FEED-A-GENE

Adapting the feed, the animal and the feeding techniques to improve the efficiency and sustainability of monogastric livestock production systems

Deliverable D2.5

Methodological tools (e.g. based on NIRS determination in faeces) for the rapid evaluation of variation in nutrient digestibility between animals

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1. Summary

In growing animals, feed efficiency is often described from the ratio between growth and corresponding feed consumption during a certain period. Feed efficiency is determined by the efficiency at digestive level and by the efficiency at a metabolic level. The efficiency measured at digestive level is difficult to assess in a large number of animals. In pigs, its measurement requires to house animals in a digestibility cage during a prolonged period to measure feed intake and totally collect the faeces that should be analysed for the nutrient contents. Because of this, this method cannot be used in selection farms of breeders. The variability in digestive ability has always been ignored whereas it has been shown to exist when animals are fed a diet with a high fibre content. The objectives of this deliverable are to identify an alternative methodology to characterize the digestive ability in pigs. For this purpose, an experiment was conducted at UMR Pegase, INRA, France with 63 pigs from Large White, Piétrain, and Duroc breeds that were fed alternatively two diets differing in crude fibre content (3.1 and 8.5% of dry matter) during four periods of three weeks each. The diets were supplemented with three molecules that may be used as indigestible marker: silicone oil, the plastic resin Kynar®, and polyethylene glycol. At each period, digestibility was measured using the gold standard (i.e., measurement of feed intake, total collection of faeces, and dedicated lab analyses to calculate the digestibility coefficients of dry matter, organic matter, energy, N, and crude fibre). At the end of each period, a sample of faeces was also collected directly from the rectum of the pigs. All samples were also analysed by Near InfraRed Spectroscopy (NIRS). The digestive ability of pigs was variable during the growing period, especially when the diet contained a high level of fibre and the digestibility of this type of diet increased when body weight increased. The utilization of indigestible markers did not give satisfying results, because of low recovery in the faeces (plastic resin or polyethylene glycol) or because of large variability in the recovery (silicone oil). The NIRS prediction of digestibility coefficients from a sample collected directly in the faeces was adequate (bias of digestibility coefficients for dry matter, organic matter, energy, and N close to 0%) when pigs are heavier than 60 kg and when they are fed a diet with a high fibre content. This type of diet is susceptible to challenge the digestive ability of pigs and allows identifying specifically the poor digesters in a population using NIRS. The method of collecting a single sample of faeces directly from the rectum of the pig and analysed by NIRS can be used in selection farms of breeders to give information on the digestive ability of individual pigs in a population.

2. Introduction

In growing animals, feed efficiency is often described from the ratio between growth and corresponding feed consumption during a certain period. Nevertheless, this ratio largely depends on the feed composition and the way animals use nutrients from the diet for growth. Feed efficiency for growth can be further partitioned in a digestive efficiency and a metabolic efficiency. Whereas the variation in metabolic efficiency because of growth characteristics (e.g., composition of bodyweight gain, ratio between viscera and body) has been characterized, the variation in digestive efficiency between animals from different breeds or from different genetic lines within a breed has received little attention. Recently, in pigs, it has been shown that this variation exists and that differences in energy digestibility between animals from different sires can differ up to 2 points of digestibility when pigs are fed a fibrous diet (Noblet et al., 2013).

In pigs, the gold standard to measure digestibility of nutrients and energy in animals is rather complex because it requires the adaptation of the animal to the diet during at least 10 days, followed during at least 7 days for accurate measurements of feed intake and simultaneous total collection of faeces, that should not be contaminated by urine. Consequently, experimental animals are most of the time of male gender and housed individually in a digestibility cage (at least during the collection period) to allow for the physical separation between faeces and urine. Apart from the collection of faeces, laboratory analyses involve additional steps, extensive labour, various laboratory equipment, and extensive associated costs. Only a limited number of animals can thus be included in digestibility studies, and this method cannot be used as a large-scale phenotyping tool to be used in selection schemes of breeders.

From a technical, laboratory point of view, predictive analyses, such as those based on Near InfraRed Spectroscopy (NIRS), are promising to reduce the time of analysis because a large variety of sample characteristics can be predicted from the NIRS spectra of a single measurement, once calibration equations have been established. First results in using this technique to predict nutrient and energy contents in faeces have been recently reported in pigs (Bastianelli et al., 2015; Schiborra et al., 2015) but they still require the measurement of feed intake and total collection of faeces to calculate satisfactorily the digestibility of nutrients and energy. Indigestible markers can be used as an alternative to the necessity to measure feed intake and total collection of faeces to assess digestibility. It involves the analysis of the marker content in diet and in a sample of faeces collected directly from the rectum of the pigs. Nevertheless, the markers currently used (e.g., titanium dioxide, chromium oxide, or ytterbium oxide) require additional laboratory analyses that are expensive and time-consuming. Analyses of these markers cannot be done by a prediction from NIRS because these markers do not have a spectral trace in NIRS wavelengths (from 400 to 2500 nm). Ideally, the simplest method to estimate the ability of a pig to digest energy and nutrient will be based on a sample of faeces collected directly from the rectum and analysed by NIRS.

One objective in task 2.2 was to develop a method to assess the ability of a pig to digest energy and nutrients from a NIRS prediction based on a sample of faeces collected from the rectum of the animal.

3. Materials and methods

The methodology was developed during an experiment conducted at UMR Pegase, INRA, France from September 2016 to May 2017 with 63 pigs from Large White, Piétrain, and Duroc breeds (n=21 pigs per breed) that were fed alternatively two diets differing in crude fibre content (3.1 and 8.5% of dry matter; annexe 1) during four periods of 21 days. The diets were supplemented with 0.5% of silicone oil, 0.5% of plastic resin (Kynar®), and 0.5% of polyethylene glycol as possible candidates for indigestible markers that can be analysed by NIRS. The pigs entered in the experiment at the end of the post-weaning phase (i.e., at 70 days of age). During each period, the pigs were adapted to their diet during two weeks. They were then housed individually during one week in a digestibility cage to measure their feed intake and to collect all their faeces. The dry matter (DM) content of the feed offered during this week was measured on a representative sample of diet. Refusals and spillages were totally collected at the end of the week, weighed and their DM content was measured. During the measurement week, the faeces were collected daily in buckets stored at 4°C. At the end of the week, faeces were thoroughly mixed, DM content was measured in two aliquots and a sample was freeze-dried and ground in a hammer meal for further lab analyses. At the end of each measurement week (i.e., on the morning of the first day after), a sample of faeces was also collected directly from the rectum of the pigs, freeze dried and ground. All samples of faeces and diets were stored at 4°C until laboratory analyses.

At the end of the experiment, samples of diet (one pooled sample per type of diet) and faeces from the total collection (one sample per pig per period) were analysed for their DM, nutrient and energy contents to calculate the digestibility coefficients of DM, nutrients and energy (refer as gold standard method). The DM content was measured from the weight loss during 24 h at 103°C. The ash content was measured from the weight of the residues after burning the sample in an oven at 550°C during 8 h. The N content was measured according to the Dumas method on an Elementar RapidN automatic analyser. The crude fibre was measured according to the Weende method, adapted to an automatic Ankom Fibersac analyser. The gross energy content was measured on an IKA adiabatic calorimeter. Samples of faeces from the total collection period and those collected directly from the rectum of the pigs were also analysed by NIRS using a Bruker MPA equipment (three spectra per sample, each being the average of 64 measurements performed with a 16 cm^{-1} resolution in a quartz cup at ambient temperature). Calibration equations for indigestible marker, nutrient concentrations and digestibility coefficients were then developed using the manufacturer's software with PLS regression using the data obtained from total collection of faeces and lab analyses as results to be predicted.

4. Results

4.1 Variability of digestibility between breeds, periods and diets

The main results obtained during the experiment are given in Table 2. According to the experimental design, body weight varied during successive periods and Piétrain pigs were heavier than Duroc pigs. As pigs got older, the weight difference between breeds became larger. The digestibility coefficients of energy and nutrients were strongly affected by diet composition and the increase in fibre content resulted in an 8 to 10-point decrease in digestibility coefficients of dry matter, organic matter, energy, and nitrogen. The digestibility coefficient of crude fibre was 11 points higher with the high-fibre diet compared to the low-fibre diet. There was no difference between breeds for the digestibility coefficients of dry matter, organic matter, energy, ether extract, and crude fibre but digestibility coefficient of N was higher in the Piétrain breed, especially when they got older. Except for N and ether extract, there was a significant interaction between diet and period for the digestibility coefficients of energy and nutrients, because of an improved ability to digest fibres in the large intestine as the pigs got older. Consequently, the experimental design was adequate to provide samples of faeces with a wide range in digestibility coefficients (e.g., digestibility coefficient for energy ranged from 70 to 90%; Figure 1), that can be used to develop predictive equations of nutrient composition in faeces and digestibility coefficients in pigs.

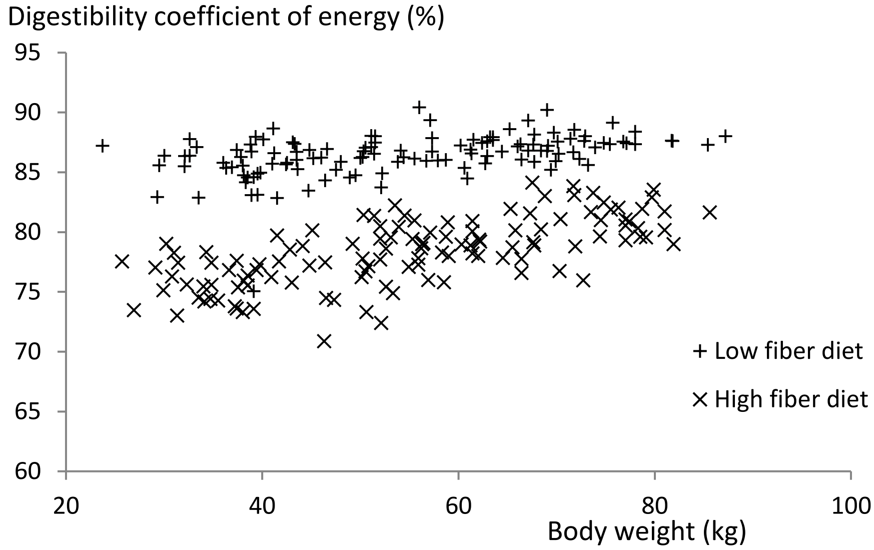


Figure 1. Effect of diet composition on digestibility coefficient of energy as affected by body weight in growing pigs (n= 246).

Table 1. Effect of diet composition, breed, and period on digestibility coefficients of energy and nutrients in growing pigs.

	Diet		Breed			Period				Statistics						
	Low fibre	High fibre	Duroc	Large White	Piétrain	1	2	3	4	RSD	D	B	P	D×B	D×P	B×P
n	123	123	76	84	86	64	60	61	61							
Body weight (kg)	54.6	54.9	50.0[a]	55.7[b]	58.4[c]	35.7[a]	48.1[b]	60.9[c]	74.1[d]	1.3	0.08	<0.01	<0.01	0.43	0.20	0.04
Feed intake (g DM/d)	1414	1406	1367[a]	1462[c]	1400[b]	1244[a]	1368[b]	1458[c]	1570[d]	38	0.09	<0.01	<0.01	0.74	0.95	<0.01
Digestibility coefficient (%)																
Dry matter	86.7	78.8	82.8	82.5	82.8	81.2[a]	82.2[b]	83.5[c]	84.0[d]	1.3	<0.01	0.71	<0.01	0.34	<0.01	<0.01
Organic matter	88.4	80.7	84.5	84.3	84.8	83.0[a]	84.0[b]	85.3[c]	85.9[d]	1.2	<0.01	0.41	<0.01	0.29	<0.01	<0.01
Energy	86.6	78.3	82.4	82.2	82.8	80.8[a]	81.9[b]	83.2[c]	83.9[d]	1.4	<0.01	0.38	<0.01	0.62	<0.01	0.02
N	86.1	76.1	80.6[a]	80.8[a]	81.9[b]	78.4[a]	80.7[b]	82.0[c]	83.3[d]	1.8	<0.01	0.04	<0.01	0.30	0.15	0.02
Ether extract	75.0	61.3	68.5	67.6	68.3	70.0	67.6	66.9	68.1	6.5	<0.01	0.59	0.06	0.77	0.16	0.24
Crude Fibber	43.6	54.6	49.5	47.7	50.2	42.4[a]	45.8[b]	52.6[c]	55.8[d]	6.9	<0.01	0.29	<0.01	0.13	<0.01	0.02

RSD, residual standard deviation; the data were analysed for the effects of diet (D), breed (B), period (P), and their interactions. Within a diet, breed or period, LS-means with different superscript letters differ (P<0.05).

4.2 Utilization of silicone oil, plastic resin, and polyethylene glycol as indigestible markers

Apart from the gold standard for digestibility measurements, indigestible markers can be used to assess digestibility from the concentrations of the marker (and nutrients) in the diet and in a faecal sample. The methodology assumes that the marker should be totally indigestible, which means that recovery in faeces should be close to 100%. The marker should also be excreted homogeneously with the faeces during the day. If this is the case, total collection of faeces is not required, and a faecal spot sample can be used. To check whether silicone oil, plastic resin, and polyethylene glycol can be used as indigestible markers, the diets were supplemented with 0.5% of each marker and the faeces collected during the seven days of the collection period were analysed by NIRS for their marker concentration.

For this purpose, NIRS calibration equations for faecal silicone oil, plastic resin, and polyethylene glycol concentrations were developed using the methodology proposed by Casasus and Albanel (2014). Briefly, known amounts of each marker were included in the faeces of non-experimental pigs that were not fed these markers. The faeces were then mixed thoroughly, humidified, freeze-dried, and ground. Equations for each marker were developed with very good accuracies, as reported in Table 2. The R^2 of prediction were all close to 100%, whereas the intercept and the slope of the relationship between theoretical and predicted concentrations of the validation dataset did not differ significantly from 0 and 1, respectively.

Table 2. Accuracy of NIRS calibration equations to predict silicone oil, plastic resin, and polyethylene glycol concentrations in the faeces of growing pigs.

		Calibration dataset			Validation dataset				
	Range (% DM)	n	Standard deviation	R^2	n	Standard deviation	R^2	Intercept[1]	Slope[1]
Silicone oil	0-11.13	771	0.25	99.5	257	0.24	99.5	0.029	0.99
Plastic resin	0-11.33	771	0.30	99.2	254	0.28	99.2	0.025	0.98
Polyethylene glycol	0-11.15	771	0.19	99.7	271	0.19	99.7	0.014	1.00

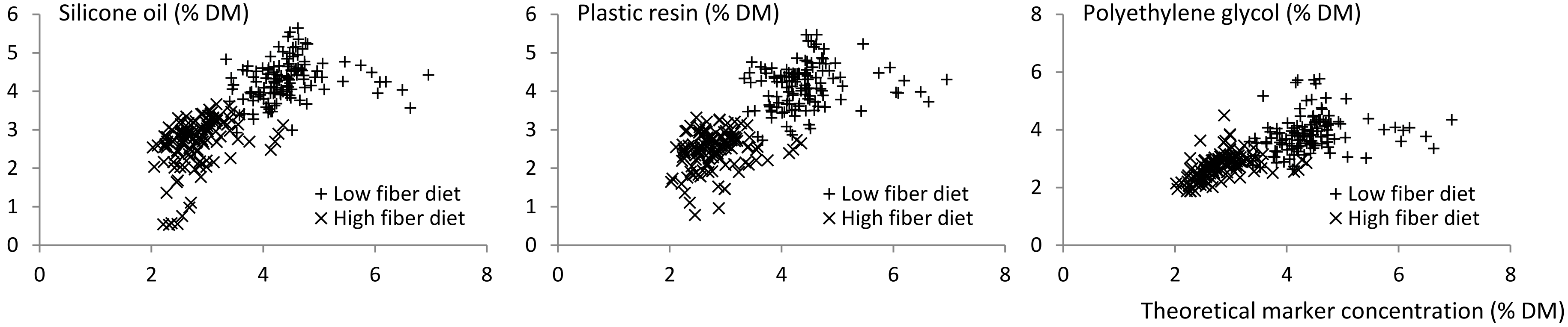
[1]Intercept and slope of the regression equation between predicted and theoretical concentrations of the marker.

These equations were then applied to samples of faeces obtained during the total collection period, assuming that it was possible to calculate the faecal concentrations in markers from their concentrations in the diet, feed intake, and faecal dry matter excretion. The best results were obtained for silicone oil. The total recovery of silicone oil did not differ between diets and breeds and averaged 97.4% (Table 3), which is a satisfactorily recovery for an indigestible marker. Nevertheless, the recovery was significantly lower during period 2 and the standard deviation of recovery was high (standard deviation: 20.7%). Additionally, the correlation between predicted and theoretical concentrations in faeces was not sufficient (Figure 2), mainly with the faeces obtained with pigs fed the low-fibre diet (i.e., when the concentration of the marker was the highest). The total recovery of plastic resin and polyethylene glycol differed significantly between diets and periods (Table 3) and the prediction of their faecal concentration (Figure 2) indicated that these markers were not good candidates as indigestible markers to be used to predict the digestive ability in growing pigs.

Table 3. Effect of diet composition, breed, and period on recovery of silicone oil, plastic resin, and polyethylene when used as markers in the faeces of growing pigs

	Diet		Breed			Period				Statistics						
	Low fibre	High fibre	Duroc	Large White	Piétrain	1	2	3	4	RSD	D	B	P	D×B	D×P	B×P
n	122	122	75	84	85	62	60	61	61							
Silicone oil	98.5	96.2	98.7	98.9	94.3	98.8[a]	92.7[b]	96.7[ab]	101.1[a]	15.5	0.26	0.48	0.03	0.06	0.12	0.07
Plastic resin	95.4	89.5	98.0	93.0	89.4	99.8[a]	90.2[b]	90.9[b]	89.0[b]	13.5	<0.01	0.32	<0.01	0.76	<0.01	<0.01
Polyethylene glycol	88.8	97.9	90.7[b]	92.4[b]	97.0[a]	92.3[b]	84.9[c]	93.4[b]	102.8[a]	12.9	<0.01	0.02	<0.01	0.04	0.63	<0.01

RSD, residual standard deviation; the data were analysed for the effects of diet (D), breed (B), period (P) and their interactions. Within a diet, breed, or period, LS-means with different superscript letters differ (P<0.05).



Intercept: 0.68% DM (P<0.01)
Slope: 0.77 (P<0.01)
R^2= 56.9 %

Intercept: 0.53% DM (P<0.01)
Slope: 0.77 (P<0.01)
R^2= 56.8 %

Intercept: 1.22% DM (P<0.01)
Slope: 0.58 (P<0.01)
R^2= 49.6 %

Figure 2. Relationships between predicted and theoretical faecal concentrations in silicone oil, plastic resin, and polyethylene glycol in growing pigs. The intercept, slope, and R^2 below each chart refer to the correlation line between predicted and theoretical concentrations. The value of the intercept was tested for the difference from 0 and the value of the slope was tested for the difference from 1.

4.3 Utilization of NIRS to predict digestive ability in growing pigs

4.3.1 Prediction of digestibility coefficients in the faeces of growing pigs

Because the equations to predict the faecal concentrations using indigestible markers were not adequate to be used to assess the digestive ability of pigs, a database with samples of faeces from the experiment conducted in the framework of Feed-a-Gene and samples of faeces from previous experiments was constituted. In this database, 831 samples of faeces from growing pigs with values of digestibility coefficients for dry matter, organic matter, energy, and N were included. Among them, 578 samples were also characterized for digestibility coefficients of crude fibre. In the database, 280 samples of faeces were obtained from direct collection from the rectum of the pigs, whereas others originated from experiments where the digestibility coefficients were measured using the gold standard method (i.e., measurement of feed intake and total collection of faeces over 6 to 10 days). , The validation dataset included 82 samples obtained from spot-sampling in the rectum of the pig to develop the NIRS prediction equations for digestibility coefficients. The results of the calibration for prediction equations for digestibility coefficients of dry matter, organic matter, energy, N, and crude fibre are presented in Table 4. The equations to predict the digestibility coefficient of dry matter, organic matter, energy, and N are good, according to the values of the ratio between the standard deviation of laboratory results and the standard error of prediction (RPD), that are close to 3. The value of RPD for the digestibility coefficient of crude fibre is lower (1.74), which can be explained by the inaccuracy of measuring crude fibre in faeces by the Weende method. Consequently, the slope of the relationship between predicted and measured digestibility coefficient is lower for crude fibre than for other nutrients (Table 4).

The NIRS equations developed with the whole database were then applied to the samples obtained during the Feed-a-Gene experiment. When the samples harvested during the total collection period are considered (Table 5), the bias in digestibility coefficient depended on diet composition and period of collection. Additionally, the bias in predicting the digestibility coefficients of dry matter and organic matter did not depend on period of collection when pigs were fed a low-fibre diet whereas it was lower when pigs fed the high-fibre diet got older. The bias in predicting the digestibility coefficient of energy decreased when pigs got older. The bias in predicting the digestibility coefficient of N averaged 1.35% when pigs were fed the high-fibre diet during period 1, but was close to 0 during other periods and with other diets. When the samples directly taken from the rectum of the pigs at the end of the collection period are considered (Table 6), the bias in predicting the digestibility coefficients were on average three times larger than the bias obtained from samples obtained during the total collection period (Figure 3). This illustrates the heterogeneity of the faeces produced during the day, even though the samples were obtained 3 weeks after the beginning of the adaptation period to the diet. Because the bias during periods 1 and 2 was larger than that measured during periods 3 and 4, the homogeneity of the faeces or the ability of the NIRS equations to predict digestibility coefficients may be better as pigs get older. The bias was also close to 0 when the diet contained high level of fibre. This suggests that the NIRS methodology can give satisfying results when pigs are heavier than 60 kg and when they are fed a diet with a high level of fibre.

Table 4. Accuracy of NIRS calibration equations to predict digestibility coefficients of energy and nutrients in the faeces of growing pigs.

		Calibration dataset			Validation dataset							
	Range (%)	n	Standard deviation	R^2	n	Standard deviation	R^2	RPD	r	Bias	Intercept[1]	Slope[1]
Dry matter	72.1-90.4	749	1.61	83.5	82	1.71	85.7	2.67	0.93	0.2	12.3	0.85
Organic matter	73.9-91.8	749	1.63	82.5	82	1.47	89.3	3.05	0.94	0.1	9.2	0.89
Energy	70.9-90.4	749	1.84	79.2	82	1.73	87.0	2.78	0.94	0.1	15.8	0.81
N	67.4-90.9	749	2.32	79.1	82	1.82	89.6	3.11	0.95	0.1	14.5	0.82
Crude fibre	24.0-73.7	497	6.75	61.4	82	6.64	66.4	1.74	0.82	0.8	18.8	0.59

RPD, ratio between standard deviation of laboratory results and standard deviation of prediction.

[1]Intercept and slope of the regression line between predicted and theoretical concentrations in marker.

Table 5. Effect of diet composition, breed, and period on the bias between measured digestibility coefficient by the total collection method and the digestibility coefficient predicted by NIRS in samples of faeces of growing pigs obtained during the total collection period.

	Diet		Breed			Period				Statistics						
	Low fibre	High fibre	Duroc	Large White	Piétrain	1	2	3	4	RSD	D	B	P	D×B	D×P	B×P
n	122	123	76	84	85	63	60	61	61							
Bias in digestibility coefficient																
Dry matter	0.12	0.24	-0.33^{b}	0.25^{a}	0.63^{a}	0.65^{a}	0.31^{ab}	-0.24^{c}	0.006^{bc}	1.23	0.44	<0.01	<0.01	0.37	<0.01	0.02
Organic matter	-0.22	0.32	-0.12	0.09	0.19	0.94^{a}	0.13^{b}	-0.59^{c}	-0.26^{bc}	1.26	<0.01	0.38	<0.01	0.90	0.08	0.05
Energy	-0.48	0.28	-0.31	-0.04	0.05	0.51^{a}	-0.06^{b}	-0.52^{b}	-0.33^{b}	1.45	<0.01	0.30	<0.01	0.90	0.13	0.08
N	-0.22	0.31	-0.37^{ab}	0.50^{a}	0.01^{b}	0.65^{a}	-0.10^{b}	-0.22^{b}	-0.14^{b}	1.85	0.03	0.01	0.03	0.62	0.09	<0.01
Crude fibre	0.65	0.64	-0.08^{b}	0.27^{ab}	1.75^{a}	3.99^{a}	1.85^{b}	-1.09^{c}	-2.16^{c}	5.80	0.99	0.07	<0.01	0.19	<0.01	<0.01

RSD, residual standard deviation; the data were analysed for the effects of diet (D), breed (B), period (P), and their interactions. Within diet, breed, or period, LS-means with different superscript letters differ (P<0.05).

Table 6. Effect of diet composition, breed, and period on the bias between measured digestibility coefficient by the total collection method and the digestibility coefficient predicted by NIRS in samples of faeces of growing pigs collected from the rectum.

	Diet		Breed			Period				Statistics						
	Low fibre	High fibre	Duroc	Large White	Piétrain	1	2	3	4	RSD	D	B	P	D×B	D×P	B×P
n	122	122	76	84	84	63	60	60	61							
Bias in digestibility coefficient																
Dry matter	-0.39	0.06	-0.58^{b}	-0.22^{b}	0.32^{a}	0.02^{b}	0.23^{a}	-0.47^{b}	-0.44^{b}	1.48	0.02	0.02	<0.01	0.63	<0.01	<0.01
Organic matter	-0.43	-0.11	-0.27	-0.02	-0.52	0.20	-0.71	-0.28	-0.30	1.84	0.06	0.18	0.17	0.74	<0.01	<0.01
Energy	-0.78	0.18	-0.29	-0.11	-0.51	0.09^{a}	-0.87^{b}	-0.16^{a}	-0.26^{ab}	1.93	<0.01	0.40	0.05	0.49	<0.01	<0.01
N	-0.99	0.55	-0.03	0.08	-0.70	1.19^{a}	-0.77^{b}	-0.38^{b}	-0.92^{b}	2.34	<0.01	0.10	<0.01	0.94	<0.01	<0.01
Crude fibre	-0.50	-3.05	-1.74	-1.58	-2.00	1.32^{a}	-2.79^{b}	-2.49^{b}	-3.13^{b}	7.23	<0.01	0.92	<0.01	0.70	0.02	<0.01

RSD, residual standard deviation; the data were analysed for the effects of diet (D), breed (B), period (P), and their interactions. Within a diet, breed, or period, LS-means with different superscript letters differ (P<0.05).

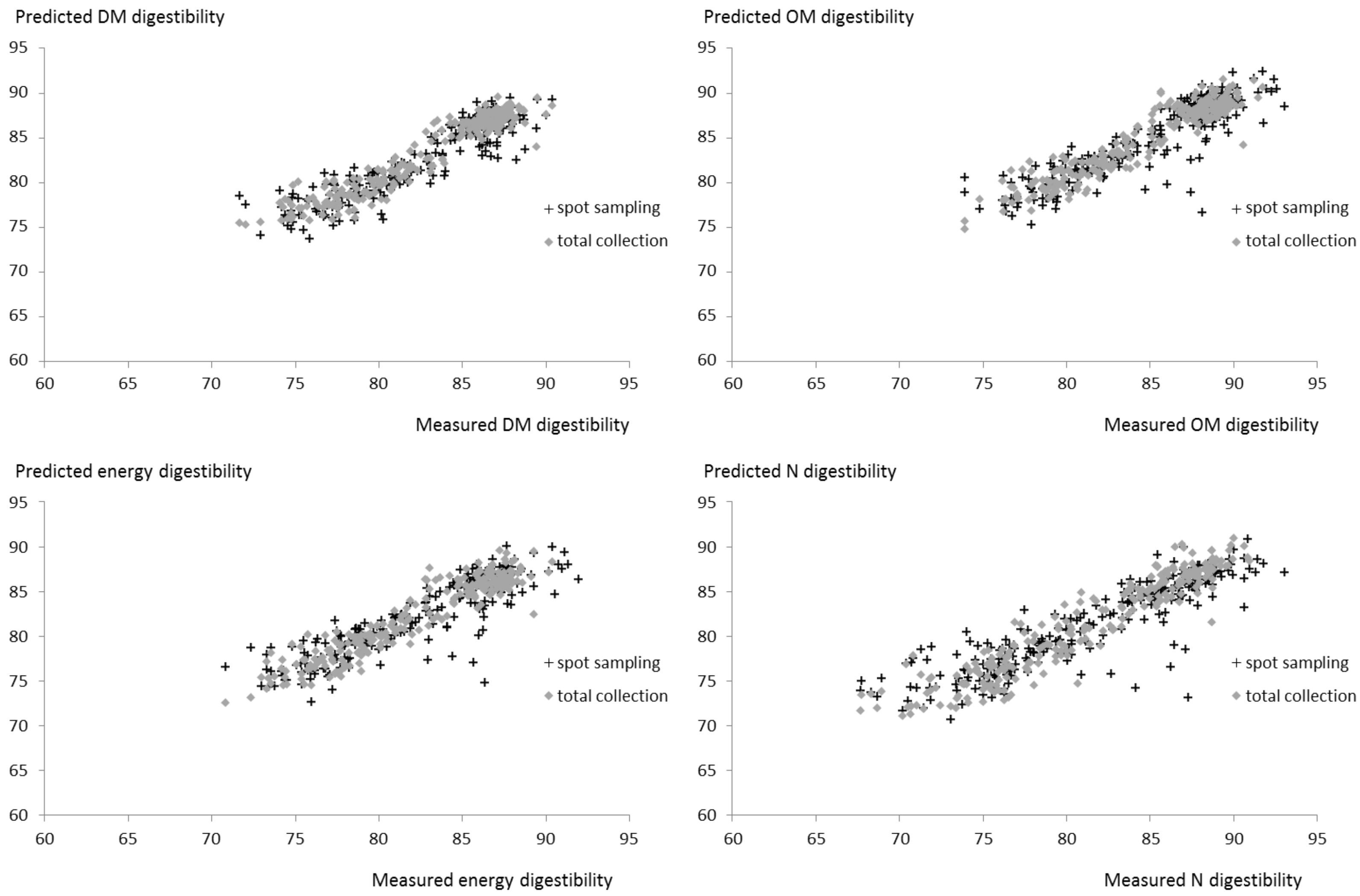


Figure 3. Prediction of dry matter (DM), organic matter (OM), energy, and N digestibility (%) from NIRS analysis of samples of faeces in growing pigs (n=245).

4.3.2 Utilization of NIRS prediction to classify pigs for their digestive ability

To detect the ability of NIRS to identify groups of pigs with a low or a high digestive ability, predicted digestibility coefficients of dry matter, organic matter, energy, N, and crude fibre from NIRS analysis of samples of faeces directly collected from the rectum of the pigs were analysed in a principal component analysis (package FactomineR in R), followed by a clustering analysis to spread the animals over six groups. The analysis was performed on samples of faeces collected at the end of periods 3 and 4.

The same approach was applied to the measured digestibility coefficients during periods 3 and 4 using the gold standard method as a reference. The clustering analysis made a clear distinction between pigs fed the high- (clusters 1 to 3) or the low- (clusters 4 to 6) fibre diet (Table 7). Cluster 1 grouped pigs fed the high-fibre diet with the highest digestibility coefficients except for the digestibility coefficient of N, whereas cluster 3 grouped the pigs with the lowest digestibility coefficients. When pigs were fed the low-fibre diet, cluster 6 grouped the pigs with the lowest digestibility coefficients whereas the digestibility coefficients of dry matter, energy, and N of pigs groups did not significantly differ between clusters 4 and 5.

Table 7. Description of clusters obtained from hierarchical classification of animals based on principal component analysis performed on measured digestibility coefficients of dry matter, organic matter, N, energy and crude fibre during total collection (periods 3 and 4).

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	RSD	P-value
n of pigs				17	26	18		
Fed low-fibre diet								
Fed high-fibre diet	28	20	13					
n of pigs								
Duroc	10	5	4	5	6	8		
Large White	7	7	7	2	11	8		
Pietrain	11	8	2	10	9	2		
Digestibility coefficients (%)								
Dry matter	81.8[c]	80.1[d]	78.0[e]	88.0[a]	87.2[a]	86.0[b]	0.8	<0.01
Organic matter	83.7[d]	82.0[e]	79.9[f]	89.8[a]	89.0[b]	87.8[c]	0.8	<0.01
Energy	81.5[c]	79.5[d]	77.5[e]	88.1[a]	87.2[a]	85.9[b]	1.0	<0.01
N	79.0[c]	78.1[c]	75.2[d]	88.8[a]	87.8[a]	85.7[b]	1.4	<0.01
Crude fibre	68.8[a]	61.2[b]	48.8[d]	54.8[c]	46.6[d]	37.7[e]	3.1	<0.01

RSD, residual standard deviation: the data (only periods 3 and 4) were analysed for the effects of cluster. LS-means with different superscript letters differ (P<0.05).

When the samples directly collected from the rectum and analysed by NIRS are considered, the hierarchical classification resulted in four groups of pigs fed the high-fibre diet and two groups of pigs fed the low-fibre diet (Table 8). Only two pigs were in cluster 4 and their digestibility coefficients did not differ from those of pigs in cluster 3. Cluster 1 grouped the pigs with the highest predicted digestibility coefficients whereas clusters 3 and 4 grouped the pigs with the lowest predicted digestibility coefficient. The pigs in cluster 1 can thus be considered as good digesters of a high-fibre diet because their predicted digestibility coefficients are the highest. In this group, 17 pigs of the 26 were also classified as good digesters from the hierarchical classification based on measured digestibility coefficients but nine pigs belonged to the second group of pigs (cluster 2 in Table 7). This result suggests that the specific character of the method combining spot-sampling of faeces directly from the rectum of the pig and NIRS prediction of digestibility coefficients is not good to identify the good digesters in a population of pigs. The pigs in clusters 3 and 4 exhibited the lowest digestive ability of a high-fibre diet and they were also classified as bad digesters in the first hierarchical classification shown in Figure 4A (i.e., measured digestibility coefficients from measured feed intake, total collection of faeces and laboratory analyses). Only two groups of pigs could be identified when pigs were fed the low-fibre diet and, except for N, the digestibility coefficients of these pigs did not significantly differ between the two groups. This is further illustrated in figure 4B using the digestibility coefficient of energy as an example. This result thus exhibited the inability of the method combining a spot-sampling of faeces directly from the rectum of the pig and a NIRS prediction of digestibility to classify animals for their digestive ability when they are fed a low-fibre diet. This may be explained by the limited variation in diet digestibility between animals when the diet does not challenge the ability of the pig (e.g., low level of dietary fibre).

Table 8. Description of clusters obtained from hierarchical classification of animals based on principal component analysis performed on predicted digestibility coefficients of dry matter, organic matter, N, energy, and crude fibre by NIRS on samples of faeces collected from the rectum of pigs during periods 3 and 4.

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	RSD	P-value
n of pigs					42	19		
Fed low-fibre diet								
Fed high-fibre diet	26	25	7	2				
n of pigs								
Duroc	7	10	2		12	7		
Large White	8	7	7	2	13	8		
Piétrain	11	8	1		17	4		
Digestibility coefficients (%)								
Dry matter	81.6^{b}	80.3^{c}	77.8^{d}	76.7^{d}	87.3^{a}	86.5^{a}	1.1	<0.01
Organic matter	83.6^{b}	82.1^{c}	79.6^{d}	78.7^{d}	89.1^{a}	88.4^{a}	1.0	<0.01
Energy	81.3^{b}	79.7^{c}	77.0^{d}	76.7^{d}	87.3^{a}	86.4^{a}	1.3	<0.01
N	79.2^{c}	77.7^{d}	75.3^{e}	73.5^{e}	87.9^{a}	86.4^{b}	1.7	<0.01
Crude fibre	67.5^{a}	61.6^{b}	48.1^{c}	43.4^{c}	47.8^{c}	43.0^{c}	6.1	<0.01

RSD, residual standard deviation: the data (only periods 3 and 4) were analysed for the effects of cluster. LS-means with different superscript letters differ (P<0.05).

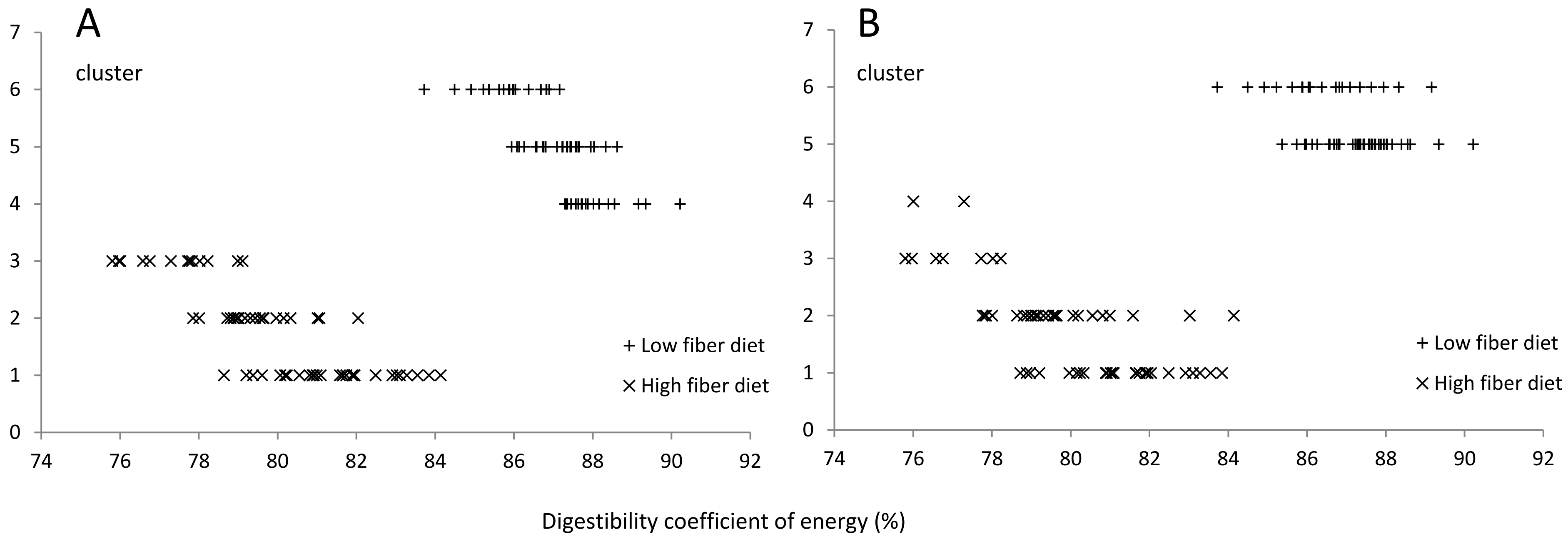


Figure 4. Hierarchical classification of animals from principal component analysis performed on measured digestibility coefficients of dry matter, organic matter, N, energy, and crude fibre during total collection (panel A) or from principal component analysis performed on predicted digestibility coefficients of dry matter, organic matter, N, energy, and crude fibre by NIRS on samples of faeces collected in the rectum of pigs (panel B).

5. Conclusions

The digestive ability of pigs is variable during the growing period, especially when the diet contains a high fibre level because the digestibility of this type of diet increases when body weight increases. The possibility to characterize the digestive ability of pigs in selection farms of breeders is limited because of the difficulty in implementing the gold standard methodology to measure digestibility. Alternatively, spot-sampling of faeces collected directly from the rectum of the pig can be used to characterize digestive ability. In our experiment, the utilization of indigestible markers did not give satisfying results, because of a low recovery of the marker in the faeces (for plastic resin or polyethylene glycol) or because of a large variability in the recovery (for silicone oil). The NIRS prediction of digestibility coefficients from a faecal sample collected directly from the rectum is adequate when pigs are heavier than 60 kg and when they are fed a diet with a high fibre content. This type of diet is susceptible to challenge the digestive ability of pigs and allows identifying specifically the poor digesters in a population using NIRS. The method combining a single sample of faeces collected directly from the rectum of the pig and analysed by NIRS can then be used in selection farms of breeders to give information on the digestive ability of pigs in their population.

6. Bibliographic references

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7. Annexes

Annexe 1: Composition of experiment diets

	Low fibre diet	High fibre diet
Ingredients, %		
Maize	34.58	17.77
Barley	17.77	17.77
Wheat	17.77	17.77
Wheat bran	2.50	15.00
Rapeseed meal	-	1.97
Soybean hulls	-	10.00
Soybean meal	15.74	9.18
Sugar beet pulp	-	5.00
Cane molasses	1.03	0.68
Corn starch	4.25	-
Sunflower oil	1.00	-
L-Lysine HCl	0.33	0.25
L-Threonine	0.15	0.10
L-Tryptophan	0.03	0.01
DL-Methionine	0.08	0.03
Sodium chloride	0.45	0.45
Calcium carbonate	0.82	0.62
Dicalcium phosphate	1.20	1.11
Vitamins and minerals premix	0.50	0.50
Titanium dioxide	0.30	0.30
Silicone oil	0.50	0.50
Plastic resin Kynar®	0.50	0.50
Polyethylene glycol	0.50	0.50
Chemical composition, %DM		
Ash	5.52	6.31
Crude protein	16.17	16.18
Ether extract	4.27	3.06
Crude fibre	3.08	8.48
NDF	10.74	22.34
ADF	3.60	10.14
ADL	0.74	1.41
Gross energy (MJ/kg DM)	18.22	18.72