



## FEED-A-GENE

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

### Deliverable D2.4

*A database with traits related to variation in amino acid utilization in pigs as affected by genotype, age, and sex for predicting variation in feed efficiency*

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## Table of contents

1. Summary .....	3
2. Introduction.....	5
3. Description of the research.....	5
4. Database .....	7
5. Access to the database .....	11
6. Conclusions.....	11
7. Annexes .....	12

# 1. Summary

## Objectives

Feed-a-gene aims to better adapt different components of monogastric livestock production systems to improve the overall efficiency and to reduce the environmental impact. This involves among others the development of new and alternative feed resources and feed technologies, the identification and selection of robust animals that are better adapted to fluctuating conditions and the development of feeding techniques that allow optimizing the potential of the feed and the animal. In the project, new traits related to feed efficiency have been developed to assess between animal variation in protein and amino acid (AA) requirements in growing pigs in response to different dietary and rearing regimes. The new traits can be used for modelling the response of animals to the nutrient supply and environmental conditions and for further developing precision feeding regimes in defined populations of growing pigs.

The database contains data on N-balance research with growing pigs aimed to establish the relationship between birth weight and genetic capacity of pigs to deposit protein, to the protein and amino acid utilization in pigs in dependence of the dietary supply of protein and amino acids (adequate and restricted). In addition, data on concentrations of nutrients, metabolites and hormones in blood with a potential relationship link to N-efficiency in pigs are included.

## Rationale

The protein/nitrogen (N) and amino acid (AA)-digestibility and metabolic utilization of N and AA for deposition in the body of pigs depend on many factors including factors related to the animal (e.g. age, sex, breed, health status), to the diet composition (ingredient and, in particular, nutrient composition), feeding strategy (e.g. ad libitum or restricted) and to environmental and management factors such as climate in the housing facilities, stocking density of animals, and animal health management. For practical diet formulation, nutrient requirement values of pigs are generally presented as “average” recommendations/standards based on results of a large number of requirement studies carried out with different designs and under different experimental conditions. This means that the match between dietary nutrient supply and nutrient requirements of a defined population of animals on a particular farm might be rather far from optimal. For that reason, it is important to get more information about the factors that could influence protein and amino acid requirements, which are currently not, or hardly, taken into account in diet formulation and to get information on new parameters linked to N-efficiency in pigs. For example, quantitative information about the effect of birth weight on N digestibility, N retention, and the metabolic utilization of protein and AA later in life is lacking. These effects could also influence the requirements for AA of pigs later in life. Considering factors such as birth weight can therefore contribute to explaining variation in growth responses and N-efficiency in populations of pigs. Differences in protein/N digestibility and N metabolism can be measured in N-balance studies. In addition, biomarkers in blood and/or urine might be very useful for in vivo estimation of amino acids requirements in animals.

The goal of this deliverable is to provide data on the effects of birth weight and genetic capacity to deposit protein on the between animal variation in faecal N-digestibility, N-retention, and N-efficiency. In addition, data are provided on concentrations of metabolites related to N- and amino acid metabolism (biomarkers of N- and AA-metabolism or N-efficiency) in plasma and urine in genetically characterized fattening pigs of the same age but having a different bodyweight. These were measured at two levels of dietary protein supply (adequate vs restricted). This information can be used to identify factors/traits that explain variation in faecal N-digestibility and N-efficiency in growing pigs and to better understand and improve the N-digestibility and N-efficiency. In addition, the results can be used for further improving the match between the dietary nutrient supply and the actual nutrient requirements of pigs, which can contribute to the further application of precision feeding concepts in practice.

**Teams involved:**

DLO

The data of the results of two N-balance experiments with pigs carried out by DLO are included in the database.

**Species and production systems considered:**

Growing pigs

## 2. Introduction

The protein/nitrogen (N) and amino acid (AA)-digestibility and metabolic utilization of N and AA in finishing pigs may differ due to variation in birth weight and genetic capacity. However, quantitative information about the effect of birth weight and genetic capacity on N digestibility, N retention and the metabolic utilization of protein and AA later in life is lacking. Such effects could also influence the requirements for AA of pigs later in life. This can contribute to explaining variation in growth responses and N-efficiency of animals in populations of pigs. Differences in protein/N digestibility and N metabolism can be measured in N-balance studies. In addition, biomarkers in blood and/or urine might be very useful for in vivo estimation of amino acids requirements in animals.

The aim of this deliverable is to provide data on the effects of birth weight and genetic capacity to deposit protein on the animal variation in faecal N-digestibility, N-retention, N-efficiency, and concentrations of metabolites related to N- and amino acid metabolism (biomarkers of N- and AA-metabolism or N-efficiency) in plasma and urine in genetically characterized fattening pigs of the same age but having a different bodyweight. These were measured at two levels of dietary protein supply (adequate vs restricted).

This information can be used to identify factors/traits that explain variation in faecal N-digestibility and N-efficiency in growing pigs and to better understand and improve the N-digestibility and N-efficiency. In addition, the results can be used for further improving the match between the dietary nutrient supply and the actual nutrient requirements of pigs and can contribute to the further application of precision feeding concepts in practice.

## 3. Description of the research

Two studies were conducted (Exp. 1 and 2) to evaluate the effects of birth weight on N-retention and N-efficiency in pigs (Exp. 1) and of birth weight and genetic capacity to deposit protein on N-retention and N-efficiency in pigs (Exp. 2) using two levels of dietary protein supply (adequate and restricted; 100 and 70%, respectively). Each animal was subjected to measurement of the response towards both dietary regimes. The composition of the experimental diets in both experiments is provided in Appendices 1 and 2.

Piglets were born at the Swine Innovation Centre Sterksel of Wageningen UR (The Netherlands). At 4 weeks of age, the piglets were weaned and at 9 weeks of age piglets were moved to the stable for growing-finishing pigs. At 14 weeks (98-days of age), in total 80 (40 in Exp. 1 and 40 in Exp 2.) preselected male pigs, considering their birth weight and litter origin (Exp 1), and birth weight, litter origin, and genetic capacity to deposit protein (Exp. 2), were transported to the experimental facilities of Wageningen University in Wageningen.

Both trials had a similar setup. Upon arrival (d 0), pigs were housed individually in metabolism cages and allowed to adjust to the housing conditions for a period of one week (experimental d 0-7). The experimental diets were provided from day 7-11, before the first balance period in which faeces and urine were collected quantitatively (day 12-17). The animals then received the other diet (day 17-23) before carrying out a second N-balance study (d 23-28). During the experimental periods, the pigs fed the protein adequate diets were fed at a feeding level of 2.8 times the maintenance requirement for energy, a level which is rather close to the level of ad libitum feed intake. The pigs on the protein-restricted

regime received the same amount of energy-supplying ingredients relative to their metabolic body weight ( $BW^{0.75}$ ), but a 30% restriction in the amount of protein supplied via the diet. The former was achieved by restricting the supply of each of the dietary protein sources (see Appendices 1 and 2) to a level of 70% relative to the supply in the protein adequate regime. Drinking water was available ad libitum.

The following measurements were performed:

- Body weight at birth, experimental day -1 (day before moving to experimental facility in Wageningen), day 7 (day before start adaptation to protein adequate/restricted diet), day 17 (final day first N-balance period), and day 28 (end of the study).
- Pigs were genotyped by Topigs Norsvin based on a hair sample per pig taken on the day of arrival in Wageningen (day 0, Exp. 1). In Exp. 2, a tissue sample for this purpose was taken at the day of birth during placement of the electronic ear tag.
- Daily feed intake during the N-balance periods
- N and gross energy (GE) content in the experimental diets
- Concentration of N and GE in faeces and N, urea and creatinine in urine
- Apparent faecal N and GE digestibility
- N-excretion via faeces and urine and N-retention
- Blood samples per pig were taken on d 62 (Exp. 2) and at the end of the first and second balance period (Exp. 1 and 2) for the analyses of insulin, glucose, urea, IGF-1,  $\alpha$ -amino N, and creatinine.

## 4. Database

The parameters of which values are reported in the database are presented in Table 1 and 2 (Experiment 1) and Tables 3 and 4 (Experiment 2).

Table 1. Parameters in the database related to performance and N-utilization in Exp. 1.

Parameter	Description	Values/units
Cage_pig	cage number	1-40
Week_balance	Week N-balance measurement	1 or 2
Pair	Litter of origin	1-20
Birthweight_level	Birth weight category	low or high
Protein_level	Protein level in diet (adequate/restricted)	ADEQU or BEPERK
BW_birth	Birth weight	kg
BW_D0	Bodyweight day 0 (start of study)	kg
Age_D0	Age start of study	d
BW_D7	Bodyweight day 7	kg
Age_D7	Age d7 study	d
BW_D17	Bodyweight day 17	kg
Age_D17	Age d17 study	d
BW_D28	Bodyweight day 28	kg
Age_D28	Age d 28 study	d
FI_g_d	Feed intake	g/d
FI_g_kg_metab_d	Feed intake	g/kg <sup>0.75</sup> /d
FI_g_kg_BW_d	Feed intake	g/kg/d
NI_g_d	N-intake	g/d
NI_g_kg_metab_d	N-intake	g/kg <sup>0.75</sup> /d
NI_g_kg_BW_d	N-intake	g/kg/d
N_level_feed	N-content diet	g N/kg
N_excr_faeces_g_d	Faecal N-excretion	g N per d
N_excr_faeces_g_kg_metab_d	Faecal N-excretion	g N per kg <sup>0.75</sup> /d
N_excr_faeces_g_kg_BW_d	Faecal N-excretion	g N per kg/d
N_excr_urine_g_d	Urinary N-excretion	g N/d
N_excr_urine_g_kg_metab_d	Urinary N-excretion	g N per kg <sup>0.75</sup> /d
N_excr_urine_g_kg_BW_d	Urinary N-excretion	g N per kg/d
N_ret_g_d	N-retention in body	g N/d
N_ret_g_kg_metab_d	N-retention in body	g N per kg <sup>0.75</sup> /d
N_ret_g_kg_BW_d	N-retention in body	g N per kg/d
N_ret_perc	N-retained as proportion of N-intake	%
N_ret_digest_NI	N-retained as proportion of dig. N	%
N_digest_perc	Faecal N-digestibility	%
DM_digest_perc	Faecal dry matter digestibility	%
GE_digest_perc	Faecal energy digestibility	%

Table 2. Parameters in the database related to nutrients and metabolites in blood and urine in Exp. 1.

<b>Parameter</b>	<b>Description</b>	<b>Values/units</b>
Cage_pig	Cage number	1-40
Week_balance	Week N-balance measurement	1 or 2
Birthweight_level	Birth weight category	low or high
Protein_level	Protein level in diet (adequate/restricted)	ADEQU or BEPERK
BW_birth	Birth weight	kg
Insulin_conc	Insulin concentration in blood	μU/ml
Blood_ureum_	Urea concentration in blood	mmol/l
Glucose	Glucose concentration in blood	mmol/l
IGF_I	IGF-1 concentration in blood	μg/l
Blood_creatin	Creatinin concentration in blood	μmol/l
Alfa_amino_N	Alfa-amino nitrogen concentration in blood	mmol/l
Urine_creatin_DEN	Creatinin concentration in urine	μmol/l
Urine_creatin_ANU	Creatinin concentration in urine	mmol/l
Urine_urea_	Urea concentration in urine	mmol/l
DP_DUMASN_FL	Concentration N in urine	g/kg



Table 3. Parameters in the database related to performance and N-utilization in Exp. 2.

Parameter	Description	Values/units
Cage_pig	Cage number	1-40
Week_balance	Week N-balance measurement	1 or 2
Birthweight_level	Birth weight category	low of high
Protein level in diet (adequate/restricted)	Protein level in diet	ADEQU or BEPERK
Breedvalue_level	Category breeding value for protein deposition (low/high)	laag or hoog
Breedvalue	Breeding value for protein deposition rel. to an average crossbred pig of the same genotype	g protein/d
BW_birth	Birth weight	kg
Bodyweight day 0 (start of study)	kg	kg
Age start of study	d	d
Bodyweight day 7	kg	kg
Age d7 study	d	d
Bodyweight day 17	kg	kg
Age d17 study	d	d
Bodyweight day 28	kg	kg
Age d 28 study	d	d
FI_g_d	Feed intake	g/d
FI_g_kg_metab_d	Feed intake	g/kg <sup>0.75</sup> /d
FI_g_kg_BW_d	Feed intake	g/kg/d
NI_g_d	N-intake	g/d
NI_g_kg_metab_d	N-intake	g/kg <sup>0.75</sup> /d
NI_g_kg_BW_d	N-intake	g/kg/d
N_level_feed	N-content diet	g N/kg
N_excr_faeces_g_d	Faecal N-excretion	g N per d
N_excr_faeces_g_kg_metab_d	Faecal N-excretion	g N per kg <sup>0.75</sup> /d
N_excr_faeces_g_kg_BW_d	Faecal N-excretion	g N per kg/d
N_excr_urine_g_d	Urinary N-excretion	g N/d
N_excr_urine_g_kg_metab_d	Urinary N-excretion	g N per kg <sup>0.75</sup> /d
N_excr_urine_g_kg_BW_d	Urinary N-excretion	g N per kg/d
N_ret_g_d	N-retention in body	g N/d
N_ret_g_kg_metab_d	N-retention in body	g N per kg <sup>0.75</sup> /d
N_ret_g_kg_BW_d	N-retention in body	g N per kg/d
N_ret_perc	N-retained as proportion of N-intake	%
N_ret_digest_NI	N-retained as proportion of dig. N	%
N_digest_perc	Faecal N-digestibility	%
DM_digest_perc	Faecal dry matter digestibility	%
GE_digest_perc	Faecal energy digestibility	%

Table 4. Parameters in the database related to nutrients and metabolites in blood and urine in Exp. 2.

<b>Parameter</b>	<b>Description</b>	<b>Values/units</b>
Cage_pig	Cage number	1-40
Week_balance	Week N-balance measurement	1 or 2
Birthweight_level	Birth weight category	low of high ADEQU or BEPERK
Protein_level	Protein level in diet (adequate/restricted) Category breeding value for protein deposition (low/high)	laag or hoog
Breedvalue_level	Breeding value for protein deposition	g protein/d
Breedvalue	Birth weight	kg
BW_birth	Insulin concentration in blood	µU/ml
Insulin_conc	Urea concentration in blood	mmol/l
Blood_ureum_	Glucose concentration in blood	mmol/l
Glucose	IGF-1 concentration in blood	µg/l
IGF_I	Creatinin concentration in blood	µmol/l
Blood_creatin	Alfa-amino nitrogen concentration in blood	mmol/l
Alfa_amino_N	Creatinin concentration in urine	µmol/l
Urine_creatin_DEN	Creatinin concentration in urine	mmol/l
Urine_creatin_ANU	Urea concentration in urine	mmol/l
Urine_urea	Concentration N in urine	g/kg
DP_DUMASN_FL		

## 5. Access to the database

The database is available (free access) for stakeholders (e.g. academia, animal production sector, and feed industry) active in the domain of feed and nutrient efficiency in pig production.

The database is available as Excel file.

The database has been published at the Zenodo platform. Further information on the database is available upon request from Alfons Jansman ([alfons.jansman@wur.nl](mailto:alfons.jansman@wur.nl)).

## 6. Conclusions

- Data of 80 pigs of parameters and traits related to N-efficiency have been collected and compiled into a database.
- The database is available in Excel and as SQL file and has been made public via the Zenodo platform.

## 7. Annexes

Appendix 1. Ingredient (%) and nutrient composition (g/kg, unless stated otherwise) in Experiment 1.

		Adequate diet	Restricted diet
Casein		3.48	2.57
Wheat gluten meal		1.91	1.41
Soya isolate		9.00	6.65
Potato protein		3.01	2.22
DL-Methionine		0.101	0.08
L-Threonine		0.009	0.007
Maize starch		24.70	26.09
Potato starch (gelatinized)		24.97	26.35
Dextrose		10.00	10.55
Oat hulls		10.00	10.55
Sugarbeet pulp		5.00	5.28
Soybean oil		3.35	3.53
Limestone		1.48	1.56
Monocalcium phosphate		1.09	1.15
NaCl		0.37	0.39
Potassium carbonate		0.63	0.67
Premix		0.50	0.53
Titanium dioxide		0.40	0.40
DM	g	914	913
Ash	g	54	55
Crude protein	g	156	119
Ether extract	g	39	40
Crude fibre	g	37	39
Starch	g	438	463
Sugars	g	117	123
NSP	g	113	118
EW	-	1.20	1.21
NE <sub>pigs</sub>	MJ	10.6	10.6
C18:2	g	17.7	18.7
Ca	g	7.98	8.37
P	g	3.88	3.76
dP	g	2.66	2.65
IP	g	0.41	0.31
Ca/dP		3.00	3.16
Mg	g	0.42	0.42
Na	g	1.80	1.84
K	g	6.55	6.42
Cl	g	2.57	2.65
EB	meq	180	174
Fe	mg	251	259
Cu	mg	25	26
Zn	mg	87	88
AID <sup>1</sup> LYSv	g	8.59	6.42
AID METv	g	3.58	2.67
AID CYSv	g	1.52	1.12
AID M+Cv	g	5.15	3.85
AID THRv	g	5.59	4.16

## Feed-a-Gene – H2020 n°633531

AID TRPv	g	1.63	1.22
AID ILEv	g	6.75	5.04
AID ARGv	g	8.01	6.00
AID PHEv	g	7.63	5.69
AID HISv	g	3.53	2.64
AID LEUv	g	12.09	9.01
AID TYRv	g	6.27	4.69
AID VALv	g	7.48	5.58
AID ALAv	g	3.97	2.92
AID ASPv	g	9.82	7.23
AID GLUv	g	23.18	17.10
AID GLYv	g	3.48	2.51
AID PROv	g	8.15	5.91
AID SERv	g	5.44	3.98

<sup>1</sup>AID: apparent ileal digestible, based on data of CVB (2016)<sup>2</sup>

<sup>2</sup>CVB (2016). CVB Veevoedertabel 2016. Chemische samenstellingen en nutritionele waarden van voedermiddelen. Wageningen, The Netherlands.

## Appendix 2. Ingredient (%) and nutrient composition (g/kg, unless stated otherwise) in Experiment 2.

		Adequate diet	Restricted diet
Casein		5.20	3.86
Wheat gluten meal		9.49	7.04
Potato protein		3.72	2.76
L-Lysine HCl		0.172	0.13
L-Threonine		0.018	0.013
Maize starch		24.70	26.16
Potato starch (gelatinized)		23.65	25.07
Dextrose		10.00	10.59
Oat hulls		10.00	10.59
Sugar beet pulp		5.00	5.30
Soya oil		3.12	3.31
Limestone		1.43	1.51
Monocalcium phosphate		1.19	1.26
NaCl		0.39	0.41
Potassium carbonate		1.02	1.08
Premix		0.50	0.53
Titanium dioxide		0.40	0.40
DM	g	912	912
Ash	g	55	57
CP	g	163	124
Ether extract	g	40	41
Crude fibre	g	36	38
Starch	g	432	456
Sugars	g	119	125
NSP	g	107	113
EW	-	1.20	1.21
NE <sub>pigs</sub>	MJ	10.6	10.6
C18:2	g	17	18
Ca	g	8.0	8.4
P	g	3.6	3.6
dP	g	2.7	2.7
IP	g	0.1	0.1
Ca/dP		3.0	3.1
Mg	g	0.37	0.38
Na	g	1.80	1.87
K	g	7.40	7.78
Cl	g	3.10	3.09
EB	meq	180	193
Fe	mg	248	257
Cu	mg	25	26
Zn	mg	80	84
AID <sup>1</sup> LYSv	g	8.54	6.41
AID METv	g	3.26	2.43
AID CYSv	g	2.10	1.56
AID M+Cv	g	5.41	4.05
AID THRv	g	5.56	4.14
AID TRPv	g	1.64	1.22
AID ILEv	g	6.74	5.04
AID ARGv	g	6.07	4.58

## Feed-a-Gene – H2020 n°633531

AID PHEv	g	8.18	6.12
AID HISv	g	3.65	2.74
AID LEUv	g	12.74	9.53
AID TYRv	g	6.87	5.15
AID VALv	g	7.92	5.92
AID ALAv	g	3.96	2.93
AID ASPv	g	7.33	5.42
AID GLUv	g	37.91	28.09
AID GLYv	g	3.76	2.73
AID PROv	g	14.77	10.84
AID SERv	g	6.55	4.82

<sup>1</sup>AID: apparent ileal digestible, based on data of CVB (2016)<sup>2</sup>

<sup>2</sup>CVB (2016). CVB Veevoedertabel 2016. Chemische samenstellingen en nutritionele waarden van voedermiddelen. Wageningen, The Netherlands.