Protein efficiency and pig welfare: no trade-offs for mitigating nitrogen pollution

Lea Roch[1,2], Esther Oluwada Ewaoluwagbemiga[1,3] and Claudia Kasper[1]*

[1]Animal GenoPhenomics, Agroscope, Tioleyre 4, 1725 Posieux, Switzerland
[2]Animal Welfare Division, Veterinary Public Health Institute, University of Bern, Länggassstrasse 120, 3012 Bern
[3]Department of Environmental Systems Science, ETH Zurich, Universitätstrasse 2, 8092 Zurich, Switzerland

*Corresponding author: claudia.kasper@agrocope.admin.ch

Abstract

Pig manure is a significant contributor to environmental pollution due to nitrogen compounds. While limiting protein in feed can mitigate this issue, this may result in damaging behaviors if the pigs' nutritional needs are unmet. One long-term strategy to reduce nitrogen pollution is to breed pigs for higher protein efficiency (PE), but concerns were raised that this may negatively affect pig welfare. To investigate whether PE is related to impaired pig welfare, this study observed 95 non-tail-docked fattening pigs around 100 days old with a 20% protein restriction. Only three pigs were observed engaging in tail biting and manipulation of regions that are particularly vulnerable to injury, and none of these behaviors were associated with PE. Pigs with higher PE tended to initiate and win more confrontations than those with lower PE. We cannot confirm or exclude the influence of PE on the manipulation of pen mates' less vulnerable regions. Such behaviors are normal and non-damaging per se, but can stress and injure pen mates if carried out excessively due to boredom or stress. Interestingly, increased engagement with straw was not associated with a reduction in potentially harmful behaviors, but we found a significant positive correlation. In conclusion, the study found no major behavioral abnormalities in pigs with higher PE. However, it highlights the importance of considering the potential trade-off between sustainability and welfare, reducing stress and providing adequate environmental enrichment to ensure the welfare of the pigs, especially if they are to become more efficient through breeding.

Pig production contributes to environmental pollution: applying pig manure to fields releases nitrogen compounds, leading to eutrophication of soils and water bodies [1,2]. Especially in areas with high livestock density and low availability of arable land, this problem has become particularly severe. Furthermore, the low self-sufficiency of protein sources for pig feed of the EU results in the need to import soybean meal for animal feed from South America. This exposes the European pig industry to increased risk of potential trade distortions, sustainability problems due to different production standards and transport, shortages and price volatility of soybeans on the world market [3,4]. Increased efforts are being made across Europe to limit pigs' protein intake to mitigate the environmental damage from pig production. Pomar and Remus [5] showed that each percentage reduction in dietary nitrogen leads to a 1.5% decrease in nitrogen excretion. However, a 20% reduction in pigs' dietary protein leads to a more pronounced increase in damaging behaviors, including tail and ear biting, especially when combined with poor sanitary conditions [6]. When restricted feeding was added to a 20% protein reduction, in combination with an imbalanced supply of essential amino acids (AAs), especially methionine, a tail biting outbreak occurred after two months [7], which subsided after increasing access to feed. Conceivably, deficiencies in certain essential AAs, such as methionine, threonine, and tryptophan, result in neurotransmitter system dysfunction, as specific AAs serve as neurotransmitters or are required for neurotransmitter synthesis [6]. Moreover, foraging behavior could increase due to the relative scarcity of AAs, potentially leading to obsessive manipulation and biting behaviors, especially when straw is unavailable as enrichment [8]. Further, AA deficiency is thought to make blood taste more attractive [6]. The trade-off between ensuring animal welfare and rendering pig production more environmentally friendly is apparent: while restricting the protein in feed can potentially reduce nitrogen emissions, it is important to examine whether this measure will affect pigs' wellbeing.

Damaging behaviors, including tail biting, are common in pig production, resulting in compromised victim welfare and economic losses. Tail biting and excessive manipulation also indicate that a pig's behavioral needs are unmet, suggesting poor wellbeing of the biters

[8]. Tail biting outbreaks are often difficult to identify, are of a multifactorial origin, and occur when stressors accumulate, including a lack of suitable occupational materials, a poor stable climate, insufficient cleanliness, an unbalanced diet, and poor health. Behavioral changes at the pen level, such as in feeding patterns, can be detected up to one month before a tail biting outbreak [9], of which tail posture is an early pen-level indicator [10]. Individually, behavioral problems caused by various stressors begin before escalating into harmful behavior leading to serious injury. For instance, a pig could proceed from gently manipulating the tail or ear of a conspecific, with no noticeable reaction from the target, to biting and inducing a bloody wound [6,11,12]. Ursinus et al. [13] found a positive correlation between the number of pen-mate manipulations, a generally natural and harmless behavior, and a high rate of tail biting. There is a delicate boundary between natural pig behaviors, including exploring and manipulating conspecifics, and deleterious behaviors, such as biting. For instance, engaging in positive social behaviors has a positive impact on growth, presumably by reducing stress through oxytocin release [12]. However, redirected “abnormal” behaviors, such as excessive belly nosing, ear biting, and “tail-in-mouth” [14], can severely disturb the receiver [15], cause permanent stress and even impact growth [12].

Individual differences in protein efficiency (PE), i.e., the ability to utilize dietary proteins [16], are heritable [17]. Therefore, it is to be expected that individual pigs will differ in how their behavior is affected by a protein reduction. Harnessing these heritable individual differences in breeding would therefore effectively reduce the long-term nitrogen pollution from pig production. The molecular basis of PE is not yet well established, and there are legitimate concerns that breeding for increased PE could induce behavioral problems. For instance, Breuer et al. [18] reported a weak but significant genetic correlation between the lean tissue growth rate and tail biting behavior in Landrace pigs, but not in the breed used in the present study (Large White pigs). Therefore, there is a certain risk of inadvertently co-selecting pigs with an increased predisposition to behavioral problems when breeding for a higher protein accretion rate.

PE is a trait arising from a combination of several processes, including gastrointestinal tract absorption and protein turnover, which occur in different tissues and organs. Genetic selection for higher PE could improve these processes or could alter the allocation of proteins toward lean tissue growth and away from other processes, including immune or endocrine system functioning, potentially compromising homeostasis and thus health and reproduction. The genetic architecture of PE or nitrogen excretion is likely complex, as multiple regions on different chromosomes were found associated with nitrogen excretion traits [19]. Some of these quantitative trait loci (QTLs) overlap with various production traits, while others are unique to nitrogen excretion, though the functions of genes within these QTLs are still unclear.

This study aimed to investigate whether PE is associated with indicators of impaired pig welfare on a phenotypic level. We investigated pigs that were all subject to dietary protein restriction. Particularly, whether the level of PE influences tail biting and other damaging behaviors is examined, as are the manipulation behaviors of pen mates. Furthermore, we explore the relationship between PE and straw rooting, which is considered a positive welfare behavior allowing animals to explore the environment, thereby reducing boredom and manipulation [15]. Ingesting straw prevents ulcers, which could lead to less tail biting [20]. Therefore, we investigated whether engagement with occupational materials (straw, metal chain, pen barriers) is linked to a reduction in tail biting or manipulation behaviors, as reported by [21]. Our study also investigated the outcome of social encounters and confrontations related to PE as an indicator of whether aggressiveness or dominance is associated with efficiency. Finally, we explore the relationship of the frequency of lesions, tail position, cleanliness, and activity with PE.

Material and Methods

Animals

The pigs were part of a larger experiment comprising 14 farrowing series (681 pigs), with the goal of estimating genetic parameters of PE [22]. In this study, 95 non-tail-docked Swiss Large White pigs (53 females and 42 castrated males) were observed between August and December 2020. They were born in two farrowing series and raised in 36.78 m^2 pens with 23 or 24 pigs in each, i.e., two pens per series. The floor space was above legal requirements, with only partial slatted flooring (as required by law in Switzerland). Pens were cleaned daily, and pigs were fed a standard starter feed after weaning until reaching an average body weight of 22.4 (± 1.6) kg. They were then mixed in groups of 24 pigs, in which they stayed until slaughter. After the starter diet, pigs were switched to a grower diet containing 128 g/kg crude protein, which corresponds to a 20% protein reduction compared to the protein content of the recommended diet in Switzerland. All AAs were reduced to the same level to avoid deficiencies and thus a nutritional imbalance. From an average body weight of 63.5 (± 2.4) kg onward, the pigs received a 20% protein-reduced finisher feed that contained 112 g/kg crude protein. The automatic feeders (Schauer Maschinenfabriken GmbH & Co KG, Prambachkirchen, Austria) were accessible to the pigs via individual identification by an RFID chip in the ear from 07:40 to 23:30. Each pen was covered with a thin layer of straw on the concrete floor, contained two mobile and two fixed straw baskets, and held four suspended metal chains as occupational materials. Following the Swiss Animal Welfare Act, it was ensured that occupation material, in our case unchopped straw, was always available. The two groups had auditory and olfactory contact. Pigs had no outdoor access. Natural light was provided through windows in the barn. The health status of the pigs on each observation day was considered, and each injured animal was treated on the same day.

Behavioral observations

Between days 98 and 115 after birth, each pig's behavior was recorded following an ethogram [23], created based on literature research and two days of observations, and refined during a preliminary study prior to this work. The first farrowing series was observed in August 2020 and the second in January 2021. Live behavioral observations were conducted in daylight between 12:45 and 16:00, when the pigs are usually most active; but might have rested during hot periods in the first farrowing series. The observer (LR) stood outside the pen to limit any influence on the animals. Observations began after a 15-min habituation period. All pigs in one pen were individually marked with colored spray and directly observed for 5 min using focal sampling [24] in a random sequence on one afternoon. Each pen was observed over four different days; thus, each pig was observed for 20 min total, resulting in approximately 65 h of observation, including scanning for lesions, tail position, and cleanliness, as described below. During observation, the observer was blind to the PE of the individual pigs, as this was only recorded several weeks later, at the time of slaughter. The total number of separate instances of nasal and oral manipulation of objects (metal chains, pen barriers, and drinkers), as well as of pen mates during the 5 min of individual observation, was recorded. These behaviors comprised biting, seizing (with the mouth, but not the teeth), and manipulating (with a closed mouth or the snout). Behaviors directed toward the head, body, ears, tail, and vulva or perineal area of a conspecific were recorded separately, and we recorded whether the focal animal performed (active) or received the behavior (passive). Due to the scarcity of manipulation behaviors directed at pen mates, we combined all active behaviors, including biting, seizing, and manipulation with the snout, toward the ears, tails, and vulva or perineal (ETV) region into one variable, termed "problematic behaviors," and those directed toward the rest of the body (excluding the ETV region) into another variable ("potentially problematic behaviors") by totaling the instances of each behavior. The same was done for behaviors received.

The outcomes of confrontations, i.e., agonistic situations arising when two pigs meet and both want an object or place simultaneously, were recorded, and the following were possible: domination, submission, or a tie, when the outcome was unclear. The number of instances of straw rooting was determined as the sum of the number of times the focal pig was engaging with the baskets and whether it performed straw rooting behaviour on the floor (never in 5 min = 0, ≥ 1 s in 5 min = 1). The total activity score was calculated as follows: for feeding, drinking, urinating, or defecating and lying on the ground, a score of one was allocated, while sitting yielded a score of two and standing a score of three. Each position (lying, sitting, standing) and each position change was counted. We included the activity score in the models to correct for the higher probability of more active pigs encountering and interacting with pen mates. In addition, all wounds and scratches (classified as mild scratches or severe wounds), the tail posture (raised corkscrew, hanging straight, or tucked between legs), and the cleanliness of each pig were recorded after each observation, using the protocol in Roch *et al.* [23].

Protein efficiency

The PE of each pig was calculated as the amount of protein in the carcass after slaughter divided by the amount of protein ingested. Pigs were slaughtered at an average live body weight of 105.2 kg (± 6. 5) at the Agroscope experimental slaughterhouse in Posieux. The intestines and viscera, as well as the hair, blood, and gallbladder, were removed, and the carcass was cut in half. Half-carcasses were scanned on a dual energy X-ray absorptiometry (DXA) device (GE Lunar i-DXA, GE Medical Systems, Glattbrugg, Switzerland) to determine the lean meat content. The following regression equation (1), which was developed in a previous study [25], was used to estimate the protein content from the lean meat content, determined by DXA:

$$\boldsymbol{protein\ content\ carcass}\ (\boldsymbol{g}) = -\mathbf{482.745} + \mathbf{0.23}\ (\mathbf{g\ lean\ tissue}\ \boldsymbol{DXA} \times \mathbf{2}) \qquad \textbf{(1)}$$

The amount of protein ingested beginning after the switch to the protein-reduced grower feed was recorded by the automated feeders. Meanwhile, the estimated protein content of the carcass at the time of the feed change was subtracted from the total weight of protein in the carcass at slaughter (for more details see [17]).

Statistical analysis

We grouped the single behaviors directed toward conspecifics into the following categories with decreasing intensity and damaging capacity by calculating the total number of occurrences: biting directed toward the ETV regions of conspecifics ("damaging behaviors"), which were too rare for statistical analysis; biting, seizing, and manipulating conspecifics' ETV regions ("problematic behaviors"); and biting, seizing, and manipulating all other body (non-ETV) regions of conspecifics, including the head ("potentially problematic behaviors"). All different behaviors directed at objects, as well as rooting in the floor straw and in the basket, were combined into a single variable (termed "object manipulation" and "straw rooting", respectively), and all analyses were conducted with the R software version 4.2.2 [26]. We identified PE outliers using the Grubbs test in the outliers package version 0.15 [27]. In consideration of these data, which were count variables with likely more zeros than expected due to an important proportion of pigs not performing the behavior in question during the observation window (i.e., zero-inflated) or data that might not satisfy the strict mean–variance relationship of a Poisson distribution (i.e., over-dispersion), we used the R package glmmTMB version 1.1.4 [28]. The effect of PE on problematic behaviors, confrontation outcomes, and straw rooting in terms of behavior counts was analyzed with generalized mixed-effects models with a Poisson, quasi-Poisson, and negative binomial family, and the latter two were chosen to account for different mean–variance relationships, i.e., over-dispersion. We included sex and activity score as a covariate in all models, and body weight at the time of observation in the models on confrontations.

We ran several models for all variables of interest: First, a full model with PE and sex as fixed effects and activity as a covariate was performed for each family (Poisson, quasi-Poisson and negative binomial), and the intercept was modelled as zero-inflated with a logit link. If the zero-inflation term was not significant, we reran the model without zero-inflation and did not use zero-inflation correction for the following models. If it was significant, zero-inflation was maintained. The three nested models included one with PE and activity, one with PE only, and one with only the intercept for each model family. Thus, in total, 12 models were run. For initiating a confrontation and its outcomes, we also included body weight at the time of the observation, because it might influence the ability to perform in confrontations. Thus, we ran 15 models for confrontations initiated, won and lost: the full model, a model with PE, body weight and activity, one with PE and weight, one with PE and one with only the intercept. After finding that activity and PE were correlated in the beginning of the analysis, we used the R package performance [29] to check the variance inflation factors for each full model. They all were close to one; therefore, we kept PE and activity in the model. Initially, the individual ID, as well as the maternal ID nested in the paternal ID, were added as random effects to correct for multiple observations and the family structure. In some cases, the models did not converge, so we removed first the maternal ID and then the paternal ID (cases are indicated in the Results section). Using the AICctab command from the bbmle package version 1.0.25 [30], the best model was chosen based on AICc (modified AIC for small sample sizes). Following Burnham and Anderson [31], we considered models with a difference in AICc (ΔAICc) of less than two and having the same evidence, and we presented them as confidence sets. For the models in the confidence set, we used the R package DHARMa [32] to diagnose any violations of distribution assumptions and model misspecifications. Plots were created using the ggplot2 package version 3.3.6 [33] with the ggstatsplot extension [34] to compute group and sex differences.

Ethical approval

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

Results

Protein efficiency

The PEs of the two farrowing series differed, but the average of the two was 0.39 ± 0.02 (Fig. 1, left; Welch two-sample t-test, t = -4.03, df = 89.87, p < 0.001). The first farrowing series had a mean PE of 0.38 ± 0.02 and the second a mean PE of 0.40 ± 0.02. To account for this difference and for the family structure in the data, we included the father and mother IDs (in addition to the individual ID) in the following models. The sexes did not differ significantly in terms of PE (Fig. 1, right; Welch two-sample t-test, t = 0.20, df = 78.28, p = 0.84). Following the outlier test, we removed two pigs with extremely high PEs (0.574 and 0.469).

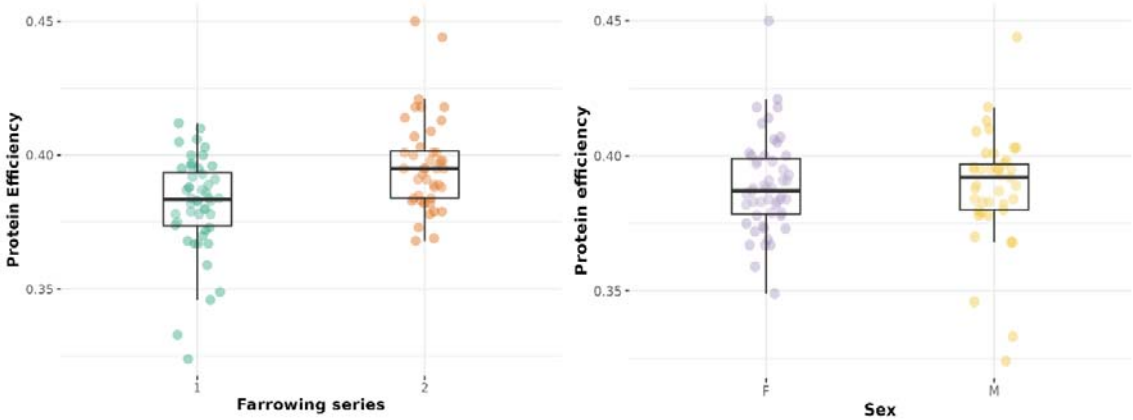

Fig. 1: Protein efficiency in the two farrowing series (left) and sexes (right). Abbreviations: F females, M castrated males.

Activity

Activity was included in the models to account for the increased likelihood of social interactions when a pig is active (non-social behavior) and moving around. The farrowing series were significantly different in their activity scores (Fig. 2, left; Welch two-sample t-test, t = -2.55, df = 90, p = 0.01), with the first group showing less activity (mean = 13.0 ± 6.6) than the second (mean = 16.4 ± 6.10), though females and castrated males were not significantly different in their activity scores (Fig. 2, left; Welch two-sample t-test, t = 1.22, df = 82.22, p = 0.23). There was a significantly positive correlation between PE and activity levels (Spearman's rho = 0.34, p < 0.001), but we did not detect signs of collinearity in the full models of each behavior (Table S1). Therefore, we used the raw activity levels to account for the influence of activity on the outcome variable in the following models.

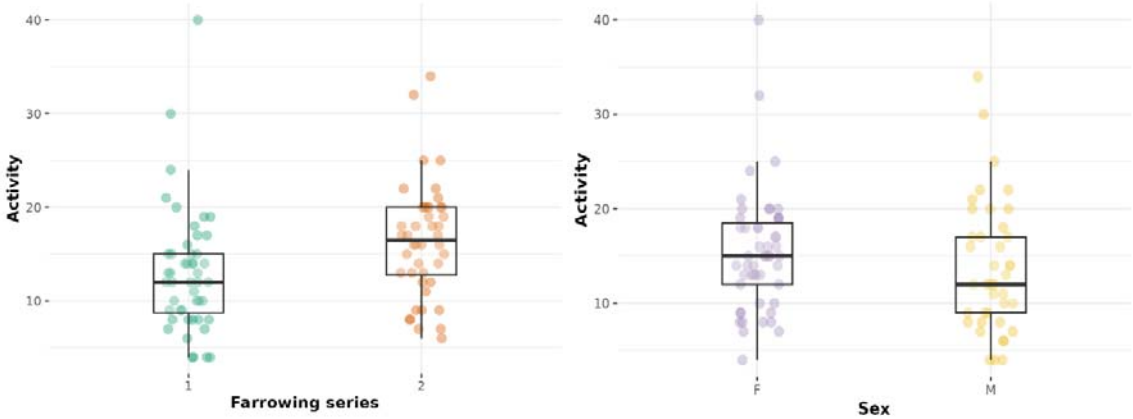

Fig. 2: Activity scores (see main text for details) in the two farrowing series (left) and sexes (right). Abbreviations: F females, M castrated males.

Damaging and potentially problematic behaviors:

Of the total 5,479 actions recorded over the four observations, 11% were directed at chains, barriers or drinkers, 27% at straw, and 61% at conspecifics. Most pig-directed behaviors targeted the body and head, i.e., less vulnerable regions, and fewer actions were directed at vulnerable regions (ETV regions; Figure 3). Of all 92 pigs, only three were involved in tail biting (3.2%): two bit pen mates and one was both a biter and a victim. Most pigs

manipulated conspecifics nasally or orally, but 16 pigs (17%) did not exhibit these behaviors in any of the observations. Only four pigs (4%) were not subjected to these behaviors, and only one pig was not involved at all (neither as performer nor receiver).

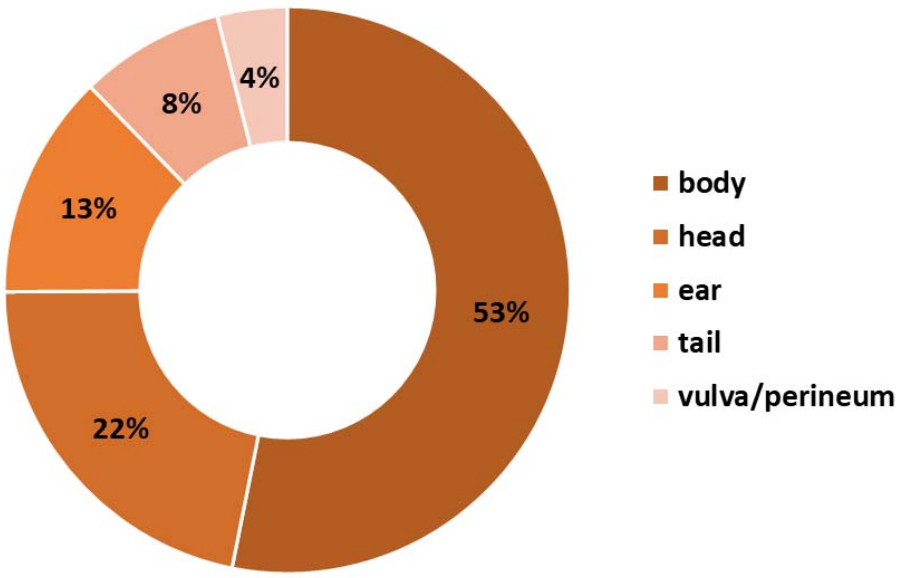

Fig. 3: The sum of all active pig-directed behaviors recorded in the four observations. On only nine occasions, pigs directed damaging behaviors toward conspecifics (i.e., biting directed at the ETV regions), and all eight biters had an average or below-average PE. Damaging behaviors were received four times by four different pigs during the focal observations, and receivers also had below-average PE.

We did not find evidence that the number of received problematic behaviors (biting, seizing, and manipulating directed toward a conspecific's ETV regions) was influenced by PE. In the two best models, PE was present but not significantly associated with the behavior (Table 1). Activity levels were also included in the two best models, and they significantly positively influenced the number of manipulations directed at a pen mate's vulnerable body regions (ETV), and sex was included in only one model, and not significant (Table 1). Two models presented similar evidence of the number of potentially problematic behaviors (biting, seizing, and manipulating with the snout) directed at a conspecific's less vulnerable body areas (non-ETV regions). Both models included PE and activity, but only one included sex. While activity was significantly positively associated with the behaviors, PE and sex were not,

but PE was close to significance (p = 0.057 and p = 0.054; Table 1). The two best models of problematic behaviors toward ETV regions only included the intercept; thus, there was no evidence that PE or sex influenced these behaviors. Concerning manipulations of the whole body received, PE, and activity were included in the two best models, but none of the variables was significant. However, the p-values for PE were again below 0.10 (Table 1). The full set of models are provided in Table S2-S5.

Table 1: Model selection for problematic (ETV) and potentially problematic (head and body) behaviours performed and received as a function of protein efficiency, sex and activity. Only models with similar evidence (ΔAICc < 2) are shown. Significant effects (p < 0.05) are highlighted in bold, and effects with 0.05 ≥ p < 0.10 in italics.

behavior	model	variable	estimate	SE	z-value	p-value	df	ΔAICc
performed ETV	QP	Intercept	-3.467	2.469	-1.404	0.160	7	0.0
		PE	4.499	6.318	0.712	0.476		
		activity	**0.165**	**0.027**	**6.088**	**<0.001**		
	QP	Intercept	-3.448	2.461	-1.401	0.161	8	1.9
		PE	4.584	6.296	0.728	0.467		
		sexM	-0.098	0.237	-0.413	0.679		
		activity	**0.164**	**0.027**	**6.070**	**<0.001**		
performed head and body	ZI-QP	Intercept	-4.698	2.134	-2.201	0.028	9	0.0
		PE	*10.279*	*5.395*	*1.905*	*0.057*		
		sexM	-0.284	0.191	-1.490	0.136		
		activity	**0.181**	**0.020**	**8.961**	**<0.001**		
	ZI-QP	Intercept	-5.091	2.216	-2.297	0.022	8	0.0
		PE	*10.987*	*5.712*	*1.923*	*0.054*		
		activity	**0.187**	**0.022**	**8.682**	**<0.001**		
received ETV	ZI-PO	Intercept	-0.173	0.279	-0.617	0.537	5	0.00
	ZI-QP	Intercept	-0.173	0.279	-0.617	0.537	5	0.00
received head and body	NB	Intercept	3.213	1.931	1.664	0.096	6	0.0
		PE	*-8.416*	*4.978*	*-1.691*	*0.091*		
	NB	Intercept	3.307	1.946	1.699	0.089	7	2.0
		PE	*-8.749*	*5.059*	*-1.729*	*0.084*		
		activity	0.009	0.027	0.357	0.721		

Abbreviations: ETV behaviours directed at ears, tails or perineal region of conspecifics, ZI zero-inflated, NB negative binomial, QP quasipoisson, PO poisson, PE protein efficiency, df degrees of freedom, ΔAICc difference in AICc to the best model

Lesions, tail posture, and cleanliness

Thirteen pigs had minor wounds (mean PE = 0.39, min = 0.33, max = 0.42), and only one pig had a part of the tail missing (PE = 0.41), where the pigs with minor wounds had a medium-range PE and the pig with the missing tail part had a PE slightly above average. The other animals had no wounds or only superficial scratches. Five pigs had a straight tail (mean PE = 0.38, min = 0.33, max = 0.41), and all others had corkscrew tails. We observed one pig having a straight tail over all four observations, another over three, two over two, and one over one observation). No pigs with tucked tails were observed, and all pigs were clean, except one that was slightly soiled (PE = 0.37) during two observations.

Initiation and outcome of confrontations

Both best models for the number of confrontations a pig initiated included PE, body weight, and activity, and one also included sex. Only activity influenced behavior significantly (Table 2), whereas PE was close to significance ($p = 0.082$ and 0.088). Sex and body weight were clearly not significant. The same variables were included in the two best models for winning a confrontation. High PE and activity levels were significantly correlated with the number of confrontations won in both models. Sex was included in one of the models but was not significantly related to winning. The best models for losing a confrontation included PE, sex, body weight and activity, but PE, sex and body weight were not significant. As in the previous models, higher activity levels significantly increased the number of confrontations lost (Table 2). The full set of models are provided in Table S6-S8.

Table 2: Model selection for initiation and outcome of confrontations as a function of protein efficiency, sex, body weight and activity. Only models with similar evidence (ΔAICc < 2) are shown. Significant effects ($p < 0.05$) are highlighted in bold, and effects with $0.05 \geq p < 0.10$ in italics.

behavior	model	variable	estimate	SE	z-value	p-value	df	ΔAICc
initiate confrontation	QP	Intercept	-6.860	2.528	-2.714	0.007	8	0.0
		PE	*10.813*	*6.211*	*1.741*	*0.082*		
		weight	0.012	0.015	0.795	0.427		
		activity	**0.174**	**0.025**	**6.949**	**<0.001**		
	QP	Intercept	-6.644	2.429	-2.735	0.006	9	0.6
		PE	*10.231*	*5.998*	*1.706*	*0.088*		
		sexM	-0.296	0.238	-1.244	0.213		
		weight	0.015	0.015	0.996	0.319		
		activity	**0.172**	**0.024**	**7.052**	**<0.001**		
win confrontation	QP	Intercept	-9.676	3.008	-3.217	0.001	8	0.0
		PE	**15.086**	**7.349**	**2.053**	**0.040**		
		weight	0.025	0.018	1.416	0.157		
		activity	**0.184**	**0.030**	**6.192**	**<0.001**		
	QP	Intercept	-9.711	3.004	-3.233	0.001	9	1.2
		PE	**14.979**	**7.403**	**2.023**	**0.043**		
		sexM	-0.257	0.273	-0.939	0.348		
		weight	0.029	0.018	1.585	0.113		
		activity	**0.181**	**0.029**	**6.209**	**<0.001**		
lose confrontation	QP	Intercept	-1.626	2.666	-0.610	0.542	8	0
		PE	-0.683	6.475	-0.106	0.916		
		weight	-0.009	0.017	-0.536	0.592		
		activity	**0.155**	**0.026**	**5.884**	**<0.001**		
	QP	Intercept	-1.696	2.659	-0.638	0.524	9	1.7
		PE	-0.683	6.492	-0.105	0.916		
		sexM	-0.153	0.240	-0.637	0.524		
		weight	-0.006	0.017	-0.357	0.721		
		activity	**0.154**	**0.026**	**5.851**	**<0.001**		

Abbreviations: QP quasipoisson, PE protein efficiency, df degrees of freedom, ΔAICc difference in AICc to the best model

Straw rooting

Three models for the number of straw rooting instances showed similar evidence and were thus included in the confidence set. They all included PE and activity, and two included sex (Table 3). Sex was significantly associated with the number of straw rooting instances in the two models in which it was included, with females rooting more frequently than castrated males. Higher activity levels were significantly associated with more straw rooting, but PE was not significant in any of the models. The full set of models is provided in Table S9.

Table 3: Model selection of number of straw rooting bouts as a function of protein efficiency, sex and activity. Only models with similar evidence (ΔAICc < 2) are shown. Significant effects ($p < 0.05$) are highlighted in bold, and effects with $0.05 \geq p < 0.10$ in italics.

model	variable	estimate	SE	z-value	p-value	df	ΔAICc
QP	Intercept	-2.166	1.976	-1.096	0.273	8	0.0
	PE	4.340	5.037	0.862	0.389		
	sexM	**-0.345**	**0.175**	**-1.973**	**0.048**		
	activity	**0.127**	**0.018**	**7.123**	**<0.001**		
NB	Intercept	-1.280	2.174	-0.589	0.556	8	0.5
	PE	1.588	5.538	0.287	0.774		
	sexM	**-0.384**	**0.189**	**-2.035**	**0.042**		
	activity	**0.158**	**0.025**	**6.254**	**<0.001**		
QP	Intercept	-2.615	2.037	-1.284	0.199	7	1.6
	PE	5.099	5.214	0.978	0.328		
	activity	**0.130**	**0.018**	**7.205**	**<0.001**		

Abbreviations: NB negative binomial, QP quasipoisson, PE protein efficiency, df degrees of freedom, ΔAICc difference in AICc to the best model

Linking object manipulation and straw rooting with problematic behaviors

The number of oral or nasal actions (biting, seizing, or manipulating) directed at metal chains, pen barriers, and drinkers significantly increased with the number of similar behaviors directed at the ETV regions of conspecifics in each of the two best models, and the same was true for activity levels. Sex was included in one of the two best models, with females directing more behaviors toward objects than castrated males, but not to the point of significance ($p = 0.061$; Table 4). Behaviors directed at the ETV regions of conspecifics, as well as activity levels, were present in the three best models of straw rooting instances. In all three, the number of straw rooting instances increased significantly along the amount of biting, seizing, and manipulation with the snout directed at the ETV regions and with increasing activity levels. The evidence of sex influencing straw rooting was slightly less strong, but it was present in two of the three models, once clearly significant and once close to significance ($p = 0.052$). As found previously in the straw rooting models, females rooted significantly more often than castrated males (Table 4). The full set of models is provided in Table S10-S11.

Table 4: Model selection of number of object manipulation bouts and straw rooting bouts as a function of activity, the number of problematic behaviours performed, sex and activity. Only models with similar evidence (ΔAICc < 2) are shown. Significant effects (p < 0.05) are highlighted in bold, and effects with 0.05 ≥ p < 0.10 in italics.

behavior	model	variable	estimate	SE	z-value	p-value	df	ΔAICc
object manipu-lation	ZI-QP	Intercept	-1.174	0.282	-4.164	<0.001	9	0.0
		performed ETV	**0.392**	**0.057**	**6.822**	**<0.001**		
		sexM	*-0.404*	*0.215*	*-1.875*	*0.061*		
		activity	**0.177**	**0.025**	**7.212**	**<0.001**		
	ZI-QP	Intercept	-1.343	0.271	-4.958	<0.001	8	1.5
		performed ETV	**0.388**	**0.057**	**6.842**	**<0.001**		
		activity	**0.185**	**0.025**	**7.509**	**<0.001**		
straw rooting	NB	Intercept	-0.721	0.293	-2.464	0.014	8	0.0
		active_ETV	**0.226**	**0.063**	**3.620**	**<0.001**		
		sexM	**-0.379**	**0.187**	**-2.021**	**0.043**		
		activity	**0.143**	**0.025**	**5.713**	**<0.001**		
	QP	Intercept	-0.532	0.252	-2.114	0.035	8	1.3
		performed ETV	**0.194**	**0.052**	**3.748**	**<0.001**		
		sexM	*-0.338*	*0.174*	*-1.942*	*0.052*		
		activity	**0.114**	**0.018**	**6.253**	**<0.001**		
	NB	Intercept	-0.899	0.277	-3.246	0.001	7	1.8
		performed ETV	**0.229**	**0.063**	**3.642**	**<0.001**		
		activity	**0.146**	**0.025**	**5.784**	**<0.001**		

Abbreviations: ZI zero-inflated, NB negative binomial, QP quasipoisson, PO poisson, PE protein efficiency, df degrees of freedom, ΔAICc difference in AICc to the best model

Discussion

The purpose of this study was to investigate the relationship between PE and harmful or potentially problematic behaviors in pigs. The relatively large reduction in dietary crude protein of 20% must be considered when interpreting the results as it has been frequently associated with an increased prevalence of damaging behaviors. Conversely, the pigs in this study were given fresh, unchopped straw daily, sufficient access to feed, floor space above the legal requirements, and daily pen cleaning, and they were closely monitored for signs of damaging behavior, as well as favorable conditions, limiting stress and the risk of damaging behavior. Great care was given to formulate a diet that included balanced amounts of essential AAs, an undersupply of which, such as methionine, is suspected of fueling tail biting [6,7]. Nevertheless, the experimental diet in the project represents a relatively strict reduction that cannot be recommended for use in practice, especially if the sanitary

conditions are non-optimal [6]. Throughout the larger project, from October 2018 to June 2021, when the nearly 700 pigs were slaughtered, piggery staff reported their impression of increased nervousness in the pigs, which they attributed to the protein reduction. One tail biting outbreak was reported in February 2020, before the present behavioral observation study. This outbreak could be contained by providing the pigs with paper bags and hay several times per day. Engaging with these materials, which were novel to them, was sufficient to reduce tail biting. From then on, only 12 instead of 14 pigs were housed per feeding station.

Damaging or problematic behaviors

In the present study, we did not find strong evidence that PE is associated with an increased risk of damaging behavior toward pen mates. Only a few pigs showed damaging behavior in terms of biting the ETV regions of pen mates, and these pigs' PE did not stand out in any direction. Further, there was no evidence that PE made an individual more susceptible to receiving damaging behaviors. However, to assess animal welfare more comprehensively, it is essential to consider potentially harmful behaviors, such as other oral and nasal behaviors, directed at the ETV areas of pen mates, and even behaviors directed at the head and the body, in addition to tail (and ear) biting. Concerning these behaviors, we again did not find strong evidence of a PE influence. Furthermore, there was no evidence that PE influenced the amount of oral and nasal manipulation of ETV regions. However, we can neither rule out nor confirm the influence of PE on the manipulation of the less-vulnerable parts of a pen mate's body, as PE was close to but did not reach significance in the two best models. Furthermore, it is unclear whether the oral and nasal manipulation of conspecifics is potentially harmful, as they represent positive interactions (allogrooming [15,35]. However, if performed excessively, they can affect and stress pen mates [15] and even lead to injuries. It seems as well that PE does not play a role in how many bites or nasal/oral manipulations a pig receives to the rest of the body (non-ETV regions).

The more active a pig is in terms of non-social behaviors (body posture changes, feeding, drinking, etc.), the more it will manipulate a pen mate's vulnerable body regions or whole body. This was expected, as a higher activity score reflects that a pig is moving around and will therefore have a higher chance of encounters with pen mates in the observation period than a pig that is not moving during this time. Ursinus et al. [13] found a similar result between the rate of group activity and tail biting. Practically no evidence was found of sex affecting any of the behaviors mentioned. Sex was included in the models for performing and receiving manipulations of the body, but the terms were never significant, though this might differ when whole males are reared. For instance, Clouard et al. [35] reported that while female and non-castrated male piglets were generally equally active, male piglets engaged in more social interactions than females, including more agonistic behaviors, mounting, and social play. The increased protein requirements of boars compared to females and castrated animals could also lead to an increased frequency of damaging behavior if the diet does not meet the boars' needs [11]. However, Brunberg *et al*. [8] noted that there is no consensus about whether sexes differ in their propensity for directing damaging behavior toward conspecifics or receiving such behavior. During the study, only one severe lesion was detected during the first observation, which must have occurred shortly before the study began. The PE of the injured pig was above average (0.41), while the mean PE of animals with light lesions was identical to the overall mean (0.39). The three animals with the most manipulations received across all observations (including bites and oral/nasal behaviors toward vulnerable regions and the whole body) showed light lesions, but the remaining animals with lesions were not far from average (mean of the number of behaviors received was 5.1 ± 4.0). It is unsurprising that many injuries occurred outside the 5-min observation periods. Moreover, not all behaviors resulted in visible injuries. Concerning tail posture, which has been suggested as an indicator of tail biting [10], we could not establish a clear connection to PE, as the five animals in which a straight (hanging) tail posture was observed (all others had raised corkscrew tails) had an approximately average PE. Some pigs with

straight tails were observed as being exposed to damaging or potentially problematic behavior, but most straight-tailed pigs were not exposed to an unusual number of behaviors during the observation, therefore it is likely the straight-tail posture indicates interactions that occurred outside the observation intervals. It has to be noted that straight tails are not necessarily linked to tail biting, but also indicate a positive and/or relaxed state in pigs, especially in enriched environments [36].

The relationship between PE and potentially problematic behaviors presented here is purely phenotypic. However, in the absence of genetic studies, it might be cautiously interpreted as a proxy for genetic correlations [37,38]. Thus, when engaging in genetic selection for increased PE, a dramatic increase in tail biting due to co-selection is highly unlikely, but it is advisable to monitor pig behavior carefully to detect any deterioration.

Frequency and outcome of confrontations

Looking at the number of confrontations initiated and their outcomes, we found convincing evidence of the role of PE in how often pigs started a confrontation and how often they won it. While a pig tended to initiate more confrontations when it had a higher PE, which was close to but not significant, there was clear evidence that pigs with higher PE won confrontations more often. One might speculate that this could be explained by the greater strength of protein-efficient pigs, since they might have a higher muscle mass. However, the number of confrontations lost was not linked to PE, at least not significantly. For all three variables concerning confrontations (initiating, winning, and losing), activity was a strong predictor. Further, sex was present in all chosen models, but it was not significantly associated with the variables in question. Body weight was not linked to starting, winning or losing confrontations. As for the behaviors directed toward vulnerable regions or the rest of the body, as discussed above, it is hardly surprising that higher activity is associated with a greater involvement in confrontations, as animals that move around more are more likely to

encounter pen mates. If a pen mate occupies a place or resource the focal pig wants to use, there will be a brief confrontation until the matter is settled. Confrontations among familiar pigs in established groups are a normal part of pigs' behavioral repertoire and reflect the dominance hierarchy in the pen [35]. If the pigs can solve a confrontation rather quickly without causing much injury, encounters are not a matter of concern.

Rooting and object manipulation

The number of pig-oriented actions was much higher than the number of object-oriented actions (including metal chains, pen barriers, drinking bowls, and straw) in this study, similar to the results of Meer et al. (2017), in whose study only a chain with a hard plastic tube was available as enrichment. In our case, straw was provided daily, and the animals engaged with it extensively. However, this might have satisfied only part of their urge to interact with mobile, flexible, and deformable objects [6,39,40]. One might speculate that the provision of straw has already prevented some of the abnormal manipulations of pen mates, which would have been more pronounced in the absence of this occupational opportunity [15,35]. PE did not seem to influence the frequency of straw rooting, but we found important sex differences. Females rooted more than castrated males, which was, in this study, probably due to more engagement with the straw in the baskets (Welch two-sample t-test, $t = 1.98$, $df = 83.76$, $p = 0.05$), rather than to the time spent rooting on the floor (Welch two-sample t-test, $t = 0.73$, $df = 88.64$, $p = 0.47$). A sex difference in rooting has been reported previously [41,42]. Here, too, activity played a major role, due to the construction of the activity score, as postural changes during rooting increased activity score. In the present study, more rooting behavior did not prevent the pigs from biting or manipulating their pen mates. Conversely, pigs that rooted more also performed more ETV-directed behaviors and behaviors directed at objects, such as metal chains, pen barriers, or drinkers It is unclear whether the interaction with objects or straw leads to more interaction with pen mates or vice versa.

The positive correlation between ETV-directed behaviors and straw rooting found in this study should not be interpreted in the sense that the offer of occupational material does not have a positive influence on behavior toward conspecifics. The correlation found here contrasts with other studies reporting that offering straw resulted less nosing other pigs, aggression, and tail biting compared to when straw was not provided [21], as well as less tail damage when only fixed enrichment materials, such as chain, rubber hoses, or other hanging toys, were available (reviewed in [43]. Often, piggery staff try to curb tail biting by providing more straw, as well as destructible materials, such as paper bags, as additional enrichment. During this behavioral observation study, there was no tail biting outbreak to require such an intervention. It is important to note that the pigs in the study were kept on a very well-managed farm, where there is enough staff to react immediately and take appropriate measures when tail biting occurs. This is not always possible in practice, and a lack of occupational materials could lead to more damaging behaviors if the urge to engage with mobile and destructible materials is not satisfied. The relationship between ETV-directed behaviors and engagement with straw or objects is probably not causal; rather, it is conceivable that another variable drives both behaviors.

PE and activity across groups

The two farrowing series differed in PE. The first series, which was born in May 2020, had a PE 0.2 lower than that of the second series, which was born in September 2020. Because the total PE range covers just 0.13, a difference between groups of 0.02 is therefore approximately 16%. This difference could be due to seasonality on the one hand and genetics on the other. Protein efficiency has been shown to be moderately heritable [22]; therefore, because mothers and fathers did not overlap in the series, it is likely that some of the differences in PE between groups are genetic. In the observation month of the first series, in August 2020, the outdoor temperature fluctuated between 21.2°C and 30.4°C during the observation hours, resulting in a temperature between 23.7°C and 27.1°C in the barn. In contrast, while observing the second series in December 2020, the outdoor and

indoor temperatures ranged between 0.3°C and 14.9°C and 14.2°C and 17.5°C, respectively. As the ideal temperature for fattening pigs is between 5 and 17°C [44], the high temperature in August might have affected the animals' metabolism and thus slowed their activity. With increasing temperatures, PE decreases [22], which can be explained by the altered metabolism during heat stress. In general, pigs exposed to high temperatures have lower muscle and higher fat mass [45]. Muscle growth could be impaired due to increased redirection of AAs to the immune and hormonal systems, as both are more activated under heat stress [46], due to reduced energy availability in the muscle tissue [47], or due to increased muscle proteolysis [45].

Similarly, the activity scores differed between the farrowing series, with a mean score of 13.0 ± 6.6 in the first and 16.4 ± 6.10 in the second. The difference of 3.4 points amounts to 20% of the range in activity score, a difference that could again be attributed to temperature because pigs reduce their activity when heat-stressed [48]. In addition, a genetic contribution to the differences between groups cannot be excluded, as activity has been shown to have low to moderate heritability [49].

Activity and PE were positively correlated, but whether this represents a direct link or a causal relationship is difficult to determine. Pigs kept outdoors in winter before slaughter, where they can roam a larger area while foraging, had a higher lean percentage than pigs reared indoors, and the aerobic capacity of glycolytic muscles was higher in outdoor pigs [50]. A higher lean percentage might be reflected in a higher PE, but PE ultimately depends on the amount of protein ingested, which was unknown for the pigs in Bee et al. [50]. The correlation between activity and PE could also mean that animals with high PE are healthy and vital, and they might be able to cope better with rather drastic dietary protein reductions.

Conclusion

The potential consequences of selection for higher PE on pig welfare are thus far unknown, but a trade-off between sustainability and welfare is a concern. This study suggests that PE is not associated with detrimental behaviors, even in a scenario in which protein in feed is severely restricted. However, we cannot completely rule out the possibility that PE may be related to an increased frequency of manipulation of pen mates. While this may not necessarily be problematic, as the behavior was not directed at vulnerable body regions, it could possibly indicate misdirected foraging behavior. This is supported by our observations of the heightened activity of protein-efficient pigs. The results of this work show how important it is to provide pigs with an appropriate environment and sufficient space, in combination with close monitoring of pens via automatic early warning systems [9], especially if they are to become more efficient through breeding. Moreover, the effects of breeding for higher PE should be carefully evaluated for possible negative effects on pigs' physiology and immune systems to avoid these potential causes of damaging behavior. In addition, efforts should also be made to improve the stress susceptibility of pigs through breeding. While this has so far been hampered by low or zero heritability [18], the development of biomarkers [51] and automated phenotyping (e.g., [52]) could be successful in the future.

Data availability statement

The data that support the findings of this study are publicly available from Zenodo [23].

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Acknowledgements

We thank Guy Maïkoff and his team for pig husbandry and Hanno Würbel, Giuseppe Bee, and Catherine Ollagnier for discussions. We are grateful to Madeleine F. Scriba for helpful comments on a version of the manuscript. This work was financially supported by a grant of the Fondation Sur-la-Croix (https://www.fondation-sur-la-croix.ch/fr) to CK. This study is the result of LR's master's thesis, which was awarded the Jean-Pierre Miéville 2021 prize for an outstanding contribution of veterinary medicine to animal welfare.

Author contributions

Conceptualization: LR and CK. Data curation: LR and CK. Formal analysis: LR, EOE, and CK. Funding acquisition: LR and CK. Investigation: LR and EOE. Methodology: LR and CK. Project administration: LR and CK. Resources: CK. Software: LR, EOE, and CK. Supervision: CK. Validation: CK. Writing - original draft: LR. Writing - review & editing: LR, EOE, and CK.

Competing Interests Statement

The authors declare no competing interests.

Figure legends

Fig. 1: Protein efficiency in the two farrowing series (left) and sexes (right). Abbreviations: F females, M castrated males.

Fig. 2: Activity scores (see main text for details) in the two farrowing series (left) and sexes (right). Abbreviations: F females, M castrated males.

Fig. 3: The sum of all active pig-directed behaviors recorded in the four observations. On only nine occasions, pigs directed damaging behaviors toward conspecifics (i.e., biting directed at the ETV regions), and all eight biters had an average or below-average PE. Damaging behaviors were received four times by four different pigs during the focal observations, and receivers also had below-average PE.