Short communication: Applicability of feeding behaviour traits as high-throughput phenotyping methods for identifying protein-efficient pigs

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Short title: Feeding behaviour to select protein-efficient pigs

Abstract

The objective of this study was to explore the potential of using automatically recorded feeding behaviour as a proxy for protein efficiency (PE) by investigating the relationship between feeding behaviour and PE. A total of 402 Swiss Large White pigs were used in this experiment (204 females and 198 castrated males). Pigs were fed ad libitum on a reduced protein diet (80% of standard) from 20kg to 100kg BW. Individual daily feed intake was monitored and carcass composition at slaughter was determined by dual-energy X-ray absorptiometry (DXA). The PE was calculated as the ratio of protein in the carcass (estimated by DXA) to the total protein consumed. Feeding behaviour traits monitored were daily feed intake (DFI; g/day), feed intake per visit (FIV; g/visit), number of daily visits (NDV; visits/day), duration of visits (DUV; min/visit), feeding rate (FR; g/min), and feeder occupation (FO; min/day). Regression analysis was used to estimate the relationship between PE and feeding behaviour, while correcting for the effects of sex, experimental series and age. Weak Pearson's correlations (-0.25 to 0.12) were found between PE and feeding behaviour traits. Beta (β) estimates from this analysis for feeding behaviours were also very low (0.0093% to 0.087%). An increase in FR (g/min) will increase PE by 0.087%

and an increase in DFI (g/day) will decrease PE by 0.0093%. In conclusion, feeding behaviours are not suitable for the identification of protein-efficient pigs, as estimates are negligible.

Keyword

Swine, protein retention, nitrogen, environmental pollution, carcass

Implications

This study suggests that feeding behaviour traits recorded via automatic feeder are not reliable predictors of protein efficiency in Swiss Large White pigs receiving a protein-reduced diet. Despite the large differences in protein efficiency, only negligible changes in a range of feeding behaviours were observed. Hence, feeding behaviours are not suitable proxies for the high-throughput phenotyping of protein efficiency and the selection of live animals for use in nutrition experiments or for breeding.

Introduction

Protein is an important nutrient for livestock, necessary for growth, building and repair of tissues, production of milk, and immune functions. However, less than 50% of ingested dietary protein in pigs is converted into carcass muscle (Kasper et al., 2020a; and Millet et al., 2018). The excess dietary protein that is not converted to meat or used for maintenance cannot be stored in the body for future use but it is rather excreted as waste, thereby contributing to environmental pollution (Wang et al., 2018). Given the growing human demand for animal protein worldwide, limited arable land for livestock production, competition between humans and livestock for plant protein, and the need to preserve the

environment, there is a pressing need to maximize protein efficiency (PE) of livestock. Both genetic (Shirali et al., 2013; Kasper et al., 2020a) and non-genetic (e.g. nutrition and weight at slaughter; Ruiz-Ascacibar et al., 2017) means are currently being considered in improving protein efficiency in pigs. However, PE is a difficult trait to measure due to costs and time involved, and the necessity to anesthetize or slaughter the animals. PE can be measured chemically, which is laborious and costly. An alternative is the use of modern imaging technology, such as DXA (dual-energy X-ray absorptiometry) (Kasper et al., 2020b), which has, after the initial investment, lower operating costs, but still requires a considerable amount of human resources for image acquisition and processing. Preferably, a proxy can be used as a high-throughput phenotyping method for the selection of protein-efficient pigs, which is ideally recorded automatically, in a non-destructive fashion and/or in the course of standard rearing conditions. Such a proxy could be feeding behavior (Ding et al., 2018) that allows the evaluation and selection of animals during their lifetimes without the need for slaughter. Previous studies have reported phenotypic correlations of -0.01 to 0.35 between feed conversion ratio (FCR) and feeding behaviour traits (Carcò et al., 2018; Kavlak and Uimari 2019), and slightly higher correlations of 0.147 to 0.408 were reported between feeding behaviour traits and protein retention (Carcò et al., 2018). In addition, an increasing number of farms are using computerized feeding systems that automatically record individual feeding patterns without disturbing the animals. These feeding systems allow for easy evaluation of feeding behaviour traits and facilitates the collection of large amounts of data required for genetic experiments. Thus, if adequate and consistent correlations between feeding behaviour and protein efficiency exist, feeding behaviour can be used as a proxy trait for PE to select protein-efficient pigs.

Materials and Methods

Protein efficiency and feeding behaviours

A total of 402 Swiss large white pigs ($N_{females}$ = 204, $N_{castrated\text{-}males}$ = 198) from eight farrowing series were used. The pigs were fed ad libitum a starter diet from weaning until 20 kg BW, and grower and finisher diet from 20 to 60 and 60 to 100 kg BW, respectively. The starter diet was formulated according to the current Swiss feeding recommendations for swine, whereas the levels of digestible protein and essential amino acids were 20% lower than the recommended levels (Ruiz-Ascacibar et al., 2017). Pigs were slaughtered at a BW of approximately 100 kg and the left carcass side including the whole head were scanned with dual-energy X-ray absorptiometry (GE Lunar i-DXA, GE Medical Systems, Glattbrugg, Switzerland), which estimates, among other things, lean tissue content. The lean tissue content obtained from DXA was used in the following calibration equation to estimate the protein content retained in animal's carcass (Kasper et al., 2020). .

$$\boldsymbol{g\ protein\ carcass} = \mathbf{-482.745 + 0.23} \times (\mathbf{g\ lean\ tissue\ DXA\ x\ 2})$$

Protein efficiency of the carcass was thereafter calculated as the ratio of protein retained in the carcass (corrected for protein content at 20 kg BW) to the total protein intake.

$$\boldsymbol{protein\ efficiency} = \frac{\boldsymbol{g\ protein\ carcass - g\ protein\ start}}{\boldsymbol{g\ protein\ intake}}$$

Protein content of pigs at 20 kg BW was estimated by multiplying the actual live weight of pigs when the reduced-protein diet experiment started with a chemically determined

102 protein content per kg carcass (protein/kg carcass) of piglets slaughtered at 21.26 ± 1.59
103 kg in a previous experiment (*g protein start*) (Kasper et al., 2020).
104
105 The time of feeding, duration of feeding and quantity of feed consumed per visit was
106 recorded for each animal with an automatic feeder as described earlier (Ruiz-Ascacibar
107 et al., 2017). Record of visits with no feed intake (i.e. 0 g) were removed from the dataset.
108 In total, 702,162 visits were recorded for the 402 pigs. Feeding behaviour traits evaluated
109 were daily feed intake (DFI; g/day), number of daily visits to the feeder (NDV; visits/day),
110 feed intake per visit (FIV; g/visit), duration per visit (DUV; min/visit), feeding occupation
111 (FO; min/day), and feeding rate (FR; g/min/day). Feeding behaviour traits were computed
112 individually, and the mean of each individual was used for further analysis.
113
114 *Statistical analysis*
115 Data were analyzed with R software V 3.6.3. The relationships between protein efficiency
116 and feeding behaviour traits were estimated with linear models, and corrected for the
117 effects of sex, experimental series and age at slaughter. However, due to the high
118 correlations between some of the feeding behaviour traits (Pearson correlation
119 coefficients r = -0.84 to 0.92, $p < 0.01$), residuals of FIV (rFIV), residuals of FO (rFO) and
120 the residuals of DUV (rDUV) were used in the final regression model. rFIV was the linear
121 regression of NDV, DUV and DFI on FIV, rFO was the linear regression of DUV, DFI and
122 FR on FO, and rDUV was the linear regression of NDV, DFI, FR, FO, and FIV on DUV.
123
124
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Results

Means and standard deviation of PE and feeding behaviour traits are presented in Table 1. Pearson's correlation coefficients between PE and feeding behaviour traits were weak, and varied between -0.25 to 0.12 (Table 1). Negative Pearson's correlations with PE were observed for DFI, FIV, DUV and FO (i.e. higher daily feed intake, feed intake per visits, durations at visits, and feeding occupation is associated with lower PE), and positive correlations with PE were observed for NDV and FR (i.e. more daily visits and higher feeding rate is associated with higher PE).

Table 1: Descriptive statistics (mean ± SD) and Pearson's correlation coefficients of protein efficiency and feeding behaviour traits in pigs

Trait	Units	Mean ± SD	Pearson's r	P-value
BW start	kg	22.63 ± 1.60		
BW slaughter	kg	107.06 ± 3.97		
Protein efficiency		0.38 ± 0.03		
FR	g/min	38.44 ± 6.37	0.12	0.019
FIV	g/visits	171.21 ± 69.3	-0.13	0.007
DFI	g/day	2027.64 ± 188.79	-0.25	<0.001
FO	min/day	54.96 ± 10.16	-0.18	<0.001
NDV	visits/day	13.82 ± 5.73	0.03	0.489
DUV	min/visit	4.63 ± 1.99	-0.13	0.007

Multiple linear regression analysis revealed significant relationships of PE with FR, rFIV, DFI, and NDV (Table 2). A one-unit increase in FR was associated with an increase in PE by 0.087%, a one-unit increase in FIV decreased PE by 0.0168%, and a unit increase in DFI decreased PE by 0.0093%. Despite their significant difference from zero, these slopes were very flat (Figure 1).

146 **Table 2:** Beta estimates and standard error of feeding behaviour traits on protein efficiency using
147 multiple linear regression, corrected for sex, experimental series and age at slaughter

Term		Beta estimate	Standard error	P-value	Adjusted R^2
Intercept		7.36×10^{-1}	4.24×10^{-2}	2×10^{-16}	0.30
Feeding behaviour	FR	8.71×10^{-4}	1.92×10^{-4}	0.008	
	rFIV	-1.68×10^{-4}	3.94×10^{-5}	0.035	
	DFI	-9.28×10^{-5}	1.03×10^{-5}	2×10^{-16}	
	rFO	$-3.19\text{x}10^{-4}$	3.92×10^{-4}	0.444	
	NDV	5.00×10^{-4}	3.92×10^{-4}	0.047	
	rDUV	4.64×10^{-3}	4.03×10^{-3}	0.759	
Age		-1.22×10^{-3}	1.38×10^{-4}	2×10^{-16}	
Sex		-4.45×10^{-3}	2.71×10^{-3}	0.101	
Series	Series 2	1.03×10^{-2}	5.03×10^{-3}	0.041	
	Series 3	3.10×10^{-2}	4.80×10^{-3}	3.22×10^{-10}	
	Series 4	1.90×10^{-2}	4.88×10^{-3}	0.0001	
	Series 5	1.58×10^{-3}	4.79×10^{-3}	0.742	
	Series 6	1.54×10^{-2}	4.72×10^{-3}	0.001	
	Series 7	1.64×10^{-2}	5.00×10^{-3}	0.001	
	Series 8	-7.75×10^{-3}	2.71×10^{-3}	0.128	

148 rFIV is the model residual of FIV; rFO is the model residual of FO, and rDUV is the model residual of DUV. Due to high correlations
149 between some feeding behaviour traits, model residuals of FIV, FO and DUV were used in the final regression model to avoid
150 collinearity. Original values of FR, DFI and NDV were used in the final model.

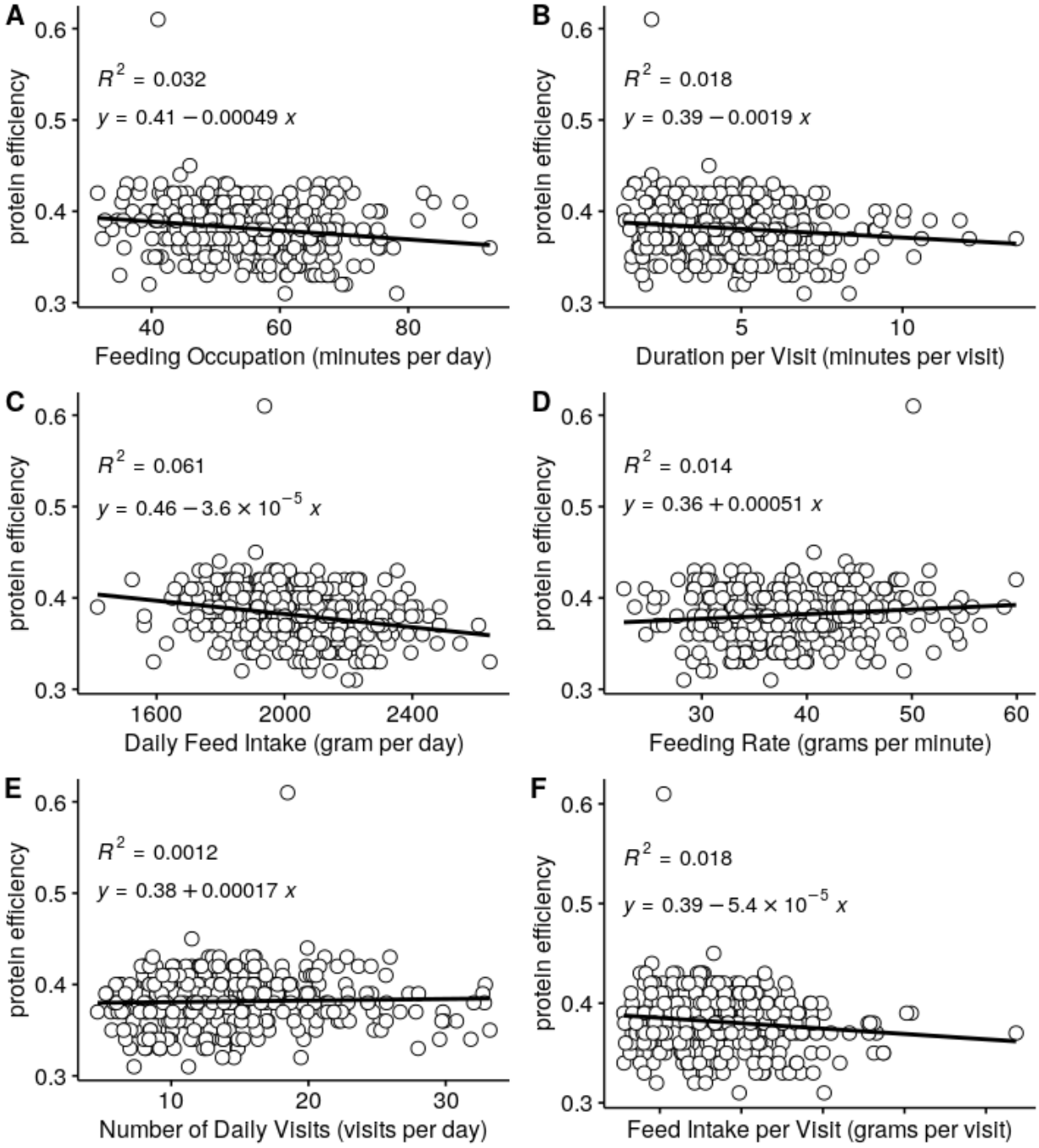

Figure 1: Simple linear regression of protein efficiency with feeding behaviour traits in pigs. Original data and regression lines are shown as dots and black lines, respectively.

157 **Discussion**

158 In this study, we investigated the potential of using automatically generated feeding
159 behaviour traits as proxies for protein efficiency for high-throughput phenotyping methods
160 in pigs. The advantage of this approach is that protein-efficient pigs may be chosen based
161 on their feeding behaviour data collected during the rearing period, and large phenotypic
162 data sets become available for genetic experiments. However, the result of this study
163 showed weak relationships between feeding behaviour traits and protein efficiency. From
164 linear regression analysis, it is clear that there was no association between PE and
165 feeding behaviour traits (Figure 1).

166

167 The mean carcass PE in this study was 0.38, which is in line with the carcass PE of Kasper
168 et al. (2020a), who investigated the potential to genetically improve protein efficiency in
169 pigs. The mean FO, DFI and FR observed in this study were slightly lower to those
170 reported by Kavlak and Uimari (2019) (FO: 61 min/d; DFI: 2100 g/d; FR: and 40 g/min)
171 and those reported by Do et al. (2013) (FO: 78min/d; DFI: 2340 g/d; FR: 31 g/min).
172 However, the mean feeding behaviour traits reported by Kavlak and Uimari (2019) were
173 recorded over five periods (i.e. growth phases) and the study of Do et al. (2013) was on
174 entire males. In our study, the focus was on the whole growth cycle (from weaning to
175 slaughter weight), castrated males and females were used and dietary protein was
176 reduced, which may influence feeding behaviour patterns.

177

178 The relationships between PE and feeding behaviour traits in this study were weak (i.e.
179 very low estimates), although regression analysis showed statistical significance of some
180 feeding behaviour traits, probably due to large sample size. For instance, a one-unit

181 increase in FR (g/min) will increase PE by 0.087% and a one-unit increase in DFI (g/day)
182 will decrease PE by 0.0093%, which is certainly of no biological relevance, and will
183 therefore have little to no impact on PE. This result is similar to the findings of Carco et al.
184 (2018), where FR was the only moderately correlated trait with protein retention among
185 other feeding behaviour traits (r = 0.41), but they concluded that FR has very little influence
186 on feed efficiency. Colpoys et al. (2016) did not find any significant relationship between
187 feeding behaviour traits and estimated protein retention. The large variations that exist
188 among individuals with respect to feeding behaviour traits (Carco et al., 2018) may
189 account for the little to no relationships found between feeding behaviour traits and protein
190 efficiency. Carco et al. (2018) described feeding behaviour as a flexible strategy each pig
191 follows to cover its nutrient requirement, which consequently may lead to variations in
192 feeding behaviour between individuals. The nutrient requirement of each individual may
193 be influenced by feed composition, among other factors such as genotype, health and
194 welfare. For example, Schiavon et al. (2018) found that a reduction in the essential amino
195 acid content of the diet increased feeding rate and feed intake. Since reduced dietary
196 protein was fed in the present study, pigs might have responded in different ways to the
197 diet in terms of feeding behaviour, which could have led to larger variations in feeding
198 behaviours. This weak to no relationship observed between PE and feeding behaviours
199 shows that differences in PE between pigs is not affected by feeding pattern. However,
200 other factors such as metabolic processes that regulate PE and genotype may influence
201 PE.
202
203 Although little to no relationship was found between feeding behaviour and protein
204 efficiency in this present study, further studies could estimate heritability of feeding

behaviours and genetic correlations with other traits, as some studies have shown high genetic correlations between some feeding behaviour traits and residual feed intake (RFI) (Ding et al., 2018). Additionally, further studies could be carried out to explore whether the influence of feeding behaviour on protein efficiency changes across growth periods.

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Declaration of interest

The authors report no conflicts of interest with any of the data presented.

Ethics approval

The experimental procedure was approved by the Office for Food Safety and Veterinary Affairs (2018_30_FR) and all procedures were conducted in accordance with the Ordinance on Animal Protection and the Ordinance on Animal Experimentation.

Data and model availability statement

The data that support the findings of this study are publicly available in Zenodo (10.5281/zenodo.4264350). The code used for models and the statistical analysis is listed in the supplementary material.

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References

Andretta, I., Pomar, C., Kipper, M., Hauschild, L., Rivest, J., 2016. Feeding behavior of growing–finishing pigs reared under precision feeding strategies. *Journal of Animal Science*, *94*(7), 3042-3050.

Carcò, G., Gallo, L., Dalla Bona, M., Latorre, M.A., Fondevila, M., Schiavon, S., 2018. The influence of feeding behaviour on growth performance, carcass and meat characteristics of growing pigs. *PLoS ONE*, *13*(10), e0205572.

Colpoys, J.D., Johnson, A.K., Gabler, N.K., 2016. Daily feeding regimen impacts pig growth and behavior. *Physiology & Behavior*, *159*, 27-32.

Ding, R., Yang, M., Wang, X., Quan, J., Zhuang, Z., Zhou, S., Li, S., Xu, Z., Zheng, E., Cai, G. and Liu, D., 2018. Genetic architecture of feeding behavior and feed efficiency in a Duroc pig population. *Frontiers in Genetics*, *9*, p.220.

Do, D.N., Strathe, A.B., Ostersen, T., Jensen, J., Mark, T., Kadarmideen, H., 2013. Genome-wide association study reveals genetic architecture of eating behaviors in pigs and its implications for humans obesity by comparative genome mapping. *PLoS ONE*, *8*(8), 1-14.

Kasper, C., Ruiz-Ascacibar, I., Stoll, P., Bee, G., 2020a. Investigating the potential for genetic improvement of nitrogen and phosphorus efficiency in a Swiss large white pig population using chemical analysis. *Journal of Animal Breeding and Genetics 137(6):545-558*.

Kasper, C., Schlegel, P., Ruiz-Ascacibar, I., Stoll, P., & Bee, G., 2020b. Accuracy of predicting chemical body composition of growing pigs using dual-energy X-ray absorptiometry. *bioRxiv*. doi: https://doi.org/10.1101/2020.09.15.286153

Kavlak, A.T., Uimari, P., 2019. Estimation of heritability of feeding behaviour traits and their correlation with production traits in Finnish Yorkshire pigs. *Journal of Animal Breeding and Genetics*, *136*(6), 484-494.

Kelly, A.K., McGee, M., Crews Jr, D.H., Fahey, A.G., Wylie, A.R., Kenny, D.A., 2010. Effect of divergence in residual feed intake on feeding behavior, blood metabolic variables, and body composition traits in growing beef heifers. *Journal of Animal Science*, *88*(1), 109-123.

Millet, S., Aluwé, M., Van den Broeke, A., Leen, F., De Boever, J., De Campeneere, S., 2018. Pork production with maximal nitrogen efficiency. A*nimal*, *12*(5), 1060-1067.

Ruiz-Ascacibar, I., Stoll, P., Kreuzer, M., Boillat, V., Spring, P., Bee, G., 2017. Impact of amino acid and CP restriction from 20 to 140 kg BW on performance and dynamics in empty body protein and lipid deposition of entire male, castrated and female pigs. *Animal*, *11*(3), 394-404.

Schiavon, S., Dalla Bona, M., Carcò, G., Carraro, L., Bunger, L., Gallo, L., 2018. Effects of feed allowance and indispensable amino acid reduction on feed intake, growth performance and carcass characteristics of growing pigs. *PLoS ONE*, *13*(4), e0195645.

Shirali, M., Duthie, C.A., Doeschl-Wilson, A., Knap, P.W., Kanis, E., van Arendonk, J.A., and Roehe, R., 2013. Novel insight into the genomic architecture of feed and nitrogen efficiency measured by residual energy intake and nitrogen excretion in growing pigs. *BMC Genetics*, *14*(1), 121.

Wang, Y., Zhou, J., Wang, G., Cai, S., Zeng, X., & Qiao, S., 2018. Advances in low-protein diets for swine. *Journal of Animal Science and Biotechnology*, *9*(1), 60.