Effect of reduced dietary protein level on finishing pigs' harmful social behaviour before and after an abrupt dietary change

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Abstract

Tail biting in pigs is a harmful behavioural disorder that negatively affects their welfare. Nutrition has played a crucial role in improving farm efficiency; however, an imbalance in key nutrients is an accepted risk factor for tail biting. The exact contributing factors and the level of risk associated with inadequate nutrition remain unclear. We aimed to assess the effect of dietary protein level on harmful social behaviours in finishing pigs before and after an abrupt dietary change. A total of 80 pigs (Duroc x (Landrace x Large White)) were assigned to this trial over two batches. Pigs were housed in mixed sex groups of 10 and assigned to one of two treatments at 12 weeks of age. These consisted of a control treatment (CON) containing 15.5% Crude Protein (CP) and a low CP treatment (LowCP) containing 13.5% CP. The abrupt dietary change occurred at 16 weeks of age (71.45kg) in batch 1 and at 15 weeks of age (67.04kg) in batch 2. This change in diet led to a 1.2% drop in CP in the CON treatment and a 1.4% drop in CP in the LowCP treatment. Behavioural observations were performed before and after the diet change and largely focused on the pigs' social behaviour. Using a "hurdle" model analysis, we investigated whether diet, diet change or sex influenced: 1) the probability that a behaviour was not performed, and 2) the frequency and/or duration when the behaviour was performed. The LowCP treatment led to an increased duration of limb-directed behaviour (P=0.03) when performed at all, and also an increased duration of ‘tail-in-mouth’ behaviour (P=0.02) in males but not females. However, LowCP did not affect the level of ear-directed behaviours in this way (P>0.05). An increase in standing frequency occurred after the abrupt diet change (P=0.04). Our study indicates that a small reduction in dietary protein and sex affect behaviour in the finishing phase, with some impacts on important harmful social behaviours. Furthermore, an effect of abrupt diet change on behaviour is suggested, which will require further confirmation.

Keywords

Finishing pig behaviour; Harmful social behaviour; Dietary protein; Dietary change

1. Introduction

With intensive pig production systems expanding throughout the world, there is growing interest in improving the lean growth potential of fattening pigs. For example, the average growth rate and feed conversion ratio of finishing pigs in the EU was reported to be 814g/d and 2.83, respectively, in 2015 and 850g/d and 2.79 in 2020 (AHDB, 2015; 2020). It is important that pigs are provided with sufficient nutrients to support this increased growth rate. Currently there is interest in reducing dietary protein supplied to fattening pigs in order to improve the efficiency of diets through better nitrogen utilisation, lower feed costs and reduced nitrogen excretion (Zhao et al., 2019).

Dietary protein supplies essential amino acids to pigs, and therefore inadequate protein intake results in suboptimal health and performance (Presto Åkerfeldt et al., 2019). It has been suggested that inadequate dietary protein can stimulate protein leverage (where a pig will attempt to regulate its protein intake by overeating a protein dilute diet) (Raubenheimer and Simpson, 2019). As a result, pigs may perform foraging and exploratory behaviour to satisfy their nutritional needs; in commercial environments where no alternative feed is available, pigs may redirect their foraging and exploratory behaviour towards penmates (Studnitz et al., 2007). There has been growing recognition of the links between insufficient dietary protein and harmful social behaviours in pigs (Van der Meer et al., 2017). Indeed, tail biting is of particular interest due to its detrimental effects on animal welfare.

Earlier studies have shown that when fed a diet low in protein, pigs' attraction to a blood-soaked tail model increased (Fraser et al., 1991; McIntyre and Edwards, 2002). While such studies indicate an association between low dietary protein and a heightened preference for blood, it appears that only one other study has investigated the effect of low dietary protein on harmful social behaviours. Van der Meer et al. (2017) lowered dietary protein during the weaner, grower and finisher phases and observed behaviour at 20 and 23 weeks of age. Their low protein treatment resulted in a significant increase in ear biting, belly nosing, other oral manipulation directed at penmates and aggression; however, only a tendency towards increased tail biting was found. In their study pigs were observed at 20 and 23 weeks of age, and at this age we could expect their protein requirement to be reduced (NRC, 2012). It is reasonable to suggest that pigs would be more affected by lower levels of dietary protein when requirements for lean tissue deposition and growth are higher. As a result, more information is required on the behavioural effects of low protein diets earlier in the finishing phase. Apart from well-known damaging behaviours such as tail and ear biting, including observations of less well-known damaging behaviours would be useful. For instance, limb biting has been identified as a welfare problem in pig farming (Bracke et al., 2012) but this behaviour is yet to be studied. In addition, information on the impact of sex on responses to low dietary protein would be beneficial. Entire male pigs and castrates have a greater potential for lean tissue deposition than females (Pauly et al., 2012), yet it is common for pigs to be reared in mixed-sex pens and offered the same diet. This suggests that adverse effects of reduced protein diets would be exacerbated in male pigs.

In addition to dietary protein level, it has been suggested that abrupt dietary transitions may also trigger harmful social behaviour in pigs (Day et al., 2002). As diet change occurs, the nutritional quality is typically also reduced, meaning pigs may experience an initial undersupply of nutrients (EFSA, 2007). As previously discussed, this undersupply of nutrients may instigate the performance of foraging and exploratory behaviour that can be redirected to penmates. Earlier studies investigating the effect of a dietary change have housed animals individually and mainly focussed on performance (Kyriazakis and Emmans, 1990). Pastorelli et al. (2012) reported that a diet change resulted in more time spent exploring the trough and more active behaviour. It is known that tail biting pigs often show increased levels of activity (Buijs and Muns, 2019), but as pigs in Pastorelli’s study were housed individually harmful social behaviours were not observed.

Moreover, the diet in this study was transitioned over three days, while in practice, diets are often changed abruptly. If abrupt dietary change triggers harmful social behaviour, it is reasonable to expect that this effect would be greater for pigs that are already on a diet that increases the risk for such behaviour (e.g. a low protein diet).

This study has three hypotheses. Firstly, we hypothesise that a low protein diet will increase harmful social behaviours in finishing pigs, particularly ear and tail biting and limb-directed behaviour. We also hypothesise that a low protein diet will result in a more marked behavioural effect in entire males than females. Lastly, we hypothesise that behavioural effects of an abrupt dietary change will be greater in pigs fed a low protein diet than a normal protein diet.

2. Methods

2.1. Ethical Note

This study was conducted at the Agri-Food and Biosciences Institute (AFBI), Hillsborough, Northern Ireland between November 2019 and April 2020. The work was carried out under Project Licence Number PPL2851 in accordance with the Animals (Scientific Procedures) Act 1986 (The Parliament of the United Kingdom, 1986).

2.2. Animals and housing

A total of 80 pigs (Duroc x (Landrace x Large White)) were assigned to this trial over two batches that were born three weeks apart. Pigs in each batch were weaned at approximately four weeks of age and reared in groups of 10 until 10 weeks of age (average weight 30.03±SEM 0.343kg). At 10 weeks of age they were transferred to the finishing accommodation and regrouped into mixed sex pens. All pigs were tail docked with veterinary authorization due to increased risk of tail biting, with approximately 50% of the tail removed within 24 hours of birth. Tail docking was performed using clippers, which were disinfected in surgical spirit between pigs. Male pigs were not castrated.

During the finishing period the pigs were housed in groups of 10 animals on plastic slatted floors. Groups were balanced for sex and body weight, and a space allowance of 0.90-0.93m^2 per pig was provided. Each group was provided with environmental enrichment in the form of a suspended wooden block and flavoured plastic biting toy (Porcichew, Nutrapet Ltd., UK). Pen temperature was set at 20 ^{o}C (19-21 ^{O}C), and housing was ventilated through fan-assisted natural ventilation. Artificial lighting was provided between 8:00 a.m. and 4.00 p.m., and pigs also had access to natural light through windows. Each pen was fitted with two nipple drinkers and feed was provided ad libitum through a single-space electronic feeding station (Schauer Compident MLP pro feeder, Prambachkirchen, Austria).

2.3. Study Design

Within each batch, two groups were assigned at random to one of two dietary treatments when pigs were 12 weeks old (43.58±SEM 0.562kg). These consisted of a control treatment (CON) and low crude protein treatment (LowCP). Within both treatments the groups were also subjected to an abrupt dietary change at 16 weeks in batch 1 (71.45kg) and at 15 weeks (67.04kg) in batch 2. In both treatments the new diet introduced ('late finisher diet') was formulated to have a lower crude protein level than the previous diet ('early finisher diet'). Details of diet formulations are provided in Table 1. Further information on the diet provided from the start of the finishing period until treatments commenced is also included in Table 1. On the days of diet change all existing feed was removed from the feeder and the new diet provided.

2.4. Behavioural assessment

Video cameras (GoPro- Hero5 Session v02.51.00, Shenzhen, China) were installed onto the ceiling above each pen on the day of recording. Pigs were spray marked with pig animal marker spray on the day that cameras were installed to allow individual identification. In batch 1 behaviour was recorded on day two before the diet change and on day seven after the diet change and in batch 2 behaviour was recorded one day before and on day four after the diet change. Following a validation trial in which pigs consumed the highest percentage of daily feed intake between 12:00 and 16:00 hours, video recordings from the first 15 minutes of each hour between 11:00 and 16:00 hours were used in the analysis. During observations, each pig's behaviour in the pen was observed continuously using the ethogram in Table 2. This equated to five 15-minute focal observations per pig per observation day. Video files were imported into BORIS version 7.9.8 (Friard and Gamba, 2016); two trained observers, who were blinded to treatment, analysed all videos and inter-rater reliability was established through a scale and reliability analysis in SPSS (Chronbach's Alpha= 0.997). Inter-rater reliability was evaluated using footage from three focal pigs observed over five 15-minute periods. For analysis, behaviours were presented as duration and frequency. If a pig ceased a behaviour at all (even momentarily) then a particular bout was deemed to have finished. This approach was applied consistently across treatments and time. Lying behaviours were collated into a ‘Lying’ group which included lying ventrally, lying laterally (left), lying laterally (right) and mixed. Similarly, tail and ear-directed behaviours were collated into ‘tail-directed behaviour’ and ’ear-directed behaviour’ groups which included tail-in-mouth behaviour, nosing/manipulating the tail and ear-in-mouth behaviour, nosing/manipulating the ear, respectively.

2.5. Statistical analysis

The unit of analysis for each behavioural variable was the mean of the five observation periods for each animal pre and post-diet change. The data tended to exhibit zero-inflation, meaning that behaviours weren't being displayed for a considerable proportion of the observation time. This necessitated the use of a hurdle model analysis (Ocepek et al., 2018). In the first part of the analysis, each responsive behaviour was modelled as a binary variable with a 1 indicating that the behaviour was not performed. This was implemented as a Bernoulli generalised linear mixed model with a logit link function. Group and Pig within Group were fitted as random effects while the fixed model consisted of a factorial arrangement of Week (referring to week pre or post-diet change), Sex and Treatment. The second part of the model was conditional on a pig performing the behaviour. A generalised linear mixed model was again fitted in this case but using a gamma distribution with a logarithm link function, which accounted for the fact that in general variables in this part tended to exhibit positive skewness. The same random and fixed effects were again fitted as in the first stage of the model. In all cases the significance of each effect ($P<0.05$) was assessed by comparing the Wald statistic for each effect against the appropriate Chi-squared distribution. For both stages of the 'hurdle' model, predictions fixed effects were calculated together with 95% confidence intervals for the predictions. Finally, the adequacy of the model fits was assessed by visual inspection of the appropriate residual plots. The separate predictions from each model were also combined to give an overall prediction for each effect for each behaviour analysed. For illustrative purposes, these are presented as supplementary material for significantly affected behaviours. An overall prediction could not be calculated for behaviours where the binomial model was not fitted (i.e., where (nearly) all observations contained the behaviour studied). However, in such cases the duration and frequency as shown by the 2nd stage of the hurdle model are very close to the true frequency and duration in the dataset as a whole (as only those rare observations lacking the behaviour would be omitted from the 2nd stage of the hurdle model).

3. Results

Main effects from the statistical analysis are presented in Table 3, while two-way interactions are presented in Table 4 (tables include test statistics and predicted values for the probability of absence, and duration and frequency of the behaviour within the subset of observations in which the particular behaviour was present).

3.1. Harmful Social Behaviours

Tail-directed behaviours

Within the subset of observations where tail-directed behaviours occurred, these behaviours were performed more frequently by females than males ($p=0.03$) (Table 3). The probability of absence of the tail-directed behaviours and the duration of these behaviours were unaffected by all of the fixed effects ($p>0.05$). The frequency of tail-directed behaviours was unaffected by week or diet ($p>0.05$).

Tail-in-mouth behaviour

Within the subset of observations containing tail-in-mouth behaviour, there was a significant interactive effect of diet and sex on its duration (Table 4). Tail-in-mouth behaviour was performed for a lower duration by males in the CON group compared to all other groups (p=0.03). The probability of absence from observations and the frequency of tail-in-mouth behaviour were unaffected by all of the fixed effects (p>0.05). In addition, the duration of time spent performing tail-in-mouth behaviour was unaffected by week (p>0.05).

Ear-directed behaviours

Within the subset of observations containing ear-directed behaviours, there was a significant effect of diet and sex: females in the LowCP group performed ear-directed behaviours more often than males in the LowCP group (p=0.02) (Table 4). There were no significant main effects on the probability that ear-directed behaviours would be absent from observations (p>0.05) (Table 3). The duration of time spent performing ear-directed behaviours was unaffected by all of the fixed effects (p>0.05), while the frequency was unaffected by week (p>0.05).

Ear-in-mouth behaviour

It was more probable that ear-in-mouth behaviour was absent from observations in the week before the diet change than in the week after the diet change (p=0.006). The probability of ear-in-mouth behaviour being absent from observations was not significantly affected by diet or sex (p>0.05). Within the subset of data where ear-in-mouth behaviour occurred, the frequency and duration of time spent performing this behaviour was unaffected by week, diet or sex (p>0.05).

Limb-directed behaviour

In the subset of data where limb-directed behaviour occurred, there was a main effect of diet: pigs in the LowCP group performed limb-directed behaviour for longer than pigs in the CON group (p=0.04) (Table 3). The duration of time spent performing limb-directed behaviour was unaffected by week and sex (p>0.05), while the frequency was unaffected by all of the fixed effects (p>0.05). Furthermore, there were no significant effects on the probability that limb-directed behaviour would not be performed (p>0.05).

3.2. General nuzzling

None of the fixed effects significantly affected the probability of general nuzzling not occurring or the frequency or duration of the behaviour when it was performed (p>0.05).

3.3 Postures

There was a main effect of week and diet on the frequency of standing (Table 3). Standing was performed more frequently after than before the diet change (p=0.04), and in the LowCP rather than the CON group (p=0.005). As lying and standing occurred in almost all observations, the 1st stage of the hurdle model could not be performed and the subset of observations where standing and lying postures occurred included nearly all data. Within this subset, the frequency and duration of lying postures were unaffected by all fixed effects (p>0.05).

4. Discussion

Using a "hurdle" model analysis, we investigated if dietary protein level and sex influenced behaviour before and after an abrupt dietary change. More specifically we analysed: 1) the probability that a behaviour was not performed, and 2) the frequency or duration of this behaviour when it was performed. This type of model provided an effective way to analyse the zero-inflated data that is often present when carrying out behavioural observations. This method of analysis has recently been applied by Ocepek et al. (2018) when evaluating whether the position of drinkers influenced areas preferred for eliminative behaviour in growing-finishing pigs. This model is particularly effective when studying harmful social behaviours as they are usually performed infrequently; they are, however, highly damaging when they occur. The second stage of the model is of particular importance as it allows us to understand the conditions that may exacerbate the performance of harmful social behaviours.

In contrast to our original hypothesis, diet did not influence the probability that behaviours involving manipulation of the tail (i.e. tail-directed behaviours and tail-in-mouth behaviour) occurred. However, we found that when such behaviours were performed, these were performed significantly longer in LowCP groups. This is in line with a previous study in which a low protein diet resulted in an increased tendency to tail bite in older groups of males (20-23 weeks (Van der Meer et al., 2017). Van der Meer et al. (2017) observed these differences in behaviour following a long-term reduction in dietary protein from weaning (24 days of age) to the end of the finishing phase (average body weight of 110kg) (starter diet:13.8%CP, grower diet:12.4%CP and finisher diet:13.2%CP). The present study is the first to show that low crude protein increases tail-in-mouth behaviour in younger males but not females (15-17 weeks). This aligns with our hypothesis that a low protein diet will result in a more marked behavioural effect in entire males than females. This may relate to the faster rate of protein deposition in males compared to females (Giles et al., 2009).

The exact mechanisms underlying the differences in tail-in-mouth behaviour remain unclear, but may be due to reduced dietary tryptophan (TRP) in the LowCP diet (LowCP early finisher: 0.05% TRP, LowCP late finisher: 0.14% TRP, CON early finisher: 0.28% TRP, CON late finisher: 0.27% TRP (Table 1)). A previous study on the effect of high dietary TRP on behaviour in female pigs found that a diet supplying 0.34% TRP compared to 0.03% TRP reduced the number of agonistic (pushing, biting and head knocking a penmate), and aggressive interactions in 3-month old gilts (Poletto et al., 2010). Harmful social behaviours were not observed in this study, however clearly TRP affected other types of penmate-directed behaviour. It is possible that in the present study the lower levels of TRP in the LowCP diet caused the increase in tail-in-mouth behaviour. Low levels of TRP may affect behaviour through limiting serotonin synthesis (Jenkins et al., 2016). A reduction in serotonin has been shown to cause a shift in neural signals towards aggression, neuroticism and impulsivity in humans (Siegel and Crockett, 2013); in pigs, this may result in harmful social behaviours. Serotonin has been shown to exacerbate neuronal responses necessary for the fine-tuning of behaviours rather than trigger or halt a specific behaviour (Bacque-Cazenave et al. (2020). Consistent with this concept, the present study has shown that the LowCP diet didn't influence the probability of tail-in-mouth behaviour occurring but seemed to exacerbate the performance of tail-in-mouth behaviour when this occurred.

Alternatively, the difference in total protein content (rather than specific amino acids) may explain the observed differences in tail manipulation. Low dietary protein leads to a compensatory increase in food intake known as protein leverage (Raubenheimer and Simpson, 2019). However, the theory of protein leverage no longer occurs at very low protein densities (Raubenheimer and Simpson, 2019). Instead, animals stop eating this unsatisfactory diet and attempt to seek higher-protein foods elsewhere. In intensive pig rearing systems where no alternative diet is available, this may result in increased foraging behaviour. In support of this, previous research has reported that diets low in protein have resulted in increased general foraging behaviours (Jensen et al., 1993). As tail biting is thought to be linked to foraging and exploratory behaviour (Taylor et al., 2010), it is reasonable to expect that low total protein would stimulate tail manipulation. However, low protein did not seem to affect other types of social exploration in the same way, as general nuzzling was not affected by diet. Nevertheless, limb-directed behaviour was affected by diet in a similar way to tail-in-mouth behaviour, which may indicate that these behaviours share a similar motivational background. This is of special interest because producers have described this behaviour as one of the main welfare problems in the pig industry (Bracke et al. (2012). Limb-directed behaviour has been described as an injurious behaviour (Bracke and Ettema (2014), yet it is seldomly included as a separate individual behaviour in behavioural studies. To the best of our knowledge, this is the first study to investigate the effects of dietary factors on this behaviour.

Females on the LowCP diet performed ear-directed behaviours significantly more often than males on the LowCP diet. However, there were no significant differences between females and males on the control diet, between LowCP and CON males or between LowCP and CON females. This contradicts our hypotheses that ear-directed behaviours would be performed more by pigs on the LowCP diet and would be especially frequent in males on this diet. In fact, ear-directed behaviours were numerically the least frequent in males on the LowCP diet. Although it is difficult to explain this unexpected effect, the fact that our hypotheses on the effect of low protein were upheld for tail-in-mouth behaviour but not for ear-directed behaviours suggest these behaviours are not always correlated in their levels of performance (Beattie et al., 2005), and could thus be affected differently by factors such as diet or sex.

Another objective of this study was to identify if an abrupt dietary change affected behaviour to a greater extent when fed a LowCP diet compared to a CON diet (the diet change involved reducing CP from 15.5% to 14.3% for CON groups and from 13.5% to 12.1% for LowCP groups, at 15-16 weeks of age). In commercial practice, dietary changes during the finishing period are common and these have been suggested to trigger harmful social behaviour (Day et al., 2002) due to an initial undersupply of nutrients (EFSA, 2007). We had expected any effect of diet change on behaviour to be exacerbated in our LowCP groups, as they would experience a greater deficit in protein after diet change than the CON groups. In contrast to our expectations, the low protein diet did not result in a greater increase in harmful social behaviour after the diet change. In fact, we found no support for the theory that dietary change triggers harmful social behaviour at all, as no post-change peak in harmful social behaviour was observed. However, our experiment did not include control groups that did not undergo an abrupt dietary change. Therefore, we cannot definitively conclude that dietary change did not trigger harmful social behaviour, as it is possible that such an effect may have been counteracted by changes in behaviour over time. Ear-in-mouth behaviour did differ between pre- and post-change observations, but the direction of the effect was opposite to our expectations: ear-in-mouth was more likely to be absent from post-change observations than from pre-change ones (as shown by the first stage of the hurdle model). The only finding that supported the theory that dietary change negatively affects behaviour was the increased post-change standing frequency, , as it may indicate increased restlessness (Rostagno et al., 2011). Under natural conditions, pigs are opportunistic omnivores and therefore would have variable diets. However, under commercial conditions, diets are highly uniform for long periods of time until often abruptly changed to a lower specification. There have been remarkably few studies on the effect of this, and this is the first time that effects on a potential indicator of restlessness have been shown, which may indicate decreased welfare in the direct post-change period. However, further research including control groups that did not undergo an abrupt dietary change is required to conclude this.

This study also aimed to assess the effect of sex on harmful social behaviours. Interactions with diet are described above, and additional sex effects were limited. When tail-directed behaviour occurred it was performed more frequently by females, and this is in line with previous research (Schrøder-Petersen et al., 2003; Zonderland et al., 2010).

5. Conclusion

This study shows that a difference in protein inclusion and sex affected harmful social behaviour in finishing pigs, whereas no evidence was found that low dietary protein exacerbated the effects of abrupt dietary change. The use of a hurdle model allowed us to identify whether the behaviour was performed at all, and to assess how often and for how long it was performed once it did occur. Protein inclusion level predominantly affected the persistence of harmful social behaviour in male but not female pigs (i.e., the amount performed when the behaviour is triggered by another cause). Harmful social behaviour was not more common after the change in diet than prior to this change, but pigs did stand more after the change. Limb-directed behaviour was identified as an understudied harmful social behaviour that may share a motivational background with tail-in-mouth behaviour. Further research is required on how dietary protein interacts with other factors such as serotonin synthesis, lean tissue deposition potential and protein leverage to collectively cause a certain level of harmful social behaviour.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

AHDB 2015. 2015 Pig Cost of Production in Selected Countries. Warwickshire.

AHDB 2020. 2020 Pig Cost of Production in Selected Countries. Warwickshire.

BACQUE-CAZENAVE, J., BHARATIYA, R., BARRIERE, G., DELBECQUE, J. P., BOUGUIYOUD, N., DI GIOVANNI, G., CATTAERT, D. & DE DEURWAERDERE, P. 2020. Serotonin in Animal Cognition and Behavior. *International Journal of Molecular Sciences,* 21.

BEATTIE, V. E., BREUER, K., O'CONNELL, N. E., SNEDDON, I. A., MERCER, J. T., RANCE, K. A., SUTCLIFFE, M. E. M. & EDWARDS, S. A. 2005. Factors identifying pigs predisposed to tail biting. *Animal Science,* 80**,** 307-312.

BRACKE, M. B. M., DE LAUWERE, C. C., WIND, S. M. M. & ZONERLAND, J. J. 2012. Attitudes of Dutch Pig Farmers Towards Tail Biting and Tail Docking. *Journal of Agricultural and Environmental Ethics,* 26**,** 847-868.

BRACKE, M. B. M. & ETTEMA, K. 2014. Pigs suffering from injurious behaviours like flank biting and tail biting are more interested to manipulate a novel rope than uninjured control animals. *Measuring Behaviour.* Wageningen, Netherlands.

BRANDT, P., HAKANSSON, F., JENSEN, T., NIELSEN, M. B. F., LAHRMANN, H. P., HANSEN, C. F. & FORKMAN, B. 2020. Effect of pen design on tail biting and tail-directed behaviour of finishing pigs with intact tails. *Animal,* 14**,** 1034-1042.

BUIJS, S. & MUNS, R. 2019. A Review of the Effects of Non-Straw Enrichment on Tail Biting in Pigs. *Animals (Basel),* 9**,** 824.

CHOU, J.-Y., D'EATH, R. B., SANDERCOCK, D. A., WARAN, N., HAIGH, A. & O'DRISCOLL, K. 2018. Use of different wood types as environmental enrichment to manage tail biting in docked pigs in a commercial fully-slatted system. *Livestock Science,* 213**,** 19-27.

DAY, J. E. L., BURFOOT, A., DOCKING, C. M., WHITTAKER, X., SPOOLDER, H. A. M. & EDWARDS, S. A. 2002. The effects of prior experience of straw and the level of straw provision on the behaviour of growing pigs. *Applied Animal Behaviour Science,* 76**,** 189-202.

EFSA 2007. The risks associated with tail biting in pigs and possible means to reduce the need for tail docking considering the different housing and husbandry systems - Scientific Opinion of the Panel on Animal Health and Welfare. 5**,** 611.

FRASER, D., BERNON, D. E. & BALL, R. O. 1991. Enhanced attraction to blood by pigs with inadequate dietary protein supplementation. *Canadian Journal of Animal Science,* 71**,** 611-619.

GILES, L. R., EAMENS, G. J., ARTHUR, P. F., BARCHIA, I. M., JAMES, K. J. & TAYLOR, R. D. 2009. Differential growth and development of pigs as assessed by X-ray computed tomography1,2. *Journal of Animal Science,* 87**,** 1648-1658.

JENKINS, T., NGUYEN, J., POLGLAZE, K. & BERTRAND, P. 2016. Influence of Tryptophan and Serotonin on Mood and Cognition with a Possible Role of the Gut-Brain Axis. *Nutrients,* 8**,** 56.

JENSEN, M. B., KYRIAZAKIS, I. & LAWRENCE, A. B. 1993. The activity and straw directed behaviour of pigs offered foods with different crude protein content. *Applied Animal Behaviour Science,* 37**,** 211-221.

KYRIAZAKIS, I. & EMMANS, G. 1990. The immediate effects of abrupt diet composition changes in young pigs. *The British Journal of Nutrition,* 64**,** 619-23.

MCINTYRE, J. & EDWARDS, S. A. 2002. An investigation into the effect of different protein and energy intakes on model tail chewing behaviour of growing pigs. *Applied Animal Behaviour Science,* 77**,** 93-104.

NRC 2012. *Nutrient Requirements of Swine*, The National Academies Press.

OCEPEK, M., GOOLD, C. M., BUSANČIĆ, M. & AARNINK, A. J. A. 2018. Drinker position influences the cleanness of the lying area of pigs in a welfare-friendly housing facility. *Applied Animal Behaviour Science,* 198**,** 44-51.

PASTORELLI, H., LE FLOC'H, N., MERLOT, E., MEUNIER-SALAÜN, M. C., VAN MILGEN, J. & MONTAGNE, L. 2012. Sanitary housing conditions modify the performance and behavioural

response of weaned pigs to feed- and housing-related stressors. *Animal (Cambridge, England),* 6**,** 1811-1820.
PAULY, C., LUGINBÜHL, W., AMPUERO, S. & BEE, G. 2012. Expected effects on carcass and pork quality when surgical castration is omitted — Results of a meta-analysis study. *Meat Science,* 92**,** 858-862.
POLETTO, R., MEISEL, R. L., RICHERT, B. T., CHENG, H.-W. & MARCHANT-FORDE, J. N. 2010. Aggression in replacement grower and finisher gilts fed a short-term high-tryptophan diet and the effect of long-term human–animal interaction. *Applied Animal Behaviour Science,* 122**,** 98-110.
PRESTO ÅKERFELDT, M., LINDBERG, J. E., GÖRANSSON, L. & ANDERSSON, K. 2019. Effects of reducing dietary content of crude protein and indispensable amino acids on performance and carcass traits of single-phase- and 2-phase-fed growing-finishing pigs. *Livestock Science,* 224**,** 96-101.
RAUBENHEIMER, D. & SIMPSON, S. J. 2019. Protein Leverage: Theoretical Foundations and Ten Points of Clarification. *Obesity* 27**,** 1225-1238.
ROSTAGNO, M. H., EICHER, S. D. & LAYJR, D. C. 2011. Immunological, Physiological, and Behavioral Effects of Salmonella enterica Carriage and Shedding in Experimentally Infected Finishing Pigs. *Foodborne Pathogens and Disease,* 8**,** 623-630.
RUTHERFORD, K. M. D., THOMPSON, C. S., THOMSON, J. R., LAWRENCE, A. B., NIELSEN, E. O., BUSCH, M. E., HAUGEGAARD, S. & SANDØE, P. 2018. A study of associations between gastric ulcers and the behaviour of finisher pigs. *Livestock Science,* 212**,** 45-51.
SCHRØDER-PETERSEN, D., SIMONSEN, H. & LAWSON, L. 2003. Tail-in-mouth Behaviour Among Weaner Pigs in Relation to Age, Gender and Group Composition Regarding Gender. *Acta Agriculturae Scandinavica,* 53.
SIEGEL, J. Z. & CROCKETT, M. J. 2013. How serotonin shapes moral judgment and behavior. *Annals of the New York Academy of Sciences,* 1299**,** 42-51.
STUDNITZ, M., JENSEN, M. B. & PEDERSEN, L. J. 2007. Why do pigs root and in what will they root?: A review on the exploratory behaviour of pigs in relation to environmental enrichment. *Applied Animal Behaviour Science,* 107**,** 183-197.
TAYLOR, N. R., MAIN, D. C. J., MENDL, M. & EDWARDS, S. A. 2010. Tail-biting: A new perspective. *The Veterinary Journal (1997),* 186**,** 137-147.
VAN DER MEER, Y. V., GERRITS, W. J. J., JANSMAN, A. J. M., KEMP, B. & BOLHUIS, J. E. 2017. A link between damaging behaviour in pigs, sanitary conditions, and dietary protein and amino acid supply. *PLoS One,* 12.
VAN STAAVEREN, N., TEIXEIRA, D. L., HANLON, A. & BOYLE, L. A. 2015. The Effect of Mixing Entire Male Pigs Prior to Transport to Slaughter on Behaviour, Welfare and Carcass Lesions. *PLOS ONE,* 10**,** e0122841.
ZHAO, Y., TIAN, G., CHEN, D., ZHENG, P., YU, J., HE, J., MAO, X. & YU, B. 2019. Effects of varying levels of dietary protein and net energy on growth performance, nitrogen balance and faecal characteristics of growing-finishing pigs. *Revista Brasileira de Zootecnia,* 48.
ZONDERLAND, J. J., BRACKE, M. B. M., DEN HARTOG, L. A., KEMP, B. & SPOOLDER, H. A. M. 2010. Gender effects on tail damage development in single- or mixed-sex groups of weaned piglets. *Livestock Science,* 129**,** 151-158.

Table 1:
Analysed composition of diets and ingredient list (including vitamins, trace elements and medications).

Item (%)		*Control Treatment*		*Low Crude Protein Treatment*	
	Grower diet (Fed at 10-12 weeks of age)*	Early finisher	Late finisher	Early finisher	Late finisher
Protein	17.50	15.50	14.30	13.50	12.10
Oil	5.50	3.89	4.11	3.80	3.71
Fibre	3.00	3.30	3.60	3.70	4.60
Ash	4.50	4.00	4.00	4.00	3.90
Lysine	1.20	1.10	1.04	1.02	0.98
Methionine	0.40	0.34	0.30	0.33	0.31
Threonine	0.78	0.67	0.62	0.62	0.59
Tryptophan	0.25	0.28	0.27	0.05	0.14
Isoleucine	0.66				
Valine	0.76				
DE (MJ/kg)	15.00				
NE (MJ/kg)	10.70				
Gross energy MJ/kg		16.48	16.31	15.93	16.22
Dry matter		88.30	88.40	88.00	88.10
Ingredient					
Barley	✓	✓	✓	✓	✓
Wheat	✓	✓	✓	✓	✓
Maize	✓	✓	✓	✓	✓
Hi-Q DDGS		✓	✓	✓	✓
Milled pollard		✓	✓	✓	✓
Rapeseed extract	✓	✓	✓	✓	✓
G.M. hipro soya	✓	✓	✓	✓	✓
Soya oil	✓	✓	✓	✓	✓
Fine limestone	✓	✓	✓	✓	✓
Mono DCP	✓	✓	✓	✓	✓
Salt	✓	✓	✓	✓	✓
Amino Acids	✓	✓	✓	✓	✓
Vitamin and mineral premix	✓				
Finisher vitamin and mineral premix		✓	✓	✓	✓
vitamin and mineral base mix	✓	✓	✓	✓	✓

The grower and finisher diets were commercially manufactured by Thompsons (Belfast, N.I.). The exact diet formulation cannot be disclosed due to commercial confidentiality, but a tick represents the presence of the material in the diet.
***Grower diet figures are based on the formulated composition.**

Table 2:
Ethogram used to assess behaviour of pigs in the finishing phase (adapted from Brandt et al. (2020); Chou et al. (2018); Rutherford et al. (2018); Van Staaveren et al. (2015).

Posture	Description
Lying Ventrally	Sternum in contact with floor and belly partially or completely concealed—body axis vertical (± 90°).
Lying laterally (right)	Recumbent, shoulder and pelvis in contact with the ground, with legs extended, body axis is > 45° away from vertical, belly exposed. Lying on right side.
Lying laterally (left)	Recumbent, shoulder and pelvis in contact with the ground, with legs extended, body axis is > 45° away from vertical, belly exposed. Lying on left side.
Mixed	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.
Standing	Pig standing upright on all four feet with the body fully lifted off the floor surface.
Penmate directed behaviour	
Tail-in-mouth	Taking the tail of a penmate into the mouth: this may range from holding the tail in its mouth to chewing/biting of the tail.
Nosing/manipulating the tail	The pig manipulates the tail of another pig by moving it around using its snout, but the tail does not enter the mouth.
Ear-in-mouth	The pig visibly has the ear of a penmate in its mouth: ranges from holding the ear in its mouth to the ear being bitten or chewed.
General nuzzling of penmates	Moving the snout across the belly, head, back or sides of another pig, this includes gentle or vigorous manipulation directed to the belly, head, back or sides of the pig.
Limb-directed behaviour	Manipulating the legs or feet of another pig including moving the legs around using the head or snout or placing legs or feet in the mouth which may range from holding in the mouth to chewing or gently manipulating.

[*] ***Postures and penmate directed behaviours were not mutually exclusive***

Table 3. Summary of main effects for the probability that behaviours would be completely absent from observations and the frequency and duration of behaviours within the subset of data where they occurred during the finishing period of pigs offered low crude protein (LowCP) (n=39) or control (CON) (n=40) diets.

Behaviour	**Fixed Effects[1]**						**Comparison between means (mean (±95%CI))**	
Tail-directed	Diet		Sex		Week			
	Wald statistic	P-Value	Wald statistic	P-Value	Wald statistic	P-Value		**Frequency[2]**
Probability of absence in sample	0.58	0.45	2.50	0.11	1.03	0.31	Males	0.56 (0.35-0.91)
Frequency when it does occur	0.77	0.38	4.58	**0.03**	0.27	0.61	Females	1 (0.66-1.55)
Duration when it does occur	1.53	0.22	3.60	0.06	0.58	0.45		
Tail-in-mouth	Diet		Sex		Week			
	Wald statistic	P-Value	Wald statistic	P-Value	Wald statistic	P-Value		**Duration**
Probability of absence in sample	0.59	0.44	2.88	0.09	2.12	0.15	Subject to interaction, see Table 4.	
Frequency when it does occur	4.17	0.05	0.25	0.62	0.25	0.62		
Duration when it does occur	6.25	**0.02**	0.62	0.44	3.50	0.07		
Ear-directed	Diet		Sex		Week			
	Wald statistic	P-Value	Wald statistic	P-Value	Wald statistic	P-Value		**Frequency**
Probability of absence in sample	0.06	0.80	0.45	0.50	1.72	0.19	Subject to interaction, see Table 4.	
Frequency when it does occur	0.01	0.93	2.06	0.15	0.57	0.45		
Duration when it does occur	0.06	0.80	0.45	0.50	1.72	0.19		
Ear-in-mouth	Diet		Sex		Week			
	Wald statistic	P-Value	Wald statistic	P-Value	Wald statistic	P-Value		**Probability of absence in sample**
Probability of absence in sample	0.21	0.64	2.08	0.15	7.41	**0.006**	Pre-change	0.4 (0.24-0.58)
Frequency when it does occur	0.47	0.50	0.12	0.74	0.00	0.95	Post-change	0.63 (0.45-0.79)
Duration when it does occur	0.20	0.65	0.33	0.56	2.80	0.09		
Limb-directed behaviour	Diet		Sex		Week			
	Wald statistic	P-Value	Wald statistic	P-Value	Wald statistic	P-Value		**Duration**
Probability of absence in sample	1.35	0.25	0.46	0.50	0.03	0.86	Control	1.02 (0.78-1.33)
Frequency when it does occur	1.45	0.23	0.01	0.94	0.07	0.79	LowCP	1.59 (1.23-2.05)
Duration when it does occur	4.46	**0.04**	0.03	0.86	1.73	0.19		
General nuzzling	Diet		Sex		Week			
	Wald statistic	P-Value	Wald statistic	P-Value	Wald statistic	P-Value		
Probability of absence in sample	NA	NA	NA	NA	NA	NA		
Frequency when it does occur	0.08	0.78	2.14	0.14	3.52	0.06		

Duration when it does occur	2.50	0.11	0.97	0.33	1.36	0.24		

Lying	Diet		Sex		Week			
	Wald statistic	P-Value	Wald statistic	P-Value	Wald statistic	P-Value		
Probability of absence in sample	NA	NA	NA	NA	NA	NA		
Frequency when it does occur	0.79	0.38	0.36	0.55	0.41	0.52		
Duration when it does occur	0.00	0.98	0.06	0.80	0.28	0.60		

Standing	Diet		Sex		Week			
	Wald statistic	P-Value	Wald statistic	P-Value	Wald statistic	P-Value		**Frequency**
Probability of absence in sample	NA	NA	NA	NA	NA	NA	Pre-change	1.23 (0.94-1.62)
Frequency when it does occur	7.73	**0.005**	1.19	0.28	4.35	**0.04**	Post-change	1.71 (1.3-2.25)
Duration when it does occur	0.01	0.93	0.01	0.91	3.38	0.07	Control	1.06 (0.77-1.45)
							LowCP	1.99 (1.45-2.75)

[1]Significant P-values for fixed effects are indicated in bold (P≤0.05)
[2]Mean values represent the average occurrence (frequency) and average percent of time (duration) each behavioural element was performed (in the subset of data where it occurred).
***The frequency and duration of each behavioural element are not normal frequencies but rather indicate each behavioural element's frequency and duration where the behaviour occurred.**

Table 4. Summary of two-way interactions for the probability that behaviours would be completely absent from observations and the frequency and duration of behaviours within the subset of data where they occurred during the finishing period of pigs in low crude protein (LowCP) (n=39) or control (CON) (n=40) diets.

Behaviour	**Fixed effects[1]**						**Predicted means of significant effects (mean (±95%CI))**	
Tail-directed behaviours	Diet x Sex		Diet x Week		Week x Sex			
	Wald statistic	P-Value	Wald statistic	P-Value	Wald statistic	P-Value		
Probability of absence in sample	0.64	0.42	0.69	0.41	0.09	0.77		
Frequency when it does occur	1.70	0.19	0.18	0.68	0.01	0.92		
Duration when it does occur	3.29	0.07	0.20	0.66	2.70	0.10		
Tail-in-mouth	Diet x Sex		Diet x Week		Week x Sex			
	Wald statistic	P-Value	Wald statistic	P-Value	Wald statistic	P-Value		**Duration[2]**
Probability of absence in sample	1.69	0.19	0.02	0.89	1.03	0.31	Males - Control	0.09 (0.02-0.36)[a]
Frequency when it does occur	0.42	0.52	0.05	0.82	0.03	0.87	Females - Control	0.58 (0.32-1.05)[b]
Duration when it does occur	5.51	**0.03**	0.26	0.44	0.07	0.79	Males - LowCP	0.86 (0.43-1.72)[b]
							Females - LowCP	0.79 (0.42-1.5)[b]
Ear-directed behaviours	Diet x Sex		Diet x Week		Week x Sex			
	Wald statistic	P-Value	Wald statistic	P-Value	Wald statistic	P-Value		**Frequency[2]**
Probability of absence in sample	0.07	0.79	0.34	0.56	0.16	0.69	Males - Control	0.92 (0.6-1.43)[ab]
Frequency when it does occur	5.54	**0.02**	2.30	0.13	0.25	0.62	Females - Control	0.76 (0.51-1.14)[ab]
Duration when it does occur	0.07	0.79	0.34	0.56	0.16	0.69	Males - LowCP	0.53 (0.35-0.82)[a]
							Females - LowCP	1.1 (0.73-1.65)[b]
Ear-in-mouth	Diet x Sex		Diet x Week		Week x Sex			
	Wald statistic	P-Value	Wald statistic	P-Value	Wald statistic	P-Value		
Probability of absence in sample	0.16	0.69	0.01	0.91	0.04	0.84		
Frequency when it does occur	1.75	0.19	1.12	0.30	0.39	0.54		
Duration when it does occur	2.89	0.09	1.16	0.28	3.50	0.06		
Limb-directed behaviour	Diet x Sex		Diet x Week		Week x Sex			
	Wald statistic	P-Value	Wald statistic	P-Value	Wald statistic	P-Value		
Probability of absence in sample	0.40	0.53	2.60	0.11	0.11	0.75		
Frequency when it does occur	0.65	0.42	1.32	0.25	0.07	0.80		
Duration when it does occur	3.82	0.05	3.85	0.05	2.18	0.14		
General nuzzling	Diet x Sex		Diet x Week		Week x Sex			
	Wald statistic	P-Value	Wald statistic	P-Value	Wald statistic	P-Value		
Probability of absence in sample	NA	NA	NA	NA	NA	NA		
Frequency when it does occur	2.33	0.13	0.00	0.98	0.48	0.49		
Duration when it does occur	3.24	0.07	2.32	0.13	0.27	0.61		
Lying	Diet x Sex		Diet x Week		Week x Sex			
	Wald statistic	P-Value	Wald statistic	P-Value	Wald statistic	P-Value		
Probability of absence in sample	NA	NA	NA	NA	NA	NA		

Frequency when it does occur	0.25	0.62	0.02	0.88	0.04	0.85
Duration when it does occur	0.01	0.92	0.12	0.73	0.00	0.95

Standing	Diet x Sex		Diet x Week		Week x Sex	
	Wald statistic	P-Value	Wald statistic	P-Value	Wald statistic	P-Value
Probability of absence in sample	NA	NA	NA	NA	NA	NA
Frequency when it does occur	2.14	0.14	2.59	0.11	1.73	0.19
Duration when it does occur	0.17	0.68	1.66	0.20	0.34	0.56

[1]Significant P-values for fixed effects are indicated in bold (P=<0.05)

[2]Mean values represent the average occurrence (frequency) and average percent of time (duration) each behavioural element was performed (in the subset of data where it occurred).

***The frequency and duration of each behavioural element are not normal frequencies but rather indicate each behavioural element's frequency and duration where the behaviour occurred.**

Pairwise comparisons for individual means are carried out on the scale of the logit link function for the binomial analysis or on the log transformed variables for the continuous part of the analysis.

[a,b,c] Means with different superscripts differ significantly at P <0.05.