Exploring the role of nutrition in expression of positive and negative social behaviours to improve pig welfare

Eleanor Hewett

Supervisors: Professor Simon Turner (SRUC), Professor Jos Houdijk (SRUC), Dr Craig Lewis (PIC), Dr Uislei Orlando (PIC), Professor Andrea Doeschl-Wilson (UoE)

Declaration

I declare that this thesis is my own composition and that the research described in it is my own work, except where acknowledged. The work described has not been submitted for any other degree or professional qualification. Any included publications are my own work, except where jointly authored publications have been included. My contribution and those of other authors to this work are clearly outlined below and throughout this thesis. I confirm that appropriate credit has been given within this thesis where reference has been made to the work of others.

Parts of this thesis have been published In Journal of Animal Science and submitted for publication in Scientific Reports and PLOS ONE. I declare that the permission to include manuscripts in this thesis has been obtained from publishers and all the co-authors.

Signed: Eleanor Hewett

Acknowledgements and Dedication

Thank you to EastBio, BBSRC and PIC for providing me this opportunity with your funding. Thank you, PIC, for providing valuable learning opportunities.

Thank you to my supervisors and thesis committee. Your support both academic and pastoral has been crucial to the completion of this thesis. Special thanks to Professor Simon Turner, who bore the burden of me and my nonsense, giving hours of his own time, with generosity and grace.

Thank you to all the technicians, farm staff and research staff who have helped and supported me. You taught me important lessons about pigs and people. Special thanks to Marianne Farish for being an all-round legend.

Thank you to all the incredible, inspirational women I met along the away. My sisters in science, my friends, my loved ones. You listened, laughed and lifted me up when I needed it most. I will be forever grateful that we all ended up in the same place at the same time.

Thank you to my family, for the long-distance love and pork free Christmases. Thank you, Juno, for getting me up on those cold, dark mornings.

I dedicate this thesis to the pigs: the porridge twins, Minny, Tiny Tim, Dobbie, Coco, Raule, Scabby, Big Ted, Bad Pig, Stevie Wonderful, Crazy Eyes, Prune Pig, Ukulele, Spooky, Disco Foot, Snoopy, Elton John, Ziggy, Naked Mole Rat, Quasimodo, Black Beauty, Tom, Baby and the thousands of others I didn’t have the pleasure to get to know personally. One day, I hope everyone will see you as I do.

Publications

Hewett, E., L. Zaragoza, C. Lewis, J. Houdijk, A. Wilson, and S. Turner. 2025. Exploring the effects of dietary lysine and tryptophan on the social behaviour of pigs. J. Anim. Sci. skaf030. doi:10.1093/jas/skaf030.

Conference Contributions

Hewett, E., L. Zaragoza, U. Orlando, C. Lewis, J. Houdijk, A. Wilson, and S. Turner. 2023. Exploring the effects of dietary amino acid levels on the social behaviour of pig. 56th Congress of the International Society for Applied Ethology, p111

Hewett, E., L. Zaragoza, U. Orlando, C. Lewis, J. Houdijk, A. Wilson, and S. Turner. 2024. 101. Exploring the effects of dietary amino acid levels on the social behaviour and performance of commercial pigs. Proceedings of the British Society of Animal Science (BSAS 2024) doi:10.1016/j.anscip.2024.02.102.

Hewett, E., J. Houdijk, A. Wilson, C. Lewis and S. Turner. 2025. Exploring the role of dietary energy in the expression of play behaviour in pigs. 58th Congress of the International Society for Applied Ethology, p113

E. Hewett, L. Zaragoza, C. Lewis, J. Houdijk, A. Wilson, S. Turner 2005. Exploring the effects of dietary amino acid levels on the behaviour and performance of pigs. 76th Annual Meeting of The European Federation of Animal Science, p81

Abstract

The pig is a highly social and intelligent animal. One of the most numerous farmed animals globally, it's domestication and intensive production have led to significant welfare issues. One of the main ways to conceptualise animal welfare argues that welfare issues arise when animals are prevented from performing their natural behaviours. Despite their domestication, pigs still retain a strong desire and motivation to perform behaviours seen in their wild counterparts. It is therefore important to have knowledge of the pigs' wild behaviour to enable us to improve their welfare. In the wild pigs live in small groups of related females, called sounders. Post-pubertal males live a solitary life and only interact with females during mating. At mating, males will fight with one another in competition for access to a female. Other than at mating, fighting is rare in wild pigs as they mutually avoid pigs in neighbouring home ranges. Within the sounder, some aggressive behaviours may be seen over access to food or other resources. Largely, sounders have a stable dominance hierarchy, with sows cooperatively raising and protecting piglets and piglets playing and foraging with piglets from other litters.

Both wild and domestic pigs display a range of positive social behaviours, including play and affiliative behaviours such as social nosing and allogrooming. Social nosing is the gentle nose touching of one pig by another and allogrooming is the gentle grooming of one pig by another using the snout and mouth. Both behaviours cause no injury. Play is a more extensively studied behaviour and is seen in pigs, as in other mammals, in early life and mimics behaviours displayed by adult pigs (e.g. fighting). Play can be broadly categorised into object play, locomotor play and play fighting. Although vigorous at times, play does not usually cause injury.

Wild and domestic pigs also perform negative social behaviours that damage or injure the actor and/or recipient of the behaviour. Fighting and biting behaviours are two of the most commonly observed negative social behaviours in domestic pigs. In wild pigs, obsessive biting behaviour of tails, ears or other body parts has not been observed in natural conditions. This behaviour seems to be exclusively shown under intensive commercial production conditions, tiggered primarily by a lack of suitable substrate to allow expression of foraging behaviour. Fighting in domestic pigs is also different to that seen in wild pigs. In domestic pigs, due to mixing with unfamiliar pigs at various points throughout the production cycle, fighting is more common and more injurious,

except by comparison to that between adult males during the breeding season. Pigs do not have the space to retreat or hide and so fights escalate and continue longer than they might in the wild. Reduced living space and limited resources can also cause increased aggressive behaviours and fighting in domestic pigs.

The social behaviour of pigs can be used as an indicator of their welfare as good animal welfare may be equated with the expression of a behavioural repertoire similar to that in the wild. Using this framework, pigs that perform high levels of play behaviour and low levels of biting and fighting behaviours are believed to have higher welfare. Positive forms of social interaction, such as social play, are a particularly sensitive indicator of animal welfare. These behaviours are displayed elastically, and their expression is more sensitive to internal and external state than behaviours essential for immediate survival. The Surplus Resource Theory suggests that animals that have access to adequate resources are more likely to engage in play behaviour than animals whose resource needs are not met. These resources can include feed/nutrients, space, safety from predators and access to siblings and the mother. When looking at negative social behaviours in the context of the Five Freedoms, hunger and thirst, discomfort (poor environment), disease, and fear and distress are markers of poor welfare and are also common risk factors in tail biting. Levels of both positive and negative social behaviours can be measured to give an indication of overall animal welfare.

Extensive research has been conducted to understand and remedy low welfare in pigs, but significant issues persist. Proposed solutions often lack commercial applicability or are not economically viable for current production systems. One strategy proposed to improve welfare is manipulation of the diet. Pigs fed well-tailored and satiating diets may be less likely to exhibit negative social behaviours. The link between diet and behaviour is complex and not fully understood but there is substantial evidence from human studies that the quantity and composition of food are important factors to consider when examining the effect of diet on health and behaviour. In this instance, the human serves as a model for the pig. Since the time of the Ancient Greek civilisation, humans have been interested in the relationship between the brain and the gut. Early studies showed that the gut physiologically responded to changes in emotional state. The field of neurogastroenterology emerged at the end of the 20th century, further exploring and explaining the bidirectional gut-brain-axis. In addition to the gut-brain-axis, we must consider the microbiota-gut-brain-axis. It is estimated that the mammalian digestive tract contains 100 trillion bacteria and studies have shown that these bacteria play an important role in human behaviour and cognition. The microbiome is responsible for performing functions the host is not capable of, for example the fermentation of dietary fibre. Short chain fatty acids produced by the fermentation of fibre cross from the gut into the blood and impact brain function. This thesis will not focus on the role or composition of the pig's microbiome, but it is important that we draw attention to it, as a major contributing factor to brain function and behaviour.

The digestive system and its microbiome are only half of the equation; the other is what the pigs eat. Feed is a largely unexplored area of production when striving to improve the welfare of pigs. There have been some studies looking at the provision of fibre to farrowing sows to reduce stereotypical behaviours, prophylactic feed additives for weaning pigs and herbal additives intended to reduce stress in finishing pigs during transport. I have focused on the feed provided to growing and finishing pigs and specifically on the energy and amino acid content and their effect on behaviour.

I began our exploration looking at the effect of dietary energy on the play behaviour of weaned pigs. In Chapter 2, I based the study around the Surplus Resource Theory which proposes that play behaviour is expressed more abundantly in juveniles that have access to necessary resources. I proposed that sufficient dietary energy was likely to be a requirement for play and therefore hypothesised that pigs on an energy-deficient diet will show reduced play. As well as being of direct applied welfare relevance, this study filled a fundamental knowledge gap by conducting the first empirical manipulation of energy provision to test the Surplus Resource Theory. A total of 128 mixed sex, weaned pigs were placed on one of two diets; a basal energy level or a lower energy level achieved by diluting the basal diet with Arbocel (basal diet: digestible energy 16.9 MJ/kg, fibre 8.5%, crude protein 18.1%, Arbocel diet: digestible energy 15.3 MJ/kg, fibre 18.4%, crude protein 16.1%). Pigs were moved to a play arena 4 times over a period of 10 days and on alternate occasions, given a sucrose supplement. I hypothesised that the provision of the sucrose supplement would increase play behaviour, particularly for pigs on the low energy diet, compared to play sessions when no sucrose supplement was given. Mixed model analysis showed that the low energy diet reduced daily weight gain ($p=0.005$) and increased feed conversion ratio ($p<0.001$). Dietary treatment and play session interacted to affect social object ($P=0.025$), solo locomotor ($P<0.001$) and social locomotor play ($P<0.001$). Dietary treatment and pen mate average sugar intake interacted to affect social object ($P=0.019$) and social locomotor play ($P<0.001$). Play session ($P<0.001$), and the interaction between dietary treatment and pen mate average sugar intake ($P=0.002$) affected total play. Overall, the high energy diet appeared to extend the developmental period of heightened engagement in social play. There was no effect of the amount of sucrose eaten by the individual itself. Our findings suggest that diet quality in weaned pigs may impact specific aspects of play behaviour with potential benefits for animal welfare.

In Chapter 3, I shifted our focus to amino acids. Previous research has highlighted the modulation of dietary tryptophan as a method of influencing the behaviour of pigs, due to its role as a precursor for the neurotransmitter serotonin which is known to affect expression of social behaviour. However previous research has tended to supplement tryptophan at levels far above the commercial norm and at the time of study design, no research had been conducted to look at the effect of lysine (the first limiting amino acid in pig diets) on pig social behaviour. The aim of our second study was therefore to explore the effects of dietary lysine (Lys) and the tryptophan to lysine ratio (Trp:Lys) on the social behaviour of commercially housed pigs. This study was conducted on a large commercial unit in the USA, using an initial cohort of 2,293 castrated males from the PIC Camborough breeding line. I started the trial with a randomised complete block design with a 2×3 factorial arrangement of the following factors: 1) Standardised ileal digestible Lys levels: 100% Lys = diets with 100% PIC requirement at the midpoint of the growth phase (Lys 100) vs 80% Lys = diets with 80% PIC requirement at the midpoint of the growth phase (Lys 80); and 2) Trp to Lys ratio of 0.210, 0.185 or 0.160. A range of positive and negative social behaviours were recorded, together with skin lesion and production data. After feeding phase 2, treatment 6 pigs (lowest Lys, 80% and lowest Trp:Lys, 0.016) were removed from the trial due to reaching their behaviour (i.e. ethical) and production end points. Chapter 3 focuses on the behaviour and lesion data whilst chapter 4 covers the analysis of the production data. In chapter 3 I report an effect of Lys ($P=0.032$) on ear biting behaviour, with pigs on the Lys 80 treatments showing a higher level of ear biting behaviour. I also found an effect of the Lys and score week interaction on the proportion of pens showing ear lesions ($P<0.001$) and a significant effect of the Lys and Trp:Lys interaction (P=0.030) and the Lys and score week interaction (P=0.0104) on the proportion of pens showing severe ear lesions. These results showed that low lysine levels increased ear biting behaviour and diets low in both Lys and Try:Lys increased the incidences of severe ear lesions. Tail and flank biting were not common in this population, but the potential remains for Lys levels to affect this behaviour in other populations and so deserves further exploration. I conclude that lysine and the interaction of Lys and Trp:Lys content of feed can affect the social behaviour of pigs, specifically ear biting, in commercial conditions.

Chapter 4 contains the production analysis from our second study. Feed intake, weight gain and feed conversion ratio were recorded over five feeding phases (the duration of behaviour data collection). As described above, after feeding phase 2, treatment 6 pigs (lowest Lys; 80% and lowest Trp:Lys; 0.016) were removed from trial due to reaching their behaviour (i.e. ethical) and production end points. The production data collected were analysed in two ways: treatments 1-6 for feeding phases 1 and 2 and treatments 1-5 for feeding phases 1-5. In the first two feeding phases, the Lys and Trp:Lys interaction (p=<0.001) and the Trp:Lys and feeding phase interaction (p=<0.001) affected ADFI. The Lys and Trp:Lys interaction (p=<0.001) and the Trp:Lys and feeding phase interaction (p=<0.001) affected ADG. ADFI and ADG were lower in treatment 6 (Lys 80, Trp:Lys 0.160) pigs for both feeding phases. FCR was affected by Lys (p=<0.001) and feeding phase (p=<0.001). After the removal of treatment 6, over the full 5 feeding phases, Lys and feeding phase (p <0.001) interacted to affect FCR. The results from both analyses suggest that in addition to Lys content and feeding phase, the combination of low Lys and low Trp:Lys had the greatest negative effects on production.

Chapter 5 builds on the work of chapters 3 and 4. This final study was designed to further explore the effect of lysine by comparing the effect of lysine levels at opposite ends of the spectrum currently available in commercial UK pig diets. A wider range of social behaviours was studied than in chapter 3 and, since the farm had a history of tail biting, greater opportunity to study the effect of lysine levels on tail biting was anticipated. An initial cohort of 723 mixed sex, Large White x Landrace x Danish Duroc grower pigs were randomly allocated across two treatments, high and low commercially available crude protein diets (HL and LL). As lysine is the first limiting amino acid, the effect of a low protein diet is mediated by the availability of lysine. For

21 days (commencing 3 days after starting on the trial diet), behaviour observations and skin lesion scores were recorded. Production metrics were collected over the full duration of the trial. I found no effect of diet on behaviour, lesions or production metrics. This is in conflict with my second study. I discuss in chapter 5 potential reasons for the conflicting results, primarily the modest difference in protein levels and the differences in production systems. I recommend additional research is required, across a diverse range of farming conditions and systems, to further explore the role of nutrition, and in particular crude protein and lysine, as a way to manipulate social behaviour in pigs.

Throughout the three studies, I identified some common challenges and areas for improvement. One important area for refinement is the practicality, reliability, accuracy and consistency of human data collection. While I maintained consistency and avoided intra-observer variation by ensuring all data collection was performed by the same individual, there are questions around the reliability and accuracy of data collected on farm and over long periods of time from video recordings. There are also discussions to be had around the emotional and cognitive load of persons performing data collection. While studying the welfare of pigs, some thought should be given to the welfare of the researcher. Long periods of time spent observing low welfare conditions can potentially have a detrimental effect on the mental health of the observer. The use of AI and other technologies may help to optimise the data collection process and improve the experience of data collection for researchers. For the sake of transparency, I must acknowledge there were potential biases introduced to trials from not blinding the observer to the treatments. Due to the nature of this PhD project, blinding was not possible as I designed trials, collected data and performed the analysis. However, independent evidence corroborated behaviour observations, production data was free from an observer effect and the final decisions to remove pigs in study 2 and treat pigs in study 3 were made by farm staff.

Other common challenges across studies 2 and 3 are those that come from carrying out research on commercial farms, with a range of stakeholders with varying priorities. Ultimately, I was at the mercy of the producer and production always took priority over research. This meant that the study design often had to be adapted to the realities of farm practices, but efforts were always taken to maximise study balance. In addition to the challenges of the commercial setting, comparison between my studies is difficult due to the different production systems. Size of farm, genetics, geographical location, management etc. are likely to have impacted on the results collected. Ideally a replication of the study carried out in the USA under UK and/or European conditions would be performed. It would also be beneficial to see how different lysine levels affect social behaviour of pigs over a range of housing and production systems. Another key discussion point is the effect of management interventions on the results collected. In all studies I outlined behavioural and welfare endpoints. Pigs which reached these endpoints were treated or removed from trial. In study 2, treatment 6 pigs were removed from trial, whilst in study 3 tail bitten pigs had their tails tarred and were treated or removed from the pen. It is possible that the effect of diet was therefore diluted as individual incidences and outbreaks of tail biting were not allowed to progress as they may have done on a commercial farm if left. There is also the episodic nature of negative social behaviours to consider. Longer term studies may have been beneficial in increasing the chances of observing such behaviours over different seasons, farrowings, health challenges and management conditions.

Beyond challenges and improvements, there is a wider discussion about the social behaviour of pigs and how researchers identify and classify those behaviours. Widening the focus of pig behaviour studies, beyond those with immediately obvious relevance to production could help us to better understand the pig as an individual and as part of a wider social network. An important next step would also be to establish the mechanistic role of the neuroendocrine and microbiome systems in this effect of diet on social behaviour. Further research into the social behaviour of pigs would also be beneficial; by understanding how they communicate and form social bonds may help us strengthen and support those bonds and improve their welfare. All of the above points are discussed in detail in the discussion chapter of this thesis, while specific discussions of the studies are included in their respective chapters.

Overall, I believe I have collected and presented sufficient evidence to show that diet does affect the social behaviour of pigs but further research into the topic is needed. The evidence presented in this thesis supports that diet has an important effect on behaviour but more specifically and less commonly studied, that dietary changes within the bounds of what is commercially available also have an effect on both positive and negative social behaviours. This presents a potentially commercially relevant and low-cost solution to reducing negative behaviours and promoting positive ones. However, simply changing the diet of intensively farmed pigs should not be seen as a silver bullet approach to improving welfare. Ideally, as over 40 years of research has shown, pigs should be given more space and provided with appropriate enrichment. However, in systems where this is not currently possible and as we transition to higher welfare systems, manipulating the diet of pigs could potentially improve their quality of life.

Lay Summary

Pig are highly social and intelligent animals. One of the most commonly farmed animals in the world, the processes of domestication and intensive farming have led to significant welfare issues. One of the ways we can think about animal welfare argues that welfare issues arise when animals are prevented from performing their natural behaviours. Despite their domestication, pigs still retain a strong desire and motivation to perform behaviours seen in their wild counterparts. It is therefore important to have knowledge of the pig's wild behaviour to enable us to improve their welfare. In the wild, pigs live in small groups of related females, called sounders. After undergoing puberty, males live a solitary life and only interact with females during mating. At mating, males will fight with one another over access to a female. Other than at mating, fighting is rare in wild pigs as they mutually avoid pigs in neighbouring home ranges. Within the sounder, some aggressive behaviours may be seen over food or other resources like access to water or sleeping areas. Largely, sounders have a stable social structure, with sows cooperatively raising and protecting piglets and piglets playing and foraging with piglets from other litters. Both wild and domestic pigs display a range of positive social behaviours, including play and affiliative behaviours such as social nosing and allogrooming. Social nosing is the gentle nose touching of one pig by another and allogrooming is the gentle grooming of one pig by another using the snout and mouth. Both behaviours cause no injury. Play is a more extensively studied behaviour and is seen in pigs, as in other mammals, in early life mimicking behaviours displayed by adult pigs (e.g. fighting). Play can be broadly categorised into object play, locomotor play and play fighting. Although vigorous at times, play does not usually cause injury.

Wild and domestic pigs also perform negative social behaviours that damage or injure the actor and/or recipient of the behaviour. Fighting and biting behaviours are two of the most commonly observed negative social behaviours in domestic pigs. In wild pigs, obsessive biting behaviour, of tails, ears or other body parts has not been observed. This behaviour seems to be exclusively shown in pigs kept under intensive farming conditions. Fighting in domestic pigs is also different to that seen in wild pigs. In domestic pigs, due to mixing with unfamiliar pigs at various points throughout the production cycle, fighting is more common and more damaging, except when compared to fighting between adult males during the breeding season. Pigs do not

have the space to retreat or hide and so fights escalate and continue longer than they might in the wild. Reduced living space and limited resources also increase fighting in domestic pigs.

The social behaviour of pigs can be used as an indicator of their welfare as good animal welfare may be equated with the expression of wild behaviours. Using this framework, pigs that perform high levels of play behaviour and low levels of biting and fighting behaviours are believed to have higher welfare. Positive forms of social interaction, such as social play, are a particularly sensitive indicator of animal welfare. These behaviours are displayed elastically, changing over time and between individuals, and their expression is more sensitive to changes inside and around the pig than behaviours that are essential for immediate survival, for example accessing resources like food or mates. The Surplus Resource Theory suggests that animals that have access to adequate resources are more likely to engage in play behaviour than animals whose resource needs are not met. These resources can include feed/nutrients, space, safety from predators and access to siblings and the mother. When looking at negative social behaviours in the context of the Five Freedoms (a widely used framework for assessing welfare in animals), hunger and thirst, discomfort (poor environment), disease, and fear and distress are markers of poor welfare and are also common risk factors in tail biting. Levels of both positive and negative social behaviours can be measured to give an indication of overall animal welfare.

Extensive research has been conducted to understand and remedy poor welfare in pigs, but significant issues persist. Proposed solutions often lack commercial applicability or are not economically viable for current production systems. One strategy proposed to improve welfare is manipulation of the diet. Pigs fed a well-balanced and filling diet may be less likely to exhibit negative social behaviours, especially if those negative social behaviours are exacerbated by nutritional deficiencies. The link between diet and behaviour is complex and not fully understood but there is substantial evidence from human studies that the quantity and composition of food are important factors to consider when examining the effect of food on health and behaviour. In this instance, the human serves as a model for the pig. Since the time of Ancient Greek civilisation, humans have been interested in the relationship between the brain and the gut. Early studies showed that the gut physically responded to changes in emotional state. The field of neurogastroenterology (the control of digestion and the gastrointestinal system through the integration of the enteric and central nervous system) emerged at the end of the 20^{th} century, further exploring and explaining the bidirectional gut-brain-axis. In addition to the gut-brain-axis, we must consider the microbiota-gut-brain-axis. It is estimated that the mammalian digestive tract contains 100 trillion bacteria and studies have shown that these bacteria play an important role in human behaviour and brain function. The microbiome is responsible for performing functions the host is not capable of like the fermentation of dietary fibre. Short chain fatty acids produced by the fermentation of fibre cross from the gut into the blood and impact brain function. This thesis will not focus on the role or composition of the pigs' microbiome, but it is important that we draw attention to it, as a major contributing factor to brain function and behaviour.

The microbiome and the pig's digestive system are only one half of the equation, the other is the food given to the pigs. Feed is a largely unexplored area of pig production when trying to find ways to improve the welfare of pigs. There have been some studies looking at the provision of fibre to farrowing pigs to reduce harmful, repetitive behaviours, feed additives designed to prevent illness in young pigs and herbal additives intended to reduce stress in pigs of all ages. I have focused on the feed provided to growing and finishing pigs (pigs nearly ready for slaughter) and specifically on the effect of the energy and amino acid content on social behaviour.

I began my exploration looking at the effect of dietary energy on the play behaviour of weaned pigs. In Chapter 2, I based our study around the Surplus Resource Theory which proposes that play behaviour is expressed more abundantly in young animals that have access to necessary resources. I proposed that sufficient dietary energy was likely to be a requirement for play and therefore hypothesised that pigs on an energy-deficient diet will show reduced play. As well as being of direct relevance to applied welfare, this study filled a fundamental knowledge gap by being the first, to my knowledge, to manipulate energy provision to test the Surplus Resource Theory.

A total of 128 mixed sex, weaned pigs were placed on one of two diets; a basal energy level diet or a lower energy level diet. Pigs were moved to a play arena and on alternate occasions given a sugar supplement in the form of sugar cubes. We hypothesised that the provision of the sugar supplement would increase play behaviour, particularly for pigs on the low energy diet, compared to play sessions when no sugar supplement was given. Statistical analysis showed the low energy diet reduced daily weight gain and increased feed conversion ratio (the measure of how efficiently livestock convert feed into weight gain). Effects were found of the dietary treatment on various forms of play, but only in combination with the date of the play session or the amount of sugar eaten by the individual's penmates. There was no effect of the amount of sugar eaten by the individual itself. Overall, the high energy diet appeared to extend the period of heightened engagement in social play to a later age. My findings suggest that diet quality in weaned pigs may impact specific aspects of play behaviour with potential benefits for animal welfare.

In Chapter 3, I shifted our focus to amino acids. Previous research has shown that changing the tryptophan levels in the diet can influence the behaviour of pigs due to tryptophan being a precursor to the brain chemical serotonin, which is known to affect social behaviour. However previous research gave tryptophan at levels far above the commercial norm and at the time of designing this study no research had been conducted to look at the effect of lysine on pig social behaviour. Lysine is the first amino acid in pig diets that is likely to become deficient, and it affects how much the other amino acids in the diet can be exploited by the animal. The aim of our second study was to explore the effects of dietary lysine (Lys) and the tryptophan to lysine ratio (Trp:Lys) on the social behaviour of pigs being raised for the meat market. This study was conducted on a large commercial unit in the USA using 2,293 castrated males. We started the trial with 6 different diets; 1) high lysine and high Trp;Lys, 2) high Lys and medium Trp:Lys, 3) high Lys and low Trp:Lys 4) low lysine and high Trp;Lys, 5) low Lys and medium Trp:Lys, 6) low Lys and low Trp:Lys. We collected data on the pigs' positive and negative social behaviours, skin lesions and feed intake and growth. Chapter 3 focuses on the behaviour and lesion data collected, whilst chapter 4 covers the analysis of the production data. In chapter 3 I report an effect of Lys on ear biting behaviour, with pigs on the low Lys treatments showing a higher level of ear biting behaviour. The proportion of pens showing ear lesions was affected by the level of lysine, but only when in combination with certain ratios or Typ:Lys and during certain weeks. These results showed that low lysine levels increased ear biting behaviour and diets low in both Lys and Try:Lys increased the incidences of severe ear lesions. Tail and body biting were not common in the pigs used in this study, but the potential remains for Lys levels to affect this behaviour in other populations and so deserves further exploration. I conclude that the lysine content of feed can affect the social behaviour of pigs, specifically ear biting, in commercial conditions.

As mentioned above, chapter 4 details the analysis of the feed intake and growth data collected during the second study. Feed intake, weight gain and feed conversion ratio were recorded over five feeding phases (the duration of behaviour data collection). After feeding phase 2, treatment 6 (lowest Lys and lowest Trp:Lys) pigs were removed from trial due to their growth slowing substantially and their levels of harmful behaviours reaching predetermined ethical limits. The feed intake and growth data collected was analysed in two ways: treatments 1-6 for feeding phases 1 and 2 and treatments 1-5 for feeding phases 1-5. In the first two feeding phases, the interaction between Lys and Trp:Lys and the interaction between Trp:Lys and feeding phase affected ADFI. The interaction between Lys and Trp:Lys and the interaction between Trp:Lys and feeding phase affected growth rate. Feed conversion ratio was affected by Lys and feeding phase. After the removal of treatment 6, over the full 5 feeding phases, the interaction between Lys x feeding phase affected FCR. The results from both analyses suggest that in addition to low Lys content and feeding phase, the combination of low Lys and low Trp:Lys has the greatest negative effects on feed intake and growth of pigs.

Chapter 5 builds on the work of chapters 3 and 4. My final study was designed to further explore the effect of dietary lysine at levels already being used on farms on the social behavior of pigs, with particular focus on tail biting. A mix of 723 male and female pigs were randomly allocated across two treatments: high and low commercially available lysine diets (HL and LL). For 21 days (commencing 3 days after starting on the trial diet), behaviour observations and lesion scores were taken. Feed intake and growth data were collected over the full duration of the trial. I found no effect of diet on behaviour, lesions or feed intake and growth data. This conflicts with my second study. I discuss in chapter 5 potential reasons for the conflicting results, primarily the small difference in lysine levels and the differences in farming systems. I recommend further research is required, across a diverse range of farming conditions and systems, to further explore the role of nutrition and in particular lysine in the manipulation of social behaviour in pigs.

Throughout the three studies, I identified some common challenges and areas for improvement. One important area for refinement is the practicality, reliability, accuracy and consistency of human data collection. While I maintained consistency and avoided variation in the data that can come from having more than one observer by ensuring all data collection was performed by the same individual, there are questions around the reliability and accuracy of data collected on farm and over long periods of time from video recordings. There are also discussions to be had around the emotional and cognitive load of persons performing data collection. While studying the welfare of pigs, some thought should be given to the welfare of the researcher. Long periods of time spent observing low welfare conditions can potentially have a negative effect on the mental health of the observer. The use of AI and other technologies may help to optimise the data collection process and improve the experience of data collection for researchers. For the sake of transparency, I must acknowledge there were potential biases introduced to trials from not blinding the observer to the treatments. As I had designed and set up the study, I knew which treatments the pigs were on. However, independent evidence corroborated behaviour observations, feed intake and growth data were free from an observer effect and final decisions to remove pigs from trial in study 2 and to treat pigs in study 3 were taken by farm staff.

Other common challenges across studies 2 and 3 are those that come from carrying out research on commercial farms, with a range of stakeholders with varying priorities. Ultimately, I was at the mercy of the producer and production always took priority over research. This meant that the study design often had to be adapted to the realities of farm practices, but efforts were always taken to try and keep the study balanced. In addition to the challenges of the commercial setting, comparison between my studies is difficult due to the different production systems. Size of farm, genetics, geographical location, management etc. are likely to have impacted the results collected. Ideally a replication of the study carried out in the USA under UK and/or European conditions would be performed. It would also be beneficial to see how different lysine levels affect social behaviour of pigs over a range of housing and production systems.

Another key discussion point is the effect of farmers' interventions on the results collected. In all studies I outlined behavioural and welfare endpoints. Pigs that reached these endpoints were treated or removed from trial. In study 2, treatment 6 pigs were removed from trial, whilst in study 3 tail bitten pigs were treated in their pen or removed from the pen and placed in hospital pens. It is possible that the effect of diet was therefore diluted, as the tail biting was not left to get as worse as it might have done on a commercial farm if we had not intervened. It would have been irresponsible and unethical not intervene in situations of low health and poor welfare so I can only speculate on the true effects of diet in these cases. There is also the episodic nature of negative social behaviours to consider. Longer term studies may have been beneficial in increasing the chances of observing such behaviours over different seasons, farrowing's, health challenges and management conditions.

Beyond challenges and improvements, there is a wider discussion about the social behaviour of pigs and how researchers identify and classify those behaviours. Widening the focus of pig behaviour studies, beyond those with immediately obvious relevance to production could help us to better understand the pig as an individual and as part of a wider social network. An important next step would also be to establish the role of the digestive system microbiome and the brain, nervous systems and hormones in this effect of diet on social behaviour. Further research into the social behaviour of pigs would also be beneficial; by understanding how they communicate and form social bonds may help us strengthen and support those bonds and improve their welfare. All of the above points are discussed in detail in the discussion chapter of this thesis, while specific discussions of the studies are included in their respective chapters.

Overall, I believe I have collected and presented sufficient evidence to show that diet does affect the social behaviour of pigs but further research into the topic is needed. The evidence presented in this thesis supports that diet has an important effect on behaviour but more specifically and less commonly studied, that dietary changes within the bounds of what is commercially available have an effect on both positive and negative social behaviours. This presents a potentially commercially relevant and low-cost solution to reducing negative social behaviours and promoting positive ones. However, simply changing the diet of intensively farmed pigs should not be seen as a silver bullet approach to improving welfare. Ideally, as over 40 years of research has shown, pigs should be given more space and provided with appropriate enrichment. However, in systems where this is not currently possible and as we transition to higher welfare systems, manipulating the diet of pigs could potentially improve their quality of life.

List of figures

List of tables

Contents

Chapter 1: General Introduction

The pig (*Sus scrofa*) is a highly social and intelligent animal with a complex repertoire of behaviours (Gieling et al., 2011). One of the most numerous farmed animals, it was estimated there were over 752 million pigs slaughtered worldwide in 2021 (USDA, 2021). Life for pigs raised for human consumption is vastly different to that of their wild counterparts, however, the pig and its' needs remain largely the same. Despite the differences in environment and breeding, domestic pigs still retain many of their wild behaviours and the desire and motivation to perform these behaviours. These behavioural needs are often not met by current intensive systems, and this is a major contributing factor to the poor welfare of domestic pigs. Problems can occur when natural behaviours are impeded (e.g. the ability of pigs to retreat from an aggressive encounter to prevent escalation) or redirected (e.g. tail biting). One of the key definitions of animal welfare states that it is achieved when an animal is able to behave naturally (Kiley-Worthington, 1989). It is therefore important that we explore and understand the natural behaviour of the pig. In an effort to improve the welfare of domestic pigs we aim to promote more natural/wild behaviours and reduce those seen as a result of suppression or redirection of natural instincts. Arguably the most significant welfare-related behavioural problem facing the pig industry is tail biting. This is a persistent and systemic issue which has been well studied but not yet eradicated. A range of potential reduction strategies have been suggested (increasing space allowance, provision of adequate and appropriate enrichment) but unfortunately, these methods are often not compatible with current production systems. One potential solution that might be commercially viable could be the manipulation of the diet. As expected, the diet of domestic pigs is far removed from that of wild pigs due to the need to support rapid growth made possible by selective breeding. As opposed to rooting for a range of vegetation, invertebrates and small vertebrates, domestic pigs are fed a nutritionally tailored, processed feed. The ability to tightly control and manipulate the diet fed to domestic pigs may offer an opportunity to improve their welfare. This thesis will explore the expression of positive and negative social behaviours within the context of welfare, and how the manipulation of feed could be used as a strategy to modulate the expression of social behaviours.

Social behaviour in mammals

According to behavioural ecology theory, sociality evolves when the net benefits of close association with conspecifics exceed the costs (Krause and Ruxton, 2002). In mammals, sociality can be beneficial for individuals because it provides greater protection from predators, improves the chances of success in locating and defending resources and creates mating opportunities. However, sociality can also be costly for individuals as it may increase resource and mating competition, expose animals to infections and in some cases increase their conspicuousness to predators (Silk, 2007a).

The social pig

Through the study of wild boar, feral pigs and experimental wild living domestic pigs, we have a comprehensive understanding of the natural behaviour of pigs. In the wild, pigs live in small groups called sounders, which are comprised of 2-4 mature females, usually related (mother and daughter or sisters), and their piglets from the most recent breeding season (Mauget, 1981; Graves, 1984; Macdonald, 1984). Sounders have a home range, rather than strictly defined and defended territories, of 100-6000 ha (Mauget, 1981; Janeau et al., 1984) which may overlap with the home range of neighbouring sounders. Territorial aggression is rarely seen between sounders as pigs practice mutual avoidance (Gabor et al., 1999). The social dynamics of the sounders are largely stable as pigs establish a dominance hierarchy primarily based on age/size, so within the sounder, aggressive behaviour between adult females is rare (Jensen and Wood-Gush, 1984; Mendl, 1994). Shortly before farrowing, the female pig will remove herself from the sounder to build a nest to farrow in. The nest provides a safe location for the farrowing female and her piglets, providing some protection from predators and ensuring her piglets do not have to compete with piglets from other litters when gaining access to milk. This separation period also allows the sow and piglets to bond, establishing recognition of each other, proposedly through nose-to-nose contact, mainly initiated by the piglets (Newberry and Wood-Gush, 1986). A short time after birth, piglets will fight amongst themselves, biting and pushing each other to gain access to the most productive teats. Once the teats have been allocated, the piglets will remain at their allocated teat throughout lactation (Newberry and Wood-Gush, 1986). Between 7-14 days after farrowing, the adult female and her piglets will rejoin the group. Adult females in the sounder often synchronise gestation and farrowing which allows mothers to co-operate in the protection of piglets (Fradrich, 1974; Mauget, 1981). It also allows the piglets to start developing social skills beyond their litter, as they play with other piglets (Petersen et al., 1989). After an initial period of exploration and socialising, inter-litter socialisation gradually declines as piglets show a strong preference of resting and playing with their littermates (Newberry and Wood-Gush, 1986). At around 6-10 months of age, on reaching puberty, male pigs disperse from the sounder in groups of up to three individuals (Fradrich, 1974; Graves, 1984; Gabor et al., 1999) becoming increasingly solitary with age until they only interact with other pigs during mating (Kurz and Marchinton, 1972). Aggression occurs between adult males during the rutting season, when they compete for access to females (Mendl, 1995). Aggression between males follows an escalatory pattern, starting with parallel walking, progressing to circling each other and then engaging in shoulder-to-shoulder pushing. This non-injurious fighting can last for some time and does not always escalate into serious, injurious fighting (Fradrich, 1974). However, in some situations, fights will escalate to involve rapid and repeated biting (Graves et al., 1978). Beyond the extensive foundational work on pig social behaviour carried out in the 1980s (briefly mentioned above) more recent research has identified evidence for more sophisticated social behaviours in pigs. Norscia et al., (2021) found yawn contagion in pigs, Rault et al., (2021) found evidence of cooperative behaviours to solve tasks and Moscovice et al., (2023) identified spontaneous helping in pigs. Studies have also shown that the presence of a familiar pig, a phenomenon termed ‘social buffering’ (Kikusui et al., 2006) or ‘social support’, can help to reduce distress in pigs during lairage and slaughter (Geverink et al., 1998; Söderquist et al., 2023).

This thesis will focus on the social behaviour of commercial housed pig post weaning. Social behaviour will be defined as any behaviour that involves two or more individuals. Social behaviours can be largely divided into positive and negative behaviours. There may be a third class of ‘neutral’ behaviour but this will also be discussed later in the thesis. Positive social behaviour is defined as behaviour that does not cause physical damage to the initiating or recipient pig and that shows active engagement/reciprocation or a lack of an active evasion response. Negative behaviour is defined as any behaviour that elicits an evasion response from the recipient pig and/or that causes physical damage to the initiating and/or recipient pig.

Positive social behaviours in pigs

Positive social behaviours in pigs are of increasing interest to researchers, with numbers of peer reviewed published articles rising steadily from 2005, where 0 papers were published, to a high point in 2021 where 22 papers were published (Scopus, 2025). While the biological and welfare benefits of positive social behaviours in pigs have yet to be fully elucidated, preferential association (Goumon et al., 2020), affiliative behaviours (Meynhardt, 1982) and play (Newberry et al., 1988) have been identified as behaviours that are likely to be under natural selection and retain some value to the animal under intensive production conditions.

Preferential association in pigs

Research has shown that pigs show preferences when interacting with conspecifics. This has been observed in wild (Podgórski et al., 2014) and domestic pigs (Durrell et al., 2004; Jowett et al., 2024) and social preference has been shown to be influenced by sex and dominance status (Goumon et al., 2020). Social preference in animals is described as a long-term social relationship between a pair of individuals characterised by high rates of non-random associations and affiliative interactions (Silk, 2007b). Potential benefits of showing preferential association include increasing reproductive success (Silk et al., 2003) and/or providing a social buffer in stressful situations (McLennan, 2012). However, studies into the effect of preferential associations on the reproductive performance of group-housed sows have been inconclusive and the relationship needs further exploration (Jowett et al., 2024).

Affiliative behaviours in pigs

In addition to preferential association, we see evidence of active affiliative behaviour in pigs, which may be expressed as social nosing (Camerlink and Turner, 2013; Portele et al., 2019) and allogrooming (Meynhardt, 1982). Social nosing is a gentle pig-directed behaviour which involves the touching of one pig by another with the snout, usually on the snout, head or body (excluding the tail, ear and ano-genital region), without causing damage (Camerlink and Turner, 2013). Social nosing has been shown to increase a pig's growth rate in certain growth phases (Camerlink et al., 2012; Camerlink and Turner, 2013). It has also been shown to be largely unrelated to harmful behaviours and was not related to dominance relationships (Camerlink and Turner, 2013). Allogrooming is when one pig gently nibbles or licks the head (including snout, ear, eye region and eye lashes) or body of another pig without causing visible skin damage to the recipient. Allogrooming has been shown to increase with pig age and group size (Camerlink et al., 2022). Thus far, there is only a small body of literature looking at allogrooming and other positive behaviours in pigs but in other animals, the presence of strong social bonds within a group has been associated with various benefits, including higher reproductive success, greater offspring survival and greater longevity (Seyfarth and Cheney, 2012; Brent et al., 2014).

Play in pigs

Pigs exhibit a complex range of play behaviours. Locomotor play is shown that includes running, pivots, flops and hops (Newberry et al., 1988; Chaloupková et al., 2007). Locomotor play can be performed individually or with conspecifics. Object directed play involves the pig manipulating, carrying or shaking an object (Newberry et al., 1988). Social play is between two or more pigs and involves nudging, pushing, climbing and non-harmful fighting. Play fighting usually involves head knocking, where piglets mutually push each other with their heads and shoulders (Donaldson et al., 2002; Chaloupková et al., 2007; Brown et al., 2015). Play in pigs is most commonly seen early in life, between 2 and 6 weeks of age, dropping off to a low level around 14 weeks (Newberry et al., 1988). Play fighting has been shown to influence social interactions later in life (Weller et al., 2019) and play behaviour in pre-weaning pigs substantially reduces the tendency of pigs to behave aggressively around food later in life (Chaloupková et al., 2007).

Negative social behaviours in pigs

As previously mentioned, fighting and aggressive behaviours are seen in wild pigs and these behaviours are also seen in domestic pigs. However, unlike in wild pigs where fighting is usually reserved for males during the mating season, domestic pigs exhibit aggressive behaviours at various points throughout production. As well as fighting, domestic pigs show other negative social behaviours, broadly classed as ‘biting’ behaviours, for example to the tail and ears. Commonly and erroneously, biting behaviours in pigs are compounded with aggressive or fighting behaviours. It must be clarified that these two classes of behaviours have very different aetiologies. Fighting and aggressive behaviours are most often seen around competition for a resource, e.g. feed or space, or when establishing dominance. Biting behaviours (discussed further in this chapter) are proposed to stem from insufficient mental stimulation (boredom) or unfulfilled behaviour needs (frustration). Nutritional deficiency may also contribute to biting behaviours. While both behaviours can have a negative impact on the health and welfare of the pig, when considering strategies to reduce either or both behaviours, we must be clear on the underlying cause of the behaviour and its associated risk factors.

Fighting and aggression in pigs

The majority of fighting between domestic pigs occurs around resources or at mixing (i.e. social regrouping) in order to establish dominance. Pigs are mixed several times during production; typically after weaning, at finishing, during transport to the abattoir and in lairage at the abattoir and in sows when weaned. Each time they are mixed, pigs will encounter unfamiliar pigs and their social group changes. Within the first 24 hours of mixing, fighting is at its highest, after which the fighting subsides as the pigs settle into their new dominance hierarchy (Meese and Ewbank, 1973; Fraser and Rushen, 1987). As in wild pigs, this fighting involves rapid and repeated biting. In common commercial pens, where space is restricted, pigs are unable to avoid conflict and display appropriate submissive, retreat or non-harmful threat behaviour (Turner et al., 2006) so fights are often prolonged and highly harmful.

Aggressive behaviours between familiar pigs may be seen when there is competition around resources (Arey and Edwards, 1998). Studies have shown that the number of skin lesions seen on pigs can be increased by a low space allowance (Turner et al., 2000). There is also evidence of an extreme aggressive behaviour in domestic pigs, known as lethal gang aggression (Camerlink et al., 2020). This presents as the killing of a conspecific in seemingly stable social groups. Little is known about this type of aggression as it is unpredictable and difficult to replicate under trial conditions. In a cross farm observational study, it was found that legal gang aggression appeared to be more common in pigs of larger group size, bedded on straw and so it has been suggested that this sudden and seemingly spontaneous aggressive behaviour may be due to the larger group size or potentially mycotoxins found in the straw bedding (Camerlink et al., 2020). Thus far, very little is known about the cause or risk factors involved in lethal gang aggression and further research is needed.

Tail biting

Tail biting is the oral manipulation (chewing, sucking, biting) of a pig’s tail by another pig that causes tissue damage. Tail biting can result in severe injuries that are likely to be painful for the pig and open to infection, having significant economic and welfare consequences. Tail biting has a complex aetiology. In the majority of cases, no single risk factor can be isolated and identified as causing tail biting, rather it is a combination of sub-optimal conditions and various risk factors that contribute to tail biting. Risk factors can be internal (to the pig); changes in health (Boyle et al., 2022), genetics (Gorssen et al., 2024), sex (Schrøder-Petersen et al., 2003), weight (Van De Weerd et al., 2005), tail length (Thodberg et al., 2018) or external; air temperature and ventilation (Geers et al., 1989; Scheepens et al., 1991), provision of environmental enrichment (Van de Weerd and Day, 2009) or group size (Misra et al., 2021). Tail biting is common in intensive systems (30% to 70% of farms in various European countries experience tail biting problems Blokhuis et al., 2007) but has also been recorded in outdoor (Walker and Bilkei, 2006) and organic (Hansson et al., 2000) systems. Tail biting has not been reported in wild pigs so is likely a product of farming conditions.

It has been proposed that there are three classifications of tail biting behaviour; 'two-stage', 'sudden-forceful' and 'obsessive', each of which may have different motivational bases (Taylor et al., 2010). Two-stage tail biting comprises of a 'pre-damage stage' and a 'damaging stage' (Schrøder-Petersen et al., 2003). During the pre-damage (or non-damage) stage, one pig gently holds the tail of another in its mouth and manipulates it causing no visible damage or distress to the recipient. This behaviour is also known as tail-in-mouth behaviour (Schrøder-Petersen et al., 2003). At some point, this non-damaging tail-in-mouth behaviour becomes damaging behaviour, as the manipulation causes a break in the skin. Sudden-forceful tail biting by contrast involves the pig's tail being seized and yanked or bitten forcefully, generally without an observed build-up of gentle manipulation (van Putten, 1969). Obsessive tail biting, also known as fanatical biting, involves a large amount of forceful tail-biting performed by one or a few individual animals that grab and yank at tails, but unlike sudden-forceful biting, obsessive tail biting is as described, obsessive and will occur repeatedly (Beattie et al., 2005; Van de Weerd and Day, 2009). Individuals appear fixated on biting tails, sometimes to the detriment of their own health and growth. Widespread tail biting on farms is often referred to as an 'outbreak'; a stage of tail-biting where injury has occurred, and blood is present that further increases the attractiveness of tails as an object to manipulate which stimulates a positive feedback loop of increasing tail biting and injury (EFSA, 2007).

Despite being prohibited under EU legislation (Council Directive 2008/120/EC) routine tail docking is performed to reduce the risk of tail biting and continues to be widely used across pig farming systems in the EU and elsewhere (De Briyne et al., 2018). However, tail docking does not completely prevent tail biting nor address the underlying cause. Tail docking has a significant negative impact on the welfare of pigs (for a review of the consequences and efficacy of tail docking, see Nannoni et al., 2014).

Having been studied for almost half a century now, comprehensive literature is available on tail biting; its risk factors, development and progression of the behaviour, biological relevance and prevention strategies (for a thorough review see O'Driscoll and Valros, 2024).

Ear biting

In addition to tail biting, pigs may also engage in ear biting. Ear biting is the oral manipulation (chewing, sucking, biting) of a pig's ear by another pig that causes tissue damage. A recent study reported a 100% farm level prevalence on 31 farms surveyed, with a median of 6.97% animals affected (van Staaveren et al., 2018) by ear biting. Comparatively little is known about ear biting, but it is suggested that it shares common risk factors with tail biting (Smulders et al., 2008) and may become more common when tails are docked to very short lengths (Goossens et al., 2008). A study surveying 58 Irish farmers found that ear biting occurred most commonly in 'grower-stage' pigs (approximately 47-81 days from weaning) (Haigh and O'Driscoll, 2019). The issue of ear biting becomes more complex with the consideration of conditions such as Porcine Ear Necrosis Syndrome (PENS). PENS is characterised by lesions on the external ear, often affecting the ear tips. These lesions can range from mild, superficial damage to severe, deep ulcers with potential for tissue loss and in extreme cases, loss of all the external ear. PENS is a complex and not fully understood condition but may contribute to, or be exacerbated by, ear biting (Park et al., 2013). From observation of the lesions alone, the cause is often unclear, however high levels of oral behaviour directed toward the ears prior to an ear biting event (Diana et al., 2019) suggest that, in some cases it is ear biting that causes the ear lesions.

Oral manipulation

In addition to tail and ear biting, pigs may engage in other forms of social oral manipulation: belly nosing, vulva biting, biting at prolapses, biting at hernias, overgrooming/eating eyelashes, genital sucking, toe/foot biting. Tail and ear biting have taken research priority over other types of social biting behaviours, so literature is scarce. However, research by Rizvi et al. (1998) showed that group size and the number of sows per drinker were significant risk factors for vulva biting in recently weaned sows, and group size, number of sows per drinker, nipple drinkers and once a day feeding were significant risk factors for dry sows. Vulva biting was reported on 58 out of 83 farms (70%) surveyed in the southwest of England (Rizvi et al., 2000). Camerlink et al. (2012) found that pigs that received more oral manipulation (which included tail biting, ear biting and foot biting) grew less well. Belly nosing is the distinctive, rhythmic up-and-down movement of one piglet rubbing the belly of another with its snout (Fraser, 1978) which can result in lesions on the recipient (Straw and Bartlett, 2001). It has been shown that belly nosing is linked to early weaning of piglets (Widowski et al., 2008; Faccin et al., 2020) but the aetiology of the behaviour is unclear (Torrey and Widowski, 2006). Flank lesions are also seen in growing pigs of various ages; 1-week post-weaning in piglets weaned at 3–4 weeks of age (Straw and Bartlett, 2001), 2 to 4 weeks after weaning (Norring et al., 2023) and in finishers of 20 weeks of age (Chou et al., 2018).

Social behaviours as indicators of welfare

When considering Kiley-Worthington's definition of welfare (good welfare comes from animals being able to perform their natural behaviours), the fourth of the Five Freedoms: freedom to express normal behaviour (FAWC, 2010) is key. This is also true of newer models of welfare that promote the provision of a life worth living for animals (FAWC, 2010). It follows that observations of behaviour can provide an insight into the animal's welfare state. Captive and domesticated animals performing natural behaviours (e.g. play, grooming, hunting) are considered to have higher welfare than those animals performing unnatural or harmful behaviours (e.g. obsessive and excessive grooming, stereotypical behaviours) or those lacking natural behaviours in their repertoire (Bracke and Hopster, 2006; Keeling and Jensen, 2017; for a critique on using behaviour to assess welfare see Watters et al., 2021).

Play has been suggested as an indicator of good animal welfare (Held and Špinka, 2011). Multiple studies have explored the link between play and positive welfare. Lawrence (1987) proposed that play is only performed when environmental conditions are 'good' and an animal's 'proximate needs' have been met. Environments with good conditions, that meet an animal's needs, would be considered to contribute to relatively higher welfare. In pigs, Barnes et al., (1976) observed that malnourished piglets spent less time playing with objects than well-nourished piglets. Worsaae and Schmidt, (1980) reported a negative correlation between plasma cortisol levels and play fighting and running in later weaned piglets. Studies have also shown that play behaviour positively relates to weight gain both pre and post weaning (Brown et al., 2015; Franchi et al., 2023).

It is widely accepted that the occurrence of tail-biting indicates that some or all pigs within a pen are experiencing reduced welfare (Schrøder-Petersen and Simonsen, 2001). The condition of the tail has been suggested as a proxy for tail biting behaviour (Zupan Šemrov and Patt, 2024), which is associated with several sustained problems in pig production. The underlying risk factors contributing to tail biting (changes in health (Boyle et al., 2022), weight (Van De Weerd et al., 2005), air temperature and ventilation (Geers et al., 1989; Scheepens et al., 1991), provision of environmental enrichment (Van de Weerd and Day, 2009), group size (Misra et al., 2021)) are often indicators of poor welfare. Tail biting itself compromises welfare, potentially resulting in severe injuries, that are likely to be painful for the pig and open to infection.

Improving pig welfare

As global pork production shows no sign of decline, with an estimated 116.45 million metric tonnes of pork products (909 million to 1.1 billion pigs) predicted to be produced in 2024/2025 (Pork | USDA Foreign Agricultural Service) our responsibility to improve pig welfare becomes increasingly important.

It could be argued that tail biting is the most prevalent behavioural welfare issue among pigs in the EU and even though the routine docking of tails is banned in the EU, the European Food Safety Authority (EFSA) (EFSA, 2007) reported that the practice of tail docking was widespread in the vast majority of EU Member States, with reports of 81–100% of pigs being tail docked. As previously mentioned, tail docking has a significant negative impact on the welfare of pigs (Nannoni et al., 2014), it is often performed without analgesia in the first few days of a pig's life and does not completely prevent tail biting nor address the underlying cause. A major improvement in pig welfare would be to reduce the reliance on tail docking, by providing conditions under which pigs could live with undocked, unbitten tails. There is a small body of literature exploring the efficacy of alternatives to tail docking in intensive systems (with slatted floors). Chou et al., (2020) suggested that the provision and regular replenishment of multiple, slat-compatible, enrichment sources can reduce tail damage to manageable levels without the need for tail docking. Wallgren et al. (2019) found that the provision of an increased straw ration decreased the number of damaged tails in finishing pigs. However, other studies have shown that even with specific training and the provision of enrichment, keeping pigs fully undocked led to an increase in mortality rates (Menegon et al., 2025).

As well as reducing harmful social behaviours to improve welfare, we can also strive to increase the opportunities for pigs to display positive social behaviours. Allowing pigs to display play more frequently or by extending their play window could improve welfare.

However, we are constrained to work within the commercial limits of the pig industry. Increasing space, better provision of enrichment, eliminating mixing etc. are currently not practical and while we should be simultaneously striving to overhaul the housing and management of pigs, we must be realistic and look for shorter-term, cost-effective changes we can make today. One such change could be nutrition. It could be relatively easy and economically feasible to fine-tune attributes of the feed to reduce negative social behaviours and promote positive ones.

Diet and Behaviour

The link between diet and behaviour is complex and not yet fully understood. The quantity and composition of food are important; energy content, amino acid profile, vitamin and mineral levels are all important factors to consider when examining the effect of food on health and behaviour. Both deficiency and excesses of specific dietary components can impact behaviour, further details of which are given in later sections. When exploring the role of nutrition in the expression of behaviour it is important to begin by considering the intricacies of the digestive system. Extensive research into the effects of nutrition on behaviour has already been carried out in humans. Similarities between the two species (both are omnivorous monogastrics) allows us to apply much of this research to pigs. Dietary components can directly or indirectly (via the microbiome-gut-brain axis) affect behaviour, and behaviour can impact, or be impacted by, an animal’s welfare state.

The gut-brain-axis

Human interest in the relationship between the brain and the gut began in ancient Greece but the first breakthrough came when William Beaumont was able to observe how emotions affected the gastric mucosal morphology and function of a patient, Alexis St. Martin, a voyager with a traumatic gastric fistula (Beaumont, 1977). Further studies in the early 20th Century showed that increased blood flow, motility, and secretion in the intestines occur when subjects experience feelings of anger, intense pleasure, or displayed aggressive behaviour. Studies also showed mucosal pallor and decreased secretion, and motor activity occurred with fear or depression: states of withdrawal or disengagement from others (Drossman, 2016). The studies clearly demonstrated that the gut physiologically responds to emotion and environmental stimuli like stress, but the studies were basic and one directional. As technology developed in the 1970s, scientists were able to better understand the motor and electrical activity of the gut. However diagnostic imaging and physiological assessment did not fully explain many patients' symptoms or do much to explain the psychological illnesses that often accompanied gastrointestinal disorders. In the latter half of the 20th century, George Engel proposed the biopsychosocial model. This new model stated that illness was a product of biological, psychological and social systems interacting at multiple levels and that it was a combination of these interacting factors that determines an illness (Engel, 1977; Engel, 1980). This new model further supported the interaction between the gut and the brain. By the end of the 20th century, new technologies and techniques, such as brain imaging, provided insight into the association between neural cells and immune functions, gut signalling, neural stimulation and pain perception. This allowed for a deeper understanding of the interactions between the gut and brain and the concept of the bidirectional brain-gut axis (Drossman, 2016). In 1999, Wood et al. outlined the fundamentals of neurogastroenterology, "a new and advancing subspecialty of clinical gastroenterology and digestive science". Neurogastroenterology focuses on the "functions, malfunctions, and malformations in the brain and spinal cord, and the sympathetic, parasympathetic and enteric divisions of the autonomic innervation of the digestive tract". The field of neurogastroenterology provided a firm foundation for gut-brain research. The majority of gut physiology is under neural control of the enteric nervous system (ENS). Although the ENS can regulate gastrointestinal tract movement largely independently of the central nervous system, motility is also modulated by the brain, other divisions of the autonomic nervous system, the gut associated immune system and the gut microbiome (Margolis et al., 2021). The gut microbiome is particularly important to the regulation of gut-brain function and is studied in its own right, as the microbiota-gut-brain-axis (Cryan and Dinan, 2012).

The microbiota-gut-brain-axis

The gut microbiome is established early in an animal's life; some research suggests as early as the foetal period (Wang et al., 2019). The microbiome continues to develop as neonates are exposed to the external environment and the wide variety of microorganism within it. The composition of the microbiome is determined by various factors; rearing environment (Inman et al., 2010), early diet (Li et al., 2012; Bian et al., 2016), current diet (Van Hul et al., 2024) and mode of food delivery (Wang et al., 2013) . Different microbes inhabit different sections of the intestines due to the variations in the microenvironment providing different niches (Zhang et al., 2018). Intestinal microbial communities include bacteria, fungi and viruses. The microbiome plays an important role in the health and wellbeing of animals, performing functions the host would not otherwise be capable of (Stokes, 2017) primarily through microbial metabolism. Gut microbes ferment dietary fibres, producing short chain fatty acids (SCFA) including acetate, propionate and butyrate (Koh et al., 2016). These SCFA and other microbial metabolites impact brain function via the vagus nerve, blood brain barrier and the immune system (O'Mahony et al., 2015; Dalile et al., 2019). SCFA bind the free fatty acid receptors of vagal nerve fibres to transmit signals to the central nervous system. SCFA can affect the maturation and function of microglia in the central nervous system. SCFA also pass through the blood brain barrier via monocarboxylate transporters and can affect the integrity of the blood brain barrier by upregulating the expression of tight junction proteins (Silva et al., 2020).

Studies exploring the role of the gut microbiota in human behaviour and cognition have highlighted that the microbiota plays a role in modulating stress response and stress related behaviours that are seen in psychiatric disorders such as anxiety and depression. Controlled studies have manipulated the gut microbiome, using infections, antibiotics and probiotics and then looked for changes in behaviour and/or cognitive ability. These studies suggest that intestinal microbiota can affect the postnatal development of the hypothalamic- pituitary- adrenal (HPA) system stress response in mice (Sudo et al., 2004). The HPA system is involved in the host stress response and regulates various processes including digestion, immune response and the regulation of mood and emotions.

The food

There are various aspects of the diet, feed delivery system and feeding environment that have already been shown to affect behaviour. Increasing feeder space (Lu et al., 2022), increasing number of feeder spaces per pig (Spoolder et al., 1999), utilisation of feeding stalls (Morrow and Walker, 1994) and provision of liquid feed (Andersen et al., 2023) have been shown to reduce ear lesions, aggressive interactions, tail biting and bite marks, respectively. Fiber content of feed has also been investigated as a strategy to reduce negative social behaviours (Gall et al., 2009). Studies have shown that increased fibre can reduce some aggressive behaviours in sows (Stewart et al., 2010). Mineral content has also been studied. There is some evidence that supplementary magnesium, in doses between <1g - >10g (from various studies analysed in a systematic review) have been seen to reduce stress measures in pigs (a range of physiological and behaviour measures across the various studies) (Bushby et al., 2021). Initially investigated in relation to meat quality (D'Souza et al., 1998), supplementary magnesium could also have important welfare implications. The energy content of feed has also been suggested to impact social behaviour. Historically, diets with a high energy content were cited as anecdotally contributing to outbreaks of tail biting (Gadd, 1967). However, later empirical studies found that high energy diets did not induce tail biting (Ewbank, 1973). Conversely, it has been suggested that energy may promote positive social behaviours. There is significant interest in the manipulation of amino acid levels as a nutritional strategy to affect behaviour in pigs. As pigs are monogastric, they lack a rumen and consequently rumen microbes to ferment ingested food into amino acids, therefore pigs must acquire amino acids from food.

Of all these dietary aspects, I have chosen to focus on the effect of energy and the amino acids lysine and tryptophan.

Lysine

Lysine is the first limiting amino acid in the majority of corn-based pig diets. Lysine is essential for determining growth and lean muscle deposition (Main et al., 2008) and lysine has also been suggested to have an effect on immune response in pigs and other monogastric species (Kornegay et al., 1993; Chen et al., 2003). Little is known about the effect of lysine on pig behaviour and the potential mechanisms by which it may act. One study found that diets fortified with lysine and arginine reduced plasma cortisol levels and reduced stress responses to transportation in pigs (Srinongkote et al., 2003). Rat studies have shown the administration of lysine to have an anxiolytic effect and to result in an increase in brain serotonin levels (Jalal et al., 2022). However, in another study it was found that when the tail of a rat was experimentally irritated, provision of lysine was found to enhance pain sensitivity and affective defence behaviours (Severyanova et al., 2019).

Lysine levels play an important role in health and immune response (Datta et al., 2001; Li et al., 2007). Multiple studies have explored the link between disease and damaging behaviour (review by Boyle et al., 2022) and the majority of the literature point towards an association between low health and negative social behaviours. Studies have linked internal inflammation to biting behaviour (Czycholl et al., 2023) and uncovered a correlation between cytokine levels and social behaviour (Munsterhjelm et al., 2017). Despite being the first limiting amino acid, only one study has explored the effect of increasing lysine levels on social behaviour (Minussi et al., 2023). When examining the effects of "indispensable amino acids", Minussi at al found that IAA supplementation (which included lysine) was more effective than additional environmental enrichment in counteracting the negative effects of a low protein diet on tail biting in pigs.

Tryptophan

Tryptophan plays a major role in regulating behavioural and physiological processes in pigs such as appetite (Zhang et al., 2007), mood (Poletto et al., 2010; Poletto et al., 2014) (however, see Stracke et al., 2017 for opposing evidence), stress hormone secretion (Adeola and Ball, 1992; Koopmans et al., 2005), activity (Koopmans et al., 2006) and immunity (Melchior et al., 2004; Floc'h et al., 2009). Tryptophan is important for protein synthesis, is metabolised through the kynurenine pathway and is the precursor of serotonin (5-hydroxytryptamine, 5-HT) and melatonin. Serotonin is a neuromediator, produced in the brain, platelets and the gut. The proportion of tryptophan used for the production of serotonin is as low as 1% (Wolf, 1974). Tryptophan transport and availability in the brain have been shown to be one of the limiting steps for serotonin synthesis. Adeola and Ball (1992) found that pigs supplemented with excess tryptophan showed increased brain serotonin concentration and Henry et al. (1996) showed the converse; a tryptophan deficiency resulted in reduced serotonin production in the brain.

The kynurenine pathway accounts for the catabolism of over 90% of ingested tryptophan (Peters, 1991; Thomas et al., 2019). The kynurenine pathway produces nicotinamide adenine dinucleotide (NAD), a cofactor involved in multiple aspects of cellular metabolism. The kynurenine pathway is associated with body defences and immune response regulation. Links between the pathway and neurodegenerative diseases, tumour proliferation, inflammation, and mood disorders are being explored (Arnone et al., 2018).

Indoleamine-2,3-dioxygenase (IDO) is a haeme-containing enzyme that catabolises compounds containing indole rings, like tryptophan. IDO is one of three enzymes that catalyse the first and rate limiting step of the kynurenine pathway. IDO is an important actor in the immune response. It is produced by cells in response to inflammation and has an immunosuppressive function. During periods of immune stress and inflammatory response, there is increased catabolism of tryptophan through the kynurenine pathway and activation of IDO (Melchior et al., 2005). This leads to reduced tryptophan availability if dietary tryptophan is insufficient.

The exact role of kynurenine metabolites in mood disorders is not fully understood but the activation of the kynurenine pathway is one of the described mechanisms by which inflammation can induce depression (Arnone et al., 2018) and there is evidence that a dysregulation of the kynurenine pathway occurs in bipolar disorder (Bartoli et al., 2021). Dysfunction of the pathway can also affect serotonin and melatonin synthesise (Zoga et al., 2014). As both the kynurenine pathway and serotonin production have been shown to affect mood, there is significant interest in the use of tryptophan as a nutritional strategy to control stress in pigs.

There is already a small body of research focusing on the effect of tryptophan on social behaviour in pigs (Meunier-Salaün et al., 1991; Koopmans et al., 2006; Shen et al., 2012; Liu et al., 2013; Poletto et al., 2014; Castilha et al., 2016; Stracke et al., 2017; Lay et al., 2021; Henry et al., 2022). However, the studies administered tryptophan at levels several fold higher than the commercial norm in pig diets, which is likely to be prohibitively costly in commercial production systems. Studies are lacking which investigate the effect of tryptophan on social behaviour within the range commonly used in industry across the US and Europe and no study has given attention to the effect on positive social behaviours.

Energy

Most dietary energy comes from dietary fat, protein, and carbohydrate. During digestion, food is broken down into its monomer subunits, proteins into amino acids, polysaccharides into sugars, and fats into fatty acids and glycerol, through the action of enzymes. After digestion, the small organic molecules derived from food enter the cytosol of the cell, where their gradual oxidation begins. Glycolysis converts molecules of glucose into two smaller molecules of pyruvate. During pyruvate formation, activated carrier molecules are produced, adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NAD) + hydrogen (H) (NADH) (Alberts et al., 2002). The conversion of ATP to adenosine diphosphate (ADP) is the principal mechanism for energy supply in biological processes. Energy is produced in cells when the terminal phosphate group in an ATP molecule is removed from the chain to produce adenosine diphosphate (ADP) when water hydrolyses ATP (Myers, 2007). The energy produced drives processes in organisms and cells, including intracellular signalling, DNA and RNA synthesis, purinergic signalling, synaptic signalling, active transport, and muscle contraction (Dunn and Grider, 2025).

There is relatively little literature exploring the effects of energy on social behaviour in pigs. Some exploration of the role of energy in tail biting has been performed but results were inconclusive (McIntyre and Edwards, 2002). McIntyre and Edwards aimed to determine if differentially altering the protein and energy intake of pig feed had an effect on preference and chewing behaviour directed at a tail models. They only found an effect of diet on preference scores in the final week of their experiment (week 5 of 5). However, a sufficiency of dietary energy has been hypothesised to be essential for the expression of play behaviour through the Surplus Resource Theory (Burghardt, 2005). Barnes et al. (1976) observed that malnourished piglets spent less time playing with objects than well-nourished piglets whilst play behaviour has been shown to positively relate to weight gain both pre- and post-weaning (Brown et al., 2015; Brown et al., 2018; Franchi et al., 2023). Studies of other animal species have explored the link between play and energy provision. Deer fawns reduced play behaviour in response to reduced milk provision (Muller-Schwarze et al., 1982) and dairy calves showed reduced play behaviour in association with a reduction in energy intake

(Krachun et al., 2010; Miguel-Pacheco et al., 2014; Rushen et al., 2016). To my knowledge, studies have not previously explored causation by empirically manipulating the diet and examining the effect on a wide repertoire of play behaviours in pigs.

Research aims and objectives

The aim of this thesis was to explore the role of nutrition in expression of positive and negative social behaviours to improve pig welfare.

The following objectives were addressed, with specific hypotheses detailed in each chapter:

Chapter 2: Explore the effects of dietary energy on pig behaviour, specifically play, to test the predictions of the Surplus Resource Theory

Chapter 3: Explore the effects of dietary lysine and tryptophan on the social behavior of pigs under USA commercial conditions

Chapter 4: Explore the effects of tryptophan to lysine ratio on the performance of fattening pigs fed at different levels of lysine under USA commercial conditions

Chapter 5: Explore effects of dietary lysine on social behaviour of UK commercial pigs fed lysine levels within the range currently available within pig diets

Chapter 2: Exploring Surplus Resource Theory and the role of dietary energy in the expression of play behaviour in commercial pigs

Chapter 2 contains the manuscript submitted for peer review for publication in Scientific Reports.

At the time of resubmitting this thesis with corrections, a reviewed manuscript has been returned to reviewers and is awaiting final decisions (Appendix 2).

A note on author contribution: Eleanor Hewett and Simon Turner designed the trial with nutritional consultations from Jos Houdijk. Eleanor Hewett collected the data and performed the analysis with guidance from Simon Turner. Eleanor Hewett drafted the manuscript which was reviewed by Simon Turner, Jos Houdijk, Craig Lewis and Andrea Doeschl-Wilson.

Introduction

There is growing interest amongst animal welfare researchers to shift the focus of animal welfare discussions to providing animals with a life worth living and promoting positive welfare. An important aspect of this effort is understanding and promoting positive social behaviour. One of the more commonly researched positive behaviours in mammals is play. Play occurs in juveniles of numerous species and the Surplus Resource Theory (SRT) (Burghardt, 2005) states that play will occur more frequently when an individual has access to necessary resources. As play is typically an energetically demanding behaviour, we predicted that dietary energy would impact play behaviour. This first study sought to empirically test the Surplus Resource Theory by manipulating dietary energy. Specifically, we hypothesised that a reduction in dietary energy would supress play behaviour in line with the prediction of the Surplus Resource Theory. Pigs display a wide range of play behaviours (object, social and locomotor) and need little encouragement to engage in play, making them excellent candidates for exploring the Surplus Resource Theory. This study also has important welfare relevance as we strive to provide farm animals with increased opportunities to engage in positive social behaviours. Play is believed to be intrinsically rewarding as well as having a potentially wide range of developmental benefits (Spinka et al., 2001).

Hence, the study has value in elucidating how manipulation of a key component of the diet could affect the animal's current and future welfare.

This first study was designed to provide pigs on one of two diets (a base level energy diet and a lower energy diet) with maximum opportunities for play by providing them access to a designated play arena, with additional enrichment. Pigs were also given a sucrose supplement to explore if temporary reversal of the adverse effects of the low energy diet was possible.

Submitted Journal Manuscript

Exploring Surplus Resource Theory and the role of dietary energy in the expression of play behaviour in commercial pigs

Eleanor Hewett*, Animal Behaviour and Welfare, School of Veterinary Medicine and Biosciences, Scotland's Rural College (SRUC), Edinburgh EH9 3JG

Jos Houdijk, Monogastric Science Research Centre, School of Veterinary Medicine and Biosciences, Scotland's Rural College (SRUC), Edinburgh, EH9 3JG

Craig Lewis, PIC Product Development, PIC, C/Pau Vila, 22 2o piso, 08174, Sant Cugat del Valles, Barcelona, Spain

Andrea Doeschl-Wilson, The Roslin Institute, The Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Edinburgh, EH25 9RG

Simon Turner, Animal Behaviour and Welfare, School of Veterinary Medicine and Biosciences, Scotland's Rural College (SRUC), Edinburgh, EH9 3JG

Abstract

The Surplus Resource Theory proposes that play behaviour is expressed more abundantly in juveniles that have access to necessary resources. We propose that sufficient dietary energy is likely to be a requirement for play and therefore hypothesise that pigs on an energy-deficient diet will show reduced play. A total of 128 mixed sex domestic pigs were randomly allocated to one of two diets: low energy or high energy. Pigs were given access to a play arena and on alternative occasions, before entering the arena, offered a sucrose supplement (sugar cubes). We hypothesised the provision of additional sucrose would increase play, particularly for pigs on the low energy diet. The low energy diet reduced daily weight gain (p=0.005) and increased feed conversion ratio (p<0.001). Dietary treatment and play session interacted on social object (P=0.025), solo locomotor (P<0.001) and social locomotor play (P<0.001). Dietary treatment and pen mate average sugar intake interacted on social object (P=0.019) and social locomotor play (P<0.001). Play session (P<0.001), and the interaction between dietary treatment and pen mate average sugar intake (P=0.002) affected total play. Overall, the high energy diet appeared to extend the developmental period of heightened engagement in social play. Our findings suggest that diet quality in weaned pigs may impact specific aspects of play behaviour with potential benefits for animal welfare.

Key words

Surplus Resource Theory, pig, play, energy, sugar

Introduction

Positive social behaviours have been shown to be critical for physical, physiological and psychological development in mammals. Positive social behaviour in animals is a relatively novel area of study, with much of the focus thus far being on play behaviour. Play or playful behaviours have been observed in an extensive range of species, including birds (Diamond and Bond, 2003), reptiles (Dinets, 2023), mammals (Špinka et al., 2001), fish (Eisenbeiser et al., 2022), cephalopods (Mather and Anderson, 1999; Kuba et al., 2014) and potentially insects (Galpayage Dona et al., 2022). Play is important for the development of cognitive, motor and social abilities(Špinka et al., 2001). Lawrence(Lawrence, 1987) proposed that play is only performed when environmental conditions are 'good' and an animal's 'proximate needs' have been met. Environments with good conditions, that meet an animals' needs, would be considered to contribute to relatively higher welfare. Burghardt's(Burghardt, 2005) Surplus Resource Theory (SRT) proposes that play behaviour is expressed in juveniles that have access to the necessary resources (e.g. a safe environment, time and adequate nutrition to support energy-demanding activities)(Graham and Burghardt, 2010). Burghardt proposed that play is most likely to evolve in young, warm-blooded animals (which is now being debated, see above for fish, reptile and insect play), with long juvenile phases and where food and protection are provided by the parent/parents.

Mammalian livestock species show a rich variety of play (e.g. cattle (Waiblinger et al., 2020), sheep (Bennett and Fewell, 1987), and pigs (Newberry et al., 1988)). Play behaviours are most commonly seen early in life and are similar to behaviours seen in adults (e.g. running, fighting), with the distinguishing features of being exaggerated, energetic, repetitive and non-damaging (Burghardt, 2005). Play can be grouped into various (overlapping) categories: locomotor, object-directed, individual and social. Locomotor play can be performed individually or with conspecifics and can involve running, pivots, flops, hops and jumps (Newberry et al., 1988; Jensen et al., 1998; Chaloupková et al., 2007). Object-directed play involves manipulating, carrying or shaking an object (Newberry et al., 1988). Social play is between two or more individuals and involves nudging, pushing, climbing and non-harmful fighting. Play fighting usually involves elements of adult fighting but without injury and in which role reversals and handicapping may be seen depending on the species (Pellis and Pellis, 1987; Pellis and Pellis, 2016; Weller et al., 2019).

Play behaviour is typically energetically demanding and likely to increase visibility to predators whilst reducing vigilance. Berghänel et al. (Berghänel et al., 2015) demonstrated that play was energetically demanding, as locomotor play accounted for up to 50% of variance in growth in macaques. Hence the Surplus Resource Theory predicts that play will occur primarily when nutritional resources are adequate and risks of injury or predation are mitigated. In this paper we focus only on the effects of diet quality on play, whilst acknowledging that other enablers of play are likely to be important.

Barnes et al.(Barnes et al., 1976) observed that malnourished piglets spent less time playing with objects than well-nourished piglets whilst play behaviour has been shown to positively relate to weight gain both pre- and post-weaning (Brown et al., 2015; Brown et al., 2018; Franchi et al., 2023). Similar observations have been made in calves, where reduced energy intake was associated with reduced play behaviour (Krachun et al., 2010; Rushen et al., 2016). These data suggest that there may be a relationship between provision of nutritional resources and play behaviour. However, studies have not previously explored causation by empirically manipulating the diet and examining the effect on a wide repertoire of play behaviours.

Using commercial pigs as our study species, we aim to test the central hypothesis of the Surplus Resource Theory through direct manipulation of dietary energy as the first limiting nutritional resource. We study the effects on a wide repertoire of play behaviours in an environment that encourages expression of play. Pigs are a social species with rich expression of play that can be stimulated experimentally. They therefore make an excellent study species to test the Surplus Resource Theory. As play has been suggested as an indicator of animal welfare (Held and Špinka, 2011), the study may also provide a first step towards developing nutritional strategies to promote expression of positive social behaviour and positive welfare.

In addition to energy derived from the diet, we aim to explore how short-term supplementary energy provision will acutely affect play behaviour of pigs. We hypothesise that pigs on an energy-deficient diet will show reduced play, which may be temporarily reversed by sucrose provision.

Methods

Ethical Review

The trial was reviewed and approved by the Scotland's Rural College (SRUC) Animal Experiments Committee (research programme number PIG RP 4-2022, animal experiment number PIG AE 11-2022) and was licensed by the Home Office (Home Office project licence number PP1403242). Authors complied with ARRIVE guidelines 2.0, Essential 10. All routine animal management procedures were performed by trained staff and health issues were treated as required. All stages of the experiment were conducted in liaison with the SRUC veterinary surgeon.

Animals and Animal Housing

A total of 128 (68 male and 60 female) (Large White × Landrace) × Danish Duroc domestic piglets were used over two farrowing batches (64 per batch). The piglets were born at SRUC's Easter Howgate Pig Unit. Teeth and tails were left intact, and males were not castrated. Piglets were weaned at 28 days, receiving a wean tag and vaccination as per normal farm procedure and allocated to mixed litter groups of 8 pigs. Groups contained on average 4 sibling pairs from different litters, of similar wean weights (to minimise aggression and bullying) and where possible each litter-pair comprised of one male and one female. Siblings were balanced across diets. Groups were housed at a space allowance of 1.28m^2 per pig (pen size 5.1 x 2.0m) on solid concrete floors with a light sawdust bedding, provided with basic enrichment (1 Porcichew and 1 old wellington boot) and food and water was available ad libitum via two bowl drinkers and two fixed plastic feeders. Pens were cleaned manually once daily. Pigs were on trial for a total of 35 days after which they were returned to farm stock.

Play arena

A separate play arena, measuring 5.1 x 5.1m was used for play test sessions in which play was stimulated and observed. The play arena had solid concrete flooring, with deep sawdust and straw cover. The following, randomly selected, additional enrichment toys were added to the play arena; a 2m length of natural fibre rope knotted in the middle, one empty feed sack, one plastic ball and one silicone dog toy. Waste was removed from the play arena between each group play session. Cameras (Canon Legria HFG25) were set at opposite corners of the play arena to capture the behaviour of all pigs in the play arena. (Figure 1).

Habituation

All pigs were habituated to human presence/touch from 5 days post farrowing to allow for individual marking. Post weaning, pigs were given 6 days to settle in their new pens and then habituated to a weigh crate and the play arena 4 times prior to test sessions, once daily over three consecutive days, with one day rest on day 4, before a final habituation session on the 5th day. One pen at a time, the social group was moved from their home pen to the play arena via the weigh crate. During the first 3 habituations, toys had been placed in the play arena before pigs entered; on the final habituation session, toys were added to the pen after the pigs had entered to mimic the test days (described below).

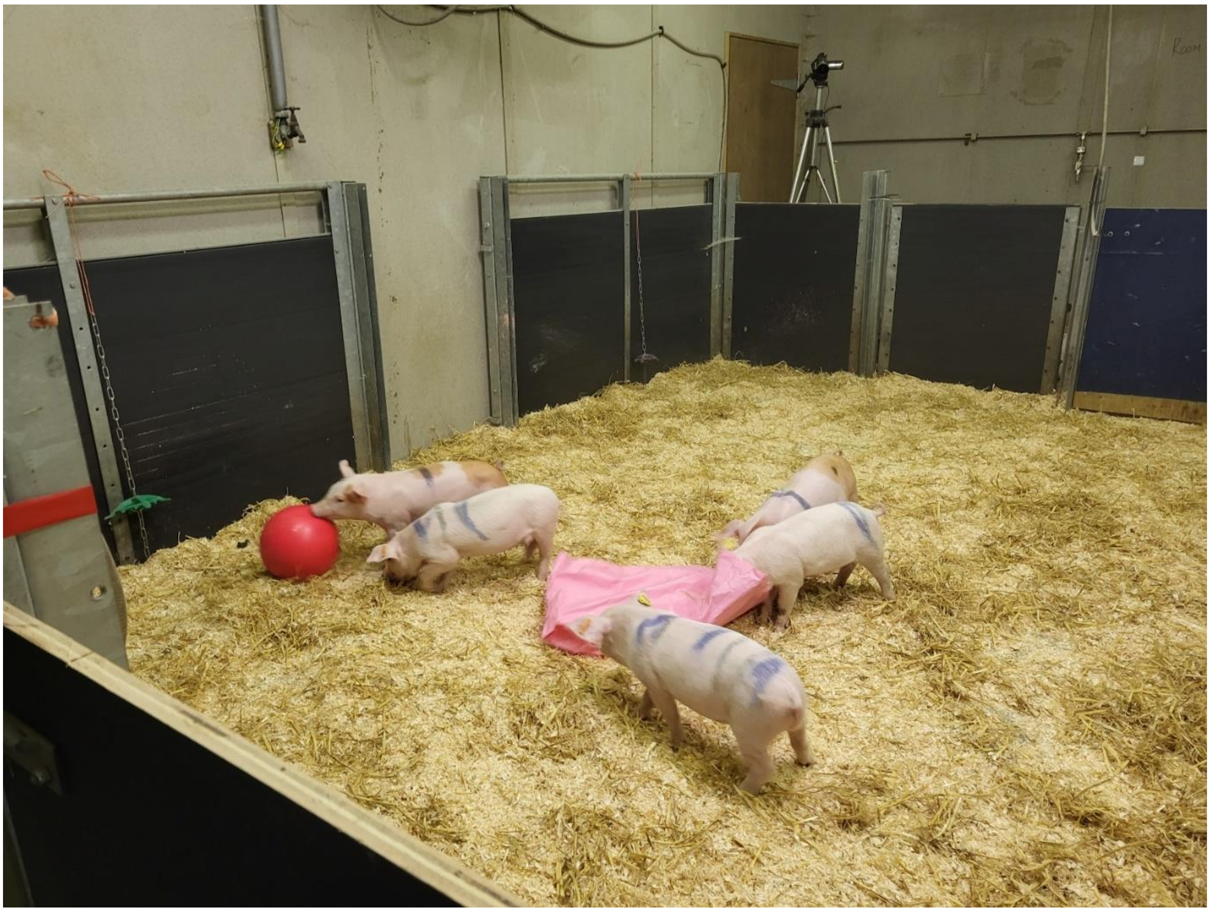


Figure 1. Play arena. A separate play arena was used, measuring 5.1 x 5.1m, with solid concrete flooring, with deep sawdust and straw cover. Photo shows pigs in play arena, interacting with toys.

Piglet measures

Piglets were weighed at weaning, at day 8 post weaning and then every time they moved to the play arena thereafter. Feed consumption was recorded on a pen level. Daily feed delivery and refusal was weighed manually. The data derived were used to estimate average daily feed intake (ADFI), average daily body weight gain (ADG) and feed conversion ratio (FCR, calculated as ADFI/ADG).

Experimental treatments

After weaning (at 28 days) pens of pigs were randomly allocated to one of two dietary treatments: a basal diet that represented the commercial norm (basal) (digestible energy 16.9 MJ/kg, fibre 8.5%, crude protein 18.1%) or a low energy diet achieved by diluting the basal diet with 15% indigestible fibre, Arbocel (digestible energy 15.3 MJ/kg, fibre 18.4%, crude protein 16.1%). Feeds were sampled daily, pooled by treatment and analysed for digestible energy through regression on chemical analysis outcomes (Table 1). The experiment was conducted over two batches, each batch consisted of 8 pens, with 8 pigs per pen.

Table 1. Ingredients and chemical analysis of the experimental diets

Ingredients, %	**Basal**	**Arbocel (calculated)**
Micronized wheat	20	17
Wheat	24.32	20.67
Cooked dehulled oats	6	5.1
Micronized maize	10	8.5
Fishmeal	1.5	1.278
AlphaSoy*	5	4.25
Full fat soya	7	5.95
Soya bean meal	3.5	2.98
Soya protein concentrate	4	3.4
Whey powder	10	8.5
Fat-filled whey (coconut)	4	3.4
Vegetable oil	1	0.85
Limestone	1.12	0.95
Monocalcium phosphate	0.66	0.56
Sodium bicarbonate	0.23	0.20
Salt	0.05	0.043
l-Lysine-HCl	0.535	0.455
dl-Methionine	0.135	0.115
l-Threonine	0.235	0.2
l-Tryptophan	0.045	0.038
L-valine	0.15	0.128
Premix	0.5	0.425
Arbocel	0	15
Pig flavour (vanilla/caramel)	0.025	0.021

Chemical analysis	**Basal**	**Arbocel**

Dry Matter (DM), g/kg	892	896
Digestible energy**, MJ/kg DM	16.89	15.32
Neutral Detergent Fibre, g/kg DM	95.29	205.36
Ether Extract, g/kg DM	63.68	58.59
Total Oil, g/kg DM	70.52	65.96
Crude Protein, g/kg DM	202.91	179.69
Ash, g/kg DM	59.42	52.46

*Enzyme-treated soya bean meal with typical crude protein content of 530 g/kg

**Digestible energy is calculated through regression: 17.47 + 0.0079 × Crude Protein + 0.0158 × Total Oil – 0.0331 × Ash – 0.014 × Neutral Detergent Fibre(McDonald et al., 2011).

Sucrose supplementation and play arena sessions

From day 10 post weaning, piglet home groups were moved from their home pen to the play arena on 4 occasions over 10 days, balanced for time of day across treatments, with two days between play sessions. Each group consistently entered the play arena in either the morning or afternoon (balanced across diet). Before entering the play arena, all pigs were weighed in a weigh crate. On alternate occasions, the piglets were given a sucrose supplement while in the weigh crate immediately before entering the play arena. All pigs were held in the weigh crate for 60 seconds, whether offered sugar or not. The amount of sugar given was relative to body weight and increased over the course of the trial. To our knowledge, this is the first trial to supplement pigs with sugar cubes. As there is no previous research on the appropriate dose, we used the UK National Health Service recommended daily intake for children: 4-6 year olds, average weight range of 8-16kg, 5 cubes and 7-10 year olds, average weight range 17-44kg, 6 cubes(Sugar: the facts, 2022). At testing, our pigs fell into these weight groups. It was expected that sugar intake would have an acute effect on play but would not be sufficient to alter longer term growth performance. Pigs chose to eat varying amounts of the sugar offered as described below.

Play arena behaviour

All 8 piglets from the home group were released into the play arena simultaneously and immediately after weighing. After 5 minutes in the play arena the above-mentioned toys were added to the arena. After a total of 20 minutes in the play arena, the pigs were returned to their home pen. The pigs' behaviour in the play arena was recorded from video images using an ethogram (Table 2) for a total of 15 minutes per pen, 5 minutes without toys and 10 minutes with toys. Time without toys was given to allow for maximum expression of locomotor play and play fighting. Each pig was continuously sampled, with the frequency of behaviours recorded.

Table 2. Ethogram used to measure behaviour of piglets taken from video recordings taken from play arena.

Behaviour	Description	Reference
Solo locomotor play	A pig is scampering, running, pivoting, head tossing, flopping or hopping. Not associated with delivery or receipt of aggression but sometimes with gentle play butt or play push	Ocepek et al., 2020
Social locomotor play	A pig is scampering, running, pivoting, head tossing, flopping or hopping together with at least one other pig. Not associated with delivery or receipt of aggression but sometimes with gentle play butt or play push	Ocepek et al., 2020; Camerlink et al., 2021
Solo object play	Pig is sniffing, touching or manipulating a toy	Defined for this study
Social object play	Pig is sniffing, touching or manipulating a toy within approximately 30cm of another pig	Defined for this study
Play fighting	Mutual ramming or pushing, without aggressive/damaging biting. Actions performed in a repetitive and energetic manner	Adapted from Camerlink and Turner, 2013 and Weller et al., 2019
Aggression	Head knocking, fighting, biting, ramming	Adapted from Camerlink and Turner, 2013

Skin lesion scoring
Skin lesion scoring was performed at various points through the trial; on days 1, 2 and 3 post weaning, upon entering the play arena (while in the weigh crate) and shortly after returning to their home pen after leaving the play arena. Lesions were recorded to reflect the level of social stress within the group, which could impact play behaviour, and to quantify whether behaviour recorded as play fighting in the arena was correctly recorded as such, rather than being injurious aggression. The following scoring system was used to record the position and number of lesions. Position of lesions: front (from the shoulder to the tip of the nose), middle (between the shoulder and the start of the back leg) and back (from the start of the back leg to the tip of the tail). The number of fresh, bright red lesions measuring longer than 1cm, with no scab formation, was categorised as follows: 0 = no lesions, 1 = 1-5 lesions, 2 = 6-10 lesions, 3 = 11-15 lesions, 4 = 16-20 lesions and 5 = >20 lesions (score system adapted from Camerlink et al., 2016).

Statistical analysis
Statistical analyses were carried out using R Software version 4.4.2 (R Core Team, 2021) and RStudio 2024.12.1+563 "Kousa Dogwood" Release for Windows. Models were constructed using packages lme4 (Bates et al., 2015) and glm2 (Marschner, 2011). Model residuals were visually inspected to confirm approximation to normality and tested for heteroscedasticity using Levene’s Test. P-values below 0.05 were considered statistically significant and P-values between 0.05 and 0.1 were considered as tendencies.

For arena behaviours, the pig was the statistical unit. Data were modelled using a generalised linear mixed model (GLMER), with link functions “log” and “logit”, for frequency of play fighting, aggression, solo object play, social object play and solo locomotor play, whilst a linear mixed model (LMER) was used for social locomotor play and total play. Not all pigs ate all of the sugar offered. We therefore accounted for the quantity of sugar eaten by the individual, and the average eaten by the pen mates of the subject animal. Inclusion of the pen mate average sugar intake recognised that play has a contagious element, as seen in elephants (Norscia et al., 2024), ravens (Wenig et al., 2021), and chimpanzees (Sandars et al., 2024), and the quantity of sugar eaten by other group members could potentially affect the play behaviour performed by a focal animal. Dietary treatment, play session, sex and individual and pen mate average sugar intake were included as fixed effects as well as first order interactions between dietary treatment and play session, dietary treatment and individual sugar intake and dietary treatment and pen mate average sugar intake. Interactions that were not significant at $p<0.05$ were removed from the models. Weight and pig nested in pen, nested in batch were included as random effects. Individual sugar intake was categorised using 4 scores; 0 = sugar not offered, 1 = sugar offered but not eaten, 2 = ate up to half the sugar offered, 3 = ate half or more of the sugar offered. For pen mate average sugar intake there were 3 scores; 0 = sugar not offered, 1 = ate up to half the sugar offered on average, 2 = ate more than half the sugar offered on average.

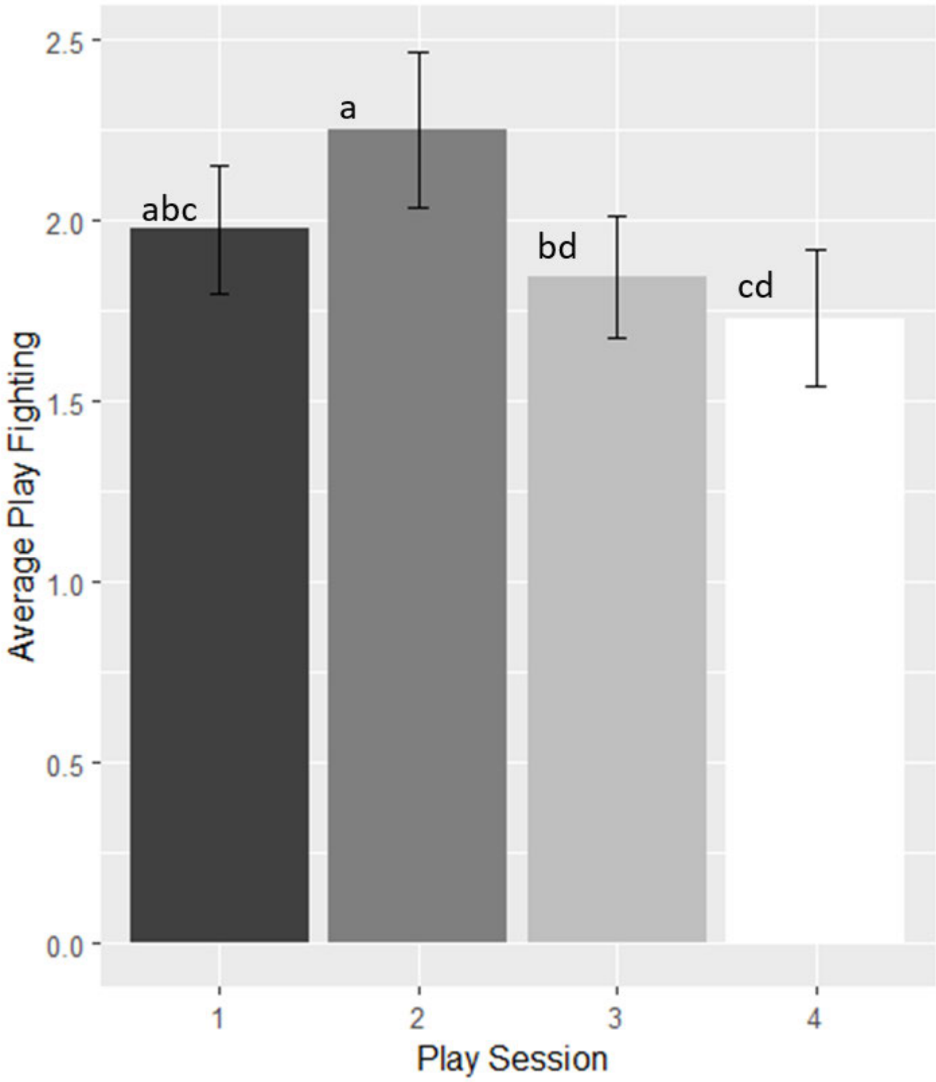
For skin lesions scores, the pig was the statistical unit, and data were coded to show lesion increases; pigs were lesion scored before and after entering the play arena and if there was an increase in lesion score pigs were coded 1, if there was no increase in lesion score, pigs were coded 0. Data were modelled using a GLMER with link function “logit”. Dietary treatment, play session, sex and individual and pen mate average sugar intake were included as fixed effects as well as first order interactions between dietary treatment and play session, dietary treatment and individual sugar intake and dietary treatment and pen mate average sugar intake. Weight and pig nested in pen, nested in batch were included as random effects.

Production data were collected and analysed at the pen level, over a period of 20 days, covering the habituation period (10 days) and 4 play sessions (10 days), with the pen being the statistical unit, averaged to give ADFI and ADG per pig. Data were modelled using an LMER, dietary treatment was included as a fixed effect and pen was included as a random effect.

Results

Play fighting was affected by sex ($P<0.001$) and play session ($P=0.030$) with the frequency of play fighting being higher in males and play fighting being at greater frequency in session 2 than in session 3 and 4 (Figure 2).

Figure 2. Effect of play session on the average frequency of play flighting per pig over 4 play sessions. Non-matching superscripts denotes statistical significance (P=0.030).



Aggression was affected by the dietary treatment x pen mate average sugar intake interaction (P=0.004). Average aggression was less in basal diet pigs of pen mate average sugar intake score 1 (ate less than half of the sugar on average) than that of Arbocel pigs of the same pen mate average intake score. For basal diet pigs, average aggression was also less in pigs of pen average sugar intake score 1 than that of pigs of scores 0 and 2 (Figure 3,a).

Sex was found to affect solo object play (P=0.021) and social object play (P=0.012), both being higher in females. Dietary treatment x play session (P=0.025) and dietary treatment x pen mate average sugar intake (P=0.019) both interacted to affect social object play. Social object play was consistent across play sessions for Arbocel pigs, with levels of social play in basal pigs being higher than that of Arbocel pigs in session 1 and 2 and then levels in basal pigs dropping to be equal to that of Arbocel pigs in session 3 and 4 (Figure 4, a). Social object play was higher in pigs fed the basal diet with a pen mate average sugar score of 1 (ate less than half of the sugar on average) compared to pigs fed the Arbocel diet with the same pen mate sugar intake. Levels of social object play were equal across pen mate sugar intake scores for basal pigs. In Arbocel pigs, levels of social object play were higher when the pen mates ate more than half the sugar on average (score 2) compared to when they ate less than half of the sugar (score 1) (Figure 3, b).

We found an effect of sex ($P=0.002$) and a dietary treatment × play session interaction ($P<0.001$) on solo locomotor play. Solo locomotor play was higher in female pigs and higher in basal diet pigs than Arbocel diet pigs in play session 3. Solo locomotor play levels stayed constant for basal pigs across play sessions but started to reduce in Arbocel pigs at session 2 (Figure 4, b).

For social locomotor play, we found an effect of sex ($P=0.05$), dietary treatment x play session ($P<0.001$), and dietary treatment x pen mate average sugar intake ($P<0.001$). The effect of individual sugar intake tended towards significant ($p=0.06$). Social locomotor play was higher in females. Social locomotor play was higher in Arbocel diet pigs in session 1 but higher in basal diet pigs in session 3 (Figure 4, c). Levels of social locomotor play were equal in Arbocel pigs in session 1 and 2 then dropped to a level equal across sessions 3 and 4. For basal pigs, levels were equal between session 1 and 2 and 1 and 3, with session 4 being lower than other sessions. Social locomotor play was higher in basal diet pigs with pen mate average sugar intake scores of 0 (not offered sugar) and 1 (ate less than half the sugar offered), than Arbocel diet pigs with the same pen mate average sugar intake scores. For pen mate average sugar intake score 2 (ate more than half the sugar offered), Arbocel diet pigs showed more social locomotor play than basal diet pigs. For Arbocel pigs, levels of social locomotor play were equal when pen mate average sugar intake was scored 0 and 1 but higher when scored 2. For basal pigs, social locomotor play increased from pen mate average sugar intake score of 0 to 1 then dropped at pen mate average sugar intake score of 2 to levels below pen mate average sugar intake of 0 (Figure 3, c).

Play session ($P<0.001$), and dietary treatment x pen mate average sugar intake ($P=0.002$) affected total play. Total play started to reduce after session 2 (Figure 5). There was a 16.74% decrease in average total play between session 2 and session 3, then a 14.61% decrease from session 3 to session 4. Total play was higher in basal diet pigs when pen mate average sugar intake was scored 0 (not offered sugar) and 1 (ate less than half the sugar offered) than Arbocel pigs with the same pen mate average sugar intake. With pen mate average sugar intake of 2 (ate more than half the sugar), levels of total play were higher in Arbocel pigs than in basal pigs. In Arbocel pigs, total play was equal in pigs with pen mate average sugar intake of 0 and 1 and higher in pens of pen mate average sugar intake 2. In basal pigs, total play was equal in pens of pen mate average sugar intake score 0 and 2, and higher in pens of pen mate average sugar intake 1 (Figure 3, d).

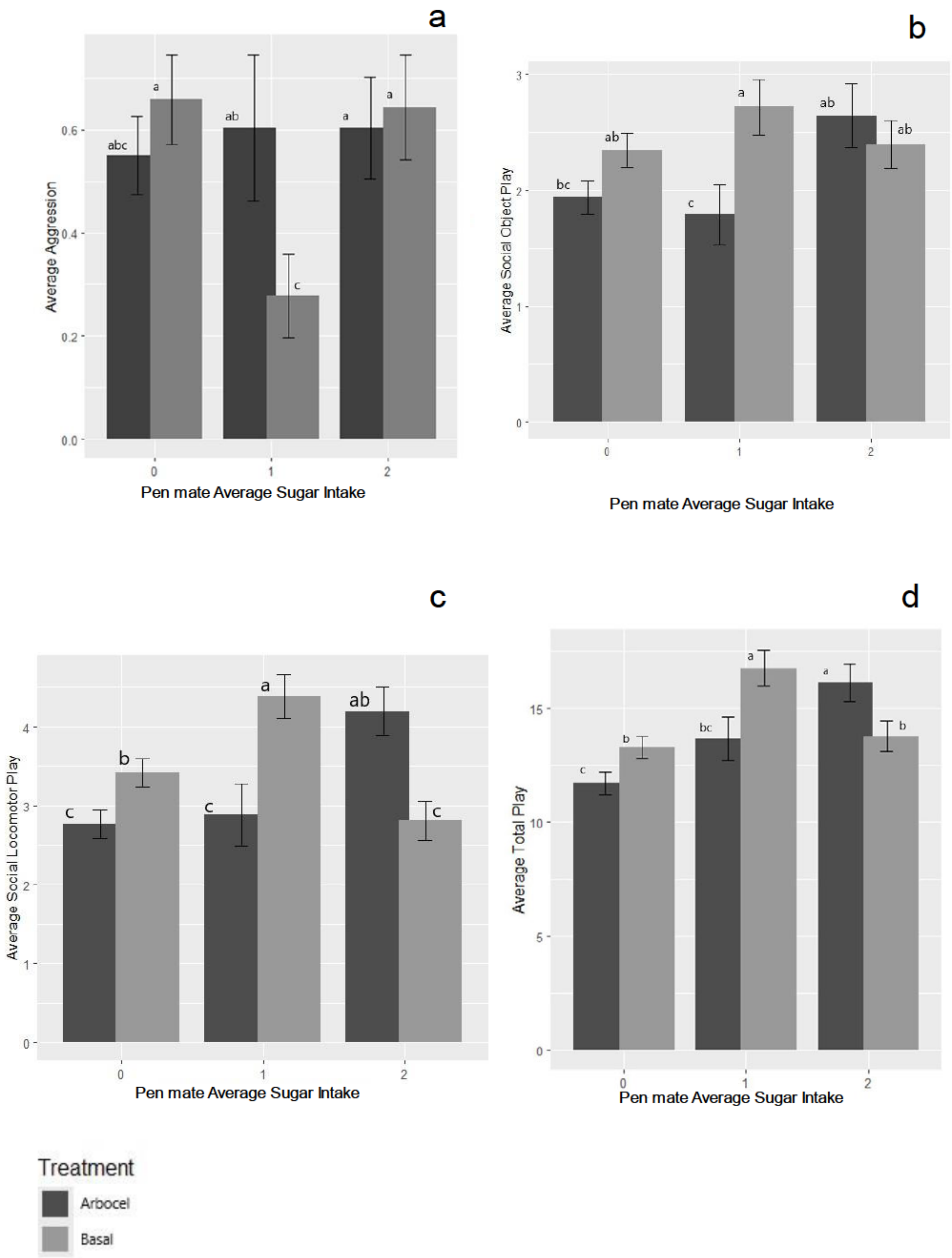


Figure 3 a) Effect of treatment x pen mate average sugar intake interaction on the average frequency of aggressive behaviour per pig on Arbocel and basal dietary treatments. Non-matching superscripts denote statistical significance (P=0.004).

b) Effect of treatment x pen mate average sugar intake on the average frequency of social object play per pig on Arbocel and basal dietary treatments.. Non-matching superscripts denote statistical significance ($P=0.019$).

c) . Effect of treatment x pen mate average sugar intake on the average frequency of social locomotor play per pig on Arbocel and basal dietary treatments. Non-matching superscripts denote statistical significance ($P<0.001$).

d) Effect of treatment x pen mate average sugar intake on the average frequency of total play per pig on Arbocel and basal dietary treatments. Non-matching superscripts denote statistical significance ($P=0.002$).

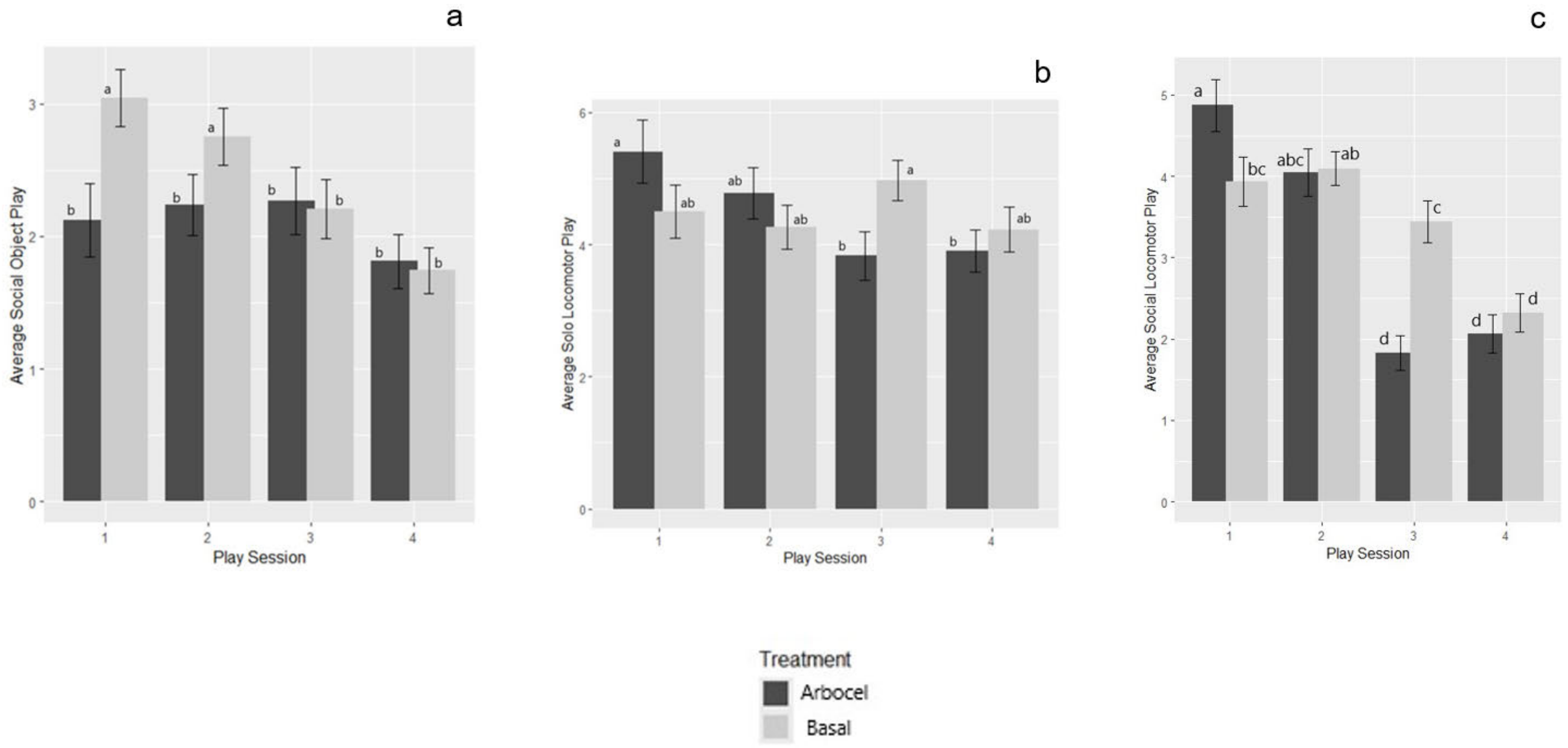


Figure 4 a) Effect of treatment x play session interaction on the average frequency of social object play per pig on Arbocel and basal dietary treatments. Non-matching superscripts denote statistical significance (P=0.025).

b) Effect of treatment x play session on the average frequency of solo locomotor play per pig on Arbocel and basal dietary treatments.. Non-matching superscripts denote statistical significance (P<0.001).

c) Effect of treatment x play session on the average frequency of social locomotor play per pig on Arbocel and basal dietary treatments. Non-matching superscripts denote statistical significance (P<0.001).

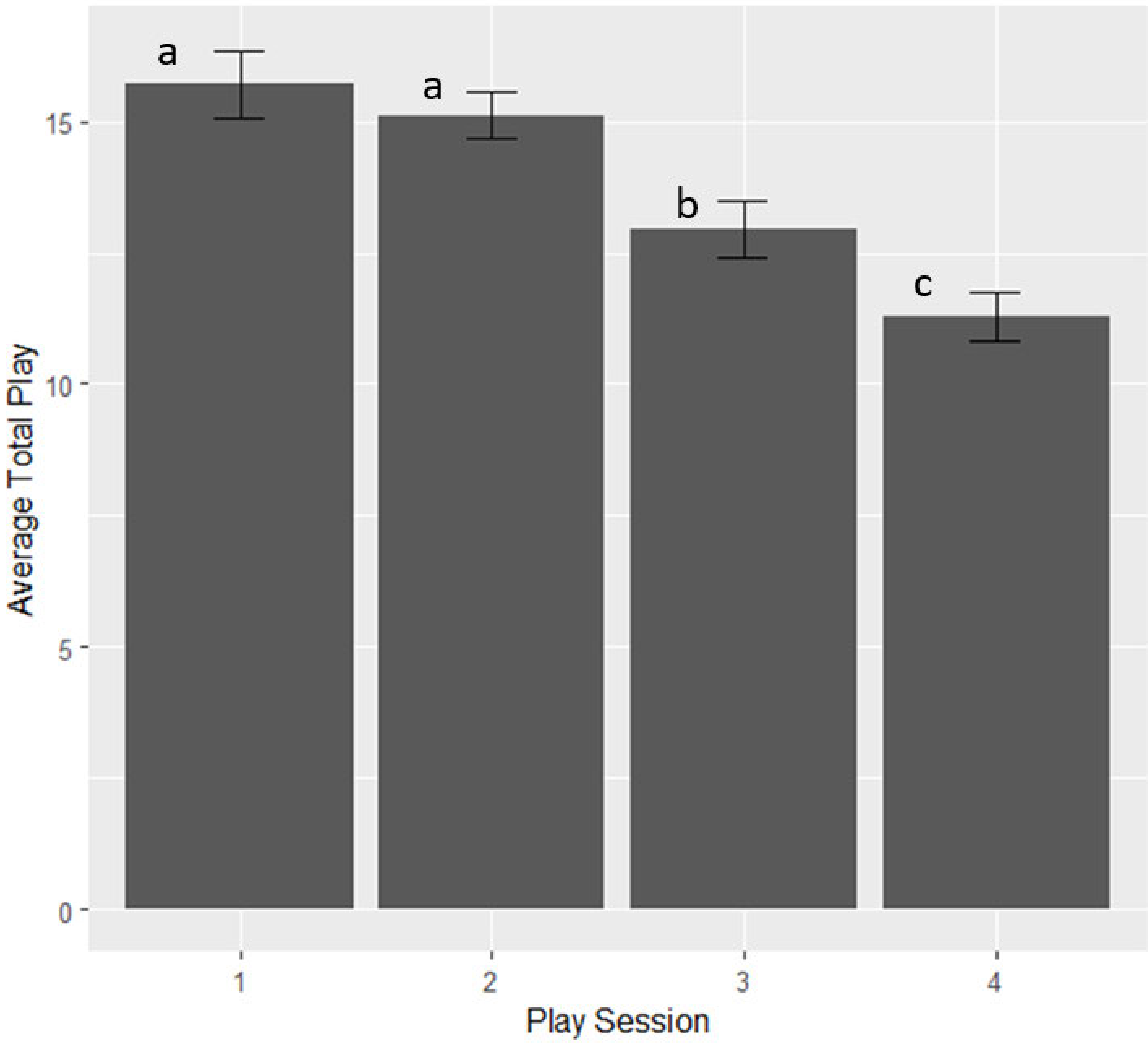


Figure 5. Effect of play session on the average frequency of total play per pig across 4 play sessions. Non-matching superscripts denote statistical significance(P<0.001).

Lesions

We found no effect of any of the variables modelled on the increase in skin lesions from pre to post play arena session. Pigs on the Arbocel diet had a mean lesion score of 0.20 (SD 0.321) before entering the play arena and a mean of 0.42 (SD 0.530) after leaving the play arena. Pigs on the basal diet had a mean lesion score of 0.17 (SD 0.291) before entering the play arena and a mean of 0.34 (SD 0.493) after leaving the play arena.

Production

We found an effect of dietary treatment on ADG (P=0.005) and FCR (P<0.001) (Table 3). ADG was higher in basal diet pigs and FCR was lower in basal diet pigs, indicating better feed efficiency. There was no effect of dietary treatment on ADFI.

Table 3. Effect of dietary treatment on ADFI, ADG and FCR of trial pigs.

Dietary Treatment				
		Average body weight at weaning (Kg)		SD
Arbocel		8.44		1.21
Basal		8.58		1.75
		ADFI (Kg)	**ADG (Kg)**	**FCR**
Arbocel				
	Mean	0.35	0.22	1.61
	SD	0.02	0.02	0.11
Basal				
	Mean	0.35	0.27	1.28
	SD	0.04	0.04	0.08

Discussion

The dilution of the diet with Arbocel resulted in a 9.5% reduction in digestible energy intake and suppressed growth rate by approximately 18.5%; this difference in effect size can be accounted for from the smaller proportion of digestible energy intake to support weight gain on the Arbocel diet compared to the basal diet after accounting for the prioritised digestible energy partitioning to maintenance (Proceedings of the British Society of Animal Science 2003, 2003). We hypothesised that dilution of the diet with fibre would suppress play and that this effect could be minimised by providing sugar immediately before entering the play arena. Our results provide some support for these hypotheses, but the effects were not consistent. We found an effect of sex on all individual play behaviours. Females played more than males, with the exception of play fighting which males expressed more of. We also found a main effect of play session on play fighting and total play and an interaction between dietary treatment and play session on social object play, solo locomotor play and social locomotor play. This interaction effect differed across behaviours but with the general trend that levels of play behaviours decreased more rapidly on the Arbocel diet as pigs aged. We found no effect of individual sugar intake (tended towards significant in social locomotor play) or effect of the interaction of dietary treatment with individual sugar intake. We did, however, find an interaction between dietary treatment and pen mate average sugar intake on aggression, social object play, social locomotor play and total play. Again, this interactive effect differed between behaviours but in general, increased pen mate average sugar intake increased play in Arbocel pigs, whilst an intermediate pen mate average sugar intake of score 1 increased play behaviours in basal pigs.

As expected, we found sex differences in play, with males performing more play fighting than females and females performing more solo object, social object, solo locomotor and social locomotor play. Our findings agree with previous studies on pigs that males perform more play fighting, while females show more locomotor play (D'Eath and Lawrence, 2004; Rauw, 2013; Brown et al., 2015). The sex effect in play behaviours and styles is also seen in other non-human mammals(Marley et al., 2022). We saw no effect of any variables modelled on lesion increase, which was generally small, suggesting that play fighting was correctly recorded as play rather than true aggression. The greater level of play fighting in males and greater expression of all other forms of play in females may reflect differential benefits derived from play as a result of the contrasting sociality of the sexes in wild boar, where females are gregarious and adult males are solitary and engage in damaging aggression during the breeding season (Stolba and Wood-Gush, 1989).

Play session affected play fighting and total play. This was expected as previous literature has found that play in pigs decreases over time, peaking around 2 to 6 weeks of age, dropping to a low level at around 14 weeks (Newberry et al., 1988). Trial pigs were between 5 and 7 weeks old during the 4 play sessions so a decrease in play was expected. The decrease in play between sessions may also reflect a reduction in novelty of the play arena experience.

An interaction between dietary treatment and play session was observed on social object, solo locomotor and social locomotor play. Social object play was higher in basal pigs in sessions 1 and 2 after which it dropped down to the same level as that of the Arbocel pigs. This suggests that the additional energy in the basal diet increased social object play but the levels dropped off as would be expected whilst the pigs fed the Arbocel diet did not show the high level of play expression when young. Solo locomotor play was only seen to be higher in basal diet pigs in play session 3 but this is likely due to a decrease in solo locomotor play in Arbocel pigs around session 3. In comparison the frequency of solo locomotor play stayed constant over the 4 sessions in pigs on the basal diet. These results go towards supporting our hypothesis, in that high levels of play are sustained over a longer period for pigs on the higher energy diet. We also saw an interaction between dietary treatment and play session on social locomotor play with play levels in Arbocel diet pigs being higher than basal diet pigs in session 1 and then play levels in basal diet pigs being higher than levels in Arbocel diet pigs in session 3. Again, this may be due to the difference in the rate at which play decreased between the two groups. Levels of social locomotor play stayed constant until after session 3 in basal pigs but fell after session 2 in Arbocel pigs. Together, our results suggest that increased dietary energy extends the developmental period over which play is expressed at a high level whereas the diet had variable effects on the rate of play during the most active first play session.

Contrary to our hypothesis, we did not see an effect of the consumption of sugar on the subject's own expression of play. It was hypothesised that sugar intake immediately before exposure to the arena would increase play, and that this effect would be most profound for pigs on the Arbocel diet. The lack of effect of individual sugar intake may be due to the amount of sugar given being too small to see an individual effect but resulting in a small, cumulative effect of the play behaviour of all pigs. Aggression, social object play, social locomotor play and total play were affected by the interaction between dietary treatment and pen mate average sugar intake, suggesting that the subject's play was sensitive to the sugar intake of its peers in a manner affected by the long-term diet. Alternatively, the finding that pen mate sugar intake affected the subject's own play behaviour may be due to social and/or play contagion. There is very little research into play and social contagion in pigs. There is research to suggest that pigs might be sensitive to emotional contagion (Reimert et al., 2013) and that they also exhibit yawn contagion (Norscia et al., 2021). Further research is needed to better understand the emotional and social contagion in pigs to help unravel the social aspect of play and the role it plays in the structure and dynamics of pig social networks.

Aggression was greater in Arbocel diet pigs with a pen mate average sugar intake score of 1 than basal diet pigs whose pen mates ate the same amount of sugar. We did not hypothesise how sugar would affect aggression, but it is possible that either a low or high energy intake could increase aggression. Specifically, it could be argued that aggression would be increased in pigs that consumed no sugar as low energy intake can increase aggression, as observed in food deprivation in Drosophila (Edmunds et al., 2021). It may also be expected that aggression would be greater in pigs that consumed more than half the sugar offered, as excess dietary energy may facilitate aggression, which is an energy-demanding activity. There is evidence of high fat diets inducing aggression in rodents (Hilakivi-Clarke et al., 1996). However, in contrast to either of these explanations, we observed that an intermediate intake of sugar by pen mates affected expression of aggression. All pigs willingly ate sugar when offered in the home pen as part of the early habituation process. It may have been that pigs that ate less than half of the sugar offered in the weigh crate did so because they were more stressed in the weigh crate or more aroused by expectation of entering the arena, either of which could have reduced motivation for the sugar. For pigs on the Arbocel diet, this increased stress or arousal, coupled with a fibre diluted diet, could have been manifest in increased aggression with the subjects once inside the play arena. Unfortunately, we did not collect any data on individual affective states of the pigs so cannot determine the likely cause of the non-linear effect of sugar intake on aggression. Further work to explore how proximate emotional states affect social behaviour and how this is moulded by nutritional provision would be beneficial.

Social object play and social locomotor play were both affected by the dietary treatment x pen mate average sugar intake interaction. Social object play was higher in basal diet pigs with a pen mate average sugar intake score of 1 compared with Arbocel diet pigs with the same sugar intake. In basal diet pigs, the levels of social object play were equal across pen mate average sugar intake scores. In Arbocel pigs, levels of social object play were the same in pen mate average sugar intake scores 0 (no sugar offered) and 1 (sugar offered but less than half consumed), 0 and 2 (sugar offered and half or more consumed) but then higher in 2 than 1. We did not specifically make hypotheses about the effect of pen mate sugar intake as it was expected that all individuals in the group would fully consume the sugar offered to them. However, the observation that the play increases in pigs fed the Arbocel diet when their pen mates ate most of the sugar offered would fit with a prediction that sugar provision promotes play when on a poorer diet. Interestingly, however, there was no difference in the subject's play when its pen mates ate most of the sugar as compared to when they were not offered sugar.

Social locomotor play was higher in basal diet pigs of pen mate average sugar intake scores 0 and 1, than Arbocel pigs of the same pen mate average sugar intake scores. Conversely, social locomotor play was higher in Arbocel diet pigs of pen mate average sugar intake score of 2 than that of pigs on the basal diet with the same pen mate average sugar intake score. Both social locomotor and total play increased in the Arbocel pigs whose pen mates consumed half or more than half of the sugar offered. This suggests that for social locomotor play, the provision of sugar to pen mates did temporarily reverse the effect of the lower energy diet for the subject, in support of our hypothesis.

An unexpected observation was that for basal diet pigs, social locomotor play and total play were higher in pigs of intermediate pen mate sugar intake scores of 1 (sugar offered but less than half consumed). We would have expected to see that pigs whose pen mates ate half or more of the sugar offered played more than those whose pen mates ate less than half of the sugar offered. It may have been that the pigs which ate less than half of the sugar were more aroused and hence distracted in the weigh crate and that this was manifest as more play in the arena which had a contagious effect on the subject's own play. In contrast to the Arbocel diet pigs where an increase in arousal led to increased aggression, an increase in arousal in the basal diet pigs could potentially have led to increased play as the pigs were not additionally metabolically stressed by a dietary energy deficit. Additional research is needed to explore the effect of contagion of affective states and their effect on play and social behaviours at a group level. Interestingly, we saw no effect of either the subject's own sugar intake or that of its pen mates on either diet on solo play behaviours. We only saw an effect of sex on solo object play (with females performing more) and effects of sex (with females performing more) and dietary treatment x play session interaction on solo locomotor play. This suggests that dietary energy, sugar and potentially stress/arousal and contagion have a stronger effect on social behaviours than on solitary behaviours.

Conclusion

Our results support previous studies that sex and age of pigs affects play behaviour. The diet affected some play behaviours in a manner supporting our hypothesis that play behaviour would be compromised on a diet diluted with fibre. In particular, a higher quality diet appeared to extend the period of heightened engagement in social play behaviours later into development than a lower quality diet. However, the effect varied between different forms of play and was apparent only during specific play sessions. Contrary to our hypothesis, individual sugar intake in the minutes before entry to the arena did not affect the amount of play on either diet. We did, however, find evidence that the sugar intake of pen mates affected the social play of the subject animals independently of their own sugar intake which could operate through a contagion effect on play. It may also be necessary to factor in the individual pigs innate "playfulness" and position in the dominance hierarchy, together with measures of arousal and affective state, into such research in the future as these could potentially affect a pig's performance of social and solo play. Our findings suggest that diet quality may impact specific aspects of play behaviour and therefore that dietary manipulation may be a way to increase the expression of play and potentially other positive forms of behaviour in commercial production with benefits for animal welfare.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Diamond, J. & Bond, A. B. A comparative analysis of social play in birds. *Behaviour* **140**, 1091–1115 (2003).

2. Dinets, V. Play behavior in ectothermic vertebrates. *Neurosci. Biobehav. Rev.* **155**, (2023).

3. Špinka, M., Newberry, R. C. & Bekoff, M. Mammalian play: Training for the unexpected. *Q. Rev. Biol.* **76**, 141–168 (2001).

4. Eisenbeiser, S., Serbe-Kamp, É., Gage, G. J. & Marzullo, T. C. Gills Just Want to Have Fun: Can Fish Play Games, Just like Us? *Animals* **12**, (2022).

5. Mather, J. A. & Anderson, R. C. Exploration, play and habituation in octopuses (Octopus dofleini). *J. Comp. Psychol.* **113**, 333–338 (1999).

6. Kuba, M. J., Gutnick, T. & Burghardt, G. M. Learning from play in octopus. in *Cephalopod Cognition* (eds. Darmaillacq, A.-S., Mather, J. & Dickel, L.) 57–71 (Cambridge University Press, Cambridge, 2014). doi:10.1017/CBO9781139058964.006.

7. Galpayage Dona, H. S. *et al.* Do bumble bees play? *Anim. Behav.* **194**, 239–251 (2022).

8. Lawrence, A. Consumer demand theory and the assessment of animal welfare. *Anim. Behav.* **35**, 293–295 (1987).

9. Burghardt, G. M. *The Genesis of Animal Play: Testing the Limits*. (2005).

10. Graham, K. L. & Burghardt, G. M. Current perspectives on the biological study of play: signs of progress. *Q. Rev. Biol.* **85**, 393–418 (2010).

11. Waiblinger, S., Wagner, K., Hillmann, E. & Barth, K. Play and social behaviour of calves with or without access to their dam and other cows. *J. Dairy Res.* **87**, 144–147 (2020).

12. Bennett, B. & Fewell, J. H. Play frequencies in captive and free-ranging bighorn lambs (Ovis canadensis canadensis). *Zoo Biol.* **6**, 237–241 (1987).

13. Newberry, R. C., Wood-Gush, D. G. M. & Hall, J. W. Playful behaviour of piglets. *Behav. Processes* **17**, (1988).

14. Jensen, M. B., Vestergaard, K. S. & Krohn, C. C. Play behaviour in dairy calves kept in pens: the effect of social contact and space allowance. *Appl. Anim. Behav. Sci.* **56**, 97–108 (1998).

15. Chaloupková, H., Illmann, G., Bartoš, L. & Špinka, M. The effect of pre-weaning housing on the play and agonistic behaviour of domestic pigs. *Appl. Anim. Behav. Sci.* **103**, (2007).

16. Pellis, S. M. & Pellis, V. C. Play-fighting differs from serious fighting in both target of attack and tactics of fighting in the laboratory rat Rattus norvegicus. *Aggress. Behav.* **13**, 227–242 (1987).

17. Pellis, S. M. & Pellis, V. C. Play fighting in Visayan warty pigs (Sus cebifrons): Insights on restraint and reciprocity in the maintenance of play. *Behaviour* **153**, (2016).

18. Weller, J. E., Camerlink, I., Turner, S. P., Farish, M. & Arnott, G. Playful pigs: early life play-fighting experience influences later life contest dynamics. *Anim. Behav.* **158**, (2019).

19. Berghänel, A., Schülke, O. & Ostner, J. Locomotor play drives motor skill acquisition at the expense of growth: A life history trade-off. *Sci. Adv.* **1**, e1500451 (2015).

20. Barnes, R. H., Levitsky, D. A., Pond, W. G. & Moore, U. Effect of postnatal dietary protein and energy restriction on exploratory behavior in young pigs. *Dev. Psychobiol.* **9**, (1976).

21. Brown, S. M., Klaffenböck, M., Nevison, I. M. & Lawrence, A. B. Evidence for litter differences in play behaviour in pre-weaned pigs. *Appl. Anim. Behav. Sci.* **172**, (2015).

22. Brown, S. M., Peters, R., Nevison, I. M. & Lawrence, A. B. Playful pigs: Evidence of consistency and change in play depending on litter and developmental stage. *Appl. Anim. Behav. Sci.* **198**, 36–43 (2018).

23. Franchi, G. A. *et al.* Play behaviour positively relates to weight gain, feeding behaviour and drinking behaviour in weaner pigs (Sus scrofa). *Appl. Anim. Behav. Sci.* **259**, 105836 (2023).

24. Krachun, C., Rushen, J. & Passillé, A. M. de. Play behaviour in dairy calves is reduced by weaning and by a low energy intake. *Appl. Anim. Behav. Sci.* **122**, 71–76 (2010).

25. Rushen, J., Wright, R., Johnsen, J. F., Mejdell, C. M. & Passillé, A. M. de. Reduced locomotor play behaviour of dairy calves following separation from the mother reflects their response to reduced energy intake. *Appl. Anim. Behav. Sci.* **177**, 6–11 (2016).

26. Held, S. D. E. & Špinka, M. Animal play and animal welfare. *Anim. Behav.* **81**, 891–899 (2011).

27. McDonald, P. *et al. Animal Nutrition*. (Pearson Higher Ed, 2011).

28. Sugar: the facts. *nhs.uk* https://www.nhs.uk/live-well/eat-well/food-types/how-does-sugar-in-our-diet-affect-our-health/ (2022).

29. Ocepek, M., Newberry, R. C. & Andersen, I. L. Which types of rooting material give weaner pigs most pleasure? *Appl. Anim. Behav. Sci.* **231**, (2020).

30. Camerlink, I., Proßegger, C., Kubala, D., Galunder, K. & Rault, J.-L. Keeping littermates together instead of social mixing benefits pig social behaviour and growth post-weaning. *Appl. Anim. Behav. Sci.* **235**, 105230 (2021).

31. Camerlink, I. & Turner, S. P. The pig's nose and its role in dominance relationships and harmful behaviour. *Appl. Anim. Behav. Sci.* **145**, (2013).

32. Camerlink, I., Peijnenburg, M., Wemelsfelder, F. & Turner, S. P. Emotions after victory or defeat assessed through qualitative behavioural assessment, skin lesions and blood parameters in pigs. *Appl. Anim. Behav. Sci.* **183**, (2016).

33. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing (2021).

34. Bates, D., Mächler, M., Bolker, B. & Walker, S. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* **67**, 1–48 (2015).

35. Marschner, I. glm2: Fitting Generalized Linear Models. 1.2.1 https://doi.org/10.32614/CRAN.package.glm2 (2011).

36. Norscia, I., Hecker, M., Aldama, B. G., Santos, S. B. & Cordoni, G. Play contagion in African elephants: The closest, the better. *Behav. Processes* **221**, (2024).

37. Wenig, K., Boucherie, P. H. & Bugnyar, T. Early evidence for emotional play contagion in juvenile ravens. *Anim. Cogn.* **24**, 717–729 (2021).

38. Sandars, G., Brooker, J. S. & Clay, Z. ChimpanSEE, ChimpanDO: Grooming and play contagion in chimpanzees. *PLoS ONE* **19**, (2024).

39. *Proceedings of the British Society of Animal Science 2003*. (British Society of Animal Science, Penicuik (GB), 2003).

40. D'Eath, R. B. & Lawrence, A. B. Early life predictors of the development of aggressive behaviour in the domestic pig. *Anim. Behav.* **67**, (2004).

41. Rauw, W. M. A note on the consistency of a behavioral play marker in piglets. *J. Anim. Sci. Biotechnol.* **4**, (2013).

42. Marley, C. L., Pollard, T. M., Barton, R. A. & Street, S. E. A systematic review of sex differences in rough and tumble play across non-human mammals. *Behav. Ecol. Sociobiol.* **76**, 158 (2022).

43. Stolba, A. & Wood-Gush, D. G. M. The behaviour of pigs in a semi-natural environment. *Anim. Prod.* **48**, (1989).

44. Reimert, I., Bolhuis, J. E., Kemp, B. & Rodenburg, T. B. Indicators of positive and negative emotions and emotional contagion in pigs. *Physiol. Behav.* **109**, 42–50 (2013).

45. Norscia, I., Coco, E., Robino, C., Chierto, E. & Cordoni, G. Yawn contagion in domestic pigs (Sus scrofa). *Sci. Rep. 2021 111* **11**, 1–12 (2021).

46. Edmunds, D., Wigby, S. & Perry, J. C. 'Hangry' *Drosophila*: food deprivation increases male aggression. *Anim. Behav.* **177**, 183–190 (2021).

47. Hilakivi-Clarke, L., Cho, E. & Onojafe, I. High-fat diet induces aggressive behavior in male mice and rats. *Life Sci.* **58**, 1653–1660 (1996).

Author Contributions

Eleanor Hewett and Simon Turner designed the trial with nutritional consultations from Jos Houdijk. Eleanor Hewett collected the data and performed the analysis with guidance from Simon Turner. Eleanor Hewett and Simon Turner drafted the manuscript which was reviewed by Jos Houdijk, Craig Lewis and Andrea Doeschl-Wilson.

Acknowledgements

We would like to acknowledge the staff and students at the EHPU for providing help and support, particularly Marianne Farish.

Funding

This work was supported by the UKRI Biotechnology and Biological Sciences Research Council (BBSRC) grant number BB/T00875X/1 and PIC as part of the EASTBIO Doctoral Training programme**.** SRUC receives funding from the Scottish Government.

Conflicts of interest

The authors declare no conflict of interest.

Discussion

During the study, the pigs' behaviour was recorded in their home pens and in the play arena. The intention was to collect as much data as possible on the pigs' play behaviours, to allow quantification of the effect of energy on unprovoked play in the home pen as well as that stimulated by the enriching environment of the play arena. Due to time constraints, I was only able to examine play behaviour in the play arena and only for the first 4 play sessions (out of a total of 8). It would have been interesting to see how play differed from the home pen to the play arena, look for effects of anticipation and novelty and quantify the effects of being weighed and handled. It is currently impossible to determine whether dietary effects on play in the arena translated to similar effects in the home pen. Whilst the prediction of the Surplus

Resource Theory was still testable using the data from the arena, the omission of the home pen data prevented an assessment of the likely welfare benefits that a heightened expression of unprovoked play may reflect. It would also be interesting to explore the social network of play, and specifically to determine if pigs have preferential playmates, to explore whether individual animals have preferred play behaviours, and to quantify the amount and spread of play contagion among pigs which has rarely been studied. Research has suggested that pigs might be sensitive to emotional contagion (Reimert et al., 2013) and play contagion has been seen in other livestock animals (Größbacher et al., 2020) but further research is needed into play contagion in pigs specifically.

I found the high energy diet appeared to extend the developmental period of heightened engagement in social play and this contributes to recent research that has been conducted to explore play behaviour in the grow-finish phase pigs. Play in pigs is most commonly seen early in life, between 2 and 6 weeks of age, dropping off to a low level around 14 weeks (Newberry et al., 1988) but studies have shown that play can be promoted and sustained until the finishing phase (Steinerová et al., 2024). Studies have also shown that regular play opportunities (novel objects and access to a larger play arena) for grow-finish pigs can improve pigs' trainability and learning. This may be due to a reduction in fear (Steinerová et al., 2025). Further exploration of the benefits of extending the play window should be conducted. Diet should also be explored as a strategy to promote play in grow-finish pigs, to aid producers that are not currently able to provide additional space or enrichment.

Chapter 3: Exploring the effects of dietary lysine and tryptophan on the social behavior of pigs

Chapter 3 contains the published manuscript of the following paper: Hewett, E., L. Zaragoza, C. Lewis, J. Houdijk, A. Wilson, and S. Turner. 2025. Exploring the effects of dietary lysine and tryptophan on the social behaviour of pigs. J. Anim. Sci. skaf030. doi:10.1093/jas/skaf030.

A note on author contribution: the study was designed by Eleanor Hewett, Simon Turner and Jos Houdijk with comments from Luis Zaragoza and Craig Lewis. Data collection was performed by Eleanor Hewett and analysis of data was performed by Eleanor Hewett with guidance from Simon Turner. Eleanor Hewett drafted the manuscript which was reviewed by Simon Turner, Jos Houdijk, Craig Lewis and Andrea Doeschl-Wilson.

Introduction

Pigs are highly social animals and exhibit a wide range of social behaviours. Some of these social behaviours have been classified as negative, in that they have a negative welfare impact on either the performer or the recipient. Biting behaviour represents a suite of negative social behaviours triggered through unstable dominance relationships (e.g. from regrouping), competition for access to limited resources, or by a barren environment leading to redirection of foraging behaviour. The redirected biting behaviour is primarily focused on the tails of other pigs but can include biting of the ears, belly, genitalia and limbs. Biting in any form can lead to skin lesions, which are open to infection and are expected to be painful to the pig (Sihvo et al., 2012). Pigs with active infections may need intervention, may be condemned at slaughter or die before infections are treated. It is therefore in the interest of both pigs and producers to reduce the incidences of biting behaviours and their associated injuries.

Extensive research has been conducted into the determinants of biting and while the most effective methods have been shown to be avoidance of regrouping, ample provision of feed, water and dry lying areas, and provision of regularly changed enrichment materials, these solutions are not widely adopted due to commercial and economical constraints. Building off previous research exploring the effects of tryptophan on pig behaviour (Meunier-Salaün et al., 1991; Koopmans et al., 2006; Shen et al., 2012; Liu et al., 2013; Poletto et al., 2014; Castilha et al., 2016; Stracke et al., 2017; Lay et al., 2021; Henry et al., 2022) I devised a study to explore the role of lysine and tryptophan in the expression of harmful behaviours. At the time of the study's design, to the best of my knowledge, I was to be the first to explore the role of lysine on social behaviour. It seemed plausible that, as the first limiting amino acid in the majority of pigs diets, lysine could have an important effect on the expression of behaviours, either through impacts on individual satiety, nutrient deficiency or impacts on the microbiome and subsequent gut-brain axis. It was important to explore the roles of lysine and tryptophan independently and interactively. As lysine is often the first limiting amino acid, its levels will dictate the availability of other amino acids. It was also important that the study and any results be commercially relevant. Furthermore, the ethogram employed allowed quantification of positive forms of social interaction as well as negative since the effects of amino acid levels on positive social behaviours are unknown.

Published Manuscript

Journal of Animal Science, 2025, **103**, skaf030 https://doi.org/10.1093/jas/skaf030
Advance access publication 6 February 2025
Animal Behavior and Cognition

Exploring the effects of dietary lysine and tryptophan on the social behavior of pigs

Eleanor Hewett,[†] Luis Zaragoza,[‡] Craig Lewis,[‖] Jos Houdijk,[$] Andrea Wilson,[¶] and Simon Turner[**]

[†]Animal Behaviour and Welfare, Animal and Veterinary Sciences Department, Scotland's Rural College (SRUC), Edinburgh EH9 3JG, UK
[‡]Global Research and Communication Manager, Pig Improvement Company (PIC), Hendersonville, TN, USA, 37075
[‖]PIC Product Development PIC, C/Pau Vila, Barcelona, Spain
[$]Monogastric Science Research Centre, Agriculture and Land-based Engineering Department, Scotland's Rural College (SRUC), Edinburgh, EH9 3JG, UK
[¶]The Roslin Institute, The Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Edinburgh, EH25 9RG, UK
[**]Animal Behaviour and Welfare, Animal and Veterinary Sciences Department, Scotland's Rural College (SRUC), Edinburgh, EH9 3JG, UK
[1]Corresponding author: ehewett@sruc.ac.uk

Abstract

Negative social behaviors between pigs can cause stress, which can compromise welfare. There has been significant interest in exploring the effect of diet on negative social behaviors and the wider social behavior repertoire of pigs. The aim of this study was to determine the effects of dietary Lysine (Lys) and Tryptophan (Trp) levels on the social behavior of commercially housed pigs. A total of 2,293 PIC Camborough barrows with a mean starting weight of 11.87 ± 1.35 kg were used in a randomized complete block design with a 2 × 3 factorial arrangement, with 16 replicates per treatment, of the following factors: 1) Standardized ileal digestible Lys levels: 100% Lys = diets with 100% PIC requirement at the midpoint of the growth phase (Lys 100) vs 80% Lys = diets with 80% PIC requirement at the midpoint of the growth phase (Lys 80); and 2) Trp to Lys ratio of 0.210, 0.185, or 0.160. Pigs were randomly allocated across the 6 treatments over 2 starting dates. Behavior and lesion data were collected. There was an effect of Lys ($P = 0.032$) on ear-biting behavior, with pigs on the Lys 80 treatments showing a higher level of ear-biting behavior. We also found an effect of the Lys score week interaction on the proportion of pens showing ear lesions ($P < 0.001$) and an effect of the Lys Trp interaction ($P = 0.030$) and the Lys score week interaction ($P = 0.0104$) on the proportion of pens showing severe ear lesions. In conclusion, the lysine content of feed can affect the social behavior of pigs, specifically ear biting, in commercial conditions.

Lay Summary

Negative social behaviors between pigs can cause stress, which can compromise welfare. There has been significant interest in exploring the effect of diet on negative social behaviors and the wider social behavior repertoire of pigs. The aim of this study was to determine the effects of dietary lysine and tryptophan levels, 2 amino acids found in pig feed, on the social behavior of commercially housed pigs. A total of 2,293 castrated male pigs were randomly allocated to 1 of the 6 treatments in a factorial arrangement: High lysine and low tryptophan, high lysine and medium tryptophan, high lysine and high tryptophan, low lysine and low tryptophan, low lysine and medium tryptophan and low lysine and high tryptophan. Behavior and lesion data were collected. There was a significant effect of lysine level on ear biting behavior, with pigs on the low lysine treatments showing a higher level of ear biting behavior. We also found a significant effect of the interaction between lysine and week on the proportion of pens showing ear lesions and a significant effect of the interaction between lysine and tryptophan and the interaction between lysine and week on the proportion of pens showing severe ear lesions. In conclusion, the lysine content of feed can affect the social behavior of pigs, specifically ear biting, in commercial conditions.

Key words: behavior, lysine, pig, tryptophan, welfare

Abbreviations: Lys, lysine; PCA,principal component analysis; PC, principal component;PEN, porcine ear necrosis; SID, standardized ileal digestible;SRUC, Scotland's Rural College;Trp, tryptophan

Introduction

Pigs (Sus scrofa) are highly social animals with a complex repertoire of social behaviors. However, intensive farming systems (often characterized by a small space allowance; Lammers and Schouten, 1985), unstimulating environment (Cox and Cooper, 2001), and competitive feeding systems (Day et al., 1995), impact the behavior of pigs and contrib- ute to a divergence from social behaviors seen in the wild (Stolba and Wood-Gush, 1989). Negative social behaviors (as defined for this paper as behaviors that involve more than one pig where one of the pigs involved shows an avoidant or aggressive response on receipt of said behavior, e.g., tail biting, ear biting, flank biting, fighting) are more commonly seen in intensive farm systems (Taylor et al., 2010). Other naturally performed, positive social behaviors are shown less commonly e.g., play (Newberry et al., 1988). Aggressive encounters induce a stress response in pigs (Marchant et al., 1995) and often result in skin lesions that are likely to be painful (Di Giminiani et al., 2017) and which are open to infection. Ear, tail, and flank biting can lead to significant lesions, necrosis and loss of tissue, infection, and

Received October 1, 2024 Accepted February 4, 2025.

abscess formation (Sihvo et al., 2012) and in the most severe cases can result in death or euthanasia of the pig or condemnation of the carcass at slaughter. Play behavior has been proposed as an indicator of high animal welfare, as research suggests that play is more commonly displayed under good environ- mental conditions (Lawrence, 1987; Lawrence et al., 2018). Studies have also shown that play behavior positively relates to weight gain both pre and postweaning (Brown et al., 2015; Franchi et al., 2023). It therefore benefits both the pig and the producer to reduce negative social behaviors and encourage positive social behaviors.

Numerous solutions to reduce negative social behaviors have been proposed, including reducing stocking density (Cho and Kim, 2011), providing enrichment (van de Weerd and Day, 2009), increasing early life socialization (Weller et al., 2019), and reducing regrouping (Camerlink et al., 2021). However, many of these strategies have economic and space constraints and are difficult to implement in current intensive farming systems, and are therefore not widely adopted (Peden et al., 2021). Consequently, we are still searching for an economically viable and commercially relevant strategy to reduce negative and promote positive, social behaviors in pigs. One area that deserves further investigation is nutrition. Literature suggests that nutrition and the feeding system may have important impacts on the behavior and welfare of pigs, including fiber content (Bakare et al., 2014; Chou et al., 2020), protein content (McAuley et al., 2022), amino acid composition (Meer et al., 2017; Minussi et al., 2023), vita- min and mineral content, e.g., magnesium and salt (Jankev- icius and Widowski, 2004) and vitamin B6 (Castilha et al., 2016), but also feeding system factors including ad libitum vs restricted feeding (D'Eath et al., 2009), wet vs dry feeding (Andersen et al., 2023) and pellet size (Brand et al., 2014).

There is increasing interest in exploring the effects of the amino acid composition of diets on the negative social behaviors of pigs, particularly lysine (Lys) and tryptophan (Trp). Lys is the first limiting amino acid in the majority of pig diets, determining growth and lean muscle deposition (Main et al., 2008). Trp is important for protein synthesis and is the primary precursor of serotonin (5-hydroxytryptamine, 5-HT) and melatonin. Trp and subsequently serotonin play major roles in regulating behavioral and physiological processes in pigs such as feed intake (Baranyiová, 1991), mood (Markus et al., 2000), and stress hormone secretion (Adeola and Ball, 1992).

There is already a small body of research focusing on the effect of Trp on social behavior in pigs (Meunier-Salaün et al., 1991; Koopmans et al., 2005, 2006, 2012; Li et al., 2006, 2011; Shen et al., 2012a, 2012b; Liu et al., 2013; Poletto et al., 2014, 2014; Castilha et al., 2016; Stracke et al., 2017; Lay et al., 2021; Henry et al., 2022). However, the studies administered Trp at levels several fold higher than the commercial norm in pig diets, which is likely to be prohibitively costly in commercial production systems. Studies are lacking which investigate the effect of Trp on social behavior within the range commonly used in industry across the United States and Europe and no study has given attention to the effect on positive social behaviors. Furthermore, despite being the first limiting amino acid, only one study has explored the effect of increasing Lys levels on social behavior (Minussi et al., 2023). The aim of this study was to explore the effect of Lys and Trp on both positive and negative social behaviors of pigs, at levels modestly above current commercial levels. We hypothesized that pigs on high Lys diets would show reduced levels of negative social behaviors and increased levels of positive social behaviors. We also hypothesized that for pigs on diets low in Lys but high in Trp, the Trp would mitigate the effect of the low Lys and those pigs would not show the increase in negative social behaviors.

Materials and Methods

Ethical review

The study was approved by the Scotland's Rural College (SRUC) Animal Experiments Committee under application PIG AE 22-2022. Routine animal care and management were performed by trained facility staff.

Animals and housing

Behavioral observations were carried out on an initial cohort of 2,293 PIC Camborough barrows, mean starting weight of 11.87 ± 1.35 kg. Pigs were housed under U.S. commercial conditions (no enrichment), in a fully slatted house, consisting of 2, tunnel ventilated barns. Each barn contained 48 pens of 2.25 × 2.25 m (with 2 additional smaller pens per barn which were used as the intensive care pens) with an initial inventory of 16 to 26 castrated male pigs per pen. Pens were fitted with one feeder, divided into 3 sections and 2 pen-mounted bowl drinkers. Pigs arrived on farm on the 8th, 11th, and 15th of August after weaning at a separate facility. Barn 1 pigs were put on treatment on the 30th of August and barn 2 on the 8th of September. Behavioral observations were taken over a period of 9 wk from the 10th of October to 11th of December.

Experimental treatments

Pigs were randomly allocated across 6 treatments in a 2 × 3 factorial arrangement in a randomized complete block design (block = wean date and weight), giving 16 replicates of each treatment, 8 per barn, of the following iso-energetic feeding treatments: 1) Standardized ileal digestible (SID) Lys levels: Lys 100 = diets with 100% PIC requirement at the midpoint of the growth phase, vs Lys 80 = diets with 80% PIC requirement at the midpoint of the growth phase; and 2) Trp to Lys ratio (Trp:Lys) of 0.210, 0.185, or 0.160. Full diet com- positions, which had a basis of corn, distiller's dried grains with solubles, and soybean meal, can be found in Appendix 1. Targeted Lys levels were attained through replacing soy- bean meal and synthetic Lys with corn, and minor modification from synthetic amino acid sources, minerals, and oil to ensure similar ratios of other essential amino acids to Lys at SID level, same levels of net energy, calcium, and standardized total tract digestible phosphorus as per recommendations of the genetic supplier (PIC, 2016) and (NRC, 2012) requirements. Within those Lys levels, targeted levels of Trp were attained by iso-energetic exchange L-tryptophan against a combination of corn and corn oil. The SID Lys levels were derived from a meta-analysis performed by PIC to determine the SID Lys requirements for the average requirement of average daily gain and feed-to-gain ratio for finishing PIC Camborough pigs under commercial conditions (Goncalves et al., 2017).

There were a total of 5 feeding phases, pigs were weighed at the end of each feeding phase. Feed delivery was recorded automatically by the feed system and feed remaining in the feeders at the end of the feeding phase was recorded to allow for calculation of feed consumption. Feed samples were taken by the research staff.

Table 1. Ethogram used to record behaviors of pigs in both live and recorded observations.

Behavior	Description	Reference
Scan sampling		
Lying together	A pig is resting, with eyes closed or opened, with the body in full contact with the floor while at least a quarter of their body is in contact with another lying pig	(Camerlink et al., 2021)
Lying alone	A pig is resting, with eyes closed or open, with the body in full contact with the floor while less than a quarter of its body is in contact with another lying pig	Adapted from (Camerlink et al., 2021)
Sitting together	Pig is sitting with back legs and rump in contact with the floor, resting on or touching another pig	Defined for this study
Sitting alone	Pig is sitting with back legs and rump in contact with the floor, not resting on or touching another pig	Defined for this study
Standing together	A pig is standing, sniffing, touching, or rooting the surroundings (e.g., floor or wall) while the front part (front shoulder to tip of nose) or all of its torso is touching another pig.	Adapted from (Camerlink et al., 2021)
Standing alone	A pig is sniffing, touching, or rooting the surroundings (e.g., floor or wall) while no part of its front body region (defined above), or less than 100% of its torso, is touching another pig	Adapted from (Camerlink et al., 2021)
Eating/drinking together	Two or more pigs are engaged in eating or drinking at the same time from adjacent feeders/drinkers	Defined for this study
Eating/drinking alone	Single pig is engaged in eating or drinking	Defined for this study
Continuous sampling		
Social nosing	Non-agonistic nosing, including allogrooming and gentle nose touching. Behavior does not escalate to aggressive behavior or elicits evasive action from recipient pig	Adapted from (Camerlink et al., 2021)
Social play	A pig is scampering, running, pivoting, head tossing, flopping, hopping nudging, pushing, climbing, or non-harmful fighting with at least one other pig. All pigs involved in social play show at least one of these play markers.	(Blackshaw et al., 1997; Brown et al., 2015; Camer- link et al., 2021)
Individual play	A pig is scampering, running, pivoting, head tossing, flopping, or hopping alone.	(Newberry et al., 1988; Donaldson et al., 2002; Brown et al., 2015)
Aggression	Head knocking, fighting, biting, ramming	Adapted from (Camerlink and Turner, 2013)
Mounting	Standing on hind legs while having front legs on other pig's body	(Camerlink and Turner, 2013)
Ear/ tail/ flank biting	Taking the ear or tail of a pen mate into the mouth or nibbling, sucking or chewing the ear, tail or body of a pen mate	Adapted from (Camerlink and Turner, 2013)
Belly nosing	Repetitive up and down snout movement on the belly of a pen mate	(Camerlink and Turner, 2013)

In discussion with veterinarians, pigs on the Lys 80 with Trp:Lys 0.160 treatment were moved to the Lys 100 with Trp:Lys 0.185 diet at the end of feeding phase 2 due to an excessive number of pens reaching the production and/or welfare endpoints, which were defined prior to the trial as the point at which a pigs growth rate was lagging more than 20% behind that of their counterparts on a standard diet or 2 animals required treatment or removal from the pen within a 7-d period of each other due to injury from social interactions.

Behavior observations

Behavior observations were taken live by a single observer and via digital recordings collected using a GoPro HERO10 Black (GoPro Incorporated, San Mateo, CA, US). Digital recordings were scored by the same observer as the live recordings after the trial had ended. Data from both the live observations and observations taken from recordings were used in the data- set. Observations were taken between the hours of 08:00 and 19:00 over a period of 9 wk from the 10th of October to the 11th of December. The order of pen observations was deter- mined in accordance with a Latin square design to reduce the impact of observation order on results. Each pen was observed 5 times over a daily period, whereby each pen was observed twice in the morning and 3 times in the afternoon (to avoid time periods when pigs were most likely to be sleeping) for 15 min each, giving a total of 75 min per day. Each pen was observed on 3 observation days, twice live and once by video recording.

Prior to the commencement of the observation, the observer entered the pen to disturb the pigs. After all pigs had been roused to standing, the observer exited the pen. On exiting the pen, the observer waited 2 min for the pigs to settle down and resume their undisturbed behavior. This process was carried out at the beginning of both live and recorded observation periods.

Behaviors of all pigs (live and recorded) were recorded using an ethogram (Table 1), using a combination of continuous observation (for short-lived behaviors) and scan sampling (for posture). For the continuous observations the frequency but not duration of behaviors was recorded. At time 0 and at intervals of 150 s after, a tally was taken by scan sampling of the number of animals lying, sitting, standing, eating, or drinking, each either alone or with another animal.

Observations were stopped if pigs were disturbed during the 15-min observation period and some observation periods were discounted due to poor video quality. A total of 3.40% of observations could not be carried out.

Table 2. Score system used for scoring ear, tail, and flank bites, both damage and freshness

Damage	Freshness
0- no evidence of damage to the skin	0- no evidence of damage to the skin
1—mild swelling, redness, and evidence of bite marks, broken skin	1—no blood
2- mild to severe swelling, redness, bite marks, broken skin, scabbing with some fresh blood	2—dry, black blood, scar for- mation
3—severe swelling, redness, open wound, fresh blood, necrosis of tissue	3—dark red blood, scabbing and some healing seen
4—more than 50% of the pig's ear is missing/ whole tail is missing and stump has become an open wound, fresh blood and necrotic tissue	4—bright red, fresh blood

Behavior observations

Five treatments remained after moving pigs from treatment Lys 80 with Trp:Lys 0.160 to treatment Lys 100 with Trp:Lys 0.185. Behavioral data were not analyzed for these pigs.

Table 3. Lesion score system used to score fight lesions

Number of lesions	Score
0	0
1 to 10	1
11 to 20	2
21 to 30	3
31 to 40	4
41 to 50	5
51+	6

Lesion scoring

Two different types of lesion scoring were performed, one to capture lesions caused by biting of the tail, ears, and flank and the other to capture lesions caused by fighting/aggressive behaviors.

Bite lesions were scored weekly for 8 wk for a total of 7 recording events (1 wk it was not possible to take lesion recordings as it was deemed unsafe for the observer to enter the pens after a feeder outage). Once a week, all pens were examined and all pigs were scored for damage and freshness of ear, tail, and flank bites (Table 2). The observer entered the pen and moved slowly through the pigs, ensuring all pigs were disturbed in order to observe as much of the pig as possible.

Fight lesion scores were collected every other week over the 8-wk period for a total of 4 recording events. All pens were examined and fight lesions were scored on 6 pigs per pen. On entering the pen, the observer randomly selected and scored 2 large pigs, 2 mid-sized pigs, and 2 small pigs. The score (Table 3) and location of lesions were recorded. Location was determined by zone: front (tip of nose to back of shoulder of font legs), middle (back of the front shoulder to front of back legs), back (front of back leg to tip of tail; Figure 1). A lesion was defined as any continuous mark on the skin of the pig, longer than 1cm. Any other skin damage e.g., ulcers, heat rash, grazes, bruises, was noted.

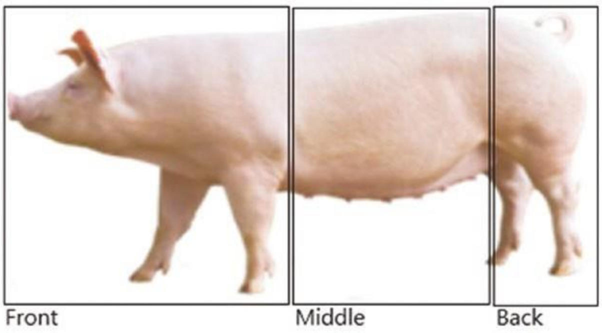


Figure 1. Body zones of the pig. Image taken from PIC, showing the Camborough pig.

Statistical Analysis

Statistical analyses were carried out using R Software version 4.2.2 (R Core Team, 2021). Models were constructed using the lme4 package (Bates et al., 2015). Pen was the experi- mental unit. Where appropriate, model residuals were visu- ally inspected to confirm approximation to normality and tested for heteroscedasticity using Levene's Test. When main effects (Lys or Trp:Lys) or interactions were not significant at $P < 0.05$, they were removed from the model. P-values below 0.05 were considered statistically significant and P-values between 0.05 and 0.1 were considered as tendencies.

Behavior scores for each pen were combined across obser- vations and divided by the total number of pigs in the pen over the 3 observation days to give one frequency for each behav- ior per pen. For posture behaviors, scores were combined together and apart as they were highly correlated. Values were standardized to a mean of zero and standard deviation of 1 and data from pens on treatments Lys 100 with Trp:Lys 0.210, Lys 100 with Trp:Lys 0.185, Lys 100 with Trp:Lys 0.160, Lys 80 with Trp:Lys 0.210 and Lys 80 with Trp:Lys 0.185 were analyzed using principal component analysis. The principal component analysis identified 2 principal components (PC); PC1 accounted for 31% of the variation and PC2 accounted for 15%. The labels for the components were determined by selecting the highest loading descriptors for the positive and negative poles. Therefore, PC1 was labeled as activity (0.45 for standing, −0.46 for lying) and PC2 was labeled as social behavior (0.37 for ear biting, -0.60 for social play).

PC1 and PC2 were analyzed using a linear mixed model with 'fill week','Ly's, 'Trp:Lys' and the 'Lys × Trp:Lys' interac- tion as fixed effects and block as a random effect. Some select behaviors of interest (aggression, a combined play score [sum of individual and social play], and ear-biting behavior) were also analyzed as response variables using linear mixed mod- els. 'Fill week', 'Lys', 'Trp:Lys' and the 'Lys × Trp:Lys' interac- tion were included as fixed effects. 'Barn' was included as a random effect.

Lesions scores

For both the lesion score data sets, data from all 6 treatments was analyzed. For treatment Lys 80 with Trp:Lys 0.160, only data collected from pigs when they were on the treatment Lys 80 with Trp:Lys 0.160 diet was used (feeding phase 1 and 2). Lesions scores were divided by the number of pigs per pen to give a score based on lesions per pig.

Bite lesions

For the ear, tail, and flank bite analysis, only ear bites occurred frequently enough for analysis. Pens were sorted into a binary data set; pens with evidence of ear biting and pens without evidence of ear biting. A second data set for pigs showing severe ear lesions (scores 2, 3, and 4) and all other pens was also established. The binary data set was analyzed using a generalized linear mixed model, with bino- mial distribution. Fixed effects were 'Lys', 'Trp:Lys', 'Lys × Trp:Lys' interaction, 'score week', 'fill date', and 'barn'. Random effects were 'block' and 'pen', with 'pen' nested in 'block'.

Fight lesions

Fight lesion scores were also sorted into a binary data set; pens with scores above and pens with a score below the median score. The binary data set was analyzed using a generalized linear mixed model, with binomial distribution. Fixed effects were 'Lys', 'Trp:Lys', 'Lys × Trp:Lys' interaction, 'score week', 'fill date' 'barn', and 'pig size'. Random effects were 'block' and 'pen', with 'pen' nested in 'block'.

Results

Morbidity and mortality

Over the 9 wk of behavioral observations, 110 pigs were removed from the trial due to morbidity (n = 66) and mortality (n = 44). Date, weight, and suspected reason for death/ removal were recorded where possible. The highest proportion of removals came from treatment Lys 80 with Trp:Lys 0.160 (33.64%) and the lowest proportion from treatment Lys 100 with Trp;Lys 0.210 (5.45%; Table 4). An additional 11 pigs died during the 9-wk observation period after being moved to the intensive care pens. As pigs were not individually identifiable, it was not possible to determine which treatment these 11 pigs had been on before moving to the intensive care pen.

Behaviors

There was an effect of fill week (which corresponds to the pigs age) on PC1 (activity; $P < 0.001$). The average PC1 (activity) score for pigs of fill week 3 was higher than the average PC1 (activity) score for pigs of fill week 1. The effect of Lys tended towards significant ($P = 0.066$; being higher at Lys 80) but there was no effect of Trp:Lys on PC1 (activity). We found no effect of Lys or Trp:Lys on PC2 (social behavior). How- ever, there was an effect of barn ($P < 0.001$) on PC2 (social behavior). For the individual behaviors of interest, there was an effect of Lys (P = 0.032) and barn ($P = 0.009$) on ear-biting behavior. Pigs on lower Lys diets, Lys 80, showed higher levels of ear biting behavior and pigs in barn 2 showed more ear biting behavior than the pigs in barn 1. We found no effect of Lys, Trp:Lys, or the Lys × Trp:Lys interaction on aggressive behavior and only an effect of fill date on the combined play behavior ($P = 0.034$).

Table 4. Number of pigs removed due to death and injury/illness from each dietary treatment

Treatment	Death	Injury/Illness	Total removed	%
Lys 100 with Trp:Lys 0.210	4	2	6	5.45
Lys 100 with Trp:Lys 0.185	8	4	12	10.91
Lys 100 with Trp:Lys 0.160	10	7	17	15.45
Lys 80 with Trp:Lys 0.210	6	9	15	13.64
Lys 80 with Trp:Lys 0.185	9	14	23	20.91
Lys 80 with Trp:Lys 0.160	7	30	37	33.64
Totals	44	66	110	100.00

Bite lesions

All ear lesions

Lys and score week (i.e., age) interacted with the number of pens showing ear lesions ($P < 0.001$). At score weeks 1 and 7, the proportion of pens showing ear lesions was lower for pigs on the Lys 100 diet compared to pigs on the Lys 80 diet. For pigs on the Lys 80 diet, the proportion of pens showing lesions was higher in score week 7 than in score week 1 (Fig-ure 2). We found no effect of fill date, barn, Trp:Lys or the Lys × Trp:Lys interaction on the number of pens showing ear lesions.

Severe ear lesions

Lys and Trp:Lys interacted on the proportion of pens with an above-median score for severe ear lesions ($P = 0.030$). Pigs on the Lys 100 with Trp:Lys 0.185 and Lys 100 with Trp:Lys 0.160 treatments had lower numbers of pens with an above-median score for severe ear lesions compared to the other Lys and Trp:Lys combinations (Figure 3). Lys and score week also interacted with the number of pens showing severe ear lesions ($P = 0.0104$). Severe ear lesion incidence increased faster over time for Lys 80 than for Lys 100 pigs, resulting in a significant difference in the final score in week 7 (Figure 4). There was no effect of fill date or barn on the proportion of pens with an above-median score for severe ear lesions.

Fight lesions

We found an effect of pig size ($P < 0.001$) and score week ($P < 0.001$) on fight lesions. Large pigs had fewer fight lesions (0.188 ± 0.0199) than small and medium pigs (0.372 ± 0.0247 and 0.326 ± 0.0239, respectively). Fight lesions were higher in score week 1 (0.389 ± 0.0288) than in score week 4 (0.198 ± 0.0235). Barn tended to affect fight lesion score ($P = 0.055$) but there was no significant effect of fill date, Lys, Trp:Lys, or the Lys × Trp:Lys interaction.

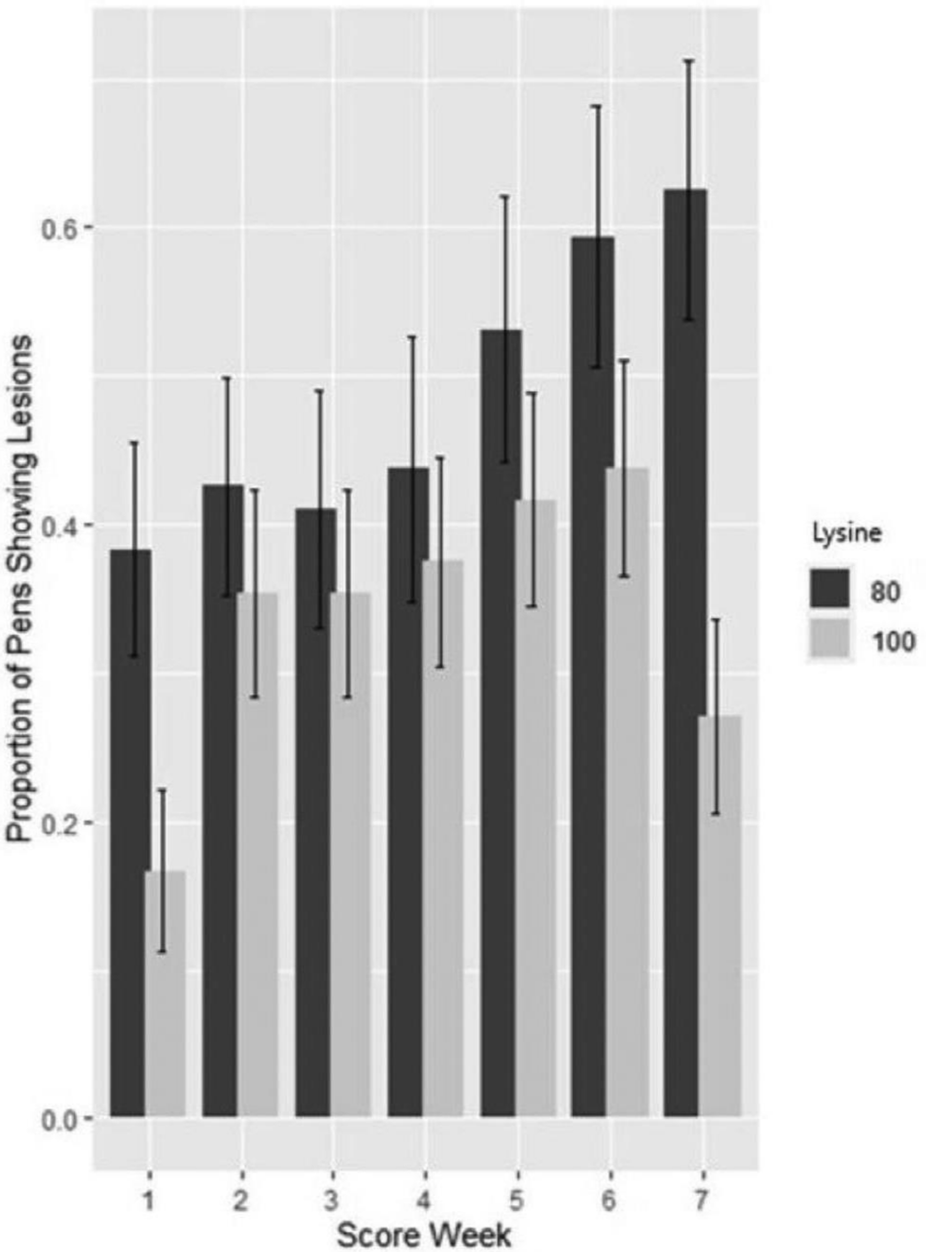


Figure 2. Effect of the interaction between lysine (Lys) level and score week (i.e., age) on the number of pens showing ear lesions ($P < 0.001$). The proportion of pens showing lesions, plotted against score week, grouped by lysine level; 100% Lys = diets with 100% PIC requirement at the midpoint of the growth phase (Lys 100) vs 80% Lys = diets with 80% PIC requirement at the midpoint of the growth phase (Lys 80). Forty-eight replicates per Lys level.

Discussion

The main aim of this study was to explore the effect of dietary Lys and Trp, given at commercially relevant levels, on the expression of both positive and negative behaviors. We found no effect of diet on play behavior. This may be due to a combination of missing the majority of the pig's play window (around 6 to 14 wk [Newberry et al., 1988]) and the housing conditions (slatted floors with no straw) not being conducive to play behaviors (Lyons et al., 1995). Other studies have also shown that nose-to-nose contact and allogrooming occurred infrequently in indoor-housed pigs (Camerlink et al., 2022). There is also evidence to suggest that social nosing is largely unrelated to negative social behaviors like tail biting (Camerlink and Turner, 2013) so it may be that the underlying mechanisms driving positive and negative social behaviors are different and strategies used to reduce one may not affect the other. Positive social behaviors are less well understood than negative social behaviors and are often harder to observe. We would benefit from further exploring the full behavioral repertoire of commercially housed pigs, looking at both positive and "neutral" social behaviors. It may be that "neutral" or "passive" social behaviors can better help us understand the social dynamics of groups and their welfare

Previous work has shown that injuries from chronic aggression occur in varying amounts between pens (Desire et al., 2015). We found no dietary effect on fight lesions or aggressive behavior, suggesting that chronic aggression in stable social groups was unaffected by the amino acid levels in this population.

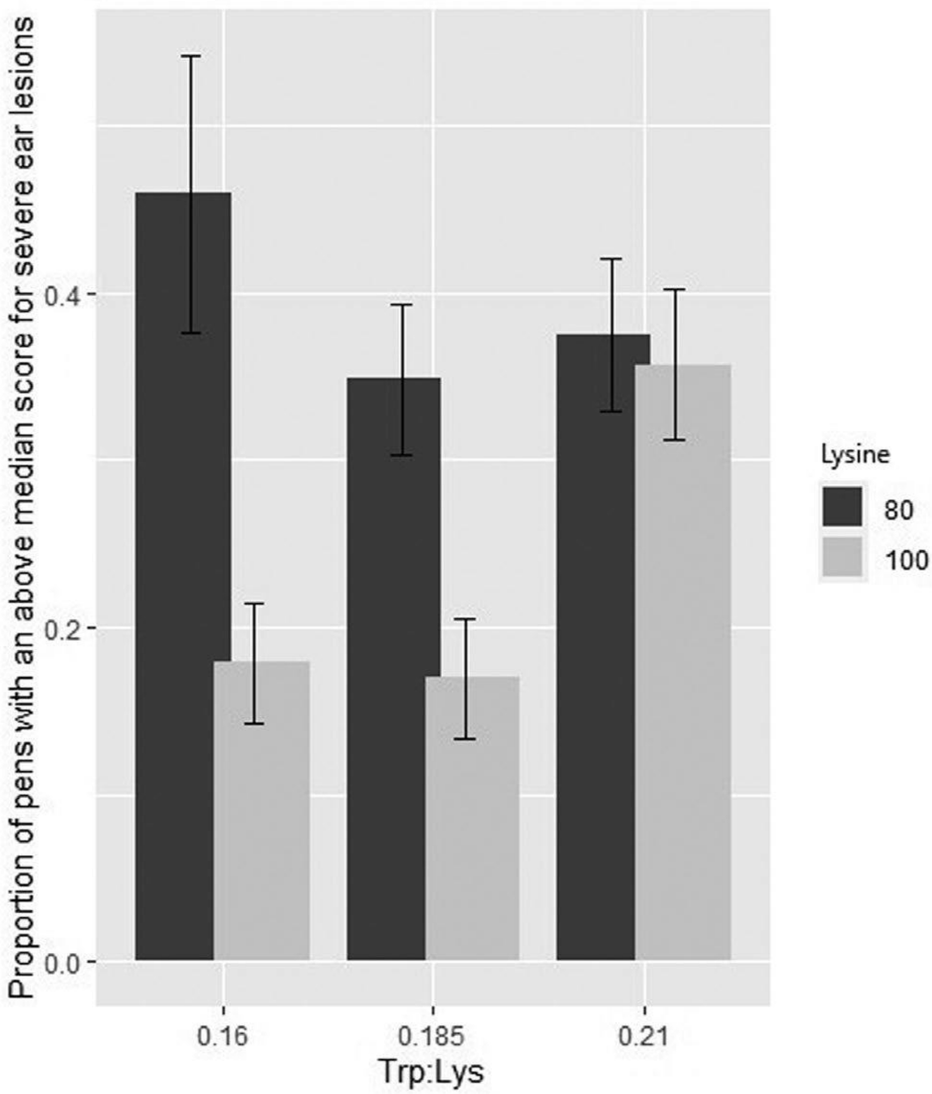


Figure 3. Effect of lysine (Lys) level and tryptophan to lysine ratio (Trp:Lys) interaction (*P* = 0.030) on the proportion of pens with an above-median score for severe ear lesions over an 8-wk period. 100% Lys = diets with 100% PIC requirement at the midpoint of the growth phase (Lys 100) vs 80% Lys = diets with 80% PIC requirement at the midpoint of the growth phase (Lys 80); and 2) Trp to Lys ratio of 0.210, 0.185, or 0.160. 48 replicates per Lys level, 32 replicates per Trp:Lys.

The regrouping period, when fighting is most common, was not observed in this study and the effect of Lys and Trp levels on this form of aggression should be studied. As mentioned before, some studies found an effect of Trp on aggression at mixing (Shen et al., 2012b; Poletto et al., 2014) but this was when Trp was supplemented at high levels and these studies did not explore the effects of Lys at mixing.

We found there to be a significant effect of Lys on increased ear-biting behavior and an effect of the interaction between

Lys and score week on ear lesions of any severity and specifically severe ear lesions. Lys is the first limiting amino acid in the majority of pig diets and so has been studied extensively in a production context but rarely in relation to the behavior and welfare of pigs. Srinongkote et al., (2003) found that diets fortified with a combination of Lys and arginine reduced plasma cortisol in pigs during transportation and Minussi et al., (2023) found that supplementing low protein diets with indispensable amino acids (L-Lysine, DL-Methionine, L-Threonine, L-Tryptophan, L-Valine, L-Isoleucine, L-Leucine and L-Histidine) reduced tail biting in pigs with intact tails. Our results, together with those of Minussi et al. (2023) and Srinongkote et al. (2003) strongly suggest an effect of Lys on pig behavior and further research is needed to explore this and to understand the mechanism of how Lys affects behavior. We speculate the increased ear biting could be due to nutritional deficiency and/or impaired immune response as a result of a Lys deficiency. Numerous studies have shown a link between low-protein diets and an increase in biting behaviors (Beattie et al., 2005; Meer et al., 2017). Studies have shown that pigs deficient in protein show increased attraction to blood (Fraser et al., 1991) and increased rooting behavior (Jensen et al., 1993). This combined attraction to blood and increased motivation to root may explain the increased ear biting.

The increased biting may also be due to low Lys levels affecting the health and immune response of pigs. Lys plays an important role in the immune response. Studies have shown that a lack of dietary Lys can impair an animal's immune function, making them more susceptible to infections (Datta et al., 2001; Li et al., 2007).

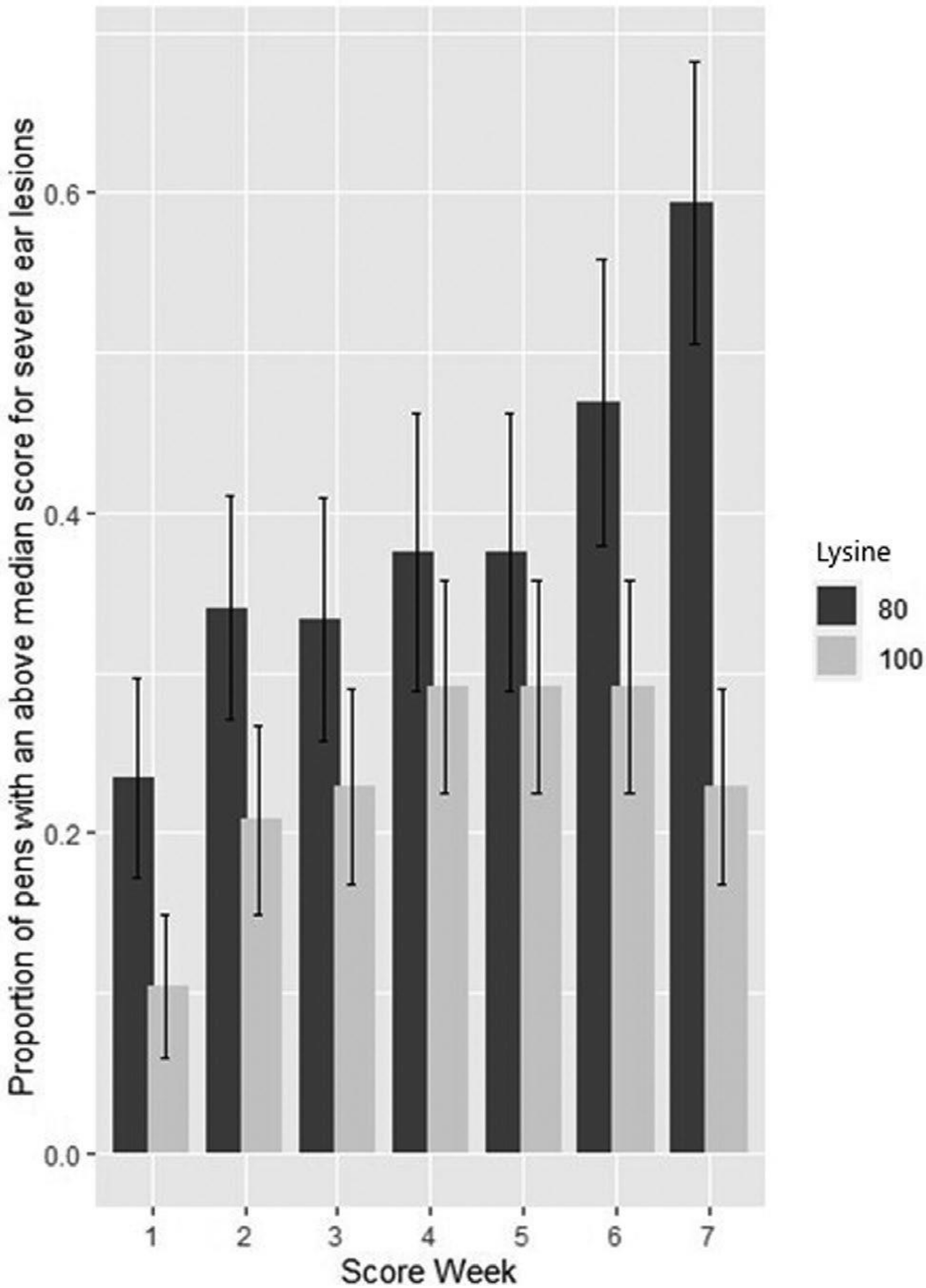


Figure 4. Effect of interaction between lysine (Lys) level and score week (*P* = 0.0104) on the number of pens showing severe ear lesions. The proportion of pens with an above-median score for severe ear lesions grouped by score week and lysine level; 100% Lys = diets with 100% PIC requirement at the midpoint of the growth phase (Lys 100) vs 80% Lys = diets with 80% PIC requirement at the midpoint of the growth phase (Lys 80). Forty-eight replicates per Lys level.

Studies in chickens estimated that the total cost of upregulating both the innate and adaptive immune system was between 7% and 10% of the animals total Lys requirements (Klasing, 2007), therefore a deficiency in Lys may limit the synthesis of immune response related proteins. Multiple studies have explored the link between disease and damaging behavior (review by Boyle et al., 2022) and the majority of the literature points towards an association between low health and negative social behaviors. Studies have linked internal inflammation to biting behavior (Czy- choll et al., 2023) and uncovered a correlation between cytokine levels and social behavior (Munsterhjelm et al., 2017).

The effect of the Lys level and score week interaction may be due to a cumulative effect of Lys level. Prolonged nutritional deficiency and/or impaired immune response as a result of a Lys deficiency could result in more damaging tail/ear biting outbreaks, as pigs on low Lys diets may not have the capacity to recover and heal from bite lesions. As we have yet to understand the mechanisms by which Lys affects behavior, the long-term effects of low Lys on behavior likewise remain to be elucidated.

Score week could have influenced the severity of ear lesions for a number of reasons; external temperature and consequent changes to wind speed in the barn (van Putten, 1969; Geers et al., 1989), emptying of slurry pits and changes to gas levels in the barn (van Putten, 1969), coincidental removal of biters (biters tend to have lower growth rates (Beattie et al., 2005) so some of these pigs may have been removed from their pen due to illness/death, removing the biter/bit- ten pigs can reduce ear/tail biting (Zonderland et al., 2008)) and the onset and development of biting outbreaks, where severity and the number of lesions tend to increase over time. Pigs on low Lys diets may be less able to cope with these environmental changes as they may already be nutritionally/ immune challenged.

Compared to ear biting, we found very little occurrence of tail biting during this study. This may have been due to the pigs' tails being docked short (approximately 3 inches). Studies have shown that when tails are docked short, ear biting occurs more often than tail biting (Goossens et al., 2008). We recommend that the effect of commercially relevant levels of Lys and Trp on tail biting be studied in populations with a higher incidence of tail biting. The high incidences of ear lesions (as opposed to more commonly observed tail lesions) could also be linked to ear necrosis. Porcine ear necrosis (PEN) is a complex and not fully understood condition but may contribute to, or be exacerbated by, ear biting (Park et al., 2013). It may be the case that initially the ear lesions are caused by PEN and these lesions become bloody and then as mentioned above, attract pigs with a nutrient deficiency to the open wound causing an increase in biting behavior. Conversely, it may be that the act of ear biting introduces pathogens to the site that cause PEN. There are suggested infectious and non-infections factors affecting PEN, potential noninfectious factors involved in PEN include slatted floors with no straw, poor air quality, high stocking density and competition for drinker and feeder space (Park et al., 2013), factors commonly found in intensive systems. It is difficult to distinguish between ear lesions caused by biting and PEN. We did observe more ear biting behavior in pigs on the low Lys diets so can be relatively confident that the lesions we observed were largely due to ear biting. There are a small number of studies exploring the effect of Lys on the skin, feathers, and fur of other species (Kratzer and Vohra, 1956; Vohra and Kratzer, 1957). In kittens, Lys-deficient diets have been linked to crusted facial lesions (Larsen et al., 2010, 2014). It may be that low Lys effects the structural integrity of the pig's skin, making the ears more susceptible to PEN or the flesh of the pigs more attractive to other pigs.

The significant interaction between Lys level and Trp:Lys on ear lesions suggests that the effect of Trp:Lys depends on Lys level. The results suggest that, in line with our hypothesis, sever ear lesions reduce at higher Trp:Lys for the Lys 80 treatment. However, the opposite was the case for the Lys 100 treatment, with the highest proportion of sever ear lesions seen with the highest Trp:Lys. Whilst it might be suggested that the latter could be due to faster-growing pigs having increased competition at the feeder or accelerated space reduction leading to increased ear biting, this was not supported by behavioral data (e.g., fight lesions). Therefore, it cannot be excluded that the increase in sever ear lesions in the Lys 100 with Trp:Lys 0.210 treatment pigs compared to their 0.185 and 0.160 counterparts were caused by factors other than negative social behaviors. From a production standpoint, previous studies have identified the optimum Trp:Lys to be between 0.17 (Susenbeth, 2006) to 0.203 (Xie et al., 2014). As this is the first trial to explore the effect of the Trp:Lys on negative social behaviors it is not clear what the optimum Trp:Lys is in relation to behavior and welfare. Further exploration of an optimal behavioral ratio is needed along with research to gain a better understanding of the interactive effect of the 2 amino acids on behavior, both positive and negative.

A key point in the trial was the removal of pigs from the Lys 80 with Trp:Lys 0.160 diet. As previously discussed, the pigs on this diet reached both their production (slowed growth rate) and welfare endpoints (as outlined in Materials and Methods). While the removal of these pigs was essential for ethical reasons, it left a gap in our research. We only had data from the first 2 feeding phases for these pigs, meaning we were unable to see how this treatment effected behavior over a longer period of time. We cannot speculate on the long-term effect of this treatment but we can say this diet had a serious negative impact on the production metrics and overall welfare of these pigs.

Conclusion

In conclusion, the Lys content of feed can affect the social behavior of pigs, specifically ear biting, in commercial conditions. The link between dietary Lys and ear lesions and ear biting behavior requires further quantification and mechanistic exploration, and further research into the effects of Lys on tail biting should be conducted, preferably on pigs with undocked tails.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

Acknowledgments

We would like to acknowledge the staff at the U.S. facility for providing general animal husbandry assistance and Victoria Lee for her help navigating R. This work was sup- ported by the UKRI Biotechnology and Biological Sciences Research Council (BBSRC) grant number BB/T00875X/1 and PIC as part of the EASTBIO Doctoral Training program. SRUC receives funding from the Scottish Government.

Conflicts of interest statement

The authors declare no conflict of interest.

Author Contributions

Eleanor Hewett (Conceptualization, Formal analysis, Investigation, Methodology, Writing—original draft), Luis Zaragoza (Conceptualization, Methodology, Writing—review & editing), Jos Houdijk (Conceptualization, Methodology, Writing—review & editing), Craig Lewis (Writing—review & editing), Andrea Doeschl-Wilson (Formal analysis, Writing—review & editing), and Simon Turner (Conceptualization, Formal analysis, Methodology, Supervision, Writing—review & editing)

Literature Cited

Adeola, O., and R. O. Ball. 1992. Hypothalamic neurotransmitter concentrations and meat quality in stressed pigs offered excess dietary tryptophan and tyrosine. J. Anim. Sci. 70:1888–1894. doi:10.2527/1992.7061888x

Andersen, I. L., M. Ocepek, S. L. Thingnes, and R. C. Newberry. 2023. Welfare and performance of finishing pigs on commercial farms: associations with group size, floor space per pig and feed type. Appl. Anim. Behav. Sci. 265:105979. doi:10.1016/j. applanim.2023.105979

Bakare, A. G., J. Madzimure, S. P. Ndou, and M. Chimonyo. 2014.

Growth performance and behaviour in grouped pigs fed fibrous diet. Asian-Australas. J. Anim. Sci. 27:1204–1210. doi:10.5713/ajas.2013.13774
Baranyiová, E. 1991. Effect of serotonin on food intake by piglets during the early postnatal period. Acta Vet. Brno 60:127–136. doi:10.2754/avb199160020127
Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw 67:1–48. doi:10.18637/jss.v067.i01
Beattie, V. E., K. Breuer, N. E. O'Connell, I. A. Sneddon, J. T. Mercer, K. A. Rance, M. E. M. Sutcliffe, and S. A. Edwards. 2005. Factors identifying pigs predisposed to tail biting. Anim. Sci. 80:307–312. doi:10.1079/asc40040307
Blackshaw, J. K., A. J. Swain, A. W. Blackshaw, F. J. M. Thomas, and K. J. Gillies. 1997. The development of playful behaviour in piglets from birth to weaning in three farrowing environments. Appl. Anim. Behav. Sci. 55:37–49. doi:10.1016/s0168-1591(97)00034- 8
Boyle, L. A., S. A. Edwards, J. E. Bolhuis, F. Pol, M. Z. Šemrov, S. Schütze, J. Nordgreen, N. Bozakova, E. N. Sossidou, and A. Valros. 2022. The evidence for a causal link between disease and damaging behavior in pigs. Front. Vet. Sci 8:771682. doi:10.3389/ fvets.2021.771682
Brand, H. van, D. Wamsteeker, M. Oostindjer, B. Kemp, J. E. Bolhuis, L. C. M. van Enckevort, A. F. B. van der Poel. 2014. Effects of pellet diameter during and after lactation on feed intake of piglets pre- and postweaning. J. Anim. Sci. 92:4145–4153. doi:10.2527/ jas.2014-7408
Brown, S. M., M. Klaffenböck, I. M. Nevison, and A. B. Lawrence. 2015. Evidence for litter differences in play behaviour in pre- weaned pigs. Appl. Anim. Behav. Sci. 172:17–25. doi:10.1016/j.applanim.2015.09.007
Camerlink, I., and S. P. Turner. 2013. The pig's nose and its role in dominance relationships and harmful behaviour. Appl. Anim. Behav. Sci. 145:84–91. doi:10.1016/j.applanim.2013.02.008
Camerlink, I., C. Proßegger, D. Kubala, K. Galunder, and J. -L. Rault. 2021. Keeping littermates together instead of social mixing benefits pig social behaviour and growth post-weaning. Appl. Anim. Behav. Sci. 235:105230. doi:10.1016/j.applanim.2021.105230
Camerlink, I., K. Scheck, T. Cadman, and J. L. Rault. 2022. Lying in spatial proximity and active social behaviours capture different information when analysed at group level in indoor- housed pigs. Appl. Anim. Behav. Sci. 246:105540. doi:10.1016/j.applanim.2021.105540
Castilha, L. D., C. P. Sangali, L. A. C. Esteves, C. F. Muniz, A. C. Furlan, R. S. Vasconcellos, and P. C. Pozza. 2016. Day-night behaviour and performance of barrows and gilts (70–100 kg) fed low protein diets with different levels of tryptophan and B6 vitamin. Appl. Anim. Behav. Sci. 180:35–42. doi:10.1016/j. applanim.2016.03.017
Cho, J. H., and I. H. Kim. 2011. Effect of stocking density on pig production. Afr. J. Biotechnol 10:13688–13692. doi:10.5897/ajb11.1691
Chou, J. Y., K. O'Driscoll, D. A. Sandercock, and R. B. D'Eath. 2020. Can increased dietary fibre level and a single enrichment device reduce the risk of tail biting in undocked growing-finishing pigs in fully slatted systems? PLoS One 15:e0241619. doi:10.1371/journal.pone.0241619
Cox, L. N., and J. J. Cooper. 2001. Observations on the pre- and post-weaning behaviour of piglets reared in commercial indoor and outdoor environments. Anim. Sci. 72:75–86. doi:10.1017/s1357729800055570
Czycholl, I., K. Büttner, D. Becker, C. Schwennen, W. Baumgärtner, W. Otten, M. Wendt, C. Puff, and J. Krieter. 2023. Are biters sick? Health status of tail biters in comparison to control pigs. Porcine Health Manag. 9:19. doi:10.1186/s40813-023-00314-0
D'Eath, R. B., B. J. Tolkamp, I. Kyriazakis, and A. B. Lawrence. 2009. "Freedom from hunger" and preventing obesity: the animal welfare implications of reducing food quantity or quality. Anim. Behav. 77:275–288. doi:10.1016/j.anbehav.2008.10.028
Datta, D., A. Bhinge, and V. Chandran. 2001. Lysine: Is it worth more? Cytotechnology 36:3–32. doi:10.1023/A:1014097121364
Day, J. E. L., I. Kyriazakis, and A. B. Lawrence. 1995. The effect of food deprivation on the expression of foraging and exploratory behaviour in the growing pig. Appl. Anim. Behav. Sci. 42:193–206. doi:10.1016/0168-1591(95)93889-9
Desire, S., S. P. Turner, R. B. D'Eath, A. B. Doeschl-Wilson, C. R. G. Lewis, and R. Roehe. 2015. Analysis of the phenotypic link between behavioural traits at mixing and increased long-term social stability in group-housed pigs. Appl. Anim. Behav. Sci. 166:52–62. doi:10.1016/j.applanim.2015.02.015
Di Giminiani, P., S. A. Edwards, E. M. Malcolm, M. C. Leach, M. S. Herskin, and D. A. Sandercock. 2017. Characterization of short- and long-term mechanical sensitisation following surgical tail amputation in pigs. Sci. Rep. 7:4827. doi:10.1038/s41598-017- 05404-y
Donaldson, T. M., R. C. Newberry, M. Špinka, and S. Cloutier. 2002. Effects of early play experience on play behaviour of piglets after weaning. Appl. Anim. Behav. Sci. 79:221–231. doi:10.1016/s0168-1591(02)00138-7
Franchi, G. A., M. L. V. Larsen, I. H. Kristoffersen, J. F. M. Winters, L. J. Pedersen, and M. B. Jensen. 2023. Play behaviour positively relates to weight gain, feeding behaviour and drinking behaviour in weaner pigs (Sus scrofa). Appl. Anim. Behav. Sci. 259:105836. doi:10.1016/J.APPLANIM.2023.105836
Fraser, D., D. E. Bernon, and R. O. Ball. 1991. Enhanced attraction to blood by pigs with inadequate dietary protein supplementation. Can. J. Anim. Sci. 71:611–619. doi:10.4141/cjas91-076
Geers, R., B. Dellaert, V. Goedseels, A. Hoogerbrugge, E. Vranken, F. Maes, and D. Berckmans. 1989. An assessment of optimal air temperatures in pig houses by the quantification of behavioural and health-related problems. Anim. Prod 48:571–578. doi:10.1017/s0003356100004098
Goncalves, M., U. Orlando, W. Cast, and M. Culbertson. 2017. Standardized ileal digestible lysine requirements for finishing PIC pigs under commercial conditions: a meta-analysis. J. Anim. Sci. 95:131–132. doi:10.2527/asasmw.2017.273
Goossens, X., L. Sobry, F. Ödberg, F. Tuyttens, D. Maes, S. De Smet, F. Nevens, G. Opsomer, F. Lommelen, and R. Geers. 2008. A population-based on-farm evaluation protocol for comparing the welfare of pigs between farms. Anim. Welf. 17:35–41. doi:10.1017/s0962728600031961
Henry, M., A. K. Shoveller, T. L. O'Sullivan, L. Niel, and R. Friendship. 2022. Effect of varying levels of dietary tryptophan on aggression and abnormal behavior in growing pigs. Front. Vet. Sci. 9:849970. doi:10.3389/fvets.2022.849970. Available from: https://www.frontiersin.org/articles/10.3389/fvets.2022.849970
Jankevicius, M. L., and T. M. Widowski. 2004. The effect of ACTH on pigs' attraction to salt or blood-flavored tail-models. Appl. Anim. Behav. Sci. 87:55–68. doi:10.1016/j.applanim.2003.12.012
Jensen, M. B., I. Kyriazakis, and A. B. Lawrence. 1993. The activity and straw directed behaviour of pigs offered foods with differ- ent crude protein content. Appl. Anim. Behav. Sci. 37:211–221. doi:10.1016/0168-1591(93)90112-3
Klasing, K. C. 2007. Nutrition and the immune system. Br. Poult. Sci. 48:525–537. doi:10.1080/00071660701671336
Koopmans, S. J., M. Ruis, R. Dekker, H. V. Diepen, M. Korte, and Z. Mroz. 2005. Surplus dietary tryptophan reduces plasma cortisol and noradrenaline concentrations and enhances recovery after social stress in pigs. Physiol. Behav. 85:469–478. doi:10.1016/J.PHYSBEH.2005.05.010
Koopmans, S. J., A. C. Guzik, J. V. D. Meulen, R. Dekker, J. Kogut, B. J. Kerr, and L. L. Southern. 2006. Effects of supplemental L-tryptophan on serotonin, cortisol, intestinal integrity, and behavior in weanling piglets,. J. Anim. Sci. 84:963–971. doi:10.2527/2006.844963X
Koopmans, S. J., F. J. van der Staay, N. le Floc'h, R. Dekker, J. T. M. van Diepen, and A. J. M. Jansman. 2012. Effects of surplus dietary L-tryptophan on stress, immunology, behavior, and nitrogen

retention in endotoxemic pigs. J. Anim. Sci. 90:241–251. doi:10.2527/jas.2010-3372
Kratzer, F. H., and P. Vohra. 1956. Graying of hair in rats fed on ration deficient in lysine. Science 124:1145. doi:10.1126/sci- ence.124.3232.1145
Lammers, G. J., and W. G. P. Schouten. 1985. Effects of pen size during rearing on later agonistic behaviour in piglets. Neth. J. Agric. Sci. 33:307–309. doi:10.18174/njas.v33i3.16846
Larsen, J. A., A. J. Fascetti, C. C. Calvert, and Q. R. Rogers. 2010. Bio- availability of lysine for kittens in overheated casein is underes- timated by the rat growth assay method. J. Anim. Physiol. Anim. Nutr. 94:e102–e108. doi:10.1111/j.1439-0396.2010.00988.x
Larsen, J. A., C. A. Outerbridge, A. J. Fascetti, and V. K. Affolter. 2014. skin lesions associated with lysine deficiency in kittens are char- acterized by inflammation. Int. J. Appl. Res. Vet. Med. 12:61–66. https://jarvm.com/articles/Vol12Iss1/index.htm.
Lawrence, A. 1987. Consumer demand theory and the assessment of animal welfare. Anim. Behav. 35:293–295. doi:10.1016/s0003- 3472(87)80236-1
Lawrence, A. B., R. C. Newberry, and M. Špinka. 2018. 15 - Posi- tive welfare: what does it add to the debate over pig welfare? In: M. Špinka, editor. Advances in Pig Welfare. Cambridge, Cambridgeshire: Woodhead Publishing. p. 415–444. Avail- able from: https://www.sciencedirect.com/science/article/pii/ B9780081010129000149
Lay, D. C., S. A. Enneking, N. C. Anderson, B. T. Richert, and A. Sap- kota. 2021. Effect of short-term high tryptophan diet fed to sows on their subsequent piglet behavior. Transl Anim Sci 5:txab127. doi:10.1093/tas/txab127. Available from:https://academic.oup.com/tas/article/5/3/txab127/6354054
Li, Y. Z., B. J. Kerr, M. T. Kidd, and H. W. Gonyou. 2006. Use of sup- plementary tryptophan to modify the behavior of pigs. J. Anim. Sci. 84:212–220. doi:10.2527/2006.841212x
Li, P., Y. -L. Yin, D. Li, S. W. Kim, and G. Wu. 2007. Amino acids and immune function. Br. J. Nutr. 98:237–252. doi:10.1017/ S000711450769936X
Li, Y. Z., S. K. Baidoo, L. J. Johnston, and J. E. Anderson. 2011. Effects of tryptophan supplementation on aggression among group- housed gestating sows. J. Anim. Sci. 89:1899–1907. doi:10.2527/ jas.2010-3125
Liu, H. W., B. M. Shi, D. S. Liu, and A. S. Shan. 2013. Supplemental dietary tryptophan modifies behavior, concentrations of salivary cortisol, plasma epinephrine, norepinephrine and hypothalamic 5-hydroxytryptamine in weaning piglets. Livestock Sci. 151:213–218. doi:10.1016/j.livsci.2012.11.003
Lyons, C. A. P., J. M. Bruce, V. R. Fowler, and P. R. English. 1995. A comparison of productivity and welfare of growing pigs in four intensive systems. Livest. Prod. Sci. 43:265–274. doi:10.1016/0301- 6226(95)00050-u
Main, R. G., S. S. Dritz, M. D. Tokach, R. D. Goodband, J. L. Nels- sen, and J. M. DeRouchey. 2008. Effects of feeding growing pigs less or more than their estimated lysine requirement in early and late finishing on overall performance. Prof. Anim. Sci. 24:76–87. doi:10.15232/s1080-7446(15)30813-5
Marchant, J. N., M. T. Mendl, A. R. Rudd, and D. M. Broom. 1995. The effect of agonistic interactions on the heart rate of group- housed sows. Appl. Anim. Behav. Sci. 46:49–56. doi:10.1016/0168- 1591(95)00636-2
Markus, C. R., B. Olivier, G. E. Panhuysen, J. V. D. Gugten, M. S. Alles, A. Tuiten, H. G. Westenberg, D. Fekkes, H. F. Koppeschaar, and E. E. de Haan. 2000. The bovine protein alpha-lactalbumin increases the plasma ratio of tryptophan to the other large neu- tral amino acids, and in vulnerable subjects raises brain serotonin activity, reduces cortisol concentration, and improves mood under stress. Am. J. Clin. Nutr. 71:1536–1544. doi:10.1093/ ajcn/71.6.1536
McAuley, M., S. Buijs, R. Muns, A. Gordon, M. Palmer, K. Meek, and N. O'Connell. 2022. Effect of reduced dietary protein level on fin- ishing pigs' harmful social behaviour before and after an abrupt dietary change. Appl. Anim. Behav. Sci. 256:105762. doi:10.1016/j. applanim.2022.105762
Meer, Y. V. D., W. J. J. Gerrits, A. J. M. Jansman, B. Kemp, and J. E. Bolhuis. 2017. A link between damaging behaviour in pigs, sanitary conditions, and dietary protein and amino acid supply. PLoS One 12:e0174688. doi:10.1371/journal.pone.0174688
Meunier-Salaün, M. C., M. Monnier, Y. Colléaux, B. Sève, and Y. Henry. 1991. Impact of dietary tryptophan and behavioral type on behav- ior, plasma cortisol, and brain metabolites of young pigs. J. Anim. Sci. 69:3689–3698. doi:10.2527/1991.6993689x
Minussi, I., W. J. J. Gerrits, A. J. M. Jansman, R. Gerritsen, W. Lambert, J. J. Zonderland, and J. E. Bolhuis. 2023. Amino acid supplemen- tation counteracts negative effects of low protein diets on tail bit- ing in pigs more than extra environmental enrichment. Sci. Rep. 13:19268. doi:10.1038/s41598-023-45704-0
Munsterhjelm, C., J. Nordgreen, F. Aae, M. Heinonen, K. Olstad, T. Aasmundstad, A. M. Janczak, and A. Valros. 2017. To be blamed or pitied? The effect of illness on social behavior, cytokine levels and feed intake in undocked boars. Physiol. Behav. 179:298–307. doi:10.1016/j.physbeh.2017.06.024
Newberry, R. C., D. G. M. Wood-Gush, and J. W. Hall. 1988. Playful behaviour of piglets. Behav. Processes 17:205–216. doi:10.1016/0376-6357(88)90004-6
NRC. 2012. Nutrient Requirements of Swine. 11th rev. ed., Washing- ton, DC: National Academic Press.
Park, J., R. M. Friendship, Z. Poljak, J. DeLay, D. Slavic, and C. E. Dewey. 2013. An investigation of ear necrosis in pigs. Can. Vet. J. 54:491–495. https://pmc.ncbi.nlm.nih.gov/articles/ PMC3624920/.
Peden, R. S. E., S. P. Turner, I. Camerlink, and F. Akaichi. 2021. An estimation of the financial consequences of reducing pig aggression. PLoS One 16:e0250556. doi:10.1371/journal.pone.0250556
PIC. 2016. PIC Nutrition and Feeding Guidelines. Hendersonville, Ten- nessee, USA.
Poletto, R., F. C. Kretzer, and M. J. Hötzel. 2014. Minimizing aggression during mixing of gestating sows with supplementation of a tryptophan-enriched diet. Physiol. Behav. 132:36–43. doi:10.1016/j. physbeh.2014.04.043
R Core Team. 2021. R: A Language and Environment for Statistical Computing. Available from: https://www.R-project.org/
Shen, Y. B., G. Voilqué, J. D. Kim, J. Odle, and S. W. Kim. 2012a. Effects of increasing tryptophan intake on growth and physiological changes in nursery pigs. J. Anim. Sci. 90:2264–2275. doi:10.2527/ jas.2011-4203
Shen, Y. B., G. Voilquí, J. Odle, and S. W. Kim. 2012b. Dietary L-tryptophan supplementation with reduced large neutral amino acids enhances feed efficiency and decreases stress hormone secre- tion in nursery pigs under social-mixing stress. J. Nutr. 142:1540– 1546. doi:10.3945/jn.112.163824
Sihvo, H. -K., O. Simola, C. Munsterhjelm, and P. Syrjä. 2012. Systemic spread of infection in tail-bitten pigs. J. Comp. Pathol. 146:73. doi:10.1016/j.jcpa.2011.11.112
Srinongkote, S., M. Smriga, K. Nakagawa, and Y. Toride. 2003. A diet fortified with l-lysine and l-arginine reduces plasma cortisol and blocks anxiogenic response to transportation in pigs. Nutr. Neuro- sci. 6:283–289. doi:10.1080/10284150310001614661
Stolba, A., and D. G. M. Wood-Gush. 1989. The behaviour of pigs in a semi-natural environment. Anim. Prod. 48:419–425. doi:10.1017/ s0003356100040411
Stracke, J., W. Otten, A. Tuchscherer, M. Witthahn, C. C. Metges, B. Puppe, and S. Düpjan. 2017. Dietary tryptophan supplementation and affective state in pigs. J. Vet. Behav. 20:82–90. doi:10.1016/j. jveb.2017.03.009
Susenbeth, A. 2006. Optimum tryptophan: lysine ratio in diets for growing pigs: analysis of literature data. Livestock Sci.101:32–45. doi:10.1016/j.livprodsci.2005.09.008

Taylor, N. R., D. C. J. Main, M. Mendl, and S. A. Edwards. 2010. Tail-biting: a new perspective. Vet. J. Lond. Engl. 1997 186:137–

147. doi:10.1016/j.tvjl.2009.08.028 van de Weerd, H. A., and J. E. L. Day. 2009. A review of envi- ronmental enrichment for pigs housed in intensive housing systems. Appl. Anim. Behav. Sci. 116:1–20. doi:10.1016/j. applanim.2008.08.001

van Putten, G. 1969. An investigation into tail-biting among fattening pigs. Br. Vet. J. 125:511–517. doi:10.1016/s0007-1935(17)48710-0

Vohra, P., and F. H. Kratzer. 1957. The role of lysine in the growth and feather pigmentation of turkey poults. J. Nutr. 63:471–476. doi:10.1093/jn/63.3.471

Weller, J. E., I. Camerlink, S. P. Turner, M. Farish, and G. Arnott. 2019. Playful pigs: early life play-fighting experience influences later life contest dynamics. Anim. Behav. 158:269–279. doi:10.1016/j.anbehav.2019.09.019

Xie, C. Y., G. J. Zhang, F. R. Zhang, S. H. Zhang, X. F. Zeng, P. A. Thacker, and S. Y. Qiao. 2014. Estimation of the optimal ratio of standardized ileal digestible tryptophan to lysine for finishing bar- rows fed low protein diets supplemented with crystalline amino acids. CZECH J. Anim. Sci. 59:26–34. doi:10.17221/7191-CJAS

Zonderland, J. J., M. Wolthuis-Fillerup, C. G. van Reenen, M. B. M. Bracke, B. Kemp, L. A. den Hartog, and H. M. Spoolder. 2008. Prevention and treatment of tail biting in weaned piglets. Appl. Anim. Behav. Sci. 110:269–281. doi:10.1016/j.applanim.2007.04.005

Discussion

A common critique I have received when presenting this research is the reliability of lesion scores as a reflection of biting. As discussed in the published paper, lesions can be caused by disease, specifically porcine ear necrosis (Malik et al., 2021), and can also be caused by damage from pen fixtures and fittings as well as from biting. To differentiate between causes of lesions, it is important to supplement lesion recording with behavioural observations. Here the direction of effect of the diet on damaging social behaviours was similar to that on lesions, confirming that biting was a cause of lesions and mediated by the diet. It would be beneficial to collect additional data to further elucidate the cause of the lesions, for example using swabs of the area and immune response data that could confirm if some lesions were from bacterial or viral necrosis. However, regardless of the cause, ear lesions are likely to reduce health and welfare of the animals, which should always be the main focus. Hence, dietary effects on ear lesions (or to other parts of the body) ought to be of interest and concern, irrespective of the mechanism of lesion formation. The work has shown that a combination of low lysine and low tryptophan has a detrimental impact on social behaviour. The study demonstrated this using a fast growing modern genotype of pigs under commercial conditions which increases the industrial relevance of the results. Demonstrating this interaction between the two most limiting amino acids and social behaviour provides a basis for future studies to quantify this effect under a wider range of conditions and genotypes and potentially create new dietary approaches to tackling the ubiquitous problem of biting behaviour. Crucially, dietary changes may be a more economically feasible approach to address these behaviours than major changes to the housing environment and hence may be more likely to be widely adopted.

It should also be noted that my intention was not to focus solely on negative social behaviours but also to explore the effects of lysine and tryptophan on positive social behaviours. Unfortunately, positive social behaviours are harder to observe than negative ones, as they are often more subtle or confined to particular growth periods (discussed in more depth in Chapter 6) and they were recorded rarely during my study. I was also limited in my behavioural observations by the large sample size in study 2 which necessitated the collection of only a modest amount of behaviour per pen.

Chapter 4: Exploring the effects of tryptophan to lysine ratio on the performance of fattening pigs fed at different levels of lysine

Introduction

As pigs (*Sus scrofa*) continue to be one of the most popular animal protein sources globally, breeding companies continue to invest in the development of faster growing, more efficient and profitable pigs. However, breeding companies have been receiving anecdotal reports from producers that they are seeing increased incidences of negative social behaviours in pigs. These negative social behaviours are an extensive and complex issue in intensive pig production systems and reduce pig welfare (Puppe et al., 1997), increase incidences of carcass condemnation and increase morbidity and mortality. Breeding companies have set out to explore producers' concerns around these negative social behaviours. Hewett et al. (2025) found that the lysine content of feed can affect the social behaviour of pigs; specifically low lysine levels can increase the incidences of ear biting. In a competitive market, producers are always looking to improve efficiency and reduce feed costs and the adoption of co-products from other industries, such as wheat and corn distillers dried grains with solubles (DDGS) from the biofuel industry (Noblet et al., 2012) is becoming more popular. However, care must be taken when using DDGS as an alternative to soybean meal (SBM) to avoid pigs being fed below their nutrient requirements, potentially causing an increase in negative social behaviours.

Studies have found that increasing inclusion of DDGS as an alternative to SBM decreased energy and protein digestibility of diets fed to weaned and growing-finishing pigs (Thacker, 2006; Avelar et al., 2010; Wang et al., 2016). In addition, the relative lysine (Lys) and tryptophan (Trp) level in corn-DDGS is ~53 and ~40% less than in SBM, respectively (Premier Nutrition Atlas, 2025). Therefore, diets containing DDGS may require fortification with additional amino acids, including Lys and Trp. Lys is the first limiting amino acid in the majority of pig diets, determining growth and lean muscle deposition (Main et al., 2008). Trp is important for protein synthesis and is the primary precursor of serotonin (5-hydroxytryptamine, 5-HT) which plays a major role in regulating feed intake (Baranyiová, 1991; Fernstrom, 1994), stress hormone secretion (Adeola and Ball, 1992), and social behaviour in pigs (Meunier-Salaün et al., 1991; Henry et al., 2022).

Genetics companies will often publish recommended requirements of dietary amino acids specific to their various genetic lines. For the Camborough barrow, PIC recommend that pigs are fed at least 80% of the recommended Lys at the midpoint of any feeding phase and a Trp to Lys ratio (Trp:Lys) of 0.185 (PIC, 2024). PIC state that "guidelines are based on published research, PIC internal research, research from universities and large-scale commercial experiments". The guidelines are updated regularly and are designed to be globally applicable. For various reasons (cost of ingredients, availability of ingredients, education and awareness of recommendations), it may not be possible for producers to follow these requirements.

Whilst it has long been known that pigs may choose a balanced diet when given access to feeds with different levels of protein (Kyriazakis et al., 1990), it was more recently demonstrated that pigs can detect multiple AA deficiencies and compensate by consuming AA-supplemented diets (Minussi et al., 2024) and reduce feed intake on rations with excess Lys (Müller et al, 2024). Thus, pigs may vary their intake to avoid AA excess and/or compensate for AA deficiencies. However, we hypothesise that when fed diets deficient in multiple AAs, pigs will only be able to compensate for the first limiting AA deficiency when fed a high fiber feed such as DDGS as they will reach a bulk constraint which creates gut fill and will be physically unable to increase feed intake further. Thus, when Lys is the first limiting amino acid and pigs are consuming a diet with low Lys levels and a low Trp:Lys ratio, they are anticipated to show reduced performance due to an inability to sufficiently increase feed intake required to overcome both Lys and Trp deficiency. In contrast, pigs fed diets high in Lys but with a low Trp:Lys ratio will be able to increase feed intake to overcome a Trp deficiency and consequently show no significant reduction in growth though perform at greater feed conversion ratio.

Materials and Methods

Ethical review

The study was approved by the Scotland's Rural College (SRUC) Animal Experiments Committee under application PIG AE 22-2022. Routine animal care and management was performed by trained facility staff.

Animals and housing

Production data was collected for 2,293 PIC Camborough barrows, mean starting weight of 11.87 ± 1.35 kg. Pigs were housed under US commercial conditions (no enrichment), in a fully slatted house, consisting of two, tunnel ventilated barns. Each barn contained 48 pens of 2.25 m x 2.25 m (with two additional smaller pens per barn which were used as hospital pens) with an initial inventory of 16 to 26 castrated male pigs per pen. Pens were fitted with one feeder, divided into three sections and two pen mounted bowl drinkers. Pigs arrived on farm on the 8th, 11th and 15th August after weaning at a separate facility. Barn 1 pigs (a mix of those that arrived on the 8^{th} and 11^{th}) were put on treatment on the 30th of August and barn 2 (a mix of those that arrived on the 11^{th} and 15^{th}) began their treatment on the 8th of September. Between arrival at the barn and starting on trial feed, pigs were fed a commercial weaner diet. Sick pigs were removed from the trial and placed in hospital pens and did not return to the trial.

Experimental treatments

Pigs were randomly allocated across 6 treatments in a 2×3 factorial arrangement in a randomised complete block design (block = wean date and weight), giving 16 replicates of each treatment, 8 per barn, of the following iso-energetic feeding treatments: 1) one of two levels of standardised ileal digestible (SID) Lys, i.e. at 100% (Lys 100) or 80% (Lys 80) of PIC requirement at the midpoint of the growth phase, and 2) one of three Trp to Lys ratio (Trp:Lys) of 0.210, 0.185 or 0.160, resulting in six treatment combinations, i.e. treatment 1 = Lys 100, Trp:Lys 0.210, treatment 2 = Lys 100, Trp:Lys 0.185, treatment 3 = Lys 100, Trp:Lys 0.160, treatment 4 = Lys 80, Trp:Lys 0.210, treatment 5= Lys 80, Trp:Lys 0.185, and treatment 6= Lys 80, Trp:Lys 0.160. Full diet compositions, which had a basis of corn, DDSG and soybean meal, can be found in Appendix 1. Targeted Lys levels were attained through replacing soybean meal and synthetic Lys with corn, and minor modification from synthetic amino acid sources, minerals and oil to ensure similar ratios of other essential amino acids to Lys at SID level, same levels of net energy, calcium and standardized total tract digestible phosphorus as per recommendations of the genetic supplier (PIC, 2024) and NRC (2012). Within those Lys levels, targeted levels of Trp were attained by iso-energetic exchange of L-tryptophan against a combination of corn and corn-oil. The SID Lys levels were derived from a meta-analysis performed by PIC to determine the SID Lys requirements for average requirement of average daily gain (ADG) and feed to gain ratio for finishing PIC Camborough pigs under commercial conditions (Goncalves et al., 2017).

There were a total of 5 feeding phases as the pigs aged, with pigs being weighed and counted at the end of each feeding phase as pen groups. Feed delivery to each pen was recorded automatically by the feed system and feed remaining in the feeders at the end of each feeding phase was recorded to allow for calculation of feed consumption. Feed samples were taken by the research staff.

In discussion with veterinarians, pigs on treatment 6 were moved to the treatment 2 diet at the end of feeding phase 2 due to an excessive number of pens reaching the production and/or welfare end points, which were defined prior to the trial as the point at which a pig's growth rate was lagging more than 20% behind that of their counterparts on a standard diet or two animals required treatment or removal from the pen within a 7-day period of each other due to injury from social interactions.

Statistical Analysis

Statistical analysis was carried out using R Software version 4.2.2. (R Core Team, 2021). Models were constructed using the lme4 package (Bates et al., 2015). Pen was the experimental unit and nested in block in models. Where appropriate, model residuals were visually inspected to confirm approximation to normality and tested for heteroscedasticity using Levene's Test. When main effects (Lys, Trp:Lys, feeding phase) or interactions thereof were not significant at $P<0.05$, they were removed from the model. P-values below 0.05 were considered statistically significant and P-values between 0.05 and 0.1 were considered as tendencies.

Due to treatment 6 being stopped after feeding phase 2, the data has been analysed in two ways: 1) data from all 6 treatments for the first 2 feeding phases using the model as stated above, 2) data from treatments 1-5 for feeding phases 1-5 using "treatment" rather than Lys and Trp:Lys as factors. Significant effects were located through Tukey ($P<0.05$). All data takes into account pigs lost through morbidity and mortality.

Results

Morbidity and mortality

Over the 5 feeding phase, 110 pigs were removed from the trial due to morbidity (n= 66) and mortality (n= 44). Date, weight and suspected reason for death/removal were recorded where possible. The highest proportion of removals came from treatment 6 (33.64%) and the lowest proportion from treatment 1 (Table 1). An additional 11 pigs died during the observation period after being moved to the hospital pens. As pigs were not individually identifiable, it was not possible to determine which treatment these 11 pigs had been on before moving to the hospital pen.

Table 1. Number of pigs removed due to death and injury/illness from each dietary treatment.

Treatment	Death	Injury/Illness	Total removed	% of total removals
1 (Lys 100, Trp:Lys 0.210)	4	2	6	5.45
2 (Lys 100, Trp:Lys 0.185)	8	4	12	10.91
3 (Lys 100, Trp:Lys 0.160)	10	7	17	15.45
4 (Lys 80, Trp:Lys 0.210)	6	9	15	13.64
5 (Lys 80, Trp:Lys 0.185)	9	14	23	20.91
6 (Lys 80, Trp:Lys 0.160)	7	30	37	33.64
Totals	44	66	110	100.00

Pen and Block

Pen nested in block was found to influence ADFI ($p<0.001$) and ADG ($p=0.004$) and block affected FCR ($p=0.02$) in the analysis of the 6 treatments. In the analysis of 5 treatments, pen nested in block had no effect on ADFI, ADG or FCR.

Table 2 shows that over the first two feeding phases, where all 6 treatments were present, Lys, Trp:Lys, feeding phase and all possible interactions between these main effects significantly impacted ADFI. ADFI was lowest on Lys 80, Trp:Lys 0.160 compared to all other treatment combinations, which did not differ, and this effect increased from a difference between treatments of 10.8% for phase 1 to 12.5% for phase 2. Lys, Trp:Lys, Lys × Trp:Lys and feeding phase also affected ADG ($p=<0.001$). Trp:Lys 0.160 reduced ADG compared to Trp:Lys 0.210 and 0.185 only at Lys 80. In addition, significant interactions with feeding phase were observed for Trp:Lys only, indicating that this reduction in ADG on Trp:Lys 0.160 increased from 6.2% for phase 1 to 8.6% for phase 2. Finally, Lys, Trp:Lys and feeding phase also affected FCR, with the Lys × Trp:Lys interaction and Lys × feeding phase observed as trends. Across Lys, FCR increased as Lys:Trp reduced from 0.210 to 0.160 ($P<0.05$), which averaged +2.0% for Lys 100 and +3.3% for Lys 80, whilst the increased FCR of Lys 80 over Lys 100 was 8.2% for phase 1 and 4.3% for phase 2.

Table 3 shows that over the full five feeding phases, where only 5 treatments were present, feeding phase alone affected ADFI ($p<0.001$), ADFI was equal across treatments in all phases, increasing over time for all treatments. Feeding phase affected ADG ($p<0.001$),in feeding phase 1, ADG was higher in treatments 1, 2 and 3 than 4 and 5. Over time the difference between treatments reduced to the point that there was no difference at phase 4. Treatment x feeding phase affected FCR ($p<0.001$). In feeding phase 1, FCR for treatments 4 and 5 were higher than treatments 1, 2 and 3, with treatment 5 FCR being 7.8% higher than treatment 1 FCR. Across the feeding phases, the difference in FCR between treatments changed. At feeding phase 5, FCR for treatments 2 and 3 were higher than FCR for treatment 5, with FCR for treatment 5 being 7.2% lower than that for treatment 2.

Table 2. Mean, standard error and associated p-values for ADFI (Kg), ADG (Kg) and FCR for treatments 1- 6 over feeding phases 1 and 2. Difference in superscript denotes significant difference within columns. FP=Feeding phase.

			ADFI (Kg)			ADG (Kg)			FCR		
		Feeding Phase	1	2	1-2	1	2	1-2	1	2	1-2
			Mean								
Treatment	Lys	Trp:Lys									
1	100	0.21	1.20^{a}	2.27^{a}	1.74^{a}	0.67^{a}	1.06^{ab}	0.86^{a}	1.80^{c}	2.14^{c}	1.97^{d}
2		0.185	1.24^{a}	2.34^{a}	1.79^{a}	0.68^{a}	1.07^{a}	0.87^{a}	1.84^{c}	2.19^{bc}	2.02^{bcd}
3		0.16	1.23^{a}	2.29^{a}	1.76^{a}	0.68^{a}	1.04^{bc}	0.86^{a}	1.82^{c}	2.20^{ab}	2.01^{cd}
4	80	0.21	1.23^{a}	2.32^{a}	1.78^{a}	0.63^{b}	1.04^{c}	0.83^{a}	1.96^{ab}	2.24^{ab}	2.10^{ab}
5		0.185	1.21^{a}	2.27^{a}	1.74^{a}	0.62^{b}	1.01^{c}	0.82^{a}	1.94^{b}	2.25^{a}	2.09^{abc}
6		0.16	1.09^{b}	2.01^{b}	1.55^{b}	0.54^{c}	0.87^{d}	0.71^{b}	2.01^{a}	2.32^{a}	2.17^{a}
			Standard Error								
			0.031	0.054	0.05	0.016	0.023	0.027	0.025	0.073	0.077
			P-vales								
		Lys	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	<0.001
		Trp:Lys	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.005	0.176	0.025
		Lys x Trp:Lys	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.678	0.104
		FP			<0.001			<0.001			<0.001
		Lys x FP			0.008			0.507			0.063
		Trp:Lys x FP			<0.001			<0.001			0.689
		Lys x Trp:Lys x FP			0.002			0.219			0.901

Table 3 Mean, standard error and associated p-values for ADFI (Kg), ADG (Kg) and FCR for treatments 1- 5 over feeding phases 1-5. Difference in superscript denotes significant difference within columns.

				ADFI						ADG						FCR					
			Feeding Phase	1	2	3	4	5	1-5	1	2	3	4	5	1-5	1	2	3	4	5	1-5
				Mean																	
Treatment	Lys	Trp:Lys																			
1	100	0.21		1.2	2.27	2.92	3.41	3.46	2.65	0.67^{a}	1.06^{ab}	1.04^{ab}	0.99	0.96	0.94	1.80^{b}	2.14^{c}	2.80^{b}	3.5	3.6^{ab}	2.77
2		0.185		1.24	2.34	2.99	3.53	3.54	2.73	0.68^{a}	1.07^{a}	1.05^{a}	1.03	0.93	0.95	1.84^{b}	2.19^{bc}	2.85^{ab}	3.45	3.87^{a}	2.84
3		0.16		1.23	2.29	2.89	3.44	3.43	2.66	0.68^{a}	1.04^{abc}	1.03^{ab}	1	0.9	0.93	1.82^{b}	2.20^{ab}	2.82^{b}	3.44	3.82^{a}	2.82
4	80	0.21		1.23	2.32	2.87	3.44	3.45	2.66	0.63^{b}	1.04^{bc}	1.00^{b}	1.04	0.95	0.93	1.96^{a}	2.24^{ab}	2.87^{a}	3.36	3.64^{ab}	2.81
5		0.185		1.21	2.27	2.9	3.5	3.51	2.68	0.62^{b}	1.01^{c}	1.00^{b}	1.04	0.98	0.93	1.94^{a}	2.25^{a}	2.91^{a}	3.4	3.59^{b}	2.82
				Standard Error																	
				0.03	0.05	0.12	0.09	0.11	0.06	0.02	0.02	0.04	0.06	0.05	0.03	0.02	0.03	0.47	0.12	0.18	0.04
				P-values																	
			Treatment	<0.001	<0.001	0.68	0.34	0.59	0.52	<0.001	<0.001	0.32	0.6	0.21	0.75	<0.001	0.03	0.01	0.052	0.1	0.35
			Feeding phase						<0.001						<0.001						<0.001
			Treatment x Feeding phase						0.95						0.26						<0.001

Discussion

Pen and block

Pen nested in block was found to have an effect on ADFI, ADG and FCR in the analysis if the full 6 treatments over 2 feeding phases. The effect of pen was expected as pens were only tested once, with one treatment. Pens were also grouped by weight and contained different numbers of pigs from the start of the trial, with numbers decreasing in some pens over the trial as pigs were removed. Research shows that body weight affects feed intake (Li and Patience, 2017) as does space allowance, with decreased space allowance resulting in poorer ADG driven by a reduction in ADFI (Thomas et al., 2017). The effect of pen and block may also be due to the position of the pen/block in the shed. Blocks in barn 2 (9-16) and blocks at the far end of both barns (7,8,15,16) were more exposed to the external environment due to the site design and local geography (tree cover etc). Production metrics could therefore have been affected by external temperature and consequent changes to internal atmospheric conditions including temperature (Ross et al., 2015) and gas levels (Ni et al., 2000). These considerations support the view that it is important to use block where possible in order to not only account for such influencing factors but also to ensure increased efficiency of data analysis, given that otherwise within-treatment variation would be considerably larger resulting in a more inflated error term than now used in the models.

6 treatments, 2 phases

Results from the analysis of the 6 treatments over the first two feeding phases supports our hypothesis that pigs on diets low in Lys and with a low Trp:Lys will have reduced performance. This is also supported by previous research (Salyer et al., 2013; Gonçalves et al., 2018; Liu et al., 2019; Clizer et al., 2023). Treatment 6 (Lys 80, Trp:Lys 0.160) pigs showed decreased ADFI, where I may have expected to see increased feed intake to attempt to compensate for the dietary shortfalls. The decreased ADFI may be due to the lack of dietary Trp and consequently serotonin, supressing feed intake. Previous studies have shown that dietary Trp deficiency induces depression of the appetite leading to reduced growth performance (Sève, 1999). It may also be that these pigs were immune suppressed due to low levels of Lys (Li et al., 2007), which caused them to be more susceptible to sub-clinical disease, which could lead to appetite suppression (Forbes, 2007). Treatment 6 (Lys 80, Trp:Lys 0.160) pigs also had a reduced ADG, which is conducive with Lys being needed for lean muscle deposition (Main et al., 2008). However, I did not see a significant negative impact of treatment 4 (Lys 80, Trp:Lys 0.210) and 5 (Lys 80, Trp:Lys 0.185) on ADG, which may imply that it is a combination of Lys level and Trp:Lys that has the greater effect on ADG, not Lys nor Trp:Lys levels in isolation. This is supported by previous research performed in both commercial (Tolosa et al., 2022) and smaller scale studies (Salyer et al., 2013; Gonçalves et al., 2018; Liu et al., 2019). As expected FCR was also affected by the treatment x feeding phase interaction. The lack of significant effect on FCR for treatment 2 (Lys 100, Trp:Lys 0.180), and 3 (Lys 100, Trp:Lys 0.165) in feeding phase 2 further suggest the aforementioned notion that it is the combination of Lys and Trp:Lys that has the greatest effect on production.

5 treatments, 5 phases

ADFI and ADG increased over time, as I would expect and as is supported by previous research (Hyun et al., 1997; Andretta et al., 2016). However, I found no effect of treatment on ADFI or ADG over the total 5 feeding phases combined, suggesting that the treatment effects on performance were largely dominated by treatment 6. I had hypothesised that pigs on a nutritionally deficient diet would increase their feed intake to compensate for deficiencies. There are two potential reasons why I didn't see this increase in feed intake; 1) the diets were not significantly deficient in Lys and Trp:Lys or 2) the pigs reached a bulk constraint and were physically unable to consume more feed.

Previous studies have identified the optimum Trp:Lys for corn–soybean meal diets to be between 0.17 (Susenbeth, 2006) to 0.203 (Xie et al., 2014) and for diets containing 30% DDGS, a Trp:Lys of 0.16 – 0.17 was found to be adequate in finishing pig diets (Kendall et al., 2007; Salyer et al., 2013). The Lys and Trp:Lys levels of treatments 1-5 were within commercially used and recommended ranges, with Trp:Lys levels being at the higher end and above recommendation. It is therefore likely that the pigs on treatments 1-5 were not subjected to significant animo acid deficiencies. This further supports the suggestion that it is the combination of low Lys and low Trp:Lys that has the most significant effect on production. In human studies, excess Trp can be used to supress appetite (Yabut et al., 2019), so it may be the case that feeding Trp:Lys above recommendation could actually reduce feed intake in pigs, even if that results in reduced Lys intake.

Despite not finding an effect of treatment or treatment x feeding phase interaction on ADFI and ADG, I found an effect of treatment x feeding phase interaction on FCR. I would expect to find an effect of feeding phase, as FCR increases over time as pigs become less feed efficient as their growth slows (Losinger, 1998; Lewis and Southern, 2000). The effect of the treatment x feeding phase interaction seen in later feeding phases may be due to pigs being at different stages of the growth curves for the different treatments. The FCR of treatment 1 (Lys 100, Trp:Lys 0.210) appeared to plateau around feeding phase 5 (Lys 80, Trp:Lys 0.180), while treatment 2 (Lys 100, Trp:Lys 0.180) and 3 (Lys 100, Trp:Lys 0.165) were still steeply rising and treatments 4 (Lys 80, Trp:Lys 0.210) and 5 (Lys 80, Trp:Lys 0.180) were also still rising but with a shallower incline (Figure 1).

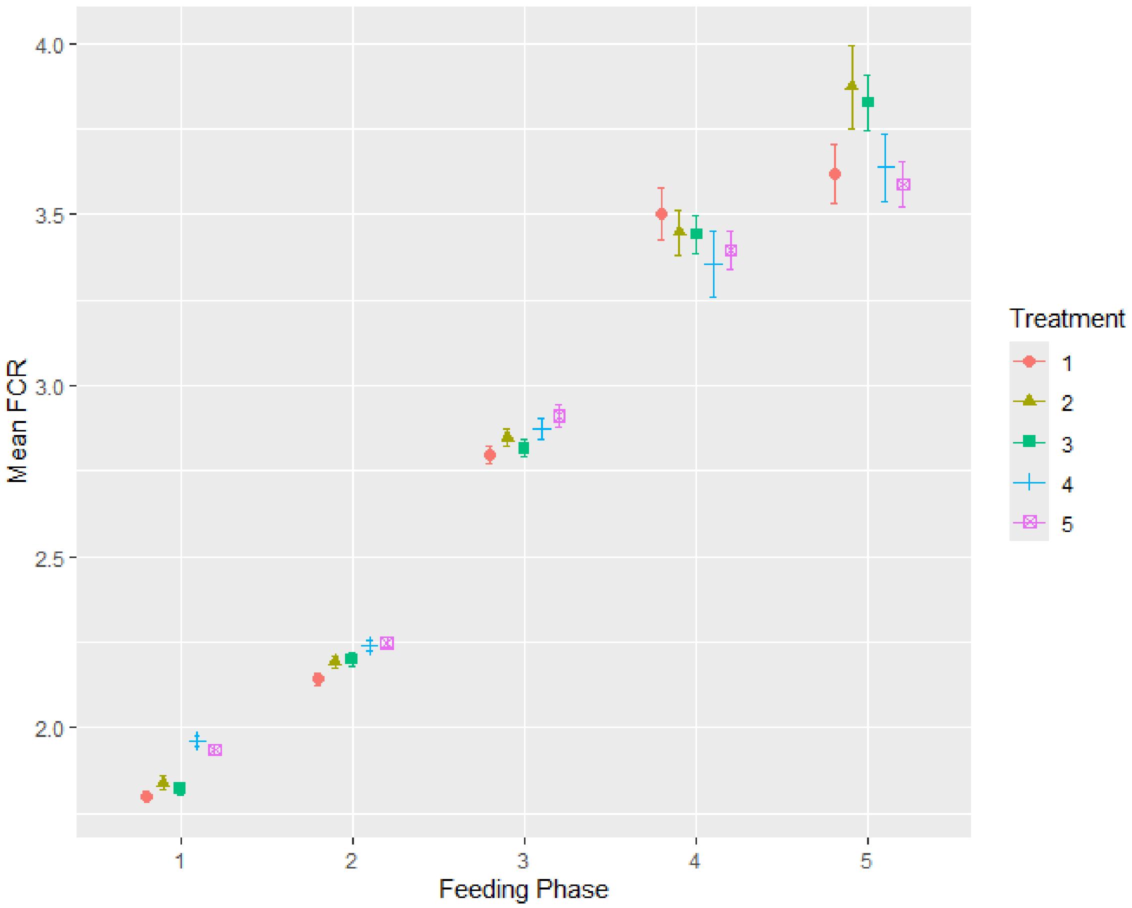


Figure 1. Mean FCR for pigs on treatments 1-5 over feeding phases 1-5.

Conclusion

Results suggest that diets low in both Lys and Trp:Lys negatively impact performance, and it is this combination that has the greatest effect on performance. To my knowledge, this is only the second study to explore the effects of low Lys and low Trp:Lys diets in a commercial setting. Extensive research has been performed to determine the optimum Lys and Trp levels of grow-finish diets but further research should be carried out exploring the interactive effect of low Lys and low Trp:Lys diets. Wider consideration should be given not only to the effects of diets low in both Lys and Trp:Lys on performance but also health and welfare.

References

Andretta, I., Pomar, C., Kipper, M., Hauschild, L., Rivest, J., 2016. Feeding behavior of growing–finishing pigs reared under precision feeding strategies1. Journal of Animal Science 94, 3042–3050. https://doi.org/10.2527/jas.2016-0392

Avelar, E., Jha, R., Beltranena, E., Cervantes, M., Morales, A., Zijlstra, R.T., 2010. The effect of feeding wheat distillers dried grain with solubles on growth performance and nutrient digestibility in weaned pigs. Anim. Feed Sci. Technol. 160, 73–77. https://doi.org/10.1016/j.anifeedsci.2010.06.009

Baranyiová, E., 1991. Effect of Serotonin on Food Intake by Piglets During the Early Postnatal Period. Acta Vet. Brno 60, 127–136. https://doi.org/10.2754/avb199160020127

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 67, 1–48. https://doi.org/10.18637/jss.v067.i01

Clizer, D.A., Tostenson, B.J., Frederick, B., Cline, P.M., Samuel, R.S., 2023. Performance response of increasing the standardized ileal digestible tryptophan:lysine ratio in diets containing 40% dried distiller grains with solubles. J Anim Sci 101, skad264. https://doi.org/10.1093/jas/skad264

Fernstrom, J.D., 1994. Dietary amino acids and brain function. J Am Diet Assoc 94, 71–77. https://doi.org/10.1016/0002-8223(94)92045-1

Forbes, J.M., 2007. Voluntary Food Intake and Diet Selection in Farm Animals. CABI.

Goncalves, M., Orlando, U., Cast, W., Culbertson, M., 2017. Standardized ileal digestible lysine requirements for finishing PIC pigs under commercial conditions: A meta-analysis. J. Anim. Sci. 95, 131–132. https://doi.org/10.2527/asasmw.2017.273

Gonçalves, M.A.D., Tokach, M.D., Bello, N.M., Touchette, K.J., Goodband, R.D., DeRouchey, J.M., Woodworth, J.C., Dritz, S.S., 2018. Dose–response evaluation of the standardized ileal digestible tryptophan : lysine ratio to maximize growth performance of growing-finishing gilts under commercial conditions. Animal 12, 1380–1387. https://doi.org/10.1017/S1751731117002968

Henry, M., Shoveller, A.K., O'Sullivan, T.L., Niel, L., Friendship, R., 2022. Effect of Varying Levels of Dietary Tryptophan on Aggression and Abnormal Behavior in Growing Pigs. Frontiers in Veterinary Science 9.

Hyun, Y., Ellis, M., McKeith, F.K., Wilson, E.R., 1997. Feed intake pattern of group-housed growing-finishing pigs monitored using a computerized feed intake recording system. Journal of Animal Science 75, 1443–1451. https://doi.org/10.2527/1997.7561443x

Kendall, D.C., Gaines, A.M., Kerr, B.J., Allee, G.L., 2007. True ileal digestible tryptophan to lysine ratios in ninety- to one hundred twenty-five-kilogram barrows1. Journal of Animal Science 85, 3004–3012. https://doi.org/10.2527/jas.2007-0013

Lewis, A.J., Southern, L.L., 2000. Swine Nutrition. CRC Press.

Li, P., Yin, Y.-L., Li, D., Kim, S.W., Wu, G., 2007. Amino acids and immune function. British Journal of Nutrition 98, 237–252. https://doi.org/10.1017/S000711450769936X

Li, Q., Patience, J.F., 2017. Factors involved in the regulation of feed and energy intake of pigs. Anim. Feed Sci. Technol. 233, 22–33. https://doi.org/10.1016/j.anifeedsci.2016.01.001

Liu, J.B., Yan, H.L., Cao, S.C., Liu, J., Li, Z.X., Zhang, H.F., 2019. The response of performance in grower and finisher pigs to diets formulated to different tryptophan to lysine ratios. Livest. Sci. 222, 25–30. https://doi.org/10.1016/j.livsci.2019.01.016

Losinger, W.C., 1998. Feed-conversion ratio of finisher pigs in the USA. Preventive Veterinary Medicine 36, 287–305. https://doi.org/10.1016/S0167-5877(98)00094-4

Main, R.G., Dritz, S.S., Tokach, M.D., Goodband, R.D., Nelssen, J.L., DeRouchey, J.M., 2008. Effects of Feeding Growing Pigs Less or More Than Their Estimated Lysine Requirement in Early and Late Finishing on Overall Performance. Professional Animal Scientist 24, 76–87. https://doi.org/10.15232/S1080-7446(15)30813-5

Meunier-Salaün, M.C., Monnier, M., Colléaux, Y., Sève, B., Henry, Y., 1991. Impact of dietary tryptophan and behavioral type on behavior, plasma cortisol, and brain metabolites of young pigs. Journal of animal science 69, 3689–3698. https://doi.org/10.2527/1991.6993689X

Ni, J.-Q., Heber, A.J., Diehl, C.A., Lim, T.T., 2000. Ammonia, Hydrogen Sulphide and Carbon Dioxide Release from Pig Manure in Under-#oor Deep Pits. Journal of Agricultural Engineering Research 77, 53–66. https://doi.org/10.1006/jaer.2000.0561

Noblet, J., Cozannet, P., Skiba, F., 2012. Nutritional value and utilization of wheat dried distillers grain with solubles in pigs and poultry, in: Biofuel Co-Products as Livestock Feed. FAO, p. 533 p.

NRC, 2012. Nutrient requirements of swine., 11th rev. ed. ed. Acad. Press., Washington, DC: Natl.

PIC, 2024. PIC®NUTRITION ANDFEEDING GUIDELINES.

Puppe, B., Tuchscherer, M., Tuchscherer, A., 1997. The effect of housing conditions and social environment immediately after weaning on the agonistic behaviour, neutrophil/lymphocyte ratio, and plasma glucose level in pigs. Livestock Production Science 48. https://doi.org/10.1016/S0301-6226(97)00006-7

R Core Team, 2021. R: A language and environment for statistical computing.

Ross, J.W., Hale, B.J., Gabler, N.K., Rhoads, R.P., Keating, A.F., Baumgard, L.H., 2015. Physiological consequences of heat stress in pigs. Anim. Prod. Sci. 55, 1381–1390. https://doi.org/10.1071/AN15267

Salyer, J.A., Tokach, M.D., DeRouchey, J.M., Dritz, S.S., Goodband, R.D., Nelssen, J.L., 2013. Effects of standardized ileal digestible tryptophan:lysine in diets containing 30% dried distillers grains with solubles on finishing pig performance and carcass traits. J. Anim. Sci. 91, 3244–3252. https://doi.org/10.2527/jas.2012-5502

Sève, B., 1999. Physiological Roles of Tryptophan in Pig Nutrition, in: Huether, G., Kochen, W., Simat, T.J., Steinhart, H. (Eds.), Tryptophan, Serotonin, and Melatonin: Basic Aspects and Applications, Advances in Experimental Medicine and Biology. Springer US, Boston, MA, pp. 729–741. https://doi.org/10.1007/978-1-4615-4709-9_95

Thacker, P.A., 2006. Nutrient digestibility, performance and carcass traits of growing-finishing pigs fed diets containing dried wheat distiller's grains with solubles. Canadian Journal of Animal Science 86, 527–529. https://doi.org/10.4141/A06-025

Thomas, L.L., Goodband, R.D., Woodworth, J.C., Tokach, M.D., DeRouchey, J.M., Dritz, S.S., 2017. Effects of space allocation on finishing pig growth performance and carcass characteristics. Transl. Anim. Sci. 1, 351–357. https://doi.org/10.2527/tas2017.0042

Tolosa, A.F., Tokach, M.D., Goodband, R.D., Woodworth, J.C., DeRouchey, J.M., Gebhardt, J.T., Wolfe, M.L., 2022. Effects of reducing the standardized ileal digestible lysine and tryptophan to lysine ratio to slow growth of finishing pigs. Translational Animal Science 6, txac057. https://doi.org/10.1093/tas/txac057

Wang, L.F., Beltranena, E., Zijlstra, R.T., 2016. Diet nutrient digestibility and growth performance of weaned pigs fed wheat dried distillers grains with solubles (DDGS). Anim. Feed Sci. Technol. 218, 26–32. https://doi.org/10.1016/j.anifeedsci.2016.05.005

Yabut, J.M., Crane, J.D., Green, A.E., Keating, D.J., Khan, W.I., Steinberg, G.R., 2019. Emerging Roles for Serotonin in Regulating Metabolism: New Implications for an Ancient Molecule. Endocrine Reviews 40, 1092–1107. https://doi.org/10.1210/er.2018-00283

Chapter 5: Exploring the effects of dietary protein on tail biting and the expression of positive social behaviours in pigs

Chapter 5 contains the manuscript submitted for peer review for publication in PLOS ONE.

Eleanor Hewett was the first named author and the co-authors were Craig Lewis, Jos Houdijk, Andrea Doeschl-Wilson, and Simon Turner. The study was designed collaboratively by Hewett, Turner and Houdijk. Data were collected by Hewett and analysis was performed by Hewett with guidance from Turner. Hewett prepared the manuscript which was reviewed by all co-authors.

At the time of resubmission of this thesis with corrections, this paper had been rejected from PLOS ONE and will be improved and resubmitted.

Introduction

Tail biting continues to be a significant issue in intensive pig production. Although banned in the EU, tail docking continues as a common preventative measure. However, there is increasing pressure to reduce routine tail docking and encourage longer tails. If producers are going to attempt to reduce their reliance on tail docking, they need an alternative, cost effective and commercially relevant solution. One such solution could be the manipulation of feed ingredients.

Study 3 was designed as a follow up to study 2 where a low lysine level, particularly in combination with low tryptophan levels, increased the incidences of ear biting in pigs. Tail biting was rare in study 2, most likely because the tails were docked shorter than allowable in the U.K. and E.U. Hence, study 3 tested whether commercially available diets of differing lysine levels would affect the social behaviour of pigs housed under UK conditions. I also hoped that a smaller sample size would allow for more detailed behavioural observations, looking at a wider range of behaviours for each pen of animals to allow quantification of the effect of lysine on play and other positive social behaviours as these behaviours proved to be rare during the sampling window achievable in the USA.

Chapter 5 contains the published manuscript of the following paper:

A note on author contribution: Eleanor Hewett and Simon Turner designed the trial with nutritional consultations from Jos Houdijk. Eleanor Hewett collected the data and performed the analysis with guidance from Simon Turner. Eleanor Hewett drafted the manuscript which was reviewed by Simon Turner, Jos Houdijk and Craig Lewis.

Submitted Journal Manuscript

Exploring the effects of dietary protein on tail biting and the expression of positive social behaviours in pigs

Eleanor Hewett, Animal Behaviour and Welfare, Scotland's Rural College (SRUC), Edinburgh EH9 3JG

Jos Houdijk, Monogastric Science Research Centre, Scotland's Rural College (SRUC), Edinburgh, EH9 3JG

Craig Lewis, PIC Product Development, C/Pau Vila, 22 2o piso, 08174, Sant Cugat del Valles, Barcelona, Spain

Simon Turner, Animal Behaviour and Welfare, Scotland's Rural College (SRUC), Edinburgh, EH9 3JG

Abstract

Harmful social behaviours compromise pig welfare and productivity and are difficult to improve without significant economic cost or labour input. For example, despite being banned by the EU, tail docking continues to be used as a routine method to reduce the incidence of tail biting and the negative impacts this has on both the pigs and their productivity. As tail docking is not completely effective and has a significant negative impact on the welfare of pigs, there is continued interest to find a cost effective and commercially applicable solution to reduce the incidence of tail biting and thus eliminate the need for tail docking. One potential solution to reduce expression of tail biting and other harmful social behaviours is the manipulation of dietary components, in particular lysine provision, as previous studies have shown that insufficient lysine increases the expression of negative social behaviors. This study aims to explore this effect by manipulating lysine levels within the range currently used in commercial UK pig diets. A total of 723 mixed sex, Large White x Landrace x Danish Duroc pigs of mean starting weight 21.41 ± 5.75 kg were used in the study. Pigs were randomly allocated across two treatments, high and low commercially available crude protein diets (HL and LL), where lysine is the first limiting nutrient. Pigs were kept on trial for an average of 26.62 days. During the first 21 days, behaviour observations and lesion scores were taken. Production metrics were collected over the full duration of the trial. We found no effect of diet on behaviour, lesions or production metrics, which conflicts with earlier studies. The lack of effect here may result from modest differences in crude protein / lysine level in the dietary treatments compared to previous studies or differences in housing and husbandry practices. Further research is required to explore the use of nutrition in general, and crude protein / lysine in particular, as a method to control tail biting across a diverse range of farming conditions and systems.

Key words

Pigs, lysine, tail biting, play, social behaviour

Introduction

Harmful social behaviours, such as fighting, tail biting and ear biting, reduce pig welfare and productivity. Tail biting, in particular, has been the focus of a considerable research effort, but persists as a significant problem in the industry globally. Despite being prohibited under UK and EU legislation (Animal Welfare Act 2006; Council Directive 2008/120/EC)), routine tail docking continues to be widely used across pig farming systems in the EU and elsewhere (De Briyne et al., 2018). Tail docking is performed to reduce the risk of tail biting, however tail docking does not completely prevent tail biting nor address the underlying cause. Tail docking has a significant negative impact on the welfare of pigs (for a review of the effects of tail docking, see (Nannoni et al., 2014)). As pressure to improve welfare standards increases, producers are looking for commercially applicable and cost-effective solutions to combat the underlying risk factors of tail biting, consequently reducing the need for routine tail docking. Despite extensive research into these risk factors and potential solutions (for review see Henry et al., 2021), the industry still lacks a widely adopted tail biting reduction strategy. Dietary manipulation could present a convenient option for reducing tail biting and other harmful social behaviours, and could be done at little extra cost or effort to the producer.

As recently demonstrated experimentally, reducing lysine provision can increase expression of negative social behaviours in commercially housed pigs, including tail biting behaviour and ear lesions (Hewett et al., 2025). Lysine is the first limiting amino acid in the majority of cereal-based pig diets. Lysine is essential for growth and lean muscle deposition (Main et al., 2008) and has also been suggested to have an effect on immune response in pigs and other monogastric species (Kornegay et al., 1993; Chen et al., 2003). However, little is known about the effect of lysine on pig behaviour and the potential mechanisms by which it may act. One study found that diets fortified with lysine and arginine reduced plasma cortisol levels and reduced stress responses to transportation in pigs (Srinongkote et al., 2003). Another study found that providing supplementary indispensable amino acids was more effective in countering the negative effects of a low protein diet on tail biting in pigs than the provision of extra environmental enrichment (Minussi et al., 2023). Studies in rats showed dietary lysine deficiency increases stress-induced anxiety by enhancing serotonin availability in the amygdala, the part of the brain involved in the fear response (Smriga et al., 2002). Additional rat studies have shown the administration of lysine to have an anxiolytic effect and an increase in brain serotonin levels (Jalal et al., 2022). Conversely, it has also been found that when the tail of a rat was experimentally irritated, provision of lysine was found to enhance pain sensitivity and affective defence behaviours (Severyanova et al., 2019).

Beyond simply reducing negative social behaviours in an effort to improve welfare of pigs, there is increased interest in promoting ‘positive welfare’ (Lawrence et al., 2023) which may be achieved through increasing the opportunity for pigs to exhibit positive social behaviours. Of the various positive social behaviours identified in pigs (e.g. play (Newberry et al., 1988), allogrooming (Camerlink et al., 2022), social nosing (Camerlink et al., 2012)), play is the most studied. However, there is limited research into the role of the diet in promoting positive welfare or positive social behaviours (effect of dietary fibre on sow behaviour (Stewart et al., 2010; Odakura et al., 2023), dietary supplementation with tryptophan and its effect on affective state (Stracke et al., 2017), effect of dietary energy on play behaviour (Hewett et al. in publication)).

The aim of this study was to empirically test the sensitivity of social behaviour to variation in dietary lysine provision, using commercially relevant crude protein levels found in UK commercial pig diets, where lysine is the first limiting nutrient. Furthermore, we sought to explore the effect of variation in crude protein levels on the expression of positive forms of social behaviour as well as harmful behaviour. The intention was not to create an artificially large contrast in lysine levels, but to compare the effect on behaviour of lysine levels within the range commercially available to farmers. We hypothesise that diets at the upper end of the commercial range of crude protein / lysine provision increase expression of positive social behaviours and decrease expression of negative social behaviours and lesions from fighting, tail and ear biting.

Methods

Ethical Review

The trial was reviewed and approved by the Scotland's Rural College (SRUC) animal ethics committee (research project number PIG RP 4-2022, experiment number AEX 2024-014 PIG). All routine animal management procedures were performed by trained staff and health issues were treated as required. All stages of the experiment were conducted in liaison with the SRUC veterinary surgeon.

Animals and housing

A total of 723 mixed sex (370 male, 353 female), Large White x Landrace x Danish Duroc pigs of mean starting weight 21.41 ± 5.75 kg, age 8 weeks were used in the study. Pigs were housed under UK commercial conditions, in the grower shed at Oatridge Farm, SRUC. Two diet treatments were used (see below) and fed in a building that comprised of four rooms, each containing two pens separated by a central passageway. One of the pens in each room was fed the high protein / lysine diet and the other fed the low protein / lysine diet. For each fresh batch of pigs entering the building, the diet was switched, such that each pen housed two batches of pigs on diet A and two on diet B to balance for effects on behaviour caused by the location of the pen in the room. However, this design was unbalanced and incomplete due to requirements for use of the experimental pens to meet commercial needs. In total, 13 pens per diet treatment were completed.

Pens were 3.20m x 3.70m, fully slatted, containing three nipple drinkers, two feed troughs with a total of 8 feeding spaces and a minimum of three different enrichment objects. Pigs were stocked at a mean average of 27.8 pigs per pen (minimum 22, maximum 33 pigs per pen). Pigs were processed after birth in accordance with standard farm practice. Their teeth were clipped and tails were docked to 2/3rds of original length with low birthweight pigs left with intact tails at the discretion of farm staff. Pigs were not castrated. Pigs were weaned at 28 days and mixed into flat deck nursery pens. After four weeks, pigs were moved from the flat decks to the grower shed without any further mixing. Sick and injured pigs were removed from trial pens to the hospital shed for treatment. Pens showing signs of biting outbreaks were treated at the discretion of farm staff, with the application of tar to affected areas (tails/ears). Additional enrichment was also provided in the effort to slow/stop biting outbreaks. Pigs with severe lesions were removed from the trial and treated in designated hospital pens. Those identified as biters were also removed by farm staff and placed in hospital pens with larger pigs. Pigs were moved from trial pens in the grower shed to the finisher shed according to standard farm practice at an average weight of 39.84kg ±13.78kg, at approximately 12 weeks of age.

Experimental treatments

Pigs were randomly allocated across two treatments; high and low commercially available lysine diets (HL and LL) fed as a dry pellet ad libitum. Diets were provided by East Coast Viners, formulated in discussion with Premier Nutrition and had a basis of wheat, barley and soya (Table 1). The pigs stayed on one diet for the duration of the trial, with no feeding phase changes. Pigs were on the trial diet for an average of 26.62 days (maximum 36 days, minimum 22 days).

Table 1. Ingredients and chemical analysis of the experimental diets

Ingredient Name, %	**Treatment HL**	**Treatment LL**
Wheat	32	32
Barley	32.9	38.7
Wheat feed	8	8
Soya bean meal	19.1	14
Vegetable oil	1.1	1.0
Limestone	0.67	0.695
Salt	0.47	0.47
Lysine HCl	0.47	0.42
Methionine	0.165	0.12
Threonine	0.2	0.165
L-Valine	0.055	0.03
Biscuit meal	4	3.5
Monodical phosphate	0.37	0.4
Trace mineral and vitamin mix	0.5	0.5
Nutrient Composition (g/kg, unless otherwise indicated)		
Dry Matter	868	869
Ash	50	43
Crude Protein (CP, N x 6.25)	166	148
Oil A (Ether Extract)	32.1	30.9
Oil B (Total Oil)	37.5	36.1
Neutral Detergent Fibre (NDF)	124	119
Digestible Energy* (MJ/kg)	13.7	13.8
Lysine (calculated)	5.6	4.8
Digestible Lysine (calculated)	5.0	4.3

*Calculated as 17.47 + (0.0079×CP) + (0.0158×Oil B) - (0.0331×Ash) - (0.0140×NDF) on a dry matter basis (McDonald et al., 2011)

Behavioural observations
Each pen was observed for a total of 80 minutes per week, 40 minutes per day (2 x 10-minute morning observation periods, 2 x 10 minute afternoon observation periods), on Wednesdays and Fridays, over a period of 3 weeks (21 days). Observations were performed by a single observer between 08:30 and 16:30, from August to December. Prior to the commencement of the observation, the observer entered the pen to disturb the pigs. After all pigs had been roused to standing, the observer exited the pen. On exiting the pen, the observer waited 2 min for the pigs to settle down and resume their undisturbed behaviour. Behaviour of all pigs was recorded live using an ethogram (Table 2), using a combination of continuous observation (for short lived behaviours) and scan sampling (for posture). For the continuous observations the frequency but not duration of behaviours was recorded. At time 0 and at intervals of 150 seconds thereafter, a tally was taken by scan sampling of the number of animals lying, sitting, standing, eating or drinking. It was not possible to identify individual animals, and therefore behaviours were analysed at the pen level.

Table 2. Ethogram used to record behaviours of pigs

Behaviour	Description	Reference
Continuous Sampling		
Tail nosing	Touching the tail of a pen mate with the snout without taking it into the mouth	(Camerlink and Turner, 2013)
Tail biting	Taking the tail of a pen mate into the mouth or nibbling, sucking or chewing on the tail	Adapted from (Camerlink and Turner, 2013)
Ear nosing	Touching the ear of a pen mate with the snout without taking it into the mouth	(Camerlink and Turner, 2013)
Ear biting	Taking the ear of a pen mate into the mouth or nibbling, sucking or chewing on the ear	Adapted from (Camerlink and Turner, 2013)
Belly nosing	Repetitive up and down snout movement on the belly of a pen mate	(Camerlink and Turner, 2013)
Oral manipulation of conspecific	Chewing part of the body of a pen mate excluding the tail or ears	(Camerlink and Turner, 2013)
Aggression	Head knocking, fighting, biting, ramming	Adapted from Camerlink and Turner, 2013
Mounting	Standing on hind legs while having front legs on another pig's body	(Camerlink and Turner, 2013)
Social nosing	Non-agonistic nose touching, including allo-grooming and gentle nose touching	Adapted from (Camerlink et al., 2021)

Play	Scampering, running, pivoting, head tossing, flopping, hopping, nudging, pushing, climbing or non-harmful fighting. Behaviours performed alone (solo play) or with at least one other pig (social play). All pigs involved in social play showed at least one of these play markers.	Adapted from (Brown et al., 2018)
Scan Sampling		
Lying	Resting, with eyes closed or opened, with the body in full contact with the floor	Defined for this study
Sitting	Sitting with black legs and rump in contact with the floor	Defined for this study
Standing	Standing on all four feet, sniffing, touching or rooting the surroundings (e.g. floor or wall)	Defined for this study
Eating/drinking	Engaged in eating or drinking, either in a standing, sitting or lying position	Defined for this study

Lesion Scores
Severity of tail and ear lesions and frequency of body lesion of each pig were scored twice weekly, for 3 weeks (Table 3).

Table 3. Score system used for scoring ear, tail and body lesions (score systems adapted from Camerlink et al., 2016; Hewett et al., 2025)

	Score
Damage - tail and ear	
No evidence of damage to the skin	0
Superficial damage to skin, redness but skin not broken	1
Mild swelling, redness and evidence of bite marks, broken skin	2
Mild to severe swelling, redness, bite marks, broken skin, scabbing with some fresh blood	3
Severe swelling, redness, open wound, fresh blood, necrosis of tissue	4
More than 50% of the pig's ear is missing/ whole tail is missing and stump has become an open wound, fresh blood and necrotic tissue	5
Number of lesions - body	
0	0
1-10	1
11-20	2
21-30	3
31-40	4
41-50	5

Production measures
Pens were weighed a maximum of three days prior to commencing the dietary treatment and again the day of ending the treatment. Feed delivery and end of trial feed refusal were recorded manually to allow for calculation of feed consumption and feed conversion ratio. Feed samples were taken by research staff.

Statistical analysis
Statistical analyses were carried out using R Software version 4.4.2 (R Core Team, 2021) and RStudio 2024.12.1+563 "Kousa Dogwood" Release for windows. Models were constructed using the lme4 package (Bates et al., 2015). Model residuals were visually inspected to confirm approximation to normality and tested for heteroscedasticity using Levene's Test. P-values below 0.05 were considered

statistically significant and P-values between 0.05 and 0.1 were considered as tendencies.

Behaviours

Histograms of behaviour frequencies were plotted and inspected to allow for model selection and elimination of infrequent behaviours. Belly nosing and oral manipulation of a conspecific were excluded from analysis due to infrequent occurrence.

For the remaining behaviours, those with the lowest frequency, tail biting, ear biting, mounting and play were organised into binary data sets; 1 = presence and 0 = absence of behaviour. Medium frequency behaviours, (tail nosing, ear nosing), were organised into grouped data sets; 0=0 counts of the behaviour, 1=1 count of the behaviour, 2=2 counts of the behaviour and 3=3 or more counts of the behaviour. The remaining behaviours were analysed in their raw form, except for aggression which was log transformed. Binary and grouped behaviour data were analysed using GLMER and raw and logged data were analysed using LMER.

A separate model was constructed for each behaviour or lesion score as a response variable. Diet and score day were included as fixed effects and all models initially included the interaction between diet and score day which was removed if not significant. Pen nested in batch was included as a random effect. For tail nosing and tail biting, initial tail lesion score recorded on score day 1 (approximately 3 days after pigs entered grower pens) was included as a random effect in the model. Similarly, for ear nosing and ear biting, initial ear lesion score was included as a random effect. Pen was the statistical unit and its inclusion in the models accounts for the combined effect of variation in number of pigs in any given pen, the difference in average starting weight of the pen and the difference in number of males and females in any given pen. Every effort was made to balance starting weight, number of pigs and sex mix across treatments but due to the constraints of conducting this trial on a commercial farm, this was not always possible.

Holm-Bonferroni corrections were performed on all P-values (diet and score day) to obtain adjusted P-value thresholds due to the large number of models produced. Adjusted P-value thresholds were used to identify significant results.

Skin Lesions

Pen was the statistical unit, with the total lesion score for each day divided by the number of pigs in the pen during that day. Diet and score day were included as fixed effects and all models initially included the interaction between diet and score day which was removed if not significant.

Pen nested in batch was included as a random effect. For tail lesions, initial tail lesion score was included as a random effect. For ear lesions, initial ear lesion score was included as a random effect. Tail and body lesion data were log transformed. All lesion scores were modelled as LMERs.

Production

Production data were collected and analysed at the pen level, averaged over the whole trial, with the pen being the statistical unit, averaged to give ADG, ADFI and FCR per pig day. Data were modelled using an LMER, diet was included as a fixed effect and pen was included as a random effect.

Results

<u>Behaviours</u>

We found no effect of diet or score day on any of the behaviours tested (tail nosing, tail biting, ear nosing, ear biting, aggression, mounting, social nosing, play, lying, sitting, standing, eating). Score day tended towards having a significant effect on tail biting (P=0.054 after Holm Bonferroni correction).

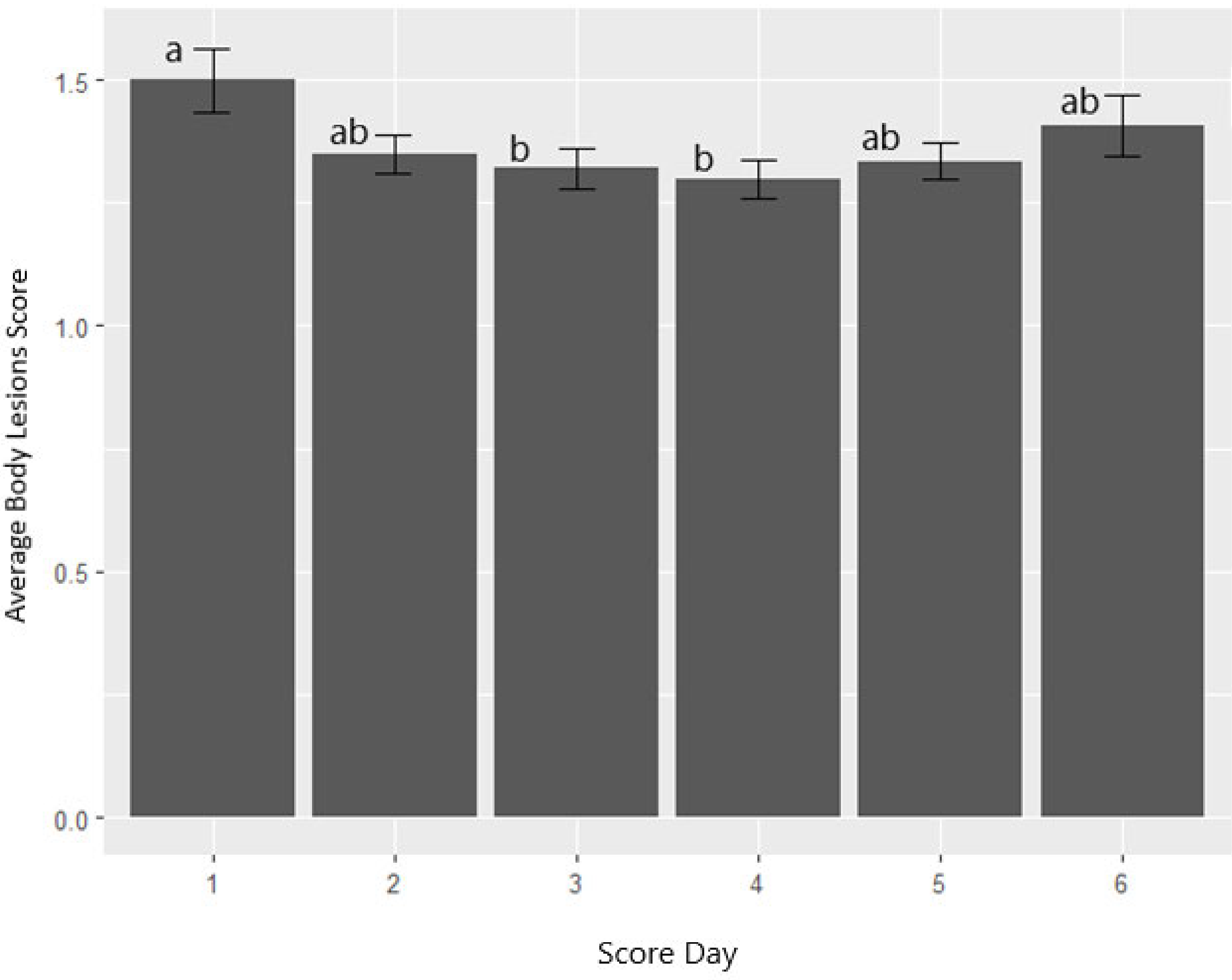
<u>Lesions</u>

We found no effect of diet or score day on average score of tail lesions.

Diet tended towards a significant effect on average score of ear lesions (p=0.095), with pigs on the higher lysine diet showing a tendency to have an increased frequency of ear lesions.

Score day affected the average body lesion score (P=0.016). The average body lesion score was lower at score days 3 and 4 than at score day 1 but equal at days 1, 2, 5 and 6 and also equal across days 2, 3, 4, 5 and 6 (Figure 1).

Figure 1. Effect of score day on average body lesions score. Non-matching superscripts denotes statistical significance (P=0.016).



Production
We found no effect of diet on ADG, ADFI or FCR (Table 4).

Table 4. Mean and standard deviation (SD) for the hight lysine diet and the low lysine diet and overall p-value of diet as fixed effect.

Diet		ADG	ADFI	FCR
High lysine	Mean	0.618	1.219	2.036
	SD	0.141	0.262	0.435
Low lysine	Mean	0.606	1.217	2.166
	SD	0.137	0.145	0.537
P-value		0.768	0.988	0.520

Discussion

We found no effect of diet on any of the behaviours tested. There was a tendency towards an increase in ear lesion severity on the high lysine diet and an effect of score day on average body lesion score. All results were in contradiction to our hypothesis that higher levels of lysine in the diet would decrease incidences of negative social behaviours and the severity of lesions, and increase incidences of positive social behaviours.

In the case of ear lesions, the tendency was that treatment HL, the higher lysine diet, showed a higher average frequency of lesions. It should be noted that the tendency was very weak and very close to the P-value threshold. We did not see an effect of diet on ear biting or ear nosing behaviour, suggesting that the slight increase in ear lesions may have bene caused by factors other than negative social behaviours. One such factor could be porcine ear necrosis (PEN). PEN is a condition that mainly occurs in intensive production systems and mostly affects weaned pigs. The syndrome manifests itself with lesions on the external portion of the ear, which either heal without intervention or become more severe resulting in partial loss of the ear (Malik et al., 2021). PEN is a complex and not fully understood condition but may contribute to, or be exacerbated by, ear biting (Park et al., 2013). It is hard to distinguish between ear lesions caused by PEN and ear lesions caused by biting through visual analysis alone. Further research into the causes of PEN and its role in ear lesions and ear biting behaviour would be beneficial. However, as we saw no increase in biting behaviour and only a very weak tendency towards an effect of diet on ear lesions, the results should be interpreted with caution.

An effect of score day on body lesions was not predicted. In the case of ear and tail lesions, outbreaks commonly occur and in the event of an outbreak we would expect to see the severity of lesions increase over time. However, body lesions, unlike ear and tail lesions, are usually caused by agonistic behaviours (Turner et al., 2006). It may have been that body lesions were higher at score day 1 as pigs had just been moved into test pens and research shows that simply moving pigs can increase aggression (D'Eath et al., 2010). Levels of body lesions then decreased as pigs settled into their new pens and then began to increase again, possibly as a result of the pigs becoming larger and competing for space and resources. However, we did not see an effect of score day on aggressive behaviour so it may have been that the body lesions were caused by non-behavioural factors like scratching on pen fixtures and fittings. The higher levels of lesions at score day 1 might have been caused during the moving and weighing process, or as pigs explored their new pens. It may also be that as pigs were not observed for on average 72 hours after entering the pen, we missed the period where most aggression occurred. We decided not to observe the first 72 hours to allow the pigs to settle, to start eating the trial diet and allow enough time for the diets to exert a possible biological effect on behaviour.

We deliberately opted to compare lysine levels at the upper and lower end of the spectrum used in UK commercial pig diets, with the range in commercial norm advised by nutritional consultants. The iso-energetic rations used differed in crude protein, and therefore in all amino acids. However, had effects been observed from feeding the high protein ration, this would be in response to the first limiting component, which under commercial conditions in wheat-soya based rations is lysine. Thus, had the high lysine diet improved social behaviour as predicted, it would have indicated that behaviour could be manipulated through appropriate diet choice within a range currently regarded by the industry as commercially feasible. Exploring the effect of animo acids on social behaviour is a relatively novel area of research, however there are studies that support the importance of amino acid manipulation in the reduction of tail biting. Minussi et al., (2023) looked at the effect of indispensable amino acids (L-Lys, DL-Met, L-Thr, L-Trp, L-Val, L-Ile, L-Leu and L-His) (IAA) on tail biting and found that for pigs on low protein diets (which has been shown to have an effect on harmful social behaviours (McAuley et al., 2022)), supplementation with IAAs was more effective at reducing tail biting that the provision of additional environmental enrichment. As previously mentioned, research carried out by Hewett et al., (2025) showed a link between lysine and tail biting behaviour and ear lesions in pigs. In the current study, the overall lack of effect of lysine on behaviour was unexpected but there are potential explanations for this, including differences between the aforementioned studies and the current study, and the small contrast in lysine levels between the dietary treatments used here.

There are several key differences between the studies performed by Minussi et al., (2023) and Hewett et al., (2025) and this current study. Research by Hewett et al., (2025) was carried out in the USA, with different dietary treatments (comparing Lys and the Trp:Lys ratio in 6 dietary treatments) and conducted on a much larger commercial farm (4000 pig wean to finish facility), with pigs housed under commercial conditions which included no enrichment provision. Both facility size and provision of enrichment have been identified as influencing expression of harmful social behaviours (Moinard et al., 2003; O'Driscoll et al., 2024). Furthermore, the genetic lines of the pigs differed in the two studies and the genotype is known to influence expression of social behaviours (Breuer et al., 2005; Turner et al., 2006; Wurtz et al., 2017). One major difference between pigs in the study conducted by Minussi et al., (2023) and this study is the length of pigs' tails, with pigs in Misussi's study having undocked tails. Docking tails has long been used as a method to reduce tail biting (Robert et al., 1987) and studies have also shown that length of docked tails affects the risk of tail biting (Scollo et al., 2016; Thodberg et al., 2018).

In this trial pigs were provided with enrichment above the minimum standard set out by UK law, in contrast to the absence of enrichment in the USA system. It is well documented that regularly exchanged enrichment objects can reduce the incidence of tail biting (Van de Weerd and Day, 2009; Buijs and Muns, 2019). Additionally, intervention at the early onset of tail/ear biting outbreaks was swift and thorough. In pens where two or more pigs were identified to have lesions with fresh blood, all pigs in the pen received tar to the tail to discourage further biting and additional enrichment was provided. Biters were identified and removed promptly along with pigs that had severe lesions (necrotic tissue or profuse bleeding). Early intervention has been shown to reduce the outbreak of tail/ear biting (Lahrmann et al., 2018). Tail/ear biting episodes were therefore not allowed to progress unchecked, which likely made it harder to identify any effect of the dietary treatment. For ethical reasons, we chose not to allow outbreaks of tail/ear biting to develop and so could not test the effect of our dietary treatments on non-managed levels of tail/ear biting. It may therefore be beneficial to repeat the experiment across a range of production facilities, over a protracted period. It has been shown that incidences of tail/ear biting varies over time and between farms, with different farms and production systems presenting different risk factors (Valros, 2023). Floor space, percentage of the floor that is slatted and feed type have been identified as some of the most important risk factors in biting outbreaks (Smulders et al., 2008). On the study farm there were relatively low numbers of tail/ear biting injuries and it may be that on other farms, with different environmental and management conditions where tail/ear biting is more prolific, a small change in lysine levels could have an effect on behaviour.

The aim of this trial was to explore the effect of commercially available levels of dietary lysine and so the difference in lysine levels between the diets was small. The experiment was designed to test commercially available feeds in the hope of finding a simple and effective solution for producers to reduce the incidences of tail biting and other harmful social behaviours, whilst promoting positive social behaviours. However, it may be that the differences between treatments was too small to see an effect of diet. The low lysine diet contained 86% of the level of lysine of the high lysine diet. The low lysine diet did not trigger an increase in feed intake, and hence pigs did not compensate for the lower amino acid level, but the difference in lysine level appears to have been too small to have affected behaviour or the resulting lesions. Follow up experiments testing a wider range of lysine levels, in different farms with varying hygiene and production systems would be recommended to fully establish the extent to which dietary lysine manipulation can benefit social behaviour.

Conclusion

Although we found no effect of dietary lysine levels on the behaviour of pigs, there is scope for further research into the area. A more extensive study, looking at a range of farms and production systems using a wider range of lysine levels would be beneficial in further exploring the effects of lysine on social behaviour of pigs. Tail biting continues to be a significant issue in pig production, and it is important that researchers and producers work together to find commercially viable strategies to improve the lives of pigs, whilst also exploring ways to promote positive forms of social interaction.

Acknowledgements

We would like to acknowledge the staff of Oatridge Pig Unit for their patience and support throughout the planning and completion of this trial.

Funding

This work was supported by the UKRI Biotechnology and Biological Sciences Research Council (BBSRC) grant number BB/T00875X/1 and PIC as part of the EASTBIO Doctoral Training programme. SRUC receives funding from the Scottish Government.

Conflicts of interest

The authors declare no conflict of interest.

References

Animal Welfare Act 2006. Statute Law Database. Available from: https://www.legislation.gov.uk/ukpga/2006/45/contents

Breuer, K., M. E. M. Sutcliffe, J. T. Mercer, K. A. Rance, N. E. O'Connell, I. A. Sneddon, and S. A. Edwards. 2005. Heritability of clinical tail-biting and its relation to performance traits. Livest. Prod. Sci. 93:87–94. doi:10.1016/j.livprodsci.2004.11.009.

Brown, S. M., R. Peters, I. M. Nevison, and A. B. Lawrence. 2018. Playful pigs: Evidence of consistency and change in play depending on litter and developmental stage. Appl. Anim. Behav. Sci. 198:36–43. doi:10.1016/j.applanim.2017.09.018.

Buijs, S., and R. Muns. 2019. A Review of the Effects of Non-Straw Enrichment on Tail Biting in Pigs. Anim. Open Access J. MDPI. 9:824. doi:10.3390/ani9100824.

Camerlink, I., G. Arnott, M. Farish, and S. P. Turner. 2016. Complex contests and the influence of aggressiveness in pigs. Anim. Behav. 121. doi:10.1016/j.anbehav.2016.08.021.

Camerlink, I., P. Bijma, B. Kemp, and J. E. Bolhuis. 2012. Relationship between growth rate and oral manipulation, social nosing, and aggression in finishing pigs. Appl. Anim. Behav. Sci. 142:11–17. doi:10.1016/j.applanim.2012.09.004.

Camerlink, I., K. Scheck, T. Cadman, and J. L. Rault. 2022. Lying in spatial proximity and active social behaviours capture different information when analysed at group level in indoor-housed pigs. Appl. Anim. Behav. Sci. 246. doi:10.1016/j.applanim.2021.105540.

Camerlink, I., and S. P. Turner. 2013. The pig's nose and its role in dominance relationships and harmful behaviour. Appl. Anim. Behav. Sci. 145. doi:10.1016/j.applanim.2013.02.008.

Chen, C., J. E. Sander, and N. M. Dale. 2003. The Effect of Dietary Lysine Deficiency on the Immune Response to Newcastle Disease Vaccination in Chickens. Avian Dis. 47:1346–1351. doi:10.1637/7008.

Council Directive 2008/120/EC. Available from: https://eur-lex.europa.eu/eli/dir/2008/120/oj/eng

De Briyne, N., C. Berg, T. Blaha, A. Palzer, and D. Temple. 2018. ‘Phasing out pig tail docking in the EU - present state, challenges and possibilities.’ Porc. Health Manag. 4:27. doi:10.1186/s40813-018-0103-8.

D’Eath, R. B., S. P. Turner, E. Kurt, G. Evans, L. Thölking, H. Looft, K. Wimmers, E. Murani, R. Klont, A. Foury, S. H. Ison, A. B. Lawrence, and P. Mormède. 2010. Pigs’ aggressive temperament affects pre-slaughter mixing aggression, stress and meat quality. Anim. Int. J. Anim. Biosci. 4:604–616. doi:10.1017/S1751731109991406.

Directive - 2008/120 - EN - EUR-Lex. Available from: https://eur-lex.europa.eu/eli/dir/2008/120/oj/eng

Henry, M., H. Jansen, M. R. Amezcua, T. L. O’sullivan, L. Niel, A. K. Shoveller, and R. M. Friendship. 2021. Tail-biting in pigs: A scoping review. Animals. 11. doi:10.3390/ani11072002.

Hewett, E., L. Zaragoza, C. Lewis, J. Houdijk, A. Wilson, and S. Turner. 2025. Exploring the effects of dietary lysine and tryptophan on the social behaviour of pigs. J. Anim. Sci. skaf030. doi:10.1093/jas/skaf030.

Jalal, K., F. Khan, S. Nawaz, R. Afroz, K. Khan, S. B. Ali, L. Hao, S. A. Khan, M. Kazi, R. Uddin, and D. J. Haleem. 2022. Anxiolytic, anti-nociceptive and body weight reducing effects of L-lysine in rats: Relationship with brain serotonin an In-Vivo and In-Silico study. Biomed. Pharmacother. 152:113235. doi:10.1016/j.biopha.2022.113235.

Kornegay, E. T., M. D. Lindemann, and V. Ravindran. 1993. Effects of dietary lysine levels on performance and immune response of weanling pigs housed at two floor space allowances. J. Anim. Sci. 71:552–556. doi:10.2527/1993.713552x.

Lahrmann, H. P., C. F. Hansen, R. B. D´Eath, M. E. Busch, J. P. Nielsen, and B. Forkman. 2018. Early intervention with enrichment can prevent tail biting outbreaks in weaner pigs. Livest. Sci. 214:272–277. doi:10.1016/j.livsci.2018.06.010.

Lawrence, A. B., R. C. Newberry, and M. Špinka. 2023. Positive welfare: What does it add to the debate over pig welfare? In: Advances in Pig Welfare, Second Edition. p. 83–112.

Malik, M., K. Chiers, F. Boyen, S. Croubels, and D. Maes. 2021. Porcine ear necrosis. Vet. J. 271:105655. doi:10.1016/j.tvjl.2021.105655.

McAuley, M., S. Buijs, R. Muns, A. Gordon, M. Palmer, K. Meek, and N. O’Connell. 2022. Effect of reduced dietary protein level on finishing pigs’ harmful social behaviour before and after an abrupt dietary change. Appl. Anim. Behav. Sci. 256:105762. doi:10.1016/j.applanim.2022.105762.

McDonald, P., R. Edwards, J. F. D. Greenhalgh, C. Morgan, L. Sinclair, and R. Wilkinson. 2011. Animal Nutrition. Pearson Higher Ed.

Minussi, I., W. J. J. Gerrits, A. J. M. Jansman, R. Gerritsen, W. Lambert, J. J. Zonderland, and J. E. Bolhuis. 2023. Amino acid supplementation counteracts negative effects of low protein diets on tail biting in pigs more than extra environmental enrichment. Sci. Rep. 13:19268. doi:10.1038/s41598-023-45704-0.

Moinard, C., M. Mendl, C. J. Nicol, and L. E. Green. 2003. A case control study of on-farm risk factors for tail biting in pigs. Appl. Anim. Behav. Sci. 81:333–355. doi:10.1016/S0168-1591(02)00276-9.

Nannoni, E., T. Valsami, L. Sardi, and G. Martelli. 2014. Tail docking in pigs: A review on its short- and long-term consequences and effectiveness in preventing tail biting. Ital. J. Anim. Sci. 13:98–106. doi:10.4081/ijas.2014.3095.

Newberry, R. C., D. G. M. Wood-Gush, and J. W. Hall. 1988. Playful behaviour of piglets. Behav. Processes. 17. doi:10.1016/0376-6357(88)90004-6.

Odakura, A. M., F. R. Caldara, M. F. de C. Burbarelli, I. C. de L. Almeida Paz, R. G. Garcia, V. M. Oliveira dos Santos, D. F. de Brito Mandu, J. M. Braz, and M. I. Lourenço da Silva. 2023. Dietary Supplementation of Eubiotic Fiber Based on Lignocellulose on Performance and Welfare of Gestating and Lactating Sows. Animals. 13:695. doi:10.3390/ani13040695.

O'Driscoll, K., M. Cupido, and J.-Y. Chou. 2024. The physical environment. In: Tail biting in pigs: A comprehensive guide to its aetiology, impact and wider significance in pig management. p. 167–194.

Robert, S., J. Dancosse, and A. Dallaire. 1987. Some observations on the role of environment and genetics in behaviour of wild and domestic forms of Sus scrofa (European wild boars and domestic pigs). Appl. Anim. Behav. Sci. 17:253–262. doi:10.1016/0168-1591(87)90150-X.

Scollo, A., B. Contiero, and F. Gottardo. 2016. Frequency of tail lesions and risk factors for tail biting in heavy pig production from weaning to 170 kg live weight. Vet. J. 207:92–98. doi:10.1016/j.tvjl.2015.10.056.

Severyanova, L. A., V. A. Lazarenko, D. V. Plotnikov, M. E. Dolgintsev, and A. A. Kriukov. 2019. L-Lysine as the Molecule Influencing Selective Brain Activity in Pain-Induced Behavior of Rats. Int. J. Mol. Sci. 20:1899. doi:10.3390/ijms20081899.

Smulders, D., V. Hautekiet, G. Verbeke, and R. Geerst. 2008. Tail and ear biting lesions in pigs: an epidemiological study. Anim. Welf. 17:61–69.

Srinongkote, S., M. Smriga, K. Nakagawa, and Y. Toride. 2003. A Diet Fortified with l-lysine and l-arginine Reduces Plasma Cortisol and Blocks Anxiogenic Response to Transportation in Pigs. Nutr. Neurosci. 6:283–289. doi:10.1080/10284150310001614661.

Stewart, C. L., L. A. Boyle, M. Mee, and N. E. O'Connell. 2010. The effect of feeding a high fibre diet on the welfare of sows housed in large dynamic groups. Anim. Welf. 19:349–357.

Stracke, J., W. Otten, A. Tuchscherer, M. Witthahn, C. C. Metges, B. Puppe, and S. Düpjan. 2017. Dietary tryptophan supplementation and affective state in pigs. J. Vet. Behav. 20:82–90. doi:10.1016/J.JVEB.2017.03.009.

Thodberg, K., M. S. Herskin, T. Jensen, and K. H. Jensen. 2018. The effect of docking length on the risk of tail biting, tail-directed behaviour, aggression and activity level of growing pigs kept under commercial conditions. animal. 12:2609–2618. doi:10.1017/S1751731118000563.

Turner, Simon P., M. J. Farnworth, I. M. S. White, S. Brotherstone, M. Mendl, P. Knap, P. Penny, and A. B. Lawrence. 2006. The accumulation of skin lesions and their use as a predictor of individual aggressiveness in pigs. Appl. Anim. Behav. Sci. 96:245–259. doi:10.1016/j.applanim.2005.06.009.

Turner, S. P., I. M. S. White, S. Brotherstone, M. J. Farnworth, P. W. Knap, P. Penny, M. Mendl, and A. B. Lawrence. 2006. Heritability of post-mixing aggressiveness in grower-stage pigs and its relationship with production traits. Anim. Sci. 82:615–620. doi:10.1079/ASC200678.

Valros, A. 2023. Managing tail biting in pigs: Preventing risk factors or docking tails? In: Advances in Pig Welfare, Second Edition. p. 261–287.

Van de Weerd, H. A., and J. E. L. Day. 2009. A review of environmental enrichment for pigs housed in intensive housing systems. Appl. Anim. Behav. Sci. 116:1–20. doi:10.1016/j.applanim.2008.08.001.

Wurtz, K. E., J. M. Siegford, R. O. Bates, C. W. Ernst, and J. P. Steibel. 2017. Estimation of genetic parameters for lesion scores and growth traits in group-housed pigs1. J. Anim. Sci. 95:4310–4317. doi:10.2527/jas2017.1757.

Discussion

It was suggested in the early design of this study that I kept tails long. As previously mentioned, there is growing pressure in the UK and EU to move away from tail docking as the primary strategy to limit tail biting. However, there are significant and serious ethical implications of a sudden cessation of tail docking. Previous research conducted by colleagues at SRUC to explore the use of 3D cameras to detect tail postures before a tail biting outbreak induced tail biting in their study pigs by leaving tails undocked, and housing them on slatted floors with minimal enrichment. This resulted in tail biting seen in 65% of their groups (R.B. D'Eath et al., 2018). For ethical reasons, I did not want to induce tail biting in my trial, but it would be beneficial to test high lysine diets on farms that already do not tail dock. It is likely that on farms that do not tail dock, they will already have systems in place to reduce tail biting as much as possible (bedded floors, increased enrichment, reduced stocking density (Kallio et al., 2018)). In situations where pigs have undocked tails and the risk factors contributing to tail biting have been significantly reduced but some biting behaviour persists, dietary manipulation, including the increase in lysine, may help to reduce biting behaviours further.

During this study, I was present in the shed almost daily (excluding weekends). One limitation to having an observer in the sheds on a daily basis, is that it does not reflect commercial norms. The observer is able to identify early onset tail/ear biting and identify biters. This triggered the early removal of biter or bitten pigs from the pen according to farm protocols and may have diminished the true effect of the diet on damaging behavioural expression. It may have been preferable for the observer instead to have conducted behavioural observations from video recordings or to have used utilise AI technologies to collect behavioural data, although these are not yet advanced enough to reliably detect subtle forms of behavioural interaction. Ideally, lesion scores could also be collected using AI, removing the presence of the observer and any bias and human error they may introduce to the study. The presence of the observer may have also inadvertently impacted on the behaviour of pigs and masked the effect of lysine. It may have been that the human presence disturbed the pigs, causing them to be restless and stressed irrespective of the dietary treatment. Alternatively, the presence of the observer and the establishment of positive human-animal relationships could have positively impacted the pigs' behaviour and welfare, whilst the lesion scoring could have been perceived as enriching. While in the shed, the observer played the radio, sang, spoke softly to the pigs and provided gentle touch when approached by pigs. Studies have shown that music can impact the behaviour of pigs (Li et al., 2019; Zapata Cardona et al., 2022; Trappe et al., 2024) and there is a significant body of literature exploring the consequences of positive human-animal relationships on the health, welfare and production of pigs (Tallet and Brajon, 2024). To avoid any potential effect of the presence of the observer, either negative or positive, it would again be preferable to conduct data collection as remotely as possible with the use of AI and/or precision livestock farming technologies.

Chapter 6: General Discussion

The aim of this thesis was to explore the role of nutrition in expression of positive and negative social behaviours to improve pig welfare. In an effort to achieve this aim, three studies, detailed above, were performed. Study 1 looked at the effects of dietary energy on play behaviour. I found the high energy diet appeared to extend the developmental period of heightened engagement in social play. However, there was no effect of the amount of supplementary sucrose eaten by the individual itself on their own play behaviour. Study 2 explored the effects of dietary lysine and tryptophan on the social behaviour of pigs, with particular focus on negative social behaviours. Results showed that low lysine levels increased ear biting behaviour and diets low in both lysine and tryptophan to lysine ratio increased the incidences of severe ear lesions. As previously discussed, tail and flank biting were not common in this population, but the potential remains for lysine levels to affect this behaviour in other populations and so deserves further exploration. I examined the production effects of study 2 separately from the effects on behaviour focusing on the first two feeding phases with all six treatments and thereafter for the remaining five feeding phases with the five treatments that were retained until the end of the experiment. The data suggest that in addition to lysine content and feeding phase, the combination of low lysine and low tryptophan to lysine ratio had the greatest negative effects on production. In study 3, while exploring the effect of lysine on social behaviours, I found no effect of diet on behaviour, lesions or production metrics under UK production conditions and with levels of lysine representing the upper and lower end of the commercially available diet spectrum. Each chapter contains a discussion specific to the chapter's study and its results. This discussion chapter will therefore focus on more general considerations around experimental limitations procedural issues and differences between studies 2 and 3, the limitations regarding the scope of the thesis and suggestions for future research.

Experimental Limitations

Data Collection

Data collection took up a significant proportion of my PhD time. I used a mixture of live data collection, on farm, and collecting data from video recordings. I spent hundreds of hours looking at pigs. Over those hundreds of hours, I began to question the reliability, accuracy and consistency of human data collection. Discussion with colleagues showed I am not alone in this. Both in animal welfare and the wider field of ethology, there are questions around the reliability and accuracy of data collected over long periods and from video recordings. Live behaviour recordings on farm presents a double challenge of retaining concentration and doing so for prolonged periods. It is well documented, from medical professionals (Cheshire, 2015) to elite sportspersons (Roberts et al., 2019), that high levels of distraction can hinder performance. I used a mixture of continuous and scan sampling which I found helped to maintain focus. I also found that the act of entering the pen to disturb the pigs and the subsequent time to let the animals settle again provided an excellent opportunity to relax my focus, move my body and reset before performing the next set of observations. In both live and video data collection there is a risk that fatigue can negatively impact data quality. The repetition and high focus needed for behavioural observations may have led to cognitive fatigue when collecting data, thus reducing my focus and performance as an observer. Visual or cognitive fatigue in relation to accuracy and reliability have been studied in a variety of fields; helicopter pilots (Stern and Bynum, 1970), ecological surveys (Lardner et al., 2019) and radiographers (Krupinski et al., 2009) to name just a few. Acute cognitive fatigue occurs when an individual spends extended periods of wakefulness and/or extended periods of effort on a single task (Varkovetski, 2015). Studies have shown that persons experiencing cognitive fatigue showed difficulties focusing on and separating the processing of current information from the distracting effects of novel incoming information (van der Linden and Eling, 2006) and reduced performance for task planning and flexibility (Boksem et al., 2006). There are also discussions to be had around the emotional cost to persons performing data collection. Compassion fatigue is well documented in animal caregivers (Stoewen, 2022). Compassion fatigue can cause chronic exhaustion, irritability, emotional numbness, feelings of hopelessness, and even physical symptoms like headaches and insomnia (COAPE, 2024). While data collection is not necessarily a husbandry or care activity, I was involved in the husbandry and care provision of the animals involved in my studies. In both study 1 and 3, I was responsible for the majority of care tasks while running the trials; feeding, mucking out and health checks. I also had extensive contact with the pigs in study 1 as I had gentled them from an early age. Over the course of my PhD, I encountered a range of pig health and welfare issues, some quite upsetting. The impact of faming on the mental health of farmers is well documented (Gregoire, 2002; Chiswell, 2023;

Casey et al., 2025) and while I only had a short exposure to the industry, the adverse effects of long periods of time spent observing upsetting conditions, potential compassion fatigue and observer fatigue could have had a detrimental effect on the quality of my data collection. The PhD by design is a solitary experience, limited by time and funding, however in an ideal world, to mitigate the effects of observer and compassion fatigue, several observers would have been employed or the sole observer would be given longer breaks between observations. The drawback of multiple observers is the introduction of inter-observer variability and by performing all observations myself I was able to maintain consistency. One option for improving the data collection experience and data quality would be to utilise A.I. technologies. A.I. and information perception technology systems (e.g. advanced sensors, Internet of Things technology and information perception algorithms) for detecting pig behaviour are becoming increasingly popular and several systems now exist (Chen et al., 2020; Ni et al., 2020; Melfsen et al., 2023). However, challenges remain in reliably identifying and tracking individual pigs in large groups at commercial stocking densities, and in detecting subtle, complex or nuanced social interactions and correctly labelling the behaviour involved (Xu et al., 2025). Further efforts and annotated datasets are needed to develop and train systems to perform accurate and reliable behavioural observations in commercially realistic intensive environments with large group sizes.

In addition to being the only observer, I was also responsible for feed delivery in study 1 and 3 and aware of the feed being delivered to each pen throughout study 2, therefore I was not blinded to the treatments. In study 1, the two feeds were bagged in coloured bags, white and pink, and referred to as such during the trial. In trial 3, diets were labelled A and B, but as the feed was delivered in tonne tote bags, which I then filled smaller bags with, I was able to see which feed was depleted quicker and so again, I was not blinded to the treatments. In all three studies, it would have been preferable to blind me to the treatments and this could easily have been done by asking someone else to label the feeds.

Observing social behaviours

One of the key challenges of studies 2 and 3 was the unpredictable occurrence of both positive and negative social behaviours. It was decided, after discussion with my supervisory team that it would be beneficial to disturb the pigs before performing the behaviour observation in an effort to encourage behaviours other than lying. In study

2 and 3, pigs were disturbed in their home pens, give some time to settle again and then their behaviours observed. I appreciate this may be a contentious strategy as disturbing the pigs before observation may have created artificial activity levels. However, there was a significant risk, especially with pigs in the late grower and finisher phases what without disturbance, I would have recorded very few behaviours of interest and thus had very little data to analyse. Ideally, I would have video recorded the pigs continuously and used AI to help identify periods of activity and collected behavioural data from those periods.

The effect of diet on tail biting was an important focus of this thesis, but in both studies 2 and 3, very little of this type of negative social behaviour was observed. As previously mentioned only ear biting occurred frequently enough for statistical analysis in study 2 and we only saw low levels of any forms of negative social behaviours in study 3 (tail biting and ear biting). The location for study 2 was chosen specifically because they had previously reported issues with tail biting. However, we did not see these high levels of tail biting and instead saw high levels of ear biting. High levels of ear biting are often seen in circumstances when tails are docked short (Goossens et al., 2008). Research into the risk factors of ear biting has shown that it shares common risk factors with tail biting (Smulders et al., 2008). It follows that strategies aimed at reducing tail biting should also be employed to reduce ear biting but we would benefit from further research to fully understand the aetiology of ear biting (Diana et al., 2019).

As a result of the low levels of tail biting in study 2 but promising results for the effect of lysine reducing negative social behaviours, study 3 was designed to be a follow-on study from study 2. Study 3 was a second attempt to explore the effect of lysine on tail biting behaviours using a smaller sample size with more detailed behavioural observations. We expected to see high levels of tail biting as there had again been reports of tail biting on the farm but unfortunately, this did not occur during the experimental period. Tail and ear biting have an episodic nature which makes their study challenging (Schrøder-Petersen and Simonsen, 2001). They often occur as outbreaks and farms can go from periods of seeing multiple outbreaks to seeing very little evidence of negative social behaviours. Various factors can affect the onset of outbreaks including the amount and suitability of enrichment, the pre-weaning environment (Ursinus et al., 2014; Hakansson and Bolhuis, 2021), time of year and external temperature (Schrøder-Petersen and Simonsen, 2001; Kim et al., 2021) and number of stockpersons present (Moinard et al., 2003). In studies 2 and 3 we did not collect data on the pre-weaning environment or any information on the mother of the piglets on trial. Ursinus et al (2014) proposed that behaviour observed at litter or pen level could act as a promising tool in predicting tail biting and tail damage. Hakansson and Bolhuis (2021) suggested that early life damaging biting behaviours, higher activity levels and social contact are connected. By observing pre-weaning behaviours, we may have been able to select litters identified as being at a higher risk of developing tail or ear biting behaviours to test our dietary treatments on. As for time of year, study 2 was carried out from October to December and study 3 was conducted from August to December. It may have been that we missed peak biting behaviour periods. Temperature has been shown to affect biting behaviour (Geers et al., 1989). Geers et al. (1989) suggested that to minimise tail biting, the optimal air temperature should be between 20-22°C. It therefore follows that lower winter (January/February) temperature and higher summer (July/ August) temperature, times of the year I was not present on farm in either study 2 or 3, might have caused an increase in tail biting behaviours. Longer term studies may have been beneficial in increasing the chances of observing biting behaviours over different seasons.

Across all studies, it was a challenge to capture positive social behaviours. The most commonly studied form of positive social behaviours is play, but preferential association (Durrell et al., 2004; Jowett et al., 2024) and affiliative behaviours (Meynhardt, 1982; Camerlink and Turner, 2013; Portele et al., 2019) are also seen in pigs. Of the different types of play exhibited by pigs I chose to look at, locomotor and object play and play fighting. These play behaviours were chosen to align my research with existing play literature, allowing for easier comparison and review of the literature. In study 1, I further divided locomotor and object play into solo and social play, this reflects the highly social nature of pigs. The difficulty in observing play behaviour is that it is most commonly seen early in life, between 2 and 6 weeks of age, dropping off to a low level around 14 weeks (Newberry et al., 1988). Studies 2 and 3 mainly focused on the growth phase out-with this play window, which may explain why very little play behaviour was observed in those two studies. It may also have been the case that the housing systems in studies 2 and 3 were not conducive to play behaviours (slatted floors with no straw) (Lyons et al., 1995). In study 1, I observed the pigs within their play window and they were housed on solid floors, with sawdust in the home pens and straw in the play arena. However, I only observed play behaviour in the play arena and not in the home pen. I saw high levels of play in the play arena, as we had hoped, but this artificially high level of play stimulated by the novelty of the play arena may have allowed detection of diet effects which would not be present within the normal environment of the home pen. Ideally, I should have compared play levels in the home pens to that seen in the play arena. It might also have been beneficial to make the behaviour measures from the play arena over an extended period of time, to get an average play measure covering initial novelty until a point where the pigs started to lose interest.

Preferential relationships between individual pigs were difficult to observer in studies 2 and 3 as I did not have individual identification of pigs or knowledge of their social networks. I did have individual identifications of the pigs in study 1 so I could have looked at preferential behaviours but sadly the scope and time of the study did not allow for this. Affiliative behaviours were also difficult to record as they are short lived and often the behaviour requires a contextual understanding. Often allogrooming and social nosing behaviours look similar to biting or oral manipulation behaviours. To differentiate between behaviours I might have needed to examine the behaviour of the recipient pig and if the behaviour cased damage. Again, in crowded pens without individual identification of pigs it was very difficult to keep track of actors and recipients and recording lesions directly after observing each social interaction was not possible.

Comparison of study 2 and 3

Comparison of results between studies 2 and 3 is difficult as the studies, although similar in design, were conducted under different conditions. In study 2 1 found evidence that lysine affects ear biting behaviour, in study 3 1 found no evidence of an effect of lysine on social behaviour. The difference in results may be a result of the different conditions and their effect on the expression of social behaviours. In study 2 I saw low levels of tail biting and high levels of ear biting. In study 3 I saw low levels of any form of biting behaviour or any tail or ear directed behaviours.

Arguably the most significant difference between study 2 and 3 was the locations. Study 2 was conducted on a very large, wean to finish commercial system in the USA (approximately 4000 pigs in total, 2000 in the barn I was working in), while study 3 was conducted on a smaller, full cycle (farrow to finish) farm in Scotland (approximately 1000 pigs). Production practices differ between the UK and USA, and legal minimum welfare standards are lower in the USA than in the UK. In study 2, pigs had no access to enrichment whereas pigs in study 3 did have access to enrichment. Extensive research has shown that provision of enrichment contributes to a reduction in incidences of tail biting (Chou et al., 2023) and enrichment may have lowered the incidence of tail and ear biting behaviour in study 3. Response by stockpersons to negative behaviours was also different across the two farms. In study 2, pigs with severely bitten tails were treated and removed from the pen and placed in hospital pens. In comparison, in study 3, efforts were made to limit the spread of tail biting. In pens where more than two pigs showed broken skin on their tails, all pigs in the pen had tar applied to their tails. Pigs with more severe lesions were treated in their home pen and if their condition did not improve, they were removed to a hospital pen. In study 2 and study 3, pigs identified as biters were removed and placed in a hospital pen with larger pigs (study 3) or destroyed (study 2). It is possible that the proactive approach to limiting tail biting during study 3 could have diluted the dietary effect as individual incidences and outbreaks of tail biting were not allowed to progress as they may have done if no intervention had been taken. In the case of study 2, the short length of tail remaining after docking likely reduced tail injury as this has previously been shown to affect injury rates (Hunter et al., 2001).

Stockpersons presence is also suspected to affect pig behaviour. Moinard et al (2003) found that as the number of pens per stockman increased by one, the risk of tail biting increased 1.06-fold. Staffing levels will affect care given to pigs and the ability to intervene in biting outbreaks. Stockpersons' behaviour towards pigs may also contribute to tail biting. Rough or negative handling from stockpersons can increase stress in pigs which may contribute to increased tail biting (Brajon et al., 2015). In studies 2 and 3 the stockpersons remained constant across the two trials but it may have been beneficial to test the trial diets across different systems with different management approaches or to control stockperson behaviour and interaction with the pigs.

Another difference between the two studies was the genetic lines used. Studies have shown that genetics (breed) contribute to negative social behaviours (Breuer et al., 2003). Breuer et al., (2005) found that tail-biting was heritable in Landrace pigs but not in Large White pigs. The pigs used in study 2 were crossbred Landrace and White Duroc castrated males whilst those in study 3 were entire male and female Large White x Landrace x Danish Duroc pigs. The addition of the Large White genetics in the study 3 pigs could have contributed to lower incidences of tail biting.

The sex of the study pigs was different across study 2 and 3. In study 2 I used castrated male pigs and in study 3, mixed sex groups, where the males had not been castrated. The sex and castration status may have contributed to the difference in incidences of biting behaviour between the two trials. Studies have shown that there is a sex effect on tail biting. Schrøder-Petersen et al., (2003) found that among pigs between 40 and 50 kg tail-in-mouth (TIM) behaviour was higher in all-female groups compared to all-male groups. Indicating female piglets are more prone to tail bite compared to male piglets, or that female piglets are more likely to become victims of tail biting .Zonderland et al., (2010) found that male piglets in mixed-sex groups developed tail damage more rapidly compared to female piglets in mixed-sex group and Kritas and Morrison, (2004) observed twice as much tail damage of castrated males (21%) compared to females (9.8%) in mixed sex groups. To my knowledge, there is no research into the effect of sex on ear biting behaviour. As tail and ear biting share common risk factors, it may follow that males may also show higher incidences of ear biting but this needs to be confirmed with further research.

Ideally, to allow for more accurate comparison between studies 2 and 3, it would have been beneficial to repeat study 2 with the diet from study 3, keeping all pig and productions conditions the same (size of farm, environmental conditions, stockpersons, sex of piglets etc.). Alternatively, I could have expanded the study and performed trials using the same dietary treatment in a range of farms and then compared the effects against the varying conditions. This may have highlighted any interaction between diet and those additional factors. Despite the outlined experimental limitations, I am confident that the results of study 2 suggest an effect of lysine on pig behaviour warrants further exploration. My results are consistent with those found by Minussi et al., (2023) and Srinongkote et al. (2003) but as of yet, there has been little exploration into the mechanisms by which lysine affects behaviour.

Thesis limitations and future research

The scope of this thesis was relatively narrow in comparison to the myriad ways in which nutrition could affect behaviour. I chose to focus my research efforts on dietary energy and dietary content of the amino acids lysine and tryptophan in pigs from post-weaning to finishing. I chose to examine dietary energy as it offered a good opportunity to test the effects of the diet on play behaviour, lysine as it is the first limiting amino acid in the majority of pig diets and there is very little research into its effects on behaviour and tryptophan because existing literature (Meunier-Salaün et al., 1991; Koopmans et al., 2006; Shen et al., 2012; Liu et al., 2013; Poletto et al., 2014; Castilha et al., 2016; Stracke et al., 2017; Lay et al., 2021; Henry et al., 2022) indicates it affects behaviour, but its efficacy has not been thoroughly tested at commercially relevant levels. There are some significant topics which I was unable to explore. Such topics include but are not limited to: the role of other dietary components (such as fibre) in expression of social behaviour, the role of the microbiome in the expression of social behaviour, the role of the diet in expression of social behaviour at different growth phases of pigs and the role of positive and negative social behaviours in the construction and maintenance of social networks in pigs.

There are numerous other dietary components that warrant exploration; for example fibre, magnesium and herbal additives. As mentioned in my introduction there is literature exploring the effects of fibre on behaviour (Gall et al., 2009) but predominantly in sows where it affects gut fill and therefore hunger, and the risk of gastric ulceration, as well as occupying time and allowing more natural foraging behaviour (Stewart et al., 2010). Thus far studies have been unsuccessful in finding a link between fibre and tail biting. Historically, Ewbank, (1973) failed to induce tail biting using a high energy, low fibre diet and more recently, Chou et al., (2020), found that a higher fibre diet in a relatively barren environment did not help reduce tail biting or tail lesions. It may be beneficial to explore the effects of differing fibre levels across a range of housing systems, including relatively better enriched environments (environments that typically have lower incidences of tail biting but still some biting) or in systems with pigs with undocked tails.

Minerals may also affect behaviour and there is already evidence of an effect of magnesium on behaviour. Supplementing magnesium in doses between <1g - >10g has been shown to reduce stress measures in pigs (a range of physiological and behaviour measures across various studies) (systematic review: Bushby et al., 2021). Thus far the focus of research around the use of magnesium supplementation has been on reducing stress measures and to my knowledge, no research has been conducted into the use of magnesium to promote positive social behaviours. Further research may also be needed into the optimum dose and delivery method of magnesium supplementation.

I also neglected any exploration into the role of the microbiome. It would be beneficial to explore the bi-directional interaction of host and microbiome and the outcomes for social behaviour. In humans, research is ongoing into 'microbiome engineering', which involves manipulating the composition and function of the microbiome in the hope of developing new therapies for a range of diseases (Singh and Rastogi, 2024). Poor health has been identified as a risk factor in negative social behaviours (Boyle et al., 2022) so improving the overall health of pigs may help to reduce the incidence of biting behaviours. Various methods are already being employed in microbiome engineering in pigs, including synthetic biology, microbiota modulation, probiotics, prebiotics, feed enzymes, faecal microbiota transplantation, and dietary supplements (Veerapagu et al., 2025). The current focus of this research is on improving the health of animals and health is intrinsically linked to behaviour and welfare. Specific consideration, however, should be given to how processes of microbiome engineering might affect behaviour, either positively or negatively.

I also chose not to collect any data on any potential health outcomes of the dietary manipulations. Unfortunately, the effects of diet specifically on the health of pigs was out of scope for this PhD. Health status is often inferred from behaviour and growth data; studies have shown that pigs in poor health are more likely to engage in negative social behaviours (Boyle et al., 2022) as well as showing slower and less efficient growth (Cornelison et al., 2018). There is also research to suggest sick animals are less likely to perform positive social behaviours like play (Aubert, 1999) and in humans it has been shown that prolonged nutritional deficit and low energy can negatively impact the immune response (Munteanu and Schwartz, 2022; Jeppesen et al., 2024). Therefore, it would have been beneficial to all trials to have collected some health data, looking at how diet affect immune response and now immune response affected behaviour. When looking at behaviour, there are so many interlinked contributing factors, it was difficult to examine all of them within the constraints of the PhD.

A major limitation of this thesis was that I only focused on the weaner to finish growth phases, but research has shown that early and in-utero conditions and experiences can affect both the microbiome and the development of social behaviours, and hence maternal nutrition may also mediate offspring behaviour. To get a full and detailed understanding of how feed might affect behaviour it may be prudent to explore how maternal diet can affect neonatal offspring and then follow those offspring through their full production cycle.

Another major limitation was only viewing the behaviours in isolation and not exploring the full social network of the pigs. Social network research is complex and there was insufficient time for me to delve into this area. However, future studies ought to investigate how being a biter or bitten pig might be triggered by the social position of an individual within the group, or how it may subsequently affect an individual's social status. Studies have shown that biting is increased by social stress due to competition which is more severely experienced by pigs of low social status due to their low resource-holding power (O'Connell et al., 2004). In addition to social position potentially having a role in the onset of tail biting, I propose that tail biting itself leads to a breakdown of the individual's social network, as other pigs become frustrated with the behaviour. This could potentially lead to exacerbation of the biting behaviour as the individual no longer benefits from being part of the social network (allogrooming, social synchrony etc.). There is some recent research in this area; Wilder et al., (2021) showed that the group structure of tail-biting pigs influences tail posture (an early indicator or tail biting) and St. Charles et al. (2024) found that pigs with increased weighted out-degree centrality tended to have increased odds of receiving higher tail injury scores. Further research would be beneficial into the social status of bitten and biter pigs and whether tail biting and social position are reciprocally linked.

Further to expanding our understanding of how social behaviours affect the social network of pigs, I propose that we have yet to fully explore the full repertoire of social behaviours in pigs. We focus predominantly on negative and to a lesser extent positive social behaviours but I propose that there is an intermediate class of behaviours that are neutral or intermediate in their impact on the recipient that are largely ignored by ethologists. These behaviours may come before a positive or negative interaction or be performed in isolation from positive or negative behaviours. They may be involved in information gathering, communication or initiating social interactions. Exploration of these subtle, more nuanced behaviours could help us to better understand how pigs communicate with conspecifics, how behavioural interactions escalate or de-escalate and how we might facilitate better communication between group members to allow for more positive interactions.

In the context of tail biting and tail docking, I think we should further explore the role of the pig's tail in communication. Studies have shown that the pig's tail can provide useful information about the individual's physical and emotional experience at that moment (Camerlink and Ursinus, 2020) and act as an early warning sign for tail biting outbreaks (R. D'Eath et al., 2018) but there seems to be little research into if/how pigs use their tails to communicate with each other. Pigs have a wide field of vision, 310 degrees around them, but have poor depth perception and limited colour vision (receptive to primarily blue and green wavelengths) (Dalmau et al., 2009) so how clearly they can see the tails of other pigs needs confirmation. However, on farms where tail docking is routine, the chances of other pigs seeing and being able to infer any message from the tail is likely to be reduced. In dogs, tail docking in the UK has been banned (unless for medical treatment or if the dog is a certified working animal and is not more than 5 days old when the docking is performed), with one of the reasons for this given by the British Veterinary Association that it "deprives dogs of an important form of canine expression". Without this potential communication tool, communication between pigs may be less effective, which could result in increased aggressive interactions. Widening the focus of pig behaviour studies, beyond those with immediately obvious relevance to production could help us to better understand the pig as an individual and as a part of a wider social network.

Research Application

Throughout this thesis dietary manipulation has been presented as a potential solution to problems related to negative social behaviours and while nutrition is an important risk factor in incidences of biting behaviours, it is one of many risk factors. Research has repeatedly shown that reducing stocking density and provision of suitable enrichment are key in reducing negative social behaviours and in promotive positive ones. Changes in nutrition should not be used instead of changes to management and housing but rather used in complement. Historically the focus of diet research has been on growth and production but it is exciting to see it being considered in relation to behaviour and welfare. As we shift from seeing pigs just as a commodity and there is increasing interest in their experience, diet could play a key part in providing commercial pigs with a life worth living. In circumstances where housing and management have been improved, pigs have space and enrichment to explore, a poor diet could act as a limiting factor. In these cases, it would be essential to optimise the diet to allow the potential positive effects of the other improvements to be realised. Wider study of the gut-brain-axis and the role of the microbiome is essential in fully understanding the role of diet in expression of behaviours but as previously mentioned, as pigs share a similar digestive system with humans, we have the advantage of being able to build on human research and apply it to pigs which should help to find answers sooner.

Conclusion

In conclusion, I have demonstrated that lysine has an effect on the social behaviour of pigs in one commercial environment, although no effect was found in a different environment with a different genotype of animals. This effect needs to be confirmed and quantified under a wide range of production systems in which tail and ear biting is more prevalent to determine the welfare and commercial benefit of altering lysine provision in pig diets. It would also be of benefit to determine the mechanism by which lysine affects behaviour as this is not as clear as for other amino acids with a proven role in neurotransmitter synthesis. Additionally, the provision of each amino acid cannot be considered in isolation as effects of the interaction between lysine and tryptophan level on behaviour were also found. I have also demonstrated that, in agreement with the Surplus Resource Theory, a high energy diet may facilitate the extension of the developmental period of heightened engagement in social play. However, the results were not straight forward potentially due to individual differences in pigs' response to dietary energy. Greater energy provision appeared to extend the age at which pigs played, but did not substantially affect the absolute quantity of play when the pigs were younger. Further research is needed into the interactions between diet and affective state, play and other social behaviour contagion and the role of social networks in exhibition of play behaviours. It would also be beneficial to explore the mechanistic role of the neuroendocrine system and microbiome in the display of play and other positive social behaviours. It is my hope that this thesis has displayed the complex and fascinating social behaviour of pigs, the role that nutrition may play in shaping this, and that it has highlighted the importance of our responsibility to strive to provide these highly social creatures with a life worth living.

References

Andretta, I., C. Pomar, M. Kipper, L. Hauschild, and J. Rivest. 2016. Feeding behavior of growing–finishing pigs reared under precision feeding strategies1. J. Anim. Sci. 94:3042–3050. doi:10.2527/jas.2016-0392.

Animal Welfare Act 2006. Statute Law Database. Available from: https://www.legislation.gov.uk/ukpga/2006/45/contents

Aubert, A. 1999. Sickness and behaviour in animals: a motivational perspective. Neurosci. Biobehav. Rev. 23:1029–1036. doi:10.1016/S0149-7634(99)00034-2.

Avelar, E., R. Jha, E. Beltranena, M. Cervantes, A. Morales, and R. T. Zijlstra. 2010. The effect of feeding wheat distillers dried grain with solubles on growth performance and nutrient digestibility in weaned pigs. Anim. FEED Sci. Technol. 160:73–77. doi:10.1016/j.anifeedsci.2010.06.009.

Baranyiová, E. 1991. Effect of Serotonin on Food Intake by Piglets During the Early Postnatal Period. Acta Vet. Brno. 60:127–136. doi:10.2754/avb199160020127.

Barnes, R. H., D. A. Levitsky, W. G. Pond, and U. Moore. 1976. Effect of postnatal dietary protein and energy restriction on exploratory behavior in young pigs. Dev. Psychobiol. 9. doi:10.1002/dev.420090505.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. J. Stat. Softw. 67:1–48. doi:10.18637/jss.v067.i01.

Bennett, B., and J. H. Fewell. 1987. Play frequencies in captive and free-ranging bighorn lambs (Ovis canadensis canadensis). Zoo Biol. 6:237–241. doi:10.1002/zoo.1430060306.

Berghänel, A., O. Schülke, and J. Ostner. 2015. Locomotor play drives motor skill acquisition at the expense of growth: A life history trade-off. Sci. Adv. 1:e1500451. doi:10.1126/sciadv.1500451.

Boksem, M. A. S., T. F. Meijman, and M. M. Lorist. 2006. Mental fatigue, motivation and action monitoring. Biol. Psychol. 72:123–132. doi:10.1016/j.biopsycho.2005.08.007.

Boyle, L. A., S. A. Edwards, J. E. Bolhuis, F. Pol, M. Z. Šemrov, S. Schütze, J. Nordgreen, N. Bozakova, E. N. Sossidou, and A. Valros. 2022. The Evidence for a Causal Link Between Disease and Damaging Behavior in Pigs. Front. Vet. Sci. 8. doi:10.3389/fvets.2021.771682.

Brajon, S., J.-P. Laforest, R. Bergeron, C. Tallet, M.-J. Hötzel, and N. Devillers. 2015. Persistency of the piglet’s reactivity to the handler following a previous positive or negative experience. Appl. Anim. Behav. Sci. 162:9–19. doi:10.1016/j.applanim.2014.11.009.

Breuer, K., M. E. M. Sutcliffe, J. T. Mercer, K. A. Rance, V. E. Beattie, I. A. Sneddon, and S. A. Edwards. 2003. The effect of breed on the development of adverse social behaviours in pigs. Appl. Anim. Behav. Sci. 84:59–74. doi:10.1016/S0168-1591(03)00147-3.

Breuer, K., M. E. M. Sutcliffe, J. T. Mercer, K. A. Rance, N. E. O'Connell, I. A. Sneddon, and S. A. Edwards. 2005a. Heritability of clinical tail-biting and its relation to performance traits. Livest. Prod. Sci. 93:87–94. doi:10.1016/j.livprodsci.2004.11.009.

Breuer, K., M. E. M. Sutcliffe, J. T. Mercer, K. A. Rance, N. E. O'Connell, I. A. Sneddon, and S. A. Edwards. 2005b. Heritability of clinical tail-biting and its relation to performance traits. In: Livest. Prod. Sci. Vol. 93. Elsevier. p. 87–94.

Brown, S. M., M. Klaffenböck, I. M. Nevison, and A. B. Lawrence. 2015. Evidence for litter differences in play behaviour in pre-weaned pigs. Appl. Anim. Behav. Sci. 172. doi:10.1016/j.applanim.2015.09.007.

Brown, S. M., R. Peters, I. M. Nevison, and A. B. Lawrence. 2018. Playful pigs: Evidence of consistency and change in play depending on litter and developmental stage. Appl. Anim. Behav. Sci. 198:36–43. doi:10.1016/j.applanim.2017.09.018.

Buijs, S., and R. Muns. 2019. A Review of the Effects of Non-Straw Enrichment on Tail Biting in Pigs. Anim. Open Access J. MDPI. 9:824. doi:10.3390/ani9100824.

Burghardt, G. M. 2005. The genesis of animal play: Testing the limits. Available from: https://books.google.com/books?hl=en&lr=&id=kWR6AgAAQBAJ&oi=fnd&pg=PR9&ots=HTpk8jqrd5&sig=JRmIrI2FrKL1mprGET2bZOGYdvI

Camerlink, I., G. Arnott, M. Farish, and S. P. Turner. 2016a. Complex contests and the influence of aggressiveness in pigs. Anim. Behav. 121. doi:10.1016/j.anbehav.2016.08.021.

Camerlink, I., P. Bijma, B. Kemp, and J. E. Bolhuis. 2012. Relationship between growth rate and oral manipulation, social nosing, and aggression in finishing pigs. Appl. Anim. Behav. Sci. 142:11–17. doi:10.1016/j.applanim.2012.09.004.

Camerlink, I., M. Peijnenburg, F. Wemelsfelder, and S. P. Turner. 2016b. Emotions after victory or defeat assessed through qualitative behavioural assessment, skin lesions and blood parameters in pigs. Appl. Anim. Behav. Sci. 183. doi:10.1016/j.applanim.2016.07.007.

Camerlink, I., C. Proßegger, D. Kubala, K. Galunder, and J.-L. Rault. 2021. Keeping littermates together instead of social mixing benefits pig social behaviour and growth post-weaning. Appl. Anim. Behav. Sci. 235:105230. doi:10.1016/j.applanim.2021.105230.

Camerlink, I., K. Scheck, T. Cadman, and J. L. Rault. 2022. Lying in spatial proximity and active social behaviours capture different information when analysed at group level in indoor-housed pigs. Appl. Anim. Behav. Sci. 246. doi:10.1016/j.applanim.2021.105540.

Camerlink, I., and S. P. Turner. 2013. The pig's nose and its role in dominance relationships and harmful behaviour. Appl. Anim. Behav. Sci. 145. doi:10.1016/j.applanim.2013.02.008.

Camerlink, I., and W. W. Ursinus. 2020. Tail postures and tail motion in pigs: A review. Appl. Anim. Behav. Sci. 230:105079. doi:10.1016/j.applanim.2020.105079.

Casey, H., A. O'Connor, S. O'Keeffe, A. D. O'Hagan, J. McNamara, and S. O'Connor. 2025. Depression in farming families: A systematic review. J. Rural Stud. 119. doi:10.1016/j.jrurstud.2025.103810.

Castilha, L. D., C. P. Sangali, L. A. C. Esteves, C. F. Muniz, A. C. Furlan, R. S. Vasconcellos, and P. C. Pozza. 2016. Day-night behaviour and performance of barrows and gilts (70–100 kg) fed low protein diets with different levels of tryptophan and B6 vitamin. Appl. Anim. Behav. Sci. 180:35–42. doi:10.1016/J.APPLANIM.2016.03.017.

Chaloupková, H., G. Illmann, L. Bartoš, and M. Špinka. 2007. The effect of pre-weaning housing on the play and agonistic behaviour of domestic pigs. Appl. Anim. Behav. Sci. 103. doi:10.1016/j.applanim.2006.04.020.

Chen, C., J. E. Sander, and N. M. Dale. 2003. The Effect of Dietary Lysine Deficiency on the Immune Response to Newcastle Disease Vaccination in Chickens. Avian Dis. 47:1346–1351. doi:10.1637/7008.

Chen, Y.-R., C.-T. Ni, K.-S. Ng, C.-L. Hsu, S.-C. Chang, C.-B. Hsu, and P.-Y. Chen. 2020. An AI-based System for Monitoring Behavior and Growth of Pigs. In: Proc. - Int. Comput. Symp., ICS. Institute of Electrical and Electronics Engineers Inc. p. 91–95.

Cheshire, Williamp. 2015. Multitasking and the neuroethics of distraction. Ethics Med. 31:19–25.

Chiswell, H. 2023. Psychological Morbidity in the Farming Community: A Literature Review. J. Agromedicine. 28:151–176. doi:10.1080/1059924X.2022.2089419.

Chou, J. Y., K. O'Driscoll, D. A. Sandercock, and R. B. D'Eath. 2020. Can increased dietary fibre level and a single enrichment device reduce the risk of tail biting in undocked growing-finishing pigs in fully slatted systems? PLoS ONE. 15. doi:10.1371/journal.pone.0241619.

Chou, J.-Y., H. van de Weerd, and I. Camerlink. 2023. Gaining and maintaining interest: Recent advances in enrichment for pigs. In: Advances in Pig Welfare, Second Edition. p. 289–308.

Clizer, D. A., B. J. Tostenson, B. Frederick, P. M. Cline, and R. S. Samuel. 2023. Performance response of increasing the standardized ileal digestible tryptophan:lysine ratio in diets containing 40% dried distiller grains with solubles. J. Anim. Sci. 101:skad264. doi:10.1093/jas/skad264.

COAPE. 2024. Compassion fatigue in animal workers. COAPE. Available from: https://coape.org/compassion-fatigue-in-animal-workers/

Cornelison, A. S., L. A. Karriker, N. H. Williams, B. J. Haberl, K. J. Stalder, L. L. Schulz, and J. F. Patience. 2018. Impact of health challenges on pig growth performance, carcass characteristics, and net returns under commercial conditions. Transl. Anim. Sci. 2:50–61. doi:10.1093/tas/txx005.

Council Directive 2008/120/EC. Available from: https://eur-lex.europa.eu/eli/dir/2008/120/oj/eng

Dalmau, A., P. Llonch, and A. Velarde. 2009. Pig vision and management/handling. Available from: https://www.pig333.com/articles/pig-vision-and-management-handling_981/

De Briyne, N., C. Berg, T. Blaha, A. Palzer, and D. Temple. 2018. ‘Phasing out pig tail docking in the EU - present state, challenges and possibilities.’ Porc. Health Manag. 4:27. doi:10.1186/s40813-018-0103-8.

D’Eath, R. B., M. Jack, A. Futro, D. Talbot, Q. Zhu, D. Barclay, and E. M. Baxter. 2018. Automatic early warning of tail biting in pigs: 3D cameras can detect lowered tail posture before an outbreak. A. Yildirim, editor. PLOS ONE. 13:e0194524. doi:10.1371/journal.pone.0194524.

D’Eath, R. B., and A. B. Lawrence. 2004. Early life predictors of the development of aggressive behaviour in the domestic pig. Anim. Behav. 67. doi:10.1016/j.anbehav.2003.06.010.

D’Eath, R. B., S. P. Turner, E. Kurt, G. Evans, L. Thölking, H. Looft, K. Wimmers, E. Murani, R. Klont, A. Foury, S. H. Ison, A. B. Lawrence, and P. Mormède. 2010. Pigs’ aggressive temperament affects pre-slaughter mixing aggression, stress and meat quality. Anim. Int. J. Anim. Biosci. 4:604–616. doi:10.1017/S1751731109991406.

D’Eath, R., M. Jack, A. Futro, D. Talbot, Q. Zhu, D. Barclay, and E. Baxter. 2018. Automatic early warning of tail biting in pigs: 3D cameras can detect lowered tail posture before an outbreak. PLoS ONE. 13. doi:10.1371/journal.pone.0194524. Available from: http://www.mendeley.com/research/automatic-early-warning-tail-biting-pigs-3d-cameras-detect-lowered-tail-posture-before-outbreak-1

Diamond, J., and A. B. Bond. 2003. A comparative analysis of social play in birds. Behaviour. 140:1091–1115. doi:10.1163/156853903322589650.

Diana, A., L. Carpentier, D. Piette, L. A. Boyle, D. Berckmans, and T. Norton. 2019. An ethogram of biter and bitten pigs during an ear biting event: first step in the development of a Precision Livestock Farming tool. Appl. Anim. Behav. Sci. 215:26–36. doi:10.1016/j.applanim.2019.03.011.

Dinets, V. 2023. Play behavior in ectothermic vertebrates. Neurosci. Biobehav. Rev. 155. doi:10.1016/j.neubiorev.2023.105428.

Directive - 2008/120 - EN - EUR-Lex. Available from: https://eur-lex.europa.eu/eli/dir/2008/120/oj/eng

Durrell, J. L., I. A. Sneddon, N. E. O’Connell, and H. Whitehead. 2004. Do pigs form preferential associations? Appl. Anim. Behav. Sci. 89:41–52. doi:10.1016/j.applanim.2004.05.003.

Edmunds, D., S. Wigby, and J. C. Perry. 2021. ‘Hangry’ *Drosophila*: food deprivation increases male aggression. Anim. Behav. 177:183–190. doi:10.1016/j.anbehav.2021.05.001.

Eisenbeiser, S., É. Serbe-Kamp, G. J. Gage, and T. C. Marzullo. 2022. Gills Just Want to Have Fun: Can Fish Play Games, Just like Us? Animals. 12. doi:10.3390/ani12131684.

Ewbank, R. 1973. Abnormal Behaviour and Pig Nutrition. An Unsuccessful attempt to Induce Tail Biting by Feeding a High Energy, Low Fibre Vegetable Protein Ration. Br. Vet. J. 129:366–369. doi:10.1016/S0007-1935(17)36440-0.

Fernstrom, J. D. 1994. Dietary amino acids and brain function. J. Am. Diet. Assoc. 94:71–77. doi:10.1016/0002-8223(94)92045-1.

Forbes, J. M. 2007. Voluntary Food Intake and Diet Selection in Farm Animals. CABI.

Franchi, G. A., M. L. V. Larsen, I. H. Kristoffersen, J. F. M. Winters, L. J. Pedersen, and M. B. Jensen. 2023. Play behaviour positively relates to weight gain, feeding behaviour and drinking behaviour in weaner pigs (Sus scrofa). Appl. Anim. Behav. Sci. 259:105836. doi:10.1016/J.APPLANIM.2023.105836.

Gall, M. L., L. Montagne, M. C. Meunier-Salaün, and J. Noblet. 2009. Use of fibres in pig feed: Consequences on nutrition, health and behaviour. Prod. Anim. 22.

Galpayage Dona, H. S., C. Solvi, A. Kowalewska, K. Mäkelä, H. MaBouDi, and L. Chittka. 2022. Do bumble bees play? Anim. Behav. 194:239–251. doi:10.1016/j.anbehav.2022.08.013.

Geers, R., B. Dellaert, V. Goedseels, A. Hoogerbrugge, E. Vranken, F. Maes, and D. Berckmans. 1989. An assessment of optimal air temperatures in pig houses by the quantification of behavioural and health-related problems. Anim. Prod. 48:571–578. doi:10.1017/S0003356100004098.

Gonçalves, M. A. D., M. D. Tokach, N. M. Bello, K. J. Touchette, R. D. Goodband, J. M. DeRouchey, J. C. Woodworth, and S. S. Dritz. 2018. Dose–response evaluation of the standardized ileal digestible tryptophan : lysine ratio to maximize growth performance of growing-finishing gilts under commercial conditions. Animal. 12:1380–1387. doi:10.1017/S1751731117002968.

Goncalves, M., U. Orlando, W. Cast, and M. Culbertson. 2017. Standardized ileal digestible lysine requirements for finishing PIC pigs under commercial conditions: A meta-analysis. J. Anim. Sci. 95:131–132. doi:10.2527/asasmw.2017.273.

Goossens, X., L. Sobry, F. Ödberg, F. Tuyttens, D. Maes, S. De Smet, F. Nevens, G. Opsomer, F. Lommelen, and R. Geers. 2008. A population-based on-farm evaluation protocol for comparing the welfare of pigs between farms. Anim. Welf. 17:35–41.

Graham, K. L., and G. M. Burghardt. 2010. Current perspectives on the biological study of play: signs of progress. Q. Rev. Biol. 85:393–418. doi:10.1086/656903.

Gregoire, A. 2002. The mental health of farmers. Occup. Med. 52:471–476. doi:10.1093/occmed/52.8.471.

Größbacher, V., A. B. Lawrence, C. Winckler, and M. Špinka. 2020. Negative play contagion in calves. Sci. Rep. 10. doi:10.1038/s41598-020-78748-7.

Hakansson, F., and J. E. Bolhuis. 2021. Tail-biting behaviour pre-weaning: Association between other pig-directed and general behaviour in piglets. Appl. Anim. Behav. Sci. 241:105385. doi:10.1016/j.applanim.2021.105385.

Held, S. D. E., and M. Špinka. 2011. Animal play and animal welfare. Anim. Behav. 81:891–899. doi:10.1016/j.anbehav.2011.01.007.

Henry, M., H. Jansen, M. R. Amezcua, T. L. O'sullivan, L. Niel, A. K. Shoveller, and R. M. Friendship. 2021. Tail-biting in pigs: A scoping review. Animals. 11. doi:10.3390/ani11072002.

Henry, M., A. K. Shoveller, T. L. O'Sullivan, L. Niel, and R. Friendship. 2022. Effect of Varying Levels of Dietary Tryptophan on Aggression and Abnormal Behavior in Growing Pigs.

Front. Vet. Sci. 9. Available from:
https://www.frontiersin.org/articles/10.3389/fvets.2022.849970

Hewett, E., L. Zaragoza, C. Lewis, J. Houdijk, A. Wilson, and S. Turner. 2025. Exploring the effects of dietary lysine and tryptophan on the social behaviour of pigs. J. Anim. Sci. skaf030. doi:10.1093/jas/skaf030.

Hilakivi-Clarke, L., E. Cho, and I. Onojafe. 1996. High-fat diet induces aggressive behavior in male mice and rats. Life Sci. 58:1653–1660. doi:10.1016/0024-3205(96)00140-3.

Hunter, E. J., T. A. Jones, H. J. Guise, R. H. Penny, and S. Hoste. 2001. The relationship between tail biting in pigs, docking procedure and other management practices. Vet. J. Lond. Engl. 1997. 161:72–79. doi:10.1053/tvjl.2000.0520.

Hyun, Y., M. Ellis, F. K. McKeith, and E. R. Wilson. 1997. Feed intake pattern of group-housed growing-finishing pigs monitored using a computerized feed intake recording system. J. Anim. Sci. 75:1443–1451. doi:10.2527/1997.7561443x.

Jalal, K., F. Khan, S. Nawaz, R. Afroz, K. Khan, S. B. Ali, L. Hao, S. A. Khan, M. Kazi, R. Uddin, and D. J. Haleem. 2022. Anxiolytic, anti-nociceptive and body weight reducing effects of L-lysine in rats: Relationship with brain serotonin an In-Vivo and In-Silico study. Biomed. Pharmacother. 152:113235. doi:10.1016/j.biopha.2022.113235.

Jensen, M. B., K. S. Vestergaard, and C. C. Krohn. 1998. Play behaviour in dairy calves kept in pens: the effect of social contact and space allowance. Appl. Anim. Behav. Sci. 56:97–108. doi:10.1016/S0168-1591(97)00106-8.

Jeppesen, J. S., H. G. Caldwell, L. O. Lossius, A. K. Melin, L. Gliemann, J. Bangsbo, and Y. Hellsten. 2024. Low energy availability increases immune cell formation of reactive oxygen species and impairs exercise performance in female endurance athletes. Redox Biol. 75:103250. doi:10.1016/j.redox.2024.103250.

Jowett, S. L., Z. E. Barker, and J. R. Amory. 2024. The effect of preferential associations on the reproductive performance of group-housed sows. Appl. Anim. Behav. Sci. 278:106376. doi:10.1016/j.applanim.2024.106376.

Kallio, P. A., A. M. Janczak, A. E. Valros, S. A. Edwards, and M. Heinonen. 2018. Case control study on environmental, nutritional and management-based risk factors for tail-biting in long-tailed pigs. Anim. Welf. 27:21–34. doi:10.7120/09627286.27.1.021.

Kendall, D. C., A. M. Gaines, B. J. Kerr, and G. L. Allee. 2007. True ileal digestible tryptophan to lysine ratios in ninety- to one hundred twenty-five-kilogram barrows1. J. Anim. Sci. 85:3004–3012. doi:10.2527/jas.2007-0013.

Kim, Y. J., M. H. Song, S. I. Lee, J. H. Lee, H. J. Oh, J. W. An, S. Y. Chang, Y. B. Go, B. J. Park, M. S. Jo, C. G. Lee, H. B. Kim, and J. H. Cho. 2021. Evaluation of pig behavior changes related to temperature, relative humidity, volatile organic compounds, and illuminance. J. Anim. Sci. Technol. 63. doi:10.5187/jast.2021.e89.

Koopmans, S. J., A. C. Guzik, J. V. D. Meulen, R. Dekker, J. Kogut, B. J. Kerr, and L. L. Southern. 2006. Effects of supplemental L-tryptophan on serotonin, cortisol, intestinal integrity, and behavior in weanling piglets,. J. Anim. Sci. 84:963–971. doi:10.2527/2006.844963X.

Kornegay, E. T., M. D. Lindemann, and V. Ravindran. 1993. Effects of dietary lysine levels on performance and immune response of weanling pigs housed at two floor space allowances. J. Anim. Sci. 71:552–556. doi:10.2527/1993.713552x.

Krachun, C., J. Rushen, and A. M. de Passillé. 2010. Play behaviour in dairy calves is reduced by weaning and by a low energy intake. Appl. Anim. Behav. Sci. 122:71–76. doi:10.1016/j.applanim.2009.12.002.

Kritas, S. K., and R. B. Morrison. 2004. An observational study on tail biting in commercial grower-finisher barns. J. Swine Health Prod. 12:17–22.

Krupinski, E. A., K. S. Berbaum, and R. Caldwell. 2009. Impact of visual fatigue on observer performance. In: Medical Imaging 2009: Image Perception, Observer Performance, and Technology Assessment. Vol. 7263. SPIE. p. 539–546. Available from: https://www.spiedigitallibrary.org/conference-proceedings-of-spie/7263/72631O/Impact-of-visual-fatigue-on-observer-performance/10.1117/12.813794.full

Kuba, M. J., T. Gutnick, and G. M. Burghardt. 2014. Learning from play in octopus. In: A.-S. Darmaillacq, J. Mather, and L. Dickel, editors. Cephalopod Cognition. Cambridge University Press, Cambridge. p. 57–71. Available from: https://www.cambridge.org/core/books/cephalopod-cognition/learning-from-play-in-octopus/661AB1112BA965B8D096AF0DA97FBB80

Lahrmann, H. P., C. F. Hansen, R. B. D´Eath, M. E. Busch, J. P. Nielsen, and B. Forkman. 2018. Early intervention with enrichment can prevent tail biting outbreaks in weaner pigs. Livest. Sci. 214:272–277. doi:10.1016/j.livsci.2018.06.010.

Lardner, B., A. A. Y. Adams, A. J. Knox, J. A. Savidge, and R. N. Reed. 2019. Do observer fatigue and taxon bias compromise visual encounter surveys for small vertebrates? Wildl. Res. 46:127–135. doi:10.1071/WR18016.

Lawrence, A. 1987. Consumer demand theory and the assessment of animal welfare. Anim. Behav. 35:293–295. doi:10.1016/S0003-3472(87)80236-1.

Lawrence, A. B., R. C. Newberry, and M. Špinka. 2023. Positive welfare: What does it add to the debate over pig welfare? In: Advances in Pig Welfare, Second Edition. p. 83–112.

Lay, D. C., S. A. Enneking, N. C. Anderson, B. T. Richert, and A. Sapkota. 2021. Effect of short-term high tryptophan diet fed to sows on their subsequent piglet behavior. Transl. Anim. Sci. 5. doi:10.1093/TAS/TXAB127. Available from: https://academic.oup.com/tas/article/5/3/txab127/6354054

Lewis, A. J., and L. L. Southern. 2000. Swine Nutrition. CRC Press.

Li, P., Y.-L. Yin, D. Li, S. W. Kim, and G. Wu. 2007. Amino acids and immune function. Br. J. Nutr. 98:237–252. doi:10.1017/S000711450769936X.

Li, Q., and J. F. Patience. 2017. Factors involved in the regulation of feed and energy intake of pigs. Anim. FEED Sci. Technol. 233:22–33. doi:10.1016/j.anifeedsci.2016.01.001.

van der Linden, D., and P. Eling. 2006. Mental fatigue disturbs local processing more than global processing. Psychol. Res. 70:395–402. doi:10.1007/s00426-005-0228-7.

Liu, H. W., B. M. Shi, D. S. Liu, and A. S. Shan. 2013. Supplemental dietary tryptophan modifies behavior, concentrations of salivary cortisol, plasma epinephrine, norepinephrine and hypothalamic 5-hydroxytryptamine in weaning piglets. Livest. Sci. 151:213–218. doi:10.1016/J.LIVSCI.2012.11.003.

Liu, J. B., H. L. Yan, S. C. Cao, J. Liu, Z. X. Li, and H. F. Zhang. 2019. The response of performance in grower and finisher pigs to diets formulated to different tryptophan to lysine ratios. Livest. Sci. 222:25–30. doi:10.1016/j.livsci.2019.01.016.

Losinger, W. C. 1998. Feed-conversion ratio of finisher pigs in the USA. Prev. Vet. Med. 36:287–305. doi:10.1016/S0167-5877(98)00094-4.

Lyons, C. A. P., J. M. Bruce, V. R. Fowler, and P. R. English. 1995. A comparison of productivity and welfare of growing pigs in four intensive systems. Livest. Prod. Sci. 43:265–274. doi:10.1016/0301-6226(95)00050-U.

Main, R. G., S. S. Dritz, M. D. Tokach, R. D. Goodband, J. L. Nelssen, and J. M. DeRouchey. 2008. Effects of Feeding Growing Pigs Less or More Than Their Estimated Lysine Requirement in Early and Late Finishing on Overall Performance. Prof. Anim. Sci. 24:76–87. doi:10.15232/S1080-7446(15)30813-5.

Malik, M., K. Chiers, F. Boyen, S. Croubels, and D. Maes. 2021. Porcine ear necrosis. Vet. J. 271:105655. doi:10.1016/j.tvjl.2021.105655.

Marley, C. L., T. M. Pollard, R. A. Barton, and S. E. Street. 2022. A systematic review of sex differences in rough and tumble play across non-human mammals. Behav. Ecol. Sociobiol. 76:158. doi:10.1007/s00265-022-03260-z.

Marschner, I. 2011. glm2: Fitting Generalized Linear Models. 1.2.1. doi:10.32614/CRAN.package.glm2. Available from: https://CRAN.R-project.org/package=glm2

Mather, J. A., and R. C. Anderson. 1999. Exploration, play and habituation in octopuses (Octopus dofleini). J. Comp. Psychol. 113:333–338. doi:10.1037/0735-7036.113.3.333.

McAuley, M., S. Buijs, R. Muns, A. Gordon, M. Palmer, K. Meek, and N. O'Connell. 2022. Effect of reduced dietary protein level on finishing pigs' harmful social behaviour before and after an abrupt dietary change. Appl. Anim. Behav. Sci. 256:105762. doi:10.1016/j.applanim.2022.105762.

McDonald, P., R. Edwards, J. F. D. Greenhalgh, C. Morgan, L. Sinclair, and R. Wilkinson. 2011. Animal Nutrition. Pearson Higher Ed.

Melfsen, A., A. Lepsien, J. Bosselmann, A. Koschmider, and E. Hartung. 2023. Describing Behavior Sequences of Fattening Pigs Using Process Mining on Video Data and Automated Pig Behavior Recognition. Agric. Switz. 13. doi:10.3390/agriculture13081639.

Meunier-Salaün, M. C., M. Monnier, Y. Colléaux, B. Sève, and Y. Henry. 1991. Impact of dietary tryptophan and behavioral type on behavior, plasma cortisol, and brain metabolites of young pigs. J. Anim. Sci. 69:3689–3698. doi:10.2527/1991.6993689X.

Meynhardt, H. 1982. Schwarzwild-Report – Mein Leben unter Wildschweinen. Neumann-Verl. Leipz.

Minussi, I., W. J. J. Gerrits, A. J. M. Jansman, R. Gerritsen, W. Lambert, J. J. Zonderland, and J. E. Bolhuis. 2023. Amino acid supplementation counteracts negative effects of low protein diets on tail biting in pigs more than extra environmental enrichment. Sci. Rep. 13:19268. doi:10.1038/s41598-023-45704-0.

Moinard, C., M. Mendl, C. J. Nicol, and L. E. Green. 2003. A case control study of on-farm risk factors for tail biting in pigs. Appl. Anim. Behav. Sci. 81:333–355. doi:10.1016/S0168-1591(02)00276-9.

Munteanu, C., and B. Schwartz. 2022. The relationship between nutrition and the immune system. Front. Nutr. 9:1082500. doi:10.3389/fnut.2022.1082500.

Nannoni, E., T. Valsami, L. Sardi, and G. Martelli. 2014. Tail docking in pigs: A review on its short- and long-term consequences and effectiveness in preventing tail biting. Ital. J. Anim. Sci. 13:98–106. doi:10.4081/ijas.2014.3095.

Newberry, R. C., D. G. M. Wood-Gush, and J. W. Hall. 1988. Playful behaviour of piglets. Behav. Processes. 17. doi:10.1016/0376-6357(88)90004-6.

Ni, C.-T., K.-S. Ng, Y.-R. Chen, S.-C. Chang, C.-B. Hsu, P.-Y. Chen, and S.-C. Liao. 2020. A System for Analyzing Pig's Behavior with AI. In: 2020 IEEE International Conference on Consumer Electronics - Taiwan (ICCE-Taiwan). p. 1–2. Available from: https://ieeexplore.ieee.org/document/9258228/

Ni, J.-Q., A. J. Heber, C. A. Diehl, and T. T. Lim. 2000. Ammonia, Hydrogen Sulphide and Carbon Dioxide Release from Pig Manure in Under-#oor Deep Pits. J. Agric. Eng. Res. 77:53–66. doi:10.1006/jaer.2000.0561.

Noblet, J., P. Cozannet, and F. Skiba. 2012. Nutritional value and utilization of wheat dried distillers grain with solubles in pigs and poultry. In: Biofuel co-products as livestock feed. FAO. p. 533 p. Available from: https://hal.science/hal-01210314

Norscia, I., E. Coco, C. Robino, E. Chierto, and G. Cordoni. 2021. Yawn contagion in domestic pigs (Sus scrofa). Sci. Rep. 2021 111. 11:1–12. doi:10.1038/s41598-020-80545-1.

Norscia, I., M. Hecker, B. G. Aldama, S. B. Santos, and G. Cordoni. 2024. Play contagion in African elephants: The closest, the better. Behav. Processes. 221. doi:10.1016/j.beproc.2024.105092.

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Acad. Press., Washington, DC: Natl.

Ocepek, M., R. C. Newberry, and I. L. Andersen. 2020. Which types of rooting material give weaner pigs most pleasure? Appl. Anim. Behav. Sci. 231. doi:10.1016/j.applanim.2020.105070.

O'Connell, N. E., V. E. Beattie, and B. W. Moss. 2004. Influence of social status on the welfare of growing pigs housed in barren and enriched environments. Anim. Welf. 13:425–431. doi:10.1017/S0962728600028682.

Odakura, A. M., F. R. Caldara, M. F. de C. Burbarelli, I. C. de L. Almeida Paz, R. G. Garcia, V. M. Oliveira dos Santos, D. F. de Brito Mandu, J. M. Braz, and M. I. Lourenço da Silva. 2023. Dietary Supplementation of Eubiotic Fiber Based on Lignocellulose on Performance and Welfare of Gestating and Lactating Sows. Animals. 13:695. doi:10.3390/ani13040695.

O'Driscoll, K., M. Cupido, and J.-Y. Chou. 2024. The physical environment. In: Tail biting in pigs: A comprehensive guide to its aetiology, impact and wider significance in pig management. p. 167–194.

Pellis, S. M., and V. C. Pellis. 1987. Play-fighting differs from serious fighting in both target of attack and tactics of fighting in the laboratory rat Rattus norvegicus. Aggress. Behav. 13:227–242. doi:10.1002/1098-2337(1987)13:4%3C227::AID-AB2480130406%3E3.0.CO;2-C.

Pellis, S. M., and V. C. Pellis. 2016. Play fighting in Visayan warty pigs (Sus cebifrons): Insights on restraint and reciprocity in the maintenance of play. Behaviour. 153. doi:10.1163/1568539X-00003346.

PIC. 2024. PIC®NUTRITION ANDFEEDING GUIDELINES. Available from: https://www.pic.com/resources/pic-nutrition-and-feeding-guidelines-metric/

Poletto, R., F. C. Kretzer, and M. J. Hötzel. 2014. Minimizing aggression during mixing of gestating sows with supplementation of a tryptophan-enriched diet. Physiol. Behav. 132. doi:10.1016/j.physbeh.2014.04.043.

Portele, K., K. Scheck, S. Siegmann, R. Feitsch, K. Maschat, J.-L. Rault, and I. Camerlink. 2019. Sow-Piglet Nose Contacts in Free-Farrowing Pens. Animals. 9:513. doi:10.3390/ani9080513.

Proceedings of the British Society of Animal Science 2003. 2003. British Society of Animal Science, Penicuik (GB).

Puppe, B., M. Tuchscherer, and A. Tuchscherer. 1997. The effect of housing conditions and social environment immediately after weaning on the agonistic behaviour, neutrophil/lymphocyte ratio, and plasma glucose level in pigs. Livest. Prod. Sci. 48. doi:10.1016/S0301-6226(97)00006-7.

R Core Team. 2021. R: A language and environment for statistical computing. Available from: https://www.R-project.org/

Rauw, W. M. 2013. A note on the consistency of a behavioral play marker in piglets. J. Anim. Sci. Biotechnol. 4. doi:10.1186/2049-1891-4-33.

Reimert, I., J. E. Bolhuis, B. Kemp, and T. B. Rodenburg. 2013. Indicators of positive and negative emotions and emotional contagion in pigs. Physiol. Behav. 109. doi:10.1016/j.physbeh.2012.11.002.

Robert, S., J. Dancosse, and A. Dallaire. 1987. Some observations on the role of environment and genetics in behaviour of wild and domestic forms of Sus scrofa (European wild boars and domestic pigs). Appl. Anim. Behav. Sci. 17:253–262. doi:10.1016/0168-1591(87)90150-X.

Roberts, L. J., M. S. Jackson, and I. H. Grundy. 2019. Choking under pressure: Illuminating the role of distraction and self-focus. Int. Rev. Sport Exerc. Psychol. 12:49–69. doi:10.1080/1750984X.2017.1374432.

Ross, J. W., B. J. Hale, N. K. Gabler, R. P. Rhoads, A. F. Keating, and L. H. Baumgard. 2015. Physiological consequences of heat stress in pigs. Anim. Prod. Sci. 55:1381–1390. doi:10.1071/AN15267.

Rushen, J., R. Wright, J. F. Johnsen, C. M. Mejdell, and A. M. de Passillé. 2016. Reduced locomotor play behaviour of dairy calves following separation from the mother reflects their response to reduced energy intake. Appl. Anim. Behav. Sci. 177:6–11. doi:10.1016/j.applanim.2016.01.023.

Salyer, J. A., M. D. Tokach, J. M. DeRouchey, S. S. Dritz, R. D. Goodband, and J. L. Nelssen. 2013. Effects of standardized ileal digestible tryptophan:lysine in diets containing 30% dried distillers grains with solubles on finishing pig performance and carcass traits. J. Anim. Sci. 91:3244–3252. doi:10.2527/jas.2012-5502.

Sandars, G., J. S. Brooker, and Z. Clay. 2024. ChimpanSEE, ChimpanDO: Grooming and play contagion in chimpanzees. PLoS ONE. 19. doi:10.1371/journal.pone.0312467.

Schrøder-Petersen, D. L., and H. B. Simonsen. 2001. Tail Biting in Pigs. Vet. J. 162:196–210. doi:10.1053/TVJL.2001.0605.

Schrøder-Petersen, D. L., H. B. Simonsen, and L. G. Lawson. 2003. Tail-in-mouth Behaviour Among Weaner Pigs in Relation to Age, Gender and Group Composition Regarding Gender. Acta Agric. Scand. Sect. — Anim. Sci. 53:29–34. doi:10.1080/09064700310002017.

Scollo, A., B. Contiero, and F. Gottardo. 2016. Frequency of tail lesions and risk factors for tail biting in heavy pig production from weaning to 170 kg live weight. Vet. J. 207:92–98. doi:10.1016/j.tvjl.2015.10.056.

Sève, B. 1999. Physiological Roles of Tryptophan in Pig Nutrition. In: G. Huether, W. Kochen, T. J. Simat, and H. Steinhart, editors. Tryptophan, Serotonin, and Melatonin: Basic Aspects and Applications. Springer US, Boston, MA. p. 729–741. Available from: https://doi.org/10.1007/978-1-4615-4709-9_95

Severyanova, L. A., V. A. Lazarenko, D. V. Plotnikov, M. E. Dolgintsev, and A. A. Kriukov. 2019. L-Lysine as the Molecule Influencing Selective Brain Activity in Pain-Induced Behavior of Rats. Int. J. Mol. Sci. 20:1899. doi:10.3390/ijms20081899.

Shen, Y. B., G. Voilqué, J. D. Kim, J. Odle, and S. W. Kim. 2012. Effects of increasing tryptophan intake on growth and physiological changes in nursery pigs. J. Anim. Sci. 90:2264–2275. doi:10.2527/JAS.2011-4203.

Sihvo, H.-K., O. Simola, C. Munsterhjelm, and P. Syrjä. 2012. Systemic Spread of Infection in Tail-bitten Pigs. J. Comp. Pathol. 146:73. doi:10.1016/j.jcpa.2011.11.112.

Singh, V., and M. Rastogi. 2024. Future and challenges of microbiome engineering. In: N. Srivastava, S. A. Ibrahim, and M. H. A. Nasr, editors. Microbiome Engineering: The New Dimension of Biotechnology. CRC Press. p. 263–280.

Smulders, D., V. Hautekiet, G. Verbeke, and R. Geerst. 2008. Tail and ear biting lesions in pigs: an epidemiological study. Anim. Welf. 17:61–69.

Spinka, M., R. C. Newberry, and M. Bekoff. 2001. Mammalian Play: Training for the Unexpected. Q. Rev. Biol. 76:141–168. doi:10.1086/393866.

Špinka, M., R. C. Newberry, and M. Bekoff. 2001. Mammalian play: Training for the unexpected. Q. Rev. Biol. 76:141–168. doi:10.1086/393866.

Srinongkote, S., M. Smriga, K. Nakagawa, and Y. Toride. 2003. A Diet Fortified with l-lysine and l-arginine Reduces Plasma Cortisol and Blocks Anxiogenic Response to Transportation in Pigs. Nutr. Neurosci. 6:283–289. doi:10.1080/10284150310001614661.

St. Charles, K. M., K. L. VanderWaal, J. E. Anderson, L. J. Johnston, and Y. Z. Li. 2024. Evaluating social network metrics as indicators of tail injury caused by tail biting in growing-finishing pigs (Sus scrofa domesticus). Front. Vet. Sci. 11. doi:10.3389/fvets.2024.1441813.

Steinerová, K., S. E. Parker, J. A. Brown, and Y. M. Seddon. 2024. The promotion of play behaviour in grow-finish pigs: The relationship between behaviours indicating positive experience and physiological measures. Appl. Anim. Behav. Sci. 275:106263. doi:10.1016/j.applanim.2024.106263.

Steinerová, K., S. E. Parker, and Y. M. Seddon. 2025. Regular play opportunities during the grow-finish phase improve pig trainability and learning in a cognitive bias test. Appl. Anim. Behav. Sci. 283. doi:10.1016/j.applanim.2024.106502.

Stern, J. A., and J. A. Bynum. 1970. Analysis of visual search activity in skilled and novice helicopter pilots. Aerosp. Med. 41:300–305.

Stewart, C. L., L. A. Boyle, M. Mee, and N. E. O'Connell. 2010. The effect of feeding a high fibre diet on the welfare of sows housed in large dynamic groups. Anim. Welf. 19:349–357.

Stoewen, D. L. 2022. Compassion Fatigue in the Animal Care Community. In: S. Loue and P. Linden, editors. The Comprehensive Guide to Interdisciplinary Veterinary Social Work. Springer International Publishing. p. 47–89.

Stolba, A., and D. G. M. Wood-Gush. 1989. The behaviour of pigs in a semi-natural environment. Anim. Prod. 48. doi:10.1017/S0003356100040411.

Stracke, J., W. Otten, A. Tuchscherer, M. Witthahn, C. C. Metges, B. Puppe, and S. Düpjan. 2017. Dietary tryptophan supplementation and affective state in pigs. J. Vet. Behav. 20:82–90. doi:10.1016/J.JVEB.2017.03.009.

Sugar: the facts. 2022. nhs.uk. Available from: https://www.nhs.uk/live-well/eat-well/food-types/how-does-sugar-in-our-diet-affect-our-health/

Thacker, P. A. 2006. Nutrient digestibility, performance and carcass traits of growing-finishing pigs fed diets containing dried wheat distiller's grains with solubles. Can. J. Anim. Sci. 86:527–529. doi:10.4141/A06-025.

Thodberg, K., M. S. Herskin, T. Jensen, and K. H. Jensen. 2018. The effect of docking length on the risk of tail biting, tail-directed behaviour, aggression and activity level of growing pigs kept under commercial conditions. animal. 12:2609–2618. doi:10.1017/S1751731118000563.

Thomas, L. L., R. D. Goodband, J. C. Woodworth, M. D. Tokach, J. M. DeRouchey, and S. S. Dritz. 2017. Effects of space allocation on finishing pig growth performance and carcass characteristics. Transl. Anim. Sci. 1:351–357. doi:10.2527/tas2017.0042.

Tolosa, A. F., M. D. Tokach, R. D. Goodband, J. C. Woodworth, J. M. DeRouchey, J. T. Gebhardt, and M. L. Wolfe. 2022. Effects of reducing the standardized ileal digestible lysine and tryptophan to lysine ratio to slow growth of finishing pigs. Transl. Anim. Sci. 6:txac057. doi:10.1093/tas/txac057.

Turner, Simon P., M. J. Farnworth, I. M. S. White, S. Brotherstone, M. Mendl, P. Knap, P. Penny, and A. B. Lawrence. 2006. The accumulation of skin lesions and their use as a predictor of individual aggressiveness in pigs. Appl. Anim. Behav. Sci. 96:245–259. doi:10.1016/j.applanim.2005.06.009.

Turner, S. P., I. M. S. White, S. Brotherstone, M. J. Farnworth, P. W. Knap, P. Penny, M. Mendl, and A. B. Lawrence. 2006. Heritability of post-mixing aggressiveness in grower-stage pigs and its relationship with production traits. Anim. Sci. 82:615–620. doi:10.1079/ASC200678.

Ursinus, W. W., C. G. Van Reenen, B. Kemp, and J. E. Bolhuis. 2014. Tail biting behaviour and tail damage in pigs and the relationship with general behaviour: Predicting the inevitable? Appl. Anim. Behav. Sci. 156:22–36. doi:10.1016/j.applanim.2014.04.001.

Valros, A. 2023. Managing tail biting in pigs: Preventing risk factors or docking tails? In: Advances in Pig Welfare, Second Edition. p. 261–287.

Van de Weerd, H. A., and J. E. L. Day. 2009. A review of environmental enrichment for pigs housed in intensive housing systems. Appl. Anim. Behav. Sci. 116:1–20. doi:10.1016/j.applanim.2008.08.001.

Varkovetski, M. 2015. The reduction of directed attention fatigue through exposure to visual nature stimuli: Exploring a natural therapy for fatigue. SURG J. 8:5–13. doi:10.21083/surg.v8i2.3057.

Veerapagu, M., K. R. Jeya, and A. Sankara Narayanan. 2025. Chapter 16 - Gastrointestinal microbiome engineering in pig. In: D. Dhanasekaran, A. Sankara Narayanan, and P. Sarkar, editors. Human and Animal Microbiome Engineering. Academic Press. p. 265–290. Available from: https://www.sciencedirect.com/science/article/pii/B9780443223488000167

Waiblinger, S., K. Wagner, E. Hillmann, and K. Barth. 2020. Play and social behaviour of calves with or without access to their dam and other cows. J. Dairy Res. 87:144–147. doi:10.1017/S0022029920000540.

Wang, L. F., E. Beltranena, and R. T. Zijlstra. 2016. Diet nutrient digestibility and growth performance of weaned pigs fed wheat dried distillers grains with solubles (DDGS). Anim. FEED Sci. Technol. 218:26–32. doi:10.1016/j.anifeedsci.2016.05.005.

Weller, J. E., I. Camerlink, S. P. Turner, M. Farish, and G. Arnott. 2019. Playful pigs: early life play-fighting experience influences later life contest dynamics. Anim. Behav. 158. doi:10.1016/j.anbehav.2019.09.019.

Wenig, K., P. H. Boucherie, and T. Bugnyar. 2021. Early evidence for emotional play contagion in juvenile ravens. Anim. Cogn. 24:717–729. doi:10.1007/s10071-020-01466-0.

Wilder, T., J. Krieter, N. Kemper, and K. Büttner. 2021. Network analysis of tail-biting in pigs - indirect effect on tail posture. Livest. Sci. 251. doi:10.1016/j.livsci.2021.104668. Available from: https://elib.tiho-hannover.de/receive/tiho_mods_00005429

Wurtz, K. E., J. M. Siegford, R. O. Bates, C. W. Ernst, and J. P. Steibel. 2017. Estimation of genetic parameters for lesion scores and growth traits in group-housed pigs1. J. Anim. Sci. 95:4310–4317. doi:10.2527/jas2017.1757.

Xu, J., Y. Ying, D. Wu, Y. Hu, and D. Cui. 2025. Recent advances in pig behavior detection based on information perception technology. Comput. Electron. Agric. 235. doi:10.1016/j.compag.2025.110327.

Yabut, J. M., J. D. Crane, A. E. Green, D. J. Keating, W. I. Khan, and G. R. Steinberg. 2019. Emerging Roles for Serotonin in Regulating Metabolism: New Implications for an Ancient Molecule. Endocr. Rev. 40:1092–1107. doi:10.1210/er.2018-00283.

Zonderland, J. J., M. B. M. Bracke, L. A. den Hartog, B. Kemp, and H. A. M. Spoolder. 2010. Gender effects on tail damage development in single- or mixed-sex groups of weaned piglets. Livest. Sci. 129:151–158. doi:10.1016/j.livsci.2010.01.018.

Appendix

Chapter 3 and chapter 4

Appendix 1

Table A1. Ingredient composition of experimental diets (as-fed basis) for diets fed from 11.9 to 29.5 kg (Phase 1)

Ingredient name, %	**T1[1]**	**T3**	**T4**	**T6**
Corn	54.79	54.83	60.64	60.66
Distiller's dried grain with solubles	20.00	20.00	20.00	20.00
Soybean meal	21.00	21.00	15.73	15.77
L-Lysine HCl	0.64	0.64	0.49	0.49
DL-Methionine	0.22	0.22	0.12	0.12
L-Threonine	0.24	0.24	0.15	0.15
L-Tryptophan	0.07	0.01	0.05	0.00
L-Isoleucine	0.07	0.07	0.01	0.01
L-Valine	0.14	0.14	0.05	0.05
Monocalcium phosphate	0.50	0.50	0.57	0.57
Limestone	1.28	1.28	1.43	1.43
Salt	0.41	0.41	0.42	0.41
Phytase (Axtra® 2500)[2]	0.03	0.03	0.03	0.03
Vitamin premix[3]	0.15	0.15	0.15	0.15
Trace mineral premix[3]	0.15	0.15	0.15	0.15
Corn oil	0.30	0.33	0.00	0.00
Nutrient composition	**T1**	**T3**	**T4**	**T6**
SID Lys, % (calculated)	1.23	1.23	0.99	0.99
SID Trp, % (calculated)	0.26	0.20	0.21	0.16
SID Trp:Lys (calculated)	0.21	0.16	0.21	0.16
Net energy, kcal/kg (calculated)	2400	2400	2400	2400
Dry matter, %	86.18	86.44	86.40	85.62
Crude protein, %	20.14	20.57	18.38	18.43
Lys, %	1.29	1.30	1.04	1.08
Trp, %	0.26	0.22	0.20	0.18
Neutral detergent fiber, %	12.0	11.9	11.7	11.5
Ether extract, %	3.87	3.96	3.64	3.44
Ash, %	4.38	4.11	4.74	4.69
Ca, %	0.57	0.57	0.78	0.88
P, %	0.56	0.56	0.59	0.56
STTD P, % (calculated)	0.29	0.29	0.29	0.29

[1]T2 and T5 were prepared by mixing equal proportions of T1 and T3, and of T4 and T6, respectively.
[2]Phytase released 0.12% avP; 0.105% STTD P, and 0.24 total Ca.
[3]The combined vitamin and trace mineral premix provided per kg feed: 2,640 IU vitamin A, 880 IU vitamin D, 26.4 IU vitamin E, 2.75 mg vitamin K, 22 µg vitamin B12, 30.8 mg niacin, 17.6 mg pantothenic acid, 5.5 mg riboflavin, 242 mg/kg zinc (zinc sulfate), 242 mg/kg Fe (iron sulfate), 88 mg/kg Mn (manganese sulfate), 26.4 mg/kg Cu (copper sulfate), and 0.66 mg/kg Se (sodium selenite), and 450 phytase units per kilogram of diet.

Appendix 1.

Table A2. Ingredient composition of experimental diets (as-fed basis) for diets fed from 29.5 to 49.6 kg (Phase 2)

Ingredient name, %	**T1[1]**	**T3**	**T4**	**T6**
Corn	49.25	49.28	56.48	56.57
Distiller's dried grain with solubles	30.00	30.00	30.00	30.00
Soybean meal	17.14	17.14	10.64	10.64
L-Lysine HCl	0.56	0.56	0.47	0.47
DL-Methionine	0.13	0.13	0.05	0.05
L-Threonine	0.17	0.17	0.11	0.11
L-Tryptophan	0.06	0.00	0.04	0.00
L-Isoleucine	0.02	0.02	0.00	0.00
L-Valine	0.09	0.09	0.03	0.03
Monocalcium phosphate	0.24	0.24	0.32	0.32
Limestone	1.27	1.27	1.32	1.29
Salt	0.34	0.34	0.35	0.35
Phytase (Axtra® 2500)[2]	0.03	0.03	0.03	0.03
Vitamin-mineral premix[3]	0.15	0.15	0.15	0.15
Corn oil	0.56	0.58	0.00	0.00
Nutrient composition	**T1**	**T3**	**T4**	**T6**
SID Lys, % (calculated)	1.11	1.11	0.89	0.89
SID Trp, % (calculated)	0.23	0.18	0.19	0.14
SID Trp:Lys (calculated)	0.21	0.16	0.21	0.16
Net energy, kcal/kg (calculated)	2418	2418	2418	2418
Dry matter, %	86.21	86.44	86.76	86.36
Crude protein, %	21.03	20.99	18.67	18.56
Lys, %	1.20	1.19	1.01	0.99
Trp, %	0.24	0.20	0.19	0.16
Neutral detergent fiber, %	13.1	12.5	12.7	12.8
Ether extract, %	4.70	4.65	4.47	4.08
Ash, %	4.50	4.48	4.35	4.27
Ca, %	0.61	0.65	0.64	0.60
P, %	0.49	0.50	0.51	0.50
STTD P, % (calculated)	0.26	0.26	0.26	0.26

[1]T2 and T5 were prepared by mixing equal proportions of T1 and T3, and of T4 and T6, respectively.

[2,3] See Table A1

Appendix 1.

Table A3. Ingredient composition of experimental diets (as-fed basis) for diets fed from 49.6 to 62.6 kg (Phase 3)

Ingredient name, %	**T1[1]**	**T3**	**T4**	**T5**
Corn	54.37	54.40	60.47	60.51
Distiller's dried grain with solubles	30.00	30.00	30.00	30.00
Soybean meal	12.62	12.62	7.04	7.04
L-Lysine HCl	0.50	0.50	0.42	0.42
DL-Methionine	0.08	0.08	0.01	0.01
L-Threonine	0.13	0.13	0.08	0.08
L-Tryptophan	0.05	0.00	0.04	0.02
L-Isoleucine	0.01	0.01	0.00	0.00
L-Valine	0.06	0.06	0.00	0.00
Monocalcium phosphate	0.03	0.03	0.10	0.10
Limestone	1.19	1.19	1.30	1.28
Salt	0.35	0.35	0.36	0.36
Phytase (Axtra® 2500)[2]	0.03	0.03	0.03	0.03
Vitamin-mineral premix[3]	0.15	0.15	0.15	0.15
Corn oil	0.44	0.47	0.00	0.00
Nutrient composition	**T1**	**T3**	**T4**	**T5**
SID Lys, % (calculated)	0.98	0.98	0.77	0.77
SID Trp, % (calculated)	0.20	0.15	0.16	0.12
SID Trp:Lys (calculated)	0.21	0.16	0.21	0.16
Net energy, kcal/kg (calculated)	2442	2442	2442	2442
Dry matter, %	86.90	87.02	86.99	86.23
Crude protein, %	18.95	19.18	17.13	17.00
Lys, %	1.02	1.08	0.96	0.86
Trp, %	0.21	0.19	0.18	0.19
Neutral detergent fiber, %	12.8	13.3	12.7	13.7
Ether extract, %	4.24	4.34	3.73	3.87
Ash, %	4.24	4.08	4.06	3.74
Ca, %	0.62	0.62	0.57	0.54
P, %	0.47	0.49	0.52	0.52
STTD P, % (calculated)	0.21	0.21	0.21	0.21

[1]T2 was prepared by mixing equal proportions of T1 and T3.

[2,3] See Table A1

Appendix 1.

Table A4. Ingredient composition of experimental diets (as-fed basis) for diets fed from 62.6 to 80.3 kg (Phase 4)

Ingredient name, %	**T1**[1]	**T3**	**T4**	**T5**
Corn	70.53	70.71	72.87	72.95
Distiller's dried grain with solubles	15.00	15.00	15.00	15.00
Soybean meal	12.03	11.87	9.82	9.76
L-Lysine HCl	0.40	0.41	0.26	0.27
DL-Methionine	0.07	0.08	0.00	0.00
L-Threonine	0.12	0.12	0.04	0.04
L-Tryptophan	0.04	0.00	0.02	0.00
L-Isoleucine	0.02	0.02	0.00	0.00
L-Valine	0.08	0.08	0.00	0.00
Monocalcium phosphate	0.04	0.04	0.07	0.07
Limestone	1.03	1.04	1.28	1.28
Salt	0.46	0.46	0.46	0.46
Phytase (Axtra® 2500)[2]	0.03	0.03	0.03	0.03
Vitamin-mineral premix[3]	0.15	0.15	0.15	0.15
Corn oil	0.00	0.00	0.00	0.00
Nutrient composition	**T1**	**T3**	**T4**	**T5**
SID Lys, % (calculated)	0.82	0.82	0.65	0.65
SID Trp, % (calculated)	0.17	0.13	0.14	0.10
SID Trp:Lys (calculated)	0.21	0.16	0.21	0.185
Net energy, kcal/kg (calculated)	2459	2459	2459	2459.
Dry matter, %	85.38	85.91	85.80	85.52
Crude protein, %	15.61	15.32	14.40	14.09
Lys, %	0.88	0.84	0.67	0.70
Trp, %	0.17	0.14	0.15	0.14
Neutral detergent fiber, %	9.8	9.1	10.0	9.4
Ether extract, %	3.96	3.94	3.83	3.70
Ash, %	3.07	3.23	3.10	3.26
Ca, %	0.40	0.50	0.44	0.50
P, %	0.35	0.36	0.36	0.37
STTD P, % (calculated)	0.17	0.17	0.17	0.17

[1]T2 was prepared by mixing equal proportions of T1 and T3.

[2,3] See Table A1

Appendix 1.

Table A5. Ingredient composition of experimental diets (as-fed basis) for diets fed from 80.3 to 103.3 kg (Phase 5)

Ingredient name, %	T1[1]	T3	T4	T5
Corn	79.02	79.05	79.57	79.58
Distiller's dried grain with solubles	9.75	9.75	9.75	9.74
Soybean meal	8.66	8.65	8.51	8.51
L-Lysine HCl	0.40	0.40	0.22	0.22
DL-Methionine	0.07	0.07	0.00	0.00
L-Threonine	0.12	0.12	0.03	0.03
L-Tryptophan	0.04	0.01	0.01	0.00
L-Isoleucine	0.04	0.04	0.00	0.00
L-Valine	0.09	0.09	0.00	0.00
Monocalcium phosphate	0.00	0.00	0.00	0.00
Limestone	0.94	0.94	0.95	0.95
Salt	0.50	0.50	0.50	0.50
Phytase (Axtra® 2500)[2]	0.03	0.03	0.03	0.03
Vitamin-mineral premix[3]	0.15	0.15	0.15	0.15
Corn oil	0.19	0.20	0.29	0.30
Nutrient composition	**T1**	**T3**	**T4**	**T5**
SID Lys, % (calculated)	0.72	0.72	0.57	0.57
SID Trp, % (calculated)	0.15	0.11	0.12	0.11
SID Trp:Lys (calculated)	0.21	0.16	0.21	0.185
Net energy, kcal/kg (calculated)	2502	2502	2502	2502
Dry matter, %	84.92	84.93	85.34	85.64
Crude protein, %	13.86	13.51	13.79	13.82
Lys, %	0.80	0.75	0.69	0.67
Trp, %	0.15	0.13	0.14	0.14
Neutral detergent fiber, %	9.4	8.0	8.3	8.6
Ether extract, %	4.02	3.66	5.21	4.02
Ash, %	3.03	3.11	3.00	2.96
Ca, %	0.45	0.46	0.50	0.34
P, %	0.30	0.29	0.29	0.29
STTD P, % (calculated)	0.14	0.14	0.14	0.14

[1]T2 was prepared by mixing equal proportions of T1 and T3.

[2,3] See Table A1

Appendix 2.

Exploring Surplus Resource Theory and the role of dietary energy in the expression of play behaviour in pigs

Eleanor Hewett*, Animal Behaviour and Welfare, School of Veterinary Medicine and Biosciences, Scotland's Rural College (SRUC), Edinburgh EH9 3JG

Jos Houdijk, Monogastric Science Research Centre, School of Veterinary Medicine and Biosciences, Scotland's Rural College (SRUC), Edinburgh, EH9 3JG

Craig Lewis, PIC Product Development, PIC, C/Pau Vila, 22 2o piso, 08174, Sant Cugat del Valles, Barcelona, Spain

Andrea Doeschl-Wilson, The Roslin Institute, The Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Edinburgh, EH25 9RG

Simon Turner, Animal Behaviour and Welfare, School of Veterinary Medicine and Biosciences, Scotland's Rural College (SRUC), Edinburgh, EH9 3JG

Abstract

Surplus Resource Theory proposes that play behaviour is expressed more abundantly in juveniles that have access to necessary resources. We propose that sufficient dietary energy is likely to be a requirement for play and therefore hypothesise that pigs on an energy-deficient diet will show reduced play. A total of 128 mixed sex pigs of 28 days old, were randomly allocated across two diets: control (CE) or low energy (LE). Pigs were given access to a play arena, for 20 minutes, 4 times over 10 days and on alternative occasions, before entering the arena, offered a sucrose supplement. We hypothesised the provision of sucrose would increase play, particularly for pigs on the LE diet. Dietary treatment and play session interacted on social object ($P=0.025$), solo locomotor ($P<0.001$) and social locomotor play ($P<0.001$). Dietary treatment and pen mate average sugar intake interacted on social object ($P=0.019$) and social locomotor play ($P<0.001$). The interaction between dietary treatment and pen mate average sugar intake ($P=0.002$) affected total play. Overall, the CE diet appeared to extend the developmental period of heightened engagement in social play. Our findings suggest that diet quality in weaned pigs may impact specific aspects of play behaviour with potential benefits for animal welfare.

Key words

Surplus Resource Theory, pig, play, energy, sugar

Introduction

Positive social behaviours have been shown to be critical for physical, physiological and psychological development in mammals. Positive social behaviour in animals is a relatively novel area of study, with much of the focus thus far being on play behaviour. Play or playful behaviours have been observed in an extensive range of species, including birds [1], reptiles [2], mammals [3], fish [4], cephalopods [5,6] and potentially insects [7]. Play is important for the development of cognitive, motor and social abilities[3]. Lawrence[8] proposed that play is only performed when environmental conditions are 'good' and an animal's 'proximate needs' have been met. Environments with good conditions, that meet an animals' needs, would be considered to contribute to relatively higher welfare. Burghardt's[9] Surplus Resource Theory (SRT) proposes that play behaviour is expressed in juveniles that have access to the necessary resources (e.g. a safe environment, time and adequate nutrition to support energy-demanding activities)[10]. Burghardt proposed that play is most likely to evolve in young, warm-blooded animals (which is now being debated, see above for fish, reptile and insect play), with long juvenile phases and where food and protection are provided by the parent/parents.

Mammalian livestock species show a rich variety of play (e.g. cattle [11], sheep [12], and pigs [13]). Play behaviours are most commonly seen early in life and are similar to behaviours seen in adults like hunting and fighting, with the distinguishing features of being exaggerated, energetic, repetitive and non-damaging [9]. Play can be grouped into various (overlapping) categories: locomotor, object-directed, individual and social. Locomotor play can be performed individually or with conspecifics and can involve running, pivots, flops, hops and jumps [13–15]. Object-directed play involves manipulating, carrying or shaking an object [13]. Social play is between two or more individuals and involves nudging, pushing, climbing and non-harmful fighting. Play fighting usually involves elements of adult fighting but without injury and in which role reversals and handicapping may be seen depending on the species [16–18].

Play behaviour is typically energetically demanding. Berghänel et al. [19] demonstrated that play was energetically demanding, as locomotor play accounted for up to 50% of variance in growth in macaques. Hence the Surplus Resource Theory predicts that, under natural, wild conditions, play will occur primarily when nutritional resources are adequate and risks of injury or predation are mitigated. In this paper we focus only on the effects of diet quality on play, whilst acknowledging that other enablers of play are likely to be important.

Pigs are a social species with rich expression of play that can be stimulated experimentally. They therefore make an excellent study species to test the Surplus Resource Theory. Play has been suggested as an indicator of animal welfare [20], in domestic pigs. Barnes et al.[21] observed that malnourished piglets spent less time playing with objects than well-nourished piglets whilst play behaviour has been shown to positively relate to weight gain both pre- and post-weaning [22–24]. Studies have also shown that pigs reared with increased opportunities to play had enhanced resilience to disease [25]. There is increasing interest among researchers, producers and consumer to provide commercial pigs with a "life worth living". One avenue being explored to achieve this goal is the promotion of positive social behaviours like play. Therefore, this study may also provide a first step towards developing nutritional strategies to promote expression of positive social behaviour and improve welfare for commercial pigs. Thus far, studies have not yet explored causation by empirically manipulating the diet and examining the effect on a wide repertoire of play behaviours. We aim to test the central hypothesis of the Surplus Resource Theory through direct manipulation of dietary energy as the first limiting nutritional resource. We will study the effects on a wide repertoire of play behaviours in an environment that encourages expression of play. We hypothesise that pigs on an energy-deficient diet will show reduced play.

In addition to energy derived from the diet, we aim to explore how acute supplementary energy provision will affect play behaviour of pigs. In humans, carbohydrate supplementation has been shown to benefit endurance performance [26], providing an ergogenic effect [27]. We hypothesise that provision of sugar before exercise, play, will have a similar effect on pigs and that the play deficit seen in low energy pigs may be temporarily reversed by sucrose provision.

Methods

Ethical Review

The trial was reviewed and approved by the Scotland's Rural College (SRUC) Animal Experiments Committee (research programme number PIG RP 4-2022, animal experiment number PIG AE 11-2022) and was licensed by the Home Office (Home Office project licence number PP1403242). Authors complied with ARRIVE guidelines 2.0, Essential 10. All routine animal management procedures were performed by trained staff and health issues were treated as required. All stages of the experiment were conducted in liaison with the SRUC veterinary surgeon.

Animals and Animal Housing

A total of 128 (68 male and 60 female) (Large White × Landrace) × Danish Duroc domestic piglets were used over two farrowing batches (64 per batch, each batch consisted of 8 pens, with 8 pigs per pen.

The piglets were born at SRUC's Easter Howgate Pig Unit. Teeth and tails were left intact, and males were not castrated. From 21 days, pigs were provided with small amounts of creep feed. Piglets were weaned at 28 days, receiving a wean tag and vaccination as per normal farm procedure and allocated to mixed sex litter groups of 8 pigs. Groups contained on average 4 sibling pairs from different litters, of similar wean weights (to minimise aggression and bullying) and where possible each litter-pair comprised of one male and one female. Siblings were balanced across diets. Groups were housed at a space allowance of 1.28m^2 per pig (pen size 5.1 x 2.0m) on solid concrete floors with a light sawdust bedding, provided with basic enrichment (1 Porcichew and 1 old wellington boot) and food and water was available ad libitum via two bowl drinkers and two fixed plastic feeders. Pens were cleaned manually once daily. Pigs were on trial for a total of 35 days after which they were returned to farm stock.

Play arena

A separate play arena, measuring 5.1 x 5.1m was used for play test sessions in which play was stimulated and observed. The play arena had solid concrete flooring, with deep sawdust and straw cover and was located in the same building as the home pens. . Additional enrichment toys were added to the play arena; 1 2m length of natural fibre rope knotted in the middle, one empty feed sack, one plastic ball and one silicone dog toy. Waste was removed from the play arena between each group play session. Cameras (Canon Legria HFG25) were set at opposite corners of the play arena to capture the behaviour of all pigs in the play arena. (Figure 1).

Habituation

All pigs were habituated to human presence/touch from 5 days post farrowing to allow for individual marking. The researchers sat in the creep area of the pen, speaking softly to the piglets, allowing them to approach in their own time. After approaching, piglets were offered the opportunity to explore the researcher and when piglets appeared confident, gentle handling was performed, stroking and tickling the piglets as per individual piglet preference. Post weaning, pigs were given 6 days to settle in their new pens and then habituated to a weigh crate and the play arena 4 times prior to test sessions, once daily over three consecutive days, with one day rest on day 4, before a final habituation session on the 5th day. One pen at a time, the social group moved from their home pen to the play arena via the weigh crate. Pigs were released from their home pen and encouraged by the researcher to walk down to the play arena. During the first 3 habituations, toys had been placed in the play arena before pigs entered; on the final habituation session, toys were added to the pen after the pigs had entered to mimic the test days (described below). The first habituation of the play arena lasted 5 minutes and the duration of subsequent sessions increased by an additional 5 minutes till they reached the full 20 minutes as used for the tests.

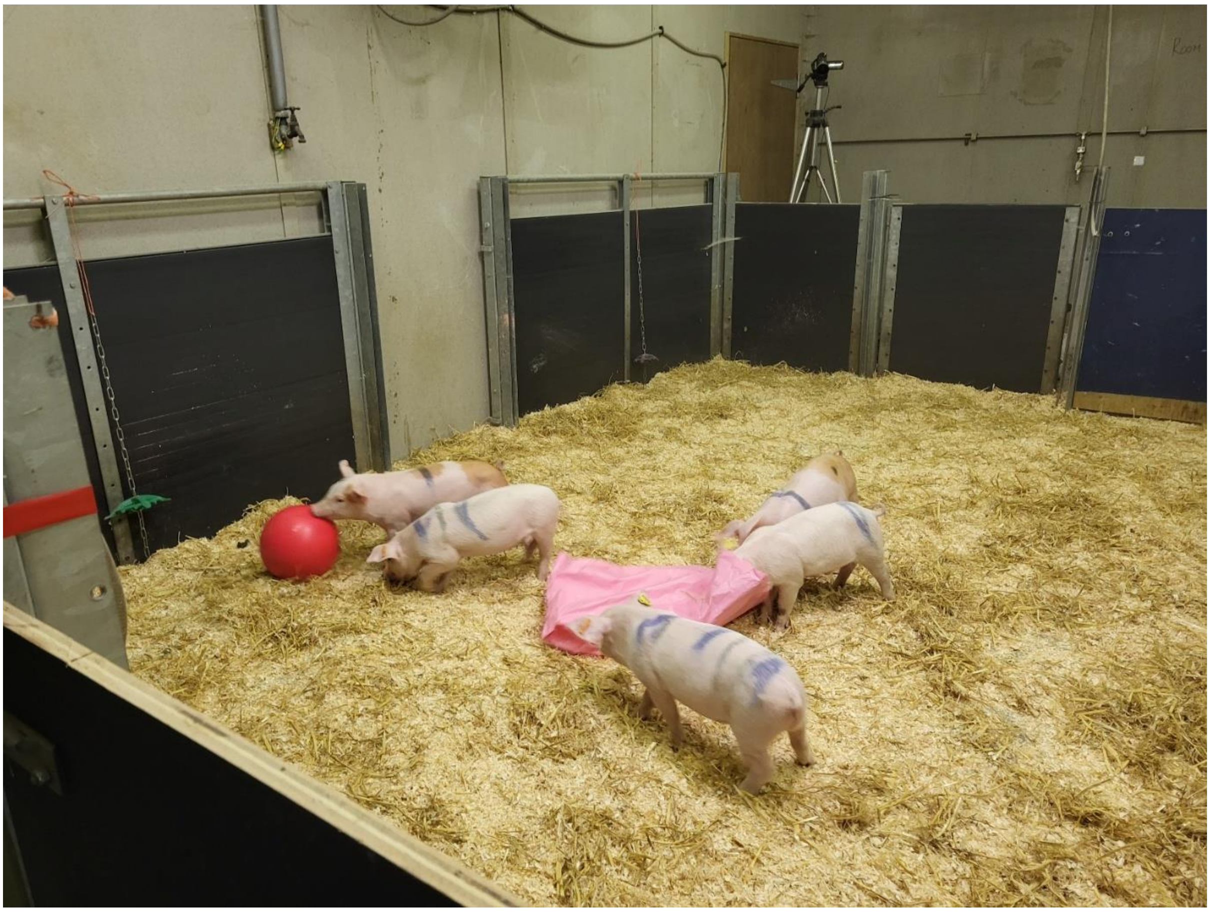


Figure 1. Play arena. A separate play arena was used, measuring 5.1 x 5.1m, with solid concrete flooring, with deep sawdust and straw cover. Photo shows pigs in play arena, interacting with toys.

Experimental treatments

After weaning (at 28 days) pens of pigs were randomly allocated to one of two dietary treatments: a control diet that represented the commercial norm (CE) (digestible energy 16.9 MJ/kg, fibre 8.5%, crude protein 18.1%) or a low energy (LE) diet achieved by diluting the control diet with 15% indigestible fibre, Arbocel (digestible energy 15.3 MJ/kg, fibre 18.4%, crude protein 16.1%). 15% dilution was chosen to ensure LE pigs would reach their bulk constraint. Full diet compositions, which had a basis of wheat, maize and soya, can be found in Appendix 1. Feeds were sampled daily, pooled by treatment and analysed for digestible energy through regression on chemical analysis outcomes (Table 1).

Table 1. Ingredients and chemical analysis of the experimental diets; a control diet that represented the commercial norm (CE) and a lower energy (LE) diet achieved by diluting the control diet with 15% indigestible fibre, Arbocel.

Ingredients	Control Diet (CE) (%)	Low Energy Diet (LE) (calculated) (%)
Micronized wheat	20	17
Wheat	24.32	20.67
Cooked dehulled oats	6	5.1
Micronized maize	10	8.5
Fishmeal	1.5	1.28
AlphaSoy*	5	4.25
Full fat soya	7	5.95
Soya bean meal	3.5	2.98
Soya protein concentrate	4	3.4
Whey powder	10	8.5
Fat-filled whey (coconut)	4	3.4
Vegetable oil	1	0.85
Limestone	1.12	0.95
Monocalcium phosphate	0.66	0.56
Sodium bicarbonate	0.23	0.20
Salt	0.05	0.04
l-Lysine-HCl	0.54	0.46
dl-Methionine	0.14	0.12
l-Threonine	0.234	0.2
l-Tryptophan	0.045	0.04
L-valine	0.15	0.13
Premix	0.5	0.425
Arbocel	0	15
Pig flavour (vanilla/caramel)	0.025	0.021

Chemical analysis	Control Diet (CE)	Low Energy Diet (LE)
Dry Matter (DM), g/kg	892	896
Digestible energy**, MJ/kg DM	16.89	15.32
Neutral Detergent Fibre, g/kg DM	95.29	205.36

Ether Extract, g/kg DM	63.68	58.59
Total Oil, g/kg DM	70.52	65.96
Crude Protein, g/kg DM	202.91	179.69
Ash, g/kg DM	59.42	52.46

*Enzyme-treated soya bean meal with typical crude protein content of 530 g/kg

**Digestible energy is calculated through regression: 17.47 + 0.0079 × Crude Protein + 0.0158 × Total Oil - 0.0331 × Ash - 0.014 × Neutral Detergent Fibre[28].

Sucrose supplementation and play arena sessions

From day 10 post weaning at 38 days old, piglet home groups were moved from their home pen to the play arena on 4 occasions over 10 days, balanced for time of day across treatments, with two days between play sessions. Movement of pigs was facilitated by primary researcher, who remained constat throughout the trial. Pigs were moved using pig boards initially but as the trial progressed, arm movements form the researcher was sufficient. Each group consistently entered the play arena in either the morning or afternoon (balanced across diet). Before entering the play arena, all pigs were weighed in a weigh crate. On alternate occasions, the piglets were given a sucrose supplement while in the weigh crate immediately before entering the play arena. All pigs were held in the weigh crate for 60 seconds, whether offered sugar or not. The amount of sugar given was relative to body weight and increased over the course of the trial. To our knowledge, this is the first trial to supplement pigs with sugar cubes. As there is no previous research on the appropriate dose, we used the UK National Health Service recommended daily intake for children: 4-6 year olds, average weight range of 8-16kg, 5 cubes and 7-10 year olds, average weight range 17-44kg, 6 cubes[29]. At testing, our pigs fell into these weight groups. It was expected that sugar intake would have an acute effect on play but would not be sufficient to alter longer term growth performance. Pigs chose to eat varying amounts of the sugar offered as described below.

Play arena behaviour

All 8 piglets from the home group were released into the play arena simultaneously and immediately after weighing. To allow time for maximum locomotor play, after the initial 5 minutes in the play arena the toys: rope, sack, ball and dog toy were added to the arena. After a total of 20 minutes in the play arena, the pigs were returned to their home pen. The pigs' behaviour in the play arena was recorded continuously from video images using an ethogram (Table 2) for a total of 15 minutes per pen, 5 minutes without toys and 10 minutes with toys. Time without toys was given to allow for maximum expression of locomotor play and play fighting. Each pig was continuously sampled, with the frequency of behaviours recorded.

Table 2. Ethogram used to measure behaviour of piglets taken from video recordings taken from play arena.

Behaviour	Description	Reference
Solo locomotor play	A pig is scampering, running, pivoting, head tossing, flopping or hopping. Not associated with delivery or receipt of aggression but sometimes with gentle play butt or play push	Ocepek et al., 2020
Social locomotor play	A pig is scampering, running, pivoting, head tossing, flopping or hopping together with at least one other pig. Not associated with delivery or receipt of aggression but sometimes with gentle play butt or play push	Ocepek et al., 2020; Camerlink et al., 2021

Solo object play	Pig manipulates object with snout, mouth or paw. Includes sniffing, rooting, gripping with teeth, shaking of head while holding object, dragging object, pawing, pushing or moving object.	Adapted from Lidfors et al., 2021
Social object play	Pig manipulates object with snout, mouth or paw. Includes sniffing, rooting, gripping with teeth, shaking of head while holding object, dragging object, pawing, pushing or moving object. within approximately 30cm of another pig	Adapted from Lidfors et al., 2021
Play fighting	Mutual ramming or pushing, without aggressive/damaging biting. Actions performed in a repetitive and energetic manner	Adapted from Camerlink and Turner, 2013 and Weller et al., 2019
Aggression	Head knocking, fighting, biting, ramming which elicits retaliatory or retreat behaviour from the recipient	Adapted from Camerlink and Turner, 2013

Skin lesion scoring

Skin lesion scoring was performed at various points thought the trial; on days 1, 2 and 3 post weaning, upon entering the play arena (while in the weigh crate) and shortly after returning to their home pen after leaving the play arena. All pigs were lesions scored. Lesions were recorded to reflect the level of social stress within the group, which could impact play behaviour, and to quantify whether behaviour recorded as play fighting in the arena was correctly recorded as such, rather than being injurious aggression. The following scoring system was used to record the position and number of lesions. Position of lesions: front (from the shoulder to the tip of the nose), middle (between the shoulder and the start of the back leg) and back (from the start of the back leg to the tip of the tail). The number of fresh, bright red lesions measuring longer than 1cm, with no scab formation, was categorised as follows: 0 = no lesions, 1 = 1-5 lesions, 2 = 6-10 lesions, 3 = 11-15 lesions, 4 = 16-20 lesions and 5 = >20 lesions (score system adapted from Camerlink et al., 2016).

Growth measures

Piglets were weighed individually at weaning (28 days), at day 8 post weaning and then every time they moved to the play arena thereafter. Feed consumption was recorded on a pen level. Daily feed delivery and refusal was weighed manually. The data derived were used to estimate average daily feed intake (ADFI), average daily body weight gain (ADG) and feed conversion ratio (FCR, calculated as ADFI/ADG).

Statistical analysis

Statistical analyses were carried out using R Software version 4.4.2 [34] and RStudio 2024.12.1+563 "Kousa Dogwood" Release for Windows. Models were constructed using packages lme4 [35] and glm2 [36]. Model residuals were visually inspected to confirm approximation to normality and tested for heteroscedasticity using Levene's Test. P-values below 0.05 were considered statistically significant and P-values between 0.05 and 0.1 were considered as tendencies.

For arena behaviours, the pig was the statistical unit. Data were modelled using a generalised linear mixed model (GLMER), with link functions "log" and "logit", for frequency of play fighting, aggression, solo object play, social object play and solo locomotor play, whilst a linear mixed model (LMER) was used for social locomotor play and total play. Not all pigs ate all of the sugar offered. We therefore accounted for the quantity of sugar eaten by the individual, and the average eaten by the pen mates of the subject animal. Dietary treatment, play session, sex and individual and pen mate average sugar intake were included as fixed effects as well as first order interactions between dietary treatment and play session, dietary treatment and individual sugar intake and dietary treatment and pen mate average sugar intake. Interactions that were not significant at $p<0.05$ were removed from the models. Weight and pig nested in pen, nested in batch were included as random effects. Individual sugar intake was categorised using 4 scores; 0 = sugar not offered, 1 = sugar offered but not eaten, 2 = ate up to half the sugar offered, 3 = ate half or more of the sugar offered. For pen mate average sugar intake there were 3 scores; 0 = sugar not offered, 1 = ate up to half the sugar offered on average, 2 = ate more than half the sugar offered on average.

For skin lesions scores, the pig was the statistical unit, and data were coded to show lesion increases; pigs were lesion scored before and after entering the play arena and if there was an increase in lesion score pigs were coded 1, if there was no increase in lesion score, pigs were coded 0. Data were modelled using a GLMER with link function "logit". Dietary treatment, play session, sex and individual and pen mate average sugar intake were included as fixed effects as well as first order interactions between dietary treatment and play session, dietary treatment and individual sugar intake and dietary treatment and pen mate average sugar intake. Weight and pig nested in pen, nested in batch were included as random effects.

Growth measures data were collected and analysed at the pen level, over a period of 20 days, covering the habituation period (10 days) and 4 play sessions (10 days), with the pen being the statistical unit, averaged to give ADFI and ADG per pig. Data were modelled using an LMER, dietary treatment was included as a fixed effect and pen was included as a random effect.

Results

Total Play

Play session (P<0.001), and dietary treatment x pen mate average sugar intake (P=0.002) affected frequency of total play. Total play, combined across both treatments, started to reduce after session 2 There was a 16.74% decrease in average total play between session 2 and session 3, then a 14.61% decrease from session 3 to session 4. Total play was higher in CE diet pigs when pen mate average sugar intake was scored 0 (not offered sugar) and 1 (ate less than half the sugar offered) than LE pigs with the same pen mate average sugar intake. With pen mate average sugar intake of 2 (ate more than half the sugar), levels of total play were higher in LE pigs than in CE pigs. In LE pigs, total play was equal in pigs with pen mate average sugar intake of 0 and 1 and higher in pens of pen mate average sugar intake 2. In CE pigs, total play was equal in pens of pen mate average sugar intake score 0 and 2, and higher in pens of pen mate average sugar intake 1 (Figure1).

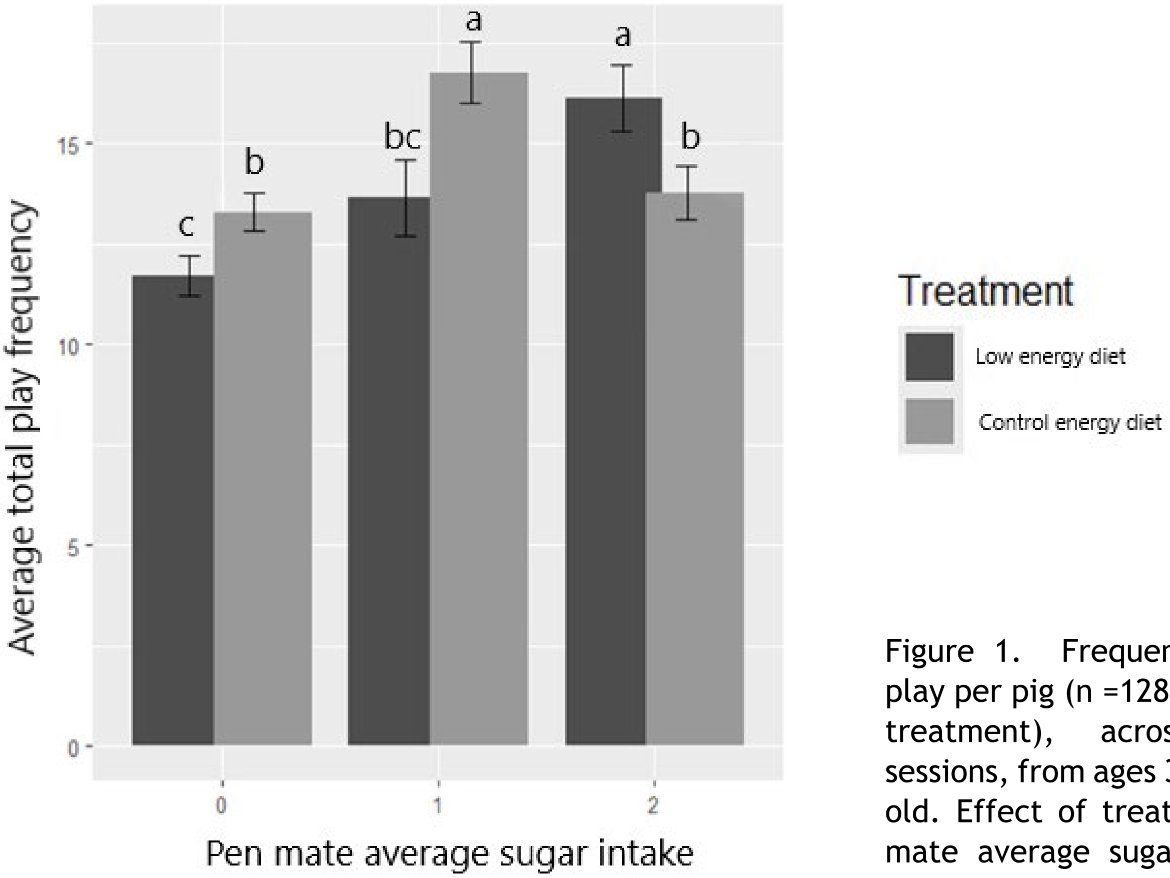


Figure 1. Frequency of total play per pig (n =128, 64 pigs per treatment), across 4 pay sessions, from ages 38 to 48 days old. Effect of treatment x pen mate average sugar intake on the average frequency of total play per pig on low energy (LE) and control energy (CE) dietary treatments. Non-matching superscripts denote statistical significance (P=0.002). Predicted means and lower and upper 95% confidence intervals are presented.

Locomotor play

There was an effect of sex (P=0.002) and a dietary treatment × play session interaction (P<0.001) on frequency of solo locomotor play. Solo locomotor play was higher in female pigs. Solo locomotor play was higher in CE diet pigs in play session 3 only. Solo locomotor play levels stayed constant for CE pigs across play sessions but started to reduce in LE pigs at session 2 (Figure2).

For social locomotor play, there was an effect of sex (P=0.05), dietary treatment x play session (P<0.001), and dietary treatment x pen mate average sugar intake (P<0.001). The effect of individual sugar intake tended towards significant (P=0.06). Social locomotor play was higher in females. Social locomotor play was higher in LE diet pigs in session 1 but higher in CE diet pigs in session 3 (Figure 3). Average frequency of social locomotor play was equal between LE and CE pigs at play session 4. Levels of social locomotor play reduce between play session 2 and 3 for LE pigs and between play session 3 and 4 for CE pigs.

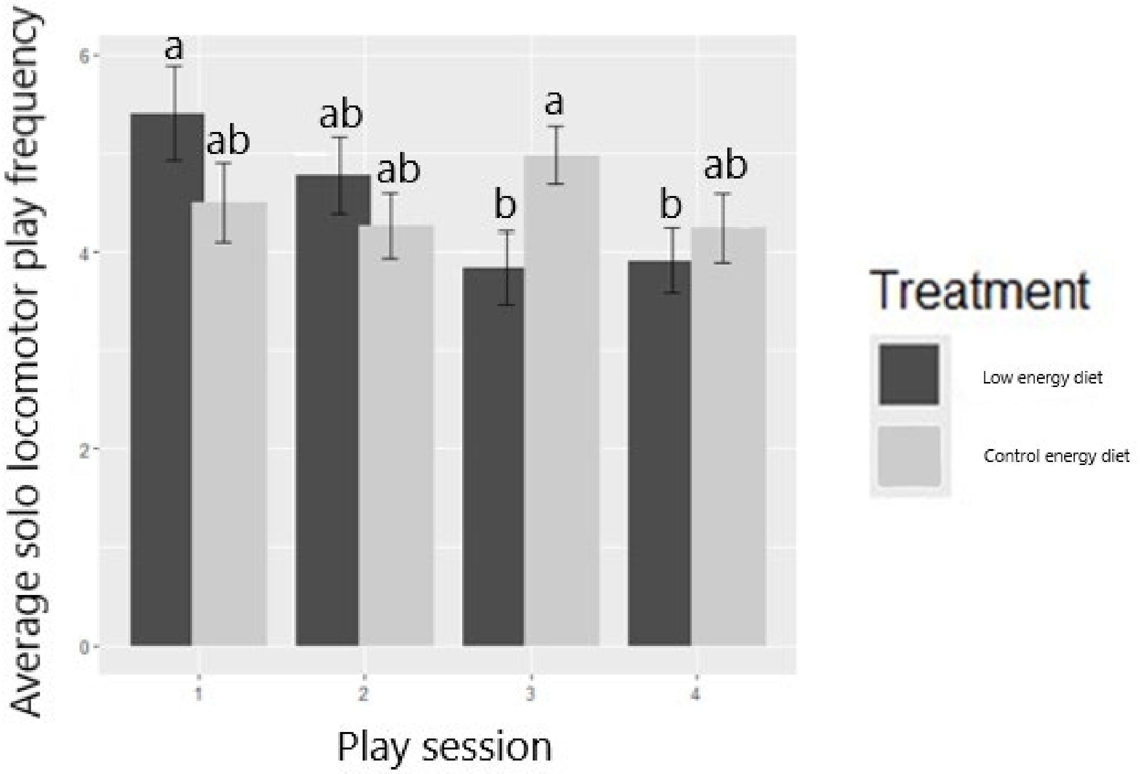


Figure 2. Frequency of solo locomotor play per pig (n =128, 64 pigs per treatment), across 4 pay sessions, from ages 38 to 48 days old. Effect of treatment x play session on the average frequency of solo locomotor play per pig on low energy (LE) and control energy (CE) dietary treatments.. Non-matching superscripts denote statistical significance (P<0.001). Predicted means and lower and upper 95% confidence intervals are presented.

Frequency of social locomotor play was higher in CE diet pigs with pen mate average sugar intake scores of 0 (not offered sugar) and 1 (ate less than half the sugar offered)than that of LE diet pigs with the same pen mate average sugar intake scores. For pen mate average sugar intake score 2 (ate more than half the sugar offered), LE diet pigs showed more social locomotor play than CE diet pigs. For LE pigs, levels of social locomotor play were equal when pen mate average sugar intake was scored 0 and 1 but higher when scored 2. For CE pigs, social locomotor play increased from pen mate average sugar intake score of 0 to 1 then dropped at pen mate average sugar intake score of 2 to levels below pen mate average sugar intake of 0 (Figure 4).

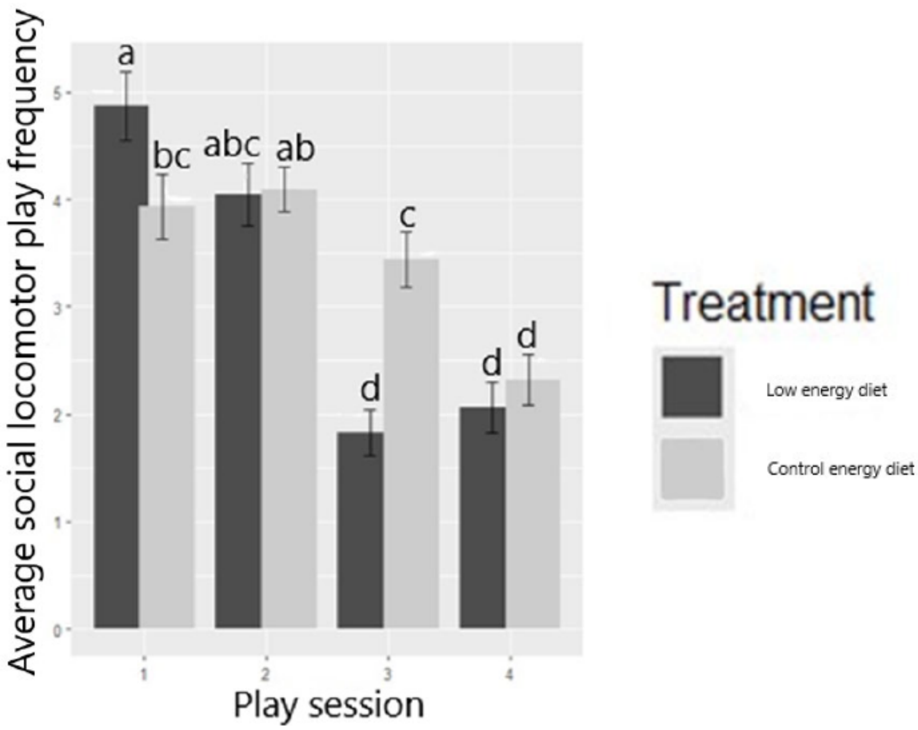


Figure 3. Frequency of social locomotor play per pig (n =128, 64 pigs per treatment), across 4 pay sessions, from ages 38 to 48 days old. Effect of treatment x play session on the average frequency of social locomotor play per pig on low energy (LE) and control energy (CE) dietary treatments. Non-matching superscripts denote statistical significance (P<0.001). Predicted means and lower and upper 95% confidence intervals are presented.

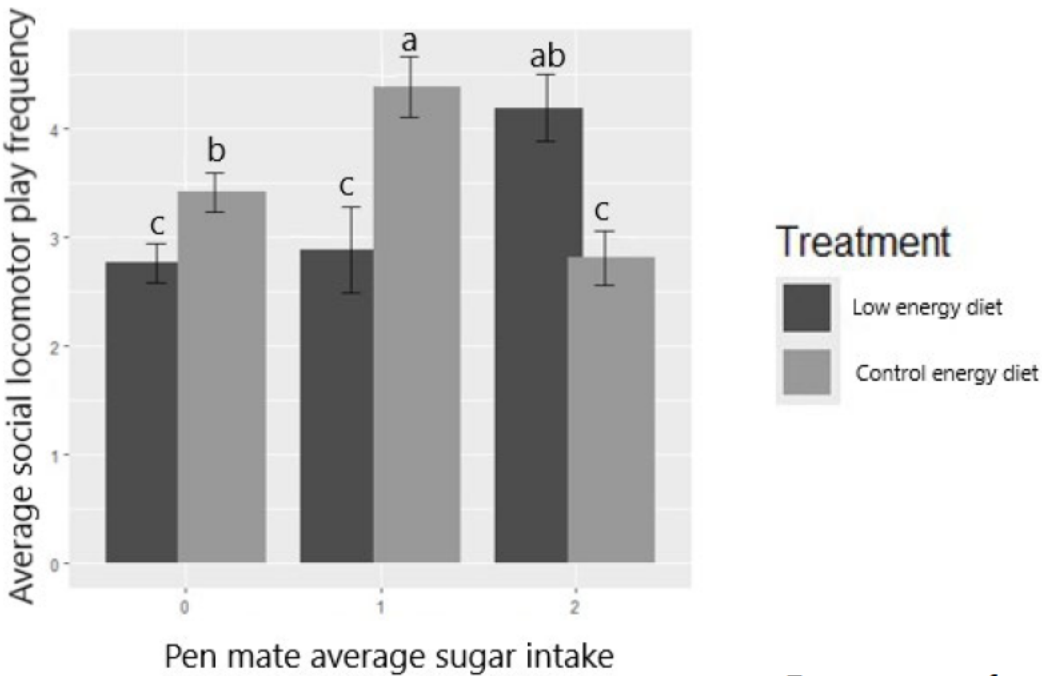


Figure 4. Frequency of social locomotor play per pig (n =128, 64 pigs per treatment), across 4 pay sessions, from ages 38 to 48 days old. Effect of treatment x pen mate average sugar intake on the average frequency of social locomotor play per pig on low energy (LE) and control energy (CE) dietary treatments. Non-matching superscripts denote statistical significance (P<0.001). Predicted means and lower and upper 95% confidence intervals are presented.

Object play

Sex was found to affect frequency of solo object play (P=0.021) and social object play (P=0.012), both being higher in females. Dietary treatment x play session (P=0.025) and dietary treatment x pen mate average sugar intake (P=0.019) both interacted to affect social object play. Social object play was consistent across play sessions for LE pigs, with levels of social play in CE pigs being higher than that of LE pigs in session 1 and 2 and then levels in CE pigs dropping to be equal to that of LE pigs in session 3 and 4 (Figure 5).

Social object play was higher in pigs fed the CE diet with a pen mate average sugar score of 1 (ate less than half of the sugar on average) compared to pigs fed the LE diet with the same pen mate sugar intake. Levels of social object play were equal across pen mate sugar intake scores for CE pigs. In LE pigs, levels of social object play were higher when the pen mates ate more than half the sugar on average (score 2) compared to when they ate less than half of the sugar (score 1) (Figure 6).

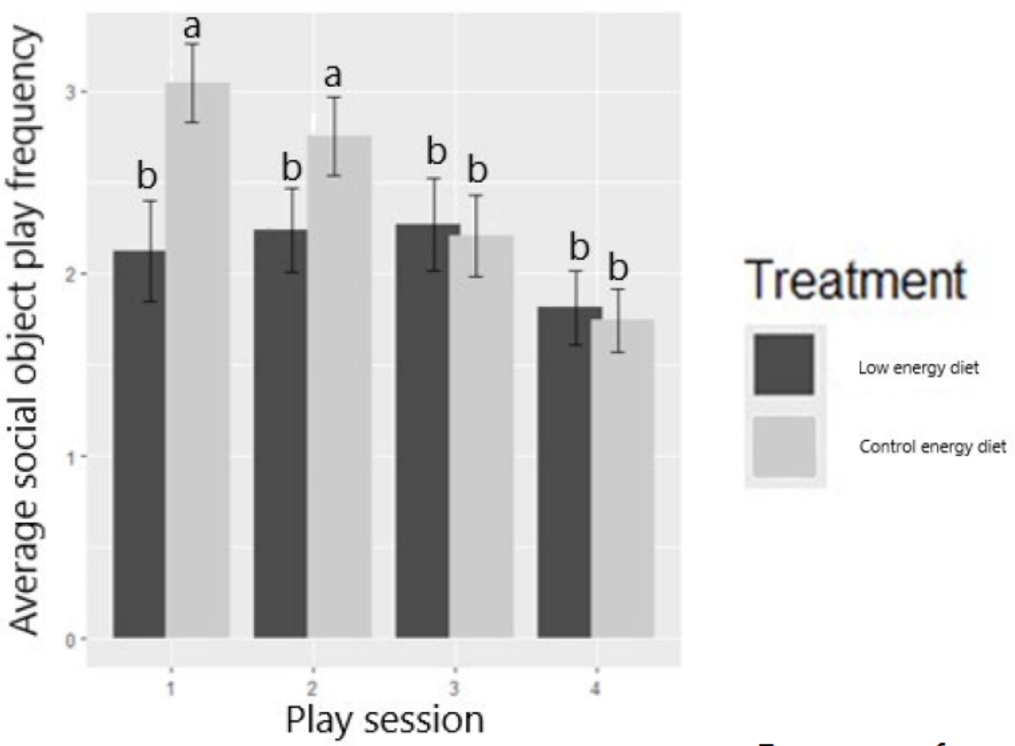


Figure 5. Frequency of social object play per pig (n =128, 64 pigs per treatment), across 4 pay sessions, from ages 38 to 48 days old. Effect of treatment x play session interaction on the average frequency of social object play per pig on low energy (LE) and control energy (CE) dietary treatments. Non-matching superscripts denote statistical significance (P=0.025). Predicted means and lower and upper 95% confidence intervals are presented.

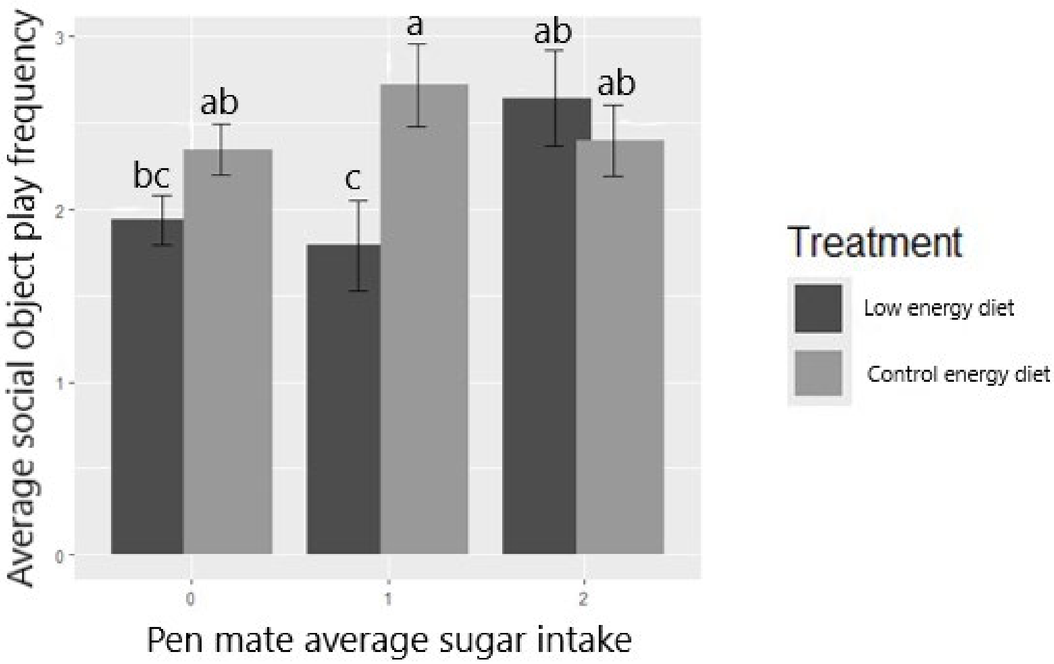


Figure 6. Frequency of social object play per pig (n =128, 64 pigs per treatment), across 4 pay sessions, from ages 38 to 48 days old. Effect of treatment x pen mate average sugar intake on the average frequency of social object play per pig on low energy (LE) and control energy (CE) dietary treatments. Non-matching superscripts denote statistical significance (P=0.019). Predicted means and lower and upper 95% confidence intervals are presented.

Play fighting

Frequency of play fighting was affected by sex (P<0.001) and play session (P=0.030) with the frequency of play fighting being higher in males and play fighting being at greater frequency in session 2 than in session 3 and 4 (Figure 7). ().

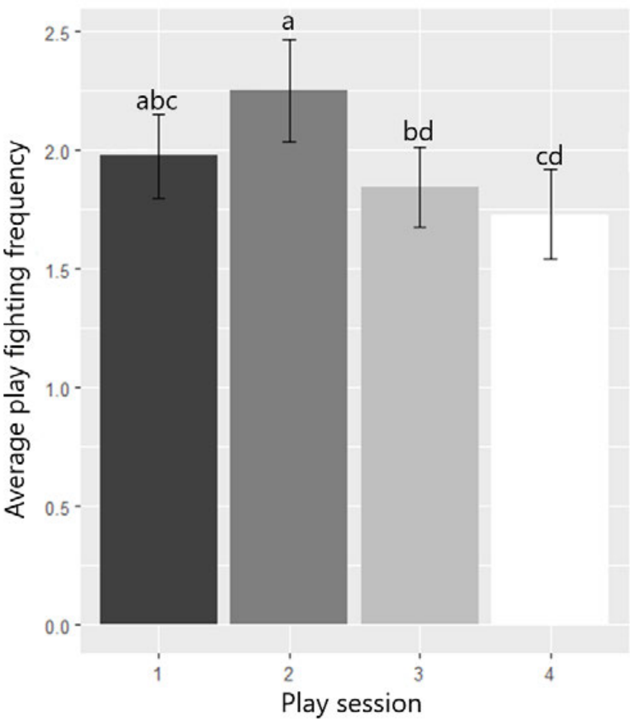


Figure 7. Frequency of play fighting per pig (n =128, 64 pigs per treatment), across 4 pay sessions, from ages 38 to 48 days old. Effect of play session on the average frequency of play flighting per

pig over 4 play sessions. Non-matching superscripts denotes statistical significance (P=0.030). Predicted means and lower and upper 95% confidence intervals are presented.

Aggression

Aggression was affected by the dietary treatment x pen mate average sugar intake interaction (P=0.004). Frequency of average aggression was less in CE diet pigs of pen mate average sugar intake score 1 (ate less than half of the sugar on average) than that of LE pigs of the same pen mate average intake score. For CE diet pigs, average aggression was also less in pigs of pen average sugar intake score 1 than that of pigs of scores 0 and 2 (Figure 8).

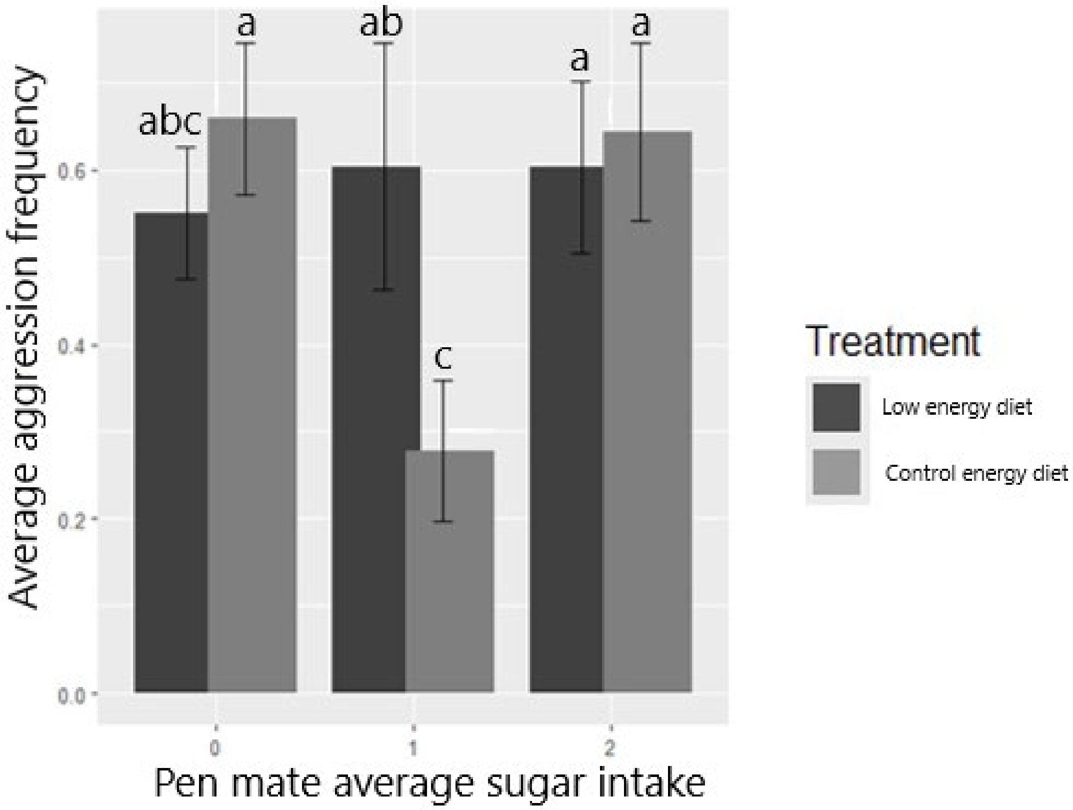


Figure 8. Frequency of aggression per pig (n =128, 64 pigs per treatment), across 4 pay sessions, from ages 38 to 48 days old. Effect of treatment x pen mate average sugar intake interaction on the average frequency of aggressive behaviour per pig on low energy (LE) and control energy (CE) dietary treatments. Non-matching superscripts denote statistical significance (P=0.004). Predicted means and lower and upper 95% confidence intervals are presented.

Lesions

No effect of any of the variables modelled was found on the increase in skin lesions from pre to post play arena session. Pigs on the LE diet had a mean lesion score of 0.20 (SD 0.321) before entering the play arena and a mean of 0.42 (SD 0.530) after leaving the play arena. Pigs on the CE diet had a mean lesion score of 0.17(SD 0.291) before entering the play arena and a mean of 0.34 (SD 0.493) after leaving the play arena.

Growth Measures

Dietary treatment was found to affect ADG (P=0.005) and FCR (P<0.001) (Table 3). ADG was higher in CE diet pigs and FCR was lower in CE diet pigs, indicating better feed efficiency. There was no effect of dietary treatment on ADFI.

Table 3. Average body weight of pigs on control energy diet and low energy diet at weaning and at play session 4. Effect of dietary treatment (control energy and low energy) on ADFI, ADG and FCR of trial pigs (n=128). Non-matching superscripts withing a row denote statistical significance ADG (P=0.005) and FCR (P<0.001)

	Control Energy Diet (CE)	**Low Energy Diet (LE)**
Average body weight at weaning (SD) (Kg)	8.37 (1.78)	8.31 (1.21)
Average body weight at play session 4 (SD) (Kg)	13.73 (2.46)	12.54 (1.57)
ADFI (SD) (Kg)	0.35 (0.04)	0.35 (0.02)
ADG (SD) (Kg)	0.27 (0.04) [a]	0.22 (0.02) [b]
FCR (SD)	1.28 (0.08) [a]	1.61 (0.11) [b]

Discussion

The aim of this study was to test the central hypothesis of the Surplus Resource Theory through direct manipulation of dietary energy as the first limiting nutritional resource. We hypothesised that pigs on an energy-deficient diet would show reduced play and that the provision of sugar before exposure to an environment conducive to play would have an ergogenic effect, temporarily reversing the effects of chronic energy deficit. The results provide some support for these hypotheses, but the effects were not consistent. We found a main effect of play session on play fighting and total play and an interaction between dietary treatment and play session on social object play, solo locomotor play and social locomotor play. This interaction effect differed across behaviours but with the general trend that levels of play behaviours decreased more rapidly on the LE diet as pigs aged. We found no effect of individual sugar intake (tended towards significant in social locomotor play) or effect of the interaction of dietary treatment with individual sugar intake. We did, however, find an interaction between dietary treatment and pen mate average sugar intake on aggression, social object play, social locomotor play and total play. Again, this interactive effect differed between behaviours but in general, increased pen mate average sugar intake increased play in LE pigs, whilst an intermediate pen mate average sugar intake of score 1 increased play behaviours in CE pigs.

As expected, the study identified a sex difference in play, with males performing more play fighting than females and females performing more solo object, social object, solo locomotor and social locomotor play. Our findings agree with previous studies on pigs that males perform more play fighting, while females show more locomotor play [22,37,38]. We saw no effect of any variables modelled on lesion increase, which was generally small, suggesting that play fighting was correctly recorded as play rather than true aggression. Play session affected play fighting and total play. This was expected as previous literature has found that play in pigs decreases over time, peaking around 2 to 6 weeks of age, dropping to a low level at around 14 weeks [13]. Trial pigs were between 5 and 7 weeks old during the 4 play sessions so a decrease in play was expected. The decrease in play between sessions may also reflect a reduction in novelty of the play arena experience. The dilution of the diet with Arbocel resulted in a 9.5% reduction in digestible energy intake and suppressed growth rate by approximately 18.5%; this difference in effect size can be accounted for from the smaller proportion of digestible energy intake to support weight gain on the LE diet compared to the CE diet after accounting for the prioritised digestible energy partitioning to maintenance [39].

The results of average frequency of solo locomotor and social locomotor play go towards supporting our hypothesis, in that heightened levels of play are sustained over a longer period for pigs on the higher energy diet. Our results may also suggest that pigs on the LE diet prioritise social locomotor play in the initial play session whereas pigs on the CE diet prioritise social object play. It may have been the case that the distinction between object exploration and object play was not made clear enough as therefore some behaviour scored as social object play would have more accurately been scored as object exploration and exploration of objects was in search of foodstuffs and that pigs on the LE diet being less interested in objects due to feeling physically more satiated by the higher fibre content of their diet. A more detailed ethogram would have allowed for more accurate scoring of behaviours. The difference in play priorities may also be due to the energy cost of different types of play. It might be expected that locomotor play has a higher energy cost than object play. It then appears counterintuitive that pigs on a lower energy diet would engage in higher levels of more costly, locomotor play. It may be that locomotor play is more beneficial to pigs. As prey animals, engaging in locomotor play might help develop skills and physical strength to evade predators. Therefore, with finite energy resources, pigs prioritise play behaviours that improve their survival chances. Further research into the energetic cost of different forms of play is needed along with exploration of if/how animals prioritise typed of play. Some discussion of this can be found in the literature but research into the energetic costs of different types of play is generally lacking [40]

Sugar Intake

Contrary to our hypothesis, we did not see an effect of the consumption of sugar on the subject's own expression of play. It was hypothesised that sugar intake immediately before exposure to the arena would increase play, and that this effect would be most profound for pigs on the LE diet. The lack of effect of individual sugar intake may be due to the amount of sugar given being too small to see an individual effect but resulting in a small, cumulative effect of the play behaviour of all pigs. It may also have been due to the time it takes for the sugar to be digested and utilised by the pig. Studies have shown that in sows, blood glucose levels peak approximately 40 minutes after ingestion [41]. Surprisingly, not all pigs ate the sugar that was offered to them in the weigh crate, despite eating sugar offered to them in their home pen as part of their habituation. It may have been that pigs that ate less than half of the sugar offered in the weigh crate did so because they were more stressed in the weigh crate or more aroused by expectation of entering the arena, either of which could have reduced motivation for the sugar. Studies have shown that pigs subjected to chronic social or restraint stress showed a reduced preference for sucrose at low concentrations but not at higher concentrations [42]. It would be beneficial to test palatability of sucrose in response to acute stress.

Aggression, social object play, social locomotor play and total play were affected by the interaction between dietary treatment and pen mate average sugar intake, suggesting that the subject's play was sensitive to the sugar intake of its peers in a manner affected by the long-term diet. The finding that pen mate sugar intake affected the subject's own play behaviour may be due to social and/or play contagion. The inclusion of the pen mate average sugar intake was to account for the proposed contagious element of play as seen in elephants [43], ravens [44], and chimpanzees [45], and the quantity of sugar eaten by other group members could potentially affect the play behaviour performed by a focal animal. There is research to suggest that pigs might be sensitive to emotional contagion [46,47] and that they also exhibit yawn contagion [48] but thus far there is a lack of empirical evidence of play contagion in pigs. Further research is needed to better understand the emotional and social contagion in pigs to help unravel the social aspect of play and the role it plays in the structure and dynamics of pig social networks. We did not hypothesise how sugar would affect aggression, but it is possible that either a low or high energy intake could increase aggression. Specifically, it could be argued that aggression would be increased in pigs that consumed no sugar as low energy intake has been linked to an increase in aggression, as observed in food deprived Drosophila [49] and self-reported by human study subjects[50] . It may also be expected that aggression would be greater in pigs that consumed more than half the sugar offered, as excess dietary energy may facilitate aggression, which is an energy-demanding activity. There is evidence of high fat diets inducing aggression in rodents [51]. However, in contrast, we observed that an intermediate intake of sugar by pen mates affected expression of aggression, not individual sugar intake. For pigs on the LE diet, potential increase in stress or arousal caused by the movement into the play arena, coupled with the low energy diet, could have been manifest in increased aggression once inside the play arena. Unfortunately, data was not collected on the affective state of individual pigs so cannot determine the likely cause of the non-linear effect of sugar intake on aggression. Further work to explore how proximate emotional states affect social behaviour and how this is moulded by nutritional provision would be beneficial.

We did not specifically make hypotheses about the effect of pen mate sugar intake as it was expected that all individuals in the group would fully consume the sugar offered to them. However, the observation that the play increases in pigs fed the LE diet when their pen mates ate most of the sugar offered would fit with a prediction that sugar provision promotes play when on a lower energy diet. Interestingly, however, there was no difference in the subject's play when its pen mates ate most of the sugar as compared to when they were not offered sugar. Both social locomotor and total play increased in the LE pigs whose pen mates consumed half or more than half of the sugar offered. This suggests that for social locomotor play, the provision of sugar to pen mates did temporarily reverse the effect of the lower energy diet for the subject, in support of our hypothesis.

An unexpected observation was that for CE diet pigs, social locomotor play and total play were higher in pigs of intermediate pen mate sugar intake scores of 1 (sugar offered but less than half consumed). We would have expected to see that pigs whose pen mates ate half or more of the sugar offered played more than those whose pen mates ate less than half of the sugar offered. It may have been that the pigs which ate less than half of the sugar were more aroused and hence distracted in the weigh crate and that this was manifest as more play in the arena which had a contagious effect on the subject's own play. In contrast to the LE diet pigs where an increase in arousal led to increased aggression, an increase in arousal in the CE diet pigs could potentially have led to increased play as the pigs were not additionally metabolically stressed by a dietary energy deficit. Additional research is needed to explore the effect of contagion of affective states and their effect on play and social behaviours at a group level. Interestingly, we saw no effect of either the subject's own sugar intake or that of its pen mates on either diet on solo play behaviours. We only saw an effect of sex on solo object play (with females performing more) and effects of sex (with females performing more) and dietary treatment x play session interaction on solo locomotor play. This suggests that dietary energy, sugar and potentially stress/arousal and contagion have a stronger effect on social behaviours than on solitary behaviours.

Stimulating increased play behaviours through diet could be a valuable tool for promoting positive welfare in commercial pigs. The concept of positive welfare goes beyond ensuring good physical health and the prevention and alleviation of suffering and strives to provide animals with opportunities to experience positive mental states resulting from rewarding experiences [52]. Play has been suggested as an indicator of positive affective states and positive emotions[53]. Application of the findings of this paper should be performed with caution as although a common commercial breed was used in this study, the environmental and handling conditions were not those commonly seen in intensive commercial settings. It would be beneficial to repeat the study under commercial conditions, larger groups, smaller pens and on fully slatted floors. Repeating the study, focusing on the effect of dietary energy without access to a specific, larger play arena is essential to test commercial relevance. It may also be beneficial to test at which production stage the pigs might benefit most from increased energy provision. As previously mentioned, play is most commonly seen in the juvenile phases [13] but studies have found that pigs can be encouraged to play into the grow-finish phase [54]. If play has the benefit of preparing pigs for later life[3], it may be more beneficial to promote play earlier on in development, however there is a concern that increased play behaviour can be at the detriment of growth[19]. Caution should be taken when exploring dietary manipulation as a strategy to improve welfare, it is well established that the provision of adequate space and suitable enrichment are essential to improving welfare and changes to diet should not replace this.

Conclusion

Our results support previous studies that sex and age of pigs affects play behaviour. The diet affected some play behaviours in a manner supporting our hypothesis that play behaviour would be compromised on a diet diluted with fibre. In particular, a higher energy diet appeared to extend the period of heightened engagement in social play behaviours later into development than a lower energy diet. However, the effect varied between different forms of play and was apparent only during specific play sessions. Contrary to our hypothesis, individual sugar intake in the minutes before entry to the arena did not affect the amount of play on either diet. We did, however, find evidence that the sugar intake of pen mates affected the social play of the subject animals independently of their own sugar intake which could operate through a contagion effect on play. It may also be necessary to factor in the individual pigs innate "playfulness" and position in the dominance hierarchy, together with measures of arousal and affective state, into such research in the future as these could potentially affect a pig's performance of social and solo play. Our findings suggest that diet quality may impact specific aspects of play behaviour and therefore that dietary manipulation may be a way to increase the expression of play and potentially other positive forms of behaviour in commercial production with benefits for animal welfare.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Diamond, J. & Bond, A. B. A comparative analysis of social play in birds. *Behaviour* **140**, 1091–1115 (2003).

2. Dinets, V. Play behavior in ectothermic vertebrates. *Neurosci. Biobehav. Rev.* **155**, (2023).

3. Špinka, M., Newberry, R. C. & Bekoff, M. Mammalian play: Training for the unexpected. *Q. Rev. Biol.* **76**, 141–168 (2001).

4. Eisenbeiser, S., Serbe-Kamp, É., Gage, G. J. & Marzullo, T. C. Gills Just Want to Have Fun: Can Fish Play Games, Just like Us? *Animals* **12**, (2022).

5. Mather, J. A. & Anderson, R. C. Exploration, play and habituation in octopuses (Octopus dofleini). *J. Comp. Psychol.* **113**, 333–338 (1999).

6. Kuba, M. J., Gutnick, T. & Burghardt, G. M. Learning from play in octopus. in *Cephalopod Cognition* (eds Darmaillacq, A.-S., Mather, J. & Dickel, L.) 57–71 (Cambridge University Press, Cambridge, 2014). doi:10.1017/CBO9781139058964.006.

7. Galpayage Dona, H. S. *et al.* Do bumble bees play? *Anim. Behav.* **194**, 239–251 (2022).

8. Lawrence, A. Consumer demand theory and the assessment of animal welfare. *Anim. Behav.* **35**, 293–295 (1987).

9. Burghardt, G. M. *The Genesis of Animal Play: Testing the Limits.* (2005).

10. Graham, K. L. & Burghardt, G. M. Current perspectives on the biological study of play: signs of progress. *Q. Rev. Biol.* **85**, 393–418 (2010).

11. Waiblinger, S., Wagner, K., Hillmann, E. & Barth, K. Play and social behaviour of calves with or without access to their dam and other cows. *J. Dairy Res.* **87**, 144–147 (2020).

12. Bennett, B. & Fewell, J. H. Play frequencies in captive and free-ranging bighorn lambs (Ovis canadensis canadensis). *Zoo Biol.* **6**, 237–241 (1987).

13. Newberry, R. C., Wood-Gush, D. G. M. & Hall, J. W. Playful behaviour of piglets. *Behav. Processes* **17**, (1988).

14. Jensen, M. B., Vestergaard, K. S. & Krohn, C. C. Play behaviour in dairy calves kept in pens: the effect of social contact and space allowance. *Appl. Anim. Behav. Sci.* **56**, 97–108 (1998).

15. Chaloupková, H., Illmann, G., Bartoš, L. & Špinka, M. The effect of pre-weaning housing on the play and agonistic behaviour of domestic pigs. *Appl. Anim. Behav. Sci.* **103**, (2007).

16. Pellis, S. M. & Pellis, V. C. Play-fighting differs from serious fighting in both target of attack and tactics of fighting in the laboratory rat Rattus norvegicus. *Aggress. Behav.* **13**, 227-242 (1987).

17. Pellis, S. M. & Pellis, V. C. Play fighting in Visayan warty pigs (Sus cebifrons): Insights on restraint and reciprocity in the maintenance of play. *Behaviour* **153**, (2016).

18. Weller, J. E., Camerlink, I., Turner, S. P., Farish, M. & Arnott, G. Playful pigs: early life play-fighting experience influences later life contest dynamics. *Anim. Behav.* **158**, (2019).

19. Berghänel, A., Schülke, O. & Ostner, J. Locomotor play drives motor skill acquisition at the expense of growth: A life history trade-off. *Sci. Adv.* **1**, e1500451 (2015).

20. Held, S. D. E. & Špinka, M. Animal play and animal welfare. *Anim. Behav.* **81**, 891–899 (2011).

21. Barnes, R. H., Levitsky, D. A., Pond, W. G. & Moore, U. Effect of postnatal dietary protein and energy restriction on exploratory behavior in young pigs. *Dev. Psychobiol.* **9**, (1976).

22. Brown, S. M., Klaffenböck, M., Nevison, I. M. & Lawrence, A. B. Evidence for litter differences in play behaviour in pre-weaned pigs. *Appl. Anim. Behav. Sci.* **172**, (2015).

23. Brown, S. M., Peters, R., Nevison, I. M. & Lawrence, A. B. Playful pigs: Evidence of consistency and change in play depending on litter and developmental stage. *Appl. Anim. Behav. Sci.* **198**, 36–43 (2018).

24. Franchi, G. A. *et al.* Play behaviour positively relates to weight gain, feeding behaviour and drinking behaviour in weaner pigs (Sus scrofa). *Appl. Anim. Behav. Sci.* **259**, 105836 (2023).

25. Steinerová, K. *et al.* Rearing pigs with play opportunities: the effects on disease resilience in pigs experimentally inoculated with PRRSV. *Front. Vet. Sci.* **11**, 1460993 (2024).

26. Vandenbogaerde, T. J. & Hopkins, W. G. Effects of Acute Carbohydrate Supplementation on Endurance Performance. *Sports Med.* **41**, 773–792 (2011).

27. Jeukendrup, A. E. Carbohydrate feeding during exercise. *Eur. J. Sport Sci.* **8**, 77–86 (2008).

28. McDonald, P. *et al. Animal Nutrition.* (Pearson Higher Ed, 2011).

29. Sugar: the facts. *nhs.uk* https://www.nhs.uk/live-well/eat-well/food-types/how-does-sugar-in-our-diet-affect-our-health/ (2022).

30. Ocepek, M., Newberry, R. C. & Andersen, I. L. Which types of rooting material give weaner pigs most pleasure? *Appl. Anim. Behav. Sci.* **231**, (2020).

31. Camerlink, I., Proßegger, C., Kubala, D., Galunder, K. & Rault, J.-L. Keeping littermates together instead of social mixing benefits pig social behaviour and growth post-weaning. *Appl. Anim. Behav. Sci.* **235**, 105230 (2021).

32. Camerlink, I. & Turner, S. P. The pig's nose and its role in dominance relationships and harmful behaviour. *Appl. Anim. Behav. Sci.* **145**, (2013).

33. Camerlink, I., Peijnenburg, M., Wemelsfelder, F. & Turner, S. P. Emotions after victory or defeat assessed through qualitative behavioural assessment, skin lesions and blood parameters in pigs. *Appl. Anim. Behav. Sci.* **183**, (2016).

34. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing (2021).

35. Bates, D., Mächler, M., Bolker, B. & Walker, S. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* **67**, 1–48 (2015).

36. Marschner, I. glm2: Fitting Generalized Linear Models. 1.2.1 https://doi.org/10.32614/CRAN.package.glm2 (2011).

37. D'Eath, R. B. & Lawrence, A. B. Early life predictors of the development of aggressive behaviour in the domestic pig. *Anim. Behav.* **67**, (2004).

38. Rauw, W. M. A note on the consistency of a behavioral play marker in piglets. *J. Anim. Sci. Biotechnol.* **4**, (2013).

39. *Proceedings of the British Society of Animal Science 2003*. (British Society of Animal Science, Penicuik (GB), 2003).

40. Mallpress, D. E. W. & Špinka, M. The practicality of practice: A model of the function of play behaviour. *Ecol. Evol.* **13**, e10521 (2023).

41. Kemp, B. *et al*. Glucose tolerance of pregnant sows is related to postnatal pig mortality. *J. Anim. Sci.* **74**, 879–885 (1996).

42. Figueroa, J., Solà-Oriol, D., Manteca, X., Pérez, J. F. & Dwyer, D. M. Anhedonia in pigs? Effects of social stress and restraint stress on sucrose preference. *Physiol. Behav.* **151**, 509–515 (2015).

43. Norscia, I., Hecker, M., Aldama, B. G., Santos, S. B. & Cordoni, G. Play contagion in African elephants: The closest, the better. *Behav. Processes* **221**, (2024).

44. Wenig, K., Boucherie, P. H. & Bugnyar, T. Early evidence for emotional play contagion in juvenile ravens. *Anim. Cogn.* **24**, 717–729 (2021).

45. Sandars, G., Brooker, J. S. & Clay, Z. ChimpanSEE, ChimpanDO: Grooming and play contagion in chimpanzees. *PLoS ONE* **19**, (2024).

46. Reimert, I., Bolhuis, J. E., Kemp, B. & Rodenburg, T. B. Indicators of positive and negative emotions and emotional contagion in pigs. *Physiol. Behav.* **109**, (2013).

47. Steinerová, K., Parker, S. E., Brown, J. A. & Seddon, Y. M. The promotion of play behaviour in grow-finish pigs: The relationship between behaviours indicating positive experience and physiological measures. *Appl. Anim. Behav. Sci.* **275**, 106263 (2024).

48. Norscia, I., Coco, E., Robino, C., Chierto, E. & Cordoni, G. Yawn contagion in domestic pigs (Sus scrofa). *Sci. Rep. 2021 111* **11**, 1–12 (2021).

49. Edmunds, D., Wigby, S. & Perry, J. C. 'Hangry' *Drosophila*: food deprivation increases male aggression. *Anim. Behav.* **177**, 183–190 (2021).

50. Swami, V., Hochstöger, S., Kargl, E. & Stieger, S. Hangry in the field: An experience sampling study on the impact of hunger on anger, irritability, and affect. *PloS One* **17**, e0269629 (2022).

51. Hilakivi-Clarke, L., Cho, E. & Onojafe, I. High-fat diet induces aggressive behavior in male mice and rats. *Life Sci.* **58**, 1653–1660 (1996).

52. Rault, J.-L. *et al*. A consensus on the definition of positive animal welfare. *Biol. Lett.* **21**, 20240382 (2025).

53. Mellor, D., Patterson-Kane, E. & Stafford, K. J. *The Sciences of Animal Welfare*. (John Wiley & Sons, 2009).

54. Steinerová, K., Parker, S. E. & Seddon, Y. M. Regular play opportunities during the grow-finish phase improve pig trainability and learning in a cognitive bias test. *Appl. Anim. Behav. Sci.* **283**, (2025).

Author Contributions

Eleanor Hewett and Simon Turner designed the trial with nutritional consultations from Jos Houdijk. Eleanor Hewett collected the data and performed the analysis with guidance from Simon

Turner. Eleanor Hewett and Simon Turner drafted the manuscript which was reviewed by Jos Houdijk, Craig Lewis and Andrea Doeschl-Wilson.

Acknowledgements

We would like to acknowledge the staff and students at the EHPU for providing help and support, particularly Marianne Farish.

Funding

This work was supported by the UKRI Biotechnology and Biological Sciences Research Council (BBSRC) grant number BB/T00875X/1 and PIC as part of the EASTBIO Doctoral Training programme. SRUC receives funding from the Scottish Government.

Conflicts of interest

The authors declare no conflict of interest.