



Genetic analyses of nutrient digestibility measured by fecal near-infrared spectroscopy in pigs

Kristine Hov Martinsen,[†] Signe Lovise Thingnes,[†] Sini Elena Wallén,[‡] LivTorunn Mydland,[‡] Nils Kristian Afseth,[‡] Eli Grindflek,[†] and Theodorus Hendrikus Elisabeth Meuwissen[‡]

[†]Norsvin, Department of Research, Storhamargata, NO-2317 Hamar, Norway

[‡]Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, NO-1432 Ås, Norway

[‡]Nofima AS – Norwegian Institute of Food, Fisheries and Aquaculture Research, Department of Raw Materials and Process Optimization, N-1431 Ås, Norway

[†]Corresponding author: kristine.martinsen@norsvin.no

Abstract

Improved nutrient digestibility is an important trait in genetic improvement in pigs due to global resource scarcity, increased human population and greenhouse gas emissions from pork production. Further, poor nutrient digestibility represents a direct nutrient loss, which affects the profit of the farmer. The aim of this study was to estimate genetic parameters for apparent total tract digestibility of nitrogen (**ATTDn**), crude fat (**ATTDcfat**), dry matter (**ATTDdm**), and organic matter (**ATTDom**) and to investigate their genetic relationship to other relevant production traits in pigs. Near-infrared spectroscopy was used for prediction of total nitrogen content and crude fat content in feces. The predicted content was used to estimate apparent total tract digestibility of the different nutrients by using an indicator method, where acid insoluble ash was used as an indigestible marker. Average ATTDdm, ATTDom, ATTDn, and ATTDcfat ranged from 61% to 75.3%. Moderate heritabilities were found for all digestibility traits and ranged from 0.15 to 0.22. The genetic correlations among the digestibility traits were high (>0.8), except for ATTDcfat, which had no significant genetic correlation to the other digestibility traits. Significant genetic correlations were found between ATTDn and feed consumption between 40 and 120 kg live weight (F40120) (-0.54 ± 0.11) and ATTDdm and F40120 (-0.35 ± 0.12) and ATTDom and F40120 (-0.28 ± 0.13). No significant genetic correlations were found between digestibility traits and loin depth at 100 kg, nor backfat thickness at 100 kg (**BF**), except between BF and ATTDn (-0.31 ± 0.14). These results suggested that selection for improved feed efficiency through reduced feed intake within a weight interval, also has led to improved ATTDdm, ATTDom, and ATTDn. Further, the digestibility traits are heritable, but mainly related to feed intake and general function of the intestines, as opposed to allocation of feed resources to different tissues in the body.

Lay Summary

Improved nutrient digestibility is an important trait in genetic improvement of pigs due to global resource scarcity, increased human population and greenhouse gas emissions from pork production. The main aim of this study was to investigate whether nutrient digestibility traits in pigs are heritable, and if they are genetically linked to other production traits. The results showed that digestibility of dry matter, organic matter, nitrogen, and crude fat are heritable, and can be selected for in a pig breeding program. The traits are genetically linked to other relevant production traits, such as feed intake, but not to carcass traits, such as loin depth. The results suggest that nutrient digestibility are traits that can be selected for, and that the traits are under indirect selection through other traits in the pig breeding program. The results also indicate that the nutrient digestibility traits express how well the animal utilizes consumed feed, rather than allocating feed to different tissue deposition.

Key words: feed efficiency, genetic variance, near-infrared spectroscopy, nitrogen digestibility, pig

Abbreviations: AIA, acid insoluble ash; ATTD, apparent total tract digestibility; ATTDcfat, apparent total tract digestibility of crude fat; ATTDdm, apparent total tract digestibility of dry matter; ATTDn, apparent total tract digestibility of nitrogen; ATTDom, apparent total tract digestibility of organic matter; BF, backfat thickness at 100 kg live weight; CT, computer tomography; D40120, days from 40 to 120 kg live weight; FCR, feed conversion ratio; F40120, feed consumption from 40 to 120 kg live weight; LDP, loin depth at 100 kg live weight; NIRS, near-infrared spectroscopy

Introduction

Livestock products provide 34% of the protein consumed in human diets and provide a significant contributor to the global food supply (FAO, 2004). Hence, efficient use of nutrients in animal production is important to accommodate human population growth, climate changes that generally hamper food production, mitigating greenhouse gas emissions and uncertainties around food production. Proteins are costly nutrients in animal feed and efficient use of proteins from feed ingredients is therefore important for

future animal production. Pigs are already efficient in protein deposition, and according to Norwegian Environment Agency (2023) the Norwegian pig production account for less than 1% of the total greenhouse gas emissions from Norway. However, by genetically improving nutrient efficiency in pigs, production efficiency can be improved even further. This improvement will impact aspects of environmental sustainability in pork production, as feed production is the most significant contributor to environmental impact from pork production (Bonesmo et al., 2012). The improvement

Received February 24, 2023 Accepted June 30, 2023.

© The Author(s) 2023. Published by Oxford University Press on behalf of the American Society of Animal Science. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com.

could allow for reduced need for protein in feed, reduced greenhouse gas emissions from feces and reduced eutrophication related to spreading of manure (Møller et al., 2022). The Norwegian maternal line has been selected for improved feed efficiency since 1958 and is highly feed efficient (Gjerlaug-Enger et al., 2012). However, to further improve feed efficiency, reduce the loss of nutrients and carbon footprint of pig production, nitrogen digestibility can be a trait of interest for genetic selection. Previous studies have shown that between breed variation exists for digestibility of different nutrients (Kanengoni et al., 2002; Barea et al., 2011; Urriola and Stein 2012). However, within breed variation is less investigated. This is partly due to the quantity of records needed for estimation of additive genetic variance, as recording of digestibility traits is labor intensive. A few studies have shown that genetic variation in different nutrient digestibility traits exists (Noblet et al., 2013; Hardie et al., 2014; Déru et al., 2021). Déru et al. (2021) estimated heritabilities for fecal digestibility of energy, nitrogen, and organic matter on a European conventional diet to be 0.26, 0.27, and 0.27, respectively.

Measuring digestibility on a large-scale can be challenging, as this previously has been measured by using respiratory chambers and total excreta collection. Near-Infrared spectroscopy (NIRS) is a rapid and non-destructive technique used to estimate chemical composition of analytes. In recent years, NIRS has been frequently studied for the prediction of feces composition and digestibility of both herbivores (Dixon and Coates, 2009; Villamuelas et al., 2017) and omnivores (Steyaert et al., 2012). For pig digestibility, it has been shown that NIRS is a feasible technique both for predicting the chemical composition of feces and for prediction of apparent total tract digestibility (ATTD) of different feed components (Bastianelli et al., 2015; Schiborra et al., 2015; Nirea et al., 2018). Recently, Paternostre et al. (2021) showed that a combination of NIRS feed and feces spectra resulted in increased prediction performance of nutrient digestibility compared to using the individual calibration models. The aim of this study was to estimate genetic parameters for ATTD of nutrients measured by fecal NIRS and investigate genetic relationships to other production traits in pigs.

Materials and Methods

Approval of the Animal Care and Use Committee was not required for this study as the data was collected with a non-invasive method or were acquired from an existing database. However, all animals were cared for according to laws and regulations controlling experiments with live animals in Norway (the Animal Protection Act of 20 December 1974, and the Animal Protection Ordinance concerning experiments with animals of 15 January 1996).

Animals, fecal sampling, and chemical analyses

The data material for these analyses were collected at Norsvins boar testing station in Norway. Boars are housed in pens of 11. In each pen there is one feed station (FIRE; Osborne Industries Inc., Osborne, KS, USA), where individual feed intake and weight is recorded at each visit. The animals are fed ad libitum on conventional concentrate containing 9.68 MJ NE and 11.11 g SID lysine per kilogram

feed in phase 1, and 9.59 MJ NE and 9.11 g SID lysine per kilogram feed in phase 2 (from 65 kg live weight). More details regarding the diets can be found in Table 1. The test interval is from 40 to 120 kg live weight. As a standard, the test is terminated, and Computer Tomography (CT) scans performed when boars reach ~120 kg. Through image analysis of the scans, CT provides information directly on the selection candidate for the traits lean meat (kg) and fat (kg) on the carcass. In the process of CT scanning, fecal samples were collected for a random sample of Duroc boars (terminal sire line) ending the test. Individual fecal samples were sampled as one grab sample during the test, in pens where the animals are kept individually for 24 h before and after CT scanning. At this stage, all animals had a body weight of ~120 kg. The samples were stored at -20 °C before pre-processing at the Livestock Production Research Centre, Norwegian University of Life Sciences (The Livestock Production Research Centre, 2022; The Livestock Production Centre, 2022). The samples were freeze dried, homogenized, and grounded (1 mm sieve) prior to chemical analyses, performed at LabTek, Norwegian University of Life Sciences (The Livestock Production Centre, 2022) and NIRS analysis, performed at Norwegian Institute of Food, Fisheries and Aquaculture Research (Norwegian Institute of Food, Fisheries and Aquaculture Research., 2022). In total, 1,975 fecal samples were sampled from randomly selected Duroc boars from June 2019 to April 2021. Feed samples were taken from the feeding station in each pen. All feed and fecal samples were analyzed for dry matter (DM) and acid insoluble ash (AIA), whereas all feed samples also were analyzed for total nitrogen content (Kjeldahl-N) (Kjeldahl, 1883) and crude fat (Accelerated Solvent Extraction). A subset of the fecal samples (in total 300 samples) was selected and used for developing calibration models for total nitrogen content and crude fat in fecal samples. For these 300 samples, analysis for total nitrogen content (Kjeldahl-N) (Kjeldahl, 1883) and crude fat (Accelerated Solvent Extraction) were performed. Average fecal content of total nitrogen content (Kjeldahl-N) and crude fat (Accelerated Solvent Extraction) was 26.8 ± 3.0 g/kg and 50.8 ± 11.3 g/kg, respectively, for the 300 samples used for the calibration curves. Subsequently, the obtained

Table 1. Diet composition

Ingredients ¹	Unit	Phase 1	Phase 2
		Value	Value
Dry matter	%	87.80	87.72
Water	%	12.20	12.28
Crude protein	%	19.15	15.85
Fat_HCl	%	5.53	4.73
Crude fiber	%	5.07	5.78
Ash	%	5.39	4.86
Starch and sugar	%	39.88	43.28
Energy	MJ NE/kg	9.68	9.59
SID lysine	g/kg	11.11	9.11

¹The finisher diets are grain based with barley, wheat, and oats as the main raw materials. Soy is the main source of protein together with small amounts of fish meal.

calibration models were used to predict crude fat and total nitrogen content in the remaining fecal samples.

Fecal near-infrared spectroscopy

Fecal NIRS were recorded on all fecal samples for total nitrogen content and crude fat content. NIR spectra were acquired using an XDS Rapid Content Analyzer (Foss, Hilleroed, Denmark) equipped with a quartz halogen lamp and a PbS detector. The spectra were collected in reflectance mode with a ceramic reference standard over the spectral region of 400 to 2500 nm, with a digital resolution of 0.5 nm. All spectra were acquired in a standard sample cell with a quartz window. For each spectrum acquisition, the sample cell was automatically moved over the NIR sampling window for maximum sampling area exposure. Each spectrum resulted as the average of 32 scans, and all samples were measured in duplicates. Partial least squares regression was used for calibration and prediction, and segmented cross-validation employing 20 segments were used in the calibration stage. Subsequently, the calibration models were used to predict nitrogen and fat contents in the remaining fecal samples. Prior to regression and prediction, all NIR spectra were pre-processed employing first-order derivative and subsequent normalization using the Standard Normal Variate approach (Barnes et al., 1989).

Estimation of apparent total tract digestibility

Apparent total tract digestibility was calculated according to Maynard and Loosli (1969), specified in Model 1. Acid insoluble ash was used as an internal marker, occurring naturally in the feed. All nutrients were measured as fraction of DM. Apparent total tract digestibility was estimated as

$$\text{ATTD} (\%) = 100 - \left(100 \times \left(\left(\frac{\text{nutrient in faeces, kg DM}}{\text{nutrient in feed, kg DM}} \right) \times \left(\frac{\text{marker in feed, g DM}}{\text{marker in faeces, kg DM}} \right) \right) \right). \quad (1)$$

Digestibility and production traits

The digestibility traits included in the genetic analyses were apparent total tract digestibility of dry matter (ATTDDm), organic matter (ATTDom), nitrogen (ATTDn), and crude fat (ATTDCfat), whereas recorded ATTDm, ATTDom, ATTDn, or ATTDCfat that were more than four standard deviations from the mean were discarded from the dataset. The following production traits were included in the genetic analyses; feed consumption from 40 to 120 kg (F40120), days from 40 to 120 kg (D40120), loin depth at 100 kg (LDP) and back fat at 100kg (BF).

Genetic analyses

The traits were analyzed using linear animal models, and estimation of variance components and genetic correlations were performed using the DMU software package (Madsen and Jensen, 2013). The fixed effects used in the models were determined based on a general linear model analysis in R (R Core Team, 2021).

For all traits, heritability (b^2) was defined as $b^2 = \sigma_a^2 / (\sigma_a^2 + \sigma_e^2)$, where σ_a^2 is the additive genetic variance and σ_e^2 is the residual variance of the trait.

The coefficient of genetic variation (CV) was calculated as $\text{CV} = (\sigma_a / \bar{\mu}) \times 100$, where σ_a is the additive genetic standard deviation and $\bar{\mu}$ is the trait mean in the dataset.

For the genetic analyses of ATTDDm, ATTDom, ATTDn, and ATTDCfat the following model was used in univariate analyses:

$$y_{ijklmnopq} = \bar{\mu} + \text{MOB}_i + \text{OP}_j + \text{DAY}_k + \text{HYS}_l + \text{LITT_NO}_m + \text{animal}_n + \text{group}_o + e_{ijklmnopq}. \quad (2)$$

The fixed effects included in the model were birth month (MOB, $i = 1$ to 12), operator taking the fecal sample (OP, $j = 1$ to 12), day of sampling (DAY, $k = 1$ to 75), herd-year-season born (HYS, $l = 1$ to 130) and dam's litter number (LITT_NO, $m = 1$ to 3). The animal's additive genetic effect (animal) and group (group, $o = 1$ to 186 for ATTDDm and ATTDom, and $o = 1$ to 181 for ATTDn and ATTDCfat) were included as random effects.

For the genetic analyses of F40120, D40120, LDP, and BF the following model was used in trivariate analyses with ATTD traits:

$$y_{ijklmno} = \bar{\mu} + \text{MOB}_i + \text{HYS}_j + \text{LITT_NO}_k + \text{animal}_l + \text{group}_m + e_{ijklmno}. \quad (3)$$

The fixed effects included in the model were birth month (MOB, $i = 1$ to 12), herd-year-season born (HYS, $j = 1$ to 603) and dam's litter number (LITT_NO, $k = 1$ to 3). The animal's additive genetic effect (animal) and group (group, $m = 1$ to 1022) were included as random effects.

Pedigree information was traced back for 10 generations and in total there were 33,348 animals in the pedigree, included as a pedigree relationship matrix **A**. These analyses were performed using the DMU software (Madsen and Jensen, 2013). Genetic and environmental trait correlations were estimated by applying the above models in bivariate analyses.

Results

Descriptive statistics

Table 2 shows descriptive statistics of the dataset. The average feed intake from 40 to 120 kg was 171.4 ± 13.7 kg, with a high variation among animals. The boars used on average 75.0 ± 8.8 d from 40 to 120 kg and showed an average daily gain of 1.06 kg/d. The boars average backfat thickness and loin depth at 100 kg was 5.9 ± 1.5 mm and 57.0 ± 6.4 mm, respectively. The average apparent total tract digestibility of different nutrients varied between 61% to 75.3%, depending on nutrient (Table 2). The average ATTDDm and ATTDom were $73.2 \pm 4.5\%$ and $75.3 \pm 4.2\%$, respectively. Whereas ATTDn and ATTDCfat were $71.5 \pm 5.8\%$ and $61.0 \pm 8.9\%$, respectively. The calibration results obtained for total nitrogen content and crude fat were as follows: Total nitrogen: cross-validated $R^2 = 0.73$, estimation error (RMSECV) = 1.6 g/kg; and crude fat: cross-validated $R^2 = 0.44$, estimation error (RMSECV) = 8.5 g/kg.

Variance components and heritabilities

Estimates of variance components and heritabilities for all digestibility traits are presented in Table 3. In all traits except for ATTDCfat, additive genetic variance explained more of

Table 2. Descriptive statistics for feed consumption from 40 to 120 kg (F40120), days from 40 to 120 kg (D40120), loin depth at 100 kg (LDP), and back fat at 100kg (BF)

Trait	<i>n</i> ¹	Mean	SD ¹	Min ¹	Max ¹
ATTDdm, %	1,950	73.2	4.6	47.2	84.4
ATTDom, %	1,949	75.3	4.2	58.2	86.6
ATTDn, %	1,890	71.5	5.8	47.2	85.5
ATTDCfat, %	1,893	61.0	8.9	24.9	85.1
F40120, kg	16,009	171.4	13.7	100.3	274.7
D40120, d	16,118	75.0	8.8	45.9	140.3
BF, mm	10,681	5.9	1.5	2.9	13.0
LDP, mm	10,887	57.0	6.4	30.6	77.6

¹Number of animals (*n*), standard deviation (SD), minimum value (min), and maximum value (max).

. Apparent total tract digestibility of nitrogen (ATTDn), apparent total tract digestibility of crude fat (ATTDCfat), apparent total tract digestibility of dry matter (ATTDdm), and apparent total tract digestibility of organic matter (ATTDom).

Table 3. Variance components ± standard error (SE), heritabilities (*h*²), and coefficient of variation (CV) for apparent total tract digestibility of dry matter (ATTDdm), organic matter (ATTDom), nitrogen (ATTDn), and crude fat (ATTDCfat)

	Additive genetic variance (σ_a^2) ± SE	Pen variance (σ_{pen}^2) ± SE	Residual variance (σ_e^2) ± SE	<i>h</i> ²	CV (%)
ATTDdm, %	2.6 ± 0.8	1.9 ± 0.5	9.1 ± 0.6	0.22	2.2
ATTDom, %	2.3 ± 0.6	1.4 ± 0.4	8.1 ± 0.5	0.22	2.0
ATTDn, %	4.9 ± 1.5	2.3 ± 0.8	20.0 ± 1.3	0.20	3.1
ATTDCfat, %	3.8 ± 1.3	6.1 ± 1.4	21.7 ± 1.2	0.15	3.2

Table 4. Genetic correlations and standard errors between apparent total tract digestibility of dry matter (ATTDdm), organic matter (ATTDom), nitrogen (ATTDn), and crude fat (ATTDCfat) and production traits

	F40120 ¹	D40120 ²	LDP ³	BF ⁴
ATTDdm	-0.35 (0.12)	0.07 (0.14)	-0.17 (0.13)	-0.10 (0.14)
ATTDom	-0.28 (0.13)	0.10 (0.14)	-0.19 (0.13)	-0.01 (0.14)
ATTDn	-0.54 (0.11)	0.11 (0.14)	-0.11 (0.13)	-0.31 (0.14)
ATTDCfat	-0.20 (0.16)	-0.05 (0.17)	0.25 (0.14)	-0.18 (0.16)

¹Feed consumption from 40 and 120 kg live weight, kg

²Days from 40 to 120 kg live weight

³Loin depth at 100 kg, mm

⁴Back fat at 100 kg, mm

the phenotypic variance than the pen variance (Table 3). Moderate heritability estimates were found for ATTDdm, ATTDom, ATTDn, and ATTDCfat. The lowest heritability was found for ATTDCfat (0.15), whereas the heritability estimated for ATTDdm and ATTDn was 0.22 and 0.20, respectively. The heritability of ATTDom was estimated to 0.22 (Table 3). The coefficients of variation for the four different digestibility traits were low, ranging from 2.0 (ATTDom) to 3.2 (ATTDCfat) (Table 3). The results suggested that there was little genetic variation expressed in the traits in the current dataset.

Genetic correlations

The estimated genetic correlations from the multivariate analysis are presented in Table 4. Genetic correlations were estimated among digestibility traits (ATTDdm, ATTDom, ATTDn, and ATTDCfat) and relevant production traits (F40120, D40120, BF, and LDP) measured on boars at the

testing station. Overall, the genetic correlations were low to moderate, and mostly not significant. Significance was tested based on the estimate ± 1.96 × SE, which signifies a 95% confidence interval for the estimate (*P* < 0.05). The genetic correlations among ATTDn, ATTDdm, and ATTDom were high and ranged from 0.83 ± 0.68 to 0.99 ± 0.002. The genetic correlations between ATTDCfat and the other ATTD traits were low to zero, ranging from -0.10 ± 0.23 to 0.028 ± 0.22.

Four genetic correlations were found to be significantly different from zero, between ATTDdm and F40120 (-0.35), ATTDn and F40120 (-0.54), ATTDom and F40120 (-0.28), and ATTDn and BF (-0.31). These results suggested that the animals with a lower feed intake over a fixed growth period would be expected to have a higher apparent total tract digestibility of DM, organic matter, and nitrogen. Further, animals with a genetic potential for lower BF, would have a higher ATTDn. No significant genetic correlations were found between D40120 and digestibility traits, nor LDP and digestibility traits.

Discussion

The current study found heritable variation existing for different digestibility traits in pigs and hence confirms that it is possible to select for improved digestibility of nutrients such as nitrogen and crude fat to minimize nutrient loss in pig production. In a global perspective, such a selection could reduce the use of protein rich feed ingredients to pigs, and hence reduce the competition for feed resources between human and animal consumption. Further, improved utilization of nutrients in feed can reduce the demands to nutrient level in the feed, as the animals can utilize the nutrients more efficiently than earlier. This again, can allow increased

use of local feed resources, use of novel feed ingredients, and reduced greenhouse gas emissions from the feces. Still, most of the nitrogen excretion from pig manure are from urine and diet dependent (Jørgensen et al., 2013), but a reduction in nutrient loss in feces could contribute to less emissions.

Various studies have investigated the genetic background of different traits related to improved feed efficiency and digestibility and found similar results (Do et al., 2013; Déru et al 2020, 2021; Santiago et al. 2021; Bouquet et al. 2022). Improved digestibility of nutrients will increase the resource utilization of the animal. Selection for changed body composition, such as carcass leanness and reduced backfat has been a strategy to obtain feed efficient animals in traditional pig breeding for decades. Other traits, such as gross feed intake, feed conversion ratio and residual feed intake has also been used in selective breeding programs (Patience, 2012). These traits provide selection responses that are related to the allocation of feed resources to different tissues. This has ensured that feed is used for growth of lean tissue. However, to improve feed efficiency further, it is important to know how well the pig can utilize the nutrients in the feed. Therefore, a more direct trait of proportion of nutrients absorbed in the intestines can provide new insight to the individual feed efficiency of pigs. The digestibility traits in the current study, such as ATTDn or ATTDcfat, can further improve the efficiency of the animals, and hence reduce nutrient loss to the environment, which is becoming an increasing global pollution issue in the pork production.

Apparent total tract digestibility with indicator method

In practical situations, the measure of total feed intake and feces output of an animal can be challenging, both from a cost (including labor) perspective, but also from an animal welfare perspective. Further, pigs are kept in groups, which makes it even more challenging. Apparent total tract digestibility based on spot sampling and using an indicator is therefore a less labor-intensive and more animal friendly method, compared to total collection method, where all feces and urine are collected (Adeola, 2000). However, it is important to ensure that the sample is representative, with a proper sample weight. A disadvantage is that variation in digestibility over time exists (increasing with age) and this information could be lost when an indicator method with one grab sample is used (Le Goeff and Noblet, 2001; Bastianelli et al., 2015). Jang et al. (2014) reported that the total collection method gave higher estimated ATTD than the indicator method in pigs, suggesting that the indicator method could underestimate the digestibility values. However, this underestimation could be handled by pooling multiple spot samples (Moughan et al., 1991; Jang et al. 2014). Moore (1958) reported that errors from using one grab sample could be improved by pooling multiple grab samples. However, to fit the practical routines at the testing station and avoid animal stress and labor, one grab sample was performed in the current study. Jang et al. (2014) found similar estimates for apparent total tract digestibility of different nutrients within treatment but between different day of grab sampling, supporting the current methodology.

Acid insoluble ash as an indigestible marker

To predict ATTD_{dm} and ATTD_n based on a spot fecal sample, AIA was used as a marker. Brestenský et al. (2017) and

Prawirodigdo et al. (2019) suggested that AIA was a suitable marker to use in digestibility studies for pigs. In a review by Sales and Janssens (2003), most studies comparing total collection method with indicator method where AIA was used as a marker, found similar results between the two methods. McCarthy et al. (1977) found low daily variation in fecal AIA content, suggesting that use of one grab sample would be feasible. However, caution must be made during sampling, to avoid contamination. In the current study, rooting material was held back for three days prior to sampling to avoid contamination of feces with AIA from other sources than the feed. Due to welfare regulations in Norway, activation material must be provided. The hay was therefore substituted by non-printed newspaper. Non-published results of chemical analysis of regular printed newspaper, showed an average level of 0.25 g AIA/kg newspaper. The level of AIA was assumed to be related to the ink of the newspaper, and this level of AIA would influence the estimate of digestibility based on how much is eaten by the animal, but in a minor manner. The paper provided in the current study was therefore non-printed newspaper, which was assumed to have negligible influence on the digestibility estimates in the study.

Thonney et al. (1985) indicated that the level of dietary AIA should be minimum 7.5 g/kg DM in cattle to avoid inaccurate measurements. The current study had an average of 3.2 g AIA/kg DM, which might be a reason for lower digestibility estimates than previous literature (Moughan et al. 1991; Brestenský et al., 2017; Déru et al., 2021). However, for pigs, no such minimum level was found in literature. It is notable, however, that Prawirodigdo et al. (2021) had a lower dietary AIA than the current study. Further, the method for chemical analysis could influence the level of digestibility estimates, but as the same method are used across animals, it is not expected to affect the variation in digestibility estimates. Bastianelli et al. (2015) proposed to use direct prediction of digestibility of different nutrients using NIRS. Then, the need for a marker would be minimized, as it is only needed for the fecal samples used for developing the calibration curves.

Near-infrared spectroscopy

There are a few aspects of the NIRS calibrations that need to be addressed. First, the choice of freeze-drying all samples prior to NIRS analysis was done out of two reasons: 1) to avoid practical sampling uncertainties related to changing moisture contents of feces; and 2) to potentially pre-concentrate minor feces components and thus make them more visible in the NIRS spectra. The downside of this choice is that by removing water, it also removes one of the main chemical components in the feces contributing to gross component modelling. It is well known that when using NIRS for prediction of e.g., proteins or fats, the water component in the NIRS spectra is indirectly used in the models, since more water in one sample might lead to less protein or fat in the same sample (Eskildsen et al., 2021). Doing NIR measurements on wet feces, Paternostre et al (2021) obtained good calibrations for all gross components in the feces samples. In the current study, the protein calibration model has comparatively lower predictive performance (see under “Results – descriptive statistics”), but the model can still be regarded as feasible for screening studies. For the fat calibration, on the other hand, the predictive performance is too low for screening purposes (see under “Results – descriptive statistics”). It should be noted that for the crude fat reference measurements of the current

study, accelerated solvent extraction was used, and using other extraction approaches could most likely provide more accurate numbers for fat contents in feces. The calibration range of fat contents is also significantly lower than for protein contents, which also could be one explanation why the protein calibration performs better than the respective crude fat calibration. The calibration results of the current study are comparable to the results provided in Nirea et al. (2018), that also performed NIR measurements on freeze-dried feces.

Genetic parameters

The variance components estimated in this study suggested that all digestibility traits are heritable and can be selected for. Few studies have previously carried out genetic analyses of digestibility traits in pigs. However, Déru et al. (2021) found similar results for digestibility coefficients of energy, organic matter and nitrogen. Déru et al. (2021) estimated a heritability for digestibility coefficients for nitrogen to be 0.27. This result was higher than in the current study, but on a smaller dataset, a different breed and for animals fed a conventional diet. The same study found an even higher heritability for nitrogen digestibility on a high-fiber diet (0.56), due to higher genetic variation. Increasing fiber content in the diet might increase the genetic variation in the trait but could at the same time reduce the digestibility of nutrients (Zhang et al., 2013). Déru et al. (2021) also corrected for the feed intake of the animal in the genetic model, to avoid that the variation in feed intake affects the estimated genetic variance in digestibility of nutrients. The current study did not correct for individual feed intake, as this was tested. Adding daily feed intake as a fixed covariate in the model lead to minor reduction in variance components and genetic correlations among ATTD traits and F40120. This suggests that there was no systematic relationship between digestibility and feed intake in the current dataset. The current dataset is highly standardized in weight and environment, as the data is recorded on a boar testing station.

Saintilan et al. (2013) estimated significant heritabilities for nitrogen retention in pig breed. Similarly, Kasper et al. (2020) estimated heritabilities of 0.36, for nitrogen efficiency of the carcass. Both studies reflect on traits which is partly dependent of the nitrogen intake and absorption in the intestines. Genetic variation existing in these traits might therefore support the current results in genetic differences in ATTDn.

Genetic correlations between the digestibility traits and production traits were identified and suggested that the current selection strategy is improving the digestibility of nitrogen and DM. The genetic correlations suggested that ATTDn and ATTDdm are a part of the genetic mechanism involved in F40120 and that improved ATTDn and ATTDdm also would improve F40120. The results are in accordance with Déru et al. (2021), which estimated a high negative genetic correlation between nitrogen digestibility and daily feed intake (-0.59) in animals on conventional diet, and favorable correlations between nitrogen and DM digestibility and feed conversion ratio (FCR). Vigors et al. (2016) found that animals selected for low residual feed intake also had significantly lower FCR and higher ATTDn and ATTDdm, than animals selected for high residual feed intake, supporting the genetic parameters found in the current study and Déru et al. (2021). A significant correlation was also found between ATTDn and BF, suggesting that animals with a high digestibility of nitrogen, had lower back fat at 100 kg. As highlighted earlier, feed intake influ-

ences the digestibility of nutrients, and hence the deposition of tissue. The lack of correction for feed intake in the current study might have led to overestimation of this genetic correlation. A low feed intake would enhance the digestibility of nutrients and result in less fat deposition in an animal, and hence might lower this genetic correlation. The current study did not find any significant genetic correlations between ATTDdm, ATTDn and D40120 or LDP. This highlights that digestibility traits are heritable, but mainly related to feed intake and general function of the intestines, as opposed to allocation of feed resources to different tissue in the body.

In conclusion, the current study found that the different ATTD traits are heritable and possible to select for. Based on the genetic correlations, selecting for improved growth and reduced feed intake within a specific weight interval are improving ATTDn and ATTDdm. For further improvements of the digestibility traits, inclusion of the studied traits in the breeding goal would be beneficial.

Acknowledgments

The authors would like to thank the technicians at Norsvin Delta for sampling and Labtek at NMBU for chemical analyses. This study was funded by the Norwegian Research Council, Fund for Research Fees for Agricultural Products and Agricultural Agreement Research Fund (FFL/JA), through project number 268127.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Literature Cited

- Adeola O. 2000. Digestion and balance techniques in pigs. In: Lewis J.A., Southern L.L., editors. *Swine nutrition*. 2nd ed. Boca Raton (USA): CRC Press; p. 903–916.
- Barea, R., R. Nieto, F. Vitari, C. Domeneghini, and J. Aguilera. 2011. Effects of pig genotype (Iberian v. Landrace x Large White) on nutrient digestibility, relative organ weight and small intestine structure at two stages of growth. *Animal*. 5:547–557. doi:10.1017/S1751731110002181.
- Barnes, R. J., M. S. Dhanoa, and S. J. Lister. 1989. Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *Appl. Spectrosc.* 43:772–777. doi:10.1366/0003702894202201.
- Bastianelli, D., L. Bonnal, Y. Jaquelin-Peyraud, and J. Noblet. 2015. Predicting feed digestibility from NIRS analysis of pig faeces. *Animal*. 9:781–786. doi:10.1017/S1751731114003097.
- Bonesmo, H., S. M. Little, O. M. Harstad, K. A. Beauchemin, A. O. Skjelvåg, and O. Sjelmo. 2012. Estimating farm-scale greenhouse gas emission intensity of pig production in Norway. *Acta Agric. Scand. A*. 62:318–325. doi:10.1080/09064702.2013.770913.
- Bouquet, A., V. Déru, B. Blanchet, P. Garnier, L. Flatrès-Grall, B. Ligonésche, C. Carillier-Jacquin, E. Labussière, and H. Gilbert. 2022. Digestive efficiency traits in growing pigs are genetically correlated with sow litter traits in the Large White breed. *Animal*. 16(100447):1–7. doi:10.1016/j.animal.2021.100447.
- Brestenský, M., S. Nitrayová, J. Heger, and P. Patrás. 2017. Chromic oxide and acid-insoluble ash as markers in digestibility studies with growing pigs and sows. *J. Anim. Physiol. Anim. Nutr.* 101:46–52. doi:10.1111/jpn.12503.
- Déru, V., A. Bouquet, C. Hassenfratz, B. Blanchet, C. Carillier-Jacquin, and H. Gilbert. 2020. Impact of high-fibre diet on genetic parameters of production traits in growing pigs. *Animal*. 14:2236–2245. doi:10.1017/S1751731120001275.

- Déru, V., A. Bouquet, E. Labussière, P. Ganier, B. Blanchet, C. Carillier-Jacquin, and H. Gilbert. 2021. Genetics of digestive efficiency in growing pigs fed a conventional or high-fibre diet. *J. Anim. Breed. Genet.* 138:246–258. doi:10.1111/jbg.12506.
- Dixon, R., and D. Coates. 2009. Near infrared spectroscopy of faeces to evaluate the nutrition and physiology of herbivores. *J. Near Infrared Spectrosc.* 17:1–31. doi:10.1255/jnirs.822.
- Do, D. N., A. B. Strathe, J. Jensen, T. Mark, and H. N. Kadarmideen. 2013. Genetic parameters for different measures of feed efficiency and related traits in boars of three pig breeds. *J. Anim. Sci.* 91:4069–4079. doi:10.2527/jas.2012-6197.
- Eskildsen, C. E., T. Næs, P. B. Skou, L. E. Solberg, K. R. Dankel, S. A. Basmoen, J. P. Wold, S. S. Horn, B. Hillestad, N. A. Poulsen, M. Christensen, T. Pieper, N. K. Afseth, and S. B. Engelsen. 2021. Cage of covariance in calibration modelling: regressing multiple and strongly correlated response variables onto a low rank subspace of explanatory variables. *Chemometr. Intell. Lab. Syst.* 213:104311. doi:10.1016/j.chemolab.2021.104311
- Food and Agriculture organization of the United Nation (FAO). 2004. Protein sources for the animal feed industry. [Accessed 26 November 2022]. <https://www.fao.org/3/y5019e00.htm>.
- Gjerlaug-Enger, E. J., J. Kongsro, J. Ødegård, L. Aass, and O. Vangen. 2012. Genetic parameters between slaughter pig efficiency and growth rate at different body tissue estimated by computer tomography in live boars of Landrace and Duroc. *Animal.* 6:9–18. doi:10.1017/S1751731111001455.
- Hardie, L. C., N. V. L. Serão, R. R. Rowland, J. F. Patience, J. C. M. Dekkers and N. K. Gabler. 2014. Proc. 10th World Congress of Genetics Applied to Livestock Production.
- Jang, Y. D., M. D. Lindemann, J. H. Agudelo-Trujillo, C. S. Escobar, B. J. Kerr, N. Inocencio, and G. L. Cromwell. 2014. Comparison of direct and indirect estimates of apparent total tract digestibility in swine with effort to reduce variation by pooling of multiple day fecal samples. *J. Anim. Sci.* 92:4566–4576. doi:10.2527/jas.2013-6570.
- Jørgensen, H., T. Prapasongsa, V. T. K. Vu, and H. D. Poulsen. 2013. Models for quantifying excretion of dry matter, nitrogen, phosphorus and carbon in growing pigs fed regional diets. *J. Anim. Sci. Biotechnol.* 4:42. doi:10.1186/2049-1891-4-42.
- Kanengoni, A. R., K. Dzama, M. Chimonoyo, J. Kusina, and S. M. Maswaure. 2002. Influence of level of maize cob meal on nutrient digestibility and nitrogen balance in Large White, Mukota and LW x M F1 crossbred pigs. *Anim. Sci.* 74:127–134. doi:10.1017/S1357729800052292.
- Kasper, K., I. Ruiz-Ascacibar, P. Stoll, and G. Bee. 2020. Investigating the potential for genetic improvement of nitrogen and phosphorus efficiency in a Swiss large white pig population using chemical analysis. *J. Anim. Breed. Genet.* 137:545–558. doi:10.1111/jbg.12472.
- Kjeldahl, J. 1883. A new method for the determination of nitrogen in organic matter. *Zeitschrift für Analytische Chemie.* 22:366–382. doi:10.1007/BF01338151.
- Le Goff, G., and J. Noblet. 2001. Comparative total tract digestibility of dietary energy and nutrients in growing pigs and adult sows. *J. Anim. Sci.* 79:2418–2427. doi:10.2527/2001.7992418x.
- Madsen, P., and J. Jensen. 2013. *A user's guide to DMU. Version 6, release 5.2.* Tjele, Denmark: University of Aarhus, Center for Quantitative Genetics and Genomics Dept. of Molecular Biology and Genetics, Research Centre Foulum.
- Maynard, L. A., and J. K. Loosli. 1969. *Animal nutrition.* 6th edition. New York, US: McGraw-Hill Book Company.
- McCarthy, J. F., J. P. Bowland, and F. X. Aherne. 1977. Influence of method upon the determination of apparent digestibility in the pig. *Can. J. Anim. Sci.* 57:131–135. doi:10.4141/cjas77-015.
- Møller, H., S. Samsonstuen, M. Øverland, I. S. Modahl, and H. F. Olsen. 2022. Local non-food yeast protein in pig production-environmental impacts and land use efficiency. *Livest. Sci.* 260(104925):1–10. doi:10.1016/j.livsci.2022.104925.
- Moore, J. H. .1958. The effect of diurnal variations in composition of the faeces of pigs on the determination of digestibility coefficients by the chromium-oxide method. *Br. J. Nutr.* 12:24–34. doi:10.1079/bjn19580006.
- Moughan, P. J., W. C. Smith, J. Schrama, and C. Smits. 1991. Chromic oxide and acid-insoluble ash as faecal markers in digestibility studies with young growing pigs. *N. Z. J. Agric. Res.* 34:85–88. doi:10.1080/00288233.1991.10417796.
- Nirea, K. G., M. P. de Nanclares, A. Skugor, N. K. Afseth, T. H. E. Meuwissen, J. Hansen, L. T. Mydland, and M. Øverland. 2018. Assessment of fecal near-infrared spectroscopy to predict faeces chemical composition and apparent total-tract digestibility of nutrients in pigs. *J. Anim. Sci.* 96:2826–2837. doi:10.1093/jas/sky182.
- Noblet, J., H. Gilbert, Y. Jaguelin-Peyraud, and T. Lebrun. 2013. Evidence of genetic variability for digestive efficiency in the growing pig fed fibrous diet. *Animal.* 7:1259–1264. doi:10.1017/s1751731113000463.
- Norwegian Environment Agency. 2023. Greenhouse gas emissions 1990-2021, National Inventory Report. Norwegian Environment Agency, p. 718. Oslo, March 2023. Available from: <https://www.miljodirektoratet.no/publikasjoner/2023/mars-2023/greenhouse-gas-emissions-1990-2021/>
- Norwegian Institute of Food, Fisheries and Aquaculture Research. 2022. [Accessed November 2022]. <https://www.nofima.com>.
- Norwegian University of Life Sciences. 2022. [Accessed December 2022]. <https://www.nmbu.no/tjenester/laboratorietjenester/labtek>.
- Paternostre, L., V. Baeten, B. Ampe, S. Millet, and J. De Boever. 2021. The usefulness of NIRS calibrations based on feed and faeces spectra to predict nutrient content, digestibility and net energy of pig feeds. *Anim. Feed Sci. Technol.* 281:115091. doi:10.1016/j.anifeedsci.2021.115091.
- Patience J.F. 2012. *Feed efficiency in swine.* Wageningen, the Netherlands: Wageningen Academic Publishers.
- Prawirodigdo, S., N. J. Gannon, B. J. Leury, and F. R. Dunshea. 2021. Acid insoluble ash is a better indigestible marker than chromic oxide to measure apparent total tract digestibility in pigs. *Anim. Nutr.* 7:64–71. doi:10.1016/j.aninu.2020.07.003.
- Prawirodigdo, S., N. J. Gannon, B. J. Leury, and F. R. Dunshea. 2019. Basal diet and indigestible marker influence apparent digestibilities of nitrogen and amino acids of cottonseed meal and soybean meal in pigs. *Anim. Nutr.* 5:234–240. doi:10.1016/j.aninu.2019.02.001.
- R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Saintilan, R., I. Mérour, L. Brossard, T. Tribout, J. Y. Dourmad, P. Sellier, J. Bidanel, J. van Milgen, and H. Gilbert. 2013. Genetics of residual feed intake in growing pigs: Relationships with production traits, and nitrogen and phosphorous excretion traits. *J. Anim. Sci.* 91:2542–2554. doi:10.2527/jas.2012-5687.
- Sales, J., and G. P. J. Janssens. 2003. Acid insoluble ash as a marker in digestibility studies: a review. *J. Anim. Feed Sci.* 12:383–401. doi:10.22358/jafs/67718/2003.
- Santiago, K. G., S. H. Kim, B. I. Lopez, D. H. Lee, Y. G. Cho, Y. N. Song, and K. S. Seo. 2021. Estimation of genetic parameters for feeding pattern traits and its relationship to feed efficiency and production traits in duroc pigs. *Agriculture.* 11:850. doi:10.3390/agriculture11090850.
- Schiborra, A., M. Bulang, A. Berk, A. Susenbeth, and E. Schlecht. 2015. Using faecal near-infrared spectroscopy (FNIRS) to estimate nutrient digestibility and chemical composition of diets and faeces of growing pigs. *Anim. Feed Sci. Technol.* 210:234–242. doi:10.1016/j.anifeedsci.2015.10.011.
- Steyaert, S. M. J. G., F. J. Hutter, M. Elfstrom, A. Zedrosser, K. Hacklander, M. H. Le, W. M. Windisch, J. E. Swenson, and T. Isaksson. 2012. Faecal spectroscopy: a practical tool to assess diet quality in an opportunistic omnivore. *Wildlife Biol.* 18:431–438. doi:10.2981/12-036.
- The Livestock Production Research Centre. 2022. [Accessed November 2022]. <https://www.nmbu.no/en/about/livestock-production-research-centre>.

- Thonney, M. L., B. A. Palhof, M. R. DeCarlo, D. A. Ross, N. L. Firth, R. L. Quaas, D. J. Perosio, D. J. Duhaime, S. R. Rolins, and A. Y. M. Nour. 1985. Sources to variation in dry matter digestibility measured by acid insoluble ash marker. *J. Dairy Sci.* 68:661–668. doi:[10.3168/jds.S0022-0302\(85\)80872-9](https://doi.org/10.3168/jds.S0022-0302(85)80872-9).
- Urriola, P. E., and H. H. Stein. 2012. Comparative digestibility of energy and nutrients in fibrous feed ingredients fed to Meishan and Yorkshire pigs. *J. Anim. Sci.* 90:802–812. doi:[10.2527/jas.2010-3254](https://doi.org/10.2527/jas.2010-3254).
- Vigors, S., T. Sweeney, C. J. O’Shea, A. K. Kelly, and J. V. O’Doherty. 2016. Pigs that are divergent in feed efficiency, differ in intestinal enzyme and nutrient transporter gene expression, nutrient digestibility and microbial activity. *Animal*. 10:1848–1855. doi:[10.1017/S1751731116000847](https://doi.org/10.1017/S1751731116000847).
- Villamuelas, M., E. Serrano, J. Espunyes, N. Fernandez, J. R. Lopez-Olvera, M. Garel, J. Santos, M. A. Parra-Aguado, M. Ramanzin, X. Fernandez-Aguilar, et al. 2017. Predicting herbivore faecal nitrogen using a multispecies near-infrared reflectance spectroscopy calibration. *PLoS One* 12:e0176635. doi:[10.1371/journal.pone.0176635](https://doi.org/10.1371/journal.pone.0176635).
- Zhang, W., D. Li, L. Liu, J. Zang, Q. Duan, W. Yang, and L. Zhang. 2013. The effect of dietary fiber level on nutrient digestibility in growing pigs. *J. Anim. Sci. Biotechnol.* 4:17–23. doi:[10.1186/2049-1891-4-17](https://doi.org/10.1186/2049-1891-4-17).