Exploring the hidden welfare problem of gastric ulceration in sows:

Behaviour and saliva composition as possible methods of diagnosis

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Declaration

I declare that I have composed this thesis. The work described is my own, I have made a substantial contribution to the work, and all assistance received is acknowledged clearly in each introduction to the chapters. The work has not been submitted for any other degree or professional qualification. Any included publications are my own work, except where clearly indicated throughout the thesis about my own and the co-authors' contributions and summarised and identified on the declarations page of the thesis.

Laura C Salazar Hofmann

To my parents, sister and nephew

To Anita and Francisca, and friends

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Abstract

Gastric ulcers are highly prevalent in the pig industry and evidence suggests probably affect pigs in all productive stages. Gastric ulcers affect pig welfare as they cause some degree of discomfort or pain (observed by a change in behaviour) as well as impacting performance. Gastric ulceration is a hidden problem as it is difficult to identify in living pigs and there is no method of diagnosis that is both non-invasive and affordable. In this thesis, I hypothesised that re-directed oral behaviours (e.g. sham chewing, biting bars) and changes in saliva characteristics may be of use for the diagnosis of gastric ulceration in pigs. Re-directed oral behaviours have been largely related with chronic hunger in sows which appear as a way to cope with a diet and environment that does not fulfil sows' nutritional and behavioural needs. However, these behaviours have also been observed in finishing pigs as well as in gilts and sows fed *ad libitum*. Furthermore, there is some evidence that the behaviour of finishing pigs changes with the presence of gastric ulcers. Saliva composition has been reported to change with various illnesses or conditions but also, recently, with the presence of gastric ulcers in finishing pigs.

Chapter 2 describes oral behaviours present in finishing pigs and compares them to the behaviour of sows. All oral behaviours studied were observed in finishing pigs, and the rate was the same as compared to sows. The behaviours which were least frequent and performed by the least number of animals in both finishing pigs and gestating sows were self-directed oral behaviours. Self-directed oral behaviours are much more difficult to explain in finishing pigs as opposed to oral behaviours that involve the interaction with an object or conspecific.

Chapter 3 explored the relationship between self-directed oral behaviours (chewing movements, wind sucking, tongue playing and jaw stretching) and the presence of gastric ulcers in finishing pigs. All self-directed oral behaviours, but jaw stretching, were observed in both pigs with healthy and ulcerated stomachs (video observations). All observed behaviours were the same between both groups.

Chapter 4 explores the relationship between re-directed oral behaviours (live observations) as well as salivary composition and pH with gastric ulceration in gestating and lactating sows. All sows were found to have some level of ulceration and the prevalence of gastric ulcers was 67.57%. The rate of re-directed oral behaviours was not affected by overall stomach score or lesion score during either gestation or lactation. Salivary pH was not affected by stomach integrity. Saliva composition changed with the overall stomach score and lesion score in gestating and lactating sows. Lipoxin A4, Succinic acid and L-Histidine were identified as possible biomarkers of gastric ulceration.

Chapter 5 is a systematic literature review of the variation of re-directed oral behaviours according to housing system, diet and feeding practices, and environmental enrichment in gestating gilts and sows. All of the results of the included studies can be explained by 'chronic hunger' theory or their housing environment. Although the design of these studies was to test factors which relate to hunger and re-directed foraging, rather than the health of the upper digestive system.

This thesis shows that oral behaviours do not have a clear link with gastric ulceration in finishing pigs, or in gestating and lactating sows. However, oral behaviours were observed in finishing pigs and some of these remain unexplained (e.g. self-directed oral behaviours). Also, all re-directed oral behaviours in gestating gilts and sows can be explained by chronic hunger or housing environment as shown by the systematic literature review. Possibly, the remaining unexplained oral behaviours may be a response to an environment that is still insufficient for the pig to fulfil its behavioural needs and/or other conditions affecting the upper digestive system. Saliva composition is linked to gastric ulceration in gestating and lactating sows, and possible biomarkers were identified in this thesis. More studies are needed to identify and validate biomarkers for gastric ulceration in pigs.

Lay summary

The pig industry has been intensified during the past decades to increase productivity. As part of this, pigs are often offered diets that are finely ground to facilitate digestion and absorption of nutrients and energy. However, as a result of this practice, the stomach contents become more fluid which facilitates the contact of the stomach fluids with a part of the stomach lining called the pars oesophagea, an area that is located proximal to the oesophagus and lacks protective mucosal glands. As a result, the pars oesophagea is irritated, and an ulcer may develop. Gastric ulcers are highly prevalent in the pig industry, may have an impact on the welfare of the pigs as well as their performance, but they are difficult to diagnose in the living pig.

This thesis explores ways of diagnosing gastric ulcers in the living pig that are easy, affordable and non-invasive. The second and third chapter of the thesis explores the possibility of using oral behaviours (usually observed in gestating sows under chronic hunger caused by restricted feed rations) as an indicator of gastric ulceration. The fourth chapter explores re-directed oral behaviours and the change in saliva composition and pH in gestating and lactating sows as ways of diagnosing gastric ulcers.

In my second chapter, the description of behaviour in finishing pigs (which are given an unlimited amount of food) confirmed that they show oral behaviours. This may mean that there might be an alternative explanation different than chronic hunger. Interestingly, self-directed oral behaviours (e.g. sham chewing, windsucking), that were assumed to appear solely as a result of chronic hunger in gestating sows, are also observed in finishing pigs who are not feed-restricted. These behaviours were observed little and performed only by few animals. This may show that self-directed oral behaviours might not be related to explorative, normal feeding or foraging behaviour, but to other conditions affecting the health of the gastro-intestinal tract such as gastric ulcers.

However, by studying the association between self-directed oral behaviour (chewing movements, wind sucking, tongue playing and jaw stretching; video observations) and the presence of gastric ulcers in finishing pigs, no relationship was found. Possible explanations for the occurrence of oral behaviours in finishing pigs are explored in the text.

The relationship between saliva composition and pH as well as behaviour (live observations) with gastric ulceration was studied in gestating and lactating sows. This study confirms the presence of gastric ulcers in lactating sows. No relationship was found between gastric ulceration and re-directed oral behaviours as well as salivary pH. However, promising results were found regarding saliva composition. The metabolite profile of gestating and lactating sows changed with stomach integrity. Metabolites were identified as possible biomarkers of gastric ulceration, but further studies need to be done.

Finally, a systematic literature review was performed to investigate how re-directed oral behaviours vary in gestating gilts and sows with the different factors as reported in the chronic hunger literature. These were factors related to housing conditions, diet composition and feeding practices as well as environmental enrichment. All results can be explained by 'chronic hunger' theory or their housing environment.

This thesis confirmed that finishing pigs do perform oral behaviours but did not find an association between these oral behaviours and gastric ulceration in finishing pigs or in sows. Salivary pH was not related with gastric ulceration. However, saliva composition showed good potential as a method of diagnosis for gastric ulcers. Further studies should include further investigation of the cause of oral behaviours in finishing pigs and refining and validating salivary biomarkers for the diagnosis of gastric ulcers in pigs.

Publications

Peer-reviewed conference proceedings

Salazar, L. C., Baxter, E., Lawrence, A., D'Eath, R. 2020. Are hunger-related oral behaviours also caused by other factors in pigs?. In: ISAE Regional UK meeting

Salazar, L. C., Baxter, E., Lawrence, A., D'Eath, R. 2021. Ad-lib fed finishing pigs perform oral behaviours usually associated with hunger in dry sows. In: Dwyer, C., Harris, M., Rahman, S. A., Waiblinger, S., Rodenburg, T. B. (Ed) Proceedings of the 54th Congress of the ISAE.

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Other conference publications

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Other peer-reviewed journal papers published during the PhD

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Teixeira, D. L., Salazar, L.C., Enriquez-Hidalgo, D., Boyle, L.A., 2020. Assessment of animal-based pig welfare outcomes on farm and at the abattoir: A case study. Front. Vet. Sci. 7, 6–11. https://doi.org/10.3389/fvets.2020.576942

Figueroa, J., Luna, D., Salazar, L. C., Morales, Pia, Valdivia, C., Müller, M., Dwyer, D. 2021. Effects of trial and error and social learning on flavour palatability in nursery pigs. Appl. Anim. Behav. Sci. 236: 105265; https://doi.org/10.1016/j.applanim.2021.105265

Guzmán-Pino, S. A., Luna, D., Salazar, L. C., Poblete, P., Figueroa, J. 2021. Effect of switching sows' positions during lactation on suckling behaviour and performance of co-mingling piglets. Appl. Anim. Behav. Sci. 241: 105391; https://doi.org/10.1016/j.applanim.2021.105391

Teixeira, D. L., Salazar, L.C., Enriquez-Hidalgo, D., Hötzel, M.J., 2023. Pigs' capacity to experience feelings and to suffer from tail lesion, ear lesion and lameness: Exploring citizens and pig farm and abattoir workers' knowledge and perceptions. PLoS One 18, 1–14. https://doi.org/10.1371/journal.pone.0286188

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ADF	Acid Detergent Fibre
BMDB	Bovine Metabolome Database
CI	Confidence Interval
DPD	Dental and Periodontal Diseases
GLMM	Generalized Linear Mixed Model
HILIC-z	zwitterionic-phase Hydrophilic Interaction Chromatography
HMDB	Human Metabolome Database
IM-qTOF	Ion Mobility quadrupole Time-Of-Flight
LMM	Linear Mixed Models
PC	Principal Component
PCA	Principal Component Analysis
PICO	Population, Intervention, Comparator, and Outcome
PO	pars oesophagea
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
REML	Restricted Maximum Likelihood
RHIMMS	Rapid HILIC-Z Ion Mobility Mass Spectrometry
SRUC	Scotland’s Rural College
UHPLC	Ultra-High-Performance Liquid Chromatography

Chapter 1 General introduction

1.1 Introduction

The intensification of the pig industry has resulted in the breeding of commercial lines of pigs for rapid lean growth and for dam lines for increased reproduction including litter size (Turner et al., 2017a). This allows farmers to produce more meat more efficiently at a lower cost. The problem is that this has created animals with higher appetite (Kirkwood and Aherne, 1985) and bigger final adult size than before (Moustsen et al., 2011). Therefore, if food was to be provided *ad libitum*, gilts and sows would have excessive fat deposition and thereby an increased risk for lameness problems and a reduced longevity (Jørgensen and Sørensen, 1998). To avoid this, farmers feed-restrict breeding sows and gilts routinely (~50% of their voluntary feed intake; Read et al., 2020). As a result, animals remain highly motivated for food which has been shown by operant tests for food motivation (boars: Lawrence et al., 1988; sows: Tokareva et al., 2021). This indicates that they may not be satiated by their ration and remain hungry.

Since feed-restricted gilts and sows are highly motivated for food, they continue to search for food (appetitive phase) and try to eat (consummatory phase) far after feed is exhausted (D'Eath et al., 2018). The frustration of appetitive and consummatory behaviours is shown as re-directed oral behaviours (e.g. sham chewing, manipulating pen, excessive manipulation of drinker) (Appleby and Lawrence, 1987), and increased activity (Read et al., 2020). Behaviour that is directed towards the available resources after the meal has finished can become repetitive and relatively invariant over time, namely stereotypical (Lawrence and Terlouw, 1993).

However, it may be possible that other factors could also cause the expression of re-directed oral behaviours. It is true that when gilts are fed *ad libitum*, they show less re-directed oral behaviour as compared to when they are feed-restricted (Brouns et al., 1994; de Leeuw and Ekkel, 2004). However, they still perform some of these behaviours. Also, according to an unpublished undergraduate dissertation, re-directed oral behaviours (previously related to hunger) in pigs start to appear before feed restriction is in place (e.g. in growing and finishing pigs who are fed *ad libitum*; Dubarry, 2019). This suggests that feed restriction cannot be the only cause in pigs. Also, oral behaviours (e.g. crib-biting) have been associated with gastric ulceration in horses (Wickens and Heleski, 2010) as well as with feeding practices (Redbo et al., 1998), and feeding practices with gastric ulceration (van den Boom, 2022). It is unknown to what extent there is a relationship between oral behaviours and other health conditions of the gastro-intestinal tract in pigs.

1.2 Re-directed behaviours and stereotypies in pigs

As defined in the 'The encyclopedia of applied animal behaviour and welfare' (Marchant-Forde and Mills, 2010), re-directed behaviour occurs when the pig is not able to perform a motivated behaviour due to a lack of available resources. Re-directed behaviours together with appetitive behaviours, displacement behaviours and intention movements are thought to be the source behaviours for the development of stereotypies. Stereotypies are behaviours that are repetitive, invariant and have no obvious goal or function (Lawrence and Terlouw, 1993), and they are usually associated with impaired welfare, although the extent of this is unknown (Mason and Latham, 2004). It is not entirely clear how re-directed behaviours become stereotypical and how fast this may happen in pigs. However, it is described as a gradual process in which the source behaviour pattern becomes stereotypical over time.

Stereotypies can undergo three major developmental changes: increasing performance, increasing invariance and emancipation (Marchant-Forde and Mills, 2010). Briefly, stereotypies can increase in frequency and duration leading to animals using more of their time budget performing them. Together with this, stereotypies become more invariant and may become more or less complex than the initial source behaviours. Finally, ‘emancipation’ can occur where stereotypies become separated from the initial triggering stimuli, meaning that they may be elicited by other stimuli as well.

Usually, in the pig literature on oral stereotypies in relation to hunger, when authors are unsure of whether the observed behaviour is stereotypical or not, they are named as re-directed behaviours. This is because it is unknown how repetitive and invariant they were (Brouns et al., 1994). When more time is allowed to score the behaviour, a distinction can be made between re-directed and stereotypical behaviours (Appleby and Lawrence, 1987; Rushen, 1984; Schouten et al., 1991; Tatemoto et al., 2020).

1.2.1 Defining oral behaviours studied in this thesis

Oral behaviours are defined for this thesis to include any activity of the pig that involves the movement of the mouth which could be in interaction with an object/penmate or not, but excluding damaging behaviour (such as tail- or ear-biting; Diana et al., 2019; Valros, 2017) or social aggressive behaviour (Camerlink et al., 2014; Turner et al., 2017b). Oral behaviours can include interactions with substrate, objects and penmates including licking, nosing rooting or biting. Biting behaviour in the case of interaction with penmates is non-aggressive, non-damaging and short-lived.

Re-directed oral behaviours are defined here as oral behaviours that were meant to be directed towards something else but because the pig could not interact with the original object, they direct the behaviours to something else. Re-directed oral behaviours are not necessarily repetitive and invariant as stereotypies. When referring to the behaviour in finishing pigs this will be named as ‘oral behaviours’ as it is unknown whether these are re-directed in finishing pigs. When referring only to sows’ behaviour these will be named as re-directed oral behaviours when appropiate.

Oral stereotypies are defined as re-directed oral behaviours that have become repetitive and invariant and seemingly have no function. In the studies of this thesis no distinction was made as to how repetitive and invariant a behaviour was. Therefore, behaviours studied here are referred to using the broader category of re-directed oral behaviours and not oral stereotypies.

Self-directed oral behaviours are a type of oral behaviour that do not involve the interaction with anything else and are generally considered as an oral stereotypy in the literature (de Leeuw et al., 2005; Spoolder et al., 1995). This includes sham (or vacuum) chewing, jaw or (mouth) stretching, wind sucking and tongue playing (de Leeuw et al., 2008, 2005; Spoolder et al., 1995; Zonderland et al., 2004). Sham chewing may elicit salivating, so presence of saliva is also considered as self-directed oral behaviour in this thesis. Lastly, behaviours studied in the present thesis have normally been observed in adult female pigs under chronic hunger.

1.3 Hunger-related oral behaviours

Re-directed oral behaviours can develop due to unsatisfied behavioural and nutritional needs (Figure 1.1). In the case of hunger and feeding behaviour, appetitive and consummatory behaviours are stimulated by an increase in feeding motivation which can occur because of external cues such as food-related stimuli indicating an opportunity to eat and/or internal cues concerning the physiological state of the animal (Appleby and Lawrence, 1987). Positive feedback from the act of eating food can also increase feeding motivation in the short term at least, before satiation occurs triggering the end of the meal (Terlouw et al., 1993). A set-point or long-term goal as well as the negative feedback coming from the intake of food regulate feed intake, experienced by humans and, probably, non-human animals as the sensation of hunger. This set-point will vary according to the individuals' characteristics (e.g. age, reproductive status, and season); depending on the status of the individual it will need more or less energy and nutrients to satisfy its needs (D'Eath et al., 2009; Tolkamp and D'Eath, 2016). Feeding motivation is stimulated by hunger or the chance to eat, and down-regulated by the mechanical action that the digested food exerts on the gut, and the micronutrients and hormones, resulting from digestion, sensed by the brain (Meunier-Salaün and Bolhuis, 2015). Meal timing, number of meals and the type and amount of fibre in the feed also modulates the motivation for food.

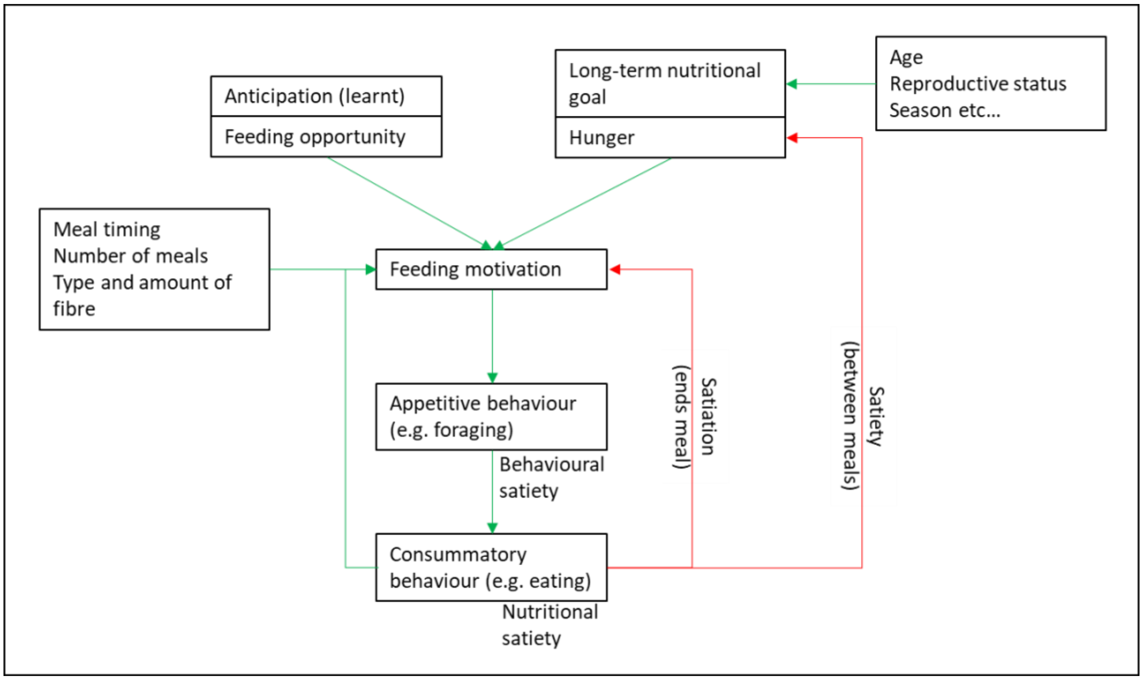

Figure 1.1. Feeding motivation model. Based on D'Eath et al. (2018). Red arrows mean negative feedback and green arrows mean positive feedback for feeding motivation.

However, re-directed oral behaviours may also become stereotypical and unrelated to the initial source and in the long-term, this pattern of behaviour can be observed long after feeding behaviour has ended (Lawrence and Terlouw, 1993; Mason and Mendl, 1997). Authors have suggested that non-specific behavioural arousal, neural sensitization and environment factors are involved in the development and maintenance of stereotypies (Lawrence and Terlouw, 1993). The form that stereotypies take may relate to species-specific evolutionary adaptive origins and/or husbandry-related factors (Mason and Mendl, 1997). For example, feeding-related stereotypies may include oral-nasal components in pigs resembling their natural foraging by rooting, grazing etc, or their expression could be influenced by how the start of a feeding event is signalled in that particular farm (Mason and Mendl, 1997). Tatemoto et al. (2022) suggests that initially stereotypies, in general, may help the animal to cope with a stressful situation, but that with time this may cause impairment in the functioning of the brain by changing the structure of it.

1.3.1 Factors that affect the expression of re-directed oral behaviours

Studies of re-directed oral behaviour in gestating sows have focussed on the idea that chronic hunger and lack of foraging substrates leading to frustrated motivation to feed without an appropriate behavioural outlet might be the primary and only causes. These studies have looked at the effect of feeding practices (e.g. number of meals, feed level), diet composition (e.g. fibre type and level of inclusion, energy content), housing conditions and environmental enrichment in the form of foraging substrate on the performance of re-directed oral behaviours.

Because post-feeding re-directed oral behaviours are a sign of unsatisfied appetitive and/or consummatory behaviour, they increase/diminish depending mainly on the absence/presence of food (Douglas et al., 1998; Rushen, 1985; Terlouw et al., 1993). Therefore, the time spent performing re-directed oral behaviours increases with the number of meals in a day, and the effect is higher in sows eating a concentrate conventional diet than in sows eating a high-fibre diet (Robert et al., 2002).

In terms of diet composition, re-directed oral behaviours are mainly affected by the fibre and energy content. In general, an increase in fibre and energy content reduces arousal pre-feeding (Robert et al., 2002, 1997; Rushen et al., 1999), as well as the performance of re-directed oral behaviours shortly after eating and hours after the feed is finished (Terlouw and Lawrence, 1993; Zonderland et al., 2004). However, the duration of the effect will vary depending on the type of fibre (soluble vs insoluble).

Soluble fibres (e.g. sugar beet and potato pulp) are mostly highly fermentable in the hindgut while insoluble fibres (e.g. pectin, wheat bran and oat hulls) pass mostly unchanged through the guts. This means that fermentable soluble fibres are broken down in the hindgut and as a result short-chain fatty acids are obtained (Bach Knudsen, 2001) which are a source of energy (Rérat et al., 1987). Thus, this has an effect on the level of activity and performance of re-directed oral behaviours: high-fibre diets based on fermentable fibre are better at improving satiation (Danielsen and Vestergaard, 2001; Jørgensen et al., 2010) and have a longer lasting effect on behaviour for up to 7 h after the meal (Souza da Silva et al., 2012).

Energy content of the diet and/or feed allowance reduces the expression of re-directed oral behaviour and activity level in general. Terlouw et al. (1991) studied re-directed oral behaviours (chain manipulation, drinking, activities directed towards the trough/floor, self-directed oral behaviours) in a 2 x 2 factorial study including feed allowance (2.2 kg vs 4.0 kg of food daily) and housing type (individual stalls vs group pens). They observed an overall decrease in all the behaviours with higher feed allowance. In the case of self-directed oral behaviours [sum of tongue sucking, mouth (or jaw) stretching and vacuum (or sham) chewing], there was a *numerically* small increase with higher feed allowance in loose-housed gilts (0.004 ± 0.002 vs 0.002 ± 0.001 proportion of observation ± SE). However, two studies show that energy is effective in reducing feeding motivation right after feeding. Robert et al. (1997) compared the behaviour of gilts receiving high fibre diets that were based on oat hulls and oats. The high fibre diets had either similar or lower energy (experimental groups) as compared to a concentrate diet (control group). They found that chain manipulation was lower before the meal when a high fibre diet (with similar energy as the concentrate) was provided as compared to a high fibre diet with low energy content. Chain manipulation did not differ between both groups after the meal. This shows that both diets were efficient in reducing hunger right after the meal due to the high bulk of the feed but in the long term both factors (energy and fibre) might be important to reduce chain manipulation (if there is a direct relationship between hunger and chain manipulation). Bergeron and Gonyou (1997) compared the expression of re-directed oral behaviours between sows (second-parity) receiving a standard concentrate diet, high energy concentrate diet and a standard concentrate diet with hanging chains placed above the feeder. When comparing sows' behaviour receiving high energy diet against standard concentrate diet, sham chewing was lower in high energy sows. This was observed right after the meal (0845 to 1200h) but not during the afternoon observations (1230 to 1545 h). This may show that energy levels are effective in improving satiation only right after feeding but in order for it to last other adequate resources needs to be provided as well.

Findings are sometimes contradictory when comparing diets with different levels of fibre but the same level of energy (iso-energetic). Some authors have still found a reduction in re-directed oral behaviours when offering high-fibre diet as compared to low-fibre diets, however others have found no difference in terms of behaviour between sows on a low- and high-fibre diet (Ramonet et al., 1999; Whittaker et al., 1999, 1998). The latter is explained by animals having a set-point for energy and nutrients. Thus, independent of high fibre diet satiating the animal mechanically, they remain hungry since the energy requirements are also not met (D'Eath et al., 2018, 2009; Lawrence et al., 2004).

Environmental factors that give sows a more natural outlet of their behavioural needs may also affect the amount of re-directed oral behaviours. This by replacing part of the 'less normal' looking behaviour e.g. self-directed and object-directed oral behaviours for behaviours that 'seem more normal' in that they resemble the foraging of pigs in natural environments e.g. rooting on the floor/substrate. This can be observed when providing straw, wood shavings or hanging items like wood. When providing straw, Whittaker et al. (1998) observed a decrease in fixture- and self-directed oral behaviours, and drinking (floor interaction was not measured); Spoolder et al. (1995) observed a decrease in fixture-directed oral behaviours, but an increase in ground oral interaction; and Fraser (1975) found a decrease in fixture-related oral behaviours. Jensen et al. (2015) found that a better outlet of behavioural needs was observed when sows were provided with *ad libitum* straw as opposed to limited access to straw, showing no change in manipulating equipment with different diets when on *ad libitum* straw. When providing wood shavings, de Leeuw and Ekkel (2004) showed a reduction in behaviours directed towards the equipment and self-directed oral behaviours and an increase of oral behaviours re-directed towards the floor/substrate; and de Leeuw et al. (2003) showed a decrease in self-directed oral behaviours during the afternoon observations and an increase of oral behaviours re-directed towards the ground/substrate during the noon observations. Finally, Li et al. (2022) observed a decrease in self-directed oral behaviours when providing pine or scented wood tied around the trough.

Similarly, bulkiness of the feed can also mask re-directed oral behaviours. It is true that bulky feed improve satiation due to the higher fibre content. However, a reduction in re-directed oral behaviours and oral stereotypies could also be explained by a longer time needed to consume the feed (Bergeron et al., 2000; Holt et al., 2006; Ramonet et al., 1999; Robert et al., 2002). However, Li et al. (2013) did not find differences in feeding behaviours and re-directed oral behaviours between sows fed a concentrate diet based on corn-soybean-based and sows fed distillers' dried grains with solubles. This could be explained by the relatively low acid detergent fibre (ADF) and little difference between treatments (3.10 vs 7.16% ADF).

It is important to note that what and how a particular re-directed oral behaviour is affected by the studied factors may differ from study to study. This is likely because of the different set up of each study. For instance, behaviours directed towards the floor are affected differently by the provision of substrate (straw) depending on the set up. In some cases, there was an increase in this behaviour (de Leeuw et al., 2003; de Leeuw and Ekkel, 2004; Spoolder et al., 1995) but in other cases a decrease or no effect was observed (Jensen et al., 2015). This probably happened due to the place where substrate was provided. In the first case, it was provided on the ground and in the latter case in racks.

1.4 Could apparently hunger-related oral behaviours in pigs actually be caused by other factors?

There is evidence to question the single origin of re-directed oral behaviours. Re-directed oral behaviours, normally observed in studies on chronic hunger in gestating gilts and sows, have also been observed in growing and finishing pigs (Dubarry, 2019). Dubarry (2019) found a significant development in sham chewing, tongue playing, jaw stretching, wind-sucking, and trough directed behaviours in growing and finishing pigs. Younger animals are routinely fed *ad libitum*, hence oral behaviours should be unrelated to hunger, and other factors may be eliciting their performance.

To add to the argument, gilts or sows continue to show re-directed oral behaviours even after being fed to '*ad libitum'* amounts (four ration of 2.5 kg daily for three days of standard pelleted dry sow diet; total intake 9.4 kg/d; Alvas, 2018; Read et al., 2020), and when fed a completely *ad libitum* on a high fibre diet (Brouns et al., 1994; de Leeuw and Ekkel, 2004). The remaining re-directed oral behaviours could be explained by the emancipation of stereotypies acquired as a result of feed restriction during gilt rearing. However, it is unknown how fast pigs can develop oral stereotypies, or how long they take to reach emancipation from the original cause.

Some results have some room for an alternative explanation. Sham chewing has been shown to be affected by the provision of straw (Fraser, 1975; Stewart et al., 2011). However, Stewart et al. (2008) found no effect when offering 0.3 kg straw/sow/day. They argued that the lack of effect was due to a low level of fibre inclusion in the diet (amount of fibre is not reported). However, it may be that no difference was found between sows with and without access to straw because of a possible confounding variable. The social stress the animals were submitted (mixing and aggression) could have favoured the development of gastric ulceration (Hessing et al., 1992). For instance, Fraser (1975) provided 0.5 to 1 kg/gilt/day of straw to gilts on a concentrate diet (2.8% crude fibre) but without any source of stress, and observed a significant decrease in the frequency of sham chewing as compared to gilts without access to straw.

My suggestion is that re-directed oral behaviours could be categorized into two groups; self-directed oral behaviours (e.g. sham chewing) and out-directed behaviours (e.g. licking the trough) (Figure 1.2). In the context of chronic hunger, re-directed oral behaviours appear as a way to cope with the lack of feed and/or a barren environment. The pig will try to fulfil their behavioural and nutritional needs by re-directing their usual oral behaviour towards the available resources. These re-directed oral behaviours may appear as out- or self-directed. Some of these behaviours will help the pig possibly reduce stomach pain/discomfort by itself (not needing to interact with anything) or possibly find food and this way improving satiation.

Any source of pain or discomfort in the upper digestive system may elicit the same type of behaviours (Figure 1.2). These may, as well, be self- or out-directed behaviours. For instance, it could be hypothesised that self-directed oral behaviours such as sham chewing may help reduce discomfort from stomach ulceration. Salivation has been observed to occur with sham chewing (personal observation), and an increased saliva production is observed in crib-biting horses with gastric ulcers (Moeller et al., 2008). Out-directed behaviour in pigs with health problems affecting the upper digestive system, especially the stomach, could be explained by an attempt to find feed to help neutralize gastric fluids, and this way improve discomfort/pain to a degree. Another example may be the relief of pain coming from oral disorders by bar-biting as suggested by Alvas (2018) and Engblom et al (2008). It seems plausible that pigs rub injured tissue to reduce pain as observed in surgically castrated piglets without pain management (Llamas Moya et al., 2008).

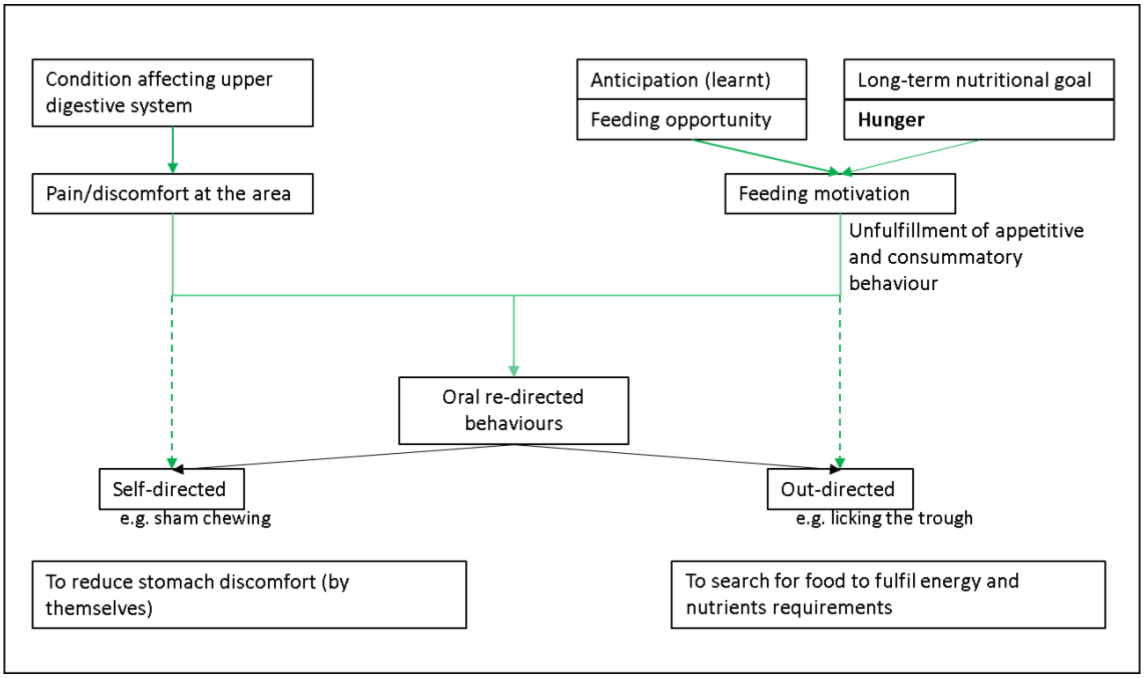

Figure 1.2. Possible different roles for re-directed oral behaviours in pigs. Green arrows mean positive feedback. Dashed arrows represent a loose connection; both self- and out-directed oral behaviours may be related to either pain/discomfort at the area or feeding motivation.

The idea of re-directed oral behaviours fulfilling different functions is supported by the differences observed in the type of oral behaviours performed before and after feeding (Rushen, 1984). They found that before feed was delivered, the behaviours that were the most common were head-waving, bar-biting, and rubbing the snout against the cage, particularly in older sows. After the meal was consumed the most commonly observed behaviours were drinker manipulation, and for some sows, rubbing. The former translates into the possibility that some oral behaviours observed in sows may be more related to reducing stomach discomfort by itself. If this is true, animals suffering from other conditions causing stomach discomfort should show similar oral behaviours.

1.5 Are re-directed oral behaviours related to gastric ulceration?

As suggested before, there may be the possibility that some re-directed oral behaviours observed due to hunger in sows may aim to reduce stomach discomfort (see section 1.4). A possibility is that gastric ulcers (see section 1.5.1) may also be eliciting oral behaviours usually related with hunger in pigs (see section 1.5.2).

1.5.1 Gastric ulceration in pigs

Gastric ulceration is an important issue in the pig industry. This affects a high number of pigs within most productive stages, impacting the productivity and animal welfare. Gastric ulcers are not only observed in finishing pigs but have recently been found to affect pigs in other productive stages as well. Studies have reported a prevalence of 26% in nursery pigs (Peralvo-Vidal et al., 2021a), 20.70 to 71.90% in finishing pigs (Cybulski et al., 2021a; Gottardo et al., 2017), and 45.5% in sows (that were culled mostly due to reproductive issues; Cybulski et al., 2021b).

Gastric ulcers are important in terms of the economy of the system as they affect pigs' weight gain and can result in death in severe cases. Some studies have reported a reduced growth rate in pigs with gastric ulcers (Ayles et al., 1996b; Elbers et al., 1995a; Hedde et al., 1985). Most recently, Dunlop et al. (2021) followed pigs (> 1000 pigs) from weaning to market weight at which point they were slaughtered and surveyed right at arrival to the abattoir. They found a significant reduction in growth rate and feed efficiency in pigs with gastric ulcers as compared to pigs with healthy stomachs. There was also an economic loss to the farmer of US$15.90 and $10.60/pig in pigs with gastric ulcers slaughtered at 157.5 days of age and at 76.27 kg carcass weight, respectively. However, there are also some studies in which no effect of gastric ulcers on weight gain was found (Dirkzwager et al., 1998; Guise et al., 1997; Wondra et al., 1995).

Gastric ulcers have an impact on animal welfare. Gastric ulcers have shown to cause some level of discomfort in humans (Barkun and Leontiadis, 2010) and have been associated with a change in behaviour in non-human animals, such as pigs (Dybkjær et al., 1994; Peralvo-Vidal et al., 2021a; Rutherford et al., 2018), horses (Wickens and Heleski, 2010) and rats (Murison and Skjerve, 1992). Adding to this, it is difficult to diagnose in the living pig as clinical symptoms are general (Friendship, 2004). This makes it a hidden animal welfare problem: to the eyes of the farmer there is no difference between pigs with a healthy and mild to moderately ulcerated stomach or other conditions, and, for them, they keep eating and growing and reach slaughter weight eventually.

Pigs are particularly prone to develop gastric ulcers in the pars oesophageal region (Figure 1.3) as compared to other parts of the stomach (Friendship, 2004). This region has a stratified squamous epithelium without mucosal glands, which are present in the rest of the stomach and secrete mucus to protect the stomach’s wall against acid, bile and enzymes (Yen, 2001). Since the pars oesophageal region does not have this protection, pigs are particularly susceptible to develop gastric ulcers when the stomach content is fluid (Friendship, 2004). If contact between acidic fluid stomach contents and the pars oesophageal is chronic, it can get damaged.

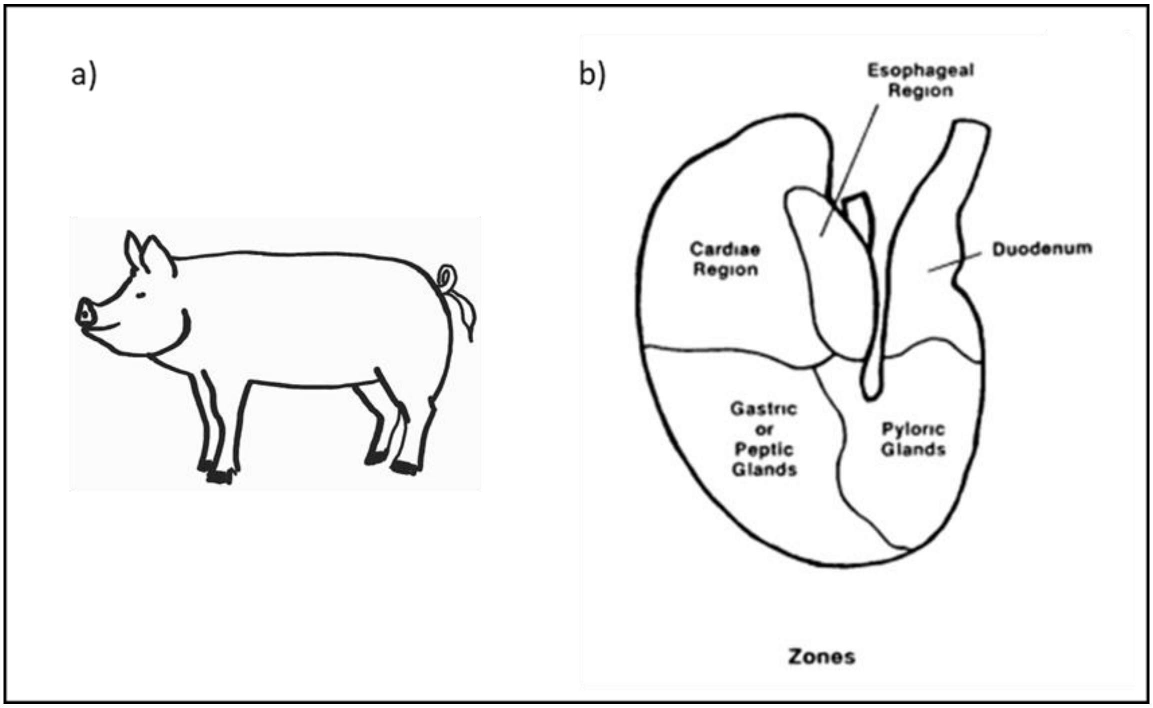

Figure 1.3. a) Direction of the pig and b) diagram of the functional regions of the stomach. The pars oesophagea is located in the area named oesophageal region proximal to the opening of the oesophagus. Pig diagram self-made (free to be used), and stomach diagram modified from doctoral thesis (van Hees, 2022)

Any factor that favours the contact of digestive fluids with the pars oesophagea may result in gastric ulceration. As reviewed by Canibe et al. (2016), factors having a negative impact on the pars oesophagea included any factor reducing the firmness of stomach content and/or factors that favours an empty stomach. Factors that reduce the firmness of the stomach content are small particle size (Vukmirović et al., 2017), pelleted vs non-pelleted feed (Wondra et al., 1995), absence of straw (Jensen et al., 2017) and *ad libitum* feeding (Robertson et al., 2002). Factors that may reduce feed intake and hence empty the stomach are mixing with unfamiliar pigs and being middle rank pigs (Hessing et al., 1992), fasting, transportation and time spent at the abattoir (Lawrence et al., 1998), and unstable environmental temperature (Riker et al., 1967). The review also suggests that being male (Di Martino et al., 2013), some genotypes (Iberian pigs) (Ramis et al., 2007), and the presence of pathogens (Archobacter and Helicobacter sp.) (Appino et al., 2006; de Oliveira et al., 2010; de Witte et al., 2018) may increase the susceptibility for development of gastric ulcers, but evidence for these is more ambiguous. Canibe et al. (2016) also suggested that some food additives may protect the stomach wall and therefore reduce the susceptibility to develop gastric ulcers [melatonin (Ayles et al., 1996a) and Vitamin U (Elbers et al., 1995b)]. Recently, Peralvo-Vidal et al. (2021b) studied the risk factors in nursery pigs and identified two main risk factors: weight at birth and pen fouling. Piglets weighing less than 1.11 kg had 2.11 times higher odds of having a lesion in the pars oesophagea and (weakly) pen with the presence of fouling.

Gastric ulceration progresses from healthy tissue to keratinization, as described by Thomson and Friendship (2012). Keratinization causes a thickening of the mucosa as response to the irritation. The thickened tissue is then fissured and peeled off which results in erosion of the mucosa. Erosion then progresses into ulceration. In more severe cases, the oesophagus can lose its flexibility and shrink at the entrance of the stomach, as a result of a severely damaged pars oesophagea. A visual guide is found in Kopinski and McKenzie (2007). It is unknown how long this process may take, however, Thomson and Friendship (2012) mention that a completely healthy pars oesophagea may get ulcerated in less than 24 h. Healing may take about seven days (Lawrence et al., 1998). Although Lawrence et al. (1998) assessed the effect of different management on different pigs.

1.5.2 Association between gastric ulcers and behaviour in pigs and other species

Gastric ulcers have been associated with a change in behaviour in non-human animals, such as pigs, horses and rats. Only four published studies have explored this association in pigs (Dybkjær et al., 1994; Hartnett et al., 2023; Peralvo-Vidal et al., 2021a; Rutherford et al., 2018). Dybkjær et al. (1994) studied the effect of stress, through behavioural indicators [belly-nosing, manipulating belly, ears and tail, and chain-chewing (Dybkjær, 1992)], on the prevalence of gastric ulcers at slaughter in growing pigs. They measured behaviour in 25-kg pigs and then sampled their stomach at slaughter weight at the abattoir. They found that pigs that behaved in a certain way (e.g. manipulating penmates) were more likely to have gastric ulcers at the moment of post-mortem inspection at the abattoir. Rutherford et al. (2018) studied the effect of gastric ulcers on pain-related behaviour (posture, activity, penmate manipulation and aggression) in finishing pigs. They observed pig behaviour either one or two days before euthanasia at the farm or slaughter at the abattoir, and then compared both study groups retrospectively according to the results of the post-mortem stomach inspection. They found differences in behaviour between pigs with and without gastric ulcers: the former spent more time standing and less time lying on their left side as well as being more active. Peralvo-Vidal et al. (2021a) studied the effect of gastric ulceration on feeding behaviour in nursery pigs. Pigs with ulcerated stomachs tended to eat for longer per feeder visit, and were visiting the feeder less often but, by the end of the day, spent the same time feeding than pigs without stomach ulcers. However feeding behaviour and gastric ulceration were weakly related. Lastly, Hartnett et al. (2023) compared the behaviour between pigs with lower and higher scores for gastric ulceration, with a gap of 5 or 12 days between behavioural observations and post-mortem stomach assessment. Pigs with higher scores laid more on their left side, and tended to show less tail-in-the mouth behaviour walk more. Because of the gap between both points, the authors suggest that it may have been that the behaviours rather than being an effect of gastric ulceration were a cause.

From these three studies, it can be concluded that gastric ulcers in pigs may be more likely to develop in pigs experiencing stress (as measured by behavioural indicators) and that the presence of gastric ulcers may cause some level of stomach discomfort as shown by a change in behaviour. However, none of these studies show a clear causative effect of gastric ulceration on pigs’ behaviour (because of the time gap between behavioural and stomach assessment), but also they do not test which way around the effect is;

gastric ulcer affecting the behaviour and/or behaviour favouring the development of gastric ulcers. Additionally, none of these studies included self-directed oral behaviours.

Similarly, in horses, gastric ulcers have been found to be related with a change in general behaviour. Horses with ulcerated stomachs pawed more and ate quicker after feed delay, and showed higher increase in faecal cortisol metabolites after a Novel Object test (Malmkvist et al., 2012). More interestingly, gastric ulceration has been related to an increase in crib-biting behaviour [oral stereotypy where the animal rests its incisor teeth, flexes the strap muscles of the neck, and makes an audible grunting sound (Houpt, 2012)] (Garcia et al., 2015; Mills and Macleod, 2002; Nicol et al., 2002). For instance, Nicol et al. (2002) studied the effect of gastric ulcers on the performance of crib-biting in horses. After an endoscopic examination of stomach health, they found that crib-biting horses had more ulcerated and inflamed stomachs than horses that do not show this behaviour, and that crib biting decreases to a greater extent in ulcerated horses as compared to unaffected horses when giving antacid after observing a significant improvement of stomach health.

Moreover, as studied in horses, oral behaviours in pigs, especially self-directed oral behaviour such as sham chewing, could be related to gastric ulcers by causing an increase in the production of saliva (personal observation). In horses, an increased level of saliva secretion has been observed in crib-biters as compared to non-crib-biters (n = 18) (Moeller et al., 2008). This may increase stomach pH, and therefore reduce the stomach discomfort caused by gastric ulcers. However, in a study where saliva production was measured in *only two horses* through a fistula placed in the parotid salivary duct, no significant relationship between crib-biting and saliva production was found, and no significant increase in saliva production was detected after crib-biting (Houpt, 2012). Also, Wickens et al. (2013) did not find any difference in the number or severity of ulcers, prevalence of hyperkeratosis, or baseline gastric pH between crib-biters and non-crib-biters. Although, during this study, horses had continued access to pasture and hay, which have a protective effect in pigs (Herskin et al., 2016; Holinger et al., 2018b, 2018a).

In rats, studies investigating the association between gastric ulceration and behaviour has focused on understanding how gastric ulcers affect how rats respond to stress. Sines (1975) studied the effect of differences in behaviour among rats on the development of stress-induced gastric ulcers under two different scenarios (1h in a circular treadmill or 'drums' + 4h of feed deprivation vs 10 h of restraint). Rats were classified as showing low, medium and high activity. They found that the interaction between the level of activity and type of stressor had an effect on the severity of gastric ulcers, but not each variable by themselves. Also, Murison and Skjerve (1992) found that the attack latency of rats with stress-induced gastric ulcers (e.g. restraint-in-water) in an intruder test is negatively correlated to the amount of gastric ulcers. This means that rats with more stress-induced ulcers attack more quickly than rats with a lower number of gastric ulcers. The presence of gastric ulcers may affect rats' behaviour or rats that are more susceptible to develop gastric ulcers due to stress behave differently than rats that are less susceptible. Overmier et al. (1997) found that the emotionality of rats influences the development of gastric ulcers after an episode of stress. It seems that rats' personality influences the way they behave and respond to stressful events and that this has an effect on the development of gastric ulcers, and these indirectly affect their behaviour.

It can be concluded that behaviour and stomach health are related. The relationship between oral behaviour and gastric ulceration in pigs is still to be explored but the evidence from other species shows that there might be a connection. The causative relationship between behaviour and gastric ulcers could occur in either direction: behaviour increasing the susceptibility for gastric ulceration and/or stomach health affecting the behaviour. Adding to this, stomach health and behaviour may be affected by stress, and personality or coping style may influence how animals react to stress.

1.6 Some considerations in the study of gastric ulcers

The study of the association between gastric ulcers and behaviour is made more difficult by the lack of knowledge on how fast an ulcer develops and heals (see section 1.7), the many risk factors that favour the development of gastric ulcers (see section 1.5.1), and the variability observed between individuals.

Good animal welfare practices such as the provision of enough straw (10 to 500 g/pig/day) has a protective effect on the stomach and therefore reduces the likelihood of gastric ulceration in growing pigs (Jensen et al., 2017). This may be a problem when studying whether re-directed oral behaviours can be elicited by factors other than hunger. Well-protected stomachs may not get damaged and therefore oral behaviours may not be observed. Also, the mechanical effect of straw may improve satiation and therefore reduce the expression of re-directed oral behaviours. In fact, Rutherford et al. (2018), whose study was done in straw-bedded finishing pigs, found a relatively low number of pigs with gastric ulcers even though pigs were fed with a high ulcerogenic diet.

The development of gastric ulcers varies from individual to individual. This could be related to individual differences in the propensity to develop ulcers, or in susceptibility to risk factors, or to variation in behaviour and coping style under stressful situations. For instance, Rutherford et al. (2018) observed a high individual variation in response to a high ulcerogenic diet. They suggested that the development of gastric ulcers is influenced by the coping style and therefore the behaviour of pigs. In fact, Hessing et al. (1994) found a higher prevalence of gastric ulceration in non-resisting pigs in a backtest (e.g. test to predict pigs' coping styles by counting the amount of escape attempts). Also Bolhuis et al. (2006) found that the coping style of the pigs influenced the protective effect of the straw; non-resisting pigs, but not pigs that were resisting, showed a decrease in gastric ulceration when straw was provided. Dybkjær et al. (1994) found that the likelihood of developing gastric ulcers was related to how pigs behave (e.g. stress-related behaviours such as pen mate manipulation). All these findings show that there is an individual variation in the development of gastric ulcers and that this may be affected by the coping style of the animals and therefore their behaviour.

Also, behaviours could be favouring the development of gastric ulcers rather than the other way around, as discussed before (see section 1.5.2). The direction of cause and effect could be investigated by observing the behaviour of pigs with completely healthy stomachs as assessed by gastroscopy. To check a possible confounding effect of gastroscopy on the development of gastric ulcers, some pigs may be left aside to then compared ulcer development between pigs submitted and not submitted to gastroscopy. The behaviour would be assessed for a few weeks. The health status of the stomach could be maintained by feeding them with a diet with coarse particle size and antacid. On a second stage, completely healthy pigs could be provided with a standard commercial diet adequate for their age and productive stage. After assessment of the stomach health by gastroscopy, pigs are observed for a few weeks. This way behaviours that favour the development of gastric ulcers could be identified during the first phase, and behaviours occurring due to gastric ulcers once they have been identified. These may be oral behaviours but also other types of behaviour.

1.7 Assessment of the health of the pars oesophagea

Nowadays, stomach health can only be assessed post-mortem (at the abattoir) or by gastroscopy in pigs under general anaesthesia. Both methods involve factors that can obscure the results. The accuracy of both might depend on the gap between the measurement of the variable of interest and the assessment of the stomach, and all the things that may happen in-between both timepoints. However, their real impact on the results is dependent on the speed of healing and/or development of gastric ulcers in pigs. A worsening in gastric ulcers can be observed relatively fast. Gastric ulcers can increase in severity after a 24-h fasting period from hyperkeratosis to erosion and some level of ulceration and hyperkeratinisation can be induced by a long (160-km) journey in finishing pigs (Lawrence et al., 1998). A better way to assess stomach health would be in the living pig, non-invasively and in real-time.

In the following sections, I will describe the current post-mortem assessment of stomachs for gastric ulcers (see section 1.7.1) and describe the possible use of saliva as a biofluid for metabolite identification for the diagnosis of gastric ulcers in pigs (see section 1.7.2). Behaviour as an indicator of gastric ulcers in pigs has been discussed previously (see section 1.5.2).

1.7.1 Post-mortem assessment of stomach integrity

Stomach integrity in pigs has been assessed macroscopically at the abattoir in epidemiological studies (Mushonga et al., 2017; Omotosho et al., 2016; Penny et al., 1972; Swaby and Gregory, 2012). This has also been used extensively to assess stomach integrity in studies looking at risk factors for gastric ulceration [e.g. particle size (Ball et al., 2015; Wondra et al., 1995); pelleting (Robertson et al., 2002; Wondra et al., 1995); straw (Di Martino et al., 2013; Holinger et al., 2018b, 2018a; Jensen et al., 2017; Krauss et al., 2018); stress (Hessing et al., 1992; Holinger et al., 2018a); barren environment (Ramis et al., 2005; Vitali et al., 2018); transport (Lawrence et al., 1998); bacteria (de Oliveira et al., 2010); and fasting (Lawrence et al., 1998)]. Finally, post-mortem assessment has also been used for studying the association between general behaviour and gastric ulcers in pigs (Dybkjær et al., 1994; Hartnett et al., 2023; Peralvo-Vidal et al., 2021a; Rutherford et al., 2018).

The outcome of the stomach assessment in all of these studies may be obscured by the movement of animals (e.g. transport, mixing animals, fasting). These may affect the stomachs' integrity pre-slaughter in an unpredictable way, affecting the strength of any expected relationships between on-farm experiences and ulcers. Notwithstanding, many studies have used this method and the results have been shown to behave as expected regarding the factors being tested (Di Martino et al., 2013; Holinger et al., 2018b, 2018a; Jensen et al., 2017; Ramis et al., 2005; Wondra et al., 1995). Even Ramis et al. (2005), who fasted pigs for at least 12 h and kept them for 4h at the lairage in the abattoir still found fewer gastric ulcers in pigs housed in enriched vs barren environments. Additionally, in those studies where the factors being tested had an unexpected effect the authors claimed other risk factors different than transport being involved [e.g. animal management, health and dietary factors (Robertson et al., 2002); inadequate proportion of particle size (Holinger et al., 2018b; Krauss et al., 2018); other stress factors (Krauss et al., 2018)]. Therefore, post-mortem assessment of stomachs for the study of gastric ulcers is fairly accurate, however, care needs to be taken.

1.7.1.1 Scoring systems for the assessment of stomach integrity

Scoring systems for the assessment of the changes in the mucosa of the pars oesophagea due to gastric ulceration could be classified broadly into two categories: those that score the pars oesophagea according to the worst lesion present and those that also include a scoring for each of the lesions separately. Within these scoring systems there may be some that do not include chronic changes.

Scoring systems where the worst lesion present gives the score can be more or less detailed but also researchers may then regroup pigs according to what they are researching. For instance, Dunlop et al. (2021) and Ayles et al. (1996a) scored stomachs into four categories, namely normal, keratinization, erosion and ulcer, leaving out any details of the severity of each of these categories. Similarly, Swaby and Gregory (2012) included chronic lesions e.g. stenosis, however the categories lack detail and put together lesions such as erosions and mild ulcers. Also, their most severe category includes stomachs that had developed ulcers, with haemorrhage and stenosis present.

Other studies had used similar scoring scales but then had regrouped or selected pigs according to the needs of their research question. Peralvo-Vidal et al. (2021a) used a scoring system based on Nielsen and Ingvartsen (2000) which scores the stomachs according to the severity of the most severe lesions, from parakeratosis to the contraction of the oesophagus. This scoring system includes in the same score ulcer and/or scarring. Peralvo-Vidal et al. (2021a) then grouped pigs into two categories: pigs that did not have any lesions in the pars oesophagea and pigs with any type of lesion e.g. erosions, ulcers, scars, and oesophageal stenosis. The latter was then divided into a subgroup of pigs that only had severe ulcers in the pars oesophagea. This group could also have had pigs that had stomachs with scar formation. Madsen et al. (2022) used a scoring system that measured the progression from healthy tissue to different levels of ulceration (extension and depth of the lesion) in the pars oesophagea. They then combined pigs with healthy stomachs with pigs that had some level of keratinization into one group and compared to pigs with active ulcers.

Few studies try to describe the coexistence of different lesions. Regarding this, Wondra et al. (1995) additionally to the usual scoring system (4-point scoring scale), they also scored the stomachs according to the level of keratinization, which they did not do for the remaining type of lesions e.g. erosion, ulcer. Lawrence et al. (1998) also used a 4-point scoring scale but since they observed that many stomachs qualified for more than one category and had varying degrees of severity, they developed a scoring system in which the most predominant type of lesion would give the score and then there was a .1 increment in the score representing the increased in the abnormality. However, these two studies do not describe well which type of lesions were present and what was their severity.

Rutherford et al. (2018) came closer to this. They used a 10-point scoring scale to describe the stomachs where 10 corresponded to a stomach with contraction of the oesophagus. Additional to this, to facilitate the scoring of the lesions, they used a separate scoring system that would score each of the type of lesions separately. The 10-point scoring scale was then based on the worst lesion present, and its severity described by this score i.e. scoring scale describing each lesion present. They then use the 10-point scoring scale for statistical purposes only. Because of the aim of the study (association between pain-related behaviour and gastric ulcers), the only relevant scores were ulcer scores including different degrees of ulceration.

Scoring systems for gastric ulceration are useful to describe the population and have a sense of the overall condition of the stomachs. The problem with all of these scoring systems are: 1) that in one single point of the scoring scale more than one type of lesion may be included e.g. in a 10-point scale usually ulcers and scar formation are combined into one; and 2) the scoring system is based on scoring the worst lesion e.g. keratinization < erosion < ulcer < contraction of oesophagus, missing out on all the less severe lesions that may be present in the same stomach. This results in a poor description of how the development of the gastric ulceration occurs and how the different lesions coexist. This might be important as different combinations of these lesions may cause different sensations in the animals as well as different changes in the physiology.

Post-mortem assessment of the stomachs is good for a first approach since it is relatively cheap and therefore a high number of animals can be assessed. Also, for the type of studies being proposed in this report (association between behaviour and gastric ulcers in pigs) the risk factors mentioned before may not affect the results. The coping style of a pig influences its behaviour and predicts to some extent how a pig will respond to a stressful challenge. Thus, in theory, pigs that behave in a certain way should behave consistently over time and therefore it may be the case that whatever the time samples are taken it will be fairly similar between times. However, because there may be factors that affect some pigs more than others (e.g. location in the truck), this method is not reliable where the link between behaviour and gastric ulceration needs to be assessed.

In this case, it would be the best to use on-site euthanasia or gastroscopy as a gold standard method.

1.7.2 Saliva and metabolomics

Saliva has a role in digestion (Pedersen et al., 2018) and, more important to this thesis, in the protection of the oesophagus (Kongara and Soffer, 1999; Sreebny, 2000), and arguably the pars oesophagea. Saliva is secreted by the salivary glands in the mouth as described in Zhang et al. (2016). The salivary glands are highly permeable and are surrounded by capillaries. This facilitates the exchange of molecules from the blood to the saliva, allowing for the measurement of compounds that could be used as biomarkers of disease. This topic has been extensively reviewed in human medicine (Nunes et al., 2015; Song et al., 2023; Zhang et al., 2016). Additionally, saliva is particularly attractive for diagnosis as it is relatively easy to collect and less invasive and time consuming as compared to blood sampling.

Compounds identified in the saliva have found to change with different conditions. Salivary compounds have been observed to change for various human diseases including chronic kidney disease (Thorman et al., 2010), gastric cancer (reviewed by Koopaie et al. (2022); Song et al., 2023), gastroesophageal reflux disease (Skoczylas et al., 2014) and peptic ulcer (Boghori et al., 2014). The variation in saliva composition due to disease has been reported in various non-human animals. For example, in rats: acute renal disease (Kovalčíkova et al., 2018), in horses: Equine Gastric Ulcer Syndrome (Muñoz-Prieto et al., 2022), in pigs: septic and non-septic inflammatory response (López-martínez et al., 2022), changes in insulin (Ortín-Bustillo et al., 2021) and cortisol blood concentration (Cook et al., 1996), and has been recently reviewed for its use in pigs (Cerón et al., 2022). Importantly, in a recent study, changes in saliva composition has been found in pigs with gastric ulceration (Madsen et al., 2022). Madsen et al. (2022) compared saliva composition between finishing pigs with active ulcers and pigs with healthy stomachs, including pigs with keratinization of the pars oesophagea. This analysis allowed for the identification of metabolites that may be used as potential biomarkers for active ulceration in farmed pigs. This study did not investigate the progression of the condition or how different types of lesions may be affecting the saliva composition.

Saliva sampling for metabolomics analysis in pigs is made more difficult by the likelihood of contamination. This because of the housing conditions of pigs e.g. access to food and substrates which may be chewed, presence of faeces and urine which pigs root, nose and lick (Madsen et al., 2022). Also, the distance from the farm to the laboratory makes it a priority to make sure samples remain cold throughout until they arrive at the laboratory for preparation for stabilization of the samples (Nam et al., 2023). Samples should be analysed immediately or stored at -80^{o} C if not being analysed right away (Cerón et al., 2022).

Saliva composition can be studied by using the techniques used within the field of metabolomics, which is one of the 'omics' science field. Omics studies the biological molecules present in a sample identifying a large number of molecules at the same time (Institute, 2012). Metabolomics studies the metabolome which is the whole set of metabolites that conform a biological sample (Gertsman and Barshop, 2018; Institute, 2012). The metabolite profile can be studied using different methods depending on the type of sample, concentration and properties of the metabolites and the amount of sample available. Techniques used within metabolomics are reviewed by Segers et al. (2019). For the identification of a wide range of metabolites (untargeted analysis) the method that is most commonly used is liquid chromatography–mass spectrometry (Gertsman and Barshop, 2018). There are different methodologies for liquid chromatography and mass spectrometry, and the best choice depends on the sample type and aims of the study (Segers et al., 2019). Liquid chromatography–mass spectrometry method is based on the differing characteristics and properties of the compounds that will interact with the instrumentation in different ways allowing for their identification. Mixtures need to be separated before going into the mass spectrometer (Furlani et al., 2021). Liquid chromatography separates mixtures into proteins, nucleic acids, or small molecules (Ahmed et al., 2023). The sample (liquid mobile phase) moves along a column (solid stationary phase), and molecules are separated according to their differing physicochemical properties in their interaction with the column (Furlani et al., 2021). Mass spectrometer ionizes the molecules, which is then transferred into the mass analyser and finally detected (peak intensities). The information is then sent to a computer for its interpretation (Furlani et al., 2021). Databases are used for the identification of the features detected in this process.

1.8 Conclusion

Re-directed oral behaviours have been widely studied in the context of dry sows and chronic hunger due to restricted feeding. However, there has been less consideration of other factors that could also be eliciting the performance of re-directed oral behaviours. Some evidence (e.g. early onset of oral behaviours in non-feed-restricted pigs, association between oral behaviours and gastric ulcers in horses) shows that gastric ulceration may be affecting the expression of re-directed oral behaviour in pigs. I suggest that gastric ulcers may be a cause for oral behaviours since gastric ulcers may cause some level of stomach discomfort shown by a change in behaviour, and they have been shown to increase oral behaviours in other animals.

Risk factors for gastric ulcers are well investigated however the impact on welfare and productivity is more ambiguous. Also, there are no methods of diagnosis in the living pig that would allow for a better understanding. Saliva composition has been explored recently in finishing pigs. This approach as not been tested in adult female pigs and how salivary composition changes with changes in the pars oesophagea. This thesis explores the use of oral behaviours and saliva composition and pH as a possible method of diagnosis of gastric ulcers in pigs.

1.9 Thesis outline

Gaps in knowledge identified in this literature review are outlined in the previous section (see section 1.8). I hypothesise that if there are explanations other than hunger for the occurrence of re-directed oral behaviours, then these behaviours would be observed in finishing pigs which are routinely fed *ad libitum*. Oral behaviours would increase with the presence of gastric ulcers in finishing pigs and adult female sows, and the progression in gastric ulceration would change salivary composition and pH.

In this thesis I aim to describe oral behaviours in finishing pigs and sows, and how they related to gastric ulcers. The description of oral behaviours was done in finishing pigs by live observation, and in sows through observation and literature review. The relationship between oral behaviours and gastric ulcers was investigated in finishing pigs and in sows. Finally, I aimed to identify potential salivary biomarkers of gastric ulcers and change in salivary pH due to gastric ulceration in sows.

In Chapter 2 I describe oral behaviours in finishing pigs routinely fed *ad libitum*. In Chapter 3 I investigate the association between gastric ulcers and oral behaviours, specifically, self-directed oral behaviours, in finishing pigs. In Chapter 4 I investigate the association between re-directed oral behaviours and gastric ulcers in gestating and lactating sows and explore the use of saliva metabolites and changes in salivary pH as a possible diagnostic method of gastric ulceration. In Chapter 5 I present the results of a systematic literature review on the variation of re-directed oral behaviours with diet and feeding practices, housing conditions and environmental enrichment in gestating gilts and sows.

Briefly, the oral behaviours included in this thesis include object-, pig- and self-directed behaviours (Table 1.1). Self-directed oral behaviours included snout twitching, wind sucking, tongue playing, jaw stretching and sham chewing as related with chronic hunger in sows [Alvas, 2018 (MSc disseration); de Leeuw and Ekkel, 2004]. Additionally, I included feeding-related behaviours which are oral behaviours directed towards the expected foraging target. All behaviours that imply the interaction with something are referred to as out-directed oral behaviours. For Chapter 2 and 3, sham chewing and chewing on straw were combined into chewing movements as it was difficult in practice to distinguish between them. For Chapter 4 this was not necessary as in sows sham chewing is more evident.

Table 1.1. Behaviour categories of oral behaviours recorded in this thesis in each chapter.

Behaviour category	**Chapter 2**	**Chapter 3**	**Chapter 4**
Self-directed	Jaw stretching, wind sucking, snout twitching, tongue playing, presence of saliva, sham chewing	Jaw stretching, wind sucking, tongue playing	Jaw stretching, wind sucking, snout twitching, tongue playing, presence of saliva, sham chewing
Sham chewing and straw chewing	Chewing movements[1]	Chewing movements[1]	
Object-directed	Chewing, licking, rooting and nosing equipment[2]		Foraging[3] pen fixtures[4], trough or wall, bite pen fixtures or trough, chew on something other than straw
Pig-directed	Licking and nosing penmate		Licking, nosing, rooting and biting[5] penmate
Feeding-related	Drinking, rooting floor, straw chewing		Drinking, foraging floor, straw chewing, bite floor[6], foraging straw, feeding

[1]Sham and straw chewing had to be combined for chapter 2 and 3 as they were difficult to distinguished
[2]Equipment includes wall, trough, fence, bars and drinker
[3]Foraging includes licking, rooting or nosing. When this is directed towards resources different than the floor and straw this is classified as re-directed.
[4]Pen fixtures includes fence, bars and drinker
[5]Biting penmate is non-aggressive and short-lived so does not include damaging behaviours
[6]The animal is using their teeth to scrape edible particles out of the floor

1.10 References

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Chapter 2 Ad-lib fed finishing pigs perform oral behaviours usually associated with chronic hunger in gestating sows

2.1 Abstract

Re-directed oral behaviours are observed in gestating sows due to chronic hunger and may be a coping mechanism for an environment in which their nutritional and behavioural needs are not satisfied. However, these behaviours may have causes other than chronic hunger such as underlying health conditions. To test the possibility that re-directed oral behaviours may have an alternative explanation, I observed food-restricted first- and second-parity gestating sows (sows hereafter) and *ad libitum* fed finishing pigs housed in straw bedded pens. I hypothesised that: 1) The rate of oral behaviours would be higher in sows; 2) These behaviours would also be observed in finishing pigs despite being fed *ad libitum*; and 3) Some of the behaviours vary together. Live behavioural observations were carried out on 95 finishing pigs and 42 sows in the morning and afternoon, resulting in 25 min/focal animal. All occurrences of self-directed oral behaviour (sham chewing, jaw stretching, tongue playing, wind sucking and snout twitching), equipment-directed (rooting, licking, biting and nosing equipment) and pig-directed oral behaviours (nosing and licking) as well as feeding-related behaviours (drink, root floor, straw chewing) were scored using continuous behaviour sampling. The effect of production stage on these behaviours was analysed with Generalized Linear Mixed Model (GLMM), and the relationship between behaviours within each production stage was investigated with Principal Component Analysis (PCA) and Spearman's correlation. Additionally, the proportion of pigs performing each behaviour at least once was calculated. The proportion of a given behaviour of the total amount of behaviour was also estimated. All behaviours were observed in finishing pigs, and unexpectedly, the

rate of behaviour did not differ from sows. Amongst the finishing pigs, the lowest rate of behaviours (means ± CI) and performed at least once by the fewest pigs (%) were jaw stretching [0.002 ± 5.502 (2.11%)], wind sucking [0.004 ± 4.741 (5.26%), snout twitching [0.010 ± 1.902 (10.53%)] and tongue playing [0.017 ± 1.994 (12.63%)]. Spearman's correlation showed positive moderate correlation between some of the behaviours; within pig-directed (r_s = 0.305, p = 0.003) and within equipment-directed oral behaviours (r_s = 0.302 to 0.335, p = 0.001 to 0.003) in finishing pigs, and within equipment-directed oral behaviours in sows (r_s = 0.450 to 0.453, p = 0.003). In sows, nose equipment was correlated negatively with straw chewing (r_s = -0.470, p = 0.002). The correlation coefficients were slightly lower in finishing pigs (r_s= +0.302 to +0.335 vs r_s = +0.450 to -0.470; $\alpha_{adjusted}$ < 0.00357). None of the self-directed oral behaviours correlated. PCAs explained 37% and 35.7% of the oral behaviour variation within finishing pigs or within gestating sows, respectively. The PCAs for finishing pigs and for sows showed differences. This chapter confirms the performance of oral behaviours, usually observed in gestating gilts and sows under chronic hunger, in ad-libitum-fed finishing pigs. Oral behaviours tend not to correlate highly in both stages. These results suggest that not all re-directed oral behaviours in pigs occur due to chronic hunger. We propose that some re-directed oral behaviours may be associated with underlying health conditions of the upper digestive system such as gastric ulcers. This is explored in Chapter 3 and 4. Furthermore, this could question the origin of hunger-related oral behaviours in gestating sows as the single origin.

2.2 Introduction

Until now, the predominant explanation for re-directed oral behaviours which develop in gestating gilts and sows is because of unsatisfied behavioural and nutritional needs (e.g. due to their routine feed-restricted diet). An insufficient amount of feed prevents feeding motivation from being downregulated by satiation/satiety. This means that gestating sows remain highly motivated for food in a relatively barren environment where a natural outlet of these needs - a foraging environment or substrate - is not possible. As a result, re-directed oral behaviours develop such as, but not restricted to, sham chewing, rooting on the empty trough, bar biting [reviewed by D'Eath et al. (2018)]. These can become stereotypical with time i.e. invariant in performance and unrelated to the initial source (Lawrence and Terlouw, 1993; Mason and Mendl, 1997), however, how this happens and how fast is still unclear (see section 1.2). It is known that the frequency of re-directed oral behaviours is affected by diet composition (e.g. type of fibre and level of inclusion, energy content) and feeding practices (e.g. number of meals, food allowance) in gestating sows [reviewed by D'Eath et al. (2018), see section 1.3.1].

However, there is evidence that other factors can elicit oral behaviours in other species. In horses, crib-biting (a type of oral behaviour) is not related to chronic hunger but has been linked with gastric ulceration among others. In a study, horses that received antacid mixture (16 horses single- and 17 double-dosed with antacid) showed a decrease in the count of crib-biting before and after feeding, showing a clear continued decline in the rate in a period of five weeks (Mills and Macleod, 2002). In another study, foals were examined for gastric ulceration by gastroscopy before entering the study and being allocated to either a control diet or treatment diet which included antacid (Nicol et al., 2002). None of the healthy foals performed crib-biting, and this behaviour declined to a greater extent in foals with gastric ulceration that received antacid. Also, similar to re-directed oral behaviours in feed-restricted gestating sows, crib-biting in horses has been related to husbandry practices as well. Stereotypies (cribbing, weaving and box-walking) increase with the amount of concentrate and decrease with the amount of roughage provided (Redbo et al., 1998). In another study, *ad libitum* provision of concentrate reduced the performance of crib-biting behaviour, which increased or returned back to baseline when fed a smaller amount in ration of concentrate throughout the day (n = 10; McCall et al., 2012).

In the case of pigs, however, no studies have been done on the association between oral behaviours, usually observed in feed-restricted gestating sows, and factors other than hunger and feeding practices. There is some evidence that this could be the case in pigs. De Leeuw and Ekkel (2004) measured re-directed oral behaviours in sows (age/parity not reported) fed either *ad libitum* or restricted, with or without substrate. They still observed hunger-related oral behaviours in sows fed *ad libitum* both with and without substrate. Similar results were found by Alvas (2018) and Read et al. (2020). In these studies, gilts and sows were fed 10 kg/d (usual restricted feeding at 2.5 kg/d) with a total feed intake of 9.4 kg/d. Both gilts and sows still performed re-directed oral behaviours although at a reduced rate. These results show that there may be an alternative explanation for the occurrence of these behaviours in pigs, however, because of the nature of the later studies, the behaviours could still be explained by the presence of emancipated hunger-related oral stereotypies.

To avoid the presence of emancipated oral stereotypies due to chronic hunger, finishing pigs were observed in the present study. Because they are fed *ad libitum* (ad-lib) on a concentrate diet as well as provided with fresh straw daily, any oral behaviours they may perform are unlikely to be related with chronic hunger and/or correspond to emancipated oral stereotypies. Also, younger sows were used to reduce the likelihood of emancipated stereotypies. In this study, oral behaviours in ad-lib fed finishing pigs and restricted-fed first- and second-parity sows were compared. I hypothesised that if factors other than chronic hunger also elicit the performance of re-directed oral behaviours in pigs, these could also be observed in animals fed ad-lib on a concentrate diet such as finishing pigs. It was expected that sows will show more oral behaviours than finishing pigs as the latter are affected by chronic hunger. Additionally, behaviours directed towards objects would occur together as well as behaviours directed towards penmates.

Finally, when referring to the behaviour in finishing pigs these will be named as ‘oral behaviours’ as it is unknown whether these are re-directed in finishing pigs. When referring only to sows’ behaviour these will be named as re-directed oral behaviours.

2.3 Materials and methods

This study was carried out from September to December 2020 at the Pig Research Centre of Scotland’s Rural College (SRUC), Scotland, and lasted for eight weeks. Due to a disruption to water supply during week 5 of this study it was decided not to include week 5 observations. The breeding stock comprises, on average 108 sows, of which 80 are gestating. On average there are 200 finishing pigs at any one time. The project was reviewed and approved by SRUC’s Animal Ethical Committee.

2.3.1 Animals and housing

In this study, 95 finishing pigs and 42 sows were observed (first and second parity). There were 73 terminal line (42 females, 31 males; Dam: Large White x Landrace, Sire: Hampshire) and 22 dam line finishing pigs (17 females, 5 males; Dam: Large White x Landrace, Sire: Landrace), and 24 first- and 18 second-parity feed-restricted sows (sows hereafter). Finishing pigs and sows were observed in their home pens four days a week for only one week. Farm management was maintained as usual but unnecessary disruptions during observations were avoided by the stockpersons.

Focal finishing pigs were selected according to the estimated time for slaughter. They were observed from approximately 2 weeks prior to slaughter. Focal pigs were observed in their home commercial pens (6.1 x 2.35 m) provided with straw, *ad libitum* access to a pelleted feed (Table 2.1) in a trough (0.9 m long) and two drinkers in the pen as usual.

Each pen held between 5 to 13 pigs (9.5 ± 2.6 pigs). Lighting was on between 0800 and 1600 h, and temperature was 21.20 ± 4.47 ºC and humidity; 65.26 ± 13.49%. Pens were selected according to the size of the pigs and group size.

Focal sows (Large White × Landrace) were selected randomly according to their parity and time to parturition (to make sure they would be available for at least one week). Focal sows were observed in their home commercial pens (6.45 x 3.6 m) provided with straw, with access to a restricted low-calorie feed in a trough once a day (0.9 m long) and unrestricted access to one drinker. Each pen held between 3 to 6 sows (5.6 ± 0.8 pigs). Lighting was on between 0700 and 1600 h and temperature was 17.45 ± 4.81 ºC and humidity; 60.9 ± 14.34 %.

Table 2.1. Diet ingredients for finishing pigs and sows

Finishing pigs	Sows
Barley	Barley
Wheat feed	Wheat feed
Peas	Wheat
Extracted sunflowers	Malt residuals
High pro soybean extract	Biscuit meal
Biscuit meal	Calcium carbonate
Calcium carbonate	Trace elements and vitamin supplements
Fat blend	Fat blend
Trace elements and vitamin supplements	Sodium chloride
Sodium chloride	Lysine hydrochlorine
Lysine hydrochlorine	Vitamin E
Monodical phosphate	Threonine
Threonine	
Methionine	

2.3.2 Study design

Finishing pigs and sows were included in the study in batches every week on Mondays as they became available. Behavioural observations were carried out over four days within one week after which the animals continued in their home pens and followed the usual production cycle.

2.3.3 Behavioural observations

Behavioural observations were done live from outside the pen. All selected pigs were observed over the morning (0800 to 1000) and afternoon (1200 to 1500) from Tuesday to Friday for one week. On any observation day finishing pigs were observed in the morning and sows in the afternoon or vice versa, and this was balanced within the four-day observation period. This meant that each focal pig was observed two days in the morning and two days in the afternoon. Pigs were observed twice during the morning and three times during the afternoon observations every ~ 1 h. Each observation lasted for 2.5 min per focal pig, resulting in 25 min/pig by the end of the observation period for both finishing pigs and sows.

Self-, equipment-, pig- directed and feeding-related behaviours (Table 2.2) were scored by continuous behaviour (focal) sampling. Only frequency of behaviours was recorded, and each bout of a certain behaviour was recorded as a new event. A bout of behaviour started with the beginning of a given behaviour and finished when the pig performed a different behaviour. All events were recorded e.g. if the pig changed from rooting on the floor to chewing and then back to rooting on the floor each behaviour would be recorded as a new event regardless of the time between events. The presence of saliva was recorded only once each 2.5-min observation period. To adjust the total time of observation, time spent visible and active as well as time the pig was disrupted by environmental factors were scored alongside (Table 2.3). Additionally, the frequency of staring at the observer was recorded as an estimation of observer disruption of the pigs. For behavioural scoring, a tablet device (Sony, model SGPT12, Android version 4.1.1) with Pocket Observer 3.2® software (Noldus, version 3.2.40) was used.

On the day before observations started (Mondays), pigs were selected and individually identified with spray markers and re-marked by the end of each observation day. Before observations, a 10-min period was allowed for habituation to the observer. The order of observation for focal pigs and pens were assigned randomly at the beginning of each day and then maintained throughout the day. The observer would move down the list of focal pigs according to visibility (e.g. if the pig was not visible at that moment, the observer would come back to this animal later).

Table 2.2. Description of self-, equipment- and pig-directed oral behaviours, and feeding-related oral behaviours and other behaviours scored in finishing pigs and sows.

Behaviours	Description
Self-directed (point events[1])	
Jaw stretching	Focal animal is opening and closing its mouth while stretching the lower jaw.
Wind sucking	Focal animal is sucking on the tongue with the lower lip open in a V- shape, whilst the snout is against a surface. Windsucking was also scored if the observer could see the focal pig had its snout against any surface but the mouth could not be seen.
Snout twitching	Focal animal protrudes and then shrinks her snout repeatedly whilst the mouth is closed, and the head is still with no lateral or horizontal movement. This behaviour does not include snout twitching when a pig is clearly sniffing (e.g. sow in feeder looking at the observer) or when the focal animal is inactive with eyes closed and the snout twitching is in coordination with breathing.
Tongue playing	Focal animal is continuously moving the tongue while it is partly outside the mouth, with or without sham chewing. This did not include tongue playing after drinking water from the nipple or liquid from the floor.
Presence of saliva	Focal animal has saliva surrounding the mouth. This was recorded as yes/no once per 2.5-min observation period.
Sham chewing	The focal animal is chewing with apparently nothing in the mouth and may or may not have saliva around its mouth. This did not include sham chewing during fighting as part of aggressive behaviour, or when observed immediately after drinking water from the nipple drinker.
Equipment-directed (point-events)	
Biting equipment	Focal animal is biting the equipment (trough, fence, bars, drinker)
Licking equipment	Focal animal has is tongue against any equipment (wall, trough, fence, bars, drinker) while moving the head in different directions.
Rooting equipment	Focal animal is massaging the equipment (wall, trough, fence, bars, drinker) with repeated backwards and forwards movements of the snout and head, or up and down movements of the snout and head. This behaviour could also involve rapid events of nosing, licking or sniffing.
Nosing equipment	Focal animal has the snout in close proximity to equipment (wall, trough, fence, bars, drinker).
Pig-directed (point-events[1])	
Licking penmate	Focal animal has its tongue against a penmate. The tongue is moving in different direction against a penmate. The pigs might also be moving the head in different directions
Nosing pig	Focal animal has the snout in close proximity to a pig (penmate or from an adjacent pen). This does not include rear and belly nosing, or nudging.

[1]Point events are behaviours scored as frequencies.

Cont. Table 2.2. Description of self-, equipment- and pig-directed oral behaviours, and feeding-related oral behaviours and other behaviours scored in finishing pigs and sows.

Feeding-related oral behaviours (point-events[1])	
Drinking	Focal animal is holding the water-dispensing nipple in its mouth.
Rooting floor	Focal animal is massaging the floor or straw on the floor with repeated movements of the snout and head. This behaviour could also involve rapid events of nosing, licking or sniffing.
Straw chewing	Focal animal is chewing on straw. It was also counted as straw chewing when the observer can either see the straw hanging from the mouth while the pig is chewing; and/or the focal pig was rooting/sniffing/manipulating or any behaviour directed towards the straw immediately before chewing
Other behaviours (point events[1])	
Staring at the observer	The focal animal has its gaze directed to the observer while doing nothing
Chewing movements	Focal animal is chewing. This includes sham and straw chewing.

[1]Point events are behaviours scored as frequencies.

Table 2.3. Description of other behaviours scored for adjusting total time spent observing pigs.

Behaviours	**Description**
Visibility (state events[1]; mutually exclusive)	
Visible	The focal animal is visible to be scored for oral behaviours
Not visible	The focal animal is not visible to be scored for oral behaviours
Activity (state events[1]; mutually exclusive)	
Active	Pig is engaging in an activity included or not in this ethogram. It is also recorded as active if the pig is lying with head not resting on a surface.
Inactive	Pig is resting and not engaging in any form of activity (as defined above). The head must be resting on a surface and no movement indicative of an active behaviour should be observed (e.g. twitching and kicking while sleeping are not considered as active). The focal pig may have its eyes closed or open.
Disruption (state events[1]; mutually exclusive)	
Disruption	Any event that could have caused change in the behaviour of the focal animal (e.g. people entering the shed, random noise in the shed or farm)
No disruption	Nothing is disrupting animals' behaviour

[1]The duration and frequency of the behaviour are measured.

2.3.4 Health assessment and animal management

Additional to the farm staff assessment of the animals, during selection and observation days, any obvious easy-to-assess symptoms of ill-health were recorded by the experimental staff (lameness, coughing and sneezing events, rectal prolapse, hernias and twisted snouts). Also, information about any veterinary treatments were obtained from the farm's recordings.

Routines were maintained as usual. The farm manager provided information about any change in the normal routines (e.g. change of diet, change of schedule, treatments, sick/dead animals, movement of animals) when appropriate.

2.3.5 Statistical analysis

Observations where visibility, activity and/or behaviour were not reliable were excluded from the analysis. Also, behavioural observations from week 5 in finishing pigs were not included due to a possible change in behaviour due to a disruption to the water supply in this facility. The same animals observed during week 5 were then observed in the following week.

The experimental units were the individual pigs. There were seven finishing pigs/pen (5 to 10 pigs/pen) and five sows/pen (3 to 6 pigs/pen). No distinction was made between first- and second-parity sows as the numbers were too small. The frequency of behaviour was reported as rate of behaviours i.e. count of behaviour/time spent visible, active and not disrupted. Results are reported as back-transformed means (by the model) and confidence interval (CI). CI was calculated by transforming log-scale s.e. into CI by multiplying by $z = 1.96$. Then anti-log was performed on this value. None of the GLMM models included 'Pig ID' nested in 'Group ID' as the models did not run and each row was a different pig.

Because the dataset was zero-inflated (i.e. high number of observations recorded as zero), the effect of productive stage on rate of behaviour was analysed by GLMM with Poisson distribution and logarithm as link function (Genstat software, version 19). Dispersion parameter was fixed at 1. The fixed factor included 'Productive stage' (finishing pigs vs sows), and the random factor included 'Group ID'. The effect of genetic line and sex was investigated within female finishing pigs and commercial finishing pigs, respectively. This because of the number of pigs in each category. For both GLMM was used with Poisson distribution and logarithm as link function and dispersion parameter fixed at 1 (Genstat software, version 19). The fixed factor included ‘Genetic line’ or ‘Sex’, respectively, and the random factor included in both models was ‘Group ID’. Finally, the study of whether behaviours were occurring in the same animal or not was not possible because of the low number of data points for each pig and the high number of zeros. This was described instead.

The proportion of animals performing a behaviour at least once and proportion of behaviour from the total number of behaviours were also estimated. The proportion of animals was calculated by dividing the total number of pigs performing a given behaviour by the total amount of focal pigs. The proportion of behaviour was calculated by dividing the total rate of a given behaviour by the total rate of all behaviours. Presence of saliva was not included here since this behaviour was measured differently than the rest of the behaviours (yes/no per 2.5-min observation period). Chewing movements is not included here as well since it includes both sham and straw chewing and therefore might be more complicated to interpret.

PCA with type of matrix as correlation, and Spearman’s correlation were carried out using Minitab 17. PCA for finishing pigs and Spearman’s correlation for finishing pigs and sows did not include staring at the observer, chewing movements, feed and presence of saliva. PCA for sows did not include lick penmate and wind sucking since only one non-pregnant first-parity sow did these behaviours resulting in a high number of zeros. Spearman’s correlation coefficient and p-value was not possible to estimate for wind sucking and lick penmate in sows. This because behaviours occurred in the same one sow (sow ID GF193, parity 1).

Health-related measures were not included in any of the models as health conditions were relatively rare, sporadic and short-lived, and so unlikely to affect pig behaviour in a systematic way. No pig showed signs of long-lasting health problems.

2.4 Results

2.4.1 Comparison between finishing pigs and sows

All oral behaviours in the ethogram were observed to occur in finishing pigs (Table 2.4). There were no significant differences between the finishing pigs' and sows' behaviour (after Bonferroni correction; $\alpha_{adjusted} > 0.00294$). However, the rate of most behaviours were *numerically* higher in sows as compared to finishing pigs. Only nose pig (0.369 ± 1.393 vs 0.027 ± 6.287; $F_{(7.51)} = 133$, $p = 0.007$) and staring at the observer (0.043 ± 2.649 vs 0.038 ± 4.722) were *numerically* higher in finishing pigs. Genetic line ($p > 0.05$) and sex ($p > 0.05$) did not affect the behaviour within female finishing pigs and commercial finishing pigs, respectively.

The variation of the rate of behaviour as expressed by the CI, was noticeably high (above 8.000) among self-directed oral behaviours within finishing pigs and sows (Table 2.4). This was high for jaw stretching in finishing pigs and sows, as well as wind sucking and snout twitching in finishing pigs. Table 2.5 shows pig IDs that were observed doing those behaviours during their week of observation. None of the pigs that were observed to do these behaviours, were observed doing so on different days. Additionally, when observed doing the behaviours the number of behaviours observed was low (1 or 2 times).

Table 2.4. Comparison of rate of self-, equipment and pig-directed and feeding-related oral behaviours, and other behaviours between finishing pigs and sows (back-transformed mean ± CI).

		Finishing pigs			**Dry sows**					
Categories	**Behaviours**	Mean	CI 95%		Mean	CI 95%		**F**	**d.f.**	**p-value**
			Lower	Upper		Lower	Upper			
Self-directed	Jaw stretching	0.002	0.022	1853.070	0.016	0.095	105.719	0.6	133	0.440
	Wind sucking	0.008	0.101	91.346	0.048	0.179	33.415	1.37	34.9	0.250
	Snout twitching	0.009	0.121	64.186	0.050	0.273	14.780	1.8	133	0.182
	Tongue playing	0.019	0.240	17.364	0.039	0.226	20.658	0.44	133	0.508
	Presence of saliva	0.025	0.284	12.642	0.119	0.469	6.272	3.95	133	0.049
	Sham chewing	0.045	0.406	6.690	0.100	0.424	7.230	1.32	133	0.252
Equipment-directed	Chew equipment	0.031	0.329	9.686	0.044	0.246	17.727	0.14	133	0.713
	Lick equipment	0.031	0.330	9.650	0.080	0.369	8.918	1.36	133	0.246
	Root equipment	0.047	0.416	6.427	0.121	0.473	0.416	2.11	133	0.149
	Nose equipment	0.241	0.845	2.841	0.306	0.786	3.965	0.46	133	0.499
Pig-directed	Lick penmate	0.034	0.316	10.576	0.065	0.293	13.424	0.52	25.2	0.479
	Nose pig	0.369	1.038	2.768	0.027	0.163	1.038	7.51	133	0.007
Ingestive/Foraging	Drink	0.209	0.793	2.925	0.333	0.827	3.893	1.8	133	0.182
	Root floor	0.304	0.941	2.768	0.416	0.948	0.941	1.07	133	0.303
	Straw chewing	0.475	1.200	2.848	0.930	1.852	4.684	9.46	133	0.003
Other	Staring at the observer	0.043	0.394	7.042	0.038	0.220	21.770	0.02	133	0.903
	Chewing movements	0.096	0.575	3.927	0.104	0.435	6.962	0.02	133	0.892

*Significance at $p < 0.05$, but after Bonferroni correction $\alpha_{adjusted}$= 0.00294

[1]Presence of saliva

Table 2.5. Finishing pigs' and sows' ID performing behaviours identified as having high variation (CI > 8.000) in their four days of observation within the week of observation. Hyphen = no sow was available for observation during that week; blank = no pig was observed doing a given behaviour. Finishing pigs ID starting with GF correspond to the dam line.

Stage	**Behaviour***	**Week of observation****						
		3	**4**	**6**	**7**	**8**	**9**	**10**
Finishing pigs	Jaw					6110(1)		3076(1)
	Wind	2955(1)		3208(1)	GF247(1)	6110(1)		
	Snout	2913(1)	1346(1)	3171(1)	3211(1) GF246(1) GF249(1)	6109(1)	GF243(1)	
Sows	Jaw		OF120(1)	-	OF191(1)	OF170(1)		GF191(1)

*Jaw = jaw stretching, wind = wind sucking, snout = snout twitching
**Numbers in brackets indicate number of days a pig was observed doing a behaviour. Week 5 observations in finishing pigs were not included due to a water shortage, and there were no sows available for observation during week 5.

2.4.2 Proportion of animals and behaviour performed

Self-directed oral behaviours (most) were performed at least once in a lower percentage of animals in both finishing pigs and sows as opposed to the rest of the behaviours (Table 2.6). In particular, jaw stretching, wind sucking, snout twitching and tongue playing were observed in a relatively low percentage of animals (13 and 29% of finishing pig and sows, respectively). However, sham chewing was performed at least once by a relatively high percentage of animals (33.68 and 54.76% of finishing pigs and sows, respectively). Also, all of these behaviours corresponded to the lowest percentage of behaviour out of the total amount of behaviours for finishing pigs (4.67%) and sows (12.35%) (Table 2.6).

Table 2.6. Percentage of animals performing a behaviour at least once and percentage of occurrence of a given behaviour. Behaviours sorted from low to high percentage of animals according to finishing pigs.

Behaviours*	**Finishing pigs (%)**		**Sows (%)**	
	% of animals[1]	**% of behaviour[2]**	**% of animals[1]**	**% of behaviour[2]**
Jaw stretching	2.11	0.15	9.52	0.61
Wind sucking	5.26	0.49	2.38	4.66
Snout twitching	10.53	0.49	28.57	1.88
Tongue playing	12.63	1.06	21.43	1.47
Lick equipment	15.79	1.7	38.1	2.96
Chew equipment	15.79	1.7	21.43	1.64
Lick penmate	23.16	1.89	2.38	3.17
Root equipment	29.47	2.58	52.38	4.53
Sham chewing	33.68	2.47	54.76	3.73
Drink	70	11.43	74.29	12.49
Root floor	78.95	16.63	90.48	15.56
Nose equipment	78.95	13.22	83.33	11.45
Straw chewing	83.16	25.98	100	34.82
Nose pig	87.37	20.2	19.05	1.01

[1]Number of animals performing a behaviour at least once/total number of focal animals

[2]Total frequency of behaviours/Total time visible, active and not disrupted

*Presence of saliva is not included here as self-directed behaviour as this was scored differently (yes/no event on each 2.5-min observation period). Chewing movements is not included here as well since it includes both sham and straw chewing.

2.4.3 PCA and correlation between behaviours within finishing pigs

According to the eigenvalues, there were six Principal Components (PC) above eigenvalue 1, however, the last three components explained a marginal additional variation. Hence, PC1, 2 and 3 were selected (Table 2.7). Figure 2.1 shows the first and second component.

For PC1 (16.6% of the variation), all the behaviours directed towards the equipment varied together (except for chew equipment). PC1 also included sham chewing. For PC2 (11% of the variation), foraging behaviours (root floor and straw chewing) were found to vary together. The third component (9.4% of the variation) corresponds to split of activity. This component showed that pig-directed behaviours (nose and lick penmate) increased while feeding-related behaviours (drinking and root floor) decreased during a day. Lastly, none of the self-directed oral behaviour varied together.

These results are to a degree consistent with Spearman's correlations between behaviours. After Bonferroni correction ($\alpha_{adjusted} < 0.00357$), from a total of 14 behaviours, only behaviours directed towards the equipment and pigs were significantly correlated but the correlation was moderate. Lick and chew equipment ($r_s = 0.302$; $p = 0.003$), lick and nose equipment ($r_s = 0.335$, $p = 0.001$), root and nose equipment ($r_s = 0.332$, $p = 0.001$), and lick and nose penmate were correlated positively ($r_s = 0.305$, $p = 0.003$). None of the self-directed oral behaviours correlated.

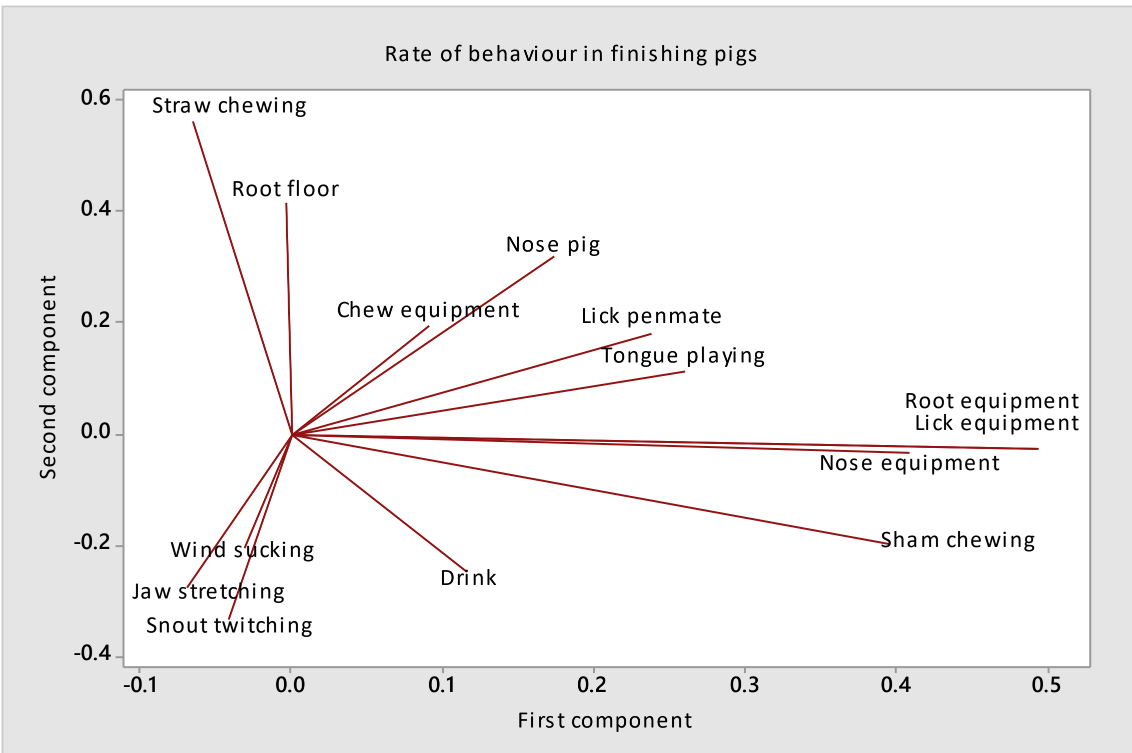

Figure 2.1. Principal Component Analysis for first and second component for finishing pigs.

2.4.4 PCA and correlation between behaviours within sows

According to the eigenvalue, PC1, 2, 3, and 4 explain the variation of the data (Figure 2.2). However, PC3 and 4 were not considered here as the behaviours within each component could not be explained as a group. Therefore, only PC1 and 2 were left (Table 2.7). PC1 and 2 explained 19.4 and 16.3% of the variation, respectively. All equipment-directed behaviours were varying together, similar to finishing pigs, as shown on the first component (19.4% of the variation). This means that when the sow wanted to interact with the equipment all these behaviours were performed. Snout-twitching and tongue-playing behaviour contributed to the second component (16.3% of the variation). None of the other self-directed behaviours were included into this component.

PC1 is consistent with Spearman's correlations between behaviours directed to the equipment. After Bonferroni corrections ($\alpha_{adjusted} < 0.00357$), root equipment was correlated positively with chew ($r_s = 0.453$, $p = 0.003$) and lick equipment ($r_s = 0.450$, $p = 0.003$), although moderately. Not in line with PCA output, none of the self-directed behaviours were correlated. Interestingly, nose equipment was correlated negatively with straw chewing ($r_s = -0.470$, $p = 0.002$).

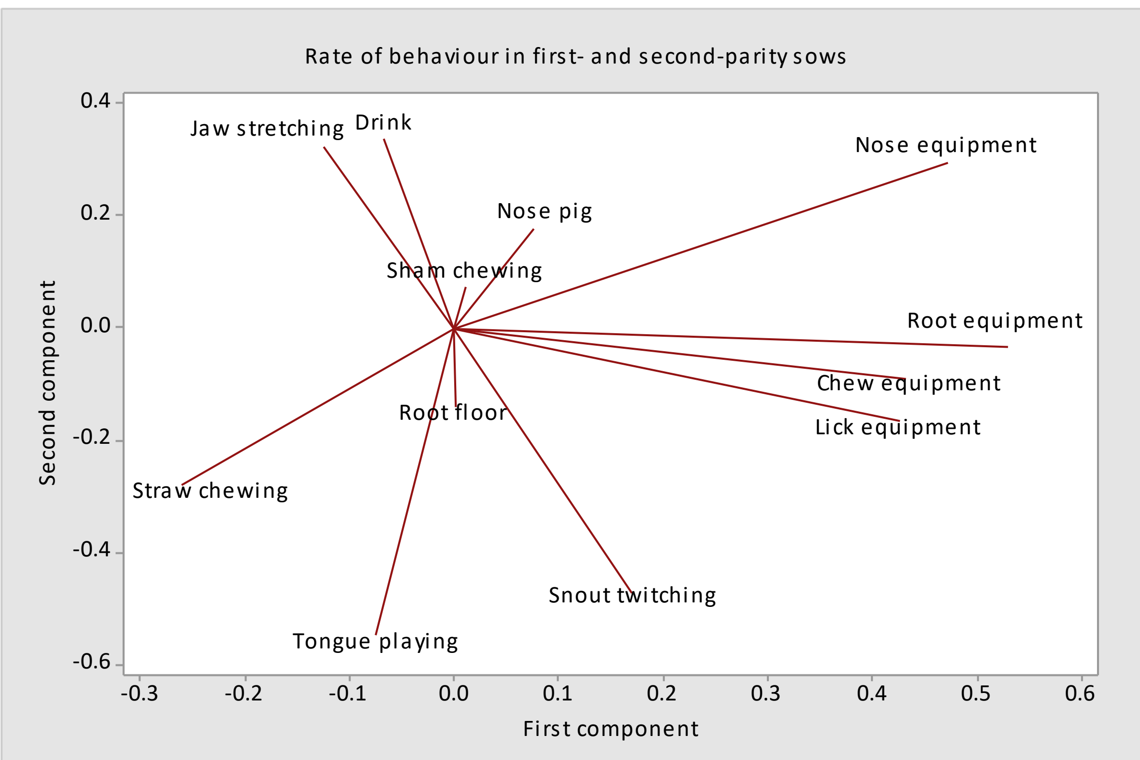

Figure 2.2. Principal Component Analysis for first and second component for first- and second-parity sows.

Table 2.7. Principal Component Analysis showing factor loadings for oral behaviours for each principal component (PC) for finishing pigs and sows. Factor loadings with an absolute value greater than 0.350 and 0.400 were included within a PC for finishing and sows, respectively (in bold).

Categories	Behaviours	Finishing pigs			Sows	
		PC1	**PC2**	**PC3**	**PC1**	**PC2**
Self-directed	Jaw stretching	-0.070	-0.275	0.127	-0.125	0.325
	Wind sucking	-0.032	-0.204	-0.061	not included[2]	
	Snout twitching	-0.041	-0.332	0.107	0.170	**-0.470**
	Tongue playing	0.260	0.113	0.072	-0.075	**-0.548**
	Presence of saliva	not included[1]			not included[1]	
	Sham chewing	**0.396**	-0.197	-0.023	0.012	0.075
Equipment-directed	Chew equipment	0.091	0.193	-0.13	**0.432**	-0.089
	Lick equipment	**0.492**	-0.027	0.029	**0.427**	-0.164
	Root equipment	**0.494**	-0.028	0.192	**0.529**	-0.032
	Nose equipment	**0.409**	-0.033	0.083	**0.471**	0.298
Pig-directed	Lick penmate	0.238	0.181	**-0.430**	not included[2]	
	Nose pig	0.174	0.318	**-0.352**	0.076	0.179
Feeding-related	Drinking	0.116	-0.247	**0.486**	-0.066	0.339
	Root floor	-0.003	**0.413**	**0.504**	0.001	-0.140
	Straw chewing	-0.065	**0.562**	0.321	-0.261	-0.278
Others	Staring at the observer	not included[3]			not included[3]	
	Chewing movements	not included[4]			not included[4]	
Eigenvalues		2.3295	1.5368	1.3080	2.3278	1.962
Proportion of variance explained		0.166	0.110	0.093	0.194	0.163
Cumulative proportion of variance explained		0.166	0.276	0.370	0.194	0.357

[1]Presence of saliva was not included as it was measured different than the rest of the behaviours.
[2]Wind sucking and lick penmate were excluded as they had too many zeros (only 1 sow was performing these behaviours).
[3]Staring at observer was excluded as it is not an oral behaviour.
[4]Chewing movements was not included as this includes both sham and straw chewing.

2.5 Discussion

All oral behaviours included in this study were observed in finishing pigs. These behaviours have been studied and observed only in sows relating to chronic hunger (see D'Eath et al., 2018). Since finishing pigs are fed with a concentrate diet and fed *ad libitum*, the presence of oral behaviours is unlikely to be related to chronic hunger. Therefore, I suggest that there might be alternative explanations for the development of oral behaviours in pigs.

2.5.1 Possible explanations for the presence of oral behaviours in finishing pigs

The presence of oral behaviours in finishing pigs is somewhat not surprising. Oral behaviours studied in here included the manipulation of conspecifics and objects as well as self-directed behaviours. Manipulative behaviours are easy to explain in finishing pigs. The interaction with conspecific might correspond to normal social interactions, and oral behaviours directed towards objects could correspond to the 'normal' repertoire of explorative behaviour. However, the presence of self-directed oral behaviours in finishing pigs, are much more difficult to explain and might be conflicting with the current knowledge.

A speculation is that different type of oral behaviours may fulfil different roles. This idea is supported by differences in the type of oral behaviours performed before and after feeding in gestating sows (Rushen, 1984; Stewart et al., 2008); different oral behaviours being affected differently by the same factor in sows (de Leeuw et al., 2005); self-directed oral behaviours reduced by the provision of straw (e.g. possibly improving stomach health) (Stewart et al., 2011; Whittaker et al., 1999); and object-directed behaviours reduced by the provision of substrate showing that they may be linked to exploratory motivation (de Leeuw and Ekkel, 2004). The last two examples are potentially explained by the effect of straw ingestion on satiety.

I suggest three different categories of oral behaviours: 1) object-directed behaviours that appear in the form of foraging behaviour (e.g. rooting the empty trough or floor) might be triggered by hunger and/or ‘normal’ exploratory motivation, 2) object-directed behaviours that appear as object manipulation (e.g. biting the bars) that could be triggered by dental health problems and/or ‘normal’ explorative behaviour, and 3) self-directed oral behaviours (e.g. wind-sucking, snout twitching) that could be triggered by stomach discomfort due to hunger or conditions affecting the upper digestive system. In this thesis I only explore the third option (Chapter 3 and 4). Explorative behaviour and dental issues as a motivation for oral behaviours are discussed further in section 6.6.

Conditions affecting upper digestive system such as ulceration of the stomach may elicit self-directed oral behaviours. The latter could be supported by the fact that gastric ulceration may cause stomach discomfort in pigs as it is known to be painful in humans (Barkun and Leontiadis, 2010) but also are associated with a change in pigs’ behaviour [longer time walking or standing, and more postural changes (Rutherford et al., 2018)]. Moreover, in the present study self-directed oral behaviours had the lowest rate, were performed by the least proportion of individuals, and represent the smallest proportion of behaviour in finishing pigs. Therefore, these could be identified as rare/’abnormal’ behaviours. This together with the likelihood that gastric ulcers prevalence might be low at the research farm (12.8% of pigs with gastric ulcers; Rutherford et al., 2018), may support the hypothesis of gastric ulcers as a possible factor that could elicit oral behaviours in pigs (tested in Chapter 3 and 4). However, other factors could be eliciting this type of behaviours as well, such as other health issues affecting the upper digestive system, still poor environment for their behavioural needs and/or still inadequate feeding practices in quality and variety of the feed (see section 3.5.2 and 6.6). The present thesis, however, was not designed to distinguish between these different hypotheses, thus further research must be done.

2.5.2 No difference was found between the rate of oral behaviour

No significant difference between finishing pigs' and sows' behaviour is unexpected. We hypothesized that sows would have a higher rate since they are feed-restricted and therefore highly motivated for feed as opposed to finishing pigs which were fed *ad libitum*, as usual. Adding to this, sows may also show emancipated oral stereotypies. The reduction of re-directed oral behaviours with the increase in food allowance has been clearly demonstrated in gestating gilts and sows (Appleby and Lawrence, 1987; Bergeron et al., 2000; Brouns et al., 1994; de Leeuw and Ekkel, 2004; Spoolder et al., 1995; Terlouw et al., 1991; Terlouw and Lawrence, 1993; Zonderland et al., 2004), as well as in gilts fed '*ad libitum'* (4 times their usual feed allowance) (Alvas, 2018; Read et al., 2020). Lower frequency of oral behaviours were also observed in earlier stages of the productive cycle as opposed to gestating gilts in a longitudinal study (Dubarry, 2019).

The lack of significant difference between the finishing pigs' and sows' behaviour in the present study could be explained by the method used for behavioural observations (Alvas, 2018; Dubarry, 2019; Read et al., 2018). Alvas (2018) and Read et al. (2020) studied the effect of '*ad libitum*' feeding on behaviour in gilts and sows. Both studies used the same animals. Animals were fed 2.5 kg/d as usual on day 1, and then four rations of 2.5 kg a day was offered on day 2, 3 and 4 (maximum intake was 9.4 kg/d). Alvas (2018) did not find an effect of '*ad libitum*' feeding on chew and lick equipment and sham chewing. They did find either a significant decrease (all nosing behaviours, trough manipulation, chewing on straw, snout twitch, wind sucking and tongue playing) or increase (drinking and jaw stretching) in behaviours in '*ad libitum*' as compared to control animals. Similarly, Read et al. (2020) found a significant effect of '*ad libitum*' feeding on all the observed behaviours. However, in these studies all pigs were at a similar stage of production (i.e. in the breeding herd) and were observed continuously for longer than in the present study. Alvas (2018) observed sows for 30 min before and 30 min after feeding time (0800 h) by continuous focal observation for each animal on ‘control’ days (when the sows were still feed restricted) and on the final ‘treatment’ day after they had experienced three days of ‘*ad libitum*’ feed. Read et al. (2020), on top of this, observed animals from 0930 to 1000, and 1430 to 1500 h, although, by using scan sampling at an interval of 5 min (resulting in 28 scans/per day, four days of observation).

Dubarry (2019) found a significant difference of the behaviours between productive stages (growing and finishing pigs, gilt rearer and gestation), as animals moved through a completely *ad libitum* diet to a restricted diet. As the animal progressed through the production cycle a trend emerged with increases in the performance of tongue playing, jaw stretching, sham chewing, wind sucking and trough-directed oral behaviours. Snout twitching tended to increase, and bar-biting was not significantly different between stages but present in all of them. Probably the difference in the method of behavioural observation had an impact on the results. Even though Dubarry (2019) observed pigs by scan sampling at 2-minute intervals, this would be for 30 consecutive minutes but also for 24 weeks (once a week). The present study only followed pigs for one week and observed them for 2.5 min every 1 h. However, differences and similarities between finishing pigs and dry sows must be taken carefully because of the age difference.

Another working hypothesis could be that finishing pigs were more active and changed in behaviour much often during those 2.5 min of observations than sows. Anecdotally, it seemed as though finishing pigs were doing more things when active during the 2.5 min-period of observation than dry sows. Apparently, little research has been done regarding the changes in behaviour according to age. In pigs, the only study on time budget that was found relates to the development and change in play behaviour under semi-natural conditions where a decrease in the frequency starting from 6 weeks of age was observed (Newberry et al., 1988). In rats this is also incipient. Sudakov et al. (2021) studied the effect of age on behaviour in adult Wistar rats from 2 to 5 months of age. Rats decreased the level of motor and exploratory activities and increased their level of anxiety as they aged. Young pigs are more motivated to engage in exploratory behaviour whereas sows have a higher motivation for feed. Both needs may be somewhat frustrated resulting in similar levels of oral behaviours in the present study. Although differences between stages were found by Dubarry (2019), it would be interesting to explore this hypothesis further.

2.5.3 High variation within self-directed oral behaviours in finishing pigs and sows

Some of the self-directed oral behaviours observed in this study were relatively highly variable within finishing pigs (jaw-stretching, wind-sucking and snout twitching) and sows (jaw-stretching) as shown by the CI. The high variation could mean that some pigs were doing a behaviour little and others a lot. It could also mean that pigs performing a given behaviour could have been doing this behaviour a lot one day but then never again, or were doing this behaviour consistently across the days. Unfortunately, it was not possible to explore this in the present study due to the short duration of the behavioural observations. However, the higher variation in these behaviours as compared to equipment- and pig-directed behaviours, and feeding-related behaviours observed in this study could be explained by the fact that the former was the least frequent but also performed by the least percentage of animals. The method of observation might not have been enough to catch all of the occurrences, and rather just caught by chance randomly, ending up with this artificial variation. Possibly if observed long enough and frequently enough a much better pattern would have appeared.

Another explanation for the high degree of variation could be that self-directed oral behaviours might be less ‘normal’ than other behaviours that seem to be more related to the natural behaviour of the pig e.g. explorative behaviour. In fact, this higher variation in self-directed oral behaviours as opposed to more ‘normal’ manipulative behaviours has also been observed in studies on chronic hunger in gestating adult females (Bernardino et al., 2021; de Leeuw et al., 2003; de Leeuw and Ekkel, 2004). For instance, de Leeuw et al. (2003) studied the effect of floor feeding and presence of foraging substrate on re-directed oral behaviours. Similarly, they found that the SEM for sham chewing was higher than drinking and pen manipulation (but not floor manipulation which could have been affected by the feed and provision of straw) (1.54 to 2.13 vs 0.27 to 0.32 and 0.40 to 0.42 SEM, respectively). If re-directed oral behaviours are in fact related with an environment that does not satisfy the pigs' needs, the variation in the expression of these behaviours may be explained by the variation in coping styles pigs show towards stress factors (Camerlink et al., 2014; Hessing et al., 1994).

2.5.4 Oral behaviours that varied together within finishing pigs and sows

Functionally, it was expected that behaviours involving the interaction with the resources in the pen and penmates would vary together and correlate highly among them. This is because, as observed, when pigs interact with an object or penmate they do so in a variety of ways, including biting, licking, nosing. This was observed to some extent in both finishing pigs and sows. Equipment interaction in finishing pigs occurred in a variety of ways e.g. licking, rooting and nosing, but correlated weakly or moderately. Interestingly, these behaviours varied together with sham chewing. This could be as a result of interacting with the equipment, small particles of food or other things might have been picked up and elicit chewing behaviour which appeared as sham chewing. Finishing pigs also rooted on the floor and straw chew together. Throughout the day, they would either interact with their penmates (which correlated moderately) or engage in behaviours related to feeding e.g. drinking water and rooting the floor.

In sows, interaction with the equipment was also done in a variety of ways and therefore behaviours correlated (moderately) e.g. chewing, licking, rooting and nosing. Also, interesting, straw chewing and nose equipment were correlating negatively (moderately). This could be explained by the location of both resources in the pen. Straw is provided as a bedding material in the bedding area which is separated by the dunging area from the feeder stalls. This way when sows are manipulating the feeder they are not foraging on straw.

Interestingly, snout twitching and tongue playing were varying together in sows. These behaviours could be responding to the level of satiation. Sows will decrease the performance of these behaviours when they are satiated and increase when they are hungry (as explained by hunger theory). No relationship was found between self-directed oral behaviours in finishing pigs. This could have been due to the relatively lower percentage of pigs performing these behaviours at least once and the lower percentage of behaviour from the total amount of behaviours performed as compared to in sows.

2.5.5 Low number of oral behaviours happening and/or varying together

Overall, few behaviours were varying together (PCA) or correlated (Spearman's correlation) within finishing pigs and sows. However, as expected, behaviours directed towards the equipment were varying together and correlated although weakly-to-moderately in finishing pigs and sows, respectively. Unexpectedly, self-directed oral behaviours did not vary together and did not correlate in finishing pigs. This was not the case in sows. Wind sucking and tongue playing varied together (but did not correlate).

The lack of correlation between self-directed oral behaviours or them varying together could have resulted because of the relatively little time of observation that would not allow for picking up this type of patterns, as already discussed. The behaviours that were more frequent among pigs and/or happened more frequently as opposed the ones that did not were the ones that were also found to contribute to the principal components and/or correlate.

Another working hypothesis is that pigs may start to adopt self-directed oral behaviours when they are younger and then continue to perform them as well as adopting new ones. This means that more and more behaviours may start to appear and may vary together/correlate later in life. In the present study, sows were performing proportionately more self-directed oral behaviours than finishing pigs (numerically). Additionally, for jaw stretching, snout twitching, tongue playing and sham chewing, a higher proportion of sows were performing these behaviours as compared to finishing pigs (numerically).

Increasing frequency of self-directed oral behaviours with age has been observed before (Dubarry, 2019). Similar results have been observed in adult female pigs with increasing parity number in studies where the effect of fibre level (Li et al., 2013; Robert et al., 1993; Zhang et al., 2022) or the provision of substrate (lucerne hay) on oral behaviours was tested (Edwards et al., 2019). Zhang et al. (2022) evaluated the behaviour and affective state of sows with strong/weak Pupil Light Reflex. Sham chewing was significantly higher in sows with higher parity number as compared to gilts independent of the Pupil Light Reflex ($p < 0.05$). Li et al. (2013) studied the effect of distillers' dried grains with solubles on re-directed oral behaviours (object biting, sham chewing, and nose rubbing) in sows kept in individual stalls during the second and third parity. The behavioural time budget (%) used for the performance of stereotypies increased significantly between the second and third parity (22.0 ± 2.2 vs 29.9 ± 2.8, respectively; $p = 0.01$). Edwards et al. (2019) studied the effect of the provision of lucerne hay to gestating sows close to their parturition. There was a significant increase in sham chewing between parity 0 and 1 (0.4 vs 3.1 proportion of behaviour; $p < 0.001$) but then it decreased to parity 0 levels. In other studies, where the effect of parity was not tested, a *numerical* increase of these behaviours were observed with the increase in parity number (Robert et al., 1993; Terlouw et al., 1991; Terlouw and Lawrence, 1993). However, no effect of parity number (Alvas, 2018) or no clear *numerical* change between parities has also been observed (Spoolder et al., 1995; van der Peet-Schwering et al., 2003).

2.5.6 Limitations of the study

The nature of the study, which was aimed at testing whether finishing pigs were performing oral behaviours, did not allow for more complete analysis of the data. The main limitations of the study were: 1) Low number of animals available (e.g. finishing pigs soon to be sent to the abattoir; first- and second-parity sows), 2) low frequency and short-lived nature of self-directed oral behaviours, and 3) time constraints. These made a number of things more difficult: the analysis of the consistency of the behaviours within the same animal, the effect of genetic line across both sex in finishing pigs as well as the effect of parity number and stage of gestation in sows. Possibly, if the observation method would have involved more time per pig for more than one week, then more significant results, and different relationships between behaviours would have been detected. However, because of practicalities it was not possible to do it in a different way.

2.5.7 Future steps

The presence of oral behaviours in finishing pigs opens the doors for new research questions to better understand the origin of re-directed oral behaviours in gestating sows but also in general. Possible future research questions could be origin and development of re-directed oral behaviours in general, and whether these remain in the pig or are acute events related to something specific; and the effect of sex and genetic line on the development of self-directed oral behaviours in pigs. This also opens the door to questioning the cause of re-directed oral behaviours. It may be the case that causes other than hunger are eliciting these types of behaviours as well. Different types of oral behaviours may relate to different causes. Finally, unrelated to oral behaviours, this study may also highlight the importance of knowing how the rate of behaviour differs between juvenile and adult animals/pigs.

2.6 Conclusion

Finishing pigs, that were fed *ad libitum* on a concentrate diet as per normal husbandry practice, performed oral behaviours normally observed in gestating sows fed on a restricted diet. It is especially interesting to note the occurrence of self-directed oral behaviours in finishing pigs. This study opens the door for new research on the understanding of the development of re-directed oral behaviours. A working hypothesis is that some of these rare self-directed oral behaviours may be caused by gastric ulcers.

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Chapter 3 A study of association between gastric ulcers and self-directed oral behaviours in finishing pigs

3.1 Abstract

Oral behaviours typically thought to be due to hunger in gestating sows have been observed in *ad libitum* fed gilts and sows, as well as in finishing pigs (Chapter 2). Self-directed oral behaviours could be related to health problems affecting the upper digestive system (i.e. oesophagus, stomach). An association between oral behaviours (crib-biting behaviour) and gastric ulcers has been found in horses. Also, changes in general behaviour are associated with and gastric ulcers in pigs. To investigate the relationship between self-directed oral behaviours and gastric ulcers (in the pars oesophagea) seventeen finishing pigs (male = 6, female = 11) were observed. Videos from a previous study on gastric ulcers and pain-related behaviours were used (Rutherford et al., 2018). Finishing pigs were housed in small groups on straw with *ad libitum* access to water and commercial finishing diets appropriate for their age. Behaviours scored were self-directed oral behaviours (wind sucking, tongue playing, jaw stretching and sham chewing), straw chewing, activity level and posture in finishing pigs. Sham and straw chewing were combined as chewing movements. Additionally, the time spent with the head in the drinker and feeder were recorded. Behaviour scoring was done by using continuous behaviour sampling of approximately 4-h video recording/pig (2-h morning and 2-h afternoon recordings). Only time periods where pigs were visible, active and not showing alert behaviour (suggesting a disturbance) were included when calculating rate of behaviour (counts/min). The adjusted rate of self-directed oral behaviours (chewing movements, tongue playing and wind sucking) and percentage of pigs performing the behaviours at least once were compared between pigs having healthy or ulcerated stomachs using GLMM and Fisher's exact test, respectively. The effect of stomach health on postures (except kneel), head in the feeder, head in the

drinker and active was investigated using Linear Mixed Models (LMM). The effect on kneel was analysed using Fisher's exact test as the residuals were not possible to normalise. Jaw-stretching behaviour was not observed in any of the pigs. There were no significant differences between the rate of self-directed oral behaviours between both groups (chewing movements : $Wald_{(1)} = 0.02$, $p = 0.901$; tongue playing: $F_{(1.13)} = 0.03$, $p = 0.861$; and wind sucking: $Wald_{(1)} = 1.3$, $p = 0.254$). Percentage of pigs performing tongue playing and wind sucking did not differ between groups either ($p > 0.05$). There were no significant different between both groups in terms of the time spent being active, in different postures, and with their head in the feeder and drinker ($p > 0.05$). These findings provide no evidence that gastric ulceration affects the oral behaviours studied. However, these findings are limited by the quality of the videos and the location of the cameras making it difficult to identify other self-directed oral behaviours such as snout twitching. There was also no information on the health of mouth and oesophagus which could have an impact on behaviour. Possible alternative explanations for the occurrence of self-directed oral behaviours in finishing pigs are: an insufficiently enriched housing environment; inadequate diet and feeding practices; and/or being related to different health-conditions of the upper digestive system. Future steps should investigate how oral behaviours, usually related to chronic hunger, develop and whether the number of pigs performing these increases with age; how oral behaviours relate to other conditions affecting the upper digestive system e.g. tooth cavities, oesophagitis; and the relationship between other behaviours and gastric ulcers.

3.2 Introduction

Re-directed oral behaviours (e.g. sham chewing, biting bars) are thought to be caused by chronic hunger in gestating sows. They are thought to result from a lack of resources to fulfil the sows' behavioural and nutritional needs (D'Eath et al., 2018). However, some studies on chronic hunger show unexpected results, as a fraction of oral behaviours were still observed in gilts and sows fed '*ad libitum'* (Alvas, 2018; Read et al., 2020), and when completely *ad libitum* (high fibre diet) (Brouns et al., 1994; de Leeuw and Ekkel, 2004). This may be explained, among other causes, by the occurrence of emancipated stereotypies (where stereotypic oral behaviour still occurs despite the original cause being removed; Lawrence and Terlouw, 1993); however, this is less likely to be the case in gilts as they have spent less time under a restricted diet. Moreover, finishing pigs also perform oral behaviours (Chapter 2). Because finishing pigs were fed *ad libitum* on a concentrate diet and provided with large amounts of fresh straw daily it is less likely that the behaviours appeared due to chronic hunger and/or corresponded to emancipated stereotypies. For these reasons, it is plausible to think that hunger may not always be the only cause of re-directed oral behaviours in pigs.

Object- and self-directed oral behaviours were observed in finishing pigs (Chapter 2). While behaviours involving the manipulation of objects may be a part of their 'normal' repertoire of explorative behaviour, other behaviours are more difficult to link with exploration. This is the case of behaviours that are not directed towards a resource e.g. self-directed (wind sucking, tongue playing, snout twitching and jaw stretching). Interestingly, in Chapter 2, self-directed oral behaviours were the least frequent but also the lowest percentage of pigs were performing these behaviours. Therefore, they were regarded as rare and/or abnormal. Additional to self-directed oral behaviours, foraging, chewing and/or eating straw may be related to gastric ulceration. This is because it has been observed that the provision of straw acts as a protective factor for gastric ulceration in growing and finishing pigs (Di Martino et al., 2013; Herskin et al., 2016; Jensen et al., 2017).

A possible explanation for self-directed oral behaviours in pigs (other than hunger) is that they could be caused, in part, by health problems affecting the upper digestive system e.g. oesophagus and/or stomach health. In horses, oral behaviours such as crib biting have been related to gastric ulcers (Wickens and Heleski, 2010) and salivary production (Moeller et al., 2008). Crib biting is defined as *'placing the incisor teeth on a fixed object, then pulling backward, contracting the neck muscles, and drawing air emitting an audible grunt'*; reviewed by Nicol (1999); Wickens and Heleski (2010). Additionally, behaviour has been reported to be different between pigs with healthy stomachs and pigs with gastric ulcers. Rutherford et al. (2018) found that pigs with gastric ulcers spend longer time walking or standing, and show more postural changes, and tend to spend less time lying on their left side. Peralvo-Vidal et al. (2021) observed affected pigs visit the feeder less but spend more time each time. Hartnett et al. (2023) found affected pigs to spend more time lying on the left side and tend to walk more and tail-in-mouth less. Also, behaviours linked to stress are (weakly) associated with the likelihood of having acute fundic ulcers in the stomach at slaughter (Dybkjær et al., 1994). It is plausible to hypothesize that self-directed oral behaviours may be related to gastric ulcers in pigs.

To date, there are only a few studies on the association between gastric ulcers and pigs' behaviours (Dybkjær et al., 1994; Hartnett et al., 2023; Peralvo-Vidal et al., 2021a; Rutherford et al., 2018) and no study has been done on the relationship between gastric ulcers and self-directed oral behaviours. Finding a connection between self-directed oral behaviours and gastric ulcers would help to better understand previous research on chronic hunger and feeding practices, but also, would add knowledge to the effect of gastric ulcers on pigs' behaviour. Potentially, it may also help identify affected pigs in farms.

Briefly, gastric ulcers (see section 1.5.1) are prevalent in the pig industry (Cybulski et al., 2021b, 2021a; Gottardo et al., 2017; Peralvo-Vidal et al., 2021c, 2021b), and of importance as they affect productivity (Dunlop et al., 2021) and might be of animal welfare concern (Barkun and Leontiadis, 2010; Rutherford et al., 2018). Gastric ulcers in pigs are mostly found in an area of the stomach named pars oesophagea (Friendship, 2004). This is a rectangular area proximal to the oesophagus which lacks protective mucosal glands.

The aim of this study was to investigate the relationship between the presence of gastric ulcers in the pars oesophagea and the rate of self-directed oral behaviours (wind sucking, tongue playing, jaw stretching and sham chewing), straw chewing, activity level and posture in finishing pigs. Additionally, the relationship between gastric ulcers and the percentage of pigs performing these behaviours at least once, and the relationship between gastric ulcers and the time spent with the head in the drinker and feeder were studied.

3.3 Materials and methods

This study used video footage from a previous study where the behaviour of finishing pigs with and without gastric ulceration was compared in relation to pain-related behaviour e.g. laying on left side (see in annexe section, Table 3.7) (for more details see Rutherford et al., 2018). This study found 12.82% pigs being affected with gastric ulcers in the present research farm (Rutherford et al., 2018).

3.3.1 Animals

In the present study, seventeen finishing pigs (male = 6, female = 11; Dam: Large White x Landrace, Sire: Hampshire) were observed to compare the frequency of oral behaviours between pigs with no signs of damage of the pars oesophagea (healthy stomach = 10; 7 female and 3 male pigs) and pigs with different levels of gastric ulceration (ulcerated stomach = 7; 4 female and 3 male pigs). According to the scoring system used in Rutherford et al. (2018) (Table 3.1), healthy stomachs corresponded to score 0 and ulcerated stomachs to score 6, 7 and 8. However, score-6 pigs were not included in Rutherford et al. (2018) and here. This was because they were regarded as probably not showing a clear change in their behaviour due to the small change in the mucosa. Also, pigs with other health problems were excluded, and the selection was blinded for sex and weight.

Pigs were housed either in groups of 2 to 3 pigs or in groups of more then 3 pigs (mean (SD) = 3.47 (0.87) pig/pen) in straw bedded pens. Groups of two to three pigs were housed in 2.85 m x 3.7 m pens. Groups of more than three pigs were provided with pens double the size (2.85 m x 7.4 m). Pelleted feed (Commercial finisher diet - ForFarmers UK Limited; see section 2.3.1, Table 2.1 for ingredients) and water (one nipple drinker) were provided *ad libitum*, and lighting was on between 0600 and 1800h. Video recordings were made from cameras (Gamut CCTV) and the footage was recorded onto a digital system (GeoVision™ software; GeoVisionUK, Herts, UK). Cameras were positioned above each pen. Small pens had one camera and large pens had two cameras on each end of the pen. In the case of the large pens, only the camera where the mouth of the focal pig was visible the most was included into the analysis. This was because both cameras overlapped, and behaviour was scored for each video separately.

Pigs were euthanized on-site. On the day of euthanasia, pigs were moved in their whole groups to a different pen. Individual pigs were then sedated before being given an overdose of barbiturates (Euthatal) via injection to the heart. Following confirmation of death, stomachs were dissected out whole and transferred to the SAC Consulting Veterinary Services for gastric ulcer scoring. Relevant stomach scores used in Rutherford et al. (2018) are described in Table 3.1.

Table 3.1. Description of stomach scoring system used in Rutherford et al. (2018). Only scores relevant to the present study are described here i.e healthy or included ulcers in their definition. Milder (keratinization and erosion; score 1 to 5) and chronic lesions (contraction of the pars oesophagea; score 9) were not included in this description.

Score	Description
0	Normal stomach with a white and shiny pars oesophagea without visible lesions
6	Superficial ulceration (nerves and blood vessels exposed and potentially damaged) with a diameter of less than 0.5 cm in pars oesophagea OR palpable scar tissue in pars oesophagea consisting of one or more peripheral fibrous strands
7	Deep ulcers with a diameter of less than 0.5 cm or more superficial ulceration with a diameter on 0.5–2.0 cm in pars oesophagea OR palpable scar tissue in pars oesophagea with fibrous strands producing an almost complete circular structure that may be slightly flexible
8	Deep ulcers with a diameter of at least 0.5 cm or more superficial ulceration with a diameter of more than 2 cm in pars oesophagea OR palpable scar tissue in pars oesophagea with fibrous strands producing a circular, rigid structure

*For the description of the remaining scores (1 to 5, and 9) check Rutherford et al. *(*2018)

3.3.2 Study design

3.3.2.1 Behavioural observations

Behaviour was scored from the video footage using Observer XT14®. This totalled to ~ 4 h of observation per pig (~ 2 h recorded in the morning and ~ 2 h in the afternoon). The frequency of oral behaviours (Table 3.2) was scored using focal sampling and behaviour sampling and were observed continuously during the whole duration of the video. No distinction was made as to how repetitive and invariant these behaviours were. Additionally, the duration of visibility, activity, postures and disrupted behaviour were recorded continuously (Table 3.3 and 3.4). Visibility, activity and disrupted behaviour were recorded to adjust the total time of observation.

Interobserver reliability was tested by re-watching 10% of the total active time. As a result, some behaviours had to be combined to improve the percentage of agreement. A new behaviour 'chewing movements' was made by combining straw and sham chewing as well as chewing uncertain.

Table 3.2. Description of self-directed oral behaviours scored on this study.

Behaviours	**Description**
Self-directed oral behaviours (point events[1])	
Chewing movements	The focal pig is performing chewing movements, and this includes sham or straw chewing. Recorded as chewing when the animal is not doing anything else e.g. chewing while rooting is recorded as rooting, not chewing. The behaviours do not include chewing movements performed after drinking water from the nipple, displaying aggressive behaviour, yawning, when the focal pig has had its head in the feeder, or licking a penmate.
Wind sucking	The focal animal is sucking on the tongue with the lower lip open in a V- shape, whilst the snout is against a surface. Wind sucking also includes when the focal pig has its snout against any surface for a while making pressure, but the mouth cannot be seen.
Tongue playing	The focal animal protrudes its tongue repeatedly out of the mouth
Jaw stretching	The focal animal opens and closes the mouth while stretching the lower jaw from side to side for several seconds. This will not include jaw stretching after drinking water from the nipple.

[1]Point events are behaviours scored as frequencies.

Table 3.3. Description of postures scored on this study.

Behaviours	Modifiers	Description
Postures (state events[1]; mutually exclusive) Change in posture was recorded when the pig starts to move to change into the next position. If spending > 2 s on a transitional posture, then it was recorded as doing so		
Kneel		Rump raised off ground by rear legs, front legs flexed, head close to the ground. [Posture must be maintained for at least 3 s – i.e. not recorded during transition from lying to standing].
Sit		Rump in contact with the ground, front of body raised up by extension of front legs. When pig is transitioning from laying to standing this was not recorded as sitting.
Lay	Left and right	Recumbent, shoulder and pelvis in contact with the ground, with legs extended, body axis is>45° away from vertical, belly exposed. Lying on left AND right side, respectively.
	Ventral/mixed	The focal animal is recumbent, shoulder and pelvis in contact with the ground and it is not lying left or right. Body axis vertical (±45°), OR Mixed posture between ventral and lateral: i.e. both rear legs have been pushed out from under the body and are presented as lateral, with hip in contact with the floor. Front legs are presented as ventral.
Stand		Body is raised off the ground on all four legs. This also includes when the pig moves laterally. Standing posture was not recorded if the pig is changing from a sitting/lying posture to walking. This includes also when the pig is standing with its hands on top of a penmate or when stepping sidewise without moving its rear legs.
Walk		Pig takes more than one step forward or back. It only counts as a step when that movement of the leg allows the pig to move forward or backward away from the standing leg. This does not include when the pig is stepping sidewise without moving its rear legs. That was recorded as standing.
Other		Pig's posture is different than any of the postures listed here e.g. playing, fighting, front feet on the wall or feeder
Not identifiable		The pig is out of sight

[1]The duration and frequency of the behaviour are measured.

Table 3.4. Description of other behaviours considered in this study.

Behaviour	Description
Visibility (state events[1]; mutually exclusive)	
Visible	The observer is able to see the oral area (mouth, snout and/or cheeks) and it is possible to identify a certain behaviour with high certainty e.g. if part of the oral area is not visible and the behaviour cannot be identified well then it will be recorded as 'not visible'.
Not visible	The observer does not have enough visual information to identify a certain behaviour. It may not be visible because it can't be seen, the quality of the video doesn't allow to see much detail or the pig is out of sight.
Feeder	The pig has its whole head in the feeder
Drinker	The pig has its whole face in the drinker
Activity (state events[1]; mutually exclusive)	
Active	The pig was actively moving by walking, standing, sitting, kneeling or other. The pig was recorded as active when laying but moving its head or mouth, or having the neck engaged in an upright position.
Inactive	The pig was laying with its head resting on a surface/pig. Includes putative sleeping.
Not identifiable	The observer cannot tell because the pig is out of sight
Disturbed behaviour (state events[1]; mutually exclusive)	
Disturbed behaviour	Focal pig is directly interacting with the people, sniffing the air and wall trying to find out what is going on, and/or has its ears and head in an alert position.
No disturbed behaviour	Focal pig is not obviously alert to its environment

[1]The duration and frequency of the behaviour are measured.

3.3.3 Statistical analysis

The total count (chewing movements, tongue playing and wind sucking; jaw stretching was not observed) and duration of behaviour (active, all postures, head in the feeder and head in the drinker) were calculated for each pig, as well as the time spent visible, active and not showing disrupted behaviour, and the total duration of each observation. Chewing movements, tongue playing and wind sucking are presented as a rate (counts/min). This was calculated from total count of behaviour/time spent visible, active and no disrupted behaviour (min). Active, all postures, head in the feeder and head in the drinker are presented as total duration of a behaviour/total duration of the observation. The experimental units are the pigs and results are presented as means ± CI.

The effect of gastric ulceration was studied as presence of gastric ulcers (ulcerated stomach) vs healthy pars oesophagea (healthy stomach) for all the behaviours. The effect of the presence of gastric ulcers on the rate of self-directed oral behaviours (chewing movements, tongue playing and wind sucking) was investigated by using GLMM (REML procedure; Restricted Maximum Likelihood; Genstat 19). Stomach health status (healthy vs ulcerated stomachs) was set as a fixed factor, and group ID as a random factor. Pig ID was not nested in group ID as the model did not run but also each row corresponded to a different pig. Sex of the pig was excluded from the model as it did not have an effect on behaviour when this was added in this model. Poisson distribution was used with logarithm as link function with the dispersion parameter fixed at 1. The effect on postures (except kneel), head in the feeder, head in the drinker and active was investigated using LMM with stomach health status (healthy vs ulcerated stomachs) as a fixed factor, and group ID as a random factor. Some behaviours had to be transformed to normalize the distribution of residuals [Ln(behaviour) for active and sit; and Ln(behaviour + 1) for lay mixed/ventral, lay right and walk]. Kneel was coded as occurrence and no-occurrence of the behaviour and were analysed using Fisher's exact test (SPSS 28) as the residuals were not possible to normalise.

The relationship of presence of gastric ulcers and the percentage of pigs performing tongue playing or wind sucking at least once was analysed using Fisher's exact test (SPSS 28). Chewing movements was not possible to analyse as all pigs showed this behaviour.

There was one pig with a healthy stomach that performed wind sucking at a high rate as compared to the rest of the pigs. Statistical analysis was run with and without it, but results did not vary and remained non-significant. Hence, this pig was included in the analysis.

3.4 Results

3.4.1 Self-directed oral behaviours

All self-directed oral behaviours except for jaw stretching were observed in both pigs with healthy and ulcerated stomachs (Figure 3.1a, b, c). The rate of self-directed oral behaviours was relatively low in general. The lowest rate of behaviour was 0.0477 and the highest 2.251 behaviour/min including all oral behaviours (when excluding chewing movements, the highest was 0.334 behaviour/min). The rate of chewing movements ($Wald_{(1)}$ = 0.02, p = 0.901), tongue playing ($F_{(1.13)}$ = 0.03, p = 0.861) and wind sucking ($Wald_{(1)}$ = 1.3, p = 0.254) were not significantly different between pigs with healthy and ulcerated stomachs (Figure 3.1a, b, c). Similarly, there was no differences in the percentage of animals performing a behaviour at least once between pigs with healthy and ulcerated stomachs (tongue playing: p = 0.622; wind sucking: p = 0.593) (Figure 3.1d). Chewing movements were observed in all pigs and the percentage of pigs performing this behaviour was the highest as compared to tongue playing and wind sucking in pigs with both healthy and ulcerated stomachs (*numerically*). Wind sucking was observed in 20% of pigs with healthy stomachs (2/10) and 42% with ulcerated stomachs (3/7). Half of the pigs with healthy stomachs (5/10) and 71% of pigs with ulcerated stomach (5/7) showed tongue playing. Only pigs with ulcerated stomachs performed both wind sucking and tongue playing (2/7).

Interestingly, one healthy pig (YD1705) was observed to perform wind sucking and some of the times this behaviour looked similar to crib-biting in horses. The pig was observed doing so during a bout of intensive feeder manipulation while lying laterally.

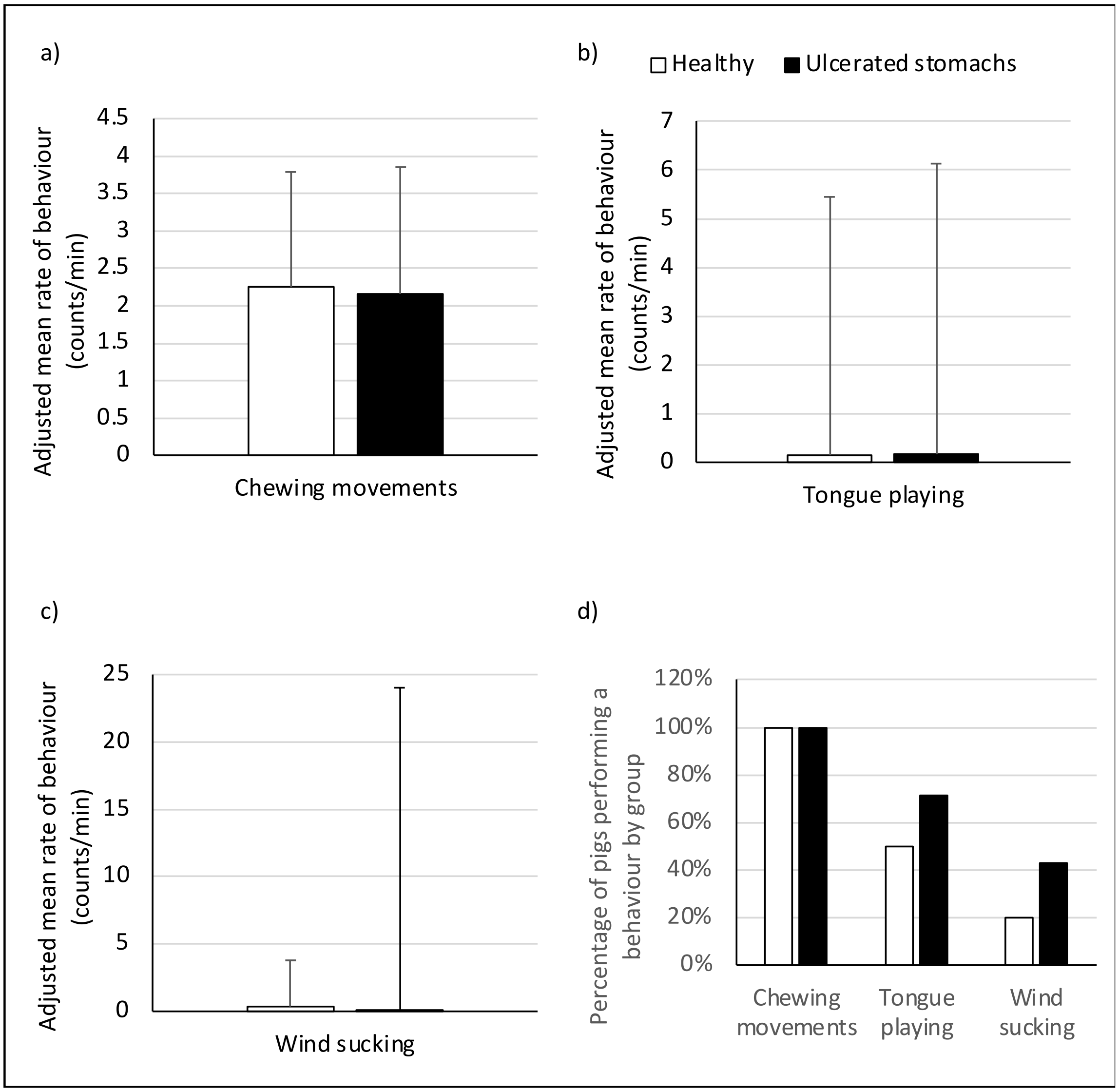

Figure 3.1. Mean rate of behaviour adjusted by time spent visible, active and no disrupted behaviour (a, b, c) (means ± CI), and (d) percentage of pigs doing a behaviour at least once by pigs with healthy stomach (clear bars) and pigs with ulcerated stomachs (shaded bars).

3.4.2 Active, postures, and head in feeder/drinker

No significant difference in proportion of time spent in an active state, in different postures, and with their head in feeder and drinker ($p > 0.05$; Table 3.5 and 3.6) was observed between pigs with healthy stomachs and pigs with ulcerated stomachs. Most behaviours were *numerically* higher in pigs with ulcerated stomachs. Only the proportion of time they spent with the head in the feeder and proportion of time spent active was *numerically* lower in pigs with ulcerated stomachs. Also, the ranking of the proportion of the behaviours are similar between groups.

Table 3.5. Mean proportion of duration of behaviours in pigs with healthy stomachs and pigs with ulcerated stomachs (mean ± CI) ranked from the lowest to the highest proportion according to healthy stomachs.

Behaviours	Healthy stomachs			Ulcerated stomachs			Statistics		
	Mean	CI 95%		Mean	CI 95%		F	d.f.	p-value
		Lower	Upper		Lower	Upper			
Sit	0.014	0.005	0.041	0.018	0.005	0.067	0.09	14.8	0.765
Head in drinker	0.031	0.011	0.051	0.035	0.014	0.057	0.27	4.4	0.627
Walk	0.059	0.024	0.096	0.077	0.038	0.117	1.34	4.1	0.311
Head in feeder	0.097	0.05	0.145	0.076	0.02	0.133	0.39	10.8	0.545
Lay right	0.376	0.202	0.574	0.434	0.224	0.681	0.15	14.2	0.709
Stand	0.463	0.252	0.675	0.529	0.304	0.754	0.65	3.9	0.467
Lay left	0.511	0.213	0.809	0.595	0.269	0.922	0.36	4.9	0.576
Lay ventral/mixed	0.640	0.355	0.986	0.829	0.495	1.238	2.52	3.9	0.190
Active	0.698	0.405	1.204	0.644	0.340	1.217	0.05	8.4	0.827

LMM, significance level at $p < 0.05$

Table 3.6. Contingency table for the occurrence and non-occurrence of kneel in pigs with healthy stomachs and pigs with ulcerated stomachs (counts).

Kneel	Healthy stomachs	Ulcerated stomachs	Grand Total	p-value*
Occurrence	7	4	11	
Non-occurrence	3	3	6	0.644
Grand Total	10	7	17	

*Fisher’s exact test significance level at $p < 0.05$

3.5 Discussion

3.5.1 Self-directed oral behaviours are not a good indicator of gastric ulcers

In the present study, no differences were observed in oral behaviours, postures, active and head in feeder and drinker between pigs with healthy and pigs with ulcerated stomachs. One possible explanation for the lack of association between self-directed oral behaviours and gastric ulceration is that there could be a learning component and persistence of the behaviour after the gastric ulcer or other condition has healed. Pigs may learn this type of behaviours by chance at first and/or by social facilitation. Some may learn that they reduce stress, boredom and/or discomfort coming from the upper digestive system. There is also the chance that these behaviours may then remain in their 'normal' behavioural repertoire as they do in gestating sows: Lawrence and Terlouw (1993) describe how sows start to perform oral behaviour re-directed towards the available resources once their meal is finished as a way of dealing with their high motivation for feed. Behavioural arousal and active behaviours may then facilitate the expression of these behaviours, which in turn leads to the sensitization of the underlying neural pathways. As a result, the behaviour is now more easily elicited and maintained. Apparently, the simpler the behaviour the easier it will be that the behaviour will remain (Lawrence and Terlouw, 1993).

However, it could just be that there is no relationship between gastric ulcers and self-directed oral behaviours. If there had been a strong association the relationship would have shown here, despite this study's small sample size. Although it is possible that with a bigger sample size and assessing the rest of the upper digestive system a relationship may have been found, the strength of any relationship must be small. For practical purposes, in order for a behaviour to be a good diagnostic indicator it should show even in a small sample size. Also, self-directed oral behaviours may have some other cause entirely.

The lack of difference between groups in terms of the time spent in different postures was unexpected. Since the subset of pigs observed on the present study came from a previous study (Rutherford et al., 2018), it was expected to find similar results, however this was not the case. Rutherford et al. (2018) found that pigs with gastric ulcers spent less time lying on the left side and more time standing than pigs without gastric ulcers, whereas in the present study none of the postures were different between groups. This could be explained by the fact that in the present study only a subset of the pigs was observed. This study only included pigs without any type of lesion in the pars oesophagea (as opposed to including pigs with mild parakeratosis as was done by Rutherford et al. (2018). This resulted in a difference of 21 pigs between both studies [n = 17 vs 38 (Rutherford et al., 2018)]. Also, the smaller sample size may have been influential in the results interpretation.

The results on posture of the present study differed from Hartnett et al. (2023) as well. Even though their sample size was small as well (n = 14), they found a relationship between gastric ulcers and lying left. Unlike the present study, they were able to pair up pigs with similar characteristics but had stomachs score of at least 2 scoring points of difference. In the present study the sample of animals was unbalanced for sex within healthy individuals. Also, characteristics that pigs were blocked for in Hartnett et al. (2023) where unknown in the present study e.g. slaughter age, weaning and end weight were unknown.

Interestingly, Hartnett et al. (2023) with Rutherford et al. (2018) results are opposite. Hartnett et al. (2023) found that pigs with ulcerated stomachs spent more time lying on the left side, whereas Rutherford et al. (2018) found that affected pigs did this less as compared to pigs with healthy stomachs (including early stages of ulceration). Rutherford et al. (2018) observed the pigs 1 or 2 days before stomach assessment, while

Hartnett et al. (2023) had a gap of 5 or 12 days between both time points. As suggested by Hartnett et al. (2023), the anatomy and positioning of the stomach in pigs as well as the gap between behavioural observations and stomach sampling may explain the differences in the results found in both studies. The stomach is located on the left side of the abdomen with the pars oesophagea more centrally. The liver is found on the right of the stomach. Rutherford et al. (2018) explained that when the pig is lying on the left side there is pressure from the liver on the damaged tissue and, if the stomach content is liquid, the content is more likely to get into contact with the pars oesophagea. In Rutherford et al. (2018), pigs were trying to reduce pain by reducing the time they spent lying on the left side, whereas Hartnett et al. (2023) results show that the increasing time spent lying on the left side increased the chances of ulceration.

3.5.2 Finishing pigs perform self-directed oral behaviours

Even though, no relationship between ulcers and self-directed oral behaviours was found here, the results are still important. They reinforce findings in Chapter 2 that oral behaviours, usually related to chronic hunger in gestation sows [reviewed by D'Eath et al. (2018)], are performed by finishing pigs as well.

Observing self-directed oral behaviours in finishing pigs is of interest as they were not feed restricted, had access to social interaction and were provided with fresh straw every day. Hence, it is unexpected to find these types of behaviours in finishing pigs. There are two possible explanations for the occurrence of these behaviours: that the housing environment, diet and/or feeding practices are still inadequate to meet the pigs' needs, and/or the presence of other health-conditions affecting the upper digestive system.

Self-directed oral behaviours may appear in finishing pigs as a way to cope with a poor housing environment inadequate in terms of environmental enrichment and diet. Pigs are omnivorous animals that forage on a wide range of feeding sources spending most of their daily budget exploring and looking for the next bite, showing a varied feeding behaviour. This includes browsing and grazing on plants, foraging on the ground surface and rooting/digging underground, and also predate small vertebrates (D'Eath and Turner, 2009). Their diet is also varied including plants, fruit, nuts, fungi, roots, fungal rhizomes as well as carrion, invertebrates and small vertebrates (Ballari and Barrios-García, 2014).

The complexity of pigs' feeding behaviour may increase the likelihood of developing self-directed oral behaviours. In fact, Lewis et al. (2022) did a systematic literature review to investigate which ungulates would be more or less suitable for captivity by investigating the likelihood of developing stereotypies, as defined by the authors, according to different risk factors. Lewis et al. (2022) found that the feeding strategy used in the wild (grazer, browser, mixed feeder, frugivore or omnivore) and the feeding practices (feeding forage, concentrates, or both) in captivity were important predictors for the performance of stereotypies in ungulates. Omnivores (including pigs as defined in this study) tended to show a higher predicted prevalence than grazers, but lower than browsers and browsers/grazers. The predicted prevalence of stereotypies is the highest in animals that are fed in meals (as opposed to *ad libitum*) and in concentrate (as opposed to forage or both). The time spent doing stereotypies was highest in omnivores, fed concentrate and it was positively correlated with diet diversity in the wild (strong trend). Adding to this, pigs are novelty seekers and have an intrinsic motivation for exploration (Day et al., 1995). Pigs (5 to 6 weeks of age) prefer to enter a test arena with a novel object and spent more time interacting with it as opposed to a pen with a familiar object (Wood-Gush and Vestergaard, 1991). Pigs will also work more for gaining access to novel rooting material as opposed to familiar rooting material (Pedersen et al., 2005).

In contrast, under indoor intensive production systems pigs receive the same diet every day (with no choice of diet) provided in the same way in the same place without the possibility to work for it (de Jonge et al., 2008). Also, in the case of the present study, they receive the same source of environmental enrichment: straw. It seems plausible that this environment and feeding practices does not provide them with enough possibilities to express their whole repertoire of foraging behaviours.

It may be that the performance of self-directed oral behaviours is affected by a combination of feeding practices and diet, health of the upper digestive system, and/or gastric ulceration in pigs. This might be mediated by learning and persistence of this behaviours (irrespective of the initial reason). There is some evidence for this in horses. Crib-biting in horses increases in poor environments (Waters et al., 2002), when they are fed on concentrate (Waters et al., 2002), and pasture is not provided (Waters et al., 2002) as well as with gastric ulceration (Patiño et al., 2020). Pigs (D'Eath and Turner, 2009; Rivero et al., 2019) and horses (Boyd et al., 1988; Duncan, 1980; Kownacki et al., 1978) are species that uses most of their daily time budget in foraging behaviours and looking for feed (Boyd et al., 1988; Kownacki et al., 1978; Rivero et al., 2019). Also, access to straw/fresh silage, feeding practices and diet composition has been found to affect the health of the stomach in pigs (reviewed by Canibe et al., 2016; Holinger et al., 2018; Jensen et al., 2017).

3.5.4 Relatively high variation in self-directed oral behaviours in finishing pigs

Self-directed oral behaviours observed in this study were relatively variable within pigs with healthy and ulcerated stomachs, similar to what was observed in Chapter 2. This was especially true for wind-sucking and tongue-playing behaviour, and wind sucking varied more within pigs with ulcerated stomachs as opposed to with healthy stomachs. The higher variation in these two behaviours as compared to chewing movements could be explained by the fact that the first ones were rarer within pigs but also because chewing movements includes 'chewing on straw' which is a common behaviour in pigs. In fact, this higher variation in oral behaviours as opposed to more 'normal' behaviours has also been observed in studies on chronic hunger in gestating adult females

(Bernardino et al., 2021; de Leeuw et al., 2003; de Leeuw and Ekkel, 2004). For instance, de Leeuw et al. (2003) studied the effect of floor feeding and presence of foraging substrate on oral stereotypies. Similarly, they found that the SEM for sham chewing was higher than drinking and pen manipulation (but not floor manipulation which could have been affected by the feed and provision of straw) (1.54 to 2.13 vs 0.27 to 0.32 and 0.40 to 0.42 SEM, respectively). If oral behaviours are in fact related with an environment that does not satisfy the pigs' needs, the variation in the expression of these behaviours may be explained by the variation in coping styles pigs show in response to stressors (Camerlink et al., 2014; Hessing et al., 1994). However, the sample size of this study is fairly small to be able to draw any strong conclusions about the variability of the results.

3.5.5 Limitations of the study

The behavioural observations were limited by the position of the cameras and the quality of the videos. Cameras were positioned and the quality of the recording were adjusted to serve Rutherford et al. (2018)'s aim of scoring easy to observe behaviours such as postures, activity level, interaction with penmates, and clear manipulation of pen fixtures. In contrast, behaviours scored in the present study are difficult to recognize from a distance as they involve the mouth of the pig only, and they are usually quick as compared to other behaviours such as walking. The characteristics of the videos made it also difficult to observe other self-directed oral behaviours such as snout twitching. Additionally, although pigs seemed not to get stressed, recordings were disrupted by the personnel cleaning neighbouring pens or moving animals. This meant that pigs would direct their attention to the people and reduce the likelihood of performing self-directed oral behaviours.

3.5.6 Future steps

This is the first study investigating the relationship between self-directed oral behaviours and gastric ulcers in pigs. Further studies should use a larger sample size and address the questions of how the oral behaviours develop and whether the number of pigs performing these increases with age; how oral behaviours relate to other conditions affecting the upper digestive system; and consider studying the relationship between other behaviours and gastric ulcers. Further studies could also measure duration of the oral behaviours rather the frequency only.

3.6 Conclusion

This chapter confirms the findings in Chapter 2: Self-directed oral behaviours usually observed in gestating sows (and usually attributed to chronic hunger) are also observed in finishing pigs (fed *ad libitum*). It was hypothesised that these could be related to gastric ulcers, however, no evidence was found for this in the present study.

This negative result on gastric ulcers leaves various other possible explanations for oral behaviours to be explored. The possible causative factors include explorative and dietary needs, and conditions affecting the upper digestive system.

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3.8 Annexe

Table 3.7. List of behaviours scored by Rutherford et al. (2018)

Behaviours	
Laying ventral, lateral or mixed	Being belly nosed
Sit	Nosing rear
Kneel	Ear biting
Stand	Mounting
Walk	In feeder
Idle	Ease quarters hind/front limbs
Root groud/pen	Back leg forward
Alert	Kick
Nose other pig	Leg switch
Belly nosing	Draw in back leg
Rub rear, head or flank	Paw
Reciprocal aggression	Back arch
Snap	Shudder
Being snapped at	

Chapter 4 A study of association of re-directed oral behaviours, salivary composition and pH and gastric ulcers in gestating and lactating sows

4.1 Abstract

Gastric ulcers are highly prevalent in the pig industry and cause discomfort/pain. However, there are no validated non-invasive methods to diagnose gastric ulcers in living pigs. In this chapter I investigated two potential diagnostic approaches: 1) re-directed oral behaviours and 2) saliva metabolic composition and salivary pH. Due to repopulation management of our research farm, thirty-eight gilts and sows (hereafter sows) designated for culling were utilised for this trial. Sows were followed through the end of their gestation until weaning when culling was done. Re-directed oral behaviours (self-, object- and pig-directed) were observed live using intervals of continuous 2.5-min observation by behaviour sampling during every ~ 1 h the last three weeks of gestation and during lactation (120 and 50 min overall/sow, respectively; n = 38). A subset of these sows was also sampled for saliva before they were moved into the farrowing facility (day 110 after service; n = 16) and then prior to euthanasia on the last day of lactation (day 27 or 28 post-partum; n = 26). At weaning, sows were humanely euthanized, and stomachs were immediately dissected post-mortem. Global metabolomics and pathway enrichment analysis were performed on saliva samples to measure metabolites. Salivary pH of gestating (n = 16) and lactating sows (n = 26) was measured. Ulceration of the pars oesophagea was assessed post-mortem using an overall stomach score, and I developed a separate scoring systems to describe presence and extension of keratinization, erosion, ulcer and/or healing tissue, and contraction of the oesophagus (n = 38). All sows had some level of change in the mucosa, and 67.57% had at least one ulcer. Behaviours during gestation and lactation were not correlated with the overall stomach score

(Spearman's correlation) and were not affected by the type of lesion present (Mann-Whitney U test). Salivary pH was also not correlated with overall stomach score and not affected by the presence of lesions. Saliva composition was different between reproductive stages. Also, metabolomic analysis identified features which significantly increased or decreased ($p < 0.05$) with increasing overall stomach score (Features are compounds that have been identified in a sample, but no further lab tests have been done for the verification). The pathway enrichment analysis identified various metabolites as part of different metabolic pathways during gestation and lactation. However, only a few may potentially be related to gastric ulceration: inosine, guanosine, adenine, thymidine and succinic acid in gestating sows, and L-Histidine and quinolinic acid in lactating sows. Additionally, lipoxin A and tromboxane B3 (both involved in the inflammatory response) increased with the presence of keratinization and erosion in lactating sows, respectively. Of these metabolites, succinic acid, L-Histidine and lipoxin A have plausible biological mechanisms linking them to gastric ulcers. To conclude, gastric ulcers are a much bigger welfare problem than previously thought as all sows in this study had a change in the pars oesophagea and 67.57% of the sows had at least one ulcer. Re-directed oral behaviours and salivary pH were not related to gastric ulceration. Although further work is needed, this study identified certain salivary metabolites which seem promising as potential biomarkers of gastric ulceration: Succinic acid, L-Histidine and lipoxin A.

4.2 Introduction

Gastric ulcers in the pars oesophagea (see section 1.5.1) are a widespread (Cybulski et al., 2021b, 2021a; Gottardo et al., 2017; Peralvo-Vidal et al., 2021c, 2021a), hidden problem in the pig industry, which are of economic (Dunlop et al., 2021) and animal welfare concern (Barkun and Leontiadis, 2010; Rutherford et al., 2018). They remain present (Helbing et al., 2022) possibly due to the lack of a real-time method of diagnosis in the living pig. Using the traditional method of collecting data and post-mortem assessment of the stomach to then compare between healthy and ulcerated individuals has some limitations. As we do not know the timeline of the development and healing of an ulcer and the belief that the progression from an erosion to an ulcer may be relatively rapid (Helbing et al., 2022; Lawrence et al., 1998), the study of the effect of any given factor is made more difficult by not knowing whether the pig had an ulcer (or not) at the time the factor was measured. A method that allows for real-time assessment of the pars oesophagea with minimum stress would help researchers to understand the problem better in terms of animal welfare and economic impact as well as finding ways of tackling it. Similarly, farmers and veterinarians would be able to identify and treat affected animals.

Oral behaviour and saliva are both potential indicators that could be used for diagnosis of gastric ulceration. As introduced in Chapter 3, oral behaviours have been shown to be caused by chronic hunger in sows, however there is some evidence that these may also be elicited by gastric ulcers (see section 3.2). It is true that in Chapter 3 no relationship between self-directed oral behaviours and gastric ulcers in finishing pigs was identified. However, this was done through video observations which could have made it difficult to identify mouth behaviours, and did not include out-directed oral behaviours.

Saliva is an easy to collect, non-invasive biofluid which could be used for the diagnosis of gastric ulcers in pigs. Its physiology allows for the assessment and measurement of a variety of analytes (Nunes et al., 2015). In non-human animals, disease-associated changes in saliva composition have been reported in rats (Kovalčíkova et al., 2018), horses (Muñoz-Prieto et al., 2022) and pigs (Cook et al., 1996; López-martínez et al., 2022; Ortín-Bustillo et al., 2021). However, most relevant for this thesis, Madsen et al. (2022) investigated the effect of gastric ulcers in pigs on salivary composition. They found differences in salivary metabolites between finishing pigs with healthy stomachs (including pigs with keratinization of the pars oesophagea) and with gastric ulcers: the latter had a lower level of salivary oxylipins (lipid mediators that play a role in inflammation). However, they did not explore changes in saliva composition with the progression of gastric ulceration and no associations were drawn with the physiological properties of saliva, such as pH due to gastric ulceration.

Saliva metabolites can be assessed by using metabolomics techniques (see section 1.7.2). In the present study, Liquid-Chromatography and Mass-Spectrometry were used. Specifically a methodology recently developed for untargeted metabolomics that is quicker than other methodologies; RHIMMS (Pičmanová et al., 2022). RHIMMS or Rapid HILIC-Z ion mobility mass spectrometry (HILIC-z; zwitterionic-phase hydrophilic interaction chromatography) is quicker but does not compromise the coverage of metabolites too much, has increased resolution and more accurate identification of small particles (Gabelica and Marklund, 2018; Gika et al., 2019).

Assessment of gastric ulcers is usually done macroscopically post-mortem by using an 11-point scale (from mild to more severe/chronic lesions). This is an easy and efficient way of assessing stomachs, however this method has a disadvantage. The scoring system lacks detail on what lesions coexist in one stomach. In this scale zero means the stomach has a healthy pars oesophagea and 10 means that it has an oesophagus with reduced diameter. On this scoring system, the most severe lesion is the one that gives the stomach the score (e.g. keratinization < erosion < ulcer < healing tissue < oesophagus contraction from less mild to severe). This is a good measure of the overall condition of the pars oesophagea, however it combines different types of healing tissue and extension of ulcers into one score. This may be a problem when studying the association between gastric ulcers and both behaviour and saliva composition, since healing tissue may have a different effect on these variables compared with an active, fresh and bleeding ulcer. In other studies, stomachs have been grouped according to the presence/absence of any types of lesion in the pars oesophagea (Peralvo-Vidal et al., 2021b, 2021c) or by the severity of the changes (Helbing et al., 2022). Even though these scores are good, they lack detail as to which type of lesion is present in the stomach e.g. keratinization, erosion, ulceration. To solve this, in this study, I created additional scores to assess the extent of each type of lesion independently (except for keratinization which was assessed qualitatively rather than its extent).

The aim of this study was to investigate a possible association between re-directed oral behaviours, and saliva composition and pH with gastric ulceration in gestating and lactating sows. Additionally, I aimed to describe stomach integrity, test the effect of farrowing environment and parity number on stomach integrity, and study the correlation between stomach integrity and parity number. Finally, I explored the overall stomach score in terms of how the lack of detail could be misleading.

The hypotheses were that gastric ulcers increase 1) pig-directed behaviours as there is a trend for increased time spent nosing other pigs (Rutherford et al., 2018); 2) self-directed behaviours as they were rare and highly variable among finishing pigs (Chapter 2 and 3); 3) straw-directed behaviours as straw has a protective effect on the pars oesophagea (Jensen et al., 2017), and 4) change the composition of saliva (Madsen et al., 2022), and its pH (because of the expected change in composition).

4.3 Materials and methods

This study was carried out over 19 weeks between July and December 2021 at SRUC Pig Research Centre near Edinburgh, Scotland, and took place during the last three weeks of gestation and lactation in selected sows. The project was reviewed and approved by SRUC's Animal Experiments Committee. This study was made possible due to a sow cull planned for management reasons in the herd.

4.3.1 Animals and housing

Thirty-eight primi- and multi-parous sows (hereafter sows) (Large White × Landrace) were used in this study (mean ± SD, 2.7 ± 1,6; range 1 to 6). Sows were housed in their home commercial pens during gestation and lactation. During gestation sows were housed in pens of 23.22 m^2 provided with fresh straw (every Monday and Friday), and access to a restricted low-energy commercial diet for gestation (Table 4.1) in an individual trough once a day (at 0720 h) and one drinker in the pen with *ad libitum* access to drinking water. Each pen held between 1 to 5 sows (mean 3.8 sows/pen). Lighting was on between 0700 and 1600.

During lactation, sows were kept either in farrowing crates (n = 17; 3.75 m^2) or PigSAFE free farrowing pens (n = 21; 8.9 m^2). For six sows housed in PigSAFE pens, the litters were socialized during lactation (as part of a separate study), by allowing two adjacent litters to mix freely through a gap in the side of the pen at 10 days of age. In both crates and pens sows were provided with a minimal amount of straw each day, *ad libitum* water and two rations according to litter size and sow body condition (at 0800 and 1530 h) of standard commercial diet for lactation (Table 4.1). Artificial lighting was on between 0800 and 1545. Farm management was maintained as usual during both stages. Unnecessary disruptions were avoided by the stockpersons during behavioural observations. Temperature and humidity for all batches are reported in Table 4.2.

Table 4.1. List of ingredients for the diet provided during gestation and lactation.

Gestation	Lactation
Barley	Barley
Wheat feed	Wheat
Wheat	Wheat feed
Malt residuals	Double low rapemeal (extracted)
Biscuit meal	Biscuit meal
Calcium carbonate	‘Hi pro’ soyabean extract.
Trace elements and vitamin supplements	Distiller’s dark grains (maize)
Fat blend	Malt residuals
Sodium chloride	Calcium carbonate
Lysine hydrochlorine	Fat blend
Vitamin E	Trace elements and vitamin supplements
Threonine	Sodium chloride
	Lysine hydrochlorine
	Monodical phosphate
	Vitamin E
	Threonine

Table 4.2. Environment temperature (Cº) and humidity (%) by batch number and productive stage (mean ± SD).

Batch	Productive stage	Temperature	Humidity
1	Gestation	18.11 ± 2.91	75.23 ± 14.67
2	Gestation	18.55 ± 3.34	82.08 ± 14.99
3	Gestation	14.71 ± 2.03	86.28 ± 13.49
4	Gestation	10.89 ± 2.10	92.52 ± 9.85
1	Lactation (crates)	23.13 ± 2.37	59.08 ± 9.22
2	Lactation (PigSAFE)	17.51 ± 0.61	77.15 ± 10.81
3	Lactation (crates)	20.12 ± 2.41	68.70 ± 11.50
4	Lactation (PigSAFE)	14.83 ± 0.41	70.27 ± 3.14

4.3.2 Study design

Thirty-eight sows were included in the trial from day 91 of gestation until the end of lactation when their piglets were weaned, and at which point sows were euthanised. Sows were observed in four batches between July and December 2021 [batch 1 (n = 12), July-August; batch 2 (n = 10) and 3 (n = 5), September-October; batch 4 (n = 11), November-December]. Behavioural observations were made during the last three weeks of gestation and during lactation (all batches). Saliva samples were taken by the end of gestation (batch 3 and 4) and on the day before (batch 2 and 4) or on the day of culling (batch 3). Batch 1 was not sampled for saliva. Stomachs were assessed post-mortem approximately 30 min after euthanasia (all batches).

4.3.3 Behavioural observations

The sows were observed live by an observer from outside the pen during both gestation and lactation. During gestation, sows were observed in the morning (0800 to 1100h) and afternoon (1200 to 1500h) from Tuesday to Thursday (or Tuesday and Wednesday in the second week of observation) for the last three weeks. Each sow was observed for 2.5 min every ~ 1h, resulting in a total of 120 min of observation per sow during gestation. During lactation, sows were observed from 1000 to 1500h on Mondays (second and third week of observation) and Fridays (first and second week of observation) for three weeks. The sows were observed for 2.5 min every ~ 1h. This resulted in a total of 50 min of observation per sow during lactation.

During these observations self-, pig- and object-directed oral behaviours (Table 4.3) were scored by continuous behaviour sampling. Only frequency of behaviours was recorded, and each bout of a certain behaviour was recorded as a new event. A bout of behaviour started with the beginning of a given behaviour and finished when the pig performed a different behaviour. All events were recorded, for example, if the sow changed from rooting on the floor to chewing and then back to rooting on the floor each behaviour would be recorded as a new event regardless of the time between events. To adjust the total time of observation, time spent visible and active as well as time being disrupted were scored (Table 4.4). A tablet device (Sony, model SGPT12, Android version 4.1.1) with Pocket Observer 3.2® software (Noldus, version 3.2.40) was used for behavioural scoring.

Identification of the sows was done by putting marks on their front and back legs, and rumps with pen markers during gestation, and numbering the pens during lactation. During gestation, new sows were identified the day before observations started (Monday) and then re-marked by the end of each observation day as well as at the beginning of the week when needed. Before each time slot of observation started, the observer would make sure that the focal sow was habituated to her presence. In the case of the lactation period, since sows are more wary of their environment, a 20 min period of habituation was allowed at the beginning of the day. Sows were also familiarised with the observer for ~ 2 min before observations started. The order of observation for focal pigs and pens were assigned randomly at the beginning of each day

Table 4.3. Description of oral behaviours scored during gestation and lactation. When foraging is directed towards resources other than the floor and straw this is classified as re-directed.

Oral behaviours	**Description**
Self-directed behaviours (point events[1])	
Sham chewing	The focal animal makes chewing movements with apparently nothing in the mouth. It is sham chewing only if the observer cannot identify what the animal is chewing on. The focal animal may or may not have saliva on its mouth.
Jaw stretching	Mouth opening and closing while stretching the lower jaw for several seconds. This may also include yawning
Tongue playing	Continuously moving the tongue while it is partly outside the mouth, with or without sham chewing
Wind sucking	Sucking on the tongue with the lower lip open in a V- shape, whilst the snout is against a pen bar, trough or conspecific
Snout twitching	The focal animal protrudes and then shrinks her snout repeatedly whilst the mouth is closed, and the head is still with no lateral or horizontal movement.
Presence of saliva	Saliva can be observed surrounding the mouth. This was recorded as yes/no by observation slot. This is not included into self-directed behaviours as it is measured differently.
Pig-directed behaviours (point events[1])	
Lick	The focal sow passes her tongue over a penmate
Root	The focal sow is massaging any part of another pig with repeated backwards and forwards movements of the snout and head
Nose	The snout of the focal sow is in contact with or in close proximity to any part of the body of other sow or piglet
Bite	The mouth of the focal sow has any part of the body of other sow or piglet in her mouth
Object-directed behaviours (point events[1])	
Bite pen fixtures	The mouth of the focal sow has the fence, bars or drinker
Bite trough	The mouth of the focal sow has the drinker
Foraging pen fixtures	The focal sow is licking, rooting or nosing the fence, bars or drinker
Foraging trough	The focal sow is licking, rooting or nosing the trough
Foraging wall	The focal sow is licking, rooting or nosing the wall
Chew	The focal sow is doing chewing movement. The observer can see how something different the straw is sticking out from the mouth or has seen the sow taking it into their mouth
Feeding-related behaviours (point events[1])	
Drink	The focal sow has the drinker in their mouth and the observer can see swallowing movements
Foraging floor	The focal sow is licking, rooting or nosing the floor
Straw chewing	The focal sow is doing chewing movements. The observer can see straw in the sows' mouth or has seen the sow taking straw into their mouth
Bite floor	The focal pig is using their teeth to scrape edible particles out of the floor
Foraging straw	The focal sow is licking, rooting or nosing the straw
Feed	The focal sow has her head in the feeder (there is feed in the feeder)

[1]Point events are behaviours scored as frequencies.

Table 4.4. Description of behaviours scored for adjusting the total time spent observing the sows during gestation and lactation.

Behaviours	Description
Activity (state events[1] mutually exclusive, exhaustive)	
Active	Pig is engaging in a behaviour included or not in this ethogram. It is also recorded as active if the pig is lying but her head is not resting on a surface (e.g. floor, pig)
Inactive	Pig is resting and not engaging in anything. The head must be resting on a surface and no movement indicative of an awake behaviour is observed (e.g. twitching and kicking while sleeping or getting comfy are not considered as active). The focal pig may have its eyes closed or open.
Visibility (state events[1] mutually exclusive, exhaustive)	
Visible	The focal animal is visible to be scored for oral behaviours
Not visible	The focal animal is not visible to be scored for oral behaviours
Disruption (state events[1] mutually exclusive, exhaustive)	
Disruption	Any event that could have caused a change in the behaviour of the focal animal (e.g. people entering the shed, random noise in the shed or farm)
No disruption	Nothing is disrupting the behaviour of the focal pig

[1]The duration and frequency of the behaviour are measured.

4.3.4 Saliva sampling and processing for storage

Saliva samples were taken at the end of gestation (batch 3 and 4), and on the day before (batch 2 and 4) or the same day of culling (batch 3) due to logistics (n = 43 samples). During gestation, samples were taken between 0815 and 0830 h after sows had eaten all their daily ration (fed at ~ 0720 h). During lactation, saliva samples were taken between 0730 and 0800 h before their morning ration (fed at 0800 h). However, due to miscommunication, batch 3 sampling occurred after feeding which occurred at 0715 h instead of the usual time at 0800 h. Saliva was collected by allowing the sows to chew on a cotton swab (Millpledge Veterinary, DX09396) until it was soaked. Collected swabs were placed in Salivette® tubes (Sarstedt Ag & Co, Germany) and placed in a styrofoam box with ice blocks at ~ 8°C before being placed in a centrifuge on site approximately 30 min after collection. Saliva was retrieved from the swab by spinning in a cooled centrifuge at ~ 4°C for 5 min at 3000 rpm. Samples were refrigerated for transport and subsequently frozen at - 80°C (~ 4 to 5 h after collection) until all samples from all batches were collected. Then the saliva samples were defrosted on the counter at room temperature to be processed for long-term storage until analysis. Metabolites were extracted by mixing the saliva sample with a solvent solution of chloroform : methanol : water (1:3:1) in a sample : solvent ratio of 1:5. The sample : solvent mixture was mixed thoroughly by vortexing at high speed for 2 min and incubated in a cooled agitator at 1,800 rpm for 1h at 4°C. The extracts were centrifuged at 14,000 rpm for 10 min at 4°C and 400 µL of the supernatant, corresponding to the metabolite extract, was transferred into a clean Eppendorf tube and stored at -80°C until metabolomic analysis. The global metabolomics analysis was performed by EdinOmics (RRID:SCR_021838) at the University of Edinburgh.

4.3.5 Metabolomics analysis of saliva samples

Global metabolomics was performed using RHIMMS method which measures the peak intensities of the metabolites. This was done by using an Agilent 1290 Infinity II series UHPLC system (Ultra-High-Performance Liquid Chromatography) coupled to an Agilent 6560 IM-qTOF (Ion Mobility quadrupole Time-of-Flight; both Agilent Technologies, Santa Clara, CA) with a Dual Agilent Jet Stream Electron Ionization source (Pičmanová et al., 2022). Agilent 1290 Infinity II series UHPLC system contained an InfinityLab Poroshell 120 HILIC-Z, 2.1 mm × 50 mm, 2.7 µm column (Agilent Technologies 689775-924, Santa Clara, CA) coupled to an InfinityLab Poroshell 120 HILIC-Z, 3.0 mm × 2.7 µm UHPLC guard column (Agilent Technologies 823750-948, Santa Clara, CA). Briefly, 1 µL sample was injected into the guard column, for chromatographic separation at a constant flow rate of 800 µL/min. Two different solvent systems of low and high pH were used to run 3.5 min gradient in positive and negative ionisation modes, respectively. In positive ionisation mode the solvent system consisted of 10 mM ammonium formate in water, pH 3 (solvent A) and 10 mM ammonium formate in water/acetonitrile (1:9), pH 3 (solvent B). Similarly, the solvent system for acquiring data in negative ionisation mode consisted of 10 mM ammonium acetate in water, pH 9 (solvent A) and 10 mM ammonium acetate in water/acetonitrile (1:9), pH 9 (solvent B). The solvent gradient for both ionisation modes consisted of 93% solvent B at the start of the run, which was reduced to 80%

solvent B in 1.8 min, and further to 70% solvent B in 0.2 min, where it was maintained for 0.3 min. At 2.35 min the column was returned to initial conditions of 93% solvent B and maintained as such until 3.5 min. The column was maintained at 30°C throughout the run. In both ionisation modes data was acquired in the 50-1700 m/z range, with an mass spectrometry acquisition rate of 0.8 frames/s. The nebulizer pressure was set to 60 psi, gas temperature to 225°C and drying gas (N_2) flow rate to 13 L/min. Sheath gas was set to 340°C with a flow rate of 12 L/min, and the instrument was operated at a capillary voltage of 3,000 V, nozzle voltage of 200 V, fragmentor voltage of 395 V, and octupole voltage of 750 V. The instrument was calibrated and tuned separately for each ionisation polarity using the ESI-L low concentration tuning mix from Agilent Technologies (Santa Clara, CA). A reference mass solution consisting of 50 mM ammonium trifluoroacetate, 5 mM purine and 1.125 mM HP-0921 was injected continuously into each sample to recalibrate for accurate mass and drift time during data processing. The ES-TOF reference mass solution kit was purchased from Agilent Technologies (Santa Clara, CA). Data acquisition and processing were performed using the Agilent MassHunter 10 software suite, details of which are available in Pičmanová et al. (2022).

4.3.6 Measurement of pH of saliva samples

After the metabolomic analysis, there were 16 and 26 saliva samples (stored at - 80°C) available from gestating and lactating sows, respectively. Only 15 sows had saliva samples from both gestation and lactation stage. The pH of the saliva samples was measured by using an electronic pH meter (PerpHecT® ROSS® Micro Combination pH Electrode and Fisher Scientific accumet® AE150) after a three-point calibration method (pH = 4, pH = 7 and pH = 10). Samples were transported in a Styrofoam box with dry ice from the storage freezer (- 80°C) to the laboratory and defrosted on dry ice and then at room temperature. The samples were assessed in a random order. Before and after measuring pH, the probe was cleaned with double distilled water. For those sows that had an extra Eppendorf tube of sample (i.e. the amount of saliva collected from the cotton swap was enough to fill in two Eppendorf tubes) the pH of both tubes was measured separately and then the values were averaged.

4.3.7 Stomach sampling and post-mortem stomach assessment for gastric ulceration

Sows were weaned and removed from their home pen as usual, and then walked into an adjacent outdoor pen with grass. For euthanasia, sows were walked individually into an adjacent building which was not occupied by any other pigs. Prior to euthanasia, sows were sedated with a mixture of 6.25 ml Azaperone (Stresnil; 40 mg/ml), 2.5 ml Medetomidine Hydrochloride (Domitor; 1 mg/ml), 25 ml Midazolam (Hypnovel; 5 mg/ml) and 25 ml ketamine (Ketamidor; 100 mg/ml) by intramuscular injection in both sides of the neck by trained personnel. Once it was established that the sow was fully sedated, trained personnel euthanised the sow by injecting a lethal dose of Pentobarbital sodium (Euthatal; 200 mg/ml. Merial) into the heart (78.03 ± 33.96 (mean ± SD)]. Total ml are given based on total live weight of 250 kg. Death was confirmed via heart rate monitoring with a stethoscope and lack of a visual evoked response. Once death was confirmed, the abdominal cavity was opened, and the stomach was cut out. All this process was done by a trained technician. The carcass was removed from the pen and the pen was cleaned with disinfectant powder (Staldran) before the next sow was brought into the facility.

The stomachs were brought to the surgery facility at the pig unit about 30 min after euthanasia. Each stomach was identified with a card with the ID of the sow. Stomachs were opened through the greater curvature, content was drained into a box for visual observation and washed gently with running cold water. Each stomach was photographed for data records. The pars oesophagea's integrity was assessed according to the overall stomach condition (overall stomach score; Table 4.5) and the type and severity of the lesions coexisting in each stomach (lesion scores; Table 4.6). When referring to both scores these will be named as stomach integrity scores or stomach integrity.

Table 4.5. Description of overall stomach score which describes the pars oesophagea (PO) in terms of the worst lesion present.

Overall stomach score	**Evaluation the white part of the stomach (keratinisation, ulcer and scarring)**	**Description**
0	No visible keratinisation; no erosion or ulcers; no scar formation	The white part of the stomach by the mouth of the oesophagus is white, shiny, smooth and elastic.
1	Keratinisation < 1 mm of thickness	Keratinisation: mucosa around the mouth of the oesophagus gradually changes structure (keratinises) into cusp regeneration
2	Keratinization > 1 mm of thickness	
3	Keratinisation is papillomatous	
4	Erosion in < 10% of the PO	Erosion: the protective layer of mucosa has disappeared resulting in direct access to the underlying sensitive tissue. There is no cliff between healthy and unhealthy tissue
5	Erosion in > 10% of the PO	
6	Ulcer in < 10% of the PO or slight scar formation (scab)	Ulcer: deep changes in the mucosa, possibly bleeding, with a cliff between healthy and unhealthy tissue Scar: old injuries partially healed during scar formation. During scar formation, fibrous tissue (fibrosis) forms and the tissue turn inelastic and contracts
7	Ulcer in 10 - 50% of the PO or scar formation with scab and slight fibrosis	
8	Ulcer > 50% of the PO or scar formation with clear fibrosis	
9	Contracted oesophagus where diameter of oesophagus is approx. 10 mm	Scar: old injuries partially healed during scar formation. During scar formation, fibrous tissue turns inelastic and contracts. In the most severe degrees, the mouth of the oesophagus contracts to a narrow, inelastic aperture.
10	Contracted oesophagus where diameter of oesophagus is below 6 mm	

Adapted from Jensen et al. (2017)

Table 4.6. Description of lesion scores which includes absence/presence of a type of lesion and its severity in the pars oesophagea (PO).

Score	Keratinization	Erosion extension	Ulcer extension	Type of healing tissue	Oesophagus contraction
0	No keratinisation	No erosion	No ulcers	No healing tissue	No oesophagus contraction
1	< 1 mm thickness	< 10% of the PO	< 10% of the PO	Scab	Diameter of ~ 10 mm
2	> 1 mm thickness	> 10% of the PO	10 to 50% of the PO	Scab and fibrosis (slight or clear fibrosis)	Diameter of > 6 mm
3	Papillomatous		> 50% of the PO	Slight or clear fibrosis	

4.3.8 Health assessment and animal management

Notes on health status were made throughout the study e.g. lameness, coughing, sneezing, skin lesions, when they were noticed during behavioural observations, saliva sampling or culling. Also, information about any veterinary treatments were obtained from farm records. This was to assist with interpretation of any unexpected results and/or outliers but was not included into the statistical analysis.

Farm husbandry routines were maintained as usual. The farm manager provided information about any change in the normal routines including changes of diet, changes of schedule, treatments, sick/dead animals, and movement of animals when appropriate.

4.3.9 Statistical analysis

A case-control study was performed to study the association between both oral behaviours and saliva characteristics (composition and pH), and gastric ulcers in sows. Thirty-eight sows were scored for rate of oral behaviours (counts of behaviour/time spent visible, active and not disrupted) and a subgroup was saliva sampled for the analysis of salivary composition and pH assessment both during gestation and lactation. The integrity of the pars oesophagea was assessed post-mortem. The experimental unit was a single sow, CI was set at 95% and significance level at 0.05. However, to correct for the multiple hypotheses tested on a single response variable, Bonferroni correction was used when needed. This is stated below for each of the statistical analysis when used.

The count of behaviours during gestation and lactation was adjusted by the time spent visible, active and not disrupted (count of behaviour/min). Also, root, nose and lick were added up into foraging maintaining modifiers (e.g. straw, trough). Foraging behaviour directed towards resources different than straw and floor were classified as re-directed foraging behaviours. Behaviours directed towards the fence, bars and drinker were summed up into 'directed to pen fixtures'. Lick, root, nose and bite penmate were summed up as pig-directed oral behaviours. Snout twitching were not observed in either gestation or lactation. Bite floor and chewing were not observed during lactation. Feeding was not scored during gestation as observations started after the morning ration was eaten. For the data on assessment of the health of the pars oesophagea, the scores assessing each lesion separately were coded as presence/absence of lesions because of low number of cases. Also, the score for oesophageal contraction was not analysed as only one sow had a contraction of the oesophagus, instead it was described.

Regression models were run to study the rate of behaviour as a predictor of the health of the pars oesophagea. However, because the sample size was too small and the number of cases were not enough, Mann-Whitney U test and Spearman's correlation were used instead (SPSS 28). Mann-Whitney U test was used to test the effect of presence/absence of a type of lesion (lesion scores) on the rate of behaviour. The relationship between overall stomach score and rate of behaviours was investigated using Spearman's correlations. Bonferroni corrections were used for both Mann-Whitney U test and Spearman's correlations.

The effect of the progression of the condition (overall stomach score) and presence/absence of lesions on saliva composition was studied by using multivariate statistical analysis and pathway enrichment analysis on the saliva metabolome using

MetaboAnalyst 5.0 web-based platform (Pang et al., 2021). The metabolomics data was log-transformed and auto-scaled to generate Partial Least Squares – Discriminate Analysis 2D score plots and box and whisker plots shown here. The peak intensities correspond to the original data as opposed to normalised as they are true to what was seen in the biological data and not affected by data analysis changes. Because there were not enough repetitions for the overall stomach score it was necessary to create pseudo-replicates to satisfy the needs of the tests which require a minimum of 3 replicates per group. This was done by copying some samples. For gestating sows, two pseudo-replicates for score 4, one for score 7 and two for score 9 were used; and for lactating sows one pseudo-replicate for score 4, and two for score 9 were used for lactating sows.

The effect of the presence of lesions (keratinization, erosion, ulcer, healing) on the salivary pH was investigated by using a two-sample t-test (Minitab 17). This was tested within gestating and within lactating sows. However, because some samples were not available anymore, it was not possible to test the effect of healing tissue in gestating sows. The correlation between the overall stomach score and salivary pH in gestating and lactating sows was tested using Spearman's correlation (Minitab 17). Differences in salivary pH between gestating and lactating sows was studied by using paired t-test (Minitab 17).

The percentage of sows with gastric ulcers, overall stomach score and lesion score were estimated. The effect of farrowing environment (farrowing crate vs PigSAFE) and parity number (parity 1 + 2 vs 3 or more parities) on stomach integrity was studied by using regression models using SPSS 28. Parity number was grouped into sows with 1 and 2 parities, and sows with 3 or more parities. This was to satisfy the assumptions of the models of minimum number of cases per level of categorical variable. The effect on the overall stomach score was studied using an ordinal regression model (cumulative odds ordinal logistic regression with proportional odds), and the effect on the presence/absence of a type of lesion (lesion score) was investigated using binomial regression model.

To study the correlation between stomach integrity (overall stomach score and ulcer extension score) and parity number of the sow a Spearman's correlation (two-tailed) was used with SPSS 28. As required in the configuration of this test in SPSS 28, both scores were set as ordinal variables (Measure), and additionally as numeric (Type). For this analysis parity number was not re-grouped. The correlation between both scoring systems (lesion score and overall stomach score) was tested the same way.

Health-related measures was not included into any of the models as these were sporadic and short-lived. No pig was showing clear signs of long-lasting health problems. It was deemed as not being important enough to affect the behaviour of the pigs.

4.4 Results

I first describe the stomach integrity and how it relates with the farrowing environment and parity number. I then explore how overall stomach score could be misleading. Lastly, I present the results on the effect of stomach integrity on behaviour and saliva characteristics.

4.4.1 Description of stomach integrity

Number of sows with a given overall stomach score are shown in Figure 4.1. The prevalence of gastric ulcers was 65.79% (25/38 sows). Almost all sows (34/38) had stomachs with signs of ulceration and/or healing tissue (score 6, 7 and 8), and none of the sows had a healthy-looking stomach (score 0) or only very early signs of ulceration (scores 1 to 3). Few sows had erosion as their worst lesion (score 4 and 5; 3/38). Lastly, only one sow had a contraction of her oesophagus (score 9). She was in a group of four sows during gestation and went into PigSAFE for farrowing and lactation. She was a third parity sow and had an erosion extension of less than 10% of the pars oesophagea, ulceration taking more than 50% of the pars oesophagea and early stages of healing (scab).

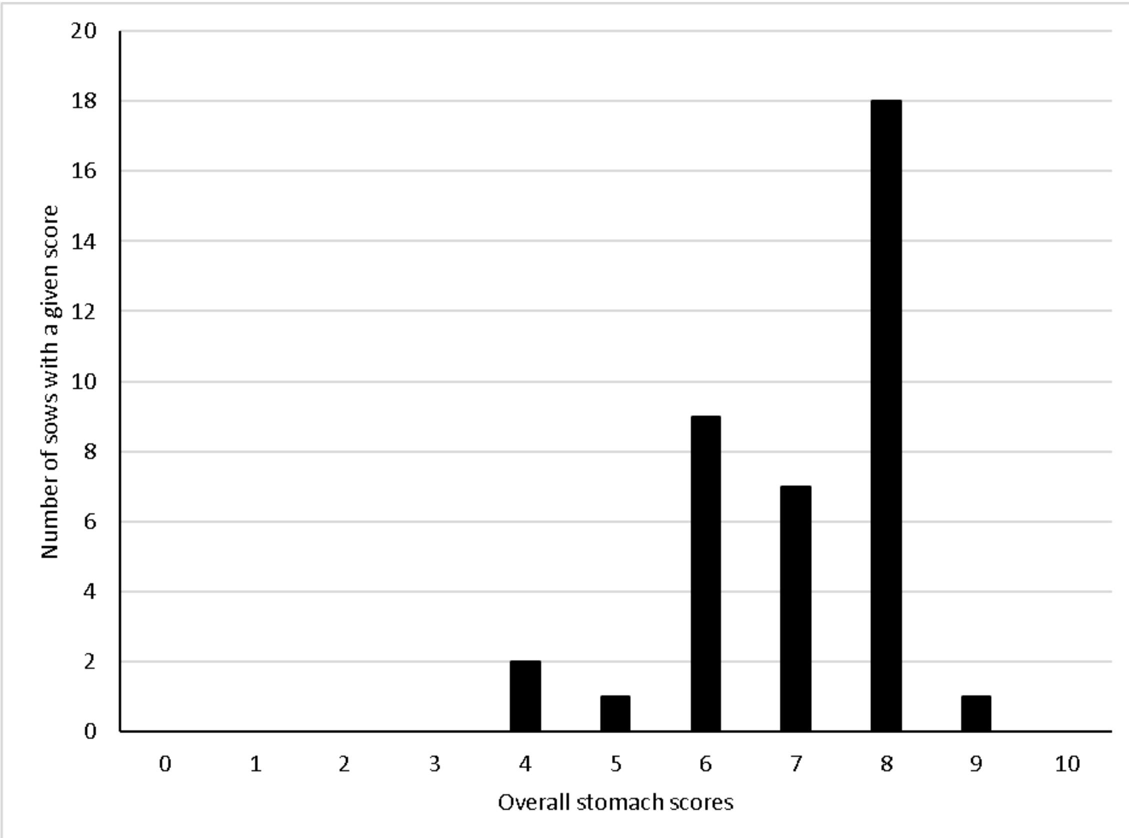

Figure 4.1. Number of sows with a given overall stomach score of the pars oesophagea (PO). Score 1: keratinization of < 1 mm of thickness; 2: keratinization of > 1 mm of thickness; 3: papillomatous appearance; 4: erosion of < 10% of the PO; 5: erosion of > 10% of the PO; 6: ulcer of < 10% of the PO or slight scar formation; 7: ulcer of 10 - 50% of the PO or scar formation with scab and slight fibrosis; 8: ulcer of > 50% of the PO or scar formation with clear fibrosis; 9: diameter of oesophagus is approx. 10 mm; 10: diameter of oesophagus is below 6 mm.

4.4.2 Relationship between stomach integrity and parity number and farrowing environment

The parity number of the sows and farrowing environment did not affect stomach integrity scores (lesion score and overall stomach score). The overall stomach score and parity number did not correlate ($r_s = -0.148$, $p = 0.376$). However, the extent of ulcers increased with increasing parity number (ulcer extension score; $r_s = 0.421$, $p = 0.009$).

4.4.3 Exploring the overall stomach score

Table 4.7 summarises the number of sows with a given lesion score. Almost all stomachs had keratinization of the pars oesophagea (31/38), and most of them had papillomatous appearance (score 3) of the pars oesophagea (29/38). Only 31.58% of the stomachs did not have ulceration of the pars oesophagea (ulcer extension score 0). Although most of the sows had small ulcers [< 10% of the pars oesophagea ulcerated (ulcer extension score 1)], 5.26 and 21.05% of the sows had ulcers covering 10 to 50% (ulcer extension score 2) or more (ulcer extension score 3) of the pars oesophagea, respectively. Most sows showed some level of healing (healing score 1 to 3). It is important to notice that there was a higher percentage of sows with score 3 healing tissue than sows with score 3 ulcer extension. Sows having scored 3 in any of these two scores, meant that they would get an overall stomach score of 8. Actually, from the total of 34 sows that scored 6 to 8 on the overall stomach score (Table 4.8), 19 sows had only signs of healing tissue and not ulcers. Furthermore, from these, nine sows had clear fibrotic tissue (healing score 3) but no ulceration.

Table 4.7. Number and percentage of sows with a certain type and severity of stomach lesion (lesion score)

Lesion score	Keratinization		Erosion extension		Ulcer extension		Type of healing tissue		Oesophagus contraction	
	n	%	n	%	n	%	n	%	n	%
0	7	18.42	16	42.11	12	31.58	8	21.05	37	97.37
1	0	0.00	15	39.47	15	39.47	9	23.68	1	2.63
2	2	5.26	5	13.16	8	21.05	9	23.68	0	0.00
3	29	76.32	-	-	2	5.26	10	26.32	-	-
Unknown[1]	0	0.00	2	5.26	1	2.63	2	5.26	0	0.00

[1]Unknown means that the observer did not take a note of the type of the lesion and extension.

Table 4.8. Number of sows with a given lesion score (ulcer extension and type of healing tissue) by overall stomach score (6 to 8) in the pars oesophagea (PO)

Overall stomach score*	Ulcer extension		Healing tissue		Only healing tissue
	Score	**Counts**	**Score**	**Counts**	**Counts**
Score 6	Score 0	1	Score 0	4	0
	Score 1	8	Score 1	4	0
	Score 2	0	Score 2	0	0
	Score 3	0	Score 3	0	0
	Unknown[1]	0	Unknown	1	0
Score 7	Score 0	0	Score 0	1	0
	Score 1	0	Score 1	4	4
	Score 2	7	Score 2	2	0
	Score 3	0	Score 3	0	0
	Unknown	0	Unknown	0	0
Score 8	Score 0	8	Score 0	0	0
	Score 1	7	Score 1	0	0
	Score 2	1	Score 2	7	6
	Score 3	1	Score 3	10	9
	Unknown	1	Unknown	1	0
Total		34		34	19

* Score 6: < 10% of the PO or slight scar formation; 7: 10 - 50% of the PO or scar formation with scab and slight fibrosis; 8: > 50% of the PO or scar formation with clear fibrosis

[1]Unknown means that the observer did not take a note of the type of the lesion and extension

4.4.4 Correlations between stomach integrity scores

All combination of stomach integrity scores were included. Overall stomach score and healing tissue score were highly correlated ($r_s = 0.815$, $p < 0.001$). Erosion extension was positively moderately correlated with keratinization score ($r_s = 0.382$, $p = 0.022$) but was negatively correlated with healing tissue type ($r_s = -0.390$, $p = 0.023$).

4.4.5 Relationship between behaviour and stomach integrity scores

Behaviour during gestation and lactation was not related to the stomach integrity scores. There was no difference in the rate of any of the observed behaviours between sows with and without a certain type of lesion (lesion score) during gestation (Table 4.9) and lactation (Table 4.10). Also, none of the observed behaviours were correlated with the overall stomach score during gestation and lactation (Table 4.11).

Table 4.9. Mean ranks of rate of behaviour during gestation by the presence (yes)/absence (no) of a certain type of lesion

Behaviours	Parakeratosis					Erosion					Ulcer					Healing tissue				
	No	Yes	U	z	p[a]	No	Yes	U	z	p[a]	No	Yes	U	z	p[a]	No	Yes	U	z	p[a]
Self-directed behaviours	26.29	17.97	61	-1.789	0.076	20.47	16.93	128.5	-1.003	0.32	16.29	20.3	182.5	1.055	0.296	17.38	18.82	121	0.342	0.751
Presence of saliva	23.86	18.52	78	-1.149	0.265	20.53	16.88	127.5	-1.035	0.305	17.88	19.54	163.5	0.438	0.666	20.44	17.95	96.5	-0.59	0.562
Pig-directed behaviours	20.64	19.24	100.5	-0.326	0.768	17.09	19.63	182.5	0.766	0.479	17.83	19.56	164	0.488	0.666	24	16.93	68	-1.812	0.099
Bite pen fixtures[1]	17.29	20	124	0.64	0.58	17.56	19.25	175	0.517	0.648	20.67	18.2	130	-0.718	0.532	15.75	19.29	134	0.935	0.421
Bite floor[2]	19	19.61	112	0.475	0.912	19.13	18	150	-1.118	0.765	20.04	18.5	137.5	-1.443	0.689	18	18.64	116	0.535	0.896
Bite trough	18	19.84	119	0.53	0.713	17.97	18.93	168.5	0.372	0.789	14.5	21.16	204	2.327	0.083	15.75	19.29	134	1.101	0.421
Drink	13.71	20.61	149	1.525	0.134	15.81	20.65	203	1.369	0.178	19.08	18.96	149	-0.032	0.987	16.13	19.18	131	0.723	0.489
Straw chewing	15.71	20.35	135	0.998	0.335	17.81	19.05	171	0.35	0.741	22.42	17.26	109	-1.33	0.192	18	18.64	116	0.152	0.896
Chewing	19.57	19.48	108	-0.03	1	18.84	18.23	154.5	-0.27	0.863	18.92	19.04	151	0.051	1	19.88	18.11	101	-0.645	0.695
Foraging[3] pen fixtures	12.14	21.16	160	1.941	0.053	15.44	20.95	209	1.561	0.124	23	17.08	102	-1.559	0.124	22.5	17.36	80	-1.219	0.236
Foraging floor	23.43	18.61	81	-1.036	0.317	18.94	18.15	153	-0.223	0.838	21.25	17.92	123	-0.876	0.395	19.75	18.14	102	-0.381	0.723
Foraging straw	17.29	20	124	0.584	0.58	18.13	18.8	166	0.191	0.863	18	19.48	162	0.389	0.713	16.75	19	126	0.533	0.614
Foraging trough	18.14	19.81	118	0.387	0.74	17.63	19.2	174	0.482	0.671	20.25	18.4	135	-0.523	0.643	16.31	19.13	129.5	0.712	0.513
Foraging wall	22.64	18.79	86.5	-0.876	0.416	19.13	18	150	-0.337	0.765	19.96	18.54	138.5	-0.397	0.713	18.44	18.52	112.5	0.02	1

[a]Significance at $p < 0.05$, but after Bonferroni correction $\alpha_{adjusted} = 0.0036$
[1]Pen fixtures includes fence, bars and drinker
[2]The animal is using their teeth to scrape edible particles out of the floor
[3]Foraging includes licking, rooting or nosing. When this is directed towards resources different than the floor and straw this is classified as re-directed.

Table 4.10. Mean ranks of rate of behaviour during lactation by the presence (yes)/absence (no) of a certain type of lesion

Behaviours	Parakeratosis					Erosion					Ulcer					Healing tissue				
	No	Yes	U	z	p[a]	No	Yes	U	z	p[a]	No	Yes	U	z	p[a]	No	Yes	U	z	p[a]
Self-directed behaviours	23.71	18.55	79	-1.114	0.282	17.34	19.43	178.5	0.59	0.56	19.33	18.84	146	-0.13	0.911	21.75	17.57	86	-0.992	0.339
Presence of saliva	18.5	19.73	115.5	0.32	0.797	19.09	18.03	150.5	-0.383	0.765	15.25	20..80	195	1.755	0.151	16.25	19.14	130	0.816	0.513
Pig-directed behaviours	14.29	20.68	145	1.379	0.179	17.94	18.95	169	0.288	0.789	21.5	17.8	120	-0.977	0.344	20.25	18	98	-0.535	0.614
Bite pen fixtures[1]	23.36	18.63	81.5	-1.175	0.317	19.03	18.08	151.5	-0.308	0.789	18.67	19.16	154	0.149	0.911	21.13	17.75	91	-0.909	0.443
Bite trough	24.86	18.29	71	-2.224	0.167	19.94	17.35	137	-1.128	0.479	17.88	19.54	163.5	0.737	0.666	18.38	18.54	113	0.063	1
Drink	20.29	19.32	103	-0.212	0.854	20.19	17.15	133	-0.88	0.404	20.54	18.26	131.5	-0.617	0.554	16.06	19.2	131.5	0.765	0.466
Feed	26.86	17.84	57	-2.098	0.053	21.56	16.5	111	-1.667	0.124	19	19	150	0	1	18.13	18.61	115	0.125	0.926
Straw chewing	16.71	2013	128	0.749	0.483	18.59	18.43	158.5	-0.049	0.962	20.67	18.2	130	-0.663	0.532	17.25	18.86	122	0.39	0.723
Foraging[2] pen fixtures	13.14	20.94	153	1.756	0.098	18	18.9	168	0.264	0.814	20.67	18.2	130	-0.683	0.532	17.13	18.89	123	0.442	0.695
Foraging floor	15	20.52	140	1.201	0.249	18.38	18.6	162	0.064	0.962	24	16.6	90	-1.973	0.053	16.75	19	126	0.54	0.614
Foraging straw	15.5	20.4	136.5	1.479	0.299	20.28	17.8	131.5	-1.247	0.369	21	18.04	**126**	**-1.081**	**0.451**	14.5	19.64	144	1.673	0.236
Foraging trough	23.14	18.68	83	-1.239	0.354	21.28	16.28	115.5	-1.794	0.158	18.88	19.6	151.5	0.065	0.962	14	19.79	148	1.801	0.18
Foraging wall	17.5	19.95	122.5	0.99	0.606	17.53	19.28	175.5	0.904	0.626	21.5	17.8	120	-1.805	0.344	18.81	18.41	109.5	-0.174	0.926

[a]Significance at $p < 0.05$, but after Bonferroni correction $\alpha_{adjusted} = 0.0038$
[1]Pen fixtures includes fence, bars and drinker
[2]Foraging includes licking, rooting or nosing. When this is directed towards resources different than the floor and straw this is classified as re-directed.

Table 4.11. Spearman’s correlation between behaviours and overall stomach score during gestation and lactation.

Behaviours	Gestation (n = 38)		Lacation (n = 38)	
	r	p[a]	*r*	p[b]
Self-directed behaviours	-0.003	0.986	0.093	0.578
Presence of saliva	-0.041	0.805	0.002	0.991
Pig-directed behaviours	-0.307	0.061	-0.277	0.093
Bite pen fixtures[1]	0.172	0.302	0.014	0.935
Bite floor[2]	0.144	0.387	Not observed	
Bite trough	-0.084	0.614	0.159	0.340
Drink	0.018	0.913	-0.094	0.574
Feed	NA		0.002	0.989
Straw	0.059	0.725	-0.129	0.439
Chew	-0.023	0.893	Not observed	
Foraging[3] pen fixtures	-0.182	0.274	0.072	0.667
Foraging floor	-0.042	0.802	-0.002	0.991
Foraging straw	0.133	0.425	0.095	0.569
Foraging trough	-0.107	0.522	0.239	0.149
Foraging wall	0.078	0.644	0.069	0.680

[a]Significance at $p < 0.05$, but after Bonferroni correction $\alpha_{adjusted} = 0.0036$
[b]Significance at $p < 0.05$, but after Bonferroni correction $\alpha_{adjusted} = 0.0038$
[1]Pen fixtures includes fence, bars and drinker
[2]The animal is using their teeth to scrape edible particles out of the floor
[3]Foraging includes licking, rooting or nosing. When this is directed towards resources different than the floor and straw this is classified as re-directed.

4.4.6 Metabolic profiling of saliva metabolome for markers of progression of gastric ulceration

Global metabolomics profiling identified differences between the saliva metabolome of gestating and lactating sows (Figure 4.2a). Within each of both production stages, several significantly changing (increasing or decreasing with p-value < 0.05) features were identified to correlate with the progression of the condition (increasing overall stomach score) (Figure 4.2b, c). Interestingly, the majority of the features decreased with progression of the condition ($p < 0.05$).

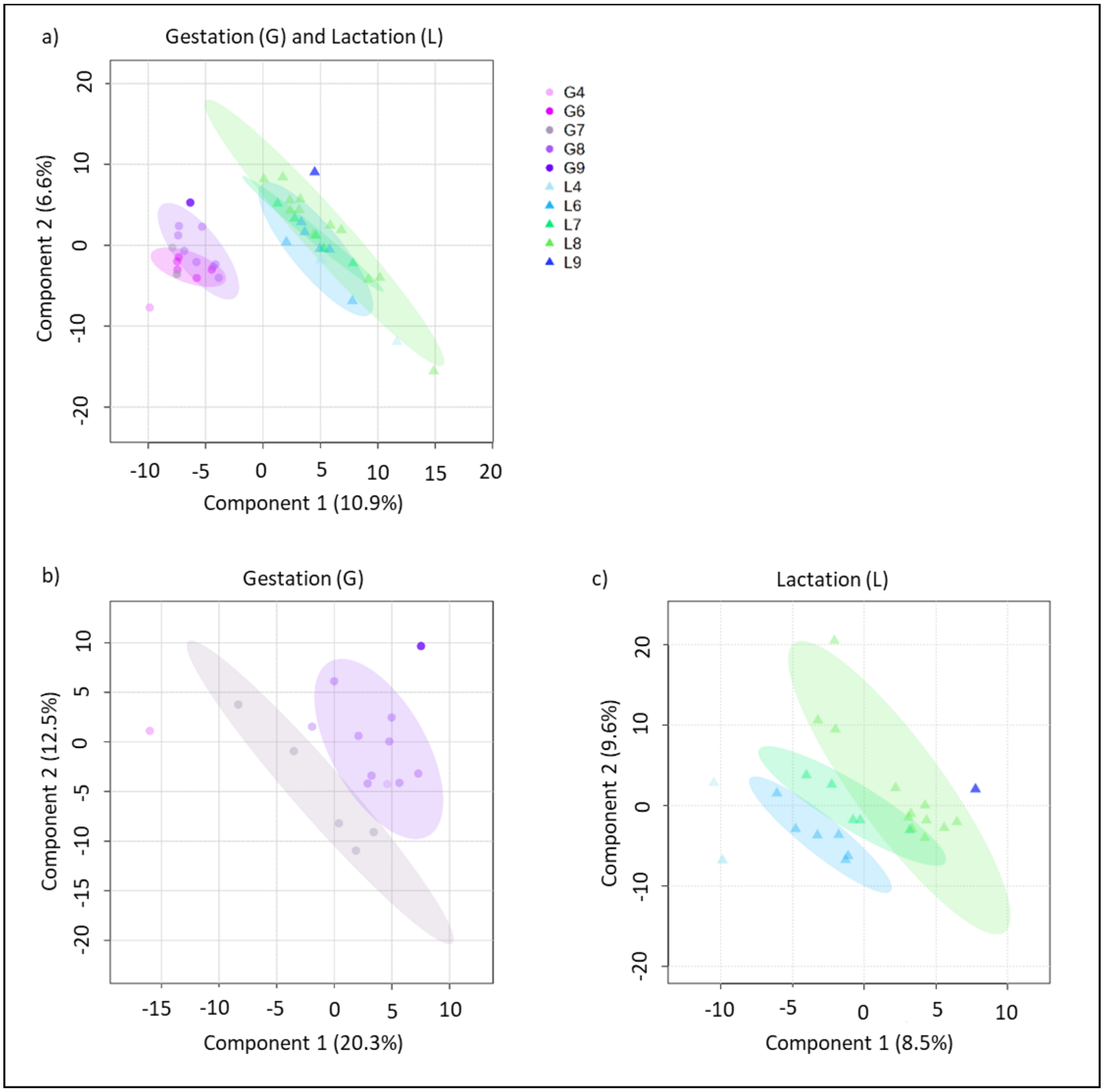

Figure 4.2. Partial Least Squares Discriminant Analysis for gestating [overall stomach score 4 (G4) to 9 (G9); circles in pink/purple] and lactating sows [overall stomach score 4 (L4) to 9 (L9); triangles in green/blue]. This shows a) differences in saliva metabolome between stages, and separation of saliva samples by overall stomach score in b) gestating and c) lactation sows. Coloured area around data points correspond to 95% confidence region. Since pseudo-replicates had to be used for statistical analysis, the variations found across the groups might not be significant.

To identify metabolic markers of disease in the saliva metabolome, we screened for features gradually increasing with the progression of the condition. We performed pathway enrichment analysis on the presence of different lesions (lesion score), that included keratinization, erosion extension, ulcer extension, and healing tissue for saliva samples taken during gestation and lactation. When keratinization was present, significant changes in primary metabolism pertaining to pyrimidine, purine, amino acid, and central carbon metabolism were identified, with the effect being most prominent in gestating sows.

During gestation, metabolites from purine metabolism [Inosine (p-value < 0.001), guanosine (p-value = 0.004), adenine (p-value = 0.004), guanine (p-value = 0.010), and deoxyguanosine (p-value = 0.045)] and pyrimidine metabolism were found to increase with the presence of keratinization [thymidine (p-value < 0.001), uridine (p-value = 0.003), cytosine (p-value = 0.008), beta-pseudouridine (p-value = 0.013), and deoxycytidine (p-value = 0.038)] (Figure 4.3). Metabolites of the tricarboxylic acid cycle were also found to increase with the presence of keratinization in gestating sows [malate (p-value = 0.010) and succinic acid (p-value = 0.014)]. Creatinine was found to increase with the presence of keratinization (p-value < 0.001) and erosion (p-value = 0.004) in gestating sows (Figure 4.4).

In addition, several oligosaccharides potentially consumed in feed or resulting from the breakdown of feed were found in the saliva of gestating and lactating sows. During gestation, these included, but are not restricted to, maltotetraose, raffinose, mannobiose, lactose, trehalose, maltulose and sophorose. Similarly, during lactation, these included raffinose, maltotriose, sophorose and sorbose, among others.

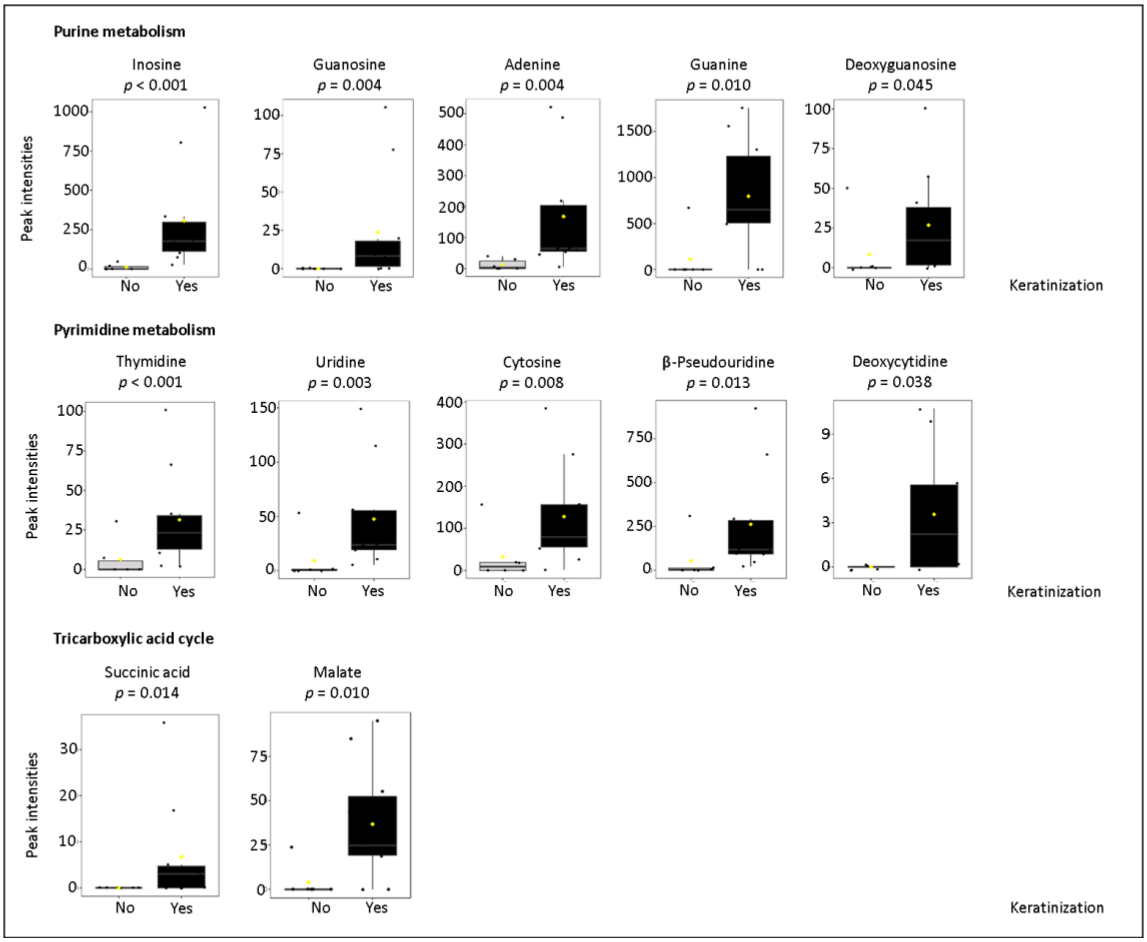

Figure 4.3. Peak intensities for features found to increase significantly ($p < 0.05$) within a metabolic pathway with the presence of keratinization in gestating sows. Numbers on these graphs are to be multiplied by 1000.

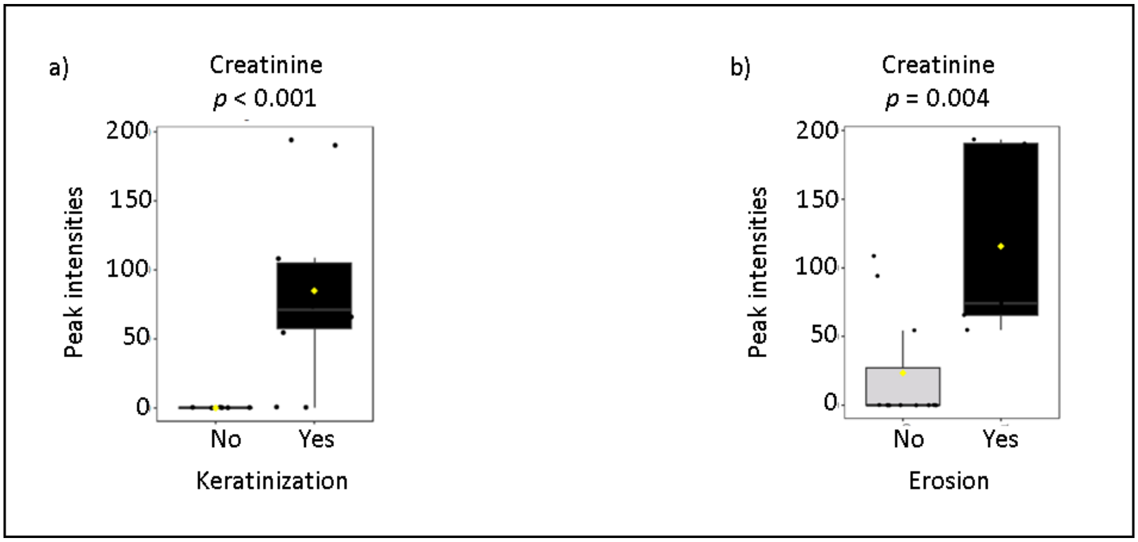

Figure 4.4. Peak intensity for creatinine in gestating sows with and without a) keratinization and b) erosion. Numbers on these graphs are to be multiplied by 1000.

The pathway enrichment analysis in lactating sows identified Aminoacyl-tRNA biosynthesis and Nicotinate and nicotinamide metabolism. Within Aminoacyl-tRNA biosynthesis, L-Histidine showed to increase with the presence of keratinization ($p = 0.040$). Within Nicotinate and nicotinamide metabolism, quinolinic acid increased with the presence of ulcers ($p = 0.003$). These are shown in Figure 4.5. It is worth noting that in lactating sows, lipoxin A4 (oxylipin) (p-value < 0.001) was found to increase with keratinization, and thromboxane B3 (p-value $= 0.006$) was found to increase with severity of erosion (Figure 4.6).

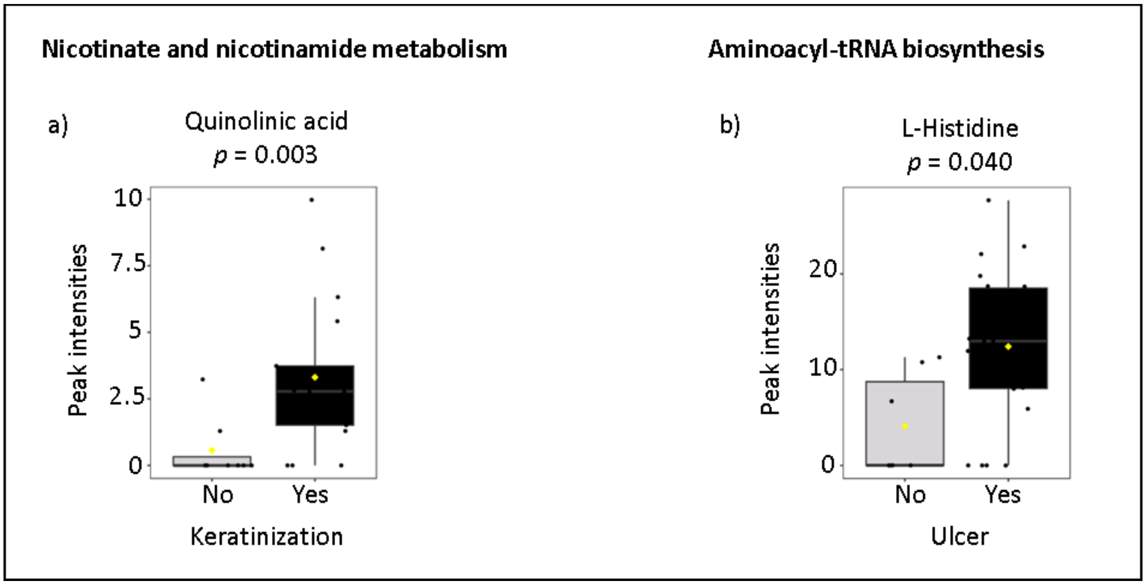

Figure 4.5. Peak intensities for features found to increase significantly ($p < 0.05$) within a metabolic pathway with the presence of a) keratinization and b) ulcer in lactating sows. Numbers on these graphs are to be multiplied by 1000.

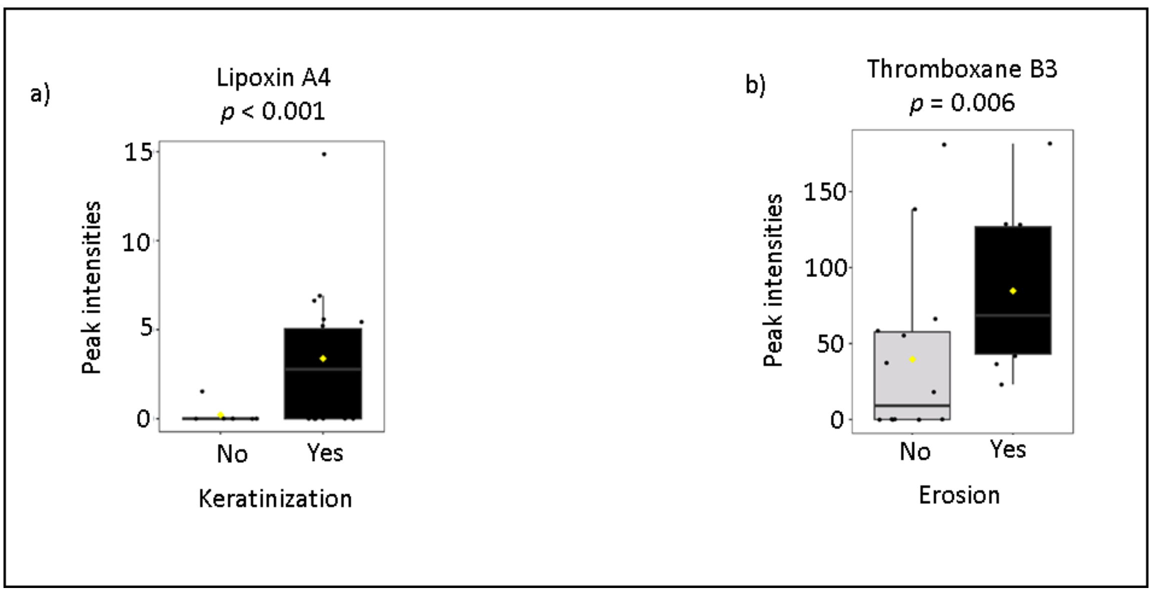

Figure 4.6. Peak intensities for features found to increase significantly ($p < 0.05$) with the presence of a) keratinization and b) erosion in lactating sows. Numbers on these graphs are to be multiplied by 1000.

4.4.7 Salivary pH of gestating and lactating sows as indicative of gastric integrity

There was no effect of the presence of lesions on the salivary pH in gestating and lactating sows. There was also no correlation between the overall stomach score and salivary pH in gestating (r_s = 0.186, p = 0.491) and lactating sows (r_s = -0.218, p = 0.284). However, when comparing salivary pH between gestating and lactating sows, the pH was significantly higher at the end of gestation (8.909 ± 0.064; Mean ± SEM) as compared to the end of lactation for the same sows (8.651 ± 0.075; t = 3.60, df = 14, p = 0.003; n = 15).

4.6 Discussion

The present study aimed to investigate whether oral behaviours and/or saliva composition and pH are related to gastric ulcers, to determine if either might be a promising basis for non-invasive diagnosis of gastric ulcers in the living pig.

4.6.1 Prevalence of gastric ulcers in lactating sows

An important finding of the present study is that it confirms existing evidence that gastric ulcers are present in sows. More than half of the sows in the current study had a gastric ulcer [65.79% vs 30% (Nielsen et al., 2013)] and none of the sows in this study had a healthy pars oesophagea. Also, the extent of ulceration increased with parity. It is worth noting that these sows were housed under relatively high welfare standards with bedding provision and stocking densities above the minimum animal welfare standards for the UK (DEFRA, 2020; Welfare of Farmed Animals (Scotland) Regulations 2010). In addition, the sows were home-bred and had access to straw bedding throughout their lives. Substrates such as straw are thought to have protective properties against development of gastric ulcers (Jensen et al., 2017), thus the extent of ulceration found is concerning. Ulcers may be painful as reported in humans (Barkun and Leontiadis, 2010) and as indicated by behavioural changes in pigs (Dybkjær et al., 1994; Hartnett et al., 2023; Peralvo-Vidal et al., 2021b; Rutherford et al., 2018). Moreover, it may be the case that pigs develop gastric ulcers throughout the production cycle, going through periods with and without ulcers (Helbing et al., 2022), deteriorating the elasticity of the pars oesophagea and in the most severe cases reducing the diameter of the entrance of the stomach making it difficult to pass feed.

4.6.2 Behaviour as predictor of gastric ulcers

The behaviours scored in this study were not good predictors of gastric ulcers, as the presence of a lesion did not affect behaviour, and the overall stomach score did not correlate with the behaviour during either gestation or lactation. None of the behaviours which had been hypothesised to be associated with ulcers were related with gastric ulcers: pig-, self- and straw-directed behaviours.

However, these results must be treated with some caution for a variety of reasons. Firstly, the sample size, and variation in stomach integrity scores, was not big enough to have all types of lesions as well as at least three repetitions for each score. Most importantly there were no healthy controls. Secondly, the rare nature of some of the behaviours such as re-directed foraging (root, nose and lick) the wall of the pen and biting trough, meant that there was a high number of zeros, which makes it more difficult to investigate whether they are predictors (or not) of gastric ulcers. Thirdly, the low representation of some of the scores or lack therefore, together with the large number of zeros in the behavioural data hindered from doing a much more complex statistical analysis that would include all the different types of lesions (and severity) into the model.

However, possibly a controlled study with a much bigger sample size and sows with a full range of ulcer scores may have been powerful enough to find an association between oral behaviours and stomach integrity. Nonetheless, for a behavioural marker to be useful under commercial conditions it must be frequent enough to be easily observed.

Given this, the behaviours scored in this study may not be a feasible method for identifying pigs with gastric ulcers.

4.6.3 Saliva composition and stomach integrity

The use of saliva metabolites as a diagnostic marker for gastric ulceration in pigs is promising. In the present study it was possible, even with a small sample size, to separate saliva samples according to the progression of gastric ulceration. Also, our results are in agreement with the only previous study exploring this hypothesis in pigs (Madsen et al., 2022). They measured metabolites in the saliva of finishing pigs receiving different diets (conventional diet in meal and pelleted, and pelleted diet with hemp hulls or hemp cake) and compared the saliva composition between pigs with ulcerated stomachs and pigs that had either healthy stomachs or signs of keratinization. They found that the latter had a higher level of oxylipins in the saliva (involved in anti-inflammatory response and pro-resolving tissue damage; Serhan et al., 2008). In our study, we found an increase in lipoxin A4 (oxylipin) with the presence of keratinization in lactating sows. Furthermore, even with differing methods of handling, processing and analysing data as well as differing study subjects as compared to Madsen et al. (2022), we found similar results.

It is noteworthy that changes in saliva composition can be observed at early stages of ulceration, which could be used for the early identification of pigs with the condition before lesions become severe. However, we need to keep in mind that in the present study pigs that had keratinization may also have had other types of lesions (erosion, ulcer and/or healing tissue). Even so, future controlled studies could compare pigs with gastric ulcers against pigs that have healthy pars oesophagea to identify early disease associated changes in saliva composition.

The website of the Human Metabolome Database (HMDB, n.d.) and the Bovine Metabolome Database (BMDB, n.d.) were used to identify possible biomarkers of gastric ulceration. Only features that had been identified in the saliva before were considered as promising for biomarkers for gastric ulceration. This was checked in both databases however the HMDB seemed to have a much more comprehensive information for the metabolites [None of the metabolites mentioned below had been detected/measured in the saliva of bovines (BMDB website)].

The metabolites that seemed the most promising according to this search were inosine, guanosine, adenine, thymidine, succinic acid and malate in gestating sows. They were found to be related with issues affecting the mouth and teeth in humans which included attachment loss of the tooth, periodontal probing depth, tooth decay, tooth alignment disorders and missing teeth. However, these metabolites were not only found in saliva but also in the urine and faeces of humans. If this is the case in pigs as well, the metabolites may have come into contact with their mouth as they tend to wet and manipulate the straw in the dunging area. Nonetheless, these features have been found in humans' faeces and urine with gastrointestinal problems, which include eosinophilic oesophagitis, gastroesophageal reflux disease, ulcerative colitis, colorectal cancer and/or irritable bowel syndrome. Hence, it seems like the above-mentioned features change with the inflammation of the digestive system. In fact, among these features, succinic acid is especially promising as it is effective at controlling intragastric pH (Chowers et al., 2012).

In the pathway enrichment analysis for lactating sows, we found only two metabolites that were increasing with the presence of a lesion. However, only one of them has been identified in saliva before (in humans; HMDB, n.d.). This metabolite was L-Histidine and increased with the presence of keratinization. L-Histidine has been related to oral cancer and periodontal diseases when found in saliva. However, it has also been found in urine of humans that suffer from eosinophilic esophagitis and/or gastroesophageal reflux disease. It is important to note that this metabolite has an anti-inflammatory role (Hasegawa et al., 2012; Niu et al., 2012).

Additional to identifying compounds coming from the saliva, compounds likely coming from the degradation of feed were also identified. Madsen et al. (2022)'s metabolomic analysis found higher amounts of different types of disaccharide, daidzen (from soybean meal) and floionolic acid (found in vegetable fats and oils) in healthy pigs and/or in pigs with hyperkeratosis as compared to pigs with active ulceration. In the present study, oligosaccharides were found in the saliva of gestating and lactating sows, and these were different.

Even though, lipoxin A4, succinic acid and L-Histidine may be promising as biomarkers of gastric ulceration it is important to investigate whether these can be found in the saliva of pigs (and do not come from urine and faeces), controlling for the presence of orodental issues and contaminants such as faeces and urine in the mouth. It is important to carefully interpret results in future studies but also to improve the sample collection and cleaning process to reduce contamination. This is important in the early stages of research to faithfully establish which salivary compounds could be related with gastric ulceration.

4.6.4 Limitations regarding the analysis of saliva composition on the present study

These results seem promising, as the saliva composition varies between stages and with changes in the condition; however, they need to be treated with caution. The sample size did not allow for enough repetitions per level of a score, and some lesion scores did not have representation at all (healthy stomachs and early stages of ulceration). The study came up as an unexpected opportunity due to need of depopulation of the breeding herd. This made optimal planning of the study more difficult but also limited the sample size to the number of animals that had to be culled. Since the present study was not planned at the beginning of my PhD studies it was impossible to get the adequate permission to cull animals as part of the research project.

Samples were contaminated with feed and there may be a chance of contamination with urine and faeces (as sows tend to wet straw in the dunging area and then chew on it). Another problem is that there is still a lot to study about the pathophysiology of the gastric ulcers as well as it is impossible to know where in the process of pathogenesis the saliva samples were taken. The latter is mostly a problem with gestating sows because of the gap between saliva collection and stomach assessment. Furthermore, there is a lot of noise regarding the study of the effect of ulcers as they do not occur alone. There may be other types of lesions such as keratinization and erosion of the tissue as well as other unidentified conditions which could be affecting behaviour and saliva composition. The sample size of sows with different types of lesions did not allow for this level of detailed analysis in the metabolomics.

These problems are somewhat inherent to the study of biomarkers in whole saliva [as in Madsen et al. (2022) and the present study]. Saliva will be contaminated with cellular debris, bacteria as well as feed and other components of the environment. This will cause changes in composition due to bacterial metabolism, and turbidity interfering with analytical techniques (Nunes et al., 2015). Hence it is important to standardise the process of collection [reviewed by Song et al. (2023)], preparation [revised by Song et al. (2023)], and analysis of the samples as well as the moment of collection. It is important to prevent and reduce contamination as much as possible, at least for early stages of biomarker research. However, the usefulness of saliva as a biological matrix for biomarker identification has increasingly been proven in humans, as reviewed by Nunes et al. (2015).

4.6.5 Salivary pH and stomach integrity

The presence of a given lesion and the overall condition of the pars oesophagea did not relate to the pH of the saliva taken during gestation or lactation. However, when testing the effect of reproductive status, the salivary pH decreased from gestation to lactation in the same sow, remaining within normal parameters (6.5 – 8.5; J. Thomson, personal communication, August 6, 2023).

The lack of effect of stomach integrity on the saliva pH during gestation and lactation could be because of the small sample size. In the present study there was no healthy control, and some type of lesions and overall stomach score did not have enough repetitions. Additionally, salivary pH is affected by other factors. In humans, salivary pH is related to the salivary flow rate (Fenoll-Palomares et al., 2004) and periodontal diseases (Baliga et al., 2013). Foglio-Bonda et al. (2017) found differences in salivary flow rate and pH between people with and without oral lesions (they do not define which types of oral lesions). Since dental issues in breeding sows are relatively common (Ala-Kurikka et al., 2019; Engblom et al., 2008; Johnson et al., 2003; Knauer et al., 2007; Malmsten et al., 2020; Tucker et al., 2011) oral lesions could have confounded the results.

Differences in salivary pH between gestating and lactating sows could be expected. The feature profile, physiological status (similar within sows of the same productive stage), feeding practices and diet differed between both productive stages. In humans the amount and composition of the saliva is affected, among other factors, by meal consumption, diet and physiological status (reviewed by Kleinberg and Jenkins, 1964; Migliario et al., 2021; Pachori et al., 2018; Schipper et al., 2007). Diet and saliva collection differed between productive stages. Although most ingredients were similar the relative amount varied between stages. Also, lactation diet included some feed ingredients: double low rapemeal (extracted), hi pro soyabean extract, distillers dark grains (maize) and monodical phosphate, which were not included in the gestation diet. The effect of these ingredients on pigs' salivary pH is unknown. Saliva collection differed in time and moment of the day with respect to feeding. Gestating sows had just been fed after a 24h period of no food while lactating sows were sampled before feed was delivered (to maintain similar collection times). Also, lactating sows are fed twice a day and some feed may remain in the feeder up until the next delivery. Finally, the difference in salivary pH could be explained by the difference in physiological status. Differences in flow rate and pH between pregnant and non-pregnant women have been found (Migliario et al., 2021).

However, the results of the current study are limited as saliva samples were defrosted and frozen again a few times, and after metabolomic analysis not all saliva samples were available for pH measurement. According to Schneyer (1956), freezing saliva will result in the formation of a persisting mucin clot that will remain after thawing. In a review by Schipper et al. (2007) it is suggested to measure pH as soon as possible after collection, however, the authors say that in their own experience storing samples at – 80°C followed by thawing did not affect the pH of their samples (They do not refer to any of their studies). Also, pH of saliva is different in different parts of the mouth (Kleinberg and Jenkins, 1964), although this might not have affected the results as all samples were taken from between the cheeks and molars as well as the molars (sows occasionally chewing on the cotton bud).

4.6.6 New scoring system for the assessment of gastric ulcers

Prior to this study, the scoring systems for the integrity of the pars oesophagea used in other studies did either not differentiate the different combination of types of lesions that may coexist in the pars oesophagea or did so but did not use that information in the analysis (Rutherford et al., 2018). Instead, they set a score according to the most severe lesion present in the par oesophagea. For example, with the overall stomach score used here, a score of 6 (ulcer taking less than 10% of the pars oesophagea or presence of scab) may have healing tissue and/or ulcer as well as it may also have or not erosion and/or keratinization. On the present study, the overall stomach score did not correlate with the parity number, but the presence and extension of an actual ulcer did. This because the sows in this study that were scored as having an overall stomach score from 6 to 8 (different level of ulcer extension and/or healing tissue) did so because of the presence of healing tissue.

The system developed here which scores each aspect separately allows to study the effect of each type of lesions as well as the extension and severity of them on the pig. The importance of the new more detailed scoring system developed here is that it will allow for a clearer study of predictors and risk factors for the different types and stages of lesion as the results will not be confounded by the presence of other lesions. The scoring system developed here is proposed for use by future researchers.

4.6.7 Future research

For the study of the relationship between gastric ulcers and saliva composition, future research should include the assessment of the health of the mouth and oesophagus as well as any other lesion found in the rest of the stomach. This is because saliva has a role in maintaining the health of the oesophagus (Kongara and Soffer, 1999; Sreebny, 2000) and lubricating the mouth (Pedersen et al., 2018). It is also important to standardize a method of saliva collection (including reducing the contamination of samples) so that results can be compared between studies. Finally, it would be helpful at this stage to do a larger scale study that would involve the assessment of gastric ulcers in the abattoir, saliva sampling before slaughter, feed analysis (ingredients and particle size) and a study of the farming conditions of the sow. This study could involve only one production stage (gestating sows) and more animals, and, if possible/necessary, the manipulation of risk factors (e.g. small and large particle size) to ensure a higher variation in the lesions observed in the stomach.

4.7 Conclusion

This study confirms the presence of gastric ulcers in lactating sows. Also, gastric ulcers may be a much bigger animal welfare problem than previously thought, as none of the sows showed a healthy-looking pars oesophagea. Re-directed oral behaviours were not related to gastric ulceration in this study. Finally, the salivary metabolome seems to be a promising method of diagnosis for gastric ulcers in pigs. Further studies need to be done in this area.

4.8 References

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Chapter 5 How do re-directed oral behaviours vary in gestating sows: a systematic literature review

5.1 Abstract

Background: Re-directed oral behaviours commonly observed in feed-restricted gilts and sows in barren environments, remain present when sows are fed *ad libitum* or diets high in fibre, and are observed in younger pigs that are routinely fed *ad libitum*. No systematic literature review has been done on the variation of hunger-related oral behaviours by diet composition and feeding practices, housing type and environmental enrichment. ***Objectives***: This systematic literature review explores how hunger-related oral behaviours vary with diet composition and feeding practices, housing type and environmental enrichment, to identify results that do not accord with the 'chronic hunger' theory. ***Data sources***: The search was done using Web of Science Core Collection database. Reference lists of the selected papers were not screened to identify more papers. ***Subjects, interventions, and study eligibility criteria***: Subjects were gestating gilts and sows, and interventions were diet, feeding practices, housing and environmental enrichment. Only peer-reviewed original research articles published in English or Spanish were included (n = 30), excluding any article that did not define the behaviours assessed and/or the design was not clear enough. ***Data extraction***: A Microsoft Excel spreadsheet was created with pre-defined categories for data extraction. In some cases (5/30), the author of the article was contacted for clarification (when possible). For comparisons between treatments and control, percentage of change was calculated when possible, otherwise the direction of effect was noted. ***Synthesis methods***: Behaviours were standardised by using umbrella terms as much as possible and then grouped into behaviour categories for the description of the data. Only

statistically significant ($p<0.05$) behaviour changes (main effects) or interactions were included. Results that apparently did not comply with the 'chronic hunger' theory were explored. Meta-analysis was not possible due to the wide variety of studies and methods of data collection. ***Results***: Almost all of the articles (29/30 articles) included in this systematic literature review can be explained by the 'chronic hunger' theory or housing environment. The exception was one study (ID 158) where three housing types were tested. These were different in the number of animals housed, pen and type of feeder. A collective pen with a protected feeder showed an increase in drinker interaction only observed after two weeks of observation (but not at the beginning of the study) as compared to the control group (conventional stall housing). There is no clear explanation of why this could have happened in the article. ***Limitations***: Relatively small sample size, differences in how behaviours were described and recorded, variation in the factor tested, and different housing environment were the main limitations of this review. This made it impossible to do a meta-analysis. Also, all studies were planned to test re-directed oral behaviours under the 'chronic hunger' theory. This means that study design and treatments were set up and described to explore chronic hunger. This makes it difficult to draw any clear conclusion. ***Conclusions and implications of key findings***: This systematic literature review offers support for the 'chronic hunger' theory as a cause for oral re-directed behaviours. It does not show evidence for possibility of alternative explanations. Since studies did not include enough information to test alternative explanations, further research is needed.

5.2 Introduction

Re-directed oral behaviours (e.g. sham chewing, manipulating pen fixtures, adjunctive drinking) are very common among feed-restricted gilts and sows housed in barren environments [reviewed by D'Eath et al. (2018)]. These behaviours are traditionally thought to develop due to unsatisfied behavioural and nutritional needs, referred to in this paper as 'chronic hunger' theory (Appleby and Lawrence, 1987; Terlouw et al., 1991a). Usually, the addition of fibre to the diet, provision of appropriate foraging material and/or increasing the feeding level reduces the performance of re-directed oral behaviours in gestating gilts and sows (Appleby and Lawrence, 1987; Brouns et al., 1994; Whittaker et al., 1999).

However gilts and sows continue to show some level of re-directed oral behaviours even after being fed '*ad libitum'* (Alvas, 2018), and when completely *ad libitum* (high fibre diet) (Brouns et al., 1994; de Leeuw and Ekkel, 2004). These behaivours are also observed in younger pigs that are routinely fed *ad libitum* (Dubarry, 2019; Chapter 2 and 3). Remaining re-directed oral behaviours in gilts and sows may correspond to emancipated oral stereotypies and/or relate to still inadequate diet in terms of quantity, fibre and energy content. However, this is unlikely to be the case in younger pigs as they are fed *ad libitum* and were provided with fresh straw every day. As proposed before, some behaviours may not be related to hunger and may be explained by other factors. Manipulative behaviour are likely to correspond to the 'normal' repertoire of exploratory behaviour in pigs. However, self-directed oral behaviours might be more difficult to explain when feed is provided *ad libitum*.

There is some evidence that re-directed oral behaviours in pigs could be associated with inadequate husbandry practices related to housing and diet (see section 3.5.2 and 6.6), and conditions affecting the upper digestive system (see section 6.6). Therefore, a systematic literature review of the literature on the effect of different factors on the performance of re-directed oral behaviours is needed to investigate whether previous studies done in feed-restricted gestating gilts and sows may show inconsistent results with the 'chronic hunger' theory.

No systematic literature review has been done to investigate the variation of hunger-related oral behaviours in gestating gilts and sows. Although valuable reviews have been done to study the effect of fibre content on behaviour (D'Eath et al., 2018, 2009; de Leeuw et al., 2008; Meunier-Salaün and Bolhuis, 2015; Tatemoto et al., 2022), these do not included factors such as type of housing, presence of enrichment and feeding practices. These previous reviews explored the effect of fibre with regards to hunger and the physiology of feeding behaviour (D'Eath et al., 2009), the effect on the general welfare of sows and piglets (D'Eath et al., 2018), the variation of behaviours with bulkiness and fermentability of the diet (de Leeuw et al., 2008), and on reproductive performance in sows (Meunier-Salaün and Bolhuis, 2015), and recently on the long-term effect of stereotypies on the sow and offspring (Tatemoto et al., 2022). There are some systematic literature reviews on adjacent topics; non-feeding oral behaviour in cattle (Ridge et al., 2020), and the risk of development of stereotypies in ungulates (Lewis et al., 2022).

In this review five types of re-directed oral behaviours are identified as affected by hunger in pigs from how authors tend to group these behaviours. These are i) adjunctive water drinking, and oral behaviours directed towards ii) pen fixtures, iii) ground, iv) pig and v) self. Generally, according to the 'chronic hunger' theory (D'Eath et al., 2018), all factors that improve the feeling of satiety (increasing fibre and/or energy content of the diet and/or increasing feed quantity, and the provision of edible substrates) will decrease the performance of these behaviours as the animal will be less motivated for feed and hence appetitive and consummatory foraging behaviours should be reduced (see section 1.3 and 1.3.1). Non-specific factors that cause behavioural arousal (e.g. loud noises; or anticipation of feeding) may also increase the performance of re-directed oral behaviours (Lawrence and Terlouw, 1993). However, it is difficult to predict which behaviour will be affected and how, as it will also depend on what is available to the pig to interact with, and time spent being feed-restricted (Jensen, 1988).

This systematic literature review aims to explore closely how re-directed oral behaviours vary in response to diet, feeding practices, housing and environmental enrichment in gestating gilts and sows. I also aimed to identify studies where the direction of the effect on re-directed oral behaviours is not consistent with the 'chronic hunger' theory. This would provide evidence that there might be an alternative explanation (different from chronic hunger) for the occurrence of re-directed oral behaviours in gestating sows.

5.3 Methodology

The literature was reviewed systematically following the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses; Moher et al., 2009), which explain the steps to achieve an unbiased literature review. The steps and number of articles excluded are summarized on a template taken from PRISMA (2020) and can be find in Figure 5.1.

5.3.1 Literature eligibility criteria

Only original research articles from peer-reviewed journals written in English or Spanish were included, and reviews, conference publications were excluded. Also, studies that were not clear in their study design and/or did not define the behaviours were not included. Only studies that investigated the effects of diet, feeding practices, housing and environmental enrichment on the performance of re-directed oral behaviours in gestating gilts and sows were included. No limit was set as regards to the year of publication. All database searches were completed prior to 28th of November of 2022.

5.3.2 Information sources

Studies were retrieved through Web of Science Core Collection. Reference lists of the selected papers were not screened to identify more papers as these should have appeared in the initial search. This decision was also made due to time constraints.

5.3.3 Search strategy and selection of publications

Search terms were selected from titles, abstracts and author keywords from a number of relevant articles as well as from the database's keywords (Web of Science Core Collection) and expanded from there. These terms were categorized according to the PICO (population, intervention, comparator, and outcome) framework. For this study only 'population' and 'outcome of interest' were used for term selection (Table 5.1). This decision was made to avoid limiting the search by certain factors. Web of Science Core Collection automatically includes both American and British spelling in its searches, so it was not necessary to run separate searches for both spellings.

Table 5.1. Description and keywords according to PICO (population, intervention, comparator, and outcome) framework.

Category[1]	**Description**	**Keywords**[2,3]
Population	Gestating gilt/sow	Sows, gilts, pigs, nulliparous sows/gilts, pregnant sows/gilt, swine, gestating sows/gilts, adult female pigs
Outcome of interest	Oral behaviours	Oral behaviours, re-directed oral behaviours, oral stereotyp*, stereotyp* behaviours, sow-stereotyped behaviours, stereotyp*, adjunctive drinking, abnormal behaviours

[1]Only 'population' and 'outcome of interest' were used for term selection to avoid limiting the search by certain factors.
[2]The asterisk substitutes different endings the word may have for inclusion in the search e.g. the term stereotyp* will include stereotypy, stereotypies, stereotypical.
[3]Web of Science Core Collection will include both American and British spelling.

To select the best search string, three options of search strings were trialled. For each of these three strings a preliminary search was done, and the list of reference was screened. During this process, only the first 30, mid-30 and last 30 articles found with each of the search strings were screened by looking at the titles, according to the eligibility criteria described above. The best string was selected according to the percentage of relevant articles retrieved. The search string for the present study is described in Table 5.2.

1Table 5.2. Search string used in 'Web of Science Core Collection' to find articles included in the present study. It includes terms for 'population' AND 'outcome of interest' (PICO)

(Sow$ OR gilt$ OR pig$ OR "nulliparous sow$" OR "nulliparous gilt$" OR "pregnant sow$" OR "pregnant gilt$" OR swine OR "gestating sow$" OR "gestating gilt$" OR "adult female pig$") AND ("Oral behaviour$" OR "re-directed oral behaviour$" OR "oral stereotyp*" OR "stereotyp* behaviour$" OR "sow-stereotyped behaviour$" OR stereotyp* OR "adjunctive drinking" OR "abnormal behaviour$")

For the search, the following options were selected on the 'Web of Science Core Collection': 'Science Citation Index Expanded (SCI-EXPANDED) --1900-present', 'Social Sciences Citation Index (SSCI) --1900-present', 'Conference Proceedings Citation Index- Science (CPCI-S) --1990-present', 'Book Citation Index– Science (BKCI-S) --2005-present' and 'Emerging Sources Citation Index (ESCI) --2015-present'. The terms were searched in the title, abstract, author keywords, and Keywords Plus (option 'topic').

Search with this search string generated 526 publications on Web of Science Core Collection (Figure 5.1). The first screening was done by only looking at the title (Identification, Figure 5.1). This identified studies that were duplicated and titles in a language other than English or Spanish (n = 9). On a second screening, articles were assessed for type of record and whether they were related or not with the research questions by looking at the title and abstract as well as the characteristics of the article (Screening, Figure 5.1). This left a list of 67 articles to be retrieved. These articles were screened a third time during data extraction. At this stage, reports were excluded due to being unrelated to the topic, having an unclear description of the study design, not describing the behaviours or the document being blurry and difficult to read (n = 37). This left a total of 30 articles to be included in this systematic literature review (Included, Figure 5.1).

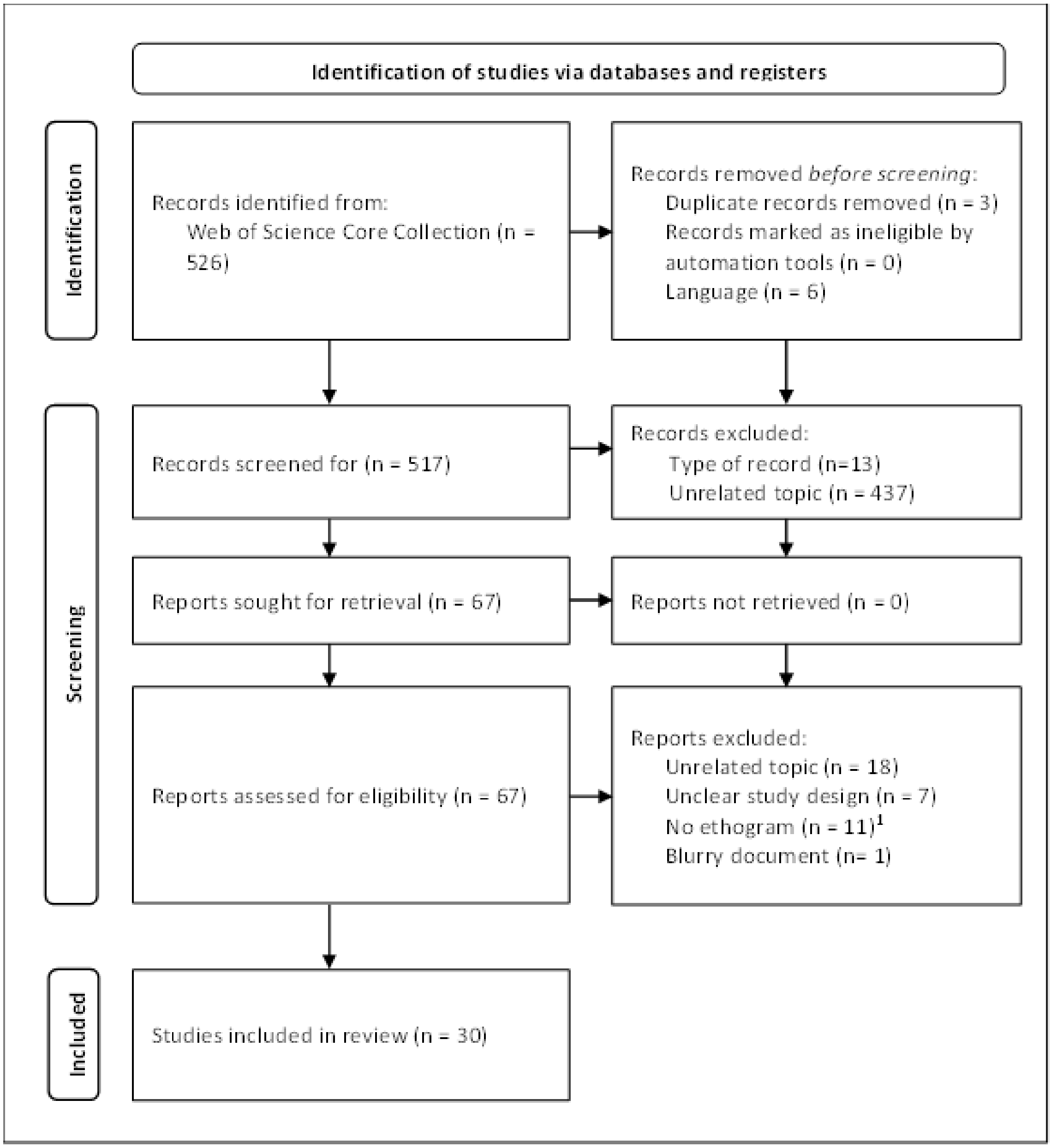

Figure 5.1. Description of the screening process and number of articles excluded/included in this systematic literature review. Template taken from PRISMA (2020). 1One of them had an ethogram but it was described in Portuguese while the article was written in English (Study ID 46).

5.3.4 Data collection process

For data extraction a template on a Microsoft Excel spreadsheet was created with categories to capture key details about the study. The template included the following information: general characteristics of the study (authors, title, factors being tested, location), study design (number of animals, type of animal, parity number, housing type, treatment description, statistical analysis, control and treatment group), detailed information about the treatments [housing (type), environmental enrichment (type, amount, location in the pen), diet (main ingredients, fibre level, energy level) and feeding practices (frequency, location in the pen)], and information about the method of behavioural observation (method, unit of measurement, time) and the effect on the behaviour [percentage of change (where available), or increase/decrease or no effect]. The data was extracted so that each row corresponded to the comparison between the control and a treatment group within each study, meaning that each study could have more than one row, depending on the number of treatments and comparisons. The data was extracted by one person and on 5 occasions, clarification was sought from the author of the paper when the article was not clear. The list of articles included, and the numerical ID assigned to them here can be found in annexe section 5.7 (Table 5.8).

5.3.5 Bias assessment of the selected papers

Bias of the selected articles was assessed by checking whether or not animals or group of animals were assigned randomly to the treatments. This was checked by looking for word relative to randomization within the material and methods section of the articles. As a result, 46.67% of the articles made specific mention of using randomization (see section 5.4.1.3).

5.3.6 Standardising behaviours under umbrella terms and behaviour categories

Behaviour categories, umbrella terms and behaviours straight from the article are described in Table 5.3. The studies included in this review differed in the behaviours included, the definitions of those behaviours, and the way in which the behaviours were grouped together. To be able to compare the results between studies with similar treatments, behaviours were regrouped into standardised umbrella terms as much as possible. Studies 243 and 264 measured sham chewing and floor interaction differently depending on whether the study group had substrate or not. For these studies, two additional umbrella terms were created for the group that was provided with substrate: 'Interaction with substrate and/or Floor interaction' and 'Chewing movements'. These umbrella terms included interaction with the floor and substrate, and sham chewing and chewing on something, respectively.

For most studies it was possible to re-group the behaviours into the umbrella terms, however, this was not possible for all the behaviours in all studies. Some studies regrouped their behaviours in a unique way that made it difficult to create an umbrella term. Additionally, some studies recorded more than one behaviour that could have been included into an umbrella term. For instance, in study ID 240, they recorded the following behaviours: 1) 'Jaw stretching' and 2) 'Tongue-playing, Sucking and Teeth-grinding and Sham-chewing'. According to the umbrella terms in Table 5.3, both behaviours should be named as 'Self-directed' behaviours. However, since we do not have the dataset for each of the studies it is not possible to add them together to create one behaviour that would go into the umbrella term.

The umbrella terms were grouped into behaviour categories. This was to facilitate the description of the results. Also, to solve the problems mentioned on the previous paragraph, behaviours that could not be fitted into any of the umbrella terms, were included within the behaviour categories when possible. Similarly, when there was more than one behaviour within a study that fit into the same umbrella term, these were added into the behaviour category directly. Behaviour categories will be capitalized, and umbrella terms will be underlined throughout the whole text. Behaviours that could not be included in any umbrella term will be mentioned between quotation marks.

Some behaviours had to be excluded: 1) behaviours that were rare, 2) behaviours that were not defined, and 3) umbrella terms that were included in less than three studies and could not be included in the behaviour categories.

Table 5.3. Behaviour categories (BC) and standardised umbrella terms created for this systematic literature review and their description. These were created to be able to compare studies (as they varied in the behaviours scored and description). Behaviours in grey are behaviours taken directly from an article e.g. not included in any umbrella term.

BC	Umbrella terms	Description
FIXTURE	Bar biting	The sow is only interacting with the bars. The definition includes biting. The original definition may have also included other behaviours such as licking, rubbing, nibbling the bars etc. Bars will be any fixed metal structure in the pen and sow needs to be able to make chewing movements on it. This may include structures such as the bars by the feeder, fence and gate.
	Chain manipulation	The sow is only interacting with the chain and it includes any type of interaction e.g. bite, chew, nose, etc
	Chain + bar manipulation	The sow is interacting in any way with chain and/or bar or fixed metal structure in the pen.
	Interaction with feeder	The sow is only interacting in any way with the empty feeder
	Resource-directed	This includes the interaction in any way with resources* available in the pen (except for food and substrate)
	Drinker manipulation	The sow is touching the drinker in any way. The definition does not include ingesting water.
	Object biting (304)	Chewing or biting any part of the stall, feeder or water bowl
	Fixture-related (431)	Standing or sitting; nosing, rubbing, licking or biting fixtures, other than chains or nipple drinkers
FIXTURE + SELF		This includes the interaction in any way with any of the resources available in the pen (except for food and substrate) and any of the self-directed behaviours described here.
DRINKING	Actual drinking	The definition explicitly says the sow is ingesting water, and only this i.e. it does not include manipulation of the drinker or that the animal is apparently drinking water.
	Apparent drinking	The definition clearly states that the animal may have or may not have been ingesting water. The definition does not include manipulation of the drinker.
	Apparent drinking + actual drinking + manipulation	The sow was ‘drinking apparently’ or ‘actually drinking’ or ‘manipulating the drinker’ (definitions of each above)

* Resources included floor, feeder or trough, bars, wooden separations of the pen, stall, drinker or water bowl, walls, faeces, chain, or any other resources different to food and substrate

Cont. Table 5.3. Behaviour categories (BC) and standardised umbrella terms created for this systematic literature review and their description. These were created to be able to compare studies (as they varied in the behaviours scored and description). Behaviours in grey are behaviours taken directly from an article e.g. not included in umbrella term.

BC	Umbrella terms	Description
GROUND	Interaction with substrate	The sow is interacting in any way with substrates provided as enrichment or bedding in the pen i.e. straw, wood shavings, wood chips, hanging chains in feeder[1]
	Floor interaction	The animal is interacting in any way with the floor and only the floor, not including loose substrates which includes any material that was provided as an enrichment treatment e.g. straw, wood shaving. The original definition may have also included interacting with slurry
	Interaction with substrate and/or Floor interaction[2]	The animal could have been interacting with the floor and/or substrate. See description for 'Interaction with substrate' and 'Floor interaction' for more detail.
	Rooting floor (511)	Snout touches the ground followed by head movements
	Licking floor (511)	Tongue touches the floor and is followed by movements with the head
	Interacting with mats (511)	Snout or tongue touches mats followed by head movements
SELF	Sham chewing	The animal is making chewing movements without anything in their mouth, although it may include chewing movements on things. The sow may or may not have saliva. One study also included the sows making a sucking noise into this behaviour. Another study also included sham chewing while sniffing the substrate
	Chewing movements	This included sham chewing and chewing on something else.
	Self-directed behaviours	This includes tongue playing, sucking, teeth grinding, sham chewing, jaw stretching and/or head waving
	Jaw-stretching (240)	Mouth opening and closing while stretching the lower jaw for several seconds
	Self directed behaviours (240)	Tongue-playing, Sucking and Teeth-grinding together with Sham-chewing
FIXTURE + PIG	Resource-directed + themselves	This includes interaction in any way with any of the resources available in the pen (except for food or substrate) and manipulation of themselves
	Resource-directed + pig-directed	This includes interaction in any way with any of the resources available in the pen (except for substrate and food) and penmates

* Resources included floor, feeder or trough, bars, wooden separations of the pen, stall, drinker or water bowl, walls, faeces, chain, or any other resources different than food and substrate.

[1]Hanging chains are considered substrate here as they are used for stimulating foraging behaviour in the pigs in the study.

5.3.7 Calculation of percentage of change between control and experimental groups

Change of a given behaviour for the treatment group was expressed as a % change relative to the same behaviour reported for the control group. This resulted in a standardised unitless comparable measure, unaffected by the observation method or data summary method used by the particular study. When the p-value was reported but where the difference lay was not reported, the pair of means that had the biggest difference were considered to be significantly different.

5.3.8 Classification of the studies

Studies were classified into categories according to the main husbandry practices that was investigated (Table 5.4). Most studies included a combination of these categories e.g. 2 x 2 factorial design. Also, some studies could not be fitted into these categories as factors could not be separated. Study ID 240 investigated the effect of diet and feeding practices (restricted concentrate vs ad lib high fibre diet). Similarly, study ID 158 compared conventional stall housing with pen group housing which also had differing feeders. Study 431 and 462 studied loose housing against tethered housing differing the possibility of interaction (restricted interaction with neighbours vs group housing).

Table 5.4. Classification of the studies according to main husbandry practices being investigated. Category of the study and description is presented here.

Category	**Description (type of factors)**
Diet	These studies include fibre level, energy level, feeding level + fibre level, fibre type and/or fibre level
Feeding practices	These studies include delayed feeding, feed level, feeding frequency, place of feed, texture
Enrichment	These studies include substrates which were hanging chain in the feeder[1], dust-free wood chips, straw, wood shavings, lucerne hay, wood
Housing	These studies include group housing, loose housing, space allowance

[1]Hanging chains are considered substrate here as they are used for stimulating foraging behaviour in the pigs in the study.

5.4 Results

5.4.1 Description of the articles included

5.4.1.1 Study demographics

Study ID and authors and year of publication as well as study demographics are described in Table 5.5. More detailed information about the articles included can be found in annexe section 5.7, Table 5.8. Regarding productive stage, in which the study was performed, this was not clearly reported for study ID 136, 243, 264 and 325. From the 30 studies included in this review, 43.33% started the experiments with gilts (parity = 0), 53.33% with only sows (parity ≥ 1), and 3.33% with both gilts and sows. There was one study (Study ID 136) where the sow age (18 months) but not the number of parities was reported and is estimated to be parity-2 sows. The housing used were either collective pens (36.67%), individual pens (6.67%), individual stalls (30%), collective pen vs individual stalls (10%) or tethered stall vs collective pen (6.67%). The remaining 10% of studies (3/30 studies) were testing a variation of the standard housing systems: standard vs width-adjustable stall vs stall with access to a small-shared area (ID 97); individual stall vs collective pen with trickle feeding vs with unprotected electronic feeder (ID 158); and individual stall with standard feeder vs with feeder + removable hanging chain (ID 358). The studies included on average (± SD) 57.60 (± 43.43) animals (range between 7 and 180 animals). Around half of the studies were performed in Europe (UK, Netherlands, Denmark, France and Spain; 56.67%), and 30% were performed in North America (Canada and USA). The remaining 13.13% were performed in China (3/30 studies) or Brazil (1/30 studies).

Table 5.5. Authors and year of publication, location, housing type, and type and number of animals used in the studies included in this systematic literature review

ID	Authors (year)	Location	Housing type	Type of pig	Parity number*	N**
97	Salak-Johnson et al. (2015)	USA	Standard or modified stall (Space)[1]	Gilts/sows	0 - 4	96
98	Jenset et al. (2015)	Denmark	Collective pen	Sows	2	30
112	Zhou et al. (2014)	China	Collective pen or individual stall	Gilts	0	28
135	Li et al. (2013)	USA	Collective pen or individual stall	Sows[2]	1 - 2	80(27)
136	Souza da Silva et al. (2013)	Netherlands	Collective pen	Sows	~ 2[3]	16
144	Stewart et al. (2013)	UK	Collective pen	Sows	3 (± 2)	128
155	Stewart et al. (2010)	UK	Collective pen	Sows	5 (± 3)	112
158	Chapinal et al. (2010)	Spain	Individual stall vs collective + feeder[4]	Sows	1 to 9	180
208	Holt et al. (2006)	USA	Individual stall	Sows	3.6 (1 to 6)	239(68)
217	de Leeuw et al. (2005)	Netherlands	Collective pen	Gilts	0	108
240	Zonderland et al. (2004)	Netherlands	Collective pen	Gilts	0	40(36)
243	de Leeuw and Ekkel (2004)	Netherlands	Individual pen	Gilts	0	96
257	van der Peet-Schwering et al. (2003)	Netherlands	Collective pen	Gilts	0	109
264	de Leeuw et al. (2003)	Netherlands	Individual pen	Gilts	0	96
269	Bergeron et al. (2002)	Canada?	Individual stall	Sows	> 1	7
277	Robert et al. (2002)	Canada?	Individual stall	Gilts	0	24
304	Bergeron et al. (2000)	Canada	Individual stall	Sows	4	21
312	Haskell et al. (2003)	UK	Individual pen	Sows	2 to 10	24

* Parity number of the animals when data collection started

** Number in brackets is the number of animals scored when a subsample of the total number of animals was observed

[1]Standard vs width-adjustable stall vs stall with access to a small-shared area

[2]Gilts and parity 1 sows were exposed to the treatments for a whole gestation period. Data was collected during the following gestation only.

[3]Parity number is not reported on this study, but their age (18 months of age)

[4]Individual stall vs collective pen with trickle feeding vs with unprotected electronic feeder

Cont. Table 5.5. Authors and year of publication, location housing type, and type and number of animals used in the studies included in this systematic literature review

ID	Authors (year)	Location	Housing type	Type of pig	Parity number*	N**
325	Ramonet et al. (1999)	France	Individual stall	Sows	5 ± 0.5	12
330	Whittaker et al. (1998)	UK	Collective pen	Sows[5]	1	48
358	Bergeron and Gonyou (1997)	Canada	Standard or modified stall (feeder)[6]	Sows	2	24
409	Spoolder et al. (1995)	UK	Collective pen	Gilts	0	96
420	Brouns et al. (1994)	UK	Collective pen	Gilts	0	54
431	Terlouw and Lawrence (1993)	UK	Tethered stall or collective pen	Sows[7]	From 1 to 2	32
433	Robert et al. (1993)	Canada	Individual stall	Gilts	0	102
445	Arellano et al. (1992)	USA	Collective pen or individual stall	Gilts	0	40
462	Terlouw et al. (1991)	UK	Tethered stall or collective pen	Gilts	0	32
506	Deng et al. (2021)	China	Individual stall	Sows	4.87 ± 1.32	84(24)
511	Bernardino et al. (2021)	Brazil	Collective pen	Gilts	0	28
520	Li et al. (2022)	China	Individual stall	Sows	3	30

* Parity number of the animals when data collection started

** Number in brackets is the number of animals scored when a subsample of the total number of animals was observed

[5]They entered the study at parity 0 however behavioural observations were done during parity 2

[6]Individual stall with standard feeder vs with feeder + removable hanging chain

[7]They entered the study at parity 0 however behavioural observation was done during parity 2 and 3

5.4.1.2 Behavioural recordings

The type of recording method and category of behaviours included in each study can be found in annexe section 5.7, Table 5.9. Studies were classified according to whether behavioural observations were made from a video or live, and the sampling rule that was used (scan or continuous). Continuous observations for the purposes of this review also include when behaviours have been recorded for a period of time by intervals e.g. for 5 min every 20 min. Most studies included live scan observations (53.33% of the total studies). There were also studies that recorded behaviour by video continuous (23.33%), video scan (16.67%) and live continuous observations (16.67%). These methods could be found alone or in combination in one single study. However, most studies included only live scan observations (46.67%). There was one study (Study ID 144) where it was not clear whether the authors performed live or video observations.

Studies varied in the type of behaviours and how they were described (see section 5.3.6). The umbrella terms that were the most recorded were <u>resource-directed behaviours</u> (56.67%), <u>sham chewing</u> (50.00%), <u>drinker manipulation</u> (23.33%), <u>self-directed behaviours</u> (23.33%) and <u>chain manipulation</u> (23.33%). There were another two studies (Study ID 243 and 264) where they measured sham chewing as chewing movements which included sham chewing and chewing on substrate.

5.4.1.3 Experimental design, type of studies and factors tested

Experimental design and treatments can be found in annexe section 5.7, Table 5.10. There were two types of experimental design: quasi-experimental, defined as studies that aim to evaluate interventions but that do not use randomization, and randomized controlled trials. Studies included in this review were either quasi-experimental studies (53.33%) or randomized controlled trials (46.67%). Quasi-experimental studies were included in this review because of the low number of articles that passed the criteria.

Studies involved 3.38 ± 0.78 treatments (mean ± SD). Most studies included in this review investigated the effect of the characteristics of the diet (33.33%), feeding practices (33.33%) and/or environmental enrichment (26.67%). The effect of housing was studied only in 20% of the studies. There were three studies where the effect of factors could not be separated. Study ID 240 investigated the effect of diet and feeding practices (Feeding level + Fibre level) however because of the characteristics of the treatment (restricted concentrate vs *ad libitum* high fibre diet) it was not possible to separate the effects. Study 462 investigated the effect of loose housing (tethered vs not tethered housing) by comparing tethered sows with loose-housed sows in groups of four in a pen [Loose housing (+Group)]. Study 158 compared three housing types differing in the amount of pigs and type of feeder (Group housing + feeder). The most frequently investigated factors were feeding level (23.33%), provision of substrate (26.67%) and level of fibre included in the diet (56.67%). Substrate included straw, dust-free wood chips, wood shavings and removable chains hanging above feeder. The latter was classified as substrate since the authors used them as a source to stimulate foraging behaviours.

5.4.2 Description of the variation in re-directed oral behaviours in gestating gilts and sows

A summary of the effect of main factors (see section 5.4.2.1) and interactions (see section 5.4.2.2) on the behaviour categories are shown in Table 5.6 and 5.7, respectively. The behaviour categories include umbrella terms as well as behaviours (straight from the articles) that could not be included under any of the umbrella terms due to their unique description. When more than one umbrella term or behaviour under the same behaviour category were tested for a given factor, if any of them was affected then the table shows an effect for the whole category whether or not the rest of the umbrella terms or behaviours remained unaffected. There were never contradictory effects.

5.4.2.1 Directions of effects that may not be possible to be explained by the 'chronic hunger' theory: Results and discussion

A summary of the effect and direction of effect of main factors on behaviour categories are shown in Table 5.6. Slightly less than half of the comparisons showed an effect (42.05% vs 57.95% of the total). In 83.19% of the significant comparisons the direction of effect was as expected by the 'chronic hunger' theory. In the following paragraphs the remaining 16.81% comparisons (highlighted in Table 5.6) that apparently show an unexpected direction of the effect are discussed. Concerned comparisons will be explored further under the 'chronic hunger' theory and characteristics of the study set up and housing environment. This will be done in order according to the list of factors in Table 5.6.

Feeding frequency was expected to increase re-directed oral behaviours. It was expected that the ingestion of feed (without satiation) would maintain feeding motivation leading to post-feeding foraging behaviours that in a barren environment will be expressed as re-directed oral behaviours (Terlouw et al., 1993). However, this was not observed in study ID 208. They assessed the effect of fibre content and feeding frequency (once or twice a day) on individually stall-housed sows (parity 1 to 6). When comparing feeding frequencies, a decrease in FIXTURE + SELF (This included 'sham chewing, bar biting, and nosing the floor and feeder') was observed with an increase in feeding times around feeding time at day 40 and 80 of gestation (17.75 and 18.5% decrease). However no effect was observed at weaning or when observed in a 24-h period (during weaning, day 40 and 80 of gestation). The authors explained that although sows received the same amount of food, sows fed twice a day spent more time feeding than sows fed only once. It is suggested that sows fed twice might have spent some time interacting with the feeder while still eating but that this was recorded as part of feeding. It may therefore be possible that the decrease in FIXTURE + SELF could be due to mis-recording of interaction with the empty feeder as feeding behaviour. If the interaction with the feeder would have been recorded as such it could be that an increase in FIXTURE + SELF may have been observed. The interaction with the feeder during feed delivery may have been higher in sows fed twice as the bulk of the ration may have been slightly smaller each time they were fed as compared to in sows fed once.

Increased energy level was expected to reduce re-directed oral behaviours as increasing energy content should improve satiation (Robert et al., 1997). However, an increase in FIXTURE (Interaction with feeder; 193.06% increase) was observed in individually-housed sows (parity 2; 1.08 m^2) around feeding time (0845 to 1200 h; feed delivery at 0815 h) (study ID 358), but no effect was observed during the following observation slot (1230 to 1545 h). This could be explained by the high level of fat that was included in the high-energy diet to reach a high-energy diet but same bulk. As the authors explain, the increase in the interaction with the feeder as compared to the control diet could have resulted from the feed sticking to the trough. This holds as high-energy sows performed less SELF (sham chewing) than low-energy sows, confirming that an increase in energy levels improves satiation and therefore reduces re-directed oral behaviours around feeding time.

An increase in fibre level was expected to reduce the performance of re-directed oral behaviours. This is because it increases the bulk of feed and therefore should improve satiation, but also there is a steadier release of glucose into the blood stream (Agyekum and Nyachoti, 2017). It is important to note that almost all comparison showed a decrease in oral behaviours with the increase in fibre content. However, there were two comparisons within one study that showed an increase in behaviour. This study (ID 433) tested the effect of increasing levels of fibre content (10.07 and 20.41% crude fibre) on GROUND in gilts followed during their first and second parity (individually stall-housed, 1.26 m^2). Floor interaction (GROUND) remained unaffected during their first parity, however, there was an increase in this behaviour during their second parity for both high and very-high-fibre diet (59 and 102.4% increase compared to low-fibre control diet, respectively). It is possible that the increase in floor interaction with higher level of fibre could have been due to feed falling from the feeder drawing the attention to it. In this study, in order to achieve similar major nutrients, a higher amount of feed was provided for the experimental diets as compared to control diet (on average 3.3 vs 2.1 kg/d). Another explanation could be that female adult pigs in this study on very-high-fibre diet were metabolically hungrier than when receiving high-fibre or control diets. This is shown by the loss in weight and backfat (values not reported) observed during first and second parity of very-high-fibre diet animals.

Similarly, feed level was expected to reduce re-directed oral behaviours as it should improve satiety (Appleby and Lawrence, 1987). Adjunctive drinking which is usually observed in feed-restricted sows should also show a reduction and therefore a general decrease in water drinking (Rushen, 1984). A reduction in re-directed oral behaviours as well as drinking behaviour was observed in almost all comparisons when feed allowance was increased. However, in two comparisons within the same study (ID 409) an increase in DRINKING (Actual drinking) was observed. These were group-housed gilts (2.46 m^2) observed during their first and second parity. The increase was only observed during a 24-h period of observation and during a 6-h period starting at feeding during their first parity (50 and 100% increase, respectively). No change was observed during their second parity, as well as around feeding time during both first and second parity. These results could be explained by the larger amount of feed provided to the experimental pigs. This will elicit more water drinking because of a dry mouth and to improve digestion (Barber et al., 1991; Pedersen and Stein, 2010). The increase in actual drinking was observed outside feeding time which is in line with previous literature (Rushen, 1985; Terlouw and Lawrence, 1993).

Texture of the feed e.g. mash vs pelleted is expected to affect re-directed oral behaviours. Mash feed takes longer to eat than the pelleted form as feed may stick to the feeder and sows may spend time licking the feeder. This could reduce the time available to perform re-directed oral behaviours in a given time period. Only one study tested this and showed inconsistent results (ID 269). They tested the effect of feed texture (mash vs pelleted) in sows (≥ 1 parity). There was an increase in FIXTURE (Drinker manipulation) after the afternoon ration when receiving the mash as compared to pelleted form. However, the experimental (28 days per diet) and observation period (total of 20 min) was very short as compared to other studies. Also, there was missing data for animals fed the very-high-fibre diet in mash form. This data came from sows that had spent a lot of time eating, so likely they would have shown little drinker manipulation, and therefore possibly reduced the mean frequency. Because of these reasons it is difficult to draw any conclusions.

Provision of substrate was expected to reduce the performance of re-directed oral behaviours. This gives the animal a more natural outlet for its behavioural needs but also, depending on the substrate, some may be consumed improving satiety (Jensen et al., 2015; Staals et al., 2007; Whittaker et al., 1999, 1998). The provision of substrate showed a decrease in almost all the tested behaviour categories, except for FIXTURE and GROUND. An increase observed in FIXTURE occurred in study ID 358 where the effect of 'substrate' (removable hanging chains above feeder during feeding time) and energy level was tested on 2-parity sows. The increase in the FIXTURE resulted from the increase in the interaction with feeder (54.76% increase) around feeding time (0845 to 1200 h; feed delivery at 0815 h) but not later (1230 to 1545 h). The interaction with the feeder could have been favoured by the presence of the chains. Hanging chains were classified as substrate as the authors of the study used these to favour the release of 'foraging' behaviours. However, chains hanging over the feeder are not fulfilling the motivation to forage as intended by the authors. Chains cannot be regarded as substrate and environmental enrichment. Environmental enrichment should be 'edible', 'changeable', 'destructible' and 'manipulable (Studnitz et al., 2007). Hence, it was not the addition of substrate that caused the increase in feeder interaction but the proximity of it with another source of manipulation.

An increase in interaction with the GROUND due to the provision of substrate was observed in three studies (ID 243, 264 and 409). These studies used dust-free wood, chips wood shaving and fresh long barley straw as substrate, respectively. The effect was significant due to an increase in the interaction with the floor and/or substrate (103.96, 48.94 and (mean) 75.24% increase in study ID 243, 264 and 409, respectively). The increase in this behaviour could be explained by the presence of the substrate on the floor. This may have directed the attention of the pigs towards the floor. Adding to this, an increase in this behaviour was not observed in another study (ID 98), where substrate was provided in racks: There was either no effect or a decrease on floor interaction.

The effect of pen and group housing (Group housing + Feeder) on behaviour is less clear. On the one side, animals may reduce the performance of re-directed oral behaviours (including adjunctive drinking) as they have more space to move around, have penmates to interact with and possibly smaller time budget to perform them. However, it may be that re-directed oral behaviours are performed at the same level as stall-housed animals but since they may be less obvious, they may remain unnoticed by the observer. One study (ID 158) compared the effect of three housing systems on behaviour in sows (1 to 9 parities). The treatments differed in size, number of animals per pen and type of feeder (conventional stall, collective pen with Trickle or FITMIX feeder). Trickle (protected feeder and simultaneous feeding of the sows) feeder pen provided feed at feeding rate and housed 10 sows. FITMIX feeder is a type of electronic feeder that is unprotected which had a lateral access to leftovers to reduce agonistic behaviours. FITMIX pen housed 20 sows. Trickle pens were provided with an individual feeder and FITMIX pens had a collective feeder shared with 20 animals. All housing systems had only one drinker per pen. Both treatment pens showed inconsistent results. On the one hand, the sows housed with a Trickle feeder showed an increase in apparent drinking + actual drinking + manipulation behaviour in the later weeks of the study, but not during the first two weeks (no change), as compared to the conventional housing. On the other hand, FITMIX sows, showed a decrease in this behaviour during both study periods. The different results however could possibly be explained by the very different set up of the treatments (different feeder type, number of animals). Probably FITMIX is more efficient in improving welfare as sows spent also more time resting and decreased time spent performing stereotypies as concluded by the authors.

However, the results in Trickle pens are difficult to explain. During the last weeks of the experiment, apparent drinking + actual drinking + manipulation increased while sham chewing and oronasal behaviours decreased as compared to stall-housed sows (oronasal behaviours defined in the study as drinking, sham chewing, and interaction with the feeder and equipment). It seems like sows were re-directing sham chewing and oronasal behaviours towards the drinker. The higher level of drinker-related behaviours as compared to conventionally stalled-housed sows could correspond to emancipated stereotypies. The authors comment that the sows used in this study had been housed in stalls previously, and that older sows showed more re-directed oral behaviours than younger sows. Additionally, sows in trickle feeder received dry feed and were fed simultaneously. As dry feed induces thirst, this meant that after feed was consumed all sows were motivated at the same time to drink water from the only drinker available. As explained by the authors, manipulation of the drinker might have increased. This was not observed in stall-housed and FITMIX sows as the first one had easy access to water, and the second ones were fed with mash-consistency feed.

It is also difficult to explain why drinker-related behaviours were higher during the last weeks as compared to stall-housed sows, but no change was observed during the first weeks of the study. The first weeks of the study was considered as a habituation period to the new housing system in this study. Probably sows were not yet used to the new way of consuming the feed and water availability. Also, not explained in the study, there could have been different reasons for this change in behaviour (e.g. temperature increase, flow rate changes) but we can only speculate.

Another two studies investigated the effect of ‘group’ housing (ID 431 and 462). They compared the behaviour between loose and tethered housing in either sows followed through parity 2 and 3 (study ID 431) or gilts (study ID 462). Since loose-housed animals were housed in groups of four and tethered pigs had reduced chances to interact with conspecifics it was not possible to separate the effect of loose from group housing. Therefore, the factor was named ‘Loose housing (+Group)’. In both studies, loose-housed pigs had a chain attached to their necks that was attached to their neck on both ends to mimic the effect of the chains in tethered pigs. Both studies showed an unexpected increase in FIXTURE. In study ID 462 there was an increase in <u>resource-directed</u> (directed towards ‘trough/floor’) in group housed gilts compared to tethered gilts (56.32% increase), but no difference on <u>chain manipulation</u> and <u>bar biting</u> was observed between both groups. Loose-housed gilts also performed less <u>self-directed behaviour</u> than tethered gilts. The increase in FIXTURE in this study may be explained by loose-housed sows being able to move around more freely and interact and explore with the environment better than tethered sows. They may have re-directed self-directed oral behaviours towards the ‘trough/floor’. In study ID 431, there was an increase in FIXTURE (the interaction with ‘Pen fixtures other than chain and nipple’) in loose-house sows as compared to tethered sows during their second and third parity (120.83 and 110% increase, respectively). This makes sense as they had access to much more space and resources to explore than tethered sows. However, there were some unexpected results regarding <u>chain manipulation.</u> This was higher in loose-housed sows as compared to tethered sows, but also this increase was only observed during their second parity. It should be noted that in this study (Study ID 431) loose-housed sows were performing the same number of self-directed behaviours as tethered sows, meaning that the increase in the exploration of the pen did not mean a reduction in self-directed behaviours, as observed in study ID 462. This could be explained by the parity of the sow as the older the sow it is more likely that oral behaviour will respond less to treatments (Lawrence and Terlouw, 1993).

An increase in space allowance is expected to reduce re-directed oral behaviours, as explained before. Unexpectedly, in one study (ID 97), female adults (parity 0 to 4) showed an increase in SELF (frequency and duration of sham chewing) when comparing a conventional stall against an adjustable (space) conventional stall. In the same study, no change in sham-chewing behaviour was observed when comparing the conventional stall against a stall with access to a small, shared space. The authors suggest that this may be because of mistakes in the design of each housing system. The higher level of sham chewing in an adjustable space conventional stall may be explained by the front gate design. This had vertical instead of horizontal bars which may have prevented the sows from biting them. They may instead have re-directed bar-biting behaviour to sham chewing. The lack of difference in sham chewing observed between conventional stalls and stall with access to a small, shared space could be explained by the extra space actually being insufficient for it to have an effect on behaviour. This space might not have been actually available for the sows to use and they might have spent most of their time in the stalls, as shown by a reduced use of the shared space as this is reduced (Mack et al., 2014).

Table 5.6. Summary of the effect of main factors on the behaviour categories. Total counts of comparison (n), number of comparisons affected (effect) and the direction of the effect (direction) are shown. Direction of effects highlighted in red are in the opposite direction to that predicted by 'chronic hunger' theory.

Factors	Studies (counts)	FIXTURE				FIXTURE + SELF				DRINKING			
		n	Effect	Direction ↓	Direction ↑	n	Effect	Direction ↓	Direction ↑	n	Effect	Direction ↓	Direction ↑
Delay in feed delivery	1	1	0	-	-	-	-	-	-	-	-	-	-
Place of feed	1	3	0	-	-	-	-	-	-	3	0	-	-
Feeding frequency	2	3	2	0	2	9	4	2	2	-	-	-	-
Energy level	1	2	1	0	1	-	-	-	-	2	0	-	-
Fibre level	15	39	16	16	0	18	4	4	0	2	0	-	-
Fibre level + Feed level	2	3	2	2	0	-	-	-	-	-	-	-	-
Fibre level (lineal relationship)	1	2	0	-	-	2	2	2	0	-	-	-	-
Fibre type	2	5	1	1	0	-	-	-	-	-	-	-	-
Mix of ingredients	1	2	0	-	-	2	0	-	-	-	-	-	-
Feed allowance	6	13	11	11	0	1	1	1	0	10	5	3	2
Texture	1	2	2	1	1	-	-	-	-	-	-	-	-
Substrate provision	7	15	9	8	1	-	-	-	-	16	1	1	0
Group housing	2	3	2	2	0	-	-	-	-	-	-	-	-
Group housing + Feeder	1	4	1	1	0	-	-	-	-	4	3	2	1
Loose housing (+Group)	2	3	3	0	3	-	-	-	-	3	1	1	0
Space allowance	1	4	0	-	-	-	-	-	-	-	-	-	-

FIXTURE includes Bar biting, Chain manipulation, Chain + bar manipulation, Interaction with feeder, Resource-directed, Drinker manipulation, 'Object biting (Study ID 304)', 'Fixture-related (Study ID 431)'; **DRINKING** includes Actual drinking, Apparent drinking, Apparent drinking + actual drinking + manipulation

Cont. Table 5.6. Summary of the effect of main factors on the behaviour categories. Total counts of comparison (n), number of comparisons affected (effect) and the direction of the effect (direction) are shown. Direction of effects highlighted in red may not be possible to explain by 'chronic hunger' theory.

Factors	Studies (counts)	GROUND				SELF				RESOURCE + PIG			
		n	Effect	Direction ↓	Direction ↑	n	Effect	Direction ↓	Direction ↑	n	Effect	Direction ↓	Direction ↑
Delay in feeding delivery	1	1	0	-	-	-	-	-	-	-	-	-	-
Place of feed	1	3	1	0	1	3	1	1	0	-	-	-	-
Feeding frequency	2	-	-	-	-	-	-	-	-	-	-	-	-
Energy level	1	2	0	-	-	2	1	1	0	-	-	-	-
Fibre level	15	9	4	2	2	21	5	5	0	2	0		
Fibre level + Feed level	2	-	-	-	-	4	1	1	0	1	1	1	0
Fibre level (lineal relationship)	1	-	-	-	-	2	1	1	0	-	-	-	-
Fibre type	2	-	-	-	-	2	1	1	0	-	-	-	-
Mix of ingredients	1	-	-	-	-	2	0			-	-	-	-
Feed allowance	6	7	5	5	0	7	3	3	0	-	-	-	-
Texture	1	-	-	-	-	-	-	-	-	-	-	-	-
Substrate provision	7	13	6	0	6	11	8	8	0	1	1	1	0
Group housing	2	-	-	-	-	3	2	2	0	-	-	-	-
Group housing + Feeder	1	-	-	-	-	4	4	4	0	-	-	-	-
Loose housing (+Group)	2	-	-	-	-	3	1	1		-	-	-	-
Space allowance	1	-	-	-	-	4	2	0	2	-	-	-	-

GROUND includes Interaction with substrate, Floor interaction, Interaction with substrate + Floor interaction, 'Rooting floor (Study ID 511)', 'Licking floor (Study ID 511)', 'Interacting with mats (Study ID 511)'; **SELF** includes Sham chewing, Self-directed behaviours, 'Jaw-stretching (Study ID 240)', 'Self-directed behaviours (240)'; **FIXTURE + PIG** includes Resource-directed + themselves, Resource-directed + pig-directed

5.4.2.2 Interactions that had a significant effect on behaviour: Results and discussion

A summary of significant interactions between treatments in factorial-designed studies on behaviour are shown in Table 5.7. From the total of interactions tested in these studies, only 18.33% of the interactions tested were significant (highlighted in Table 5.7). In the following sections, only interactions that were significant are explored and discussed in the light of the 'chronic hunger' theory and housing environment. This will be done in order according to the list of factors in Table 5.7.

The effect of the interaction 'Substrate x fibre level' on FIXTURE and SELF were tested in three and two studies, respectively. FIXTURE was affected by the interaction in study ID 98 and ID 144, but SELF was only affected in study ID 144. Study ID 98 tested the effect of fibre level and substrate provision on re-directed oral behaviours in parity-2 sows. Substrate corresponded to uncut straw provided in racks. Control-group sows were provided with a limited amount of straw on the floor, while the experimental group received additional straw in racks. Study 144 was similar but differed in study animals and substrate provision. They studied the behaviours in parity-3 (± 2) sows, and straw was provided chopped and the control group did not have access to any straw.

In study ID 98, the 'Substrate x fibre level' interaction showed an effect of increased fibre level within sows that had access to limited amount of straw but not within sows that were provided with additional straw. FIXTURE decreased with the increase in fibre level. This means that an additional amount in fibre provided in the diet appears to improve satiety when sows have limited access to straw. The increase in fibre content within sows receiving an extra amount of straw might not have had an effect as sows might have fulfilled their nutritional needs by eating straw so that the increased level of fibre did not make any difference.

In study 144, however, the interaction on FIXTURE showed that FIXTURE directed oral behaviours increased with increased dietary fibre level for sows that were provided with straw, but not for sows without access to straw. Similarly, an effect of straw provision on FIXTURE was only observed in sows receiving a high fibre diet. Both showed a decrease in FIXTURE with increased fibre level or straw provision, as expected. The effect of increasing fibre content or providing straw was expected to be greater for sows without access to straw or fed on a conventional diet (Appleby and Lawrence, 1987; Whittaker et al., 1998). This is because sows in with control treatments should be expected to be hungrier than sows with experimental treatments groups. A similar effect was observed on SELF (sham chewing). Increased fibre level had only an effect within sows provided with straw. The provision of straw, however, had an effect within both sows on low and high fibre diet. The direction of effect was as expected: Increasing fibre level or providing straw reduced the frequency of sham chewing. Again, it is difficult to explain why the behaviour of sows without access to straw was not affected by an increase in fibre level. Possibly the amount of fibre in the 'high fibre diet' (89 g/kg dry matter) and/or type of fibre (barley- and soya-hulls-based diet) was not enough to reduce the frequency of sham chewing (Stewart et al., 2010). This may indicate that there was a confounding variable that caused both treatments to show the same amount of behaviour. However, we have to keep in mind that these were multiparous sows and therefore both straw and high fibre diet was necessary to reduce sham chewing or sham chewing may have corresponded to emancipated stereotypies.

The interaction 'Place of feed x substrate' was tested only in one study and had an effect on DRINKING and GROUND (ID 264). They studied the effect of the interaction on gilts' behaviour. They found an interaction on apparent drinking in gilts during the morning observations (0700 to 0900 h) but not during the rest of the day. The interaction was apparently inconsistent. Floor-feeding increased apparent drinking as compared to trough-feeding when gilts were provided with substrate (wood shavings). However, when they were not provided with wood shavings, floor-fed gilts showed a decrease in this behaviour as compared to trough-fed gilts. If gilts were over-drinking water due to hunger or as a stereotypy, then floor-fed sows with access to wood shavings should have shown the least drinking behaviour, and not trough-fed sows with substrate. This is because it would have taken longer to eat their ration but also it would have fulfilled their behavioural needs to a greater extent than when trough-fed. So, they should show less re-directed oral behaviours because of a reduced time budget and less motivation to do so. However, the interaction could also be explained by thirst in floor-fed gilts with access to substrate driven by the ingestion of wood shavings together with the feed. This may also be backed up by the fact that floor-fed gilts with access to wood shavings were performing apparent drinking as much as floor-fed gilts without access to substrate, but when gilts were trough-fed there was a decrease in apparent drinking with the provision of substrate. This means that this extra amount of apparent drinking performed by floor-fed gilts with substrate were drinking water due to thirst.

In the same study (ID 264), GROUND was affected by the same interaction ('Place of feed x substrate'). In this study, floor interaction was recorded differently depending on the study group. For gilts in the experimental group, floor manipulation included interaction with substrate e.g. Interaction with substrate and/or Floor interaction. Here it will be named as floor manipulation for ease. During all observation periods (0700 to 0900, 1000 to 1200, 1300 to 1500 h; feeding times at 0630 and 1500 h), floor manipulation was affected by the feeding method only when no wood shavings were provided; there was an increase in floor manipulation when sows were floor fed as compared to trough fed. This was expected as the floor may appear more attractive to gilts since that is where remaining feed may be found. Similarly, there was an effect of wood shavings provision only when sows were fed in the trough, showing an increase when wood shavings were provided. This may have happened likely due to the same reason. Wood shavings may have attracted gilts towards the floor, and therefore increase their interaction with it. The authors also suggest that they might have been manipulating substrate in order to eat it (which was the case, as shown by faeces samples). As expected, during morning (0700 to 0900 h) and noon observations (1000 to 1200 h), but not during afternoon observations, trough-fed gilts without access to wood shavings showed this behaviour less than floor-fed sows with access. Again, feed provided on the floor mixed in with substrate may have drawn the attention of gilts towards the floor and therefore interact with it more than gilts that have been fed in the trough without access to substrate. Also, during the afternoon observation no difference was found between these two groups, which is in line with the 'chronic hunger' theory. Most re-directed oral behaviours concentrate around feeding time (Terlouw et al., 1993, 1991b).

The effect of the interaction 'Feed level x substrate' was investigated in two studies (ID 243 and ID 409) and had an effect on FIXTURE, GROUND and SELF. In study ID 243 dust-free, wood chips were provided on the floor and behaviour was observed in gilts. In study ID 409 fresh long barley straw was provided on the floor and the behaviour was observed in gilts followed from their first to their second parity.

In study ID 409, an interaction of 'Feed level x substrate' on FIXTURE (Chain + bar manipulation and resource-directed) was observed. Chain and bar manipulation (second parity only), and resource-directed behaviours (both parities) showed the same pattern during a 6-h observation period that started after feeding. In both cases, the interaction showed that the provision of fresh straw only had an effect in low-fed pigs, showing a decrease in the behaviour with the presence of fresh straw. The level of feeding only had an effect when no fresh straw was provided; showing a decrease in high-fed pigs. Also, within pigs without access to fresh straw the increase in feeding level decreased the performance of the behaviour. This is as expected by the 'chronic hunger' theory, as animals will perform less re-directed oral behaviours when satiety is improved (Appleby and Lawrence, 1987). Substrate such as fresh straw, will reduce the expression of re-directed oral behaviours as it can be ingested and therefore improve satiety, but also because it provides them with a much natural and interesting surface to fulfil their behavioural needs (Jensen et al., 2015; Staals et al., 2007; Whittaker et al., 1999, 1998). However, this was not observed in all cases. The level of feed included in the diet did not matter when pigs had access to fresh straw for both resource-directed behaviours and chain and bar manipulation. Similarly, the provision of fresh straw did not affect these behaviours when high feed allowance was provided. It could be that fresh straw provision and feed allowance do not have an additive effect on pigs' behaviour, and that as long as either of them are provided, satiation will be improved and will be enough to reduce re-directed oral behaviours.

The same study (ID 409) showed an effect of the interaction 'Feed level x substrate' on GROUND (Interaction with substrate). It is important to mention that Interaction with substrate was defined by the authors as nosing, chewing, rooting, biting, lifting or licking *any* available substrate (straw) in the stalls or dunging area other than feed or pen mates. Meaning that anything available to the pigs could fall under this behaviour. The interactions were as expected by the 'chronic hunger' theory. There was an increase in substrate interaction when providing substrate for both low and high fed sows. There was a decrease in substrate interaction when increasing feeding level for pigs with and without substrate. The effect of the provision of fresh straw was as expected. The increase in the interaction with the floor can be explained by the increased attention towards the floor, pigs may explore straw to find a source of food and it provides a way of fulfilling their behavioural needs (Jensen et al., 2015; Staals et al., 2007; Whittaker et al., 1999, 1998).

In the case of feed level, improved satiety caused by a higher amount of feed, should decrease the motivation to look for other sources of feed (Appleby and Lawrence, 1987). However, maybe unexpectedly, the lowest level of substrate interaction was observed in high-fed pigs without substrate instead of high-fed pigs with substrate. It is thought that the provision of substrate together with a higher amount of feed should be the most efficient in reducing re-directed oral behaviours (in this scenario). However, since substrate may increase the attention towards the floor, this could have resulted in high-fed pigs without substrate to have the lowest interaction with the resources available to them including the ground. They are less hungry than low-fed pigs and have nothing to draw their attention towards the ground.

In study ID 243 an interaction of ‘Feed level x substrate’ on SELF (Chewing movements) was found (gilts). Provision of substrate (dust-free wood chips) did not affect chewing movements (sham chewing and/or chewing on something) when unrestricted feeding. Since chewing movements included chewing on substrate for the group with access to dust-free wood chips, it could be that the lack in difference may be explained by this. However, provision of dust-free wood chips did have an effect in feed-restricted gilts. Probably, in gilts with restricted access to feed and, therefore, hungrier than their counterparts, dust-free wood chips have a much bigger impact. They may be chewing on substrate a lot increasing the overall count for chewing movements. Increasing feed level resulted in a decrease of chewing movements independent of whether substrate is provided or not. These results mostly follow what the ‘chronic hunger’ theory predicts. It is difficult to draw any conclusion as chewing movements included sham chewing and chewing on substrate.

Table 5.7. Summary of the effect of interactions on the behaviour categories. Total counts of comparison (n) and number of comparisons affected by the interaction (effect) are shown. Effect highlighted in red may not be possible to be explained by 'chronic hunger' theory.

Factor	Studies (counts)	FIXTURE		RESOURCE + SELF		DRINKING		GROUND		SELF		RESOURCE + PIG	
		n	Effect	n	Effect	n	Effect	n	Effect	n	Effect	n	Effect
Fibre level x Feeding frequency	2	3	0	9	0	-	-	-	-	-	-	-	-
Fibre level x Substrate	3	5	2	-	-	1	0	1	0	2	1	1	0
Fibre level x Texture	1	2	0	-	-	-	-	-	-	-	-	-	-
Place of feed x Substrate	1	3	0	-	-	3	1	3	3	3	0	-	-
Feed level x Loose housing (+Group)	2	5	0	-	-	5	0	-	-	5	0	-	-
Feed level x Substrate	2	5	2	-	-	7	0	7	1	1	1	-	-

Fixture includes Bar biting, Chain manipulation, Chain + bar manipulation, Interaction with feeder, Resource-directed, Drinker manipulation, Object biting (Study ID 304), Fixture-related (Study ID 431); **Drinking** includes Actual drinking, Apparent drinking, Apparent drinking + actual drinking + manipulation; **Ground** includes Interaction with substrate, Floor interaction, Interaction with substrate + Floor interaction, Rooting floor (Study ID 511), Licking floor (Study ID 511), Interacting with mats (Study ID 511); **Self** includes Sham chewing, Self-directed behaviours, Jaw-stretching (Study ID 240), Self-directed behaviours (240); **Fixture + Pig** includes Resource-directed + themselves, Resource-directed + pig-directed

5.4.3 Summary of comparisons and interactions that did not fit the predictions of the 'chronic hunger' theory

The effect of main factors and interactions on the behaviour of gilts and sows could be explained by the 'chronic hunger' theory in almost all cases. Only one comparison remains unexplained. In study ID 158 (see page 194 for more detail) three housing types were compared. These differed in the number of animals per pen and type of feeder. Collective pen with trickle (protected) pen (10 sows/pen) showed an increase in drinker interaction (apparent drinking + actual drinking + manipulation) as compared to conventional stall housing. This increase in drinker interaction was only observed after two weeks of observation but not right at the beginning of the study. There are no clear explanations in the article of why this could have happened, so we can only speculate why this could have been observed. It seems though that it does not conflict with the 'chronic hunger' theory. However, to put into context this is 1 out of 138 comparisons for main factors that was included in this systematic literature review. It is safe to say that all of the articles included in this review are consistent with the 'chronic hunger' theory and their results could be explained by the housing environment.

5.4.4 Limitations of the systematic literature review

Because of the method of information extraction where the percentage of change was calculated for each comparison between control and different treatment groups there may be a case of repeated measures. Each study contributed to more than one row, meaning that rows are not completely independent of one another. For example, if there had been a problem with the control group this would have impacted all estimations for that study.

It was difficult to draw strong conclusions as there were few similar studies of each type and it was also impossible to perform a meta-analysis. The sample size of the systematic literature review is relatively small. Only 30 out of 517 studies fulfilled the criteria and were used in this study. Also, the way in which behaviours were described, and grouped together and the way behavioural data was collected and summarised varied greatly. Characteristics of subjects (such as breed, age/parity) and housing conditions which did not form part of the treatment variables were different between articles. This study is also limited in that it did not explore the variation in re-directed oral behaviours by the parity number and did not screen through the reference list of selected articles.

These studies were set up with the 'chronic hunger' theory in mind. This means that the factors tested were all trying to satisfy the behavioural or nutritional needs of the sow under that paradigm. Also, the variables measured, and the description of the treatments were responding to the same context. This makes it difficult to identify unexplained results as there is missing information to properly make that decision. Studies designed to investigate alternative explanations for re-directed oral behaviours such as conditions affecting the upper digestive system or barren environment would have considered other factors relating to those e.g. different particle size, increasing complexity of the environment with different type of environmental enrichment with different textures and smells at different locations. They would also have included additional measurements e.g. health assessments of mouth, oesophagus, gastric, as well as type, frequency and duration of the interaction with a certain enrichment.

5.4.5 Future steps

This systematic literature review highlights the importance of doing more research in this area. This is because the lack of evidence found here may mean that because of the nature of the studies it was not possible to conclude anything. For example, it would be useful to measure re-directed oral behaviours in gilts in a 2 x 2 study investigating the effect of fibre level (expected to affect hunger) and feed particle size (expected to affect gastric ulcers), to see which behaviours are affected by different combinations of these.

5.5 Conclusion

This systematic literature review offers support for chronic hunger theory as a cause for oral behaviours in gestating gilts and sows. It does not show the possibility of alternate variables causing these behaviours. The results of all articles included in this review could be explained by 'chronic hunger' theory and/or housing environment. However, the studies included in this systematic literature review were planned with this theory in mind and therefore the future steps suggested to expand the research are merited.

5.6 References

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5.7 Annexe

Table 5.8. List of references and study ID that were included in this systematic literature review

Study ID	Authors	DOI
97	Salak-Johnson et al., 2015	https://doi.org/10.2527/jas.2015-9017
98	Jensen et al., 2015	https://doi.org/10.1016/j.applanim.2015.08.011
112	Zhou et al., 2014	https://doi.org/10.1017/S1751731114001025
135	Li et al., 2013	https://doi.org/10.4141/cjas2012-043
136	Souza da Silva et al., 2013	https://doi.org/10.1016/j.physbeh.2013.01.006
144	Stewart et al., 2011	https://doi.org/10.1017/S0962728600003274
155	Stewart et al., 2010	https://doi.org/10.1017/S0962728600001743
158	Chapinal et al., 2010	https://doi.org/10.1016/j.jveb.2009.09.046
208	Holt et al., 2006	https://doi.org/10.2527/2006.844946x
217	de Leeuw et al., 2005	https://doi.org/10.1016/j.applanim.2005.02.006
240	Zonderland et al., 2004	https://doi.org/10.1016/j.applanim.2003.12.009
243	de Leeuw and Ekkel, 2004	https://doi.org/10.1016/j.applanim.2003.12.004
257	van der Peet-Schwering et al., 2003	https://doi.org/10.1016/S0168-1591(03)00112-6
264	de Leeuw et al., 2003	https://doi.org/10.1016/S0168-1591(02)00234-4
269	Bergeron et al., 2002	https://doi.org/10.4141/A01-073
277	Robert et al., 2002	https://doi.org/10.1016/S0168-1591(02)00003-5
304	Bergeron et al., 2000	https://doi.org/10.1016/S0168-1591(00)00142-8
312	Haskell et al., 2000	https://doi.org/10.1016/S0376-6357(00)00077-2
325	Ramonet et al., 1999	https://doi.org/10.2527/1999.773591x
330	Whittaker et al., 1998	https://doi.org/10.1016/S0168-1591(98)00183-X
358	Bergeron and Gonyou, 1997	https://doi.org/10.1016/S0168-1591(96)01169-0
409	Spoolder et al., 1995	https://doi.org/10.1016/0168-1591(95)00566-B
420	Brouns et al., 1994	https://doi.org/10.1016/0168-1591(94)90157-0
431	Terlouw and Lawrence, 1993	https://doi.org/10.1016/0168-1591(93)90060-3
433	Robert et al., 1993	https://doi.org/10.1016/0168-1591(93)90119-A
445	Arellano et al., 1992	https://doi.org/10.1016/0168-1591(92)90006-W
462	Terlouw et al., 1991	https://doi.org/10.1016/S0003-3472(05)80151-4
506	Deng et al., 2021	https://doi.org/10.1016/j.aninu.2020.06.015
511	Bernardino et al., 2021	https://doi.org/10.1016/j.applanim.2021.105433
520	Li et al., 2022	https://doi.org/10.3390/ani12111355

Table 5.9. Type of behavioural recording method and category of behaviour included in the studies selected for this systematic literature review (counts of studies).

Study ID	Behaviour sampling method	FIXTURE	FIXTURE + SELF	DRINKING	GROUND	SELF	FIXTURE + PIG
97	Video continuous	2				1	
98	Video continuous	2			2		
112	Video continuous	1				1	
135	Video scan		1	1		1	
136	Live scan	1					
144	Live? Scan + continuous	1				1	
155	Video scan + continuous					1	
158	Live scan	1		1		1	
208	Video continuous		1	1			
217	Live scan	1	1	1		1	
240	Video continuous	1				3	1
243	Live scan	1		1	2	2	
257	Live scan	1				1	
264	Live scan	1		1	2	2	
269	Live continuous	3					
277	Live scan	3	1	1			
304	Live continuous	3	1	1		1	
312	Live scan				1		
325	Live scan	2				1	
330	Live scan	1		1	1	1	1
358	Live scan	2		1	1	1	
409	Live + video scan	2		1	1		
420	Live scan	2				1	
431	Live scan	3		2		1	
433	Video scan	2			1		
445	Live continuous	1				1	
462	Live scan	3		1		1	
506	Video continuous	2				1	
511	Live continuous	1			3	1	
520	Video scan			1		1	

Table 5.10. Description of the experimental design [Randomized controlled study (Randomized) and quasi-experimental studies (Non-randomized)], number of treatments, and type of study and factors being tested for each study included in this systematic literature review

Study ID	Experimental design	Treatment number	Type of study	Factors being tested (study)
97	Randomized	3	Housing	Space allowance
98	Non-randomized	4	Diet; Enrichment	Fibre level; Substrate (straw)
112	Randomized	2	Housing	Group housing
135	Randomized	4(2)	(Housing); Diet[1]	(Group housing); Fibre level (distillers' dried grain)
136	Non-randomized	4	Diet	Fibre type; Fibre level
144	Randomized	4	Diet; Enrichment	Fibre level; Substrate (straw)
155	Non-randomized	2	Diet	Fibre level
158	Randomized	3	Housing	Group housing + Feeder
208	Non-randomized	4	Diet; Feeding practices	Fibre level; Feeding frequency
217	Non-randomized	5	Diet	Fibre level
240	Non-randomized	2	Diet + Feeding practices[2]	Feeding level + Fibre level
243	Non-randomized	4	Feeding practices; Enrichment	Feed level; Substrate (dust-free wood chips)
257	Non-randomized	4(2)[3]	Diet	Fibre type
264	Randomized	4	Feeding practices; Enrichment	Place of feed; Substrate (wood shavings)
269	Randomized	3	Diet; Feeding practices	Texture; Fibre level
277	Non-randomized	4	Diet; Feeding practices	Fibre level; Feeding frequency
304	Randomized	4	Diet; Feeding practices	Fibre level; Feed level
312	Non-randomized	3	Feeding practices	Delayed feeding
325	Non-randomized	3	Diet	Fibre level
330	Non-randomized	4	Diet; Enrichment	Fibre level; Substrate (straw)
358	Randomized	3	Diet; Enrichment	Energy level; Substrate (hanging chain in the feeder[4])
409	Randomized	4	Enrichment; Feeding practices	Substrate (straw); Feed level
420	Randomized	3	Diet; Feeding practices	Fibre level; Feed level
431	Non-randomized	4	Housing; Feeding practices	Loose housing; Feed level
433	Randomized	3	Diet	Fibre level
445	Non-randomized	2	Housing	Group housing
462	Randomized	4	Housing; Feeding practices	Loose housing (+Group)[5]; Feed level
506	Non-randomized	3	Diet	Fibre level (wheat aleurone)
511	Non-randomized	2	Diet	Fibre level
520	Randomized	3	Enrichment	Substrate (wood)

[1]Included housing type however oral behaviours were only scored in sows in gestation stalls
[2]Studied the difference in behaviour between a restricted concentrate and an *ad lib* fibre diet
[3]This study tested fibre type during lactation and gestation period but I only extracted information corresponding to the gestation period
[4]Chains are not considered as substrate, however, the authors use these to stimulate foraging behaviours
[5]Loose vs tethered housing was tested in a collective pen in groups of four gilts

Chapter 6 General discussion

6.1. Main findings

In this thesis I aimed to investigate whether re-directed oral behaviours observed in gestating gilts and sows due to chronic hunger could also be related to gastric ulceration, and whether the progression of gastric ulceration changes the profile of metabolites in sows with gastric ulcers. This thesis was a starting point to explore both oral behaviours and saliva composition as tools for diagnosing gastric ulceration in pigs. The importance of this thesis lies in that gastric ulcers are of animal welfare concern and may impact the productivity of pig farms. Adding to this, gastric ulcers are prevalent in the pig industry and might affect all productive stages (Cybulski et al., 2021a, 2021b; Gottardo et al., 2017; Peralvo-Vidal et al., 2021). Most importantly, presence of gastric ulcers in the pig industry has remained virtually unchanged throughout the years (Helbing et al., 2022). The reasons for this may be that gastric ulcers are difficult to diagnose in the living pig and they still reach market weight with or without gastric ulcers. Research on the impact of gastric ulceration on productivity and welfare of the pigs remains ambiguous as 1) we do not know the timeline of development and healing of gastric ulcers, which impacts 2) the results of productivity trials where there is a gap between the measurement of the variables and finding out which pigs did and did not have a gastric ulcer. As there is not a clear picture of whether ulcers have an impact on productivity, farmers continue to feed pigs with small particle size diets because they are thought to improve feed efficiency (Ball et al., 2015; Wondra et al., 1995), despite being an important risk factor for the development of gastric ulcers (Canibe et al., 2016).

The starting point of this thesis was to confirm whether re-directed oral behaviours, observed in feed-restricted dry sows are also present in finishing pigs. The rationale was that if finishing pigs, that are routinely fed *ad libitum* and had never been subjected routinely to a restricted diet, perform this type of behaviour then it is plausible to think that oral behaviours may (at least sometimes) be caused by factors other than chronic hunger. Previously, an undergraduate student (Dubarry, 2019) described these oral behaviours in growing and finishing pigs, and in gilts at the rearer and at gestation stages. However, these results have not been yet published. Therefore, my starting point was to carry out my own investigation of this question.

In Chapter 2, I confirmed that re-directed oral behaviours are present in finishing pigs, but most interestingly, self-directed oral behaviours were present as well. These behaviours were occurring at the same rate as in dry sows, some had high variability, and they were the least frequently performed and by the fewest pigs. Self-directed oral behaviours scored in this study (jaw stretching, wind sucking, snout twitching, tongue playing, sham chewing) did not correlate or vary together. These are interesting findings as it questions the idea that re-directed oral behaviours in gestating sows originate solely as a result of feed restriction resulting in chronic hunger. Most oral behaviours observed in this study could be explained by the motivation for exploration of young pigs e.g. object- and pig-directed oral behaviours. However, self-directed oral behaviours are much more difficult to explain in finishing pigs. I selected these behaviours to explore the hypothesis of association with gastric ulcers in Chapter 3 and 4.

In Chapter 3, I further reinforced my previous finding in Chapter 2, that finishing pigs also perform self-directed oral behaviours. These behaviours occurred at a higher rate in the pigs studied in Chapter 3. Self-directed oral behaviours observed in Chapter 3 (chewing movements, tongue playing and wind sucking) were not related to the presence of ulcers in the pars oesophagea. Therefore, the occurrence of these behaviours remains unexplained. In the discussion of this chapter, I propose explanations for their occurrence (see section 3.5.2). Briefly, pigs might need a much more enriched environment and/or better feeding practices that adjust better to their needs and preferences.

Pigs are omnivores and show a wide range of foraging behaviours (D'Eath and Turner, 2009), and eat a varied diet [Ballari and Barrios-García, 2014; reviewed by Nogueira et al. (2007); Parkes et al., 2015)] that their current housing and feeding conditions do not fulfil. Furthermore, feed-restricted gilts and sows provided with straw generally show a change from the less 'normal' self-directed oral behaviours towards exploration of the substrate provided (de Leeuw et al., 2003; Spoolder et al., 1995), and they also prefer much more complex environments (Moser et al., 2019). Further research is needed to investigate if and how self-directed oral behaviours in young pigs might result from some inadequacy in current housing or feeding.

In Chapter 4, there were two important findings. Salivary composition is a potentially useful indicator of gastric ulceration in pigs as it changed with the type of lesions and the progression of the condition. Also, this study confirms previous findings of the presence of gastric ulcers in adult female pigs and shows that they might be a much bigger problem than previously thought: Sows in this study had ulcers despite being kept in group housing, stable groups, and plenty of uncut straw replenished twice a week. The present study also extended the result found with finishing pigs in Chapter 3, that re-directed oral behaviours are not related with gastric ulcers in adult sows.

Chapter 5 showed that all articles scrutinised support the chronic hunger theory as a cause for oral behaviours in gestating gilts and sows. It does not show the possibility of alternate variables causing these behaviours. However, it should be noted that all of these studies were designed to investigate aspects relevant to hunger such as feeding and enrichment, rather than risk factors relating to oral or gastro-intestinal tract health such as fine particle size feed.

This thesis shows that re-directed oral behaviours observed in feed-restricted gestating sows are present in finishing pigs. No evidence of a relationship was seen between self-directed oral behaviours and gastric ulceration, and further research should be done in this area to further understand the origin of these type of behaviours. Gastric ulceration may be possible to be diagnosed with a saliva swab test, however, more research needs to be done to identify and further validate in a range of settings reliable and consistent salivary indicators for presence/absence and for progression/types of ulcers.

6.2 Novelty of the approaches

Chapter 2 showed for the first time the presence of oral behaviours observed in feed-restricted gestating sows in pigs fed *ad libitum*. Although there is one previous record, this has not yet been published in a peer-reviewed journal (Dubarry, 2019). Chapter 3 is the first study of the relationship between self-directed oral behaviours and gastric ulcers in pigs. There are only four previous publications where the relationship between behaviour and gastric ulcers is investigated in pigs. As mentioned before, Hartnett et al. (2023) studied the effect on postures and pig-directed behaviours, Peralvo-Vidal et al. (2021) on feeding behaviour, Rutherford et al. (2018) on general behaviour that might be related with pain, and Dybkjær et al. (1994) studied the likelihood of gastric ulcers in pigs behaving in a certain way. This chapter adds to the knowledge that self-directed oral behaviours are not related with gastric ulcers.

Chapter 4 is the second study to our knowledge that investigates the relationship between saliva composition and gastric ulceration in pigs (Madsen et al., 2022). However, the present study is the first one in investigating how the saliva composition changes with the progression of the condition as well as with the presence of a certain type of lesion. It is also novel in that this is the first study investigating this in gestating and lactating sows. This study is also novel in that it also developed a new scoring system to better describe the presence and severity of the different lesions in the pars oesophagea as opposed to only scoring them by the worst lesion present as is usually done. Unfortunately, because of the small sample size and the lack of repetition of some of the lesions, it was not possible to perform more complex statistics that would also factor in the different combination of lesions and severity. This is also the first study that investigates the relationship between gastric ulcers and salivary pH in pigs.

This is also the first study to investigate the relationship between re-directed oral behaviours and gastric ulcers in gestating and lactating sows. All previous studies have centred on the effect of factors related to the diet, feeding practices, housing and environmental enrichment on re-directed oral behaviours under the chronic hunger theory.

Chapter 5 is the first systematic literature review that explores the variation of re-directed oral behaviours in gestating sows according to the diet, feeding practices, housing conditions and environmental enrichment. Only a few reviews have been done in this area but addressing slightly different things as described before (see section 5.2).

6.3 Limitations

In Chapter 2, the length of the observation period was not long enough to be able to investigate the consistency of the behaviours over time within individual pigs. Because of time constraints it was not possible to observe the pigs for longer than one week. Also, the duration of the observation slot e.g. 2.5 min every 1 hour may have hindered me from observing more occurrences of the behaviours that were low in frequency e.g. self-directed oral behaviours. The total observation time was set to favour number of animals over time of observation. This decision was made because gastric ulcers are supposed to be relatively low in prevalence (e.g. 12.82% of the pigs in the study had ulcers and/or healing tissue) at the research farm (Rutherford et al., 2018), therefore, if re-directed oral behaviours were also elicited by gastric ulcers, few animals would be expected to perform these. The more animals I observed, the more likely I was to observe these behaviours. Also, if I have had more weeks available for observations, it would have been possible to observe these animals for a longer period of time, and study how consistent these behaviours are and whether pigs pick up on new behaviours with time.

In Chapter 3, the videos were not of sufficient quality to score self-directed oral behaviours and, for example, hindered me from observing snout twitching as the snout was not easy to recognize from the background. Probably a better option would have been to observe the pigs live or situate cameras closer to the pen, ensure good lighting and quality of the image. Naturally, this was not possible because of the circumstances: This was a study that was planned during lockdown due to the COVID-19 Pandemic, when new farm studies were not possible and it made use of the recordings of a previous study. However, if this study were to be replicated in future, my recommendation would be to do live observations. This allows for a better observation of the mouth of the pig but also allows some movement of the observer to improve visibility.

Another limitation was that during video recording pigs were disrupted relatively often by the personnel moving pigs and cleaning pens nearby. Pigs showed a high curiosity and interest for the people present showed as sniffing and walking towards the person near the pen. Although the pigs were not scared or fearful, this meant that the pigs were directing their behaviour and attention towards the people. No self-directed oral behaviours were observed during disrupted behaviour.

In Chapter 4, the small sample size did not allow for enough repetitions for some of the overall stomach scores and lesion scores. However, the sample size was limited by the number of sows that were selected to be culled due to the farm repopulation, and of course the level and range of stomach scores were unknown without first culling them. The small sample size, and all stomachs having some level of change in the mucosa of the pars oesophagea meant that I did not have any healthy controls in this study as a baseline to compare salivary composition and pH, and behaviour. This also meant that it was not possible to do more sophisticated statistics that would account for the different combinations of lesions and severity present in the stomach.

Saliva sampling had limitations as well related to the nature of the study and the sampling itself. This study was possible due to the need for repopulation of the breeding herd at the research farm. The culling of the sows was scheduled for the end of lactation after weaning, and this became available when sows were at gestation stage already. This meant that the study had to be planned quickly. Unfortunately, because of this it was not possible to sample the saliva of all sows.

Obtaining clean saliva samples from pigs is difficult. Saliva samples were contaminated with feed (as shown by the features found in the saliva) and might have also been contaminated with urine and faeces (as sows tend to bring the straw to the dunging area and manipulate it there against the floor). This, however, is inherent to this type of studies. Madsen et al. (2022) also found features in saliva analysis coming from the feed. In future studies, if possible, an appropriate moment for sample collection should be selected to reduce the contamination, but most importantly this should be taken into account when processing and analysing the saliva samples, and interpreting the results. It would probably be very useful to develop a methodology that will reduce the presence of this particles in the samples at the moment of analysis as it might be difficult to obtain clean saliva samples.

It is worth mentioning as well that the study of gastric ulcers is made even more difficult by the little knowledge about the pathophysiology of gastric ulceration in pigs, especially, regarding the timeline of development and healing of the ulcers. This was a bigger problem for gestating sows in this study due to the gap between behavioural observations and saliva sampling with the moment of euthanasia and post-mortem assessment of the stomachs. The only solution to this challenge might be serial gastroscopic studies which would be challenging to perform, requiring repeated animal handling, sedation and anaesthesia. All of this could also disrupt the disease process being studied.

In Chapter 5, the variation in the definition of the re-directed oral behaviours and the way behaviours had been summed up in previous studies was the main limitation. It made it impossible to perform a meta-analysis of the data. Also, the sample size ended up being relatively small because of the high number of studies that did not define their behaviours and some studies being unclear in their materials and methods. The number of articles could have been increased slightly if female adult pigs that were not pregnant and young pigs were included as well. However, I decided to exclude them as this would have complicated the interpretation and discussion of the results further.

6.4 Next steps for saliva swabs for the diagnostics of gastric ulceration in pigs

Being able to diagnose gastric ulcers in pigs by means of a saliva swab test would have clear benefits for both research and the industry. This would allow investigation of the impact of gastric ulcers on the welfare of the animals but also a more accurate understanding of the impact on their performance. Although today research has been done in this matter, it is obscured by the time gap between the measurement of the variables and the post-mortem assessment of the stomachs. There is no clear knowledge of what the timeline of development and healing of the ulcers is as well as how exactly different factors impact the integrity of the pars oesophagea e.g. transport, movement of animals, fasting, but also the underlying coping style of the individual pig. Knowing exactly which animals have a gastric ulcer at a specific moment would help us to easily identify risk factors, study the impact on weight gain in finishing pigs, or understand how these affect the performance of the sow, but also to understand how they impact the subjective experience of the pigs. As such saliva swab test would allow us to identify how to improve the welfare of the pigs.

However, to get to this point there is still a lot of research to do. The study of Madsen et al. (2022) and this thesis show a promising start. Both studies showed that there is a change in saliva composition with gastric ulceration in both finishing pigs and adult female pigs during gestation and lactation, respectively. Even with different age groups and different methodologies, these studies found similar results. Compounds called oxylipins were higher in individuals with healthy stomachs or with keratinization, in the case of Madsen et al. (2022), and higher in individuals with keratinization, in the present thesis.

To move forward from this a number of steps are needed: 1) To reduce the gap between sampling and post-mortem assessment of the pars oesophagea; 2) To have a much larger sample size to get better representation of all types of lesions usually present as part of gastric ulceration; 3) To investigate the saliva composition of some completely healthy pigs; 4) To reduce sample contamination and/or find a methodology that will reduce the presence of these particle in the processed sample for analysis; and 5) To study the changes of saliva composition at various sampling points in the same pig by comparing with gastroscopic assessment of the pars oesophagea in pigs habituated to human interaction to reduce the effect of handling stress on the change of saliva composition. The latter would enable us to understand the changes with gastric ulcer progression but also the normal changes in saliva composition throughout the day.

6.5 Gastric ulcers and sustainable farming systems

Sustainability of the pig industry includes environmental, economic and societal factors as well as pig health and well-being (Vonderohe et al., 2022). Environmental sustainability of the system is affected by pig nutrition. Environmental impact has been reduced through precision feeding and dietary strategies to increase the use of nutrients by the pig. Particle size is one of the most important characteristics that impacts feed utilization in pigs (Ball et al., 2015; Wondra et al., 1995). The use of nutrients is improved with small particle size diets in pigs as shown by increased productivity (Ball et al., 2015; Nemechek et al., 2016). However, small particle size is also the main cause for gastric ulceration in pigs (Canibe et al., 2016). There might be a trade-off between particle size and gastric ulceration in terms of the sustainability of the system.

The current knowledge is that small particle size is the main risk factor for the development of gastric ulcers in pigs as reviewed by Canibe et al. (2016), and shown in multiple studies (Ayles et al., 1996; Hedde et al., 1985; Wondra et al., 1995). It is suggested that gastric ulcers have an impact on the welfare of pigs (Rutherford et al., 2018), and welfare of the pigs has a direct impact on sustainability by means of productivity but also by social acceptance (Velarde et al., 2015). Additionally, gastric ulcers have shown to impact weight gain and growth rate negatively, which clearly impacts the sustainability of the system by affecting the time to reach market weight (Dunlop et al., 2021). However, findings in this area are ambiguous. Some studies show a reduction in productivity (Ayles et al., 1996; Dunlop et al., 2021; Elbers et al., 1995; Hedde et al., 1985), but others show no effect of gastric ulcers on productivity (Dirkzwager et al., 1998; Guise et al., 1997; Wondra et al., 1995).

There is one large recent study where the impact of severe gastric ulcers on the economics of the farm was estimated in a commercial farm (Dunlop et al., 2021). They assessed the pars oesophagea of 901 pigs that were followed from 3.5 to 22.5 weeks of age, at which point they were transported and slaughtered at arrival at the abattoir (180 km away from the farm). They concluded that severe gastric ulcers have a negative impact on the economics of the farm; having severe gastric ulcers means a loss of US$15.90 per pig (if all pigs are processed at 157.5 days of age) as compared to pigs without any lesions ($p = 0.00001$). However, this estimation is based on an assumed live weight at slaughter (carcass weight x 1.28), assumed average daily feed intake from weaning to processing (4 % of the average body weight of each group after weaning), the 5-year average feed cost per tonne (USD), and the 5-year average market carcass value per kg (USD).

There is a trade-off between particle size and gastric ulcers. Small particle size seems to increase sustainability by improving feed utilization on the one side. However, by causing gastric ulcers, which may decrease daily weight gain and cause economic losses to the system, it may actually reduce or not improve sustainability in terms of productivity. Regardless of the effect of ulcers on productivity, even if on balance small particle diets are more profitable to use, the problem of ulcers might still be worth tackling as they might impact social sustainability negatively; they are not desirable as they also cause some level of discomfort/pain in the animal. At this point it is difficult to know exactly what would be more sustainable considering productivity, economics of farms and of the pig supply chain and the concerns that citizens and consumers in wider society have about affordable food but also animal welfare.

Further research needs to be done to answer this question. A suggestion is to carry out a systematic literature review on the effect of gastric ulcers and small particle size on performance and the cost associated with this. This would allow us to have a better understanding on how average daily gain, daily feed intake and feed conversion compares between two extreme scenarios: small particle size and high prevalence of gastric ulcers vs coarse particle size. It is important to note studies on the effect of gastric ulcers on the performance of the pigs are limited and ambiguous due to the difficulties regarding data collection. Hence, the importance, as well, of further investigating the development of a method of diagnosis that is both cheap and non-invasive in the living pig. To my knowledge there are no studies yet assessing the knowledge and opinion of the general public on gastric ulcers.

6.6 Further studies of the development of oral behaviours in pigs

It is important to fully understand the origin of oral behaviours in pigs to be able to tackle the underlying causes as well as help us understand their behavioural needs. This is part of providing housing and management which results in good welfare. Identifying and tackling the causes of re-directed oral behaviour and oral stereotypies is important as it affects not only the mother but the offspring probably through stress mediation. In the following paragraph, the relationship between mother's and offspring's behaviour, and stress will be discussed. Possible alternative explanations for these behaviours in pigs will be explored.

Recent research shows that stereotypies are not only important for the sow at the moment, but also, they might have an effect on the behaviour and resilience of the coming generations, affecting their welfare as well [reviewed by Tatemoto et al. (2022)]. Bernardino et al. (2016) proved that sows fed a diet high in fibre had piglets that showed less agonistic behaviours at weaning than piglets from sows that were fed on a low fibre diet. Later on, the same team investigated the differences in cortisol level in the sows and fear response in piglets at weaning coming from sham-chewing and non-sham-chewing sows (Tatemoto et al., 2019). They proved that piglets coming from mothers that were consistently sham chewing showed less fear in an open field test as opposed to piglets coming from sows that never performed sham chewing within a period of 6 days of observation. Sham-chewing sows had higher cortisol levels. One year later, Tatemoto et al. (2020) studied the effect of stereotypic behaviour in sows on the emotionality in the offspring. They found that piglets coming from high stereotyping sows walked more in the central and lateral areas of an open field test, and vocalized less in a novel object test than piglets coming from a low-stereotyping sow. Tatemoto et al. (2023) then studied the effect of stereotypic behaviour of sows on the brain of their offspring. There were differences regarding the amygdala, hippocampus and frontal cortex. The first one was affected by the stereotypies and the latter by the environment.

The changes observed in piglets' behaviour and emotionality from high-stereotypying mothers may be mediated by stress. Gestating sows housed in barren environments experience more stress as measured by cortisol level as compared to sows housed in a less barren environments (de Leeuw and Ekkel, 2004; Merlot et al., 2012), and their maternal behaviour is affected negatively by the environment and stress during gestation (Ringgenberg et al., 2012). Research shows that pre-natal stress can affect the offspring's behaviour. Jarvis et al. (2006) found that daughters from mothers that have been exposed to social stress through mixing at their second and third gestation trimester show worse maternal behaviour as well as a longer cortisol response to mixing. Rutherford et al. (2014) also found that pre-natally stressed sows show changes in the maternal behaviour linked to a change in the brain.

Stress may arise from the housing environment in the pig industry. There is a clear mismatch between the housing conditions in indoor intensive production systems and the original natural habitat of wild boar and feral pigs. This means that across all stages the behavioural and nutritional needs and preferences of pigs might not be completely satisfied. Re-directed oral behaviours may appear at early stages in the life of a pig due to factors related to diet and feeding practices, housing conditions. They may become more apparent in gestating gilts and sows as on top of the relatively barren environment they are also feed restricted to ~50% of their voluntary feed intake (Read et al., 2020).

Pigs that have been released or live in wild conditions have non-specialist omnivorous diet that is high in plant material. Feral pigs show a diet rich in different feed sources that seem to vary according to the availability of the sources themselves [reviewed by Nogueira et al. (2007)]. This review describes the feral pig as an omnivorous animal being mostly a plant eater, where 41-45% of the stomach content corresponds to different types of grasses. In a study done in New Zealand, the assessment of the stomach content of hunted feral pigs showed a wide variety of feeding sources: New Zealand giant stag beetle, earthworms, mammals, birds, plant material such as coniferous tree seeds, common rainforest vine fruit and stems, lowland forest tree fruit, evergreen hedge plant, roots and fungi (Parkes et al., 2015). The different sources of plant material ranged from 14.3 to 71.4% of the feed found in the stomachs. This shows a high variety in feeding sources but also that pigs have a complex repertoire of behaviour when it comes to foraging. Also, feral pigs spend a lot of their time grazing and rooting up pasture, bracken, forest floor or swamps (McIlroy, 1989; Rivero et al., 2019; Stolba and Wood-Gush, 1989), and show a variety of behaviours when foraging: trampling, wallowing, and rooting [Reviewed by Wehr et al. (2018)]. This shows that pigs kept under intensive indoor production systems where pens are enriched with straw but the environment itself does not vary, might in fact still be in a very impoverished environment compared to the environment in which feral co-specific live in and where they evolved as a species. These types of systems may not be addressing and fulfilling their behavioural needs, which may be causing the development of re-directed oral behaviours in finishing pigs. Opening the possibility that both hunger and barren environment in gestating sows both have a causative effect in re-directed oral behaviours.

The conflict between the pigs' captive environment and that of their wild counterpart is also shown in the chronic hunger studies done in gestating sows. Studies show that gilts and sows housed in a barren environment and/or on a restricted low-fibre diet start to develop re-directed oral behaviours that are directed towards themselves (e.g. sham chewing, tongue playing) or anything that is available to them (e.g. bar biting, chain manipulation). However, when they are provided with substrate such as straw or wood shavings, these behaviours are reduced and replaced by behaviours directed towards the enrichment material which appear more natural/'normal'. De Leeuw et al. (2003) tested the effect of providing substrate (wood shavings) and place of feeding (trough vs floor) on oral behaviours in gilts. The provision of substrate reduced the frequency of fixture and self-directed oral behaviours as compared to gilts that did not have any substrate. Spoolder et al. (1995) compared the effect of feeding level and substrate provision (straw) on the performance of chain and bar manipulation in gilts. Low fed sows that were not provided with substrate re-directed their foraging behaviour towards chain and bars, which were substituted for substrate manipulation when provided with this. This shows the high motivation sows have for foraging behaviour (increased due to hunger presumably) and that providing substrate allows them to express this type of behaviour. Whittaker et al. (1999) measured the effect of fibre level and substrate provision (straw) on behaviour in sows. They observed that sows that had access to substrate had a lower level of manipulation of pen fixtures and non-manipulative oral behaviours. Moser et al. (2019) tested the effect of different enrichment materials on the motivation for foraging by providing them one by one consecutively, and all together. Sows spent much more time in the pen that had all the materials as compared to when they were provided with only one. This shows that they are motivated in performing foraging behaviours but also that they like a variety.

Manipulative behaviours observed in in finishing pigs could be related with other conditions of ill-health affecting the upper digestive system (i.e. mouth, oesophagus and stomach). Dental and periodontal diseases (DPD) are present in weaned pigs, gilts and sows. DPD found in pigs include stains or caries, gingivitis, molar and incisor wear, fracture, missing or loose teeth, abscessation, gingival retraction, periodontitis, dental calculus, malalignment, supernumerary teeth (Ala-Kurikka et al., 2019; Engblom et al., 2008; Johnson et al., 2003; Knauer et al., 2007; Malmsten et al., 2020;

Tucker et al., 2011). The prevalence of these oral health issues ranged from 4 to 85% varying from study to study and type of lesion. The prevalence of some of these conditions (e.g. stains or caries, oral lesions, gingivitis, molar and incisor wear, and teeth fracture) is related with age, being higher in older animals [weaned pig (Tucker et al., 2011), adult pigs (Ala-Kurikka et al., 2019; Johnson et al., 2003)]. Johnson et al. (2003) also found that sows in outdoor systems had *numerically* fewer oral lesions and no signs of incisor wear as compared to sows in indoor systems. Engblom et al. (2008) suggest that the injuries that were observed in the incisors in their study may have appeared due to bar-biting behaviour while waiting for the feed, and Johnson et al. (2003) report that worn/broken teeth were painful as sows reacted to touch. It has been reported that piglets that are in pain scratch the affected area e.g. in studies investigating pain management for surgical castration in piglets (Llamas Moya et al., 2008), and piglets that have been teeth clipped show teeth champing (opening and closing of the mouth) (Noonan et al., 1994). This shows that there might be a causative relationship between re-directed oral behaviours and DPD, where the behaviour might be causing the oral health problems, as well as the oral health problems such as worn/fractured teeth may be eliciting behaviours such as bar-biting in an attempt to reduce pain, as suggested by Alvas (2018).

This thesis shows that re-directed oral behaviours, usually observed in feed-restricted female adult pigs, may be elicited by factors different than chronic hunger. I present evidence in support of the suggestion given in Chapter 1 that oral behaviours may fulfil different needs (see section 1.4). Briefly, I suggested that oral behaviours could be categorized into two groups; self-directed oral behaviours and resource-directed oral behaviours, and that these may fulfil different roles: 1) reducing the feeling of stomach discomfort and 2) finding food. While the present study did not support the idea of self-directed oral behaviours being related to stomach discomfort (for example caused by gastric ulcers) it showed that not all oral behaviours appear only due to chronic hunger as shown by Chapter 2 and 3. It is important to continue exploring the factors that elicit this type of behaviours. Re-directed oral behaviours may appear as a way to cope with stress in general. Improving whatever these sources of stress might be, may not only improve the sows' welfare but also their offspring.

6.7 Self-reflections and questions that could not be answered

My PhD was widely affected by the COVID-19 pandemics as three out four of my planned studies had to be changed. During my PhD, I learnt how to set up a study and carry it out by myself (Chapter 2 and 3) and continued to develop my skills to work within a team (Chapter 4). I learnt to identify hunger-related oral behaviours, how to score stomachs for gastric ulceration, and prepare saliva samples for long-term storage until analysis of the samples.

My training to identify oral behaviours included in my studies was varied. I had a sample of videos to look at to get familiarised with them provided by the research technician; and I visited the farm to get familiarised with the behaviours of the pigs and had a guided visit with one of the research technicians (Marianne Farish) to talk about the different behaviours and watch pigs together. Also, whenever I was not sure about a behaviour, I would record it to ask for clarification later. From my experience, oral behaviours are much easier to be observed live than in a video and are quite short lived mostly e.g. snout twitching. I think that short periods of continuous observation is a good method to score these types of behaviours as it gives you some time to catch the behaviour but also understand the context in which it happens. This is particularly important when trying to differentiate sham chewing from chewing on something.

I got training for stomach scoring by Dr Jill Thomson with a sample of ~ 60 stomachs collected from the abattoir. She also provided help whenever I was not sure about a score by assessing my back up pictures. Euthanasia and stomach scoring is not an easy task both mentally and physically, the later mostly in bigger pigs as in this thesis. I was quite shocked with the first sows that were euthanised and the process of extracting the stomach (even being a vet myself), but I then quickly was able to overcome and focus on the task. Certainly, the kindness of the people working with me helped. For the euthanasia of a sow and stomach extraction as well as moving animals to the euthanasia site at least four people are needed. I would strongly recommend getting mentally prepared beforehand.

There were some questions that my thesis could not answer because of time constraints or the context in which the studies were planned. Below a list of unanswered questions are given.

During my PhD, I was not able to address one of our questions: longitudinal study of re-directed oral behaviours from farrowing until gestation stage, and comparison between commercial and dam line. At the beginning of my PhD the idea was to carry out a study like this one, however, because of time constraints it was not possible. In particular, the Covid-19 pandemic meant that for a long period in the middle of my PhD, visits to the research farm were not allowed, preventing regular repeated observations of the same animals.

6.8 Future research topics

My findings in this thesis opens the door for new research questions worth exploring. The main aim was to study hunger-related oral behaviours and saliva composition as a method of diagnosis for gastric ulcers in pigs. The presence of these behaviours in finishing pigs and lack of relationship with gastric ulcers in juvenile and adult pigs opens the doors for new questions regarding chronic hunger and re-directed oral behaviours in sows. Saliva composition changing with the progression of the condition and the presence of a given lesion gives as a good starting point to keep exploring this as a method of diagnosis. Having reached this point, I believe the following questions would benefit from further investigation:

1. Origin and development of re-directed oral behaviours in general, and the extent to which frustrated exploration and foraging, or hunger play a part.
2. Origin and development of re-directed oral behaviours (frequency and duration) and development of conditions affecting the upper digestive system.

3. Consistency of re-directed oral behaviours during the life of a pig, and whether the number of pigs performing these increases with age.
4. Effect of sex and genetic line on the development of re-directed and in particular self-directed oral behaviours in pigs.
5. Relationship between saliva composition and ill-health conditions affecting the upper digestive system.
6. Optimization of protocols for saliva sample collection and analysis to reduce contamination with feedstuff.
7. Large scale study surveying pigs at the abattoir for saliva and upper digestive system conditions including gastric ulcers as well as feed analysis (last week before slaughter) and farming conditions

6.9 Conclusions

No evidence of a relationship was seen between gastric ulceration and oral behaviours usually observed in feed-restricted sows, and usually explained as being due to chronic hunger. Interestingly, these behaviours are also observed at an earlier productive stage and in animals that have never been feed-restricted before. Saliva composition changes with changes in the pars oesophagea. Finally, gastric ulceration might be a much bigger problem than previously thought before since gestating gilts and sows under good welfare conditions present ulcers.

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