
- Magazine or news article
- Other (please state)

11(h). What were the main reasons you decided to use additional magnesium? Please select all that apply.

- Stress within the herd or group
- Aggressive or harmful behaviours
- Meat quality
- Performance
- Health
- Advised by a nutritionist
- Other (please state)

11(i). Why did you stop using additional magnesium? Please select all that apply.

- Increase in stress within the herd or group
- Increase in aggressive or harmful behaviours
- Negative impact on meat quality
- Negative impact on pig performance
- Negative impact on pig health
- Advised by a nutritionist
- Found no benefit or effect of the additional magnesium
- Cost
- Other (please state)

11(j). While giving additional magnesium to your pigs, did you noticed any change in stress within the herd or group? (Stress can be defined as the animal making physiological or behavioural changes to cope with its environment, e.g. being more alert)

- Yes, I saw a large decrease in stress within the herd or group

- Yes, I saw a small decrease in stress within the herd or group
- Yes, I saw a large increase in stress within the herd or group
- Yes, I saw a small increase in stress within the herd or group
- No, I saw no change in stress within the herd or group

11(k). While giving additional magnesium to your pigs, did you noticed any change in aggressive or harmful behaviours?

- Yes, I saw a large decrease in aggressive and/or harmful behaviours
- Yes, I saw a small decrease in aggressive and/or harmful behaviours
- Yes, I saw a large increase in aggressive and/or harmful behaviours
- Yes, I saw a small increase in aggressive and/or harmful behaviours
- No, I saw no change in aggressive and/or harmful behaviours

11(l). While giving additional magnesium to your pigs, have you seen any change in performance?

- Yes, I saw a large positive effect on pig performance measures
- Yes, I saw a small positive effect on pig performance measures
- Yes, I saw a large negative effect on pig performance measures
- Yes, I saw a small negative effect on pig performance measures
- No, I saw no change in pig performance measures

11(m). While giving additional magnesium to your pigs, did you see any change in meat quality since giving additional magnesium to your pigs?

- Yes, I saw a large positive effect on meat quality
- Yes, I saw a small positive effect on meat quality
- Yes, I saw a large negative effect on meat quality
- Yes, I saw a small negative effect on meat quality
- No, I saw no change in meat quality
- Not applicable – I do not rear pigs to slaughter age
- Not applicable - I do not receive feedback on meat quality.

11(n). Based on your experience, would you recommend using additional magnesium to other producers?

- Yes
- No

Thank you for your time, please feel free to leave any additional comments or relevant information if you wish. Please do not include any identifying information such as names, addresses or contact details.

[TEXT BOX]

Pig World e-mail newsletter advert

Can nutrition reduce stress and increase performance and welfare?

Including additional dietary magnesium may reduce stress, resulting in improved pig performance and welfare. At the University of Leeds we want to understand UK pig farmers' views and experiences using magnesium. If you would like to contribute, take a quick survey here.

(UK farmers, 18+, answers anonymised, and no identifying information collected. Image: Simon Vine Photography)

Survey responses

Table B 1. Number and percentage of respondent's answers to each question. For length, unselected answers not stated in the table.

Question	Answer	Number of respondents	Percentage of respondents	Comments
Country	UK	24	96%	
	Ireland	1	4%	
How would you describe your farm?	Farrow to finish	18	72%	
	Wean to finish	2	8%	
	Grower to finish	2	8%	
	Breeding herd	3	12%	
Is your herd open or closed?	Open	8	32%	
	Closed	17	68%	

Approximately, what is the total size of your pig herd?	0 - 500 pigs	3	12%	
	500 1000 pigs	1	4%	
	1000 - 5000 pigs	11	44%	
	5000 - 10,000 pigs	7	28%	
	10,000 - 15,000 pigs	1	4%	
	More than 15,000 (please state)	2	8%	
Is your farm an indoor or outdoor pig system?	Indoor (slatted)	11	44%	
	Indoor (straw)	6	24%	
	Outdoor bred and reared indoors (slatted)	1	4%	
	Outdoor bred and reared indoors (straw)	2	8%	
	Other (please state)	5	20%	Indoor straw and slatted; indoor straw & slats; Indoor farrow, indoor finish on straw (some on slats for 6weeks after weaning) dry sows outdoors; A mixture. Sows are farrowed and served indoors but after scanning at 5 weeks they spend the rest of their pregnancy outdoors. two thirds of progeny are reared to 40kg on slatts the remainder on straw, then from

				40kg to slaughter all are on straw.; Dry sows outdoors farrow indoors on slats pigs mostly reared on slats finished on straw
Which genotype/breed is the dam line?	Large White X Landrace	18	72%	
	Large White	4	16%	
	Landrace	3	12%	
	Duroc	4	16%	
	Welsh	1	4%	
	Hampshire	1	4%	
	Gloucestershire old spot	1	4%	
	Lop	1	4%	
	Other	3	12%	Durocxlandrace; Rattlerow landroc; Middle White.
Which genotype/breed is the sire line?	Large White X Landrace	4	16%	
	Large White	3	12%	
	Landrace	1	4%	
	Duroc	14	56%	
	Welsh	1	4%	
	Hampshire	5	20%	
	Gloucestershire old spot	1	4%	
	Lop	1	4%	
	Berkshire	1	4%	
	Saddleback	1	4%	
	Other	9	36%	Pic genetics; Middle white; tempo / jsr 900; Tempo; Pietran;

				hybred currently mostly JSR Tempo; Rattlerow maximus (similar to pietrain); JSR 900; optimus rattlerow
Additional magnesium (in addition to the standard amount in feed) may reduce stress in pigs.(Stress can be defined as the animal making physiological or behavioural changes to cope with its environment, e.g. being more alert)	Yes, additional dietary magnesium reduces stress in pigs	2	8%	
	Yes, additional dietary magnesium reduces stress in pigs in some circumstances	7	28%	
	No, additional magnesium does not influence stress in pigs	1	4%	
	I am unsure whether additional dietary magnesium reduces stress in pigs	15	60%	
Additional magnesium (in addition to the standard	Yes, additional dietary magnesium	3	12%	

amount in feed) may reduce aggressive or harmful behaviours in pigs.	reduces aggressive and/or harmful behaviours in pigs		
	Yes, additional dietary magnesium reduces aggressive and/or harmful behaviours in pigs in some circumstances	6	24%
	No, additional magnesium does not influence aggressive and/or harmful behaviours in pigs	1	4%
	I am unsure whether additional dietary magnesium alters aggressive and/or harmful behaviours in pigs	15	60%
Additional magnesium (in addition to the standard amount in feed) may improve pig performance measures (e.g. average daily gain)	Yes, additional dietary magnesium improves pig performance	0	0
	Yes, additional dietary	8	32%

	magnesium improves pig performance in some circumstances		
	No	0	0
	I am unsure whether additional dietary magnesium alters pig performance	17	68%
Additional magnesium (in addition to the standard amount in feed) may improve meat quality	Yes, additional dietary magnesium improves meat quality	2	8%
	Yes, additional dietary magnesium improves meat quality in some circumstances	4	16%
	No, additional magnesium does not alter meat quality	3	12%
	I am unsure whether additional dietary magnesium improves meat quality	16	64%
Do you, or have you previously, used magnesium in pig feed or water in addition to standard amounts in	Are currently using magnesium	2	8%

the mineral pack or feed?				
	Have previously used magnesium	9	36%	
	Have never used magnesium	14	56%	
Where did you first hear about using additional magnesium?	Advice from nutritionist	2	18.18%	
	Word of mouth	3	27.27%	
	Own research	2	18.18%	
	Other (please state)	4	36.36%	Vet
What were the main reasons you decided to use additional magnesium?	Aggressive or harmful behaviours	6	54.55%	
	Stress within the herd or group	2	18.18%	
	Health	1	9.09%	
	Other (please state)	2	18.18%	vet advice to help with a health issue; Reduce constipation in pre-farrowing sows
Why did you stop using additional magnesium?	Found no benefit or effect of the additional magnesium	3	33.33%	
	Other (please state)	6	66.67%	
Since / While giving additional magnesium to your pigs, did you	No, I saw no change in stress within the herd or group	2	18.18%	

noticed any change in stress within the herd or group?(Stress can be defined as the animal making physiological or behavioural changes to cope with its environment, e.g. being more alert)			
	Yes, I saw a small decrease in stress within the herd or group	6	54.55%
	Yes, I have seen a large decrease in stress within the herd/group	1	9.09%
	Yes, I saw a large increase in stress within the herd or group	2	18.18%
Since / While giving additional magnesium to your pigs, have you noticed any change in aggressive or harmful behaviours?	No, I saw no change in aggressive and/or harmful behaviours	2	18.18%
	Yes, I have seen a large decrease in aggressive and/or harmful behaviours	1	9.09%

	Yes, I have seen a small decrease in aggressive and/or harmful behaviours	7	63.64%
	Yes, I saw a large increase in aggressive and/or harmful behaviours	1	9.09%
Since / While giving additional magnesium to your pigs, have you seen any change in in performance ?	No, I saw no change in pig performance measures	9	81.82%
	Yes, I have seen a small positive effect on pig performance measures	1	9.09%
	Yes, I saw a large positive effect on pig performance measures	1	9.09%
Since / While giving additional magnesium to your pigs, have you seen any change in meat quality?	Not applicable - I do not receive feedback on meat quality.	8	72.73%
	No, I saw no change in meat quality	3	27.27%
When adding additional magnesium, did / do you add it to:	Added magnesium to feed	7	63.64%

	Added magnesium to water	2	18.18%	
	Added magnesium to (other)	2	18.18%	Put magnesium grazing block in yard of pigs; magnesium feed block
Which type of additional magnesium do / did you previously use?	Magnesium Oxide	2	18.18%	
	Magnesium Sulphate	2	18.18%	
	Magnesium Phosphate	1	9.09%	
	Don't know type	4	36.36%	
	Other	2	18.18%	Calcium Magnesite; Emgevet in water.
At which age or stage of production did / do you use additional magnesium?	Weaners (4-7 weeks of age)	1	9.09%	
	Growers (8-12 weeks of age)	4	36.36%	
	Finishers (13 weeks – slaughter weight)	3	27.27%	
	Breeding sows	2	18.18%	
	Other (please state)	1	9.09%	growers and finishers
Approximately, how long did each pig receive / how long do you expect each pig will	< 1 week	3	27.27%	

receive additional magnesium?				
	2-4 weeks	5	45.45%	
	3-6 months	3	27.27%	
If possible, can you indicate approximately, how much additional magnesium you currently use / how much additional magnesium you previously used?	0.01 – 0.1 %	1	9.09%	
	0.1 – 0.2 %	3	27.27%	
	0.3 – 0.4 %	1	9.09%	
	Other (please state)	6	54.55%	different for age groups; do not know; Not sure; not known; By advise from the vet
Based on your experience, would you recommend using additional magnesium to other producers?	Would recommend using magnesium to others	8	33.33%	
	Would NOT recommend using magnesium to others	3	66.67%	Aggressive behaviour stopped; just used in an aggressive batch; Made a more laxative pre-lactation diet using higher levels of

		wheat middlings and soya hulls; Ongoing; It was a very basic trial and something I never considered further; Not stopped.
Thank you for your time, please feel free to leave any additional comments or relevant information if you wish. Please do not include any identifying information such as names, addresses or contact details.		like to know more; I would suggest genotype would have a large influence on stress in pigs; As with all additional products they work on some farms and not others!; Advise by vet; non scientific trial which showed some benefits; The breed of pig on site at the time of use were deliberately bread to be agressive by the breeding company so they would compete more for food in theory. we have discontinued that breed due to their behaviour issues being socially unacceptable both to each other and the people working with them and also their general health was not very robust with the reduced use of antibiotics and routine meds we all aim for; I think it is the ratios of ingredients such as calcium, phosphorus, magnesium that are important, rather

	than just adding a supplement of one mineral.

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