Variation and estimation of nitrogen utilization efficiency in a crossbred pig population

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University of Hohenheim

Faculty of Agricultural Sciences

Institute of Animal Science

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Daniel Berghaus

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Berichterstatter, 1. Prüfer:	Prof. Dr. Markus Rodehutscord
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3. Prüfer:	Prof. Dr. Jörn Bennewitz

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ABBREVIATIONS

%CV	Percent coefficient of variation
AA	Amino acids
ADFI	Average daily feed intake
ADG	Average daily gain
AIC	Akaike information criterion
AMW	Average metabolic body weight
ANOVA	Analysis of variance
ATTD	Apparent total tract digestibility
BUN	Blood urea nitrogen
BW	Body weight
CA	Crude ash
CP	Crude protein
CVB	Centraal Veevoeder Bureau
CW	Carcass weight
D_2O	Deuterium oxide
DM	Dry matter
DNI	Digestible nitrogen intake
DNUE	Digestible nitrogen utilization efficiency
EBA	Empty body ash
EBF	Empty body fat
EBP	Empty body protein
EBW	Empty body weight
EBWA	Empty body water
FBW	Final body weight
FDR	Fractional degradation rate
FFL	Fat-free lean
FFS	Fat-free substance
FNE	Fecal nitrogen excretion
FRR	Fractional retention rate
FSR	Fractional synthesis rate
FTR	Fractional turnover rate
G:F	Gain to feed
GfE	Gesellschaft für Ernährungsphysiologie
IBW	Initial body weight

IGF-I	Insulin-like growth factor 1
K-EDTA	Potassium ethylenediaminetetraacetate
LI	Lysine intake
LR	Lysine retention
LUE	Lysine utilization efficiency
MAX	Maximum value
ME	Metabolizable energy
MIN	Minimum value
N	Nitrogen
N_2O	Nitrous oxide
NADH	Nicotinamide-adenine-dinucleotide
NI	Nitrogen intake
NR	Nitrogen retention
NRC	National Research Council
NUE	Nitrogen utilization efficiency
ONR	Overall nitrogen retention
ONUE	Overall nitrogen utilization efficiency
pc	prececal
r	Correlation coefficient
R^2	Coefficient of determination
RFI	Residual feed intake
RMSE	Root mean square error
SC	Serum cortisol
SCC	Spearman's rank correlation coefficient
SD	Standard deviation
SEM	Standard error of the mean
SP	Sampling period
TBW	Total body water
TiO_2	Titanium dioxide
TNE	Total nitrogen excretion
UCE	Urinary cortisol excretion
UCrE	Urinary creatinine excretion
UNE	Urinary nitrogen excretion
UUE	Urinary urea nitrogen excretion
VDLUFA	Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten

1 INTRODUCTION

Livestock farming is facing major challenges. Forecasts predict that the world's population will rise to about 8.5 billion in 2050, with a simultaneously increasing demand for animal protein due to growing prosperity. Hence, meat production is projected to grow by around 75% (Alexandratos and Bruinsma, 2012; Lassaletta et al., 2019). This is accompanied by the progressively noticeable consequences of climate change, which endangers the cultivation of sufficient amounts of feed and the competition for use of the limited arable land for human food and alternative purposes such as bioenergy production or settlement area (FAO, 2018).

Protein-producing animals are dependent on a continuous supply of dietary crude protein (CP), amino acids (AA) in particular, to meet their requirements. However, dietary AA cannot be completely utilized to cover the net requirement, because the digestibility of feedstuffs limits the amount of intestinal AA absorption, and the continuous tissue protein turnover limits the utilization of absorbed AA. Hence, for lysine as the first limiting AA, the requirement recommendations of the Gesellschaft für Ernährungsphysiologie (GfE, 2006) assume an intermediary utilization of 63% for protein retention.

Under commercial conditions, pigs retain only 30 – 45% of the ingested CP during the fattening period (Flachowsky et al., 2018; Millet et al., 2018b). The non-retained nitrogen (N) is excreted to about one-third via feces and two-thirds via urine. Feces mainly contain undigested dietary N, endogenous N, and bacterial N, whereas urea as the product of AA degradation is the quantitatively most important fraction in the urine. Urea is of particular interest because of its reactivity under the prevailing conditions in the slurry. Urinary urea excretions lead to the formation of ammonia, nitrogen oxides, and nitrate. Especially in regions with high livestock density, these metabolites can cause environmental and potential health issues which are of concern. Improving the CP, or more precisely the N utilization efficiency (NUE) especially in the fattening period, would have the greatest impact on reducing N losses, as it contributes up to 70% of total N excretion from pig production (Dourmad et al., 1999; Millet et al., 2018b).

The N excretions can mainly be reduced by supplying adequate amounts of AA according to the pigs' requirements. However, since phase feeding is already a prerequisite for achieving the mentioned efficiencies, it is necessary to explore perspectives to improve NUE that go beyond known feeding strategies for further improving the sustainability of pork production. In this context, breeding offers a promising potential for the improvement of NUE. To assess the possibility of breeding and the estimation of reliable genetic parameters, it is necessary to first determine the variation of the trait in a large number of animals. Since the exact recording of NUE is laborious, the phenotyping of a F1 crossbred population was performed in the present thesis with an integrated approach of N balance and subsequent estimation of the N retention.

2 LITERATURE REVIEW

2.1 Environmental impact of nitrogen excretions

The efficient conversion of feed into high-quality human-edible protein is an elemental issue in livestock farming because it is related to several environmental implications. The strong increase in demand for pork has led to a 4-fold expansion of pig farming in recent decades and was accompanied by the need to supply the pigs with sufficient feed protein according to their requirement (Lassaletta et al., 2019). This resulted in a shift from the use of non-humanly edible feedstuffs to cereals and oil crops that could potentially be directly used for human consumption (Windisch et al., 2013), and the share of protein-rich oilseed meals in pig diets doubled (Lassaletta et al., 2019). To intensify feed crop production and increase the protein output per hectare, the use of N fertilizers was expanded, and more agricultural land was used for the cultivation of feedstuffs, leading to the situation that 75% of the protein from plant origin is used as animal feed (Lassaletta et al., 2016). Due to its nutritional benefits, soybean meal is especially widely used for supplying non-ruminant animals with high-quality feed protein, and approximately 85% of total soybean production is used for feeding livestock (Rauw et al., 2020). However, because of the growth conditions of soybeans, global protein-feed production mainly takes place on the American continent, whereas the world's pork production is concentrated in China (47%) and Western Europe (19%; Lassaletta et al., 2019), making those regions reliant on imports. These imports are the reason for N surpluses in the pig-producing countries, as pork production is uncoupled from agricultural land (Willems et al., 2016), and cause the destruction of sensitive tropical ecosystems through substantial deforestation (- 110 million hectares between 1990 and 2005) in the soybean producing countries (Popp et al., 2017). The N surpluses in the importing countries are of great concern as they account for N losses that can cause the contamination of waterbodies due to nitrate leaching into the groundwater when manure is applied. Animal housing is responsible for 80% of the agricultural N input into waterbodies (Westhoek et al., 2015), and nitrate concentrations above 50 mg/L water are considered potentially harmful for human health (Rotz, 2004). To counteract this issue, the European Union has approved the Nitrates Directive (European Commission, 2021) which sets an upper limit of 170 kg N/hectare and year for the application of animal manure on agricultural land and can be additionally tightened by national guidelines. In addition to nitrate, the emission of greenhouse gases from livestock farming is an increasing issue. Regarding pork production and NUE, the formation of nitrous oxide (N_2O) is of concern, as the global emissions increased by 60% to 5.7 Mt/year between 1970 and 2005 (Popp et al., 2017), its greenhouse gas potential is 296-fold higher than carbon dioxide (Flachowsky et al., 2018), and the livestock sector accounts for 53% of the anthropogenic N_2O-formation (Rauw et al., 2020).

The N_2O emissions are directly linked to the extent of animal husbandry and could be substantially reduced by improved management systems with high NUE (Popp et al., 2017). N_2O arises due to microbial processes during the storage and application of the manure, and it is formed out of ammonia and urea (Sigurdarson et al., 2018), which are excreted via the pigs' urine because of inefficient intermediary use of dietary N. Ammonia is another manure-based potent pollutant that can cause eutrophication of aquatic systems, acidification of soils and human health issues through the formation of fine particles. Livestock production accounts for 87% of total agricultural ammonia formation (Westhoek et al., 2015), and the relationship between animal N excretion and the level of ammonia emissions is generally known (Lassaletta et al., 2016; Rotz, 2004; Sajeev et al., 2018; Sigurdarson et al., 2018).

It is a consensus that pork demand and production will further increase in the near future (Lassaletta et al., 2019; Popp et al., 2017; van Vuuren et al., 2017). With sustainability in mind, there is a need to reduce N excretions and to improve the NUE of the sector by both innovative feeding strategies and genetic editing of the animals. Selection for highly efficient N utilizing animals may be the most cost-effective and impactful solution towards sustainable pork production by reducing the feed protein deficit in pork producing countries (Kim et al., 2019), but this has not yet been taken into account by breeding programs.

2.2 Nitrogen utilization efficiency in the context of feed efficiency

Improving feed efficiency (FE) of pork production always was a major goal because feed costs have steadily risen over the last decades. Regarding the volatile market for finisher pigs, producers must control feed costs, as they account for up to 70% of total production costs of fattening operations (Martinsen et al., 2015). Advances in FE can be achieved in several ways, e.g., by management factors, feeding strategies, and maintenance of good animal health, as discussed by Patience et al. (2015) but also by genetic selection.

Most commonly, FE is expressed as a ratio of one unit of body weight gain achieved by one unit of feed intake (G:F) or inversely as the feed conversion ratio (FCR), but it is not a directly measurable trait (Patience et al., 2015). Therefore, improvement of FE is not possible without the modification of performance characteristics. The ratio can be improved by either lowering feed intake at the same growth rate or increasing growth rate at the same feed intake level. Both approaches assume that a higher share of dietary energy can be used for growth, as FE mainly reflects the utilization of dietary energy for maintenance and performance. The metabolic basis of this assumption is given in the composition of growth, i.e., the ratio of lean tissue to fat tissue.

Protein has the property of binding large amounts of water, and it can be supposed, that for every gram of protein retention, three additional grams of water are retained (GfE, 2006). As a result, lean tissue has a lower energy density than the comparable amount of fatty tissue and, despite the approximately 30% poorer partial energy efficiency of protein retention (0.56 for protein retention vs. 0.74 for lipid retention), only about one-fifth of the metabolizable energy (ME) which is required for lipid retention (209 kJ) is needed for the same quantity of protein retention (42.5 kJ; National Research Council (NRC), 2012). Thus, a selection for FE by higher growth rate results in a higher proportion of protein gain and leaner carcasses. This has been indirectly exploited since Bernard and Fahmy (1970) have shown that selection for higher lean meat content and lower fat content in the carcass improves FE.

Nevertheless, improving FE by minimizing FCR due to selection for lower feed intake may also result in decreased growth rates (Gaillard et al., 2020). To avoid this difficulty, the concept of residual feed intake (RFI) as an alternative measure of FE in fattening pigs has been investigated extensively over the last 20 years, as reviewed by Gilbert et al. (2017). RFI is the difference from the observed feed intake, and the feed intake which is theoretically predicted from requirements for maintenance and growth and takes into account the composition of growth. Thus, low RFI means high FE and vice versa. The biological principles are explained in detail in Herd and Arthur (2009), but the major advantage of RFI is the fact that it is phenotypically independent of production traits and thus may represent the individual variation in the energetic efficiency of the basic metabolic processes for production and maintenance. Yet, there is still a lot of uncertainty regarding the contribution of individual physiological processes on the level of RFI, but among others, digestive capacity and the subsequent intermediary utilization of ingested N are discussed as major factors (e.g., Cruzen et al., 2013; Harris et al., 2012; Hewitt et al., 2020; Vigors et al., 2016). Despite the significant advantages of improving FE by selecting for RFI, the trait has not yet become widely accepted in practice due to the high cost and effort of accurately phenotyping the composition of growth (Gaillard et al., 2020; Gilbert et al., 2017).

Despite the breeding focus on energy efficiency, improvements in FE of course also increased the NUE. Shirali et al. (2012) e.g., observed a 12% decline in total N excretion of commercial crossbred pigs within the period from 60 – 140 kg body weight (BW) when FCR was improved by 10% and both measures were strongly correlated (r = 0.91). The authors concluded that improvement in FCR by one phenotypic standard deviation reduces total N excretion during the growing period by 700 g, and so the increase of FE is the best strategy to enhance the N utilization.

In a subsequent genome-wide association study of this pig population, the same authors revealed a genetic basis for N excretion and found overlapping quantitative trait loci for N excretion and FE, suggesting differences in N utilization and metabolism as the cause of variation in FE and N excretion (Shirali et al., 2013). Similar results were obtained by Saintilan et al. (2013), who discovered strong phenotypic as well as genetic correlations (r = 0.99 for both parameters) between FCR and N excretion in four purebred lines during a test period from 35 – 110 kg BW, indicating a favorable effect on N utilization by increasing FE. Improvement of FCR was also shown to have a positive impact on lowering greenhouse gas emissions due to decreases in N excretions when modeling the live cycle assessment of the entire process of pork production, from feed cultivation to manure application (Monteiro et al., 2021).

However, Millet et al. (2018b) defined the improvements in NUE only as a side effect of selection for energy efficiency, which is obvious, as more efficient animals consume less feed at a given production level and thus excrete relatively less N. Furthermore, both reduced feed intake and higher lean growth potential lead to higher relative lysine requirements (in g lysine/MJ ME; Gilbert et al., 2017; Millet et al., 2018b; Saintilan et al., 2015), which must be considered in diet formulation using high-quality protein sources. Feeding diets deficient in lysine resulted in a 20% greater decrease in the average daily gain of pigs selected for low RFI than for pigs selected for high RFI, compared to diets adequate to the demand of the highly efficient pigs (Gilbert et al., 2017). Thus, selection for FE is not efficient for improving NUE as clearly outlined by Lassaletta et al. (2019) when examining the global context of feed use and pork production. FE expressed as FCR significantly improved between 1970 and 2005 in intensive systems from 4.8 to 3.6 kg/kg, whereas NUE only improved marginally to an average of 23% (both measures expressed on carcass basis and not on live weight basis). The authors attributed this effect mainly to the inclusion of oil crops regarding meeting the animals' increased AA demand and preventing nutrient deficiencies. In poultry, reducing N excretion by selection for improved FE was also shown to be less efficient than selection for NUE itself (Verdal et al., 2011). Thus, Millet et al. (2018b) stated, that selection for increased NUE should be performed using diets deficient in AA concentrations to limit growth, as these diets favor animals that efficiently and thus sustainably utilize dietary N and the potential for genetic improvements of NUE would be obvious.

Nevertheless, so far only Kasper et al. (2020) systematically assessed the potential of breeding towards improved NUE and demonstrated that approximately 40% of the variation in NUE can be explained by genetic factors.

Their study was based on the phenotyping work of Ruiz-Ascacibar et al. (2017) in a purebred Large White population of 168 growing pigs divided into two feeding groups: one fed a control diet and the other one fed a diet containing only 80% of the recommended amount of CP and the four first-limiting AA during the fattening period between 20 – 140 kg. The results showed that approximately 30% of the pigs fed the test diet had the same growth rate as the pigs of the control group and the authors discussed individual differences in AA requirements and different genetic potentials of adaption to challenging feeding situations as possible reasons, which assumes differences in the underlying N metabolism.

2.3 Factors influencing nitrogen retention in growing pigs

The growth of meat-producing animals is closely related to their increase in muscle mass and thus N retention (Therkildsen and Oksbjerg, 2009). Growth is a complex, dynamic phenomenon controlled by the combined regulation of feed or AA intake and utilization (Moughan, 1991). In this context, the efficiency of protein utilization of growing pigs depends largely on the matching of AA and energy intake to AA requirements (Quiniou et al., 1996).

The total AA requirement of fattening pigs includes the maintenance requirement and the requirement for growth or protein retention. The maintenance requirement includes all necessary expenditures of N containing compounds that maintain the N balance of the body. The AA requirement can be determined in two different ways. One is the factorial method, and the other is the experimental empirical method. The factorial method is based on the calculation of all components of the requirement and needs the knowledge of the maintenance requirement, as well as the accurate determination of the level and composition of growth. In the experimental empirical method, a response performance criterion of animals fed a basal diet deficient in the test AA is compared with the performance of pigs fed graded levels of this AA. The point at which the AA supply is not further limiting the performance of the animals is considered the requirement. Since lysine is typically the first limiting AA for protein retention under conventional feeding conditions, and the total AA requirement is largely determined by protein retention, the level and change in lysine requirements over time are well understood. Recommendations for the supply of other essential AA are usually given based on the concept of ideal protein (mean AA pattern in the protein retention) relative to lysine. For more information on the determination of the AA requirements, the recommendations for AA supply, and the ideal protein, the reader is referred to GfE (2006), NRC (2012), and van Milgen and Dourmad (2015).

In this context, the AA utilization for protein or N retention (NR) is subject to a variety of animal as well as environment-related factors, which influence the digestibility and the post-absorptive metabolism of the AA for maintenance and growth and thus their efficiency. According to de Lange et al. (2012) and Moughan (1991), the main biological processes limiting AA utilization for whole-body NR in growing pigs are the bioavailability of dietary AA and N, the extent of AA losses, the AA demand for the synthesis of non-protein compounds, the use of AA for maintenance of body protein, the preferential catabolism of AA for energy supply, the animal's capacity for whole-body NR as well as the associated inevitable AA catabolism, and the catabolism of AA supplied above the requirements for maximum NR. These operations have energetic costs that limit the amount of the NR.

2.3.1 Digestibility of dietary crude protein

Since not all nutrients are absorbed in the animal's digestive tract, and 15 to 25% of the total intake is excreted via feces in conventional systems (Le Goff and Noblet, 2001), it is necessary to determine the fraction of AA that are absorbed and thus available for metabolism. Besides the AA pattern of the CP fraction, this is the value-defining factor of the feed (NRC, 2012). However, since availability is difficult to determine experimentally, the in-vivo digestibility of feedstuffs is usually applied as a practical measure of the proportion of nutrients absorbed (GfE, 2005; Stein et al., 2007). The digestibility of dietary AA and CP reflects their absorption from the gastrointestinal lumen after enzymatic hydrolysis and microbial fermentation of the ingested proteins and is based on the measurement of the amount of AA and CP that disappeared from the digestive tract (Fuller, 2012). Nutrient absorption in pigs is nearly complete by the distal ileum and unabsorbed CP components are microbially fermented in the large intestine. However, ammonia produced by fermentation can still be absorbed in the large intestine and used intermediary for the synthesis of non-essential AA under N deficiency circumstances (Mansilla et al., 2015), but is usually excreted directly as urea via urine (Windisch et al., 2000). Nevertheless, ammonia serves mainly as a substrate for microbial protein synthesis (Mosenthin et al., 1997), and thus about 70% of fecal N is of microbial origin (NRC, 2012). This changes the AA pattern excreted in the feces and therefore, no conclusions about the availability of AA to the animal can be drawn from fecal AA excretion, or so-called apparent total tract digestibility (ATTD). Therefore, their ileal or prececal (pc) digestibility must be determined (Sauer and Ozimek, 1986). The pc digestibility of CP and AA is affected by the amount of endogenous N losses. These are proteins synthesized and secreted into the lumen of the gastrointestinal tract, which serves for the digestion of nutrients (digestive enzymes and mucus proteins) but also sloughed epithelial cells, serum albumin, endogenous ammonia, and urea, which are not reabsorbed until the distal ileum.

Intestinal bacteria, as well as swallowed hair, are also included in the fraction, although strictly speaking, they are not of endogenous origin. The extent of endogenous N losses depends on age or BW, the amount of dry matter (DM) feed intake, the CP concentration in the diet and its physical structure, feed processing, and the composition of the diet, in particular dietary fiber and antinutritional factors, and the microbial colonization of the digestive tract. Since endogenous N losses are considered the main determinant for maintenance AA requirements (de Lange et al., 2012) and thus contribute to the inefficiency of utilizing dietary N for retention, they have been extensively investigated in the past and there exists a vast amount of research. For further information on the influencing factors on CP and AA digestibility, endogenous N losses, and methods for their experimental determination, the reader is referred to Agyekum and Nyachoti (2017), Gabert et al. (2001), GfE (2005), Mosenthin et al. (1997), NRC (2012), Nyachoti et al. (1997), Stein et al. (2007), and Urbaityte et al. (2009).

For the investigation of the digestibility of feedstuffs, individual variation between animals usually is not taken into account. However, with the aim of improving FE, there has been an increased focus on the individual digestive capacity over the last decade, as this trait could be potentially improved by breeding (Kyriazakis, 2011). Breed effects on nutrient digestibility have been widely reported in the literature, comparing commercial lean pig genotypes with local, unimproved, and more obese breeds (e.g., Fevrier et al., 1992; Len et al., 2009; Urriola and Stein, 2012) but the effects depend on the feedstuffs and diets used in the comparisons. The unimproved breeds have a higher capacity for ATTD of organic matter and nutrients when fed diets high in fiber content, which is associated with a larger hindgut and prolonged retention time of the digesta and thus more pronounced microbial fermentation. Given the fact that selection for fast-growing pigs is carried out in optimal environments including highly digestible diets (Mauch et al., 2018; Montagne et al., 2014), it is not surprising that lean type breeds digest low fiber diets more efficiently than local, unimproved breeds when they are fed the same diets at a comparable age and feed intake level (Barea et al., 2011; Rivera-Ferre et al., 2006). Nevertheless, Kyriazakis (2011) suggested, that improving the nutrient utilization of pigs through genetic selection seems promising, especially through more efficient total tract digestion of fiber-containing diet components, because the hindgut can contribute up to 20% of digestion (Le Goff and Noblet, 2001). Improved nutrient and fiber digestibility also offers the possibility to replace the soybean meal in the diets with regional protein sources and thus increase the degree of self-sufficiency and sustainability of the pig industry (Déru et al., 2021; Pérez de Nanclares et al., 2017).

In addition to differences between breeds, differences between boars and gilts of the same breed have been reported. CP ATTD was higher in boars, when they were housed in groups and it was higher in gilts when they were housed individually, whereas the overall effect of sex was not significant (Haer and Vries, 1993). However, Verschuren et al. (2021) observed a highly significant sex effect, even when feeding two different diets. In both treatments, gilts had a higher capacity for CP ATTD (+ 2.6 and + 2.7 percentage points, respectively) than boars at a similar feed intake level, but this was not related to growth performance or FE traits.

Furthermore, differences in CP ATTD were found between individuals of the same sex and breed when different lines, divergently selected for either high or low RFI, were compared (Harris et al., 2012; Rakhshandeh et al., 2012). In the study of Harris et al. (2012) the higher CP ATTD was further associated with a tendency (p = 0.08) for higher NR and thus higher NUE. Still, no other study could detect differences in CP ATTD of same-sex growing pigs divergently selected for RFI when they were fed conventional diets (Barea et al., 2010; Labussière et al., 2015; Renaudeau et al., 2013). However, Mauch et al. (2018) observed differences in CP ATTD between the RFI lines, when diets high in fiber content were fed. The low-RFI pigs digested dietary CP more efficiently (61.9 vs. 56.1%) but without consequence for FE traits. With exception of the study of Harris et al. (2012), it was concluded that differences in ATTD do not contribute to variation in RFI, but the relationship of ATTD and nutrient efficiency was not considered separately. Barea et al. (2010) and Labussière et al. (2015) published N balance data of their trials but similar to the non-existent differences in ATTD, no differences in NUE between the RFI lines could be seen. Renaudeau et al. (2013), however, observed a significantly greater NUE of low-RFI pigs compared to their high-RFI counterparts (46.7% vs. 34.1%) despite there being no differences in CP ATTD.

Differences between individuals of the same sex and breed in unselected lines were also reported. In an evaluation of the fattening performance of 75 male Large White x Landrace crossbred pigs, housed under commercial conditions and fed the same diets, Vigors et al. (2016) observed significant differences (83.0 vs 79.9%) in the CP ATTD, when classifying the individuals in either high or low RFI after slaughter. On the contrary, differences in apparent pc CP digestibility were nonexistent. However, Pérez de Nanclares et al. (2017) observed differences in both apparent pc CP digestibility and CP ATTD, when comparing animal individual differences of Landrace piglets fed either soybean meal or a rapeseed meal as CP supplement. The apparent pc CP digestibility ranged from 74.7 to 84.7% for the soybean meal diet and was 80.9% on average, whereas it ranged from 64.4 to 79.8% for the rapeseed diet with an average of 73.2%. The mean CP ATTD of the pigs fed the soybean meal diet was 84.4% and 77.6% for the piglets fed the rapeseed diet.

The hindgut N disappearance was within a range of 10.7 percentage points for the soybean meal diet and 16.7 percentage points for the rapeseed diet but was similar on average for both feeding groups (4.2% for the soybean meal diet and 4.7% for the rapeseed diet).

In addition to breed, sex, and line effects, Noblet et al. (2013) discovered a familial effect on differences of CP ATTD. They investigated the differences in digestive efficiency of 20 Large White pigs, originating from four unrelated boars, fed one unique experimental diet rich in fibrous feedstuffs within a 10-week fattening period from 25 – 95 kg. CP ATTD increased linearly from 78.6% in week one to 83.6% in week ten and the mean CP ATTD of the offspring of the respective boar varied from 79.8% to 82.6%. The individual values for CP ATTD ranged from 73.0 to 86.2% (Bastianelli et al., 2015). Additionally, the most digestive-efficient pigs consumed the least feed and showed the highest daily weight gains, but this observation was not statistically significant. Noblet et al. (2013) hypothesized that the differences in digestive efficiency originated from increased hindgut absorption and that digestion seems to be heritable, but the results need to be confirmed by a larger number of animals. Additionally, the absence of period x boar interactions indicates the independence of heritability of CP ATTD from age or BW.

These results were in line with the results of Ouweltjes et al. (2018). In an integrated evaluation of nine different digestibility studies following the same experimental procedure, they examined the repeatability of nutrient ATTD for individual growing pigs. This repeatability was defined as the proportion of phenotypic variance of repeated digestibility measurements of the same animal fed different diets that can be explained by the animal. Within diet and trial, the repeated CP ATTD measurements of the same pig were correlated, resulting in a repeatability of 16%, indicating that relevant differences exist between individuals fed the same diet. Nevertheless, 50% of the total phenotypic variation in CP ATTD was related to the diet of which 85% was related to dietary fiber. However, no relations to nutrient utilization were drawn in this study.

The possibility to improve CP ATTD by selection was confirmed by Déru et al. (2021), who were the first to first to systematically assess the genetics of digestive efficiency in a purebred Large White population with a defined family structure. They estimated a CP ATTD heritability of 0.27 for a conventional control diet and a heritability of 0.56 for a high fiber test diet. Additionally, significant negative genetic correlations between CP ATTD and FE traits (FCR and RFI) for both diets were observed, even when the digestibility has been adjusted for feed intake. Similar correlations were obtained by Verschuren et al. (2021) who investigated the effect of nutrient ATTD on FE traits in 105 crossbred pigs, half boars, and half gilts, fed either a corn, soybean meal-based or a wheat, barley, and co-product-based diet.

On the contrary, correlations of CP ATTD to lean meat content and carcass yield were not different from or were close to zero (Déru et al., 2021).

In addition to the level of feed intake, which is known to affect digestibility and thus should be adjusted for comparisons (Déru et al., 2021; Verschuren et al., 2021), other factors were discussed in the literature to explain the differences between breeds, lines, sex, and individual animals. These were the age of the pigs (Barea et al., 2011; Le Goff and Noblet, 2001; Noblet et al., 2013; Ouweltjes et al., 2018), the size of the gastrointestinal tract and thus variations in the transit time of the digesta (Barea et al., 2011; Montagne et al., 2014; Vigors et al., 2016), the micro-anatomy of the gastrointestinal tract surface (Barea et al., 2011; Pérez de Nanclares et al., 2017; Pluske et al., 2003), the activity of digestive enzymes and nutrient transporters (Clarke et al., 2018; Montagne et al., 2014; Pérez de Nanclares et al., 2017; Pluske et al., 2003; Vigors et al., 2016), and the composition of intestinal microbiota (Déru et al., 2021; Montagne et al., 2014; Verschuren et al., 2018; Verschuren et al., 2020; Vigors et al., 2016).

Pigs with a higher CP ATTD generally have more N available for growth and maintenance than pigs showing a lower CP ATTD. However, there is evidence that the additional N cannot be utilized efficiently by the animals, independent of breed or line.

For example, the higher CP ATTD of Landrace gilts compared to Pietrain gilts (94.4 vs. 92.2%) was completely compensated for by the increased urinary N excretion of the Landrace pigs (+ 0.05 g/kg metabolic BW; Windisch et al., 2000). Furthermore, the proportion of urea in the urinary N excretion was increased (66.4 vs. 58.3%), suggesting that the additional absorbed AA were directly degraded. Thus, they were not used for retention and no differences in NUE between the breeds were observed. Barea et al. (2011) only observed significant differences in CP ATTD between Landrace x Large White and Iberian barrows in younger pigs (30 kg) but not in older pigs (80 kg). The NUE, on the other hand, was higher in Landrace x Large White barrows in both periods (73.5 vs. 49.7% at 30 kg and 46.3 vs. 29.6% at 80 kg). They concluded that the higher growth rate of the lean-type pigs is mainly due to a better utilization efficiency of the absorbed N rather than differences in CP ATTD.

Further, when crossbred barrows of the same origin were fed diets differing in fiber source, the CP ATTD decreased significantly compared to the wheat, barley, and soybean meal control diet (71 – 75% vs. 83%; Hansen et al., 2006). The amount of N intake remained constant (60.4 – 64.2 g/d vs. 63.1 g/d) and so did the NR (29.2 – 31.9 g/d vs. 34.6 g/d) and thus NUE (49 – 51% vs. 51%). The additional absorbed N observed in the control group was completely excreted again via urine. Pérez de Nanclares et al. (2019) also did not detect differences in NR and NUE (53 – 55%) of Landrace piglets fed diets with increasing proportions of rapeseed meal.

The decrease in N absorption due to lower CP ATTD compared to the control group was compensated for by less urinary N output, and thus total N excretion was constant. Only the ratio of urinary N excretion to fecal N excretion declined from 1.51 in the control group to 1.03 in the group with the highest inclusion rate of rapeseed meal.

Thus, the overall effect of differences in ATTD on the nutrient supply of the animal seems minor, without a quantitatively significant effect. Genetically determined differences in the postabsorptive metabolism of nutrients in organs and tissues overcome the differences in ATTD between the individuals and seem to be responsible for differences in NUE (Windisch et al., 2016). The increase in intestinal N absorption is accompanied by a less efficient intermediary use and increased urinary N excretion and vice versa. However, the mentioned results were obtained under conventional feeding conditions with sufficient CP supply. A scarce CP and AA supply could possibly reveal effects of differences in the digestive capacity between individuals.

2.3.2 Body protein turnover

The aforementioned factors limiting the intermediary efficiency of AA utilization are associated with the constantly occurring protein turnover. Protein turnover is defined as the simultaneous occurring processes of protein synthesis and degradation in body tissues (Duggleby and Waterlow, 2005). Protein retention of growing animals is the result of the excess of synthesis over degradation with the rate of these processes largely exceeding the actual protein retention rate. Therefore, small changes in the level of synthesis or degradation can have large effects on the level of protein retention (Therkildsen and Oksbjerg, 2009).

For an efficient de-novo synthesis of body protein, the growing pig depends on a continuous supply of dietary AA. If a continuous supply cannot be provided, the organism can utilize AA independently of feed intake through the breakdown of body protein. Despite high energetic costs, protein turnover is therefore essential to maintain vital metabolic processes. This allows the organism the metabolic flexibility necessary to respond to changing environmental or feeding conditions. This is important, on the one hand, to regulate long-term chronic processes such as bone expansion and the associated muscle fiber elongation during growth and, on the other hand, to synthesize and recycle proteins that are needed in the short term, such as enzymes or transport proteins. Furthermore, the protein turnover enables the immune system to respond rapidly to unforeseen events such as infection or injury, and damaged or malfunctioning proteins are cleaved off the cells. In addition, it also contributes to the maintenance of body temperature due to its energetic inefficiency. It is estimated that per kg of protein synthesized, 15 – 20 MJ of energy is released as heat (Lobley, 2003).

The different body proteins have different half-lives, which are explained by their role in metabolism (Stangl, 2010). For example, enzymes have a half-life of only a few hours, whereas structural proteins as found in the skeletal musculature have a half-life of up to one year. However, there are also differences between the proteins of the skeletal muscles. In porcine satellite cells, the half-life of myofibrillar proteins is twice as high as that of sarcoplasmic proteins (Therkildsen and Oksbjerg, 2009), but with three months for actin and six months for myosin, the half-life is still high (Stangl, 2010). The different half-lives of the proteins cause differences in the turnover rates of the body tissues. The gastrointestinal tract and the liver, for example, are highly metabolically active tissues, and because of the necessary production of digestive enzymes and transport proteins, they express high rates of protein synthesis per 100 g protein mass per day (Lobley, 2003). This fractional synthesis rate (FSR) was shown to range between 74 – 93% in the liver and 59 – 78% in the duodenal mucosa, of weaned piglets (Ponter et al., 1994), and between 39 – 54% in the liver and 85 – 119% in the duodenum of growing barrows (Sève et al., 1993). Hence, even the gastrointestinal tract and the liver together account for only about 10% of the total body protein mass, they constitute 25% of the total body protein synthesis (Sève and Ponter, 1997) and account for up to 50% of total energy consumption (Gutierrez and Patience, 2012). The skeletal musculature, on the other hand, accounts for approximately 35% of the daily body protein synthesis, whereas more than half of the body protein is bound as muscle protein. The FSR of the *longissimus* muscle was measured to be 8 – 12% in weaned piglets and 3 – 5% in growing pigs (Ponter et al., 1994; Sève et al., 1993). Therefore, the absolute daily protein synthesis of the duodenum is twice as high as that of the complete skeletal musculature (407 – 466 g vs. 156 – 277 g; Sève et al., 1993). Despite accurate postmortem estimates of the turnover of individual tissues, these cannot be differentiated experimentally in the living animal. Thus, measured values for protein synthesis and degradation of total body protein are always average values of all individual proteins and tissues of the entire body (Therkildsen and Oksbjerg, 2009).

Protein degradation and synthesis are dependent on feed intake and are subject to hormonal control, which has been extensively researched and is presented in detail elsewhere (e.g., Fuller et al., 1987; Moughan, 1999; Reeds and Davis, 1992; Sève and Ponter, 1997; Stangl, 2010; Therkildsen and Oksbjerg, 2009; Weiler, 1995). Briefly, insulin, somatotropin, glucocorticoids, thyroid hormones, and androgens are involved in the regulation of body protein turnover.

Insulin is an anabolic hormone that stimulates protein synthesis at the translational stage in response to feed intake via the short-term upregulation of the activity of existing RNA. In addition, it was shown to have a depressing effect on muscle protein degradation in various species. The effect of insulin on protein syntheses depends on the type of target tissue, with glycolytic muscles being more insulin sensitive than oxidative muscles (Baillie and Garlick, 1991).

Somatotropin and the insulin-like growth factor 1 (IGF-I), whose formation is mediated by the influence of somatotropin, are also anabolic hormones and stimulate body protein synthesis at the transcriptional stage via the long-term upregulation of RNA synthesis and the stimulation of satellite cell proliferation. The anabolic effect of somatotropin and IGF-I is well established, and several studies observed an increase in body protein synthesis after somatotropin treatment (e.g., Sève et al., 1993; Tomas et al., 1992; Vann et al., 2000b). Furthermore, the administration of somatotropin can inhibit protein degradation and AA catabolism and thus has an additional anti-catabolic effect (Vann et al., 2000a).

Glucocorticoids are catabolic hormones that suppress protein synthesis at both the transcriptional and the translational sites and increase protein degradation. Protein degradation by glucocorticoids, such as cortisol, is caused by local or systemic stress and in the case of immune challenge. Stress can be triggered by starvation, which additionally reduces anabolic signaling by insulin or nutrients and thus reduces the transport of AA into the target cells. Reduced stress responsiveness to an adrenocorticotropic hormone challenge and thus lower plasma cortisol concentrations in low-RFI gilts compared to high-RFI gilts can also contribute to differences in FE (Colpoys et al., 2019). The result of immune system stimulation is a decrease in muscle protein synthesis accompanied by an increase in visceral protein synthesis and thus lower body protein retention (Rudar et al., 2017). The effect of immune system stimulation on body protein turnover was recently outlined by McGilvray et al. (2019). The starter gilts treated with lipopolysaccharides showed significantly lower protein degradation per kg of metabolic BW (MBW, 6.7 vs. 9.4 g N/kg MBW) and synthesis rates (8.1 vs. 11.6 g N/kg MBW) than the untreated gilts, resulting in lower efficiency of protein retention (69 vs. 80%). In weaned barrows, however, Rudar et al. (2017) did not observe a significant difference in protein degradation after immune system stimulation ($p = 0.38$), whereas protein synthesis, protein retention and the protein retention to synthesis ratio were significantly lower in the treated barrows ($p = 0.045$, $p < 0.001$ and $p = 0.040$, respectively).

The thyroid hormones generally increase protein turnover by increasing both protein synthesis and degradation. It is assumed that they stimulate both the transcriptional and translational level of protein synthesis but are not involved in the acute regulation.

The thyroid hormone triiodothyronine, for example, provides higher insulin concentrations and thus indirectly leads to increased protein synthesis (Millward et al., 1988), whereas it also has a proteolytic effect by stimulating the calpain pathway (Zeman et al., 1986).

The androgens testosterone and estradiol stimulate protein retention by simultaneously enhancing protein synthesis and suppressing protein degradation, and the larger muscle mass of uncastrated male animals is largely the cause of high testosterone levels. The effect of androgens appears to be indirect via the somatotropin axis, as the concentration of IGF-I in blood plasma increases because of estradiol treatment (Johnson et al., 1998). The effect of sex hormones is age-dependent since no significant production of sex hormones occurs before the onset of sexual maturity. For example, no differences in muscle protein turnover rates were observed between four-week-old boars and castrates (Skjaerlund et al., 1994).

The effect of genotype and animal age on the expression of hormone concentrations associated with protein turnover and retention is well documented in the literature (e.g., Nielsen et al., 1995; Sutherland et al., 2005; Weiler, 1995), but the results are inconclusive. In a study by Weiler et al. (1998), the superiority of protein retention of Large White boars compared to wild boars and Meishan boars could be largely explained by low levels of catabolic hormones and thus reduced protein degradation. Despite the highest plasma IGF-I concentrations, wild boars showed the lowest growth rate. The high plasma androgen levels in Meishan boars counteracted the low IGF-I and high cortisol concentrations and seemed to prevent excessive protein degradation. Elsaesser et al. (2002) also accounted the lower lean growth potential and higher carcass fat of Minipig barrows compared to German Landrace barrows to elevated plasma cortisol concentrations and a higher activity of the hypothalamus-pituitary-adrenal axis. Sève and Ponter (1997) reported the results of a comparison of plasma hormone concentrations between Large White, Pietrain, and Meishan piglets. Even though Pietrain piglets had the lowest plasma IGF-I concentrations and medium cortisol concentrations, they showed the highest muscle protein synthesis rate. Also, a significant correlation between plasma IGF-I concentrations and muscle RNA activity in Pietrain piglets was observed, but not for Large White and Meishan piglets. On the contrary, in the study of Clutter et al. (1995), the differences in growth performance of gilts divergently selected for growth performance were attributed to differences in IGF-I concentrations. With 217 ng/mL blood plasma on average, fast-growing gilts had significantly ($p < 0.05$) higher IGF-I concentrations than slow-growing gilts (145 ng/mL). Buonomo et al. (1987) observed three times higher plasma IGF-I concentrations in conventional crossbred barrows compared to miniature laboratory swine.

Based on the differences between these extreme genotypes and correlations with body size and growth rate, the authors concluded that IGF-I might be a physiologic indicator of the growth potential of pigs.

In addition to breed-specific differences, sex-specific differences in plasma IGF-I concentrations were reported, which are related to the age of the pigs and thus advancing maturity. Louveau et al. (1991) did not observe differences in IGF-I concentrations of Large White boars, gilts, and barrows at the age of 45 d, whereas, at the age of 140 d, boars had significantly higher IGF-I concentrations than gilts and barrows. Thus, the authors determined a significant ($p < 0.05$) interaction of age and sex on the plasma IGF-I level. Similar results were reported by Biereder et al. (1999). Until the age of 78 d, no significant differences in plasma IGF-I concentrations between crossbred boars, barrows, and gilts were observed. From the age of 111 d, boars had significantly higher plasma IGF-I levels than gilts and barrows. At the end of the fattening period (d 200), the IGF-I concentration of boars (379 ng/mL) was three times higher than that of castrates (130 ng/mL) and twice that of gilts (184 mg/mL). In addition, gilts had additionally higher IGF-I concentrations than barrows at the age of 180 d. Also, positive correlations between plasma IGF-I concentrations and growth rate as well as the diameter of *triceps brachii* muscle fibers were observed, which could be attributed to the differences between the sex of the pigs.

In conclusion, pig growth is dependent on the interaction between the anabolic and catabolic processes in which the respective hormones seem to play a major role. Thus, high growth potential can be concluded in the case of high secretion of anabolic hormones with simultaneously low catabolic hormone concentrations (Weiler et al., 1998).

Since protein turnover and the associated efficiency of AA utilization are thought to contribute significantly to differences in FE (Herd and Arthur 2009), it stands to reason that protein turnover also contributes to differences in NUE, even though the extent of the impact of protein turnover on AA utilization is not clear (Sève and Ponter, 1997). In addition, the results of the effect of protein turnover on FE of growing pigs are inconclusive (Gilbert et al., 2017). Cruzen et al. (2013) observed lower activities of the 20S proteasome, an important catalytic subunit responsible for proteolysis of ubiquitin-tagged proteins, in the *longissimus* muscle when comparing twelve low-RFI and twelve high-RFI gilts each after the slaughter at approximately 68 kg BW. They also found lower activities of calpains involved in calcium-dependent proteolysis. They concluded that the lower protein degradation contributes to the better FE. In contrast, in the study by Le Naou et al. (2012), no differences in total proteasome and calpain activity in the *longissimus* muscle were detected between RFI lines, either post-weaning or at final BW.

Vincent et al. (2015) observed reduced expression of proteins and genes involved in energy metabolism in a comparison of eight gilts of each of the different RFI lines. These expressions were also measured in the *longissimus* muscle after the slaughter at 115 kg BW. The authors concluded that low-RFI animals might have more energy available for protein synthesis due to lower oxidative metabolism and possibly lower cellular stress. This confirms the findings of Grubbs et al. (2013). These authors observed a positive correlation between RFI and reactive oxygen species in eight gilts per RFI line after the slaughter at 94 – 98 kg BW, also in the mitochondria of the *longissimus* muscle. In contrast, this relationship could not be established in liver mitochondria. The authors concluded that decreased production of reactive oxygen species results in less DNA damage and therefore reduces protein degradation due to decreased mitophagy.

Regarding protein synthesis, neither Cruzen et al. (2013) nor Le Naou et al. (2012) detected differences between the RFI lines. Both protein synthesis rates and protein synthesis marker expressions in the *longissimus* muscle were at similar levels. Vincent et al. (2015) on the other hand, were able to detect overexpression of several genes encoding subunits of the initiation and elongation translation factor in the *longissimus* muscle of low-RFI pigs.

However, the results above are all based on qualitative studies that measured the expression of genes or proteins associated with protein turnover in selected tissues. Quantitative information on the level of protein turnover cannot be given, and no relation to the NUE of the animals can be drawn. Furthermore, except for the study by Renaudeau et al. (2013), no difference in NUE was detected between RFI lines (Barea et al., 2010; Labussière et al., 2015). A relationship between quantitative body protein turnover and the NUE between the different RFI lines is provided by Hewitt et al. (2020). They measured the protein turnover of twelve gilts of each of the two lines in vivo after oral administration of ^{15}N-labeled glycine (see chapter 2.4.1). Half of the animals of each group received a diet that met the nutrient requirement recommendations, and the other half received a diet deficient in lysine. The results indicate no differences in body protein turnover between the two lines and the authors conclude that it does not contribute to differences in FE of the animals. However, they observed a significant 13% decrease in lysine utilization efficiency (LUE) in the low-RFI group compared to the high-RFI group, which tended to result in lower NUE of the low-RFI gilts. This difference was more pronounced in the group fed lysine-reduced diets than in the adequately fed group. However, lysine restriction had a significant effect on the level of protein turnover ($p = 0.05$) independent of the RFI line ($p = 0.64$; Hewitt et al., 2020).

Similar results were obtained by Rivera-Ferre et al. (2006) when comparing the protein turnover of two different breeds at similar BW and different lysine intake levels using the same marker method. In both the Landrace and the Iberian gilts, both synthesis and degradation were decreased by lysine restriction, whether expressed as fractional rates or in absolute terms ($p < 0.001$). Nevertheless, independent of the lysine intake level, significantly higher protein synthesis ($p = 0.004$) and degradation ($p = 0.009$) were observed in Landrace gilts compared to the Iberians. However, with about one-fifth, the proportion of the retained protein from the synthesized protein was the same for both breeds ($p = 0.759$). In the study of Hewitt et al. (2020), the efficiency of protein synthesis was also low, and the gilts retained only about one-sixth of the synthesized protein. In other studies with growing pigs, despite similar feed intake levels, a retention rate of about one-third of the synthesized protein was determined after the oral administration of ^{15}N-labeled glycine (Hellwing et al., 2007; Saggau et al., 2000; Windisch et al., 2000). Nevertheless, this illustrates that protein synthesis capacity appears to not limit the amount of protein retention, but the rate of protein degradation seems to be a major biological cause for the relatively low NUE in growing pigs. Looking at the protein or AA degradation, three different types of catabolism can be distinguished, minimum AA catabolism, AA catabolism for energetic use (preferential catabolism), and the inevitable AA catabolism.

2.3.3 Amino acid catabolism

Minimum amino acid catabolism

Besides the digestion and its associated losses (see chapter 2.3.1) as well as regrowth of skin and hair, the minimum AA catabolism associated with the basal turnover of body protein, irreversible synthesis of non-protein components, and the concomitant loss of AA and N of endogenous origin via urine is the major process causing maintenance AA requirements (Moughan, 1999). These losses must be compensated by AA ingested with feed. Since maintenance processes are assumed to have priority over growth processes (Moughan, 1991), the AA used to cover these maintenance costs are not available for the de-novo synthesis of body protein. Thus, maintenance of body functions contributes to the inefficiency of AA utilization for protein retention.

The minimum AA catabolism is the minimum breakdown rate of AA that occurs even when pigs are fed N-free diets or are in protein equilibrium, i.e., no protein retention occurs (de Lange et al., 2012; Whittemore et al., 2001). This minimum catabolism is essential to the animal organism despite the energetic costs involved. Since the body has no significant pool of free AA other than blood plasma (Remus et al., 2021), the necessary AA for synthesis processes must be taken from pre-existing body protein if not provided by the diet.

The determination of the maintenance requirement and especially the minimum catabolism is difficult in growing animals. It is therefore carried out with adult animals that are in N equilibrium and have no protein retention. It is usually expressed relative to MBW (GfE, 2006). Due to the experimental difficulty of recording minimum AA catabolism, there exist insufficient quantitative estimates of animal and diet effects on body protein turnover and minimum AA catabolism for the individual AA (NRC, 2012). Therefore, for practicality, it is assumed that the post-absorptive inefficiency of the AA (as described later in this chapter) already accounts for losses associated with basal body protein turnover. Nonetheless, there are empirical estimates of the magnitude of urinary N losses associated with basal protein turnover. In a literature review, Whittemore et al. (2001) reported a maintenance body protein turnover of 16 g/kg MBW, which is approximately 5% of total body protein. However, the efficiency of recycling the degraded protein is high (94%), resulting in losses associated with basal turnover of only 0.14 to 0.21 g N/kg MBW, which is equivalent to approximately 0.3% of the total body N amount. These urinary N losses associated with basal turnover increase by approximately 0.16 g/kg MBW during immune system stimulation. Moughan (1999) reported daily urinary losses due to basal turnover of 0.06 g N/kg MBW.

Amino acid catabolism for energetic use

Preferential AA catabolism is the breakdown and utilization of AA for energetic usage in case of insufficient energy supply to support the growing pigs desired rate of protein deposition (de Lange et al., 2012). This occurs when pigs are fed diets in which the ratio of non-protein energy to AA energy is low and should be differentiated from the catabolism of AA that are supplied in excess of requirements (Moughan, 1991).

The use of the energy ingested with feed for the various metabolic processes is shown schematically in Figure 1 (adapted from van Milgen et al., 2008). It shows the dual role of CP for protein retention or energy utilization. Under non-limiting conditions, the digestible AA from the diet are used entirely for the de-novo synthesis of body protein, either to meet maintenance requirements or for protein retention. Only the AA from CP ingested in excess of requirements are used for energetic purposes and lipid retention. Even in situations in which the dietary energy intake has a limiting effect on the level of protein retention, body fat is mobilized for energy supply rather than preferential catabolism of AA, because protein retention has priority over lipid retention (Quiniou et al., 1996).

However, a minimum level of body fat in the organism has to be ensured, regardless of lean growth (Whittemore, 1983). If, after meeting the energetic cost of maintenance and protein retention, there is insufficient ME to meet this minimum lipid retention requirement, AA deamination is triggered, and AA are degraded for energy supply. This is especially the case in young animals. Feed, and thus energy intake, is known to be an important factor determining growth performance (Bikker et al., 1995), but up to about 50 kg BW, the size of the gastrointestinal tract limits feed intake and pigs are not capable to compensate reductions in dietary energy density by increasing voluntary feed intake (Li and Patience, 2017). In addition, preferential catabolism is related to the maintenance of blood glucose levels so that AA can be degraded and used for gluconeogenesis (Moughan, 1991).

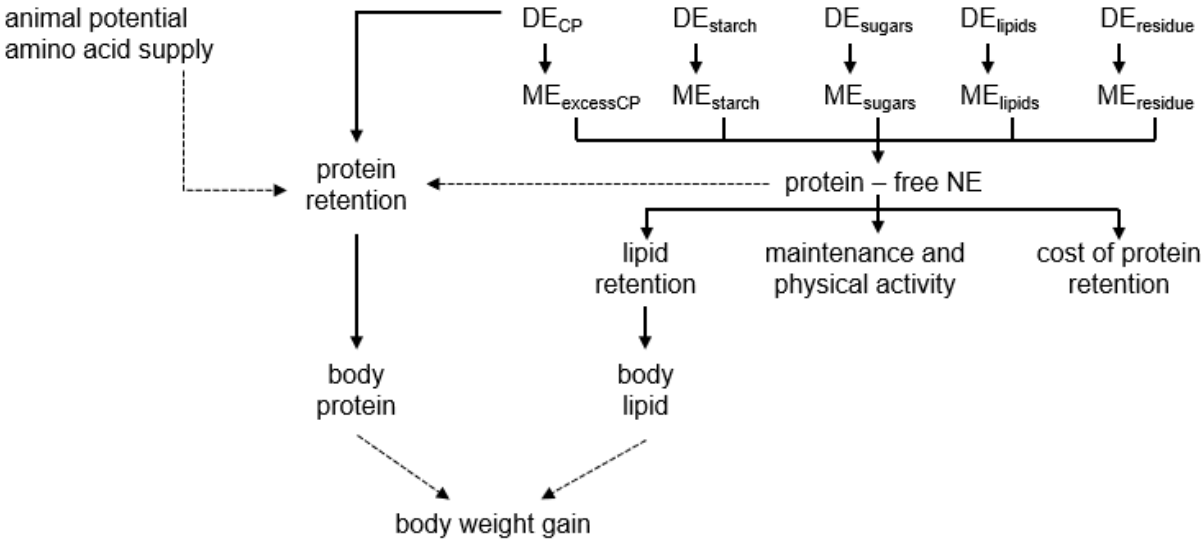

Figure 1. Main energy and nitrogen flows (solid arrows) and determinants (dashed arrows) in growing pigs. DE = digestible energy, ME = metabolizable energy, NE = net energy (adapted from van Milgen et al., 2008).

Preferential catabolism not only depletes the AA supply but is also energetically wasteful. AA must be deaminated, i.e., the amino group is cleaved off, before the remaining carbon chain can be energetically used in the mitochondria via the citric acid cycle. The cleaved amino group, in turn, must be removed from the metabolism because of the resulting toxic ammonia. A small amount of the resulting ammonia can be excreted directly via the urine in the form of ammonium. However, the majority is detoxified to urea via the urea cycle in the liver, which has an energetic cost (Patience, 2012). Since body cells have a maximum capacity for protein synthesis and AA cannot be stored for later use, AA ingested in excess of the requirement for the animals' maximum protein retention capacity will also be degraded for energetic use (Moughan, 1991). According to Le Goff and Noblet (2001), these are 31.1 kJ per additional gram of N consumed in excess of required for protein retention.

This value is independent of the physiological status of the animals and linearly dependent on the additional CP intake. Thus, the ME amount of the diet depends largely on the utilization of CP for energetic purposes or protein retention (GfE, 2006).

By feeding well-balanced diets matching the protein retention capacity of the pigs, it should be possible to avoid catabolism of AA for energetic use. Under practical conditions, however, this can hardly be avoided, since the exact determination of the protein retention capacity of the animals is not trivial and, above all, cannot be performed in real-time at the farm level (Gaillard et al., 2020). However, since it has been proven that the protein retention capacity varies greatly between individual animals of the same genotype and over time, the AA loss due to catabolism for energetic purposes can be considerable, especially in group-housed pigs (de Lange et al., 2012; Moughan, 1991). In a review of literature data, Jha and Berrocoso (2016) stated, that 25% of the CP of a typical corn and soybean meal-based diet cannot be utilized intermediary because of unbalanced AA relative to the pigs' requirements and is thus excreted as urea via urine.

Inevitable amino acid catabolism

Inevitable AA catabolism is the minimum breakdown rate of absorbed dietary AA, which determines the maximum marginal intermediary efficiency of AA utilization for body protein retention (de Lange et al., 2012). This catabolism is caused by the minimal activity of catabolic enzymes in tissues and occurs even though the intake of non-protein energy far exceeds the demand for essential metabolic processes (Moughan, 1991). The level of inevitable catabolism has been extensively studied for lysine as the first limiting AA and is estimated to vary from 3 to 40% (Mnilk et al., 1996; Moehn et al., 2000; Moughan, 1991), with an average of 25% of intermediary available lysine intake (de Lange et al., 2012; NRC, 2012). The inevitable catabolism limits the AA utilization efficiency over and above maintenance requirements. This maximum marginal efficiency of lysine utilization was estimated to be 87% in high-performing animals (Moehn et al., 2004), but marginal efficiencies of 63% and 65% are given for requirement recommendations to account for individual variation among animals (GfE, 2006; NRC, 2012). Inevitable catabolism is constant over a wide range of AA intake and decreases only in the case of serious deficiencies in AA supply (de Lange et al., 2001; Moehn et al., 2000; Moehn et al., 2004). It was thought to be independent of the energy intake and BW (age) of the animals, but further investigations revealed a declining marginal efficiency with increasing BW (NRC, 2012). Hence, marginal lysine efficiency averages 68.2% for 20 kg BW and 56.8% for 120 kg BW. Furthermore, the rate of inevitable catabolism is dependent on the protein retention capacity of the pigs and increases in absolute terms with increasing growth potential.

Relatively, however, it was shown to decrease by 0.2% for each additional gram of maximum daily protein retention rate (Moehn et al., 2000; Moehn et al., 2004). The marginal efficiency of lysine is almost entirely dependent on protein retention since no significant amounts of lysine are required for the synthesis of non-protein compounds (Moehn et al., 2003).

The maximum protein retention capacity is the protein retention an individual can theoretically achieve under optimum conditions, i.e., sufficient AA and energy supply as well as freedom from stress and disease (Schinckel and de Lange, 1996). This value is additionally dependent on the genotype and sex of the animals and is considered constant for a period of 35 – 65 kg (Schinckel and de Lange, 1996), 25 – 70 kg (Moehn and de Lange, 1998) or 45 – 100 kg (Quiniou et al., 1996). Since this value is only theoretical and cannot be achieved under practical feeding conditions, the maximum protein retention is that which is achieved by the animals under ad libitum feeding conditions (van Milgen et al., 2008). Below a BW of approximately 40 kg, the pigs feed intake capacity is generally limiting the intake of sufficient amounts of energy to express maximum protein retention capacity (Li and Patience, 2017; Moehn and de Lange, 1998). Hence, at low BW, protein retention is generally not determined by the pigs' genetic capacity, but by feed intake.

In addition, protein retention is dependent on lysine intake as the first limiting AA. Evaluating literature data, Susenbeth (1995) demonstrated that when energy intake and growth potential of the animals were not limiting, regardless of genotype, BW (age), and sex, protein retention depended solely on the amount of lysine intake. Thus, depending on the determination method of protein retention, the marginal efficiency of lysine utilization was 58 and 65%, respectively. Furthermore, the ambient temperature can limit the protein retention of growing pigs. Since protein retention is less energetically efficient than lipid retention, more heat is released as ME losses during protein retention (GfE, 2006). Pigs adapt to high temperatures by altering the composition of growth. Hence, Renaudeau et al. (2013) observed a significant decrease in protein retention during a period of heat stress in the grower period ($p < 0.001$), whereas more energy was retained as lipid. In addition, NUE was reduced from an initial 46% to an average of 35% during the test period.

Differences in the maximum protein retention capacity between sex and genotype are well known (Morel et al., 2008; Pomar et al., 2003). The protein retention of the animals is determined by the genetically determined composition (specific ratio of fat to lean) of the mature body. The higher the protein content in the mature body, the later the animals reach physical maturity and the longer they can maintain high protein retention rates (Fabian et al., 2003; van Milgen et al., 2008). Boars generally have the highest average protein retention, followed by gilts and barrows.

In addition, boars can maintain their high protein retention up to higher BW (Bikker, 1994; Quiniou et al., 1996; Schinckel and de Lange, 1996; Schinckel et al., 2008). In a recent study with comparative carcass data of growing Large White pigs of different sexes, the basic relationship was confirmed (Ruiz-Ascacibar et al., 2017), but the authors showed that boars could maintain or even slightly increase their daily protein retention up to a BW of 140 kg. Even in barrows, the maximum daily protein retention was observed at 120 kg BW. Differences in protein retention between the sexes may underlie a different distribution of energy for metabolic processes, as higher feed and thus energy intake resulted in higher lipid retention rather than higher protein retention in barrows compared to gilts (Ruiz-Ascacibar et al., 2017, Schinckel et al., 2008; Suárez-Belloch et al., 2015).

In addition to the differences between the sexes, Ruiz-Ascacibar et al. (2017) observed that about 30% of the pigs fed a low CP diet (80% of control for CP and the first five limiting essential AA) exhibited the same growth rate as the pigs of the control group. The authors discussed individual differences in the requirements for essential AA or a different genetic potential of utilizing dietary nutrients and thus dealing with limiting situations as possible reasons for their observation. Differences in protein retention within the same population at the same feed intake levels and same BW are caused by the change of specific nutrient requirements of individual animals change over time (de Lange et al., 2001; Remus et al., 2021; van Milgen et al., 2008). These changes are independent of diet composition.

In a study designed to elaborate genotype x feed interactions in different growth stages, Godinho et al. (2018) examined the growth performance of 2,230 crossbred pigs fed either a typical American (corn and soybean meal-based) or European (wheat, barley, and co-product-based) diet within the fattening period from 22 – 122 kg. The authors observed a wide range of protein retention between the pigs, ranging from 52 – 244 g/d with an average of 154 g/d for the American diet and 158 g/d for the European diet. The heritability of protein retention was independent of the growth stage but differed between the dietary treatments (0.38 for the American diet and 0.24 for the European diet). The genetic correlation of protein retention between the two treatments was 1.00, indicating that no interactions between genotype and feed exist and every pig would express the same protein retention independent from the diet it receives. The authors argued that both diets had sufficient energy and AA and thus the protein retention was not influenced by the environment.

In a cluster analysis, Remus et al. (2021) compared 95 barrows with similar feed and AA intake differing in their mean daily protein retention by 55 g between the high (213 g/d) and low (167 g/d) retaining animals. The higher protein retention resulted in significantly higher NUE (64 vs. 50%), and LUE (80.8 vs. 70.2%) compared to the pigs with the lowest protein retention. The authors attributed the differences between the groups to differences in maintenance requirements due to differences in energy and AA metabolism that resulted in improved intermediary utilization of AA for protein retention. Regardless of the protein retention capacity of the animals, the efficiency of AA utilization decreased with increasing AA intake and, hence, 74% of the variation in LUE within the group could be explained by the amount of intake. However, between the groups, 84 – 87% of the variation in LUE was explained by the group, i.e., by the differences in protein retention, and only 13% by the differences in AA intake.

It seems obvious that the efficiency of N and AA utilization is mainly determined by the amount of protein retention. The higher the protein retention of the pigs, the lower the proportion of the maintenance requirement, and more AA can be used for the productive performance (de Lange et al., 2012). Thus, an increase in protein retention should lead to an increase in the NUE.

2.4 Determination of nitrogen retention and nitrogen utilization efficiency

Similar to FE (Patience et al., 2015), there are several ways to express NUE in growing pigs. Because the measurement of NR is more objective than estimations of lean growth (de Lange et al., 2012), one practical approach to express whole-body NUE is to determine the proportion of the retained N from the ingested N. In this context, the measurement of NR is crucial and implies the recording of N excretions via urine and feces. The maximum realizable NR at a given dietary N intake is the key variable that affects the amount of NUE. The determination of the NR in the empty body of the pigs can essentially be done in two different ways. One is the direct determination of the body composition of the animals and the other is the indirect determination of the N excretion via feces and urine utilizing a mass balance (GfE, 2006).

2.4.1 Determination and estimation of body composition

The determination of the body composition of growing pigs can be done by invasive and non-invasive methods. Invasive methods require the removal and sampling of body tissues, which in growing pigs, is typically performed via the comparative slaughter method.

Determination of body composition by slaughter and subsequent chemical analysis of the carcass is the basis for determining AA requirements of growing pigs (GfE, 2006; NRC, 2012).

It serves as a reference method for assessing the suitability of other methods for determining body composition, such as dilution methods and modeling (Scholz and Mitchell, 2001), and is the method of choice when accurate data on composition and changes in NR as a function of age and sex are needed. The carcass or parts of the carcass are divided into the biological fractions of meat, bones, viscera and intestine, hair and hooves, blood, and bile (Ruiz-Ascacibar et al., 2017). The samples are weighed, homogenized, pooled, dried, and analyzed for chemical raw nutrients (Susenbeth and Keitel, 1988). When using large laboratory animals and/or large numbers of animals, this method is labor-intensive and costly (Susenbeth, 1984). A sampling of one-half of a carcass is reported to take about 110 h (Scholz, 2002). Of great importance for the accuracy of the slaughter method is the adequate homogenization and representative sampling for the individual tissues and fractions as well as an avoidance of losses due to dissection. A major limitation of the method is that it can inherently only be performed once per animal. To determine the protein retention at a particular growth stage, reference must be made to the mean results of other animals with lower BW. Thus, the results of protein retention are dependent on the selection of the reference animals (GfE, 2006; Ruiz-Ascacibar et al., 2017).

Non-invasive methods can be performed in living animals, allowing for multiple measurements. Current methods that have been proven suitable for estimation of body composition in growing pigs include imaging techniques such as X-ray computer tomography, dual-energy X-ray absorptiometry, and magnetic resonance tomography or dilution methods using stable isotopes as reviewed in Scholz (2002), Scholz and Mitchell (2001), and Simeonova et al. (2012). In addition to these non-invasive techniques, several approaches have been developed in recent years to model pig growth and protein retention based on BW development and feed intake (e.g., NRC, 2012; Schinckel and de Lange, 1996; van Milgen et al., 2008). However, the basis of any modeling is the exact determination of the body composition of the animals.

2.4.2 Nitrogen balance method

The N balance method as a basis for the determination of NUE provides information on the proportion of AA and CP ingested with the feed that is not excreted again with the feces and urine and is thus retained in the animal. The determination requires animal housing in metabolism crates so that both feed intake (and thus N intake) and total fecal and urinary N excretion can be accurately quantified. An adaptation period of three to seven days is followed by a collection period of four to six days to eliminate day-to-day variation in N excretion. The use of males facilitates the separate collection of feces and urine, as catheterization, a more invasive procedure, is not necessary (Adeola, 2001).

The main advantage of the N balance method is the possibility to perform repeated measurements on the same animal. Nevertheless, there are some restrictions in the application of the N balance technique. Due to the indirect measurement of the NR, the retention of AA can only be estimated assuming average values. In addition, measurements are made over a short period and are thus representative of only a small BW range. Furthermore, due to volatilization of fecal and urinary N, gaseous losses are considered as part of the NR, which thus may be overestimated (GfE, 2006). For example, Quiniou et al. (1995a) observed an overestimation of NR of 5.8 to 7.0% comparing the N balance technique with the comparative slaughter technique and Noblet et al. (1987) reported a 12% overestimation. Similar results were found by Susenbeth (1995). He compared the results of the effect of lysine intake on the amount of NR between N balance studies and serial slaughter trials. The evaluation was based on N balance data of 80 groups of growing pigs with 530 animals and slaughter data of 73 groups with 393 animals. The author observed that each additional gram of lysine intake resulted in a 9.0 g increase in protein retention in N balance studies and in an 8.1 g increase in protein retention in slaughter studies. Thus, the N balance method overestimated protein retention by 11% compared to the slaughter method. Moehn et al. (2000) observed that the difference in NR between the N balance and slaughter method additionally depends on the level of protein retention. The NR measured by slaughter technique at low protein retention rates was on average 84% of the protein retention measured by the N balance technique and 96% at high protein retention rates. However, the results of both techniques were closely correlated ($r = 0.91$).

2.4.3 Indicator method for digestibility determination

Instead of quantitative collection, the indicator method can also be used for the determination of fecal N excretion. In this method, an indigestible marker is added to the feed and the ratio between the concentration of the indicator in the feed and the feces can be used to determine the CP ATTD. Then, based on feed intake, CP concentration in the feed, and CP ATTD, total fecal N excretion can be calculated. Hence, animals do not necessarily need to be kept in metabolism crates for fecal collection. Substances, used as indicators, must meet certain requirements. They must be indigestible, must be excreted quantitatively in the feces, must pass the digestive tract uniformly, and must be distributed homogeneously in feed and feces. Chromium oxide or titanium dioxide (TiO_2) are usually used as indicators, and occasionally the natural markers acid insoluble ash or lignin (Adeola, 2001).

2.4.4 Deuterium oxide dilution technique

The use of the deuterium oxide (D_2O) dilution method to measure body water content and subsequent estimation of chemical body composition is an effective method in various species and animal categories (Andrew et al., 1995; Arnold et al., 1985; Brown and Taylor, 1986; Landgraf et al., 2006; Pedersen et al., 2019). The method is based on the knowledge of the relationship of the empty body water content to the fat-free body substance (FFS). The FFS shows a constant dependence on empty body mass in its composition (water, protein, and ash) but is independent of the body fat content of the animals (Susenbeth, 1984). Body water is determined by a single D_2O administration and blood sampling once an equilibrium between the marker and body water has been established. The advantages of the marker are its even distribution within the body water, its stability in the metabolism, its harmlessness for animal and user, low stress for the animal due to its application, and its cost-effective, repeatable use.

In a literature review, Susenbeth (1984) found the composition of the FFS to be independent of body fat content, growth rate and genotype of the pigs. However, females had slightly higher protein content in the FFS than males but in principle, the applicability of this method is given for animals of different sex, genotype, BW, growth rate, and fed different diets. In own investigations, Susenbeth (1984) used the D_2O dilution technique in a study of 42 castrated male German Landrace pigs over a BW range of 16 to 124 kg to compare the suitability of the dilution technique for the body composition measurement with the slaughter technique. The method proved to be very accurate, and the results showed an overestimation of body water at the time of blood sampling of only 2.5% of the animals' BW compared to the slaughter method. Empty body composition was calculated with a coefficient of variation of 1.1% for body water content, 1.7% for FFS and body fat content, and 1.0% for body protein content.

Nevertheless, other studies showed that the goodness of fit of body composition estimation equations based on the D_2O dilution technique and comparative slaughter depended on the BW and genotype of the animals. Hence, the estimation accuracy decreased from $R^2 = 0.99$ within the BW range of 18 – 109 kg to $R^2 = 0.60$ within the BW range of 109 – 145 kg (Shields et al., 1983) and was higher within a certain BW group compared to the complete fattening period (Landgraf et al., 2006). The latter authors slaughtered 48 crossbred pigs (17 females, 31 castrates) over a BW range of 20 – 140 kg, with eight animals slaughtered at each 20, 30, 60, 90, 120, and 140 kg category. They generated two sets of equations, one for the entire data set (n = 48) and one for each weight class (n = 8). For the entire data set, they observed a correlation coefficient between the results of both methods of r = 0.93 for empty body water content (standard deviation (SD) 2.9%) and r = 0.83 for the protein content in the FFS (SD 1.05%).

The correlation coefficients improved to r = 0.95 (SD = 2.62%) for empty body water content and r = 0.92 (SD = 0.77) for protein content in the FFS when the individual weight classes were considered separately.

Rozeboom et al. (1994) experimented on 58 mature, but not pregnant, gilts from two different crossbred genotypes, differing in age. The estimation accuracy was slightly different for the two crossbreds but was similar within each genotype at R^2 = 0.89 and R^2 = 0.98 for empty body water and empty body protein content, respectively. Due to noticeable differences in feed intake before slaughter, gilts of one genotype were divided into two feed intake groups (high and low) for evaluation, and estimation equations were derived for each group separately and cross-validated on the other group. The accuracy for estimating empty body protein content decreased to a medium level (R^2 = 0.44 and 0.67, respectively). The authors concluded that the accuracy of body composition estimation decreases when age, physiological status, genotype, and feed intake differ from those of the population for which the prediction equations were derived. Thus, the estimation of body composition based on the D_2O dilution technique is limited to the population in which estimation is conducted.

Another limitation of the D_2O dilution method is that it does not allow the determination of the proportions of the various tissues to the total protein content of the empty body, which changes with the age of the animals. It has been shown that the partition of the visceral organs to the total protein content of the empty body varies from 6.6 to 9.6%, that of bones varies from 8.5 to 17.4% and that of lean meat varies from 53.9 to 57.8% (Susenbeth and Keitel, 1988). However, only the protein retention in lean meat is of economic significance.

2.5 Body protein turnover measurement

Using the N balance technique for the determination of NR, however, N excretions are not only based on unutilized dietary N but endogenous N losses with the feces are also recorded, as well as N losses with the urine that originate from maintenance metabolism (Berschauer, 1977). Thus, no differentiation of the contribution of the processes of synthesis and degradation to protein retention or the quantification of their efficiency is possible via the N balance technique (Hewitt, 2020). Several methods can be applied to determine protein turnover based on the use of AA labeled with radioactive (Waterlow and Stephen, 1967) or stable (Picou and Taylor-Roberts, 1969) isotopes in various species. These tracers can be administered in two different ways, once continuously via intravenous or intragastric infusion or a single oral administration. There are also two ways to measure the N flux and thus protein turnover, a direct and an indirect way (Duggleby and Waterlow, 2005).

In the direct measurement, individual tissues are taken from the animal and the deposition of labeled AA in the tissues is measured. In the indirect measurement, the quantitative deposition of the marker in the end-product of protein metabolism is measured after a single oral dose of the labelled AA, typically ^{15}N glycine. The end-product method is also particularly well suited for repeated sampling of large numbers of animals. Thus, under standardized conditions, a reasonable estimate of body protein turnover can be made with relatively little effort and individual variation can be determined in field studies (Duggleby and Waterlow, 2005; Hinde et al., 2021). Unlike continuous infusion, it is non-invasive and does not necessarily require complete collection (Russell et al., 2003). In addition, an infusion can lead to rapid flooding of labeled AA into the AA pool, stimulating their oxidation. Subsequent rapid metabolism may underestimate protein turnover (Duggleby and Waterlow, 2005). However, a major limitation of the end-product method with a single oral administration is that it cannot determine the contribution of individual tissues (or other protein pools) to the body protein turnover (Waterlow, 2006a).

2.6 Blood urea concentration in relation to protein metabolism

As already described, AA that are not used for protein synthesis are degraded to urea. Urea is the main N-containing degradation product of the AA metabolism and the urea produced in the liver is subsequently transported with the blood to the kidneys and excreted via urine (Patience, 2012). The level of urea circulating in the blood is thus influenced by both its formation and renal diuresis. Furthermore, the blood urea concentration increases because of increased catabolic processes as in the case of feed deprivation or immune system stimulation (Wilson et al., 1972).

In ureotelic animals, urinary urea excretion is obligatory and reabsorption in the kidney occurs only to a small extent. Because of the linear dependence, urea N excretion with urine in various species can be estimated by determining blood urea nitrogen (BUN) concentrations (Kohn et al., 2005). Since urinary N and urea N excretion are closely correlated, the NR and subsequently the NUE can be accurately estimated from the estimated urea N excretion, provided that the CP ATTD is known. In a comparison between carcass data and BUN concentrations, Berschauer (1977) showed that the BUN concentration both under and overestimates protein utilization by a maximum of 6.5%.

A negative correlation between BUN concentration and NUE of growing pigs when comparing diets differing in CP content is well established and has been observed in several studies (e.g., Berschauer, 1977; Eggum, 1970; Tran-Thu, 1975; Whang et al., 2003).

Because AA ingested in excess of requirements must be deaminated, BUN concentration can also be used as a response criterion for determining AA requirement in empirical studies (e.g., Anderson et al., 1984; Coma et al., 1995; Millet et al., 2018a). However, the suitability of using BUN concentrations for predicting AA requirements depends on the relation of nonessential AA to lysine in the CP of the diet. Thus, Cameron et al. (2003) could not estimate the lysine requirement of growing pigs using BUN concentrations and observed only low correlations between BUN and protein retention.

The suitability of BUN concentration as a response criterion also results from the rapid response to diet transition in less than 24 h (Coma et al., 1995). A new equilibrium is thought to be established after two to three days (Fuller et al., 1979). However, Bergner et al. (1971) recommended that BUN concentration should not be measured until twelve days after feed transition because BUN levels were still affected by the previous diet for more than a week. An important prerequisite for using BUN concentrations to estimate NUE is the implementation of standardized conditions so that the BUN concentration depends only on the diet and the protein retention capacity of the animal. In addition, measurements should be performed over a longer period to generate reliable results (Berschauer, 1977).

The BUN concentration increases steadily during the first hours after feeding until it reaches a maximum after 4 – 6 h (Berschauer, 1977; Tran-Thu, 1975). Whereas the studies by Eggum (1970), Tran-Thu (1975), and Berschauer (1977) demonstrated a plateau after the increase in BUN concentration in growing pigs, Herrmann and Schneider (1983) did not observe a plateau in gestating sows. Even on a protein-free diet or in a state of fasting, consistently low, minimum BUN levels were observed, which were attributed to increased body protein catabolism (Bergner, 1970). Since the BUN concentration is influenced by the time of blood sampling after the last feeding, Pedersen and Boisen (2001) recommended sampling at two, four, and six hours after morning feeding to be able to reliably determine the plateau. However, since this involves additional stress for the animals, blood sampling is usually performed four to five hours after morning feeding (Herrmann and Schneider, 1983; Tran-Thu, 1975). Nevertheless, for animals with ad libitum access to feed, the time of blood sampling is of less relevance. Zervas and Zijlstra (2002b) did not observe any significant increase in BUN concentration 4 h after morning feeding, neither when diets containing low CP contents were fed (4.6 vs. 4.6 mmol/L) nor when high CP diets were fed (6.8 vs. 6.4 mmol/L). Furthermore, the accuracy of the estimation of urinary N excretion was similar for both time points (R^2 = 0.71 at 08:00 h and R^2 = 0.65 at 12:00 h).

In addition to the feeding time and the amount of AA ingested, the BUN concentration is also influenced by the AA pattern and digestibility of the CP (Berschauer, 1977; Tran-Thu, 1975; Cai et al., 1994). Thus, it was shown that supplementation of the first-limiting AA lysine improved protein utilization for retention and consequently decreased BUN concentration (Coma et al., 1995; Millet et al., 2018a). Zervas and Zijlstra (2002b) measured how BUN concentrations changed when different fiber sources were added to the diet compared to a control group. Both in the restricted fed animals and in the pigs with ad libitum access to feed, BUN concentrations of the test groups were 15 – 38% lower both at the time of morning feeding and four hours later compared to the control group. The lower BUN concentrations were attributed to the 5 – 7% decrease in CP ATTD and thus lower intestinal AA absorption. An increase in BUN concentration was also observed when phytases and xylanases were added to the diet, which could be explained by the improved CP digestibility because of increased phytate and non-starch polysaccharide degradation (Lan et al., 2017; Pomar et al., 2008).

It was also shown that phase feeding consisting of two, three, or four phases resulted in significantly lower BUN concentrations than in single-phase feeding (Lee et al., 2000). Reasons for the lower BUN concentration due to phase feeding are the lower dietary levels of CP and the better matching of the AA supply to the decreasing requirements of the animals. In addition, a comparison of conventional three-phase feeding with feeding daily tailored diets adapted to the requirements of the individual animals showed that the BUN concentrations of the individually fed animals were 22 – 33% lower during the entire fattening period (Andretta et al., 2016). Moreover, when the nutrient supply was limited to only 80% of the individual animal's requirement, BUN concentrations were again significantly ($p < 0.05$) reduced. Furthermore, low but significant correlations between BUN and N excretion ($r = 0.40$) and NUE ($r = - 0.13$) were observed. The authors concluded that BUN concentration is an important metabolic indicator for adjusting the ration to the animals' requirements.

In addition to the influence of CP and AA supply on BUN concentration, an influence of sex was reported by Suárez-Belloch et al. (2015). The increase in BUN concentration following different lysine intake levels occurred equally in castrates and gilts, but the BUN concentration of castrates was generally higher than that of gilts. This can be explained on the one hand by the significantly higher feed intake of castrates (2.50 vs. 2.32 kg/d; $p < 0.001$) and on the other hand by the feed intake behavior of castrates. Especially in restrictedly fed barrows, the increase in BUN concentration is more pronounced than in gilts as a consequence of the faster and higher feed intake (Whang and Easter, 2000). Whang and Easter (2000) observed differences in the correlation between BUN concentration, FE and lean gain in commercial gilts and barrows when blood was sampled under standardized conditions.

The correlations between BUN and lean gain were highest for gilts at 55 kg BW (r = 0.72) and for barrows at 77 kg BW (r = 0.52), both of which are within the range of the animals' maximum protein deposition capacity

Furthermore, an influence of genotype on the response of BUN to different diets was reported. In addition to the level of CP supply, the increase in BUN concentration during the fattening period was dependent on genotype, with the leaner breed showing lower BUN concentrations (Chen et al., 1995). The authors concluded that BUN concentrations could serve as an alternative to growth and carcass data for phenotyping the lean gain potential. Fabian et al. (2003) observed lower BUN concentrations at the beginning ($p < 0.05$) and at the end ($p = 0.10$) of the grower phase in a comparison of a Duroc line selected for FE to non-selected animals, resulting in lower average BUN concentrations during the fattening period (10.9 vs. 13.7 mg/dL; $p < 0.05$). The authors associated the lower BUN concentrations with more efficient utilization of AA for growth in the line selected for FE. Furthermore, when comparing typical North American with Chinese diets, Liu et al. (2015) observed a significant interaction ($p = 0.05$) between breed and diet. Hence, Landrace pigs had lower BUN concentrations when fed the North American diet (4.03 vs. 5.29 mmol/L) and pigs of a local breed had lower BUN concentrations when fed the Chinese diet (4.47 vs. 5.12 mmol/L). Nevertheless, the change in BUN concentration to different N intake levels is independent of the genotype of the animals (Windisch et al., 2000).

However, when comparing crossbred boars of the same genotype with different breeding values for protein retention, van der Peet-Schwering et al. (2021) could not detect a significant difference in BUN concentration at a BW of 59.5 kg (3.21 vs. 3.10 mmol/L; $p = 0.41$), although NUE was significantly higher in the animals with the high breeding value (55.8 vs. 52.7%; $p = 0.04$). The BUN concentrations were only dependent on daily CP and lysine supply and were significantly lower with restrictive feeding than with adequate supply (2.64 mmol/L vs. 3.67 mmol/L; $p < 0.01$).

2.7 Conclusions from literature and objectives of the own work

The NUE of growing pigs is limited by the digestibility of feedstuffs and the protein metabolism of the pigs. The inefficient AA utilization leads to N losses, which cause environmental issues. Since these losses occur even with phase feeding, improving the NUE of growing pigs through measures that go beyond known feeding strategies is necessary for sustainability reasons.

Differences in protein synthesis and retention have been reported between different genotypes (Rivera-Ferre et al., 2006) and are likely to have hormonal causes. The release of growth hormone and IGF-I promotes protein synthesis and a relationship between the plasma cortisol concentration and the protein degradation has also been reported (Weiler et al., 1998). Due to this constantly occurring protein turnover, growing pigs retain only about one-sixth (Hewitt et al., 2020) to one-third (Saggau et al., 2000) of the synthesized protein. This illustrates that protein degradation appears to limit the amount of protein retention and seems to be a major biological cause for the relatively low NUE in growing pigs

Urea is the main N-containing product of AA degradation. Consequently, higher AA utilization results in decreased urea synthesis and hence BUN concentration (Coma et al., 1995). A highly significant negative relationship between NR or NUE and BUN concentration was reported when pigs were fed diets differing in CP and AA content (e.g., Berschauer, 1977; Coma et al., 1995), but BUN concentrations also serve to reveal differences in NUE between different genotypes (Chen et al., 1995; Fabian et al., 2003). Hence, the BUN concentration could also serve to detect differences in the NUE between individuals of the same genotype.

Although several studies indicate that there is considerable variation across animals of the same genotype in the utilization of dietary CP (Noblet et al., 2013; Pérez de Nanclares et al., 2017; Ruiz-Ascacibar et al., 2017), only Kasper et al. (2020) has investigated the potential of genetic improvement of NUE. The findings of these authors indicated a genetic basis of NUE, which could explain up to 40% of the variation within this trait, but they concluded, that better methods are needed for phenotyping large numbers of animals as needed for systematic investigations. Protein turnover studies or N balance experiments are very reliable and accurate methods to determine NUE. However, such experiments are very laborious and therefore hardly suitable for phenotyping sufficient animals to estimate genetic parameters.

In this context, the first objective of the present thesis was to accurately quantify the NR and NUE of a subsample of pigs housed in metabolism crates at two different growth stages and to determine the impact of body protein turnover on these variables. In addition, it sought to determine whether improvements in NUE lead to changes in body composition. The second aim was to assess the suitability of serum hormone and BUN concentrations to establish equations for the estimation of the characteristic variables of protein metabolism as these could be easy to collect alternatives to accurately phenotype large numbers of animals with minimal effort. Subsequently, the third objective was to evaluate the individual variation of NR and NUE between animals of a F1 crossbred population fed diets scarce in lysine supply during the fattening period as these findings are intended to serve as a basis to further develop breeding and feeding strategies regarding improving NUE in successive steps.

3 MATERIALS AND METHODS

3.1 General description of the approach

The present thesis was part of the joint project "ProtiPig" of the Departments of Animal Genetics and Breeding, Livestock Functional Microbiology, Livestock Microbial Ecology and Animal Nutrition of the University of Hohenheim.

The project was designed as a pilot project and aimed for identification of influences on the NUE of growing pigs to develop breeding and feeding strategies for improved NUE in further steps. The essential prerequisite for the development of these measures was the determination of the NR of each individual pig, as it served as the reference for all other traits evaluated by participating departments. Since sufficiently large numbers of animals are required to estimate genetic parameters, a total of 508 growing pigs were used and raised under standardized conditions. As described in chapter 2.4, the standard methods for the determination of NR are the comparative slaughter technique and the N balance approach. Because both methods are very time-consuming and expensive, N balance was only carried out on a subsample of 56 of the growing pigs. This subsample served as the basis to estimate protein retention by means of BUN and serum hormone concentrations and hence NUE of all animals. The data collection took place between October 2018 and April 2021.

3.1.1 Animals and animal housing

The trial was approved by the Regierungspräsidium Tübingen (Project no. HOH52/18 TE) according to the German Animal Welfare Legislation and was conducted at the Agricultural Experimental Station of the University of Hohenheim, location *Unterer Lindenhof* in Eningen unter Achalm. The experimental station has a herd of approximately 150 Landrace sows, which is kept in a closed system, i.e., reproductive sows are replaced by own raised gilts. A two-week production rhythm is used resulting in groups of 10 – 14 sows farrowing every two weeks. The suckling period of the piglets is 28 days.

Multiphase feeding of the sows is practiced. Sows in early gestation (up to 85th day) receive 2.31 kg of gestation feed daily as default and 2.89 kg in late gestation (from the 85th day). The amount of feed is adjusted individually according to the body condition of the sows. Gestating sows are kept in the group and fed via transponder at an automatic feeder. Straw in the waiting area is available for ad libitum intake. 10 d before the calculated farrowing date, the sows are transferred to the farrowing pen and receive 3.0 kg of pre-lactation feed daily via a volumetric feeder and straw as an additional source of fiber. With farrowing, the transition to lactation feed takes place and the feed quantity is increased gradually within the first week until feed is available for ad libitum intake.

After weaning of the piglets and transfer to the service center, the sows are switched back to the gestation feed. Prior to insemination, the sows receive 3.5 kg gestation feed per day. The relatively high energy supply in comparison with the requirements triggers a flushing effect and thus increases ovulation. After successful insemination, the sows are transferred to the waiting area again. All sow diets consist of the same ingredients. These are barley, wheat, soybean meal, field peas, rapeseed cake, soybean oil, a fiber mix, and a mineral premix. The ingredients are mixed in different proportions depending on the stage of production and the energy and nutrient requirements of the sows.

For the experiment, the sows were mated to 20 different Pietrain boars. The criteria of boar selection were the location, age, and degree of relationship. Since the semen cannot be transported frozen, the boars had to be located at the reproduction station in Herbertingen. Then, the animals should be as young as possible, so that they could be used for a period of at least one year. The distributed use over this period was necessary to be able to separate possible seasonal effects from the boar effects in the evaluation. Furthermore, the boars should preferably be unrelated to increase genetic variation. Boars were intended to be used with equal frequency, so that data of almost the same number of offspring were available from each boar (see Table 26 in 4.2.4). The average number of offspring of each boar was 25 and varied from 16 to 33.

All male piglets were surgically castrated under analgesia (Metacam®) and anesthesia (Ketamidor®), supervised by a veterinarian, in their first week of life. During rearing, piglets were vaccinated against Mycoplasma *hyopneumoniae* (Stellamune One®), *Lawsonia intracellularis* (Enterisol Ileitis®), and porcine circovirus (Circo Flex®).

According to the production rhythm of the sow herd, the housing of the piglets into the experimental barn was carried out continuously every four weeks in cohorts of 25 animals each, when piglets reached the age of 77 d (± 6 d). In this process, four piglets per litter were selected out of six litters of a farrowing group. From each litter, the heaviest castrate and female, and one castrate and female corresponding to the mean litter weight, were chosen. The last piglet to complete the quantity of 25 was randomly selected out of these six litters. The variation in initial body weight (IBW) ranged from 19.0 kg to 41.0 kg (mean 29.5 kg). In total, the 508 animals were distributed among 21 cohorts. As can be seen in Figure 2, for each cohort, the trial lasted ten weeks and included starter and grower phase, but not finisher phase. Target live weight for slaughter was 90 kg. Animals were kept in the same pen from the beginning of the experiment until slaughter at a commercial slaughterhouse at the age of 149 d (± 6 d).

The experimental barn was equipped with 80 individual floor pens, arranged in three rows, so that three cohorts of different ages could be kept at the same time. Individual housing was necessary to record feed intake of each animal. The pens had a size of 2.7 m² of which 2.2 m² were concrete floor with minimal sawdust bedding and 0.5 m² were slatted area for excretion of feces and urine. The partition between the pens consisted of metal grids with 10 cm distance between the bars to enable animal interaction. Each pen was equipped with a low-pressure nipple drinker and a height-adjustable feeding through, suitable for both dry and liquid feeding. The ceramic trough had a capacity of 15 L, was rounded at the bottom, and could be completely emptied via a tilt mechanism. There were no feed storage containers at the trough. Fresh feed had to be presented daily. Pigs had free access to feed and water.

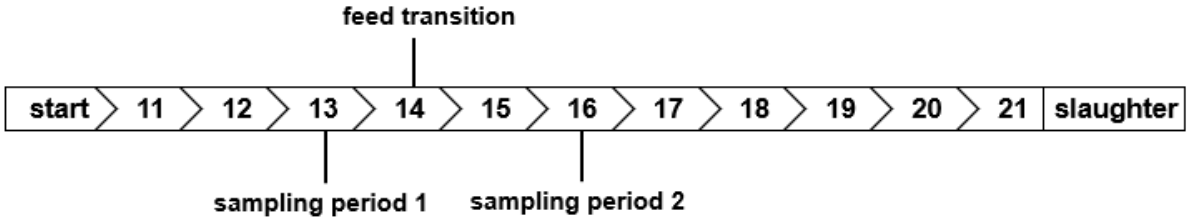

Figure 2. Timeline of the experimental fattening period for each of the 21 cohorts. The experimental period started in the 11th week of life and ended in the 21st week of life.

The stainless-steel metabolism crates (Inner dimensions: 1.55 m long, 0.80 m wide, and 1.00 m high, with tender nova flooring, Tenderfoot International GmbH, Ibbenbüren, Germany) used to carry out N balance (as described in chapter 3.1.3) were located in the same barn as the floor pens. Eight metabolism crates were available and eight castrates from seven cohorts were randomly selected for N balance measurement. The cohorts were selected that they were evenly distributed over the experimental period, so that progeny of as many different boars as possible were used and all seasons were covered. A detailed schedule of the entire experiment, including the dates of the N balance periods, is provided in Appendix 1. Pigs were only housed in the crates for adaption and sampling. Before, between and after the sampling period (SP) they were kept in their individual pens.

The barn was a commercial farm building and for this reason not completely environmentally controlled. In summer, temperatures could raise above 30°C due to a lack of air-conditioning, but a minimum temperature of 18°C was ensured by gas heaters with temperature sensor. The barn was force ventilated and additional fans ensured air circulation during high temperatures. A lighting program was not applied. Daylight was available through polycarbonate light bands on the long sides of the barn. Additional lighting during working hours from 07:00 h to 17:00 h was provided by fluorescent tubes.

3.1.2 Experimental diets and nutrient composition

Due to the background of the experiment and the need for large numbers of observations, all animals had to be fed the same diets. A two-phase fattening period was conducted, consisting of starter and grower phase, formulated to cover the requirements for 40 kg BW, 800 g average daily gain (ADG) and 1.70 kg average daily feed intake (ADFI) in the starter phase and 70 kg BW, 800 g ADG and 2.30 kg ADFI in grower phase. The diets were calculated to meet the recommendations of the GfE (2006) except for lysine, which was set to meet only 90% of the recommendations (Table 1). Thus, lysine was the intended limiting factor for protein retention, allowing the animals to reach their full genetic potential of protein utilization. The transition from starter to grower feed took place in the 14th week of life with an approximate BW of 47.0 kg. Daily feed allowance was a function of BW and provided 1.5 times the recommended amount of ME for 950 g ADG (GfE, 2006). It was adjusted weekly to the respective animal's BW and was weighed daily directly from the silo into a bucket with the corresponding pen number for each pig. The feed was administered in two equal portions at about 08:00 h and 16:00 h and the buckets were simply emptied over the old feed, so the trough was constantly filled. Old feed in the troughs was only removed if it was found to contain feces or urine. The feed was available for ad libitum intake in dry, coarse form. Feed refusals were recorded weekly to calculate ADFI. Animals were also weighed weekly to calculate ADG and G:F ratio.

To allow feeding to be as consistent as possible throughout the two-and-a-half-year experimental period, diets were formulated using as few as possible and easily accessible ingredients. Diets were composed of barley, wheat, and soybean meal as the only main ingredients in different proportions in the respective fattening phases, because these ingredients are available in large quantities with little variation in quality and nutrient density. Also, no free AA or enzymes were added to the diets. Adequate supplementation of minerals and vitamins was provided via a mineral premix, suitable for organic farming (Josera GmbH & Co. KG, Kleinheubach, Germany, Table 2).

During the SP, diets also contained 5 g/kg DM TiO_2 (VWR International GmbH, Darmstadt, Germany) as indigestible marker to determine the ATTD of dietary CP. The transition to the diet containing TiO_2 took place five days before the beginning of the SP. Calculated nutrient concentrations were confirmed by analyses (Table 1).

Table 1. Ingredient and nutrient composition of the experimental diets fed throughout the sampling periods

	Starter phase	Grower phase
Ingredient, g/kg (as fed basis)		
Barley	295	397.5
Wheat	430	430
Soybean meal	240	140
Soybean oil	2.5	5
Mineral premix	27.5	22.5
TiO_2	5	5
Calculated nutrient concentrations, g/kg DM		
ME, MJ/kg DM	14.9	14.8
Crude Protein	232	189
Lysine	10.8	8.0
Methionine + cysteine	7.2	6.2
Threonine	8.0	6.3
Valine	11.1	9.0
Leucine	17.1	13.7
Isoleucine	9.5	7.4
Histidine	6.1	5.0
Phenylalanine + tyrosine	18.8	15.1
Prececal digestible lysine g/MJ ME	0.60	0.45
Analyzed nutrient concentrations[1], g/kg DM		
Crude Protein	215	177
Lysine	10.6	7.9
Methionine + cysteine	7.0	6.1
Threonine	8.0	6.4
Valine	10.0	8.3
Leucine	16.1	13.0
Isoleucine	8.8	6.9
Histidine	5.8	4.7
Phenylalanine + tyrosine	17.7	14.4
TiO_2	5.4	5.1

Diets were formulated to meet 90% of daily prececal (pc) digestible lysine requirement for 800 g ADG and 1.7 kg ADFI in starter phase and 800 g ADG and 2.3 kg ADFI in grower phase. The recommended daily energy and lysine supply for the respective body weight and body weight gain is 23 MJ ME and 15.3 g pc digestible lysine in the starter phase and 30 MJ ME and 15.0 g pc digestible lysine in grower phase (GfE, 2006). Composition of the mineral premix is presented in Table 2.

[1]Results are presented as the means of all diets fed in the respective fattening phase during the sampling periods (n = 21 for starter phase and n = 19 for grower phase). Variation of nutrient concentrations within the diets of the respective fatting phase is described in Appendix 2.

Diets were mixed on demand in the certified feed mill of the Experimental Station and only as much feed was produced as was consumed in about two weeks. There were all four feeding variants stored in silos, because at least two cohorts of different age were kept in the barn and for every diet a variant with and without TiO_2 was necessary. A total of 173 diets were mixed throughout the complete experimental period, of which 57 were starter diets and 116 grower diets. 41 of these diets contained TiO_2 and were fed within the SP, 21 in starter phase and 20 in grower phase (see chapter 4.2). The variation in nutrient concentrations of the diets fed within the SP is given in Appendix 2.

Table 2. Composition of the mineral premix and provided nutrients per kg of complete diet on average

	per kg premix	per kg complete diet
Vitamin A, IU	215,000	5,375
Vitamin D3, IU	50,000	1,250
Vitamin E, IU (as alpha-tocopherol acetate)	4,000	100
Vitamin K3, mg	50	1.3
Vitamin B1, mg	100	2.5
Vitamin B6, mg (as pyridoxine hydrochloride)	750	18.8
Vitamin B12, µg	675	16.9
Niacin, mg	375	9.4
D-pantothenic acid, mg	90	2.3
Folic acid, mg	30	0.8
Choline chloride, mg	12,000	300
Biotin, µg	8,000	200
Zinc, mg (as zinc oxide)	1,875	46.9
Manganese, mg (as manganese oxide)	2,000	50.0
Iron, mg (as ferrous sulfate monohydrate)	1,878	47.0
Copper, mg (as copper sulfate pentahydrate)	300	7.5
Iodine, mg (as calcium iodate)	50	1.3
Selenium, mg (as sodium selenite)	10	0.3
Calcium, g	240	6.0
Phosphorus, g	50	1.3
Sodium, g	50	1.3
Magnesium, g	23	0.6

3.1.3 Sampling and measurements

Sampling took place in each of the two fattening periods. As seen in Figure 2, sampling within starter phase (SP1) was in the 13th week of life and sampling within grower phase (SP2) was in the 16th week of life. All procedures and sampling were the same in both SP and the samples were collected within five consecutive days.

Feed

To calculate the amount of feed consumed by the animals within the five days of the SP, prior to morning feeding of day one, feed residues in the trough were removed. Feed wastage in the pens was collected manually twice daily and feed refusal in the trough was weighed at the end of the SP. Bulk feed samples were obtained for analysis every week for each cohort from the daily feed allowances out of the buckets before feeding. In the SP, feed was analyzed for concentration of DM, N, AA, and TiO_2 and for concentration of DM and N in the weeks without sampling of blood and feces, to calculate the feed and N intake during the complete fattening period.

Feces

Three days prior to the SP, the sawdust was removed from the pens and no more bedding was used until the end of the SP to avoid mixing of the excreta with the sawdust. Feces collection for determination of N excretion was carried out twice daily, between 09:00 h – 10:30 h and 14:30 h – 16:00 h, resulting in a total of ten samples obtained per animal and SP. Before collection, the pens were thoroughly cleaned from old feces. Approximately a handful of feces was collected immediately after defecation, and care was taken to ensure that it was not previously combined with urine or feed. In case that animals did not defecate during this period, pigs were moved to another pen, which usually triggered defecation. Feces were collected in plastic bags, immediately frozen and stored at - 20°C until processing. Feces collection for determination of microbiota composition was carried out once per animal in each SP and is described in detail in Sarpong (yet unpublished).

Blood

On day two to four of the SP blood was collected for determination of BUN, serum cortisol (SC) and serum IGF-I concentration. Animals were restrained with a nose snare and the *vena jugularis* was punctured with a Strauss cannula (diameter 2 mm). According to Berschauer (1977), blood collection was performed five hours after morning feeding, between 13:00 h and 13:30 h, to reach the presumed plateau of BUN concentration. Because of diurnal variation of SC concentration, a constant time for blood sampling also was necessary (Hay et al., 2000).

Two times 4 mL blood per pig were collected in test tubes with silicate as clot activator (Kabe Labortechnik GmbH, Nümbrecht-Eisenroth, Germany) and subsequently centrifuged for 10 min at 1,000 x g to obtain serum. Serum was stored at - 20°C until further analysis. On one day, an additional blood sample was taken for genotyping the animals and verifying their parentage (Weishaar, 2022).

Nitrogen balance

In addition to the data collected for all pigs as described before, a combination of N balance and stable isotope tracer approach was used to measure NUE in an extended data-collection subsample of pigs, housed in metabolism crates during the SP.

After two days of adaption to the crates, total feces and urine were separately collected for consecutive four days. For urine collection, which was carried out in a similar manner as described in McGilvray et al. (2019), the metabolism crates were equipped with stainless steel trays, from which the urine flows into a collection bucket, containing 20 mL of 20% sulfuric acid to keep urine pH below 2 and prevent N losses via ammonia volatilization. To minimize contamination with spilled feed due to pig's permanent access to feed, the buckets were checked continuously, and urine was transferred into a sealed collection bottle quickly after urination. In this way, individual urine output was collected in 12-h intervals (Table 3). At the end of each interval, urine was filtered through a sieve to remove remaining feed particles, weighed and homogenized. The urine of the first 12-h interval was needed for determination of natural enrichment of ^{15}N (see below) and a 30 mL sample of the homogenized urine was directly pipetted in a plastic container and stored at - 20°C until analysis of total N, urea N, and ^{15}N concentration. From the urine of the subsequent 12-h intervals, 10% aliquots were taken, pooled for each pig, and stored in airtight bottles at 4°C. After the four-day collection period, the pooled samples were homogenized in a 5 L beaker using a magnetic stirrer and a 30 mL subsample was taken for N analysis.

Feces collection was carried out as described by Goerke et al. (2014). A Velcro ring was fixed with Leucoplast® around the anus of the animals. On this ring, a textile collection bag containing a freezer bag, in which the animals defecate directly, was attached. The collection bag was checked at least three times daily and the freezer bag was changed if necessary. Feces were then weighed and immediately frozen at - 20°C and stored until processing.

The feeding protocol was the same as for the other animals of the cohort. Spilled feed was taken from the urine collection tray, pooled for each pig, and dried at 65°C until constant weight. These losses were considered in the calculation of daily feed intake (Zhu et al., 2005).

^{15}N administration

During the four-day collection period, body protein turnover was determined by the end-product method via oral administration of a single dose of ^{15}N glycine (Rivera-Ferre et al., 2006; McGilvray et al., 2019). Urine of the first 12-h interval was used to determine the natural enrichment of urinary ^{15}N. At the beginning of the second 12-h interval (Table 3), a gelatin capsule containing 6 mg ^{15}N glycine/kg BW (corresponds to a dose of 1.18 mg ^{15}N/kg BW; isotopic purity of ≥ 99%; Eurisotop, Saint Aubin Cedex, France) was manually placed behind each pig's tongue base, to ensure the complete intake of the tracer. For the first two balance periods that were conducted, ^{15}N enrichment in every 12-h interval aliquot was determined and a cumulative excretion pattern for 84 h was created by non-linear regression (see chapter 3.3.1). More than 95% of total urinary ^{15}N excretion occurred within 72 h, which was in accordance with literature data (Hewitt et al., 2020; Rivera-Ferre et al., 2006). Based hereupon, it was decided to take the same bulk sample of the last seven consecutive 12-h urine aliquots for determination of ^{15}N enrichment, as described above for total N and urea N analysis. This corresponds to a total of 84 h following ^{15}N administration. Due to technical restrictions, a separate recording of urea ^{15}N enrichment and ammonia ^{15}N enrichment was not possible and only ^{15}N enrichment of total urinary N was determined.

Table 3. Time schedule of nitrogen balance, marker administration and blood sampling in the extended data-collection subsample during the sampling period

Time	Day 1	Day 2	Day 3	Day 4	Day 5
05:00					Water withdrawal
-08:00	Urine sampling 1	Urine sampling 3	Urine sampling 5	Urine sampling 7	
08:00	Administration ^{15}N				Administration D_2O
13:00		Blood sampling BUN, SC, IGF-I			Blood sampling D_2O
16:00				Feed withdrawal	
-20:00	Urine sampling 2	Urine sampling 4	Urine sampling 6	Urine sampling 8	
20:00	Start N balance			End N balance	

Urine samples were collected in 12-h intervals and collection for each interval was completed until 08:00 h and 20:00 h, respectively. Feces sampling was carried out time independent, but collection bags were checked at least three times daily and changed if necessary. BUN = blood urea nitrogen; SC = serum cortisol; IGF-I = insulin-like growth factor; D_2O = deuterium oxide.

Cortisol and creatinine

For examination of possible hormonal catabolic effects, urinary cortisol and creatinine excretion was analyzed. Because of the diurnal variation of metabolic cortisol release, 24-h urine samples instead of spot samples are necessary to determine the level of excretion (Hay et al., 2000). For this purpose, subsamples of the urine aliquots from the two consecutive 12-h intervals of each collection day were pooled and stored at - 20°C until further analysis.

D_2O administration

After completing quantitative collection, pigs stayed in the metabolism crates for estimation of body water content using the D_2O dilution technique (Susenbeth, 1984). Therefore, on day four, together with blood sampling for BUN, SC, and IGF-I a sample was taken to identify the basal blood level of D_2O. Then, the animals were fasted from 16:00 h on day four for 16 h and drinking water was withdrawn for 3 h starting at 05:00 h on day five (Table 3). The marker was weighed into a syringe (0.7 g D_2O solution/kg BW, isotopic purity of 51%, Chemotrade, Leipzig, Germany) and a metal straw was fixed on its tip. For marker administration at 08:00 h of day five, pigs were restrained with a nose snare and the D_2O was entered with the metal straw as deeply as possible into the pharynx to ensure the pigs would swallow the marker completely. After the application, pigs had no access to feed and water for another 5 h which is the period needed for the D_2O to be distributed within the body water (Susenbeth, 1984). Then a blood sample was collected at 13:00 h as described above in test tubes containing K-EDTA as anticoagulant and stored at - 20°C until D_2O concentration was analyzed.

Slaughter

At the end of the experimental period, pigs were slaughtered in a commercial slaughterhouse, where carcass weight, dressing percentage, backfat thickness and loin muscle thickness were recorded, as described in Weishaar (2022). The animals of the extended data-collection subsample were not slaughtered at the commercial slaughterhouse but euthanized at the experimental station and used for investigations on microbiota composition and enzyme activity in the gastrointestinal tract, as explained in detail in Kurz (yet unpublished) and Sarpong (yet unpublished).

3.2 Chemical analyses

Feed and feces

After completion of the respective SP, the feces samples were thawed at + 4°C, pooled for each pig, and homogenized. Due to quantitative sampling, feces obtained in the metabolism crates were pooled totally and thoroughly mixed with a drill stirring rod. For all other animals, a subsample of 50 g of the feces sample was taken after manual blending and the resulting 500 g feces were mixed using a standard hand blender. Then, a fresh subsample of the homogenized feces was taken for N analysis and another subsample was dried (65°C for a minimum of 48 h) and pulverized using a vibrating cup mill (Pulverisette 9, Fritsch GmbH, Idar-Oberstein, Germany) for TiO_2 and DM analysis.

All feed samples were ground through a 0.5 mm sieve (Ultra Centrifugal Mill ZM 200, Retsch GmbH, Haan, Germany) for determination of total N and DM concentration. The feed samples of the SP were subsequently pulverized for AA and TiO_2 analysis as described before. Pulverized samples were stored in sealed containers in desiccators until analyses.

Feed and feces DM (method no. 3.1) and total N (method no. 4.1.1) were analyzed according to official procedures of VDLUFA (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, 2007). Then, CP was calculated as total N multiplied by 6.25. Concentration of TiO_2 was measured using an inductively coupled plasma optical emission spectrometer (VISTA PRO, Varian Inc., Palo Alto, USA) after acid digestion, according to a modified method of Boguhn et al. (2009), as described in Zeller et al. (2015). Concentrations of AA in the feed were measured photometrically using an L-8900 AA analyzing system (VWR/Hitachi Ltd, Tokyo, Japan) after sample oxidation and acid hydrolysis, according to a modified method of Rodehutscord et al. (2004), described in detail in Siegert et al. (2017). Concentration of tryptophan was not measured.

Urine

Before analyses, frozen urine samples were gently thawed in a water bath. Total urinary N was analyzed by Kjeldahl digestion (method no. 4.1.1, VDLUFA, 2007) and urinary urea N was determined by a coupled optical-enzyme test (R-BIOPHARM AG, Darmstadt, Germany). In the first step of the reaction, the urea of the sample is hydrolyzed by urease. In the second step, the resulting ammonia converts 2-oxogluterate into L-glutamate under consumption of nicotinamide-adenine-dinucleotide (NADH) and glutamate-dehydrogenase. NADH is the measured variable and equivalent to the amount of consumed ammonia and thus equivalent to half of the amount of urea in the sample.

The extinction of NADH can be measured at 340 nm (Evolution 201 UV-visible spectrophotometer, Thermo Fisher Scientific, Waltham, USA) and using the Lambert-Beer-Law, its concentration can be calculated.

For analysis, a subsample of 1 mL was taken under stirring, and centrifuged for 10 min at 13,000 x g. Subsequently, 400 µL of the obtained supernatant were neutralized with 0.1 M sodium hydroxide and diluted to a ratio of 1:250 with deionized water. Since the concentration of ammonia is the dependent variable of the test, for every sample, two repetitions had to be set up. One for determining the concentration of urea in the sample and the other one for determining its initial ammonia concentration, to correct the result for urinary ammonia excretion. Each repetition consisted in two measurements, one for the sample and one for a blank value. The measurements proceeded according to the following scheme:

Table 4. Pipetting scheme for determination of urinary urea concentration

	Urea		Ammonia	
Volume in µL	Sample	Blank value	Sample	Blank value
Reaction solution 2 (Containing NADH and 2-oxogluterate)	400	400	400	400
Sample	40	-	40	-
Solution 3 (Containing urease)	8	8	-	-
Deionized water	760	800	768	808
Merging and incubation for five min; measurement of extinction 1 (E1)				
Solution 4 (Containing glutamate-dehydrogenase)	8	8	8	8
Merging and incubation for 20 min; measurement of extinction 2 (E2)				

The urea concentration of the sample could be calculated as:

$$\text{urinary urea (g/L)} = \left((\Delta E_1 - \Delta E_2) - (\Delta E_3 - \Delta E_4)\right) \text{x } 0.1449 \text{ x } 250$$

where ΔE_1 is the difference between E2 and E1 of the urea sample, ΔE_2 is the difference between E2 and E1 of the urea blank value, ΔE_3 is the difference between E2 and E1 of the ammonia sample, ΔE_4 is the difference between E2 and E1 of the ammonia blank value, 0.1449 is the coefficient including test volume, urea molecular weight, layer thickness of the cuvette, and extinction coefficient, and 250 accounts for dilution of the sample.

The urea concentration was converted to urea N concentration by the factor of 0.467 and daily urinary urea N excretion was calculated by multiplication with daily urine excretion.

All samples were analyzed in duplicate with a time lag between the repetitions of the same sample and a five percent deviation between the duplicates was tolerated. In each series, a control with a defined concentration of 50 mg urea/dL was run. Intraassay and interassay coefficients of variation were 2.9% and 3.0% (n = 24), respectively.

The enrichment of ^{15}N in urine samples was determined by using a combination of an elemental analyzer (EA 1108; Carlo Erba Instruments, Biberach, Germany) and an isotope mass spectrometer (MS Finnigan MAT; Thermoquest Italia S.p.A., Milan, Italy). Prior to analysis, a subsample of 10 mL was neutralized with 1 M sodium hydroxide. Then, 0.8 g silica as a carrier material was added and the sample was freeze-dried for 48 h. Subsequently, 6 mg of the freeze-dried sample was weighed into a tin capsule with a precision scale (AD-4 Autobalance; PerkinElmer Inc., Waltham, USA). Determination of urinary ^{15}N enrichment was performed in six replicates per sample.

Cortisol in urine and serum and urinary creatinine concentrations were analyzed in the Department of Behavioral Physiology of Livestock at the University of Hohenheim. Cortisol was determined in a radioimmunological assay according to the method of Claus and Weiler (1996) and creatinine was analyzed by photometry using a commercial enzyme reaction kit (KIT-No. 553-172G; mti-diagnostics GmbH, Idstein, Germany), both described in detail in Wesoly et al. (2015).

Blood

For determination of SC concentration, a modified version of the radioimmunological assay was used, as described in Engert et al. (2017). Serum of the three consecutive collection days of each SP was pooled per animal, ethanol was added for extraction of cortisol, and was centrifuged. Subsequently, a polyclonal antibody against cortisol was added to the supernatant, as well as radioactive labeled ^{3}H-cortisol as a tracer. The cortisol concentration of the sample was measured using scintillation liquid in a beta counter after incubation and centrifugation of the reaction solution.

Determination of serum IGF-I concentration was carried out at the Institute of Experimental Endocrinology at Charité Berlin using an IDS-iSYS IGF-I assay (Immunodiagnostic Systems Holdings Ltd, East Boldon, United Kingdom). This method is based on chemiluminescence technology, where the resulting light emitted by an acridinium labelled anti-IGF-I monoclonal antibody is directly proportional to the concentration of IGF-I in the sample. Although this assay is originally developed for measurement of human IGF-I, it also allows quantification of porcine IGF-I concentrations, due to their structural similarity (Hinrichs et al., 2020; Hofmann et al., 2020).

BUN concentrations were measured by photometry at IDEXX BioResearch Vet Med Labor GmbH (Ludwigsburg, Germany) in a coupled enzyme reaction assay, using a Beckman AU 480 autoanalyzer system (Beckman Coulter, Brea, USA). After overnight thawing in the refrigerator and overhead shaking at 20 rotations per minute, 10 µL of the sample was diluted with a reagent solution in a ratio of 1:76. The urea of the sample is hydrolyzed to ammonia in the presence of urease and the resulting ammonia reacts with 2-oxoglutarate to form 2 L-glutamate under the consumption von NADH. NADH is the measured variable, and its absorption is detected at two different time points and at two different wavelengths each, primarily at 340 nm and secondary at 660 nm. The decrease in NADH absorption per time unit is proportional to the urea concentration of the sample, which is automatically calculated by the analyzer using a specific system calibrator. The conversion from urea to BUN was then performed by multiplying with the factor 0.467. All samples were measured in duplicate.

Analysis of D_2O concentration of the blood samples was carried out similar as described by Claus et al. (2007). 3 mL of each sample were frozen in an - 30°C ethanol bath, subsequently vacuum distilled and the sublimated blood water was collected in cryo traps, cooled by liquid N. In the next step, extinction of the H_2O/D_2O mixture was determined by infrared spectrometry at 3960 nm (Prozess-Photometer Spectran 677IR, Bodenseewerk Geosystem GmbH, Überlingen, Germany). A standard curve was created with five different dilutions of a known D_2O solution and deionized water. Using the regression line resulting from the comparison of the concentrations of the dilutions with the corresponding extinctions, the D_2O concentration of the H_2O/ D_2O mixtures, was calculated. After every series of samples, a new standard curve was obtained. Samples were analyzed in duplicate and before measurement of a new sample, the measuring cell of the spectrometer was thoroughly rinsed with deionized water twice.

3.3 Data processing and calculations

3.3.1 Nitrogen balance data set

Nitrogen utilization efficiency

NR (g/d) was calculated as:

$$\mathrm{NR\ (g/d)} = \mathrm{NI} - \mathrm{UNE} - \mathrm{FNE}$$

Where NI is the daily N intake via feed (g), UNE is the daily urinary N excretion (g) and FNE is the daily fecal N excretion (g). Daily NI was calculated as the product of feed intake (g DM/d) and the analyzed N concentration of the feed (g/kg DM).

NUE was computed as:

$$\text{NUE}\ (\%) = \frac{\text{NR}}{\text{NI}}\ \text{x}\ 100$$

Assuming an average concentration of 7.2 g lysine per 100 g protein being retained during growth (GfE, 2006), lysine retention (LR) was calculated as:

$$\text{LR}\ (\text{g/d}) = \text{NR x}\ 6.25\ \text{x}\ \frac{7.2}{100}$$

Then, the LUE was computed as:

$$\text{LUE}\ (\%) = \frac{\text{LR}}{\text{LI}}\ \text{x}\ 100$$

Where LI is the lysine intake (g/d), calculated as ADFI (g DM) multiplied by the analyzed lysine concentration of the feed (g/kg DM).

^{15}N excretion

As described in chapter 3.1.3, the cumulative urinary ^{15}N excretion was measured for the first two balance periods. Based on the measured values, the growth constant of the excretion curve and the theoretical maximum of the ^{15}N excretion were determined via non-linear regression using the NLIN procedure in SAS (version 9.4 of the SAS system for Windows; SAS Institute Inc., Cary, NC). Thus, the ^{15}N excretion at the time t can be determined by the bounded growth function:

$$y_t = a\ \text{x}\ (1 - e^{-\ b\ \text{x}\ t})$$

Where y_t is the ^{15}N excretion (mg) at the time t (h), a is the theoretical maximum of the ^{15}N excretion (mg), e is the base of the natural logarithm (Euler's number), and b is the growth constant.

By rearranging the equation, the time at which 95% of the total ^{15}N excretion has occurred can be determined:

$$t_{95\%\ \text{max}} = \frac{\ln 0.05}{-\ b}$$

Protein turnover

For calculation of body protein turnover, the end-product method, based on the two-pool model, was chosen (Figure 3; Duggleby and Waterlow, 2005). This method assumes that the ^{15}N of a single oral dose of a labeled AA is used in equal proportions for de-novo protein synthesis and degradation via AA oxidation, as the N of all AA that enter the free AA pool.

Another assumption is, that the single dose is the only source of ^{15}N in the pool and no recycling from the protein pool occurs during the measurement period.

At these conditions, the proportions of isotopically labeled N that either go into synthesis or excretion via urine represent the total N flux into synthesis or excretion. Hence, total N flux was obtained by the following equation:

$$Q = d/\varepsilon_{(t)};\ \text{with}\ \varepsilon_{(t)} = e_x/E_x$$

where Q is the N flux (representing the body protein turnover in g/d), d is the administered dose of ^{15}N (mg), ε is the ^{15}N enrichment in the end-product (total urinary N) within the collection period t, e_x is the amount of tracer excreted in the end-product (g ^{15}N/84 h) and E_x is the amount of end-product excreted (g total N/84h) with urine.

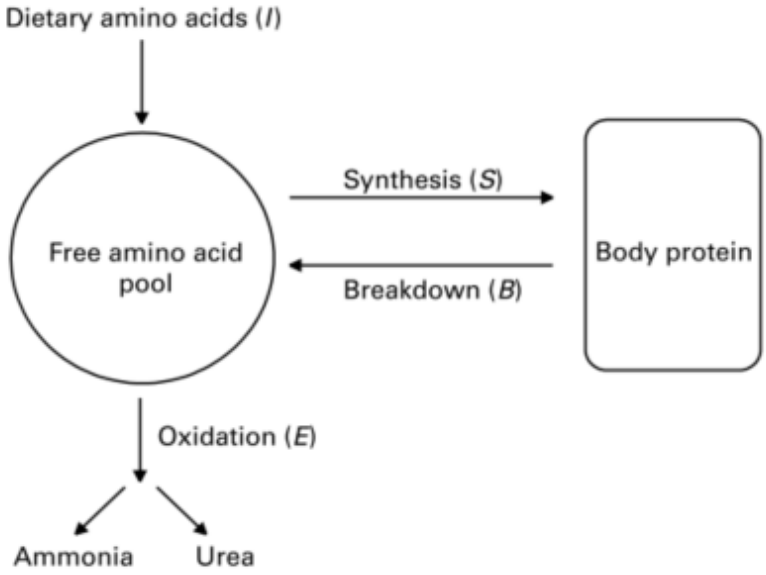

Figure 3. Two-pool model of body protein turnover (from Duggleby and Waterlow, 2005).

Under the assumption of a steady state, after calculation of body protein turnover, rates of protein synthesis and degradation could be obtained by following relation:

$$Q = S + E_T = D + I$$

where Q is the body protein turnover (g N/d), S is the rate of body protein synthesis (g N/d), E_T represents the total urinary N excretion (g/d), D is the rate of body protein degradation (g N/d) and I represents the amount of dietary N, entering the free AA pool (g/d; Duggleby and Waterlow, 2005). Values obtained for N were converted to protein by using the factor of 6.25.

The results of body protein turnover and N balance were expressed in fractional terms relative to the amount of body protein using the following equations (Rivera-Ferre et al., 2006):

$$\text{fractional turnover rate (FTR, \%)} = \frac{\text{body protein turnover (g/d)}}{\text{body protein amount (g)}} \text{ x } 100$$

$$\text{fractional synthesis rate (FSR, \%)} = \frac{\text{body protein synthesis (g/d)}}{\text{body protein amount (g)}} \text{ x } 100$$

$$\text{fractional degradation rate (FDR, \%)} = \frac{\text{body protein degradation (g/d)}}{\text{body protein amount (g)}} \text{ x } 100$$

$$\text{fractional retention rate (FRR, \%)} = \frac{\text{body protein retention (g/d)}}{\text{body protein amount (g)}} \text{ x } 100$$

The amount of protein in the body was determined as explained in the following section.

Body composition

Estimation of body composition was carried out by D_2O dilution technique. This method assumes, that D_2O is evenly distributed within body water and is used for physiological processes in the same way as normal body water. In a state of equilibrium total body water content (TBW) can be calculated as follows (Landgraf et al., 2006):

$$\text{TBW (\%)} \frac{\text{D}_2\text{O administered (g)}}{\text{D}_2\text{O concentration blood (ppm)}} \text{ x } \frac{100000}{\text{BW (kg)}}$$

Based on the result of D_2O dilution the different chemical body fractions were calculated by allometric association using the prediction equations developed by Landgraf et al. (2006). First, empty body water content (EBWA) was calculated as a function of TBW, measured by blood D_2O concentration, and the respective animal's BW at the time of blood sampling:

$$\text{EBWA (\%)} = 17.0957 \text{ x } \text{TBW}^{0.4131} \text{ x } \text{BW}^{-0.1141}$$

Then, fat-free substance (FFS) of the empty body is obtained by the following equation:

$$\text{FFS (\%)} = 3.3270 \text{ x } \text{EBWA}^{0.7730}$$

and empty body fat content (EBF) as the difference to 100%:

$$\text{EBF (\%)} = 100 - \text{FFS}$$

Due to its strong correlation to each other, CP, and crude ash (CA) in the FFS were calculated based on FFS content:

$$\mathrm{CP_{FFS}}(\%) = 972.81 \text{ x } \mathrm{FFS}^{-0.8804}$$

$$\mathrm{CA_{FFS}}(\%) = 255.77 \text{ x } \mathrm{FFS}^{-0.9619}$$

Amounts of empty body protein (EBP) and empty body ash (EBA) were obtained by conversion of the percentages by following equations:

$$\mathrm{EBP\ (kg)} = (\mathrm{EBW} - \mathrm{EBF}) \text{ x } \frac{\mathrm{CP_{FFS}}}{100}$$

$$\mathrm{EBA\ (kg)} = (\mathrm{EBW} - \mathrm{EBF}) \text{ x } \frac{\mathrm{CA_{FFS}}}{100}$$

Where EBW is calculated as BW multiplied by 0.93 and 0.96 in SP1 and SP2 respectively (Landgraf et al., 2006), to account for water in the digestive tract and urine in the bladder.

3.3.2 Complete data set

The CP ATTD was calculated using TiO_2 concentrations in feed and feces by the indicator method, using the following equation:

$$\mathrm{CP\ ATTD\ (\%)} = 100 - 100 \text{ x } \left(\frac{\mathrm{Ti\ feed}\ \left(\frac{\mathrm{g}}{\mathrm{kg}}\mathrm{DM}\right)}{\mathrm{Ti\ feces}\ \left(\frac{\mathrm{g}}{\mathrm{kg}}\mathrm{DM}\right)} \text{x} \frac{\mathrm{N\ feces}\left(\frac{\mathrm{g}}{\mathrm{kg}}\mathrm{DM}\right)}{\mathrm{N\ feed}\ \left(\frac{\mathrm{g}}{\mathrm{kg}}\mathrm{DM}\right)} \right)$$

Overall nitrogen utilization efficiency

In addition to NUE calculated from the balance data, the overall efficiency of N utilization (ONUE) was calculated using slaughter data. Based on carcass data, protein retention of the pigs in the entire experimental period was estimated. First, the EBP content of the animals at slaughter was calculated, using an equation derived from GfE (2006):

$$\mathrm{EBP\ (kg)} = \frac{\mathrm{FFL}}{100} \text{ x CW x } \frac{0.22}{\mathrm{gender\ factor}}$$

where FFL is the fat-free lean content (%), CW is the carcass weight (kg), and the gender factor is 0.563 for gilts and 0.557 for castrates. Within a BW range of 90 – 120 kg an average of 56% of total body protein is located in the skeletal muscles, not depending on the fat content. However, protein content in skeletal muscles of gilts is 0.3 percentage points higher than this average and 0.3 percentage points lower in castrates (Susenbeth and Keitel, 1988), thus, different gender factors were used.

The amount of EBP at the beginning of the experiment was estimated as followed (GfE, 2006):

$$\text{EBP (kg)} = \text{EBW} - 0.0000914 \text{ x } \text{EBW}^2$$

where EBW is the empty body weight (kg) which results from the multiplication of BW with 0.93, because at 30 kg BW the weight of the gastrointestinal tract accounts for approximately 7% of total BW (Landgraf et al., 2006).

By subtraction of EBP content at the beginning of the experiment from EBP content at slaughter, total protein retention was calculated and converted into NR by dividing protein deposition through the factor 6.25. In addition, dietary NI was calculated on weekly basis by multiplying feed intake (kg DM) with the analyzed N concentration of the feed. Total NI in the entire experimental period was calculated and ONUE could be calculated as the quotient of NR and NI.

Feed efficiency

For each of the SP and for the entire experimental period, feed efficiency was expressed as G:F ratio by dividing ADG by ADFI. As an alternative measurement to G:F ratio, RFI for the complete experimental period was computed. This difference between observed ADFI and necessary ADFI predicted from maintenance and growth requirements was estimated by multiple linear regression of ADFI on performance data. ADG was used to account for growth, carcass data, such as carcass weight, dressing percentage, lean meat content, backfat thickness, and loin muscle thickness, to address for composition of growth and average metabolic BW (AMW) to account for maintenance requirements. AMW was calculated according to a slightly modified formula presented in Saintilan et al. (2013):

$$\text{AMW (kg)} = \frac{(\text{BW}_{\text{slaughter}}^{1.75} - \text{IBW}^{1.75})}{(1.75 \text{ x } (\text{BW}_{\text{slaughter}} - \text{IBW}))}$$

where $BW_{slaughter}$ is the live body weight measured at the day of slaughter and IBW is the live body weight at the beginning of the experiment.

Multiple linear regression of ADFI on the performance data was carried out using a macro for the MIXED procedure in SAS. The macro simultaneously compares all possible combinations of the variables and ranks the models based on the Akaike information criterion (AIC) and the model with the lowest AIC provides the best fit for the present data (Bozdogan, 1987). The model with the best fit to explain the variation in ADFI (AIC = - 797, adj. R^2 = 0.70, root mean square error (RMSE) = 0.11) included AMW and ADG as variables. Based on this model, RFI was calculated as:

$$\text{RFI (kg)} = \text{ADFI} - (0.051 \text{ x } \text{AMW} + 1.21 \text{ x } \text{ADG})$$

3.4 Estimation model selection and statistical evaluation

Results of the N balance data set were analyzed by one-way ANOVA using the MIXED procedure of SAS, with the individual pig as the experimental unit, using the following model:

$$y_{ij} = \mu + \alpha_i + \beta_j + e_{ij}$$

where y_{ij} is the dependent trait, μ is the overall mean, α_i is the fixed effect of the SP, β_j is the random effect of the animal, and e_{ij} is the residual error.

Differences between the SP were determined by pairwise *t*-testing and statistical significance was declared at $p \leq 0.05$. Prior to ANOVA, normal distribution, and homogeneity of variance of the data was tested by using the UNIVARIATE procedure in SAS. In case the results of the variables within a SP were not normally distributed, the data were either square root or log transformed, or logit transformed for percentages, respectively. Results of the simple statistics are presented as the untransformed data. Since not all values were normally distributed, for correlation analysis, Spearman's rank correlation coefficients (SCC) between the variables and SP were calculated using the CORR procedure in SAS.

Multiple regressions were performed with the results of the N balance data set to estimate NR, NUE and urinary N/urea excretion for all animals of the experiment, using a macro for the MIXED procedure in SAS, as described before. Models were only considered further if all variables were significant at $p \leq 0.05$ level. Considered variables for model estimation were the blood levels of BUN, SC, and IGF-I and the data on IBW, BW, ADG, ADFI, NI, and CP ATTD. Different categories of models were established, in which various possible combinations of variables were considered. Models with only linear effects of the variables, models with linear and quadratic effects, models with linear effects and interactions between the variables and models with linear and quadratic effects and interactions. The following model base was used and was adjusted to the corresponding model category:

$$Y_i = \alpha_i + a_i + b_1 \, x \, V_1 + b_2 \, x \, V_2 + b_3 \, x \, (V_1 \, x \, V_2) + b_4 \, x \, V_1^2 + b_5 \, x \, V_2^2 + \cdots + b_{n-4} \, x \, V_{i-1} + b_{n-3} \, x \, V_i + b_{n-2} \, x \, (V_{i-1} \, x \, V_i) + b_{n-1} \, x \, V_{i-1}^2 + b_n \, x \, V_i^2 + e_i$$

Where Y_i is the value of the response trait, a_i is the intercept, α_i is the fixed effect of the SP $b_{1\text{-}n}$ are the regression coefficients of the respective variables, $V_{1\text{-}i}$ are the estimation variables, and e_i is the residual error.

This process was performed for the entire N balance data set and separately for each SP, to study whether more precise equations can result for the single SP. Observations of the same animal were considered as repeated measures. In case the intercept did not differ significantly from zero, it was not included in the model.

The next step was to validate if the variables included, and their coefficients, significantly differed from zero or whether their effect had occurred by coincidence due to sample size. For this purpose, a bootstrapping validation was conducted (Efron and Tibshirani, 1998). Bootstrapping is a statistical technique of case resampling where a series of random same-sized data sets is computed out of the original data set. With this resampling method, it is possible that the same observations are included several times in a single data set and others do not appear in the respective data set. Due to multiple repetition of the resampling an approximate normal distribution of the data is simulated. For this, a series of 1,000 data sets was created using the SURVEYSELCT procedure in SAS. For these simulated data, the obtained regressions were validated for overparameterization of the model, and the 95%-confidence interval of each variable coefficient was computed. If zero was included in this confidence interval, the coefficient did not differ significantly from zero and the variable had to be removed from the equation. Finally, a regression of the estimated values with the observed values was carried out, using the GLM procedure of SAS. For evaluating the goodness of fit of the derived estimation equations, the adjusted R^2 and the RMSE were used.

In the same way, equations for estimating body protein turnover, -synthesis and -degradation on basis of the N balance data set and hormone concentrations of both SP were created. Due to the number of possible variables, only variables that showed significant correlations with the target values were chosen for modeling.

Subsequently, NR, NUE and urinary total N and urea N excretion were estimated for all animals in both SP by the obtained equations. The results of the complete data set were evaluated by two-way ANOVA, using the following model:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + e_{ijk}$$

where y_{ijk} is the dependent trait, μ is the overall mean, α_i is the fixed effect of the period, β_j is the fixed effect of sex, $\alpha\beta_{ij}$ is the interaction between period and sex, γ_k is the random effect of the individual animal and e_{ijk} is the residual error.

For evaluating the boar effect, a simple one-way ANOVA was conducted in a similar way as for the N balance data, but instead of the fixed effect of period, the fixed effect of the boar was used in the model.

For ANOVA, Tukey adjustment was carried out because the number of observations differed between periods as well as between the offspring of different boars.

4 RESULTS

4.1 Nitrogen balance data set

The first cohort of pigs used for the determination of N balance had stress-related diarrhea during SP1, as they had no time to adapt to the metabolism crates, before starting of fecal sampling. Fecal sampling was partly incomplete, and some urine samples were mixed with feces. Thus, the results of this SP were not considered further. For the following cohorts, sampling procedure was adjusted as described in chapter 3.1.3. Furthermore, due to the Covid-19 Pandemic, SP2 of the third N balance cohort had to be cancelled. Therefore 48 observations were available for each SP.

4.1.1 Performance traits

The pigs were weighed before and after each SP for calculation of average BW and ADG within the SP. The mean BW was 40.0 kg in SP1 with the lightest animal weighing 29.8 kg and the heaviest 53.0 kg (Table 5). In SP2, which was carried out three weeks later, the BW of the animals ranged from 44.0 to 72.0 kg, with a mean of 59.4 kg.

Table 5. Performance data of the pigs in metabolism crates during both sampling periods (SP; n = 48 pigs)

	BW, kg		ADG, kg		ADFI, kg DM		G:F, kg/kg	
SP	1	2	1	2	1	2	1	2
MEAN	40.0^{b}	59.4^{a}	0.77^{b}	0.86^{a}	1.69^{b}	2.21^{a}	0.46^{a}	0.39^{b}
SD	4.47	5.25	0.12	0.11	0.18	0.27	0.07	0.05
%CV	11.2	8.84	16.2	12.7	10.5	12.4	14.9	12.1
MIN	29.8	44.0	0.50	0.64	1.33	1.41	0.26	0.32
MAX	53.0	72.0	1.00	1.17	2.11	2.88	0.58	0.51
SEM	0.99		0.02		0.05		0.01	
P-value	<0.001		<0.001		<0.001		<0.001	
SCC	0.95		0.38		0.50		0.15	
P-value	<0.001		0.017		<0.001		0.360	

SP1 = 13th week of life; SP2 = 16th week of life. Different superscript letters indicate significant differences in trait means between the SP ($p \leq 0.05$). BW = body weight; ADG = average daily gain; ADFI = average daily dry matter feed intake; G:F = gain-to-feed ratio; SD = standard deviation; %CV = percent coefficient of variation; MIN = minimum value; MAX = maximum value; SEM = standard error of the mean; SCC = Spearman's rank correlation coefficient.

ADG was within a range of 0.50 to 1.17 kg and pigs in SP2 gained significantly more weight per day compared with SP1 (0.86 kg vs. 0.77 kg). The mean ADFI was also significantly higher in SP2 (2.21 kg DM) than in SP1 (1.69 kg DM), with a minimum ADFI of 1.33 kg DM in SP1 and a maximum ADFI of 2.88 kg DM in SP2. ADG and ADFI of the individual animals correlated only moderately between the two SP (r = 0.38 for ADG and r = 0.50 for ADFI).

The G:F ratio was significantly lower in SP2 (0.39) than in SP1 (0.46) and no correlation for this trait between the SP was observed. The overall variation was 0.26 to 0.58. With exception of ADFI, the CV of all characteristics decreased from SP1 to SP2 (Table 5).

4.1.2 Variation of the nitrogen balance traits

Although the mean N concentration was lower by 6 g/kg DM in the grower diet than in the starter diet (Table 1, chapter 3.1.2), NI was higher (p = 0.001) in SP2 (62.0 g/d; Table 6) than in SP1 (59.0 g/d) because of the higher ADFI in SP2. As observed for ADFI, daily NI varied widely between the pigs (38.5 to 83.0 g/d) and the correlation between the results of both SP was similar (r = 0.43). The maximum NI was 8.6 g/d higher in SP2 (83.0 g/d) than in SP1 (74.4 g/d). The FNE varied between 5.62 and 17.1 g/d and correlated with ADFI (r = 0.60; Appendix 5), leading to a significantly higher mean value in SP2 (11.6 g/d) compared with SP1 (10.1 g/d). The CV of FNE increased from SP1 (18.0%) to SP2 (22.2%).

Table 6. Nitrogen balance data of the pigs in metabolism crates during both sampling periods (SP; n = 48 pigs)

	NI, g/d		FNE, g/d		UNE, g/d		TNE, g/d		NR, g/d	
SP	1	2	1	2	1	2	1	2	1	2
MEAN	59.0^{b}	62.0^{a}	10.1^{b}	11.6^{a}	21.0^{b}	23.7^{a}	31.1^{b}	35.3^{a}	27.9	26.7
SD	6.46	7.92	1.82	2.57	3.85	3.69	4.59	4.98	3.49	4.29
%CV	11.0	12.8	18.0	22.2	18.3	15.5	14.8	14.1	12.5	16.1
MIN	45.7	38.5	6.30	5.62	11.9	16.0	20.6	23.7	19.3	14.8
MAX	74.4	83.0	14.6	17.1	35.3	32.3	45.3	49.5	36.1	38.0
SEM	1.48		0.45		0.77		0.98		0.80	
P-value	0.001		<0.001		<0.001		<0.001		0.114	
SCC	0.43		0.66		0.65		0.61		0.16	
P-value	0.005		<0.001		<0.001		<0.001		0.336	

SP1 = 13th week of life; SP2 = 16th week of life. Different superscript letters indicate significant differences in trait means between the SP ($p \leq 0.05$). NI = nitrogen intake; FNE = fecal nitrogen excretion; UNE = urinary nitrogen excretion; TNE = total nitrogen excretion; NR = nitrogen retention; SD = standard deviation; %CV = percent coefficient of variation; MIN = minimum value; MAX = maximum value; SEM = standard error of the mean; SCC = Spearman's rank correlation coefficient.

The UNE was highly variable (11.9 to 35.3 g/d), and it was higher (p < 0.001) in SP2 (23.7 g/d) than in SP1 (21.0 g/d). Total N excretion (TNE) also was higher in SP2 (35.3 g/d) than in SP1 (31.1 g/d), since both FNE and UNE were higher in SP2, and was within a range of 20.6 to 49.5 g/d. In both SP, the CV of TNE (14.8% in SP1 and 14.1% in SP2) was smaller than the respective CV for FNE (18.0% in SP1 and 22.2% in SP2) and UNE (18.3% in SP1 and 15.0% in SP2). The results of both SP significantly correlated for FNE (r = 0.66), UNE (r = 0.65), and TNE (r = 0.61). The mean NR tended to be lower in SP2 (26.7 g/d) than in SP1 (27.9 g/d), but this difference was not significant. The CV of NR increased from 12.5% in SP1 to 16.1% in SP2 and the results did not correlate between the SP (r = 0.16).

The mean NUE in SP1 was 47.4% and the difference between the most and least efficient animal was 17.4 percentage points (56.5 vs. 39.1%; Table 7). In SP2, the mean NUE was significantly lower ($p < 0.001$), and the variation ranged from 34.5 to 53.1%. The measured values of the two SP were not correlated (r = 0.08). The mean CP ATTD was 82.9% in SP1 and 81.3% in SP2 and the values were only moderately correlated between the SP (r = 0.45). Although the difference was small, the values differed significantly between SP1 and SP2 because of the low CV of this trait (3.29% for SP1 and 4.21% for SP2).

Table 7. Nitrogen digestibility and utilization of the pigs in metabolism crates during both sampling periods (SP; n = 48 pigs)

	NUE, %		CP ATTD, %		DNI, g/d		DNUE, %	
SP	1	2	1	2	1	2	1	2
MEAN	47.4[a]	43.0[b]	82.9[a]	81.3[b]	48.9	50.4	57.2[a]	52.9[b]
SD	4.13	4.05	2.72	3.43	5.85	6.58	4.60	4.50
%CV	8.71	9.41	3.29	4.21	12.0	13.1	8.05	8.51
MIN	39.1	34.5	78.1	72.6	37.1	30.8	45.2	44.4
MAX	56.5	53.1	88.3	89.0	64.4	65.9	69.1	64.4
SEM	0.83		0.63		1.27		0.93	
P-value	<0.001		0.001		0.279		<0.001	
SCC	0.08		0.45		0.41		0.34	
P-value	0.632		0.004		0.010		0.032	

SP1 = 13th week of life; SP2 = 16th week of life. Different superscript letters indicate significant differences in trait means between the SP ($p \leq 0.05$). NUE = nitrogen utilization efficiency; CP ATTD = apparent total tract crude protein digestibility; DNI = digestible nitrogen intake; DNUE = intermediary utilization efficiency of the digested nitrogen; SD = standard deviation; %CV = percent coefficient of variation; MIN = minimum value; MAX = maximum value; SEM = standard error of the mean; SCC = Spearman's rank correlation coefficient.

Owing to the lower CP ATTD in SP2 there was no difference in the mean digestible nitrogen intake (DNI) between the SP (48.9 g/d in SP1 vs. 50.4 g/d in SP2). The DNI values varied between 30.8 g/d and 65.9 g/d and as observed for NI, results of the SP were moderately correlated (r = 0.41). The maximum DNI was similar in both SP (64.4 g/d in SP1 and 65.9 g/d in SP2). On average, the pigs utilized the digestible N more efficiently in SP1 (57.2%) than in SP2 (52.9%), but the minimum digestible N utilization efficiency (DNUE) was similar in both SP (45.2% in SP1 and 44.4% in SP2).

The mean daily LI did not differ between the SP (17.9 g/d in SP1 vs. 17.4 g/d in SP2; Table 8) because the higher ADFI in SP2 was compensated by the 2.7 g/kg DM lower lysine concentration in the grower diet (Table 1). Daily LI was within the range of 10.3 to 22.2 g. The LR ranged from 6.66 g/d to 17.1 g/d and the mean LR was 12.5 g/d in SP1 and 12.0 g/d in SP2. Since LR was calculated from NR (see chapter 3.3.1), the means also did not differ between the SP and no correlation of the values between the SP was observed (r = 0.16).

The LUE tended to be smaller in SP2 (69.0%) than in SP1 (70.2%), but the means were not significantly different (p = 0.300). The pigs showed remarkable variation in LUE, ranging from 57.1 to 89.1%, but no significant correlation between the individuals comparing the SP existed (r = 0.11), indicating that the animals were not equally efficient in both SP.

Table 8. Lysine utilization of the pigs in metabolism crates during both sampling periods (SP; n = 48 pigs)

	LI, g/d		LR, g/d		LUE, %	
SP	1	2	1	2	1	2
MEAN	17.9	17.4	12.5	12.0	70.2	69.0
SD	1.96	2.25	1.57	1.93	6.46	6.74
%CV	11.0	12.8	12.5	16.0	9.20	9.76
MIN	13.6	10.3	8.69	6.66	59.9	57.1
MAX	22.0	22.2	16.2	17.1	85.7	89.1
SEM	0.43		0.36		1.35	
P-value	0.276		0.112		0.300	
SCC	0.45		0.16		0.11	
P-value	0.003		0.322		0.513	

SP1 = 13th week of life; SP2 = 16th week of life. Lysine retention was calculated assuming an average of 7.2 g lysine per 100 g protein being deposited. Different superscript letters indicate significant differences in trait means between the SP ($p \leq 0.05$). LI = lysine intake; LR = lysine retention; LUE = lysine utilization efficiency; SD = standard deviation; %CV = percent coefficient of variation; MIN = minimum value; MAX = maximum value; SEM = standard error of the mean; SCC = Spearman's rank correlation coefficient.

The mean daily urinary urea nitrogen excretion (UUE) was higher ($p < 0.001$) in SP2 than in SP1 (18.8 g vs. 17.1 g; Table 9). The values were highly variable between the pigs and ranged between 9.1 and 30.1 g/d.

Table 9. Urinary metabolite excretion of the pigs in metabolism crates during both sampling periods (SP; n = 48 pigs)

	UUE, g/d		UCrE, g/d		UCE, mg/d	
SP	1	2	1	2	1	2
MEAN	17.1^{b}	18.8^{a}	1.64^{b}	2.54^{a}	0.20	0.21
SD	3.58	3.43	0.24	0.41	0.05	0.05
%CV	20.9	18.2	14.5	16.2	23.1	25.7
MIN	9.10	11.4	1.04	1.39	0.14	0.12
MAX	30.1	26.4	2.03	3.36	0.33	0.39
SEM	0.72		0.07		0.01	
P-value	<0.001		<0.001		0.965	
SCC	0.63		0.71		0.63	
P-value	<0.001		<0.001		<0.001	

SP1 = 13th week of life; SP2 = 16th week of life. Different superscript letters indicate significant differences in trait means between the SP ($p \leq 0.05$). UUE = urinary urea excretion; UCrE = urinary creatinine excretion; UCE = urinary cortisol excretion; SD = standard deviation; %CV = percent coefficient of variation; MIN = minimum value; MAX = maximum value; SEM = standard error of the mean; SCC = Spearman's rank correlation coefficient.

The urinary creatinine excretion (UCrE) was also significantly higher in SP2 (2.54 g/d) compared with SP1 (1.64 g/d) and individual values were within the range of 1.04 to 3.36 g/d. The daily urinary cortisol excretion (UCE) varied between 0.14 to 0.39 mg, but mean values were similar for both SP (0.20 in SP1 and 0.21 ins SP2). The individual values of all three metabolites showed a clear correlation between the two SP (r = 0.63, r = 0.71 and r = 0.63 for UUE, UCrE and UCE, respectively).

4.1.3 Relationships between the nitrogen balance traits

The UNE was linearly increased by NI across both SP (Figure 4; $R^2 = 0.60$). On average, 37% of every additionally ingested gram of N was excreted via urine. For UUE a similar relation to NI was observed ($R^2 = 0.57$) and every gram of additional NI resulted in 0.3 g urea N excretion. The proportion of the ingested N excreted via urine was significantly lower in SP1 than in SP2 (35.5% vs. 38.3%; $p < 0.001$; Figure 5), but the variation among the individual animals was similar for both SP (25.2 to 47.4% for SP1 and 28.4 to 47.1% for SP2). The UUE was strongly correlated with UNE (r = 0.97) and on average for both SP, 80% of the urinary N was bound as urea. Even if the UUE:UNE ratio was almost constant (≤ 5.0% CV), it varied from 68.7 to 86.9% and was significantly higher in SP1 at 81.3% than in SP2 at 79.2%. A moderate correlation between LI and UNE ($R^2 = 0.38$; Figure 4) as well as UUE ($R^2 = 0.40$) existed. With every gram of additional LI 1.26 g of total N and 1.02 g of urea N were excreted on average for both SP.

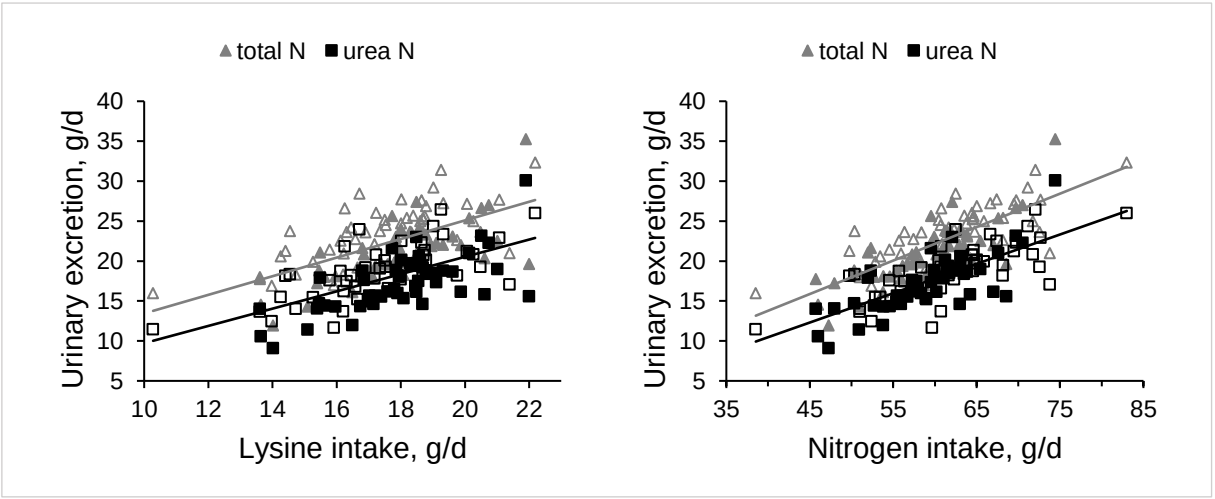

Figure 4. Urinary nitrogen (UNE) and urinary urea nitrogen (UUE) excretion of the extended data-collection subsample within both sampling periods (filled symbols = sampling period 1; open symbols = sampling period 2) as a function of lysine intake (left part; UNE = 1.26x; $R^2 = 0.38$, RMSE = 3.16; UUE = 1.02x; $R^2 = 0.40$, RMSE = 2.79) and nitrogen intake (right part; UNE = 0.37x; $R^2 = 0.60$, RMSE = 2.55; UUE = 0.30x; $R^2 = 0.57$, RMSE = 2.38)

Not only the absolute FNE and UNE was significantly higher in SP2 compared with SP1 but also FNE and UNE relative to NI. In SP2 18.7% of the ingested N was excreted via feces and 38.3% via urine, whereas in SP1 17.1% was excreted via feces and 35.5% via urine (Figure 5). However, no difference existed in the distribution of TNE. Even if there consisted considerable variation (22.2 to 46.0% FNE of TNE and 54.0 to 77.8% UNE of TNE), the mean proportion of FNE (32.6 % for SP1 and 32.8% for SP2) and UNE (67.4 and 67.2% for SP1 and SP2, respectively) was the same in both SP.

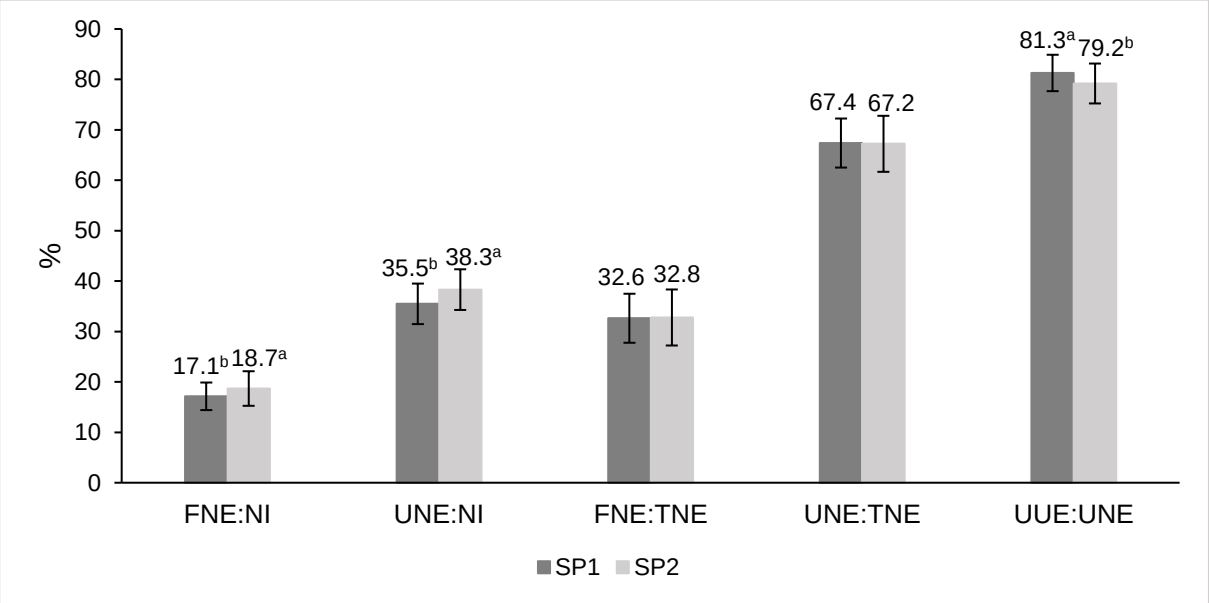

Figure 5. Nitrogen excretion pattern of the extended data-collection subsample in comparison of both sampling periods (SP). Observations of 48 animals were used in each period. Data are least square means ± standard deviation. Different superscript letters within a pair of bars indicate significant differences between the SP ($p \leq 0.05$). FNE:NI = proportion of ingested nitrogen excreted with feces; UNE:NI = proportion of ingested nitrogen excreted with urine; FNE:TNE = proportion of fecal nitrogen in total nitrogen excretion; UNE:TNE = proportion of urinary nitrogen in total nitrogen excretion; UUE:UNE = proportion of urinary urea nitrogen in total urinary nitrogen excretion.

A strong correlation between NI, DNI and LI with NR (R^2 = 0.53, R^2 = 0.61 and R^2 = 0.60, respectively) was observed when describing NR as a linear function of NI, DNI and LI (Figure 6). With every additional gram of NI, on average 0.45 g N or 2.81 g of protein were retained in both SP. When NI was corrected for the indigestible fraction, on average 0.55 g N or 3.44 g of protein were retained per gram of DNI. The average NR per gram of additionally ingested lysine was 1.54 g which resulted in 9.63 g of protein retention and a mean LUE of 69.3%. The slope of SP1 was 1.55, resulting in a mean LUE of 70.2% and did not differ significantly from the values for SP2 (slope 1.53; mean LUE 69.0%).

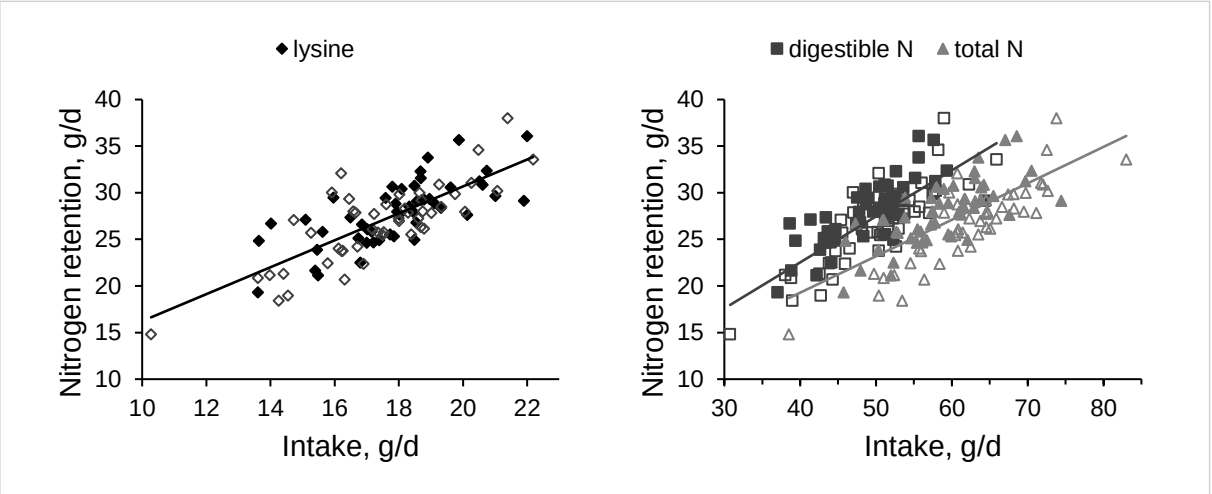

Figure 6. Nitrogen (N) retention of the extended data-collection subsample within both sampling periods (filled symbols = sampling period 1; open symbols = sampling period 2) as a function of lysine intake (y = 1.54x; R^2 = 0.60, RMSE = 2.49), digestible N intake (y = 0.55x; R^2 = 0.61, RMSE = 2.46) and total N intake (y = 0.45x; R^2 = 0.53, RMSE = 2.70). Since the intercept of the linear regressions was not significantly different from zero, the regression lines were forced through zero.

4.1.4 Blood metabolites

The mean BUN concentration of the pigs was 5.94 mmol/L in SP1 and 5.70 mmol/L in SP2 (Table 10), which was significantly different (p = 0.022). The BUN concentrations varied between 4.15 and 7.72 mmol/L and showed moderate correlation with NI and LI (r = 0.50 and r = 0.56, respectively; Appendix 5) as well as NR (r = 0.41), but no significant correlation existed with NUE.

Table 10. Estimated parameters of the concentration of blood metabolites of the pigs in metabolism crates during both sampling periods (SP; n = 48 pigs)

	BUN, mmol/L		SC, ng/mL		IGF-I, ng/mL	
SP	1	2	1	2	1	2
MEAN	5.94[a]	5.70[b]	21.5	22.9	204	196
SD	0.78	0.86	8.33	11.2	38.5	37.1
%CV	13.2	15.0	38.8	48.8	18.9	18.9
MIN	4.50	4.15	3.85	4.92	83.7	111
MAX	7.47	7.72	41.3	48.5	279	276
SEM	0.17		2.01		7.72	
P-value	0.022		0.124		0.790	
SCC	0.68		0.65		0.87	
P-value	<0.001		<0.001		<0.001	

SP1 = 13th week of life; SP2 = 16th week of life. Different superscript letters indicate significant differences in trait means between the SP ($p \leq 0.05$). BUN = blood urea nitrogen; SC = serum cortisol; IGF-I = insulin-like growth factor 1; SD = standard deviation; %CV = percent coefficient of variation; MIN = minimum value; MAX = maximum value; SEM = standard error of the mean; SCC = Spearman's rank correlation coefficient.

The correlation of BUN with UNE and UUE differed between the two SP. In SP1, BUN was highly correlated with UNE and UUE (r = 0.81 for UNE and r = 0.80 for UUE; Appendix 6), whereas in SP2 the correlation coefficients were only moderate (r = 0.55 for UNE and r = 0.52 for UUE; Appendix 7). Consistently, UNE and UUE could only be partly explained by linear regression of BUN concentration (R^2 = 0.30 for UNE and R^2 = 0.34 for UUE; Figure 7). The SC concentrations were highly variable among the individuals and reached from 3.85 to 48.5 ng/mL with a mean SC concentration of 21.5 ng/mL in SP1 and 22.9 ng/mL in SP2 (Table 10). This resulted in a CV of 38.8% for SP1 and 48.8% for SP2.

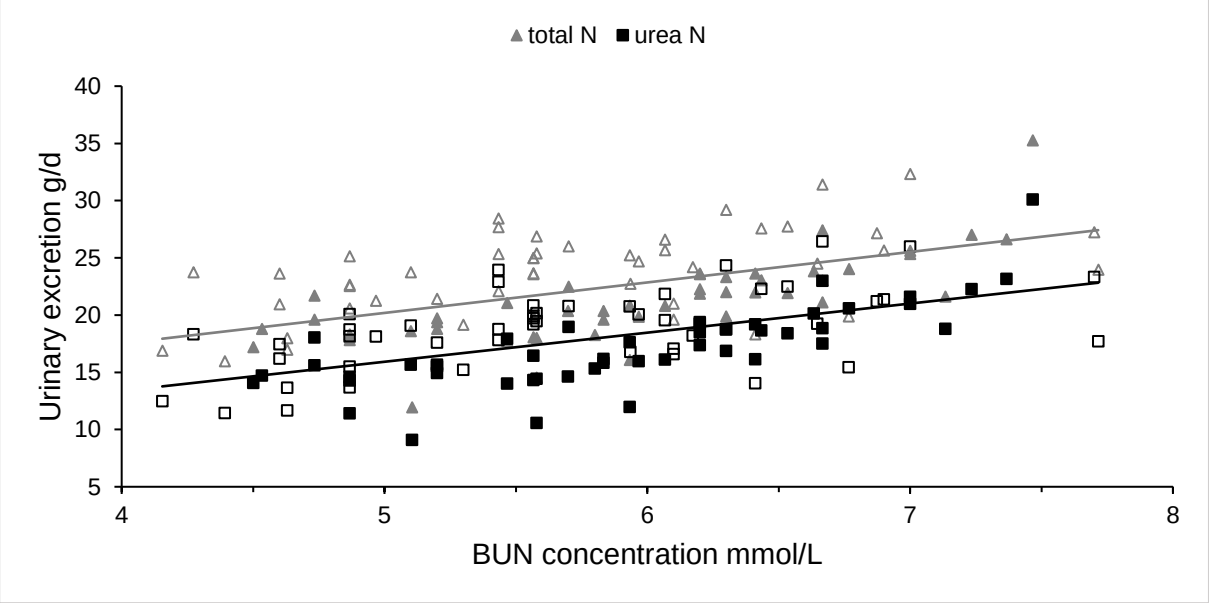

Figure 7. Urinary nitrogen (UNE) and urinary urea nitrogen (UUE) excretion of the extended data-collection subsample within both sampling periods (filled symbols = sampling period 1; open symbols = sampling period 2) as a function of blood urea nitrogen (BUN) concentration obtained five hours after morning feeding (UNE = 6.89 + 2.66x; R^2 = 0.30, RMSE = 3.34; UUE = 3.08x; R^2 = 0.34, RMSE = 2.93).

The mean IGF-I concentration was slightly lower in SP2 (196 ng/mL) than in SP1 (204 ng/mL) but the variation among the animals ranged between 84 to 279 ng/mL, so no significant differences between the SP were observed.

The concentrations of blood metabolites were highly correlated between the SP (r = 0.68 for BUN, r = 0.65 for SC and r = 0.87 for IGF-I; Table 10).

4.1.5 Protein turnover

Despite apparently successful administration of the marker, results from six animals in SP2 indicated that they did not swallow the capsule containing the ^{15}N glycine completely and therefore their results were not included in the analysis. Thus, for SP2 only 42 observations for body protein turnover were available.

Since the individual dose of ^{15}N glycine was adjusted to BW, the mean ^{15}N dose was higher in SP2 than in SP1 (63.7 vs. 42.0 mg; Table 11).

The cumulative urinary ^{15}N excretion ($U^{15}NE$) within the 84-h sampling interval was 8.37 mg on average in SP1 and 14.4 mg in SP2, ranging from 5.13 to 21.9 mg. Relative to the administered dose, 19.8% ^{15}N were excreted via urine in SP1 and 22.4% in SP2, with similar minimum values of 13.0 and 12.5% in SP1 and SP2, respectively. The maximum $U^{15}NE$ was 26.8% in SP1 and 31.8% in SP2. There also existed a low but significant correlation for the relative $U^{15}NE$ between the SP (r = 0.36).

Table 11. Amount of ^{15}N administered and excreted via urine within 84 h after administration during both sampling periods (SP)

	Dose ^{15}N, mg		$U^{15}NE$, mg		$U^{15}NE$, % of dose	
SP	1	2	1	2	1	2
MEAN	42.0[b]	63.7[a]	8.37[b]	14.4[a]	19.8[b]	22.4[a]
SD	4.91	6.97	1.93	3.42	3.17	4.08
%CV	11.7	10.9	23.0	23.8	16.0	18.2
MIN	30.7	42.0	5.13	7.50	13.0	12.5
MAX	55.9	77.2	13.2	21.9	26.8	31.8
SEM	1.26		0.58		0.77	
P-value	<0.001		<0.001		0.001	
SCC	0.95		0.62		0.36	
P-value	<0.001		<0.001		0.035	

Observations of 48 animals were available in SP1 and observations of 42 animals were available in SP2. SP1 = 13th week of life; SP2 = 16th week of life. Different superscript letters indicate significant differences in trait means between the SP ($p \leq 0.05$). $U^{15}NE$ = urinary ^{15}N excretion; SD = standard deviation; %CV = percent coefficient of variation; MIN = minimum value; MAX = maximum value; SEM = standard error of the mean; SCC = Spearman's rank correlation coefficient.

As mentioned in 3.1.3, during the first two balance periods, urine was sampled in 12-h intervals and cumulative excretion of $U^{15}NE$ was measured for a total of 84 h (Figure 8). The cumulative excretion curves were estimated by non-linear regression using the bounded growth function. According to the individual excretion curves, on average 95% of the ^{15}N was excreted after 62.9 h in SP1 and 68.2 h in SP2. The mean 95% $U^{15}NE$ was 7.79 mg or 19.5% of the administered dose in SP1 and 11.5 mg or 19.7% of the administered dose in SP2. Big differences existed in the duration of $U^{15}NE$ between the individuals. The minimum estimated time for 95% ^{15}N excretion was 52.8 h and the maximum time was 100 h.

Overall, all measures of body protein turnover were equal for SP1 and SP2 (Table 12). The mean protein turnover was 669 g/d in SP1 and 668 g/d in SP2 and the individual values were within a range of 448 to 1226 g/d. Values for protein synthesis varied from 350 to 1072 g/d with a mean of 538 g/d in SP1 and 521 g/d in SP2. The variation was the widest for protein degradation, ranging from 223 to 714 g/d in SP1 (26.8% CV) and from 181 to 885 g/d in SP2 (34.9% CV), but the means were similar in both SP (364 g/d in SP1 vs. 357 g/d in SP2).

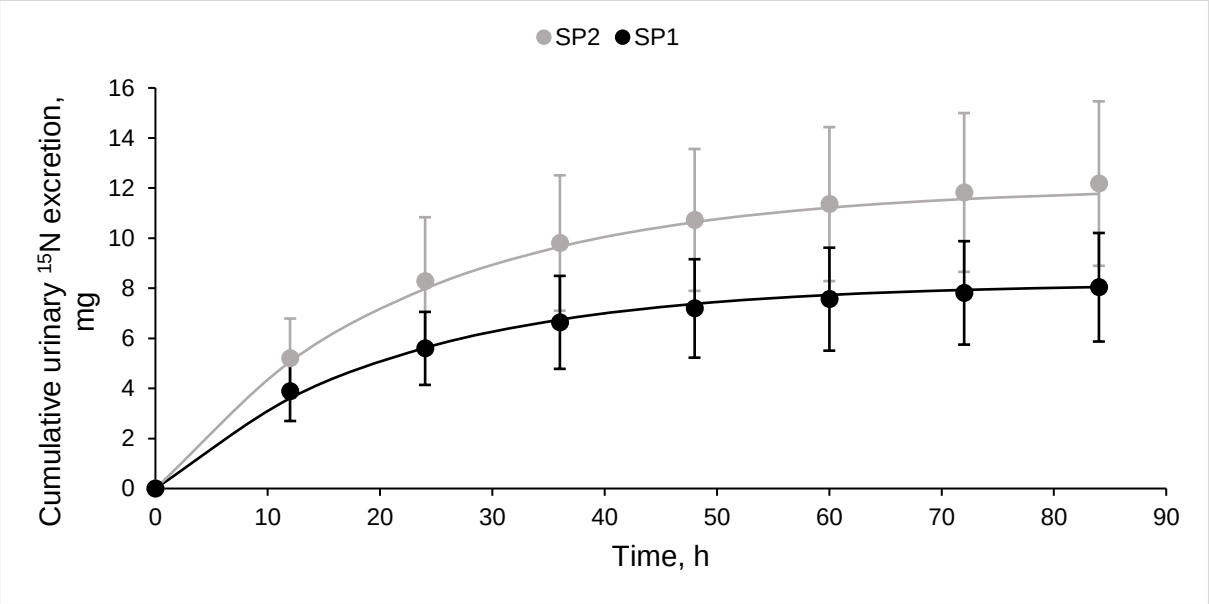

Figure 8. Cumulative curve of urinary ^{15}N excretion for 84 h of eight barrows for each of both sampling periods (SP) after oral administration of a single dose of ^{15}N glycine (6 mg/kg bodyweight). Data are least square means ± standard deviation. The mean urinary ^{15}N excretion at time t could be calculated as $y_t = 8.20 \times (1 - e^{-48.3 \times t})$ for SP1 and $y_t = 12.1 \times (1 - e^{-45.7 \times t})$ for SP2. On average, 95% of total ^{15}N excretion was achieved after 62.9 h in SP1 and 68.2 h in SP2.

Within both SP, protein turnover correlated marginally with NR (r = 0.23; Appendix 5) and protein degradation with LUE (r = - 0.22). In SP1 no correlation with NR existed, but correlations with NUE (r = - 0.32 for protein turnover and r = - 0.35 for protein degradation; Appendix 6) and LUE (r = - 0.36 for protein turnover and r = - 0.47 for protein degradation) existed. In SP2 no significant correlations existed at all (Appendix 7). Also, a positive correlation between protein turnover and BUN was observed, which was almost constant in both SP (r = 0.31 in SP1 and r = 0.35 in SP2). Comparing the SP, moderate correlations were observed for all three traits (r = 0.61 for protein turnover, r = 0.50 for protein synthesis and r = 0.51 for protein degradation; Table 12).

The protein retention to synthesis ratio was similar in both SP (33.2% for SP1 vs. 32.7% for SP2), indicating that only about one third of the synthesized protein was retained by the animals on average.

Big differences existed between the individuals within this trait (18.7% CV in SP1 and 22.3% CV in SP2). In both SP, the most efficient animal retained nearly half of the synthesized protein (47.2% in SP1 and 48.8% in SP2), whereas the least efficient animal only retained about one sixth of the synthesized protein (16.0% in SP1 and 17.0% in SP2). Only moderate to marginal correlations between the protein retention to synthesis ratio and NUE (r = 0.35; Appendix 5) as well as LUE (r = 0.46) existed within both SP. In SP2, correlations were even less pronounced and not significant, whereas they were stronger in SP1 (r = 0.51 for NUE and r = 0.64 for LUE; Appendix 6). Similar to NUE and LUE, no significant correlation of the protein retention to synthesis ratio between the SP existed.

Table 12. Body protein-turnover, -synthesis and -degradation of the pigs in metabolism crates during both sampling periods (SP) obtained by the two-pool model after a single oral dose of ^{15}N glycine

	Body protein-						Retention:	
	Turnover, g/d		Synthesis, g/d		Degradation, g/d		Synthesis, %	
SP	1	2	1	2	1	2	1	2
MEAN	669	668	538	521	364	357	33.2	32.7
SD	104	136	96.3	130	97.5	125	6.20	7.31
%CV	15.6	20.3	17.9	24.9	26.8	34.9	18.7	22.3
MIN	465	448	376	350	223	181	16.0	17.0
MAX	1034	1226	896	1072	714	885	47.2	48.8
SEM	24.3		23.9		23.5		1.42	
P-value	0.581		0.372		0.824		0.642	
SCC	0.61		0.50		0.51		0.19	
P-value	<0.001		0.002		0.002		0.291	

Observations of 48 animals were available in SP1 and observations of 42 animals were available in SP2. SP1 = 13th week of life; SP2 = 16th week of life. SD = standard deviation; %CV = percent coefficient of variation; MIN = minimum value; MAX = maximum value; SEM = standard error of the mean; SCC = Spearman's rank correlation coefficient.

Since the measurements of EBP content were not available for all animals (as explained in chapter 4.1.6), a mean EBP content of 16.6% for SP1 and 16.5% for SP2 was assumed for calculations of the fractional terms of protein turnover as this trait showed high consistency among the individuals.

The fractional rates of body protein turnover, synthesis, degradation, and retention were higher in SP1 compared to SP2 (Figure 9). On average 8.8% of the body protein mass were newly synthesized daily in SP1 and 5.9% degraded again. As a result, a protein amount equivalent to 2.8% of the total body protein mass was retained daily. In SP2 the mean FSR was 5.6% and the mean FDR was 3.8%, so FRR was only 1.8%. The CV within each trait and SP was numerically nearly identical with the absolute values (Table 12 and Table 6) and a wide variation was observed.

The values ranged from 4.7 to 15.4% for FTR, 3.4 to 13.4% for FSR, 1.8 to 10.6% for FDR and 1.2 to 3.7% for FRR. The FRR was significantly correlated with NUE (r = 0.68; Appendix 5), LUE (r = 0.44) and LI (r = 0.30) within both SP but not with NI. Considering the SP separately, the correlation between FRR and the efficiencies was r = 0.74 for NUE and r = 0.72 for LUE in SP1 (Appendix 6) and r = 0.41 for NUE and r = 0.49 for LUE in SP2 (Appendix 7). The opposite was observed for NI and LI. In SP2 higher correlations were seen (r = 0.56 for NI and r = 0.50 for LI) but no significant correlations existed at all in SP1.

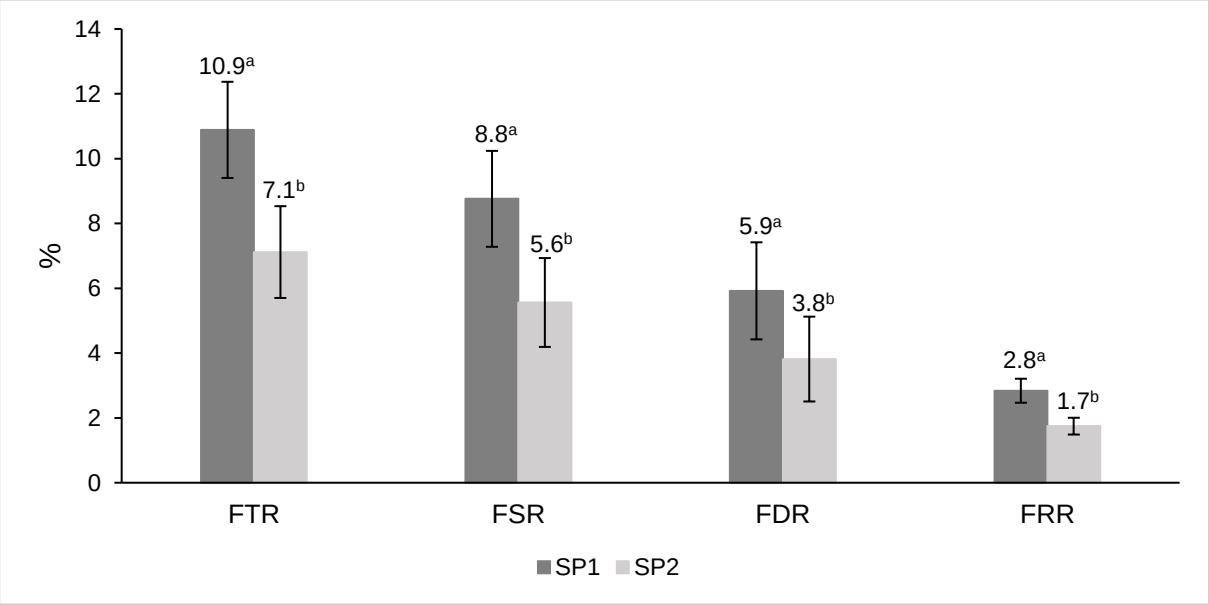

Figure 9. Fractional rates of body protein turnover (FTR), synthesis (FSR), degradation (FDR), and retention (FRR) of the extended data collection subsample in comparison of both sampling periods (SP). Observations of 48 animals were available in SP1 and observations of 42 animals were available in SP2. Data are least square means ± standard deviation. Different superscript letters within a pair of bars indicate significant differences between the SP ($p \leq 0.05$). Body protein turnover, synthesis, and degradation were measured using the end-product method after administration of a single oral dose of ^{15}N glycine (6 mg/kg bodyweight) and body protein amount was calculated assuming an average empty body protein content of 16.6% in SP1 and 16.5% in SP2.

4.1.6 Body composition

The variation of the absolute empty body composition determined by D_2O dilution technique and the difference between the individuals of the extended data-collection subsample in the two SP is presented in Table 13. Because of difficulties with some animals in administering the marker and consequently, incomplete intake, results of body composition were available for only 44 pigs in SP1 and 40 pigs in SP2. EBW, EBP and EBA showed similar variation within each SP (12.8, 13.0 and 13.1% CV in SP1 and 9.63, 9.82 and 9.92% CV in SP2 for EBP, EBA and EBW, respectively), because of biological relations and the method of calculation, amounts of EBP and EBA are mostly dependent on EBW.

The amount of EBWA was even less variable (10.4% CV in SP1 and 8.18% CV in SP2), whereas EBF mass varied widely (38.9% CV in SP1 and 25.4% CV in SP2). Due to the increased BW of the animals, means were significantly higher in SP2 compared to SP1, but the variation declined. With exception of EBF (r = 0.52) all traits of empty body composition were highly correlated between the SP (r = 0.90 for EBWA and r = 0.92 for each EBW, EBP and EBA). The mean EBW, EBP and EBA increased by 50%, whereas the mean amount of EBWA only increased by 40%. EBF mass doubled on average from SP1 (5.35 kg) to SP2 (10.6 kg).

Table 13. Empty body weight and fractions of the pigs in metabolism crates during both sampling periods (SP) obtained by deuterium oxide dilution technique

	EBW, kg		EBWA, kg		EBF, kg		EBP, kg		EBA, kg	
SP	1	2	1	2	1	2	1	2	1	2
MEAN	37.5^{b}	56.8^{a}	25.1^{b}	35.5^{a}	5.35^{b}	10.6^{a}	6.22^{b}	9.35^{a}	1.14^{b}	1.72^{a}
SD	4.93	5.63	2.62	2.91	2.08	2.70	0.79	0.90	0.15	0.17
%CV	13.1	9.92	10.4	8.18	38.9	25.4	12.8	9.63	13.0	9.82
MIN	28.4	42.7	19.5	28.0	2.19	5.24	4.74	7.06	0.86	1.29
MAX	49.5	71.0	32.2	41.7	11.7	19.2	8.15	11.6	1.49	2.14
SEM	1.15		0.60		0.52		0.19		0.03	
P-value	<0.001		<0.001		<0.001		<0.001		<0.001	
SCC	0.92		0.90		0.59		0.92		0.92	
P-value	<0.001		<0.001		0.001		<0.001		<0.001	

Observations of 44 animals were used in SP1 and observations of 40 animals were used in SP2. SP1 = 13th week of life; SP2 = 16th week of life. Different superscript letters indicate significant differences in trait means between the SP ($p \leq 0.05$). EBW = empty bodyweight; EBWA = empty body water; EBF = empty body fat; EBP = empty body protein; EBA = empty body ash; SD= standard deviation; %CV = percent coefficient of variation; MIN = minimum value; MAX = maximum value; SEM = standard error of the mean; SCC = Spearman's rank correlation coefficient.

Expressing the amounts of the fractions in relation to EBW, the mean EBWA content declined from 67.3% in SP1 to 62.7% in SP2 and the average EBF content increased from 13.9% in SP1 to 18.5% in SP2 (Figure 10). Like the absolute EBF mass, the relative EBF content was highly variable (26.9% CV in SP1 and 17.8% CV in SP2), whereas variation in EBWA content was small (5.59% CV in SP1 and 5.21% CV in SP2). Protein and ash content of the empty body were constant within both SP and variation was negligible (0.53 and 0.49% CV for EBP and 0.17 and 0.16% CV for SP1 and SP2, respectively). Nevertheless, the slight differences in the means of the traits were significant and EBP and EBA were lower in SP2 (16.5% EBP and 3.02% EBA) than in SP1 (16.6% EBP and 3.03% EBA).

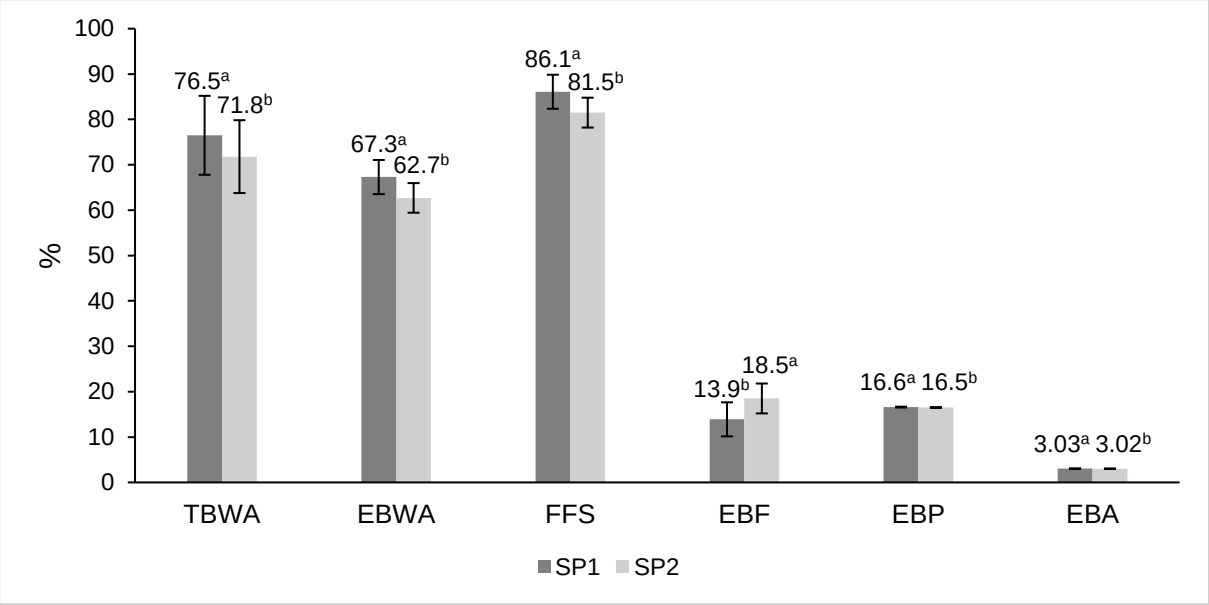

Figure 10. Percentage of body fractions of the extended data-collection subsample during both sampling periods (SP) obtained by deuterium oxide dilution technique after oral administration of a single dose D_2O (0.7 g/kg bodyweight). Observations of 44 animals were available in SP1 and observations of 40 animals were available in SP2. Data are least square means ± standard deviation. Different superscript letters within a pair of bars indicate significant differences between the SP ($p \leq 0.05$).
TBWA = total body water; EBWA = empty body water; FFS = fat free substance; EBF = empty body fat; EBP = empty body protein; EBA = empty body ash.

4.1.6 Estimation equations

From the 96 observations that were available for modeling in both SP, 56 were independent and 40 were repeated measures. Due to this data structure, independent estimation for each SP was not feasible, as the number of observations (n = 48) was considered not sufficient for robust estimations. Therefore, all models presented in the following section are models based on the entire N balance data set, i.e., on observations from both SP. For the resulting equations, it was additionally tested, whether the SP had to be included as a covariable in the model, but no significant effect of the SP was detected for any estimated model.

The results of the estimation of NR are displayed in Table 14. Mean estimated NR over both SP was 27.3 g/d. The linear model with the best goodness of fit (model no. 1; AIC = 408) included BW, NI, CP ATTD, and BUN and all regression coefficients were highly significant. With this model 73% of the variation of NR could be explained (adj. $R^2 = 0.73$) and only a small residual error existed (RMSE = 2.01 g/d, which corresponds to 7.36% of the mean).

The best linear model without the inclusion of CP ATTD (model no. 2; AIC = 438), containing BW, NI, SC, and IGF-I as variables, was only slightly inferior to model 1 (adj. $R^2 = 0.66$ and RMSE = 2.27 g/d or 8.31%).

If blood metabolite concentrations were not considered, the estimation of NR was possible using BW and NI as predictive variables (model no. 3, AIC = 449). By this model, 62% of the variation of NR could be explained with a RMSE of 2.41 g/d (8.83%).

Table 14. Equations for estimating nitrogen (N) retention (g/d) of growing pigs fed diets with marginal lysine supply in two sampling periods (SP) based on N balance data and blood metabolite concentrations

Model	Parameter	b	BCI_b	SE_b	*P*-value	AIC	adj. R^2	RMSE
1						408	0.73	2.01
	Intercept	-37.82	[-47.66; -12.46]	5.941	<0.001			
	BW	-0.147	[-0.197; -0.075]	0.019	<0.001			
	NI	0.579	[0.423; 0.675]	0.039	<0.001			
	CP ATTD	0.564	[0.250; 0.696]	0.073	<0.001			
	BUN	-1.543	[-2.504; -0.024]	0.352	<0.001			
2						438	0.67	2.27
	BW	-0.137	[-0.181; -0.083]	0.022	<0.001			
	NI	0.479	[0.393; 0.566]	0.026	<0.001			
	SC	0.055	[0.009; 0.102]	0.024	0.024			
	IGF-I	0.018	[0.007; 0.028]	0.006	0.001			
3						449	0.62	2.41
	Intercept	5.067	[1.127; 9.647]	2.153	0.021			
	BW	-0.134	[-0.179; -0.073]	0.024	<0.001			
	NI	0.478	[0.377; 0.548]	0.039	<0.001			
4						425	0.72	1.98
	Intercept	-32.60	[-51.51; -12.50]	11.39	0.005			
	ADG	-36.01	[-65.22; -14.21]	10.71	0.001			
	NI	1.568	[1.220; 2.029]	0.217	<0.001			
	BUN	4.855	[1.446; 8.305]	1.960	0.015			
	IBW x ADG	1.169	[0.527; 2.066]	0.329	<0.001			
	IBW x ADFI	-0.561	[-0.938; -0.283]	0.132	<0.001			
	ADFI x BUN	2.125	[0.606; 4.193]	0.731	0.005			
	ADFI x SC	-0.072	[-0.111; -0.022]	0.026	0.008			
	NI x BUN	-0.154	[-0.242; -0.095]	0.038	<0.001			
	SC x IGF	0.001	[0.000; 0.002]	<0.001	<0.001			

In total 96 observations of 56 animals in two SP were used for estimations, resulting in 56 independent and 40 repeated measures, evenly distributed over both SP. Mean nitrogen retention was 27.3 g/d. Equations are equally valid for both SP. SP1 = 13th week of life; SP2 = 16th week of life.
Regression coefficients (b) and standard errors (SE_b) were obtained using multiple regression. Confidence intervals of the regression coefficients (BCI_b) were generated by 1,000 bootstrap data sets.
AIC = Akaike information criterion; RMSE = root mean square error; IBW = initial body weight, kg; BW = body weight, kg; ADG = average daily gain, kg; ADFI = average daily feed intake, kg dry matter; NI = nitrogen intake, g/d; CP ATTD = apparent total tract crude protein digestibility, %; BUN = blood urea nitrogen, mmol/L; SC = serum cortisol, ng/mL; IGF-I = insulin-like growth factor 1, ng/mL.

The best goodness of fit, without using CP ATTD, was shown by model no. 4 (AIC = 425, adj. R^2 = 0.72 and RMSE = 1.98 g/d or 7.25%). It included linear effects as well as interactions between IBW, ADG, ADFI, NI, BUN, SC, and IGF-I and all estimated parameters sustained bootstrapping validation. Since IBW is included, this model is only suitable for the use of the present data set. Other models, containing interactions and quadratic effects were estimable but over parameterized and thus not robust to validation and were rejected.

Table 15. Equations for estimating nitrogen (N) utilization efficiency (%) of growing pigs fed diets with marginal lysine supply in two sampling periods (SP) based on N balance data and blood metabolite concentrations

Model	Parameter	b	BCI_b	SE_b	*P*-value	AIC	adj. R^2	RMSE
5						499	0.51	3.13
	IBW	-0.299	[-0.455; -0.007]	0.108	0.007			
	BW	-0.238	[-0.308; -0.142]	0.032	<0.001			
	NI	0.210	[0.051; 0.331]	0.062	0.001			
	CP ATTD	0.719	[0.559; 0.804]	0.051	<0.001			
	BUN	-2.214	[-2.990; -0.702]	0.524	<0.001			
	SC	0.102	[0.035; 0.159]	0.036	0.005			
	IGF-I	0.025	[0.001; 0.037]	0.009	0.010			
6						544	0.26	3.94
	Intercept	49.15	[44.16; 55.09]	2.851	<0.001			
	BW	-0.209	[-0.274; -0.122]	0.037	<0.001			
	IGF-I	0.032	[0.008; 0.046]	0.011	0.004			
7						537	0.32	3.71
	ADFI	-71.82	[-107.0; -29.02]	15.79	<0.001			
	NI	3.543	[2.152; 4.747]	0.515	<0.001			
	SC	0.108	[0.019; 0.182]	0.042	0.013			
	IGF-I	0.032	[0.011; 0.046]	0.011	0.005			
	ADFI x ADFI	15.70	[4.953; 24.49]	3.965	<0.001			
	NI x NI	-0.027	[-0.037; -0.015]	0.004	<0.001			
8						524	0.41	3.44
	BUN	15.96	[14.91; 17.53]	0.673	<0.001			
	BW x BUN	0.112	[0.059; 0.168]	0.028	<0.001			
	BW x IGF-I	-0.004	[-0.006; -0.003]	<0.001	<0.001			
	NI x BUN	-0.248	[-0.301; -0.201]	0.026	<0.001			
	NI x IGF-I	0.007	[0.006; 0.009]	<0.001	<0.001			
	BUN x IGF-I	-0.034	[-0.041; -0.029]	0.003	<0.001			

In total 96 observations of 56 animals in two SP were used for estimations, resulting in 56 independent and 40 repeated measures, evenly distributed over both SP. Mean N utilization efficiency was 45.1%. Equations are equally valid for both SP. SP1 = 13th week of life; SP2 = 16th week of life.
Regression coefficients (b) and standard errors (SE_b) were obtained using multiple regression. Confidence intervals of the regression coefficients (BCIb) were generated by 1,000 bootstrap data sets. AIC = Akaike information criterion; RMSE = root mean square error; IBW = initial bodyweight, kg; BW = bodyweight, kg; ADFI = average daily feed intake, kg dry matter; NI = nitrogen intake, g/d; CP ATTD = apparent total tract crude protein digestibility, %; BUN = blood urea nitrogen, mmol/L; SC = serum cortisol, ng/mL; IGF-I = insulin-like growth factor 1, ng/mL.

Similar to the estimation of NR, estimation equations of NUE provided the best goodness of fit if CP ATTD was included as a variable into the model. The model with the lowest AIC (499, model no. 5; Table 15) contained IBW, BW, NI, CP ATTD, BUN, SC, and IGF-I as variables and could explain 51% of the variation of NUE. The estimation error was 3.13 percentage points, corresponding to 6.94% of the mean of 45.1% NUE.

Without the inclusion of CP ATTD, only BW and IGF-I concentration remained as variables in the best linear model and the goodness of fit decreased substantially (AIC 544, $R^2 = 0.26$ and RMSE = 3.94 percentage points or 8.74% of the mean; model no. 6). Even with the inclusion of interactions and quadratic effects (e.g., models no. 7 and 8), it was not possible to generate a model with a fit similar to that of the model including CP ATTD.

Similar models and values for the goodness of fit as for the estimation of NUE were obtained if LUE was estimated and therefore are not presented here (see Appendix 8).

Due to the close correlation of UNE and UUE (r = 0.97), models for estimating UNE and UUE were the same. Only the regression coefficients differed slightly (Table 16 and Table 17). The linear models with IBW, BW, NI, BUN, SC, and IGF-I as variables showed high goodness of fit (AIC = 396, adj. $R^2 = 0.77$ for estimating UNE, model no. 9 and AIC = 397, adj. $R^2 = 0.72$ for estimating UUE, model no. 12). The residual error was low and two percentage points higher for UUE then for UNE (RMSE = 1.88 g/d or 8.39% for UNE and 1.84 g/d or 10.2% for UUE). The estimated mean for UNE over both SP was 22.4 g/d and 18.0 g/d for UUE.

Including interactions and quadratic effects into the models improved goodness of fit only to a low extent. Comparing the best models containing interactions with the best linear models, adj. R^2 increased by 0.03 for UNE (model no. 11) and 0.06 for UUE (model no. 14). The RMSE decreased to 1.72 g/d (7.68%) for UNE and 1.63 g/d (9.06%) for UUE. In contrast to the estimation of NR and NUE, CP ATTD had no significant influence on UNE ($p = 0.90$) and UUE ($p = 0.94$) and was not considered as a variable.

Simpler models, considering only BW, NI, and BUN as variables (models no. 10 and 13), could be estimated with just marginally lower goodness of fit (AIC = 411 and adj. $R^2 = 0.72$ for UNE and AIC = 412 and adj. $R^2 = 0.66$ for UUE) and acceptable RMSE (2.08 g/d or 9.29% for UNE and 2.08 g/d or 11.6% for UUE).

Table 16. Equations for estimating urinary nitrogen (N) excretion (g/d) of growing pigs fed diets with marginal lysine supply in two sampling periods (SP) based on N balance data and blood metabolite concentrations

Model	Parameter	b	BCI_b	SE_b	*P*-value	AIC	adj. R^2	RMSE
9						396	0.77	1.88
	Intercept	-8.825	[-13.19; -1.773]	2.283	<0.001			
	IBW	0.206	[0.031; 0.329]	0.063	0.002			
	BW	0.136	[0.076; 0.171]	0.018	<0.001			
	NI	0.212	[0.128; 0.306]	0.038	<0.001			
	BUN	1.617	[0.878; 2.169]	0.298	<0.001			
	SC	-0.059	[-0.099; -0.023]	0.021	0.007			
	IGF-I	-0.014	[-0.022; -0.001]	0.005	0.016			
10						411	0.72	2.08
	Intercept	-8.420	[-12.78; -3.860]	1.972	<0.001			
	BW	0.144	[0.092; 0.178]	0.019	<0.001			
	NI	0.239	[0.162; 0.348]	0.040	<0.001			
	BUN	1.587	[0.749; 2.247]	0.336	<0.001			
11						385	0.80	1.72
	BW	0.474	[0.053; 0.756]	0.177	0.009			
	ADG	25.82	[2.456; 42.05]	8.069	0.002			
	NI	-0.485	[-0.737; -0.067]	0.149	0.002			
	BW x ADFI	-0.487	[-0.643; -0.216]	0.094	<0.001			
	BW x NI	0.031	[0.021; 0.040]	0.005	<0.001			
	BW x BUN	-0.205	[-0.294; -0.132]	0.041	<0.001			
	ADG x BUN	-4.769	[-7.337; -0.505]	1.384	0.001			
	ADFI x NI	-0.392	[-0.645; -0.200]	0.099	<0.001			
	ADFI x BUN	8.061	[5.243; 10.51]	1.234	<0.001			

In total 96 observations of 56 animals in two SP were used for estimations, resulting in 56 independent and 40 repeated measures, evenly distributed over both SP. Mean urinary N excretion was 22.4 g/d. Equations are equally valid for both SP. SP1 = 13th week of life; SP2 = 16th week of life.
Regression coefficients (b) and standard errors (SE_b) were obtained using multiple regression. Confidence intervals of the regression coefficients (BCIb) were generated by 1,000 bootstrap data sets. AIC = Akaike information criterion; RMSE = root mean square error; IBW = initial bodyweight, kg; BW = bodyweight, kg; ADG = average daily gain, kg; ADFI = average daily feed intake, kg dry matter; NI = nitrogen intake, g/d; BUN = blood urea nitrogen, mmol/L; SC = serum cortisol, ng/mL; IGF-I = insulin-like growth factor 1, ng/mL.

Table 17. Equations for estimating urinary urea nitrogen (N) excretion (g/d) of growing pigs fed diets with marginal lysine supply in two sampling periods (SP) based on N balance data and blood metabolite concentrations

Model	Parameter	b	BCI_b	SE_b	*P*-value	AIC	adj. R^2	RMSE
12						397	0.72	1.84
	Intercept	-7.861	[-12.49; -1.459]	2.403	0.002			
	IBW	0.175	[0.008; 0.310]	0.065	0.009			
	BW	0.090	[0.032; 0.128]	0.018	<0.001			
	NI	0.186	[0.106; 0.271]	0.038	<0.001			
	BUN	1.525	[0.880; 2.244]	0.307	<0.001			
	SC	-0.079	[-0.119; -0.040]	0.022	<0.001			
	IGF-I	-0.011	[-0.022; -0.001]	0.006	0.053			
13						412	0.66	2.08
	Intercept	-8.077	[-13.19; -4.456]	2.021	<0.001			
	BW	0.096	[0.041; 0.132]	0.019	<0.001			
	NI	0.215	[0.141; 0.325]	0.040	<0.001			
	BUN	1.438	[0.701; 2.254]	0.346	<0.001			
14						380	0.78	1.63
	ADG	16.47	[11.38; 20.64]	2.608	<0.001			
	BW x NI	0.012	[0.007; 0.021]	0.003	0.001			
	BW x BUN	-0.095	[-0.186; -0.042]	0.036	0.010			
	ADG x BUN	-2.967	[-3.961; -1.707]	0.564	<0.001			
	ADG x SC	-0.090	[-0.131; -0.042]	0.024	<0.001			
	ADFI x NI	-0.302	[-0.536; -0.164]	0.090	0.001			
	ADFI x BUN	2.839	[1.332; 5.366]	1.013	0.006			
	ADFI x IGF-I	-0.007	[-0.012; -0.001]	0.003	0.011			
	NI x BUN	0.050	[0.036; 0.061]	0.006	<0.001			

In total 96 observations of 56 animals in two SP were used for estimations, resulting in 56 independent and 40 repeated measures, evenly distributed over both SP. Mean urinary urea N excretion was 18.0 g/d. Equations are equally valid for both SP. SP1 = 13th week of life; SP2 = 16th week of life.
Regression coefficients (b) and standard errors (SE_b) were obtained using multiple regression. Confidence intervals of the regression coefficients (BCIb) were generated by 1,000 bootstrap data sets. AIC = Akaike information criterion; RMSE = root mean square error; IBW = initial bodyweight, kg; BW = bodyweight, kg; ADG = average daily gain, kg; ADFI = average daily feed intake, kg dry matter; NI = nitrogen intake, g/d; BUN = blood urea nitrogen, mmol/L; SC = serum cortisol, ng/mL; IGF-I = insulin-like growth factor 1, ng/mL.

4.2 Complete data set

For the evaluation of the complete data set, 508 observations were available for calculations in SP1 and 458 observations in SP2. The 25 pigs of cohort no. five had to be excluded as they were not provided with the correct diet in SP2. In the cohort no. 13 sampling was not possible due to the Covid-19 Pandemic and the lockdown imposed. The observations also include the results of the extended data collection subsample of pigs, but the results of the N metabolism are all based on the estimated values, also for the N balance pigs. The measured N balance values were not used for the evaluations of the following section.

4.2.1 Performance traits

The BW of the animals in SP1 was in a range from 27.5 to 56.5 kg (Table 18) with a mean of 40.5 kg. In SP2, the mean BW of the pigs was 60.2 kg with a range from 44.0 to 87.0 kg. With exception of the maximum weight in SP2, the range and the average BW in both SP was in accordance with the results of the N balance data set, indicating it was a representative subsample of the complete data set. The high maximum weight was in cohort no. one, where SP2 was conducted for five weeks (18th week of life) after SP1. Since the pigs grew faster than assumed in the planning phase (0.90 kg mean ADG in SP1 and 0.97 kg mean ADG in SP2, compared to a calculated ADG of 0.80 kg; see 3.1.2), it was decided to conduct SP2 in the 16th week of life for the rest of the experiment.

Table 18. Performance traits of growing pigs in two sampling periods (SP), fed diets with marginal lysine supply

	BW, kg		ADG, kg		ADFI, kg DM		G:F, kg/kg	
SP	1	2	1	2	1	2	1	2
MEAN	40.5	60.2	0.90	0.97	1.86	2.51	0.49	0.38
SD	4.80	7.04	0.13	0.16	0.25	0.37	0.07	0.06
%CV	11.7	11.7	14.3	16.5	13.7	14.6	13.5	15.2
MIN	27.5	44.0	0.45	0.58	1.20	1.36	0.26	0.22
MAX	56.5	87.0	1.33	1.38	2.49	3.56	0.79	0.57
SCC	0.92		0.39		0.58		0.14	
P-value	<0.001		<0.001		<0.001		0.003	

Observations of 508 animals in SP1 and 458 animals in SP2 were available. SP1 = 13th week of life; SP2 = 16th week of life. BW = body weight; ADG = average daily gain; ADFI = average daily dry matter feed intake; G:F = gain-to-feed ratio; SD = standard deviation; %CV = percent coefficient of variation; MIN = minimum value; MAX = maximum value; SCC = Spearman's rank correlation coefficient.

The average ADFI was 1.86 kg DM in SP1 and 2.51 kg DM in SP2 and thus approximately 300 g and 500 g higher than expected in SP1 and SP2, respectively (mean ADFI was calculated to be 1.7 kg as fed in SP1 and 2.3 kg as fed in SP2). A great variation of ADFI was observed, with the lowest observed ADFI of 1.20 kg DM in SP1 and the highest observed ADFI of 3.56 kg DM in SP2 (Table 18).

Barrows consumed more feed than gilts in both SP (1.90 kg DM vs. 1.83 kg DM in SP1 and 2.61 kg DM vs. 2.41 kg DM in SP2; $p < 0.001$; Table 19). The variation in ADFI could partly explain the observed variation in ADG. The ADG ranged from a minimum of 0.45 kg in SP1 to a maximum of 1.38 kg in SP2 with barrows gaining 60 g more per day on average than gilts (0.96 kg vs. 0.90 kg; Table 19). The difference between the sexes was small in SP1 (0.91 kg vs. 0.89 kg) but bigger in SP2 (1.01 kg vs. 0.93 kg). Both traits were moderately correlated between the two SP (r = 0.39 for ADG and r = 0.58 for ADFI; Table 18).

The mean G:F ratio was 0.49 in SP1 and significantly higher than 0.38 in SP2 (Table 19). Within this trait, no difference between gilts and barrows existed. The most efficient animal gained 0.79 kg BW per kg feed intake while the least efficient animal only gained 0.22 kg BW (Table 18). The measured values of G:F ratio hardly correlated between both SP (r = 0.14).

Table 19. Two-factorial analysis of the performance traits

SP	sex	BW, kg	ADG, kg	ADFI, kg DM	G:F
2-way interactions					
1	gilts	40.2^{c}	0.89^{c}	1.83^{d}	0.49^{a}
	barrows	40.8^{c}	0.91^{bc}	1.90^{c}	0.48^{a}
2	gilts	59.5^{b}	0.93^{b}	2.41^{b}	0.38^{b}
	barrows	61.4^{a}	1.01^{a}	2.61^{a}	0.38^{b}
	pooled SEM	0.76	0.02	0.04	0.008
main effects					
1		40.5^{b}	0.90^{b}	1.86^{b}	0.49^{a}
2		60.2^{a}	0.97^{a}	2.51^{a}	0.38^{b}
pooled SEM		0.38	0.01	0.02	0.004
	gilts	49.7	0.90^{b}	2.13^{a}	0.43
	barrows	50.9	0.96^{a}	2.25^{b}	0.43
	SEM	0.77	0.01	0.03	0.004
P-values	SP	<0.001	<0.001	<0.001	<0.001
	sex	0.135	<0.001	<0.001	0.674
	SP x sex	<0.001	<0.001	<0.001	<0.001

Observations of 508 animals were available in SP1 and observations of 458 animals were available in SP2. SP1 = 13th week of life; SP2 = 16th week of life. Values in the same column and within the same subheading not sharing the same superscript letter are significantly different ($p \leq 0.05$).
SP = sampling period; BW = body weight; ADG = average daily gain; ADFI = average daily dry matter feed intake; G:F = gain-to-feed ratio; SEM = standard error of the mean.

4.2.2 Nitrogen metabolism

Nitrogen and lysine intake

Among all animals, NI ranged from 37.5 to 104 g/d and LI from 9.30 to 27.6 g/d (Table 20). Comparing the SP between each other, both traits were moderately correlated (r = 0.55 for NI and r = 0.52 for LI). With 71.0 g/d, the mean NI was significantly higher in SP2 than in SP1 (64.0 g/d), whereas the mean LI was the same in both SP (19.7 g/d in SP1 and 19.8 g/d in SP2; Table 21). Barrows ingested more N per day than gilts in SP1 (65.1 g/d vs. 62.9 g/d; $p < 0.001$) and in SP2 (73.7 g/d vs. 68.1 g/d; $p < 0.001$). For LI the effect of sex also existed in SP2 (20.6 g LI/d for barrows vs. 19.1 g LI/d for gilts) and for the average LI (20.3 g/d for barrows and 19.3 g/d for gilts). In SP1, the mean values for LI did not differ significantly (20.1 g/d for barrows and 19.4 g/d for gilts).

Table 20. Nitrogen intake, lysine intake and estimated excretion of urinary nitrogen and urea nitrogen of growing pigs in two sampling periods (SP), fed diets with marginal lysine supply

	NI, g/d		LI, g/d		UNE, g/d		UUE, g/d	
SP	1	2	1	2	1	2	1	2
MEAN	64.0	71.0	19.7	19.8	21.1	25.3	17.3	20.4
SD	8.71	11.1	2.78	2.72	3.44	4.14	3.02	3.56
%CV	13.6	15.6	14.1	13.7	16.3	16.4	17.5	17.5
MIN	41.6	37.5	13.2	9.30	12.1	12.9	8.80	9.67
MAX	87.9	104	27.6	26.0	32.5	42.3	27.3	35.4
SCC	0.55		0.52		0.70		0.68	
P-value	<0.001		<0.001		<0.001		<0.001	

Observations of 508 animals in SP1 and 458 animals in SP2 were available. SP1 = 13th week of life; SP2 = 16th week of life. Values for UNE were estimated using estimation equation no. 9 and values for UUE were estimated using estimation equation no. 12. NI = nitrogen intake; LI = lysine intake; UNE = urinary nitrogen excretion; UUE = urinary urea nitrogen excretion; SD = standard deviation; %CV = percent coefficient of variation; MIN = minimum value; MAX = maximum value; SCC = Spearman's rank correlation coefficient.

Urinary excretion

Values for UNE and UUE were obtained using the estimation equations no. 9 and no. 12 (Table 16 and Table 17). The estimated UNE showed a minimum of 12.1 g/d, a maximum of 42.3 g/d and was 21.1 g/d on average in SP1 and 25.3 g/d in SP2 (Table 20). The mean UUE was 17.3 g/d in SP1 and 20.4 g/d in SP2 and the estimated values ranged from 8.80 g/d to 35.4 g/d. Comparing SP1 with SP2, the estimated values of UNE and UUE were highly correlated (r = 0.70 for UNE and r = 0.68 for UUE).

For both UNE and UUE differences between the sexes existed (Table 21). In both SP barrows had significantly higher UNE (21.7 g/d vs. 20.6 g/d in SP1 and 26.5 g/d vs. 24.0 g/d in SP2) and UUE (17.7 g/d vs. 16.8 g/d in SP1 and 21.6 g/d vs. 19.5 g/d in SP2) than gilts.

Table 21. Two-factorial analysis of nitrogen intake, lysine intake and excretion of urinary nitrogen and urea nitrogen

SP	sex	NI, g/d	LI, g/d	UNE g/d	UUE, g/d
2-way interactions					
1	gilts	62.9^{d}	19.4^{bc}	20.6^{d}	16.8^{d}
	barrows	65.1^{c}	20.1^{ab}	21.7^{c}	17.7^{c}
2	gilts	68.1^{b}	19.1^{c}	24.0^{b}	19.5^{b}
	barrows	73.7^{a}	20.6^{a}	26.5^{a}	21.6^{a}
	pooled SEM	1.25	0.37	0.47	0.41
main effects					
1		64.0^{b}	19.7	21.1^{b}	17.3^{b}
2		71.0^{a}	19.8	25.3^{a}	20.4^{a}
pooled SEM		0.63	0.19	0.24	0.21
	gilts	65.5^{b}	19.3^{b}	22.2^{b}	18.0^{b}
	barrows	69.2^{a}	20.3^{a}	24.0^{a}	19.5^{a}
	SEM	0.66	0.19	0.27	0.23
P-values	SP	<0.001	0.331	<0.001	<0.001
	sex	<0.001	<0.001	<0.001	<0.001
	SP x sex	<0.001	<0.001	<0.001	<0.001

Observations of 508 animals were available in SP1 and observations of 458 animals were available in SP2. SP1 = 13th week of life; SP2 = 16th week of life. Values in the same column and within the same subheading not sharing the same superscript letter are significantly different ($p \leq 0.05$). Values for UNE were estimated using estimation equation no. 9 and values for UUE were estimated using estimation equation no. 12. SP = sampling period; NI = nitrogen intake; LI = lysine intake; UNE = urinary nitrogen excretion; UUE = urinary urea nitrogen excretion; SEM = standard error of the mean.

Retention

The NR of the pigs was estimated by using model no. 4 (Table 14) and subsequently LR, NUE and LUE were calculated as described in chapter 3.3.1. Daily NR was within a range from 13.6 g to 46.7 g and daily LR varied between 6.12 g and 21.0 g (Table 22). No significant differences between SP existed in the estimated NR and LR (Table 23).

For both traits a significant effect of sex and interaction between sex and SP existed. In SP2 barrows retained significantly more N and lysine than gilts (32.4 g N/d vs. 30.8 g N/d and 14.6 g lysine/d vs. 13.9 g lysine/d), whereas in SP1 the greater retention was not significant (31.4 g N/d vs. 30.8 g N/d and 14.1 g lysine/d vs. 13.9 g lysine/d). The estimated values correlated moderately between the SP (r = 0.44 for NR as well as LR).

Table 22. Estimated retention and utilization efficiency of dietary nitrogen and lysine of growing pigs in two sampling periods (SP), fed diets with marginal lysine supply

	NR, g/d		LR, g/d		NUE, %		LUE, %	
SP	1	2	1	2	1	2	1	2
MEAN	31.1	31.7	14.0	14.2	48.6	44.6	71.1	71.8
SD	4.57	5.15	2.06	2.32	2.93	2.64	5.07	5.48
%CV	14.7	16.3	14.7	16.3	6.03	5.93	7.13	7.61
MIN	15.9	13.6	7.18	6.12	37.3	35.2	50.1	54.3
MAX	46.7	44.5	21.0	20.0	57.9	52.0	87.9	88.0
SCC	0.44		0.44		0.33		0.39	
P-value	<0.001		<0.001		<0.001		<0.001	

Observations of 508 animals in SP1 and 458 animals in SP2 were available. SP1 = 13th week of life; SP2 = 16th week of life. Values for NR were estimated using estimation equation no 4. LR was calculated as NR x 6.25 x 0.072. NR = nitrogen retention; LR = lysine retention; NUE = nitrogen utilization efficiency; LUE = lysine utilization efficiency; SD = standard deviation; %CV = percent coefficient of variation; MIN = minimum value; MAX = maximum value; SCC = Spearman's rank correlation coefficient.

The estimated NR values of the complete data set were strongly linearly dependent on LI (R^2 = 0.77; Figure 11). Across both SP every additional gram of LI resulted in an additional 1.48 g NR or 9.25 g protein retention corresponding to a LUE of 66.6%.

Table 23. Two-factorial analysis of the estimated retention and utilization efficiency of dietary nitrogen and lysine

SP	sex	NR, g/d	LR, g/d	NUE, %	LUE, %
2-way interactions					
1	gilts	30.8^{b}	13.9^{b}	49.0^{a}	71.5^{b}
	barrows	31.4^{ab}	14.1^{ab}	48.3^{b}	70.6^{b}
2	gilts	30.8^{b}	13.9^{b}	45.1^{c}	72.6^{a}
	barrows	32.4^{a}	14.6^{a}	44.0^{d}	71.0^{b}
	pooled SEM	0.62	0.28	0.36	0.67
main effects					
1		31.1	14.0	48.6^{a}	71.1^{b}
2		31.7	14.2	44.6^{b}	71.8^{a}
pooled SEM		0.31	0.14	0.18	0.34
	gilts	30.8^{b}	13.9^{b}	46.9^{a}	71.9^{a}
	barrows	31.8^{a}	14.3^{a}	46.1^{b}	70.8^{b}
	SEM	0.32	0.14	0.22	0.34
P-values	SP	0.133	0.139	<0.001	0.006
	sex	0.008	0.008	<0.001	0.004
	SP x sex	0.003	0.003	<0.001	<0.001

Observations of 508 animals were available in SP1 and observations of 458 animals were available in SP2. SP1 = 13th week of life; SP2 = 16th week of life. Values in the same column and within the same subheading not sharing the same superscript letter are significantly different ($p \leq 0.05$). Values for NR were estimated using estimation equation no. 4. LR was calculated as NR x 6.25 x 0.072. SP = sampling period; NR = nitrogen retention; LR = lysine retention; NUE = nitrogen utilization efficiency; LUE = lysine utilization efficiency; SEM = standard error of the mean.

When considering the SP individually, the slope of the regression was 1.44 for SP1 and 1.51 for SP2 which corresponds to a difference of less than .5% and slopes can therefore be regarded as equal. Since the mean NR did not differ between both SP (Table 23) and R^2 and RMSE of the individual regressions were similar (R^2 = 0.76 and RMSE = 2.22 g/d for SP1 and R^2 = 0.79 and RMSE = 2.39 g/d for SP2) there is no need to differentiate between the SP.

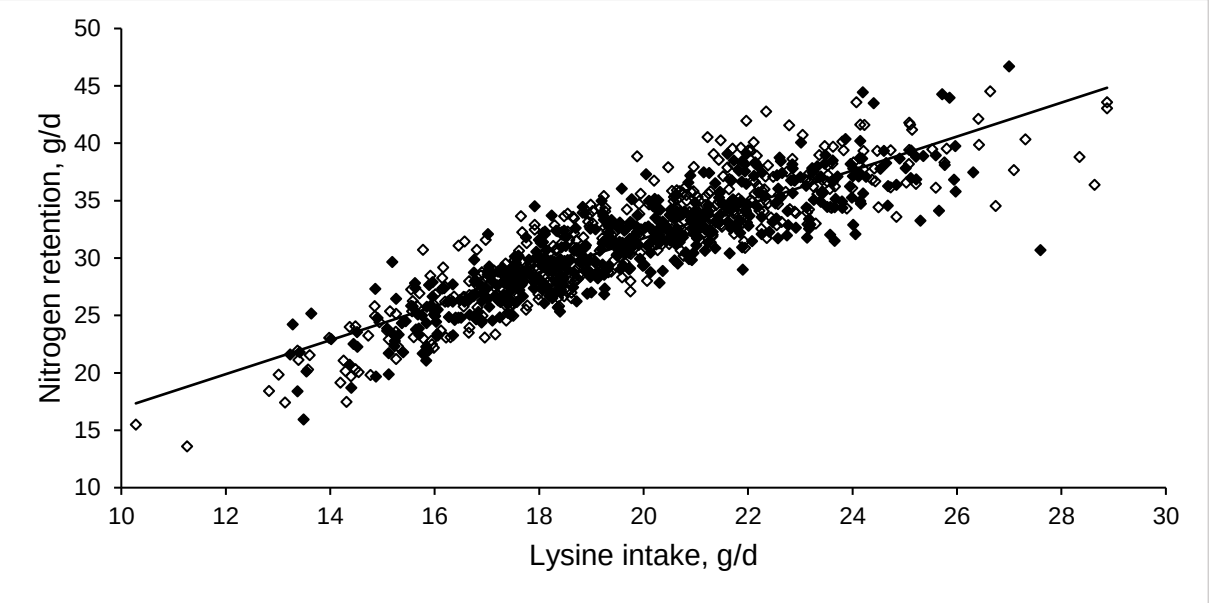

Figure 11. Estimated nitrogen (N) retention of growing pigs fed diets with marginal lysine supply within two sampling periods (filled symbols = sampling period 1; open symbols = sampling period 2) as a function of lysine intake (y = 2.14 + 1.48x; Mean N retention = 31.4 g/d, R^2 = 0.77, RMSE = 2.31 g/d). N retention was estimated using estimation equation no 4. Observations of 508 animals were available in sampling period 1 and observations of 458 animals were available in sampling period 2.

Utilization efficiency

The mean estimated NUE for all animals was 48.6% in SP1 and it was significantly lower in SP2 (44.6%; Table 23). The range of the observed values was wide with maximum values of 57.9% in SP1 and 52.0% in SP2 (Table 22). The least efficient animal retained 37.3% of the ingested N in SP1 and 35.2% in SP2. The values of NUE showed a low correlation comparing SP1 with SP2 (r = 0.33), indicating that the pigs with high NUE in SP1 not necessarily were the most efficient in SP2 and vice versa. In addition to the effect of the SP, a significant effect of sex on NUE existed (Table 23). In both SP, gilts utilized dietary N more efficiently than barrows (49.0% vs. 48.3% in SP1 and 45.1% vs. 44.0% in SP2; $p < 0.001$).

The mean LUE was 71.1% in SP1 and increased significantly to 71.8% in SP2 (Table 23), but the estimated maximum values were similar in both SP (87.9% in SP1 and 88.0% in SP2; Table 22). Estimated values of LUE correlated only slightly between the two SP (r = 0.39).

An effect of sex on LUE also existed, with gilts utilizing dietary lysine more efficiently than barrows (71.9% on average vs. 70.8%; Table 23). This difference was clearly in SP2 (72.6% for gilts vs. 71.0% for barrows), but only tended to exist in SP1 (71.5% for gilts vs. 70.6% for barrows), which cause a significant interaction effect.

4.2.3 Blood metabolites

The mean BUN concentration in the complete data set was 5.68 mmol/L in SP1 and 5.57 mmol/L in SP2 (Table 24). Although differences were small, values significantly differed from each other (Table 25). The minimum BUN concentration was almost the same in both SP (3.68 mmol/L in SP1 and 3.70 mmol/L in SP2), whereas the maximum BUN concentration was lower in SP2 (8.67 mmol/L) than in SP1 (8.90 mmol/L). A strong correlation of the measured values between SP1 and SP2 could be observed (r = 0.74).

The mean BUN concentration of gilts (5.40 mmol/L; Table 25) was significantly lower than that of barrows (5.81 mmol/L). The BUN concentration of gilts decreased significantly from SP1 (5.52 mmol/L) to SP2 (5.37 mmol/L) whereas BUN concentration of barrows remained on the same level (5.84 mmol/L in SP1 and 5.87 mmol/L in SP2).

Table 24. Concentrations of blood metabolites related to nitrogen metabolism of growing pigs in two sampling periods (SP), fed diets with marginal lysine supply

	BUN, mmol/L		SC, ng/mL		IGF-I, ng/mL	
SP	1	2	1	2	1	2
MEAN	5.68	5.57	24.8	21.1	208	210
SD	0.87	0.85	8.70	7.99	42.7	39.8
%CV	15.3	15.3	35.1	37.8	20.6	19.0
MIN	3.68	3.70	3.00	4.90	83.7	106
MAX	8.90	8.67	71.8	59.8	371	333
SCC	0.74		0.54		0.75	
P-value	<0.001		<0.001		<0.001	

Observations of 508 animals in SP1 and 458 animals in SP2 were available. SP1 = 13th week of life; SP2 = 16th week of life. BUN = blood urea nitrogen; SC = serum cortisol; IGF-I = insulin-like growth factor 1; SD = standard deviation; %CV = percent coefficient of variation; MIN = minimum value; MAX = maximum value.

The average SC concentration of the complete data set decreased significantly from 24.8 ng/mL in SP1 to 21.1 ng/mL in SP2 (Table 25), but values were highly variable, ranging from 3.00 to 71.8 ng/mL in SP1 (35.1% CV; Table 24) and from 4.90 to 59.8 ng/mL in SP2 (37.8% CV). The measured SC values in SP1 and SP2 showed a moderate correlation (r = 0.54). The decline was more pronounced in gilts (- 4.2 ng/mL) than in barrows (- 3.7 ng/mL), leading to a significant effect of sex in SP2 (19.8 ng/mL for gilts and 22.0 ng/mL for barrows). On average, SC concentrations of gilts (22.2 ng/mL; Table 25) were also significantly lower than those of barrows (24.2 ng/mL; p = 0.001).

The IGF-I concentrations were 208 ng/mL (SP1; Table 25) and 210 ng/mL blood serum (SP2) on average and no significant difference between the SP was detected. In both SP, gilts had significantly higher average IGF-I concentrations in blood serum than barrows (213 ng/mL vs. 202 ng/mL in SP1 and 223 ng/mL vs. 196 ng/mL in SP2). The IGF-I concentration of gilts increased significantly from SP1 to SP2, whereas the IGF-I concentration of barrows tended to decrease. The overall variation was very high with values ranging from 83.7 to 371 ng/mL in SP1 (20.6% CV; Table 24) and 106 to 333 ng/mL in SP2 (19.0% CV). However, values were strongly correlated in both SP (r = 0.75).

Table 25. Two-factorial analysis of blood metabolite concentrations related to nitrogen metabolism

SP	sex	BUN, mmol/L	SC, ng/mL	IGF-I, ng/mL
2-way interactions				
1	gilts	5.52^{b}	24.0^{a}	213^{b}
	barrows	5.84^{a}	25.7^{a}	202^{c}
2	gilts	5.34^{c}	19.8^{c}	223^{a}
	barrows	5.87^{a}	22.0^{b}	196^{c}
	pooled SEM	0.11	1.07	5.16
main effects				
1		5.68^{a}	24.8^{a}	208
2		5.57^{b}	21.1^{b}	210
pooled SEM		0.06	0.54	2.66
	gilts	5.40^{b}	22.2^{b}	219^{a}
	barrows	5.81^{a}	24.2^{a}	199^{b}
	SEM	0.05	0.57	2.67
P-values	SP	0.006	<0.001	0.267
	sex	<0.001	0.001	<0.001
	SP x sex	<0.001	<0.001	<0.001

Observations of 508 animals were available in SP1 and observations of 458 animals were available in SP2. SP1 = 13th week of life; SP2 = 16th week of life. Values in the same column and within the same subheading not sharing the same superscript letter are significantly different ($p \leq 0.05$). BUN = blood urea nitrogen; SC = serum cortisol; IGF-I = insulin-like growth factor 1; SEM = standard error of the mean.

4.2.4 Effect of boar on the nitrogen utilization of the offspring

Marked differences between the offspring of single boars in the mean values of the traits were detected. Due to the abundance of data, the following evaluation focuses on NR, NUE and LUE. Data for the remaining characteristics is provided in the appendix (Appendices 9-11). Since the observations of SP2 of cohort no. 5 and no. 13 were not available for evaluations, the number of offspring of the boars no. 1, 4, 7, 8, 10, 11, and 14 in SP2 was different from that in SP 1 (Table 26).

The lowest mean NR of a group of offspring was 28.9 g/d in SP1 (boar no.4; Table 27) and it was significantly lower than the mean NR of the offspring of boar no. 9 (33.1 g/d), boar no. 18 (33.7 g/d) and boar no. 17 (34.9 g/d), which showed the highest NR. The mean values of the remaining groups of offspring ranged from 29.6 to 32.6 g NR/d and did not significantly differ from the offspring of boar no. 4. In SP2, the highest NR was recorded for the offspring of boar no. 20 (36.3 g/d), which was 10.2 g/d more than the NR of the group of offspring of boar no. 10 (26.1 g NR/d), which had the lowest NR of all groups. For the offspring of boar no. 3 (30.0 g NR/d) and boar no. 9 (29.8 g NR/d) also a significantly lower NR was observed. The means of the other groups of offspring varied from 30.2 to 35.3 g NR/d without significant differences.

Table 26. Number of offspring of the boars (no. 1 to 20) used for the evaluations in the two experimental periods and slaughtered

Boar	Sampling period 1	Sampling period 2	Slaughter
1	29	20 (2)	27
2	25	25 (2)	23
3	32 (2)	32 (2)	30
4	26	18	26
5	26	26	23
6	24	24	24
7	30	26	30
8	28	24 (2)	25
9	30 (4)	30 (6)	23
10	21 (6)	12 (4)	16
11	33 (6)	21 (2)	33
12	29 (2)	29 (2)	29
13	16 (2)	16 (2)	10
14	33 (8)	29 (6)	26
15	30 (8)	30 (8)	24
16	21 (4)	21 (4)	12
17	17	17	12
18	20	20	14
19	20 (4)	20 (4)	20
20	18 (2)	18 (2)	18
Total	508	458	445
Mean	25	23	22
SD	5.5	5.5	6.6
MIN	16	12	10
MAX	33	32	33

In sampling period 1, observations of all pigs of the experiment were evaluated. Observations of cohort no. 5 and no. 13 with each 25 animals could not be used in sampling period 2. At slaughter, carcass data was not available for 62 pigs, since 56 were euthanized at the experimental station for digesta sampling and the carcasses of six animals were not clearly identifiable. One animal died during the 8th week of the experiment for unknown reasons. Values in brackets represent the number of offspring of the respective boar that were used for nitrogen balance during each sampling period. SD = standard deviation; MIN = minimum value; MAX = maximum value.

The correlation of NR between the two SP (r = 0.81; Table 28) indicated, that the offspring of the respective boars tended to retain either more or less N in both periods.

Table 27. Mean nitrogen retention, nitrogen utilization efficiency, and lysine utilization efficiency of the offspring of the used boars (no. 1 to 20) in the two sampling periods (SP)

	Sampling period 1			Sampling period 2		
Boar	NR, g/d	NUE, %	LUE, %	NR, g/d	NUE, %	LUE, %
1	29.6^{bc}	46.3^{e}	67.8^{e}	35.3^{ab}	43.1^{b}	69.4^{cde}
2	30.5^{abc}	49.5^{abc}	74.9^{abc}	33.6^{abc}	45.2^{ab}	71.9^{bcde}
3	29.4^{bc}	48.5^{bcde}	72.6^{abcd}	30.0^{cd}	43.1^{b}	70.6^{bcde}
4	28.9^{c}	47.3^{cde}	67.4^{e}	31.6^{abcd}	45.7^{ab}	74.2^{bcd}
5	30.5^{abc}	48.8^{bcde}	70.5^{bcde}	31.8^{abc}	45.2^{ab}	71.9^{bcde}
6	30.6^{abc}	47.7^{cde}	69.9^{de}	31.0^{abcd}	44.6^{ab}	70.3^{bcde}
7	31.4^{abc}	48.5^{bcde}	69.9^{de}	32.4^{abc}	44.3^{b}	71.4^{bcde}
8	30.0^{abc}	47.8^{cde}	70.0^{cde}	29.9^{bcd}	45.1^{ab}	74.5^{bc}
9	33.1^{ab}	50.6^{ab}	75.9^{a}	29.8^{cd}	44.4^{b}	74.1^{bcd}
10	30.2^{abc}	49.1^{bcd}	73.7^{abcd}	26.1^{d}	43.1^{b}	70.0^{bcde}
11	32.6^{abc}	47.2^{cde}	69.8^{de}	32.5^{abc}	43.8^{b}	69.3^{de}
12	31.8^{abc}	49.1^{bcd}	70.4^{bcde}	31.4^{abcd}	44.6^{ab}	70.1^{bcde}
13	31.9^{abc}	49.9^{abc}	71.6^{abcde}	31.4^{abcd}	45.4^{ab}	71.6^{bcde}
14	32.1^{abc}	49.5^{abc}	70.1^{cde}	31.0^{abcd}	44.7^{ab}	68.9^{e}
15	29.3^{bc}	47.7^{cde}	69.1^{de}	30.2^{bcd}	44.4^{b}	71.2^{bcde}
16	32.5^{abc}	48.9^{bcd}	70.1^{cde}	30.5^{abcd}	43.7^{b}	68.4^{e}
17	34.9^{a}	49.8^{abc}	71.2^{abcde}	35.0^{abc}	45.0^{ab}	73.1^{bcde}
18	33.7^{ab}	52.5^{a}	75.4^{ab}	33.1^{abc}	46.2^{ab}	75.3^{ab}
19	28.9^{bc}	46.6^{de}	70.3^{bcde}	31.6^{abcd}	44.4^{ab}	75.4^{ab}
20	32.2^{abc}	48.9^{bcde}	73.6^{abcd}	36.3^{a}	47.2^{a}	80.4^{a}
pooled SEM	3.98	2.40	4.17	4.69	2.43	4.70
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Observations of 508 animals were available in SP1 and observations of 458 animals were available in SP2. SP1 = 13th week of life; SP2 = 16th week of life. Different superscript letters within a column indicate significant differences between the group of offspring of the respective boar ($p \leq 0.05$). NR = nitrogen retention; NUE = nitrogen utilization efficiency; LUE = lysine utilization efficiency; SEM = standard error of the mean.

The mean values for NUE of the offspring groups ranged from 46.3 to 52.5% in SP1 and 43.1 to 47.2% in SP2 (Table 27). Due to the wider range, more differences between the groups were observed in SP1 than in SP2. The most pronounced differences existed for the offspring of boars no. 18 (52.5% NUE) and no. 9 (50.6% NUE) which utilized dietary N significantly more efficient than the offspring of boars no. 1 (46.3% NUE), no. 4 (47.3% NUE), no. 6 (47.7% NUE), no. 8 (47.8% NUE), no. 11 (47.2% NUE), no. 15 (47.7% NUE), and no. 19 (46.6% NUE).

In SP2, the offspring of boar no. 20 (47.2 % NUE) were the most efficient and only for this boar significant differences from the other groups of offspring were observed. All offspring groups with a mean NUE of ≤ 44.4% (1, 3, 7, 9, 10, 11, 15, and 16) utilized dietary N significantly less efficient than the offspring of boar no. 20. The offspring of boars no. 1 and no. 11 had low NUE values in both SP, whereas the offspring of boars no. 2, no. 13, and no. 18 were among the most efficient in SP1 and SP2. The highly significant correlation of NUE between the two SP (r = 0.72; Table 28) underlined that the offspring of the respective boars did either utilize dietary N more efficiently or less efficiently in both SP.

Table 28. Spearman correlation coefficients of nitrogen retention (NR), nitrogen utilization efficiency (NUE), and lysine utilization efficiency (LUE), comparing the mean values of the offspring of the boars between the two sampling periods (SP)

		SP 1			SP 2		
		NR, g/d	NUE, %	LUE, %	NR, g/d	NUE, %	LUE, %
SP 1	NR, g/d						
	NUE, %	0.46					
	LUE, %	n.s.	0.60				
SP 2	NR, g/d	0.81	n.s.	n.s.			
	NUE, %	n.s.	0.72	0.45	0.39		
	LUE, %	n.s.	0.42	0.79	n.s.	0.66	

Correlations with |R| ≥ 0.44 are significant at $p \leq 0.05$
Correlations with |R| ≥ 0.38 are significant at $p \leq 0.10$
n.s. = not significant

The mean LUE of the offspring groups in SP1 also was highly variable with a range of 8.4 percentage points between the offspring of the most and the least efficient boars (Table 27). The offspring of boars no. 2 (74.9%), no. 9 (75.9%), and no. 18 (75.4%) showed the highest values for LUE, whereas the offspring of boars no. 1 (67.8%) and no. 4 (67.4%) were the least efficient ones. In SP2 the range between the offspring of the most efficient boar (no. 20, 80.4% LUE) and the least efficient boar (no. 16, 68.4% LUE) extended to 12.0 percentage points. In addition, significant differences between the offspring of boars no. 19 (75,4% LUE) and no. 18 (75.3% LUE) with the offspring of boars no. 1 (69.4% LUE), no. 11 (69.3% LUE), and no. 14 (68.9% LUE) existed. Regarding LUE, offspring of boars no. 1 and no. 11 also were among the less efficient in both SP, whereas the offspring of boars no. 9, no. 18, and no. 20 utilized dietary lysine highly efficient in SP1 and in SP2. Similar to NUE, LUE showed a high correlation between the SP (r = 0.79; Table 28), indicating that the pigs were either utilizing dietary lysine more or less efficiently in both SP.

Due to the highly correlated values for NR, NUE and LUE, both SP were considered together for further evaluations. The mean NR of the offspring groups ranged from 28.7 g/d to 34.9 g/d (Figure 12). The offspring of the boars no. 1, no. 2, no.7, no. 9, no. 12, no. 13, no. 14, and no.16 did not significantly differ from any other offspring group. Nevertheless, significant differences existed between the offspring of the boars with the highest NR (34.9 g/d, 33.4 g/d, and 34.2 g/d for boars no. 17, no. 18, and no. 20, respectively) and the offspring of the boars with the lowest NR (29.7 g/d, 28.7 g/d, and 29.7 g/d for boars no. 3, no. 10, and no. 15, respectively). The mean NR of the offspring of the latter three boars (29.4 g/d) was 4.8 g/d lower than the average NR of the offspring of the three boars showing the highest NR (34.2 g/d).

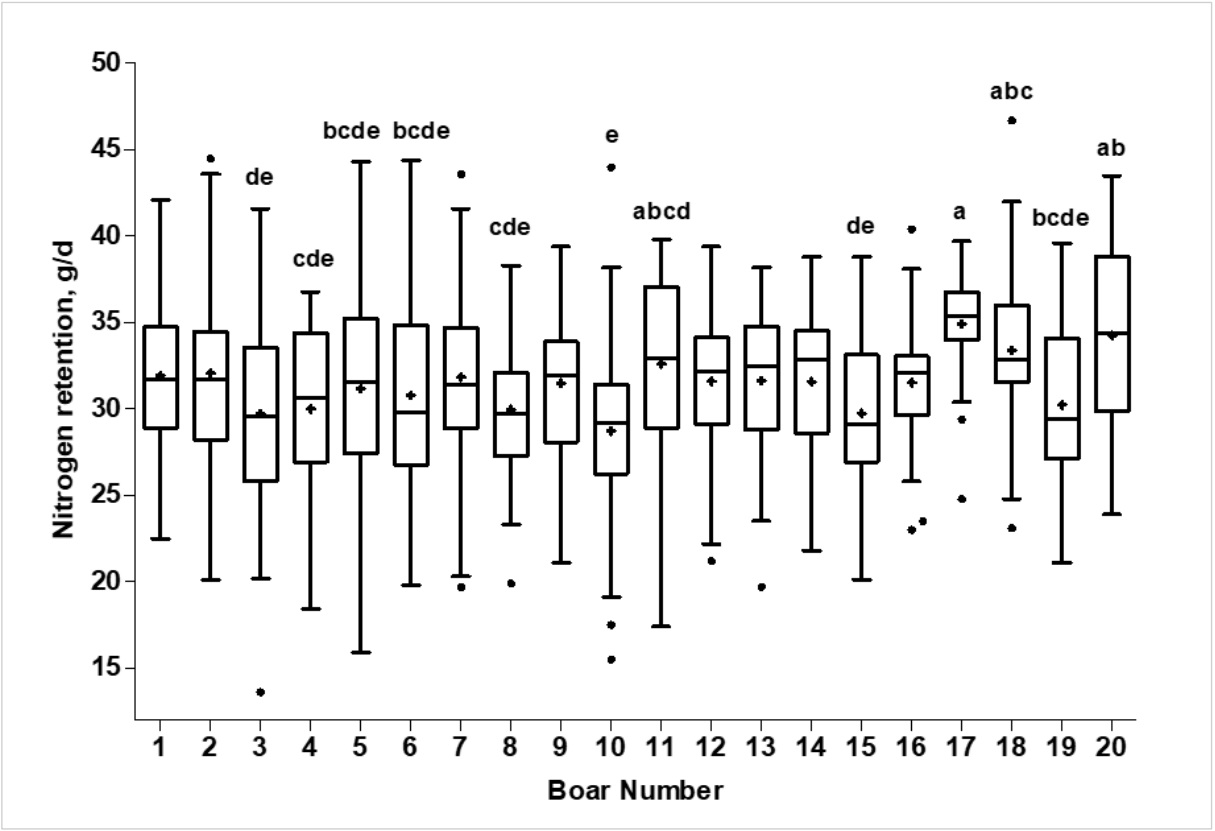

Figure 12. Distribution of the nitrogen retention of the offspring of the boars (no. 1 to 20) within both sampling periods. Observations of 508 animals were available in sampling period 1 and observations of 458 animals were available in sampling period 2. The number of observations available per boar varied between 32 (boar no. 13) and 64 (boar no. 3) and was 48 on average. Different superscript letters above the boxes indicate significant differences between the group of offspring of the respective boar ($p \leq 0.05$).

Regarding NUE within both SP (Figure 13) a large variation existed for the offspring groups of the boars, with a maximum range of 35.4 to 57.9% for the offspring group of boar no. 10. The smallest range of values (9.4 percentage points) was observed for the offspring of boar no. 20, with the least efficient offspring retaining 43.7% of the ingested N and the most efficient 53.1%.

Comparing the means of NUE of the offspring groups, the offspring of boar no. 18 (49.3% NUE) utilized dietary N most efficient and on average 4.3 percentage points better than the offspring of the least efficient boar (no. 1, 45.0% NUE). Significant differences existed also to the offspring of boar no. 3 (45.8% NUE), no. 4 (46.6% NUE), no. 6 (46.1% NUE), no. 7 (46.5% NUE), no. 8 (46.5% NUE), no. 11 (45.9% NUE), no. 15 (46.0% NUE), no. 16 (46.3% NUE), and no. 19 (45.5% NUE). The mean value of NUE for the offspring of the second highest efficient boar (no. 20, 48.0% NUE) was only significantly different from the mean of the offspring of boar no. 1, indicating that the means for NUE of the offspring groups were rather similar.

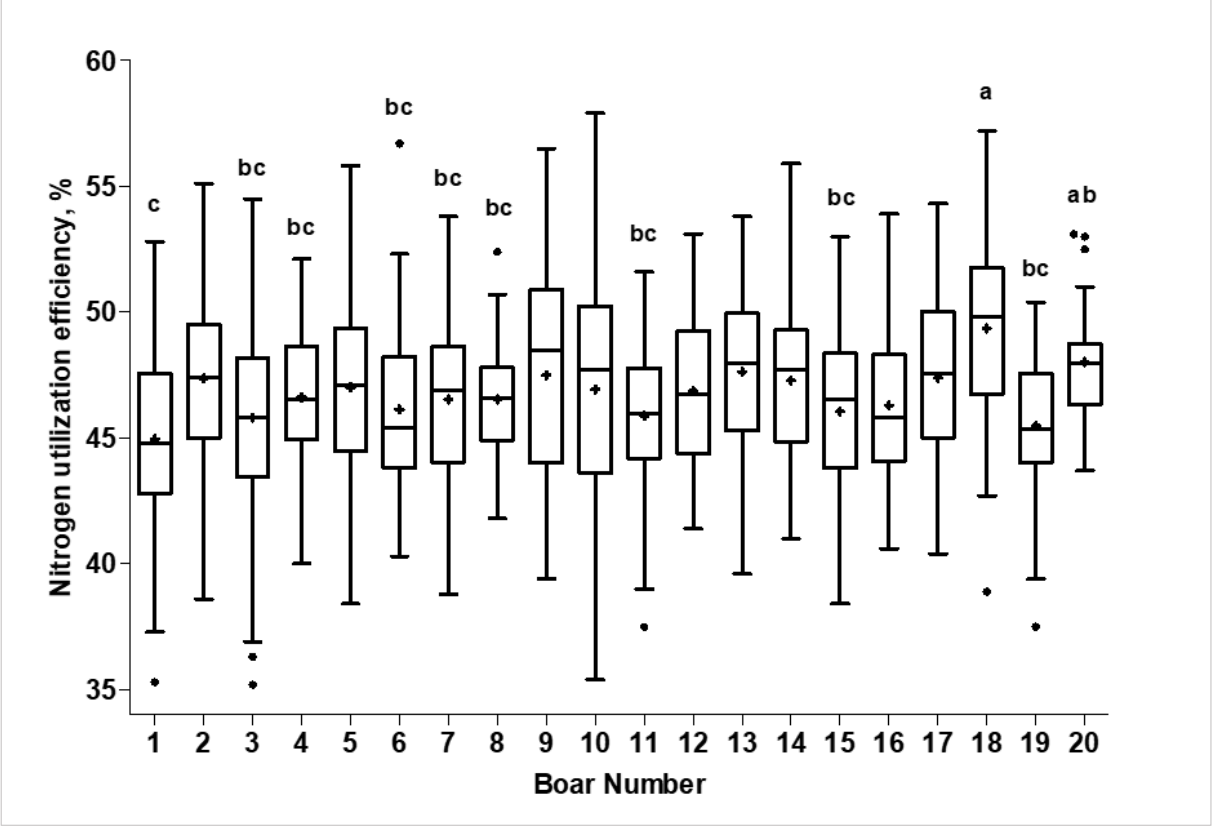

Figure 13. Distribution of the nitrogen utilization efficiency of the offspring of the boars (no. 1 to 20) within both sampling periods. Observations of 508 animals were available in sampling period 1 and observations of 458 animals were available in sampling period 2. The number of observations available per boar varied between 32 (boar no. 13) and 64 (boar no. 3) and was 48 on average. Different superscript letters above the boxes indicate significant differences between the group of offspring of the respective boar ($p \leq 0.05$).

Similar to NUE, the offspring of boar no. 1 utilized dietary lysine the least efficient within both SP (mean LUE 68.5%; Figure 14) and significantly less than the offspring of boar no. 2 (73.4% LUE), no. 9 (75.0% LUE), no. 18 (75.4% LUE), and no. 20 (77.0% LUE). The offspring of those four most efficient boars were additionally significantly more efficient in lysine utilization than the offspring of boar no. 11 (69.6% LUE), no. 14 (69.6% LUE), and no. 16 (69.3% LUE).

In comparison with the average LUE of the offspring of the four least efficient boars (69.3%), the offspring of the four most efficient boars (75.2%) utilized dietary lysine on average 5.9 percentage points more efficiently. Due to the high variation of this trait within the respective offspring groups, all mean values between 70.1% and 73.4% (boar no. 2, no. 3, no. 4, no. 5, no. 6, no. 7, no. 8, no. 10, no. 12, no. 13, no. 15, no. 17, and no. 19) did not differ significantly from each other. The observed values for LUE varied the most within the offspring of boar no. 10, ranging from 55.0 to 87.9% and with half of this variation the lowest range could be seen within the offspring of boar no. 17 (65.6 to 81.9% LUE).

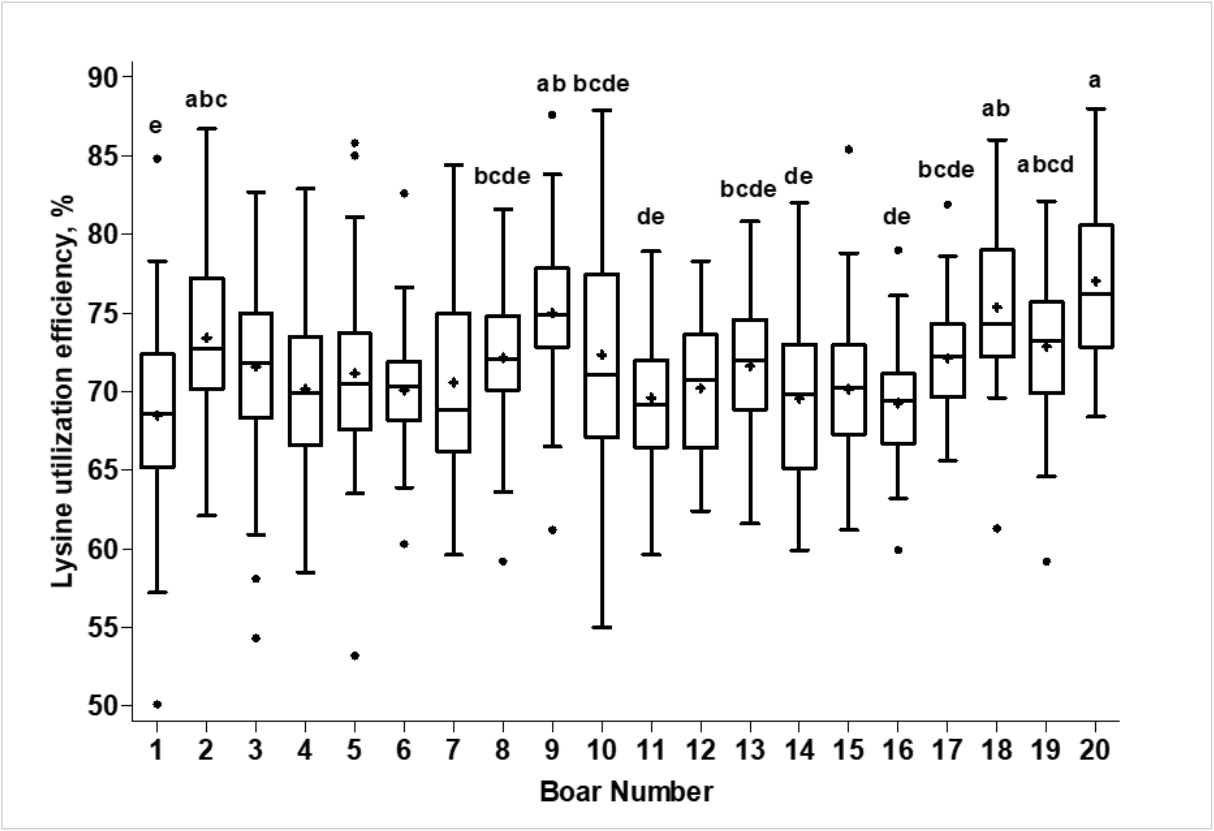

Figure 14. Distribution of the lysine utilization efficiency of the offspring of the boars (no. 1 to 20) within both sampling periods. Observations of 508 animals were available in sampling period 1 and observations of 458 animals were available in sampling period 2. The number of observations available per boar varied between 32 (boar no. 13) and 64 (boar no. 3) and was 48 on average. Different superscript letters above the boxes indicate significant differences between the group of offspring of the respective boar ($p \leq 0.05$).

4.3 Overall fattening period

Since one animal died for unknown reasons in the 8th week of the experiment, observations of 507 pigs were available for the calculation of the overall growth performance until slaughter. In addition to the 56 pigs that were euthanized on the experimental station for digesta sampling, the markings of six animals were not readable at the slaughterhouse and these carcasses could not be clearly identified. Therefore, in total, carcass data of 63 pigs was missing and observations of 445 pigs (of which were 250 gilts and 195 barrows) were used for calculations of ONR and ONUE as described in chapter 3.3.2.

4.3.1 Performance traits

Table 29 shows the overall growth performance data during the 10-week fattening period separated for the sex effect. Starting with the same mean IBW (29.4 kg) and very similar distribution of the measured values for both sexes (minimum IBW of 19.0 kg and 19.5 kg for gilts and barrows, respectively and maximum BW of 41.0 kg; CV of 13.0% for gilts and 13.1% for barrows), barrows gained 90 g more per day on average than gilts (0.96 vs. 0.87 kg/d). Therefore, mean final BW (FBW) at slaughter was significantly higher for barrows (99.3 kg) than gilts (93.0 kg) and higher than the targeted FBW of 90 kg. Since ADG was highly variable (0.63 to 1.06 kg for gilts and 0.71 to 1.17 kg for barrows), FBW ranged from 72.5 to 116 kg for gilts and 76.5 to 126 kg for barrows.

Table 29. Growth performance traits in the complete data set over the 10-week experimental period and their differences between the sex of the pigs

	IBW, kg		FBW, kg		ADG, kg	
sex	gilts	barrows	gilts	barrows	gilts	barrows
MEAN	29.4	29.4	93.0[b]	99.3[a]	0.87[b]	0.96[a]
SD	3.83	3.86	7.59	8.24	0.08	0.08
%CV	13.0	13.1	8.17	8.30	8.83	8.15
MIN	19.0	19.5	72.5	76.5	0.63	0.71
MAX	41.0	41.0	116	124	1.06	1.17
pooled SEM	0.34		0.70		<0.01	
P-value	0.971		<0.001		<0.001	

Observations of 507 animals were available for calculations. Different superscript letters indicate significant differences in trait means between the sexes ($p \leq 0.05$). IBW = initial body weight; FBW = final body weight; ADG = average daily gain; SD = standard deviation; %CV = percent coefficient of variation; MIN = minimum value; MAX = maximum value; SEM = standard error of the mean.

The mean ADFI was 2.32 kg DM for barrows and thus significantly higher than the ADFI of gilts (2.15 kg DM; Table 30). The variation in ADFI was 1.83 to 2.83 kg DM for barrows and 1.60 to 2.71 kg DM for gilts. The G:F ratio of barrows was slightly but significantly higher than G:F ratio of gilts (0.41 vs. 0.40). The observed values were in a range of 0.33 to 0.48 within both sexes.

For RFI the relationship was exactly the opposite, with a mean RFI of - 0.02 for gilts, indicating that they utilized the feed significantly more efficiently than barrows (mean RFI of 0.01). The range of calculated RFI was ± 0.30 for gilts and ± 0.28 for barrows.

Table 30. Feed intake and feed efficiency in the complete data set over the 10-week experimental period and their differences between the sex of the pigs

	ADFI, kg DM		G:F, kg/kg		RFI, kg DM	
sex	gilts	barrows	gilts	barrows	gilts	barrows
MEAN	2.15[b]	2.32[a]	0.40[b]	0.41[a]	-0.02[b]	0.01[a]
SD	0.18	0.18	0.02	0.02	0.11	0.11
%CV	8.45	7.67	6.10	5.80	.	.
MIN	1.60	1.83	0.33	0.35	-0.30	-0.28
MAX	2.71	2.83	0.47	0.48	0.30	0.28
pooled SEM	0.02		<0.01		0.01	
P-value	<0.001		<0.001		0.035	

RFI was calculated as y = ADFI - (1.205 x ADG + 0.051 x AMW; R^2 = 0.71, RMSE = 0.11 kg/d, mean ADFI = 2.24kg). Observations of 507 animals were available for calculations. Different superscript letters indicate significant differences in trait means between the sexes ($p \leq 0.05$). AMW = average metabolic weight; ADFI = average daily dry matter feed intake; G:F = gain to feed ratio; RFI = residual feed intake; SD = standard deviation; %CV = percent coefficient of variation; MIN = minimum value; MAX = maximum value; SEM = standard error of the mean.

4.3.2 Overall nitrogen utilization efficiency

The average NI in the experimental period was significantly higher for barrows (69.4 g/d) than for gilts (64.2 g/d; Table 31). In accordance with ADFI, NI varied widely between 49.6 to 82.3 g/d for gilts and 54.4 to 86.0 g/d for barrows. The overall NI was moderately correlated with NI in SP1 (r = 0.56; Appendix 12) and well correlated with NI in SP2 (r = 0.67).

Based on the carcass data recorded at the slaughterhouse, the EBP content of the pigs at slaughter was calculated (see chapter 3.3.2) and subsequently the ONR and ONUE for the entire fattening period. The overall LUE could not be calculated, as lysine concentrations of the feed were only analyzed during the SP, but not during the complete experimental period.

The mean calculated ONR was 27.7 g/d for gilts (Table 31), which was 1.8 g/d and significantly lower than the average ONR of barrows (29.5 g/d). ONR was highly correlated with overall ADG (r = 0.73; Appendix 12) and moderately correlated with NR in SP1 (r = 0.47) and SP2 (r = 0.53). Also, low but significant correlations to BUN concentration in SP1 (r = 0.17) and SP2 (r = 0.23) were calculated.

The minimum calculated ONR was 21.3 g/d for gilts and thus similar to the minimum ONR of barrows (21.5 g/d), whereas the maximum ONR of barrows (37.1 g/d) was higher than the maximum ONR of gilts (33.8 g/d; Table 31).

Despite the higher ONR, barrows significantly utilized dietary N less efficient on average than gilts (42.4% vs. 43.2%), but the maximum efficiency was highest for barrows (54.6%).

The calculated values for ONUE were within a range of 37.3 to 50.5% for gilts and 30.1 to 54.6% for barrows. No significant correlation was observed between ONUE and NUE in SP1 and only a very low correlation to NUE in SP2 existed (r = 0.15; Appendix 12). Neither BUN concentration in SP1 nor in SP2 showed a significant correlation to ONUE.

Table 31. Overall nitrogen metabolism traits in the complete data set over the 10-week experimental period and their differences between the sex of the pigs

	NI, g/d		ONR, g/d		ONUE, %	
sex	gilts	barrows	gilts	barrows	gilts	barrows
MEAN	64.2[b]	69.4[a]	27.7[b]	29.5[a]	43.2[a]	42.4[b]
SD	5.49	5.73	2.39	2.58	2.61	3.02
%CV	8.55	8.25	8.61	8.74	6.03	7.12
MIN	49.6	54.4	21.3	21.5	37.3	30.1
MAX	82.3	86.0	33.8	37.1	50.5	54.6
pooled SEM	0.61		0.27		0.33	
P-value	<0.001		<0.001		0.002	

Observations of 507 animals for NI were available and observations of 445 animals were available for calculation on ONR and ONUE (250 for gilts and 195 for barrows). Different superscript letters indicate significant differences in trait means between the sexes ($p \leq 0.05$). NI = nitrogen intake; ONR = overall nitrogen retention; ONUE = overall nitrogen utilization efficiency; SD = standard deviation; %CV = percent coefficient of variation; MIN = minimum value; MAX = maximum value; SEM = standard error of the mean.

4.3.3 Effect of boar on the nitrogen utilization of the offspring

Since the boars were not evenly distributed over the experimental period due to operational constraints, boars no. 13 to 18 were more affected by the selection of pigs for microbiota sampling and thus for their offspring relatively less carcass data from the slaughterhouse was available (Table 26). The mean estimated values for ONR varied among boars between 23.4 and 37.1 g/d and the ONR within a group of offspring was highly variable. The variation was greatest for the offspring of boar no. 7, ranging from 21.3 g/d to 35.0 g/d (Figure 15). The smallest variation was observed for the offspring of boar no. 17, where ONR varied between 26.1 g/d and 30.1 g/d. However, the mean ONR was rather constant, ranging from 26.6 g/d to 29.7 g/d, and did not differ significantly for most offspring groups. Nevertheless, the offspring of the four boars showing the highest ONR (29.7 g/d, 29.6 g/d, 29.4 g/d, and 29.7 g/d for the offspring of boars no. 1, no. 5, no. 11, and no. 14, respectively) significantly retained more N on average (+2.9 g/d) than the offspring of the two boars showing the lowest ONR (26.8 g/d and 26.6 g/d for the offspring of boars no. 15 and no. 19).

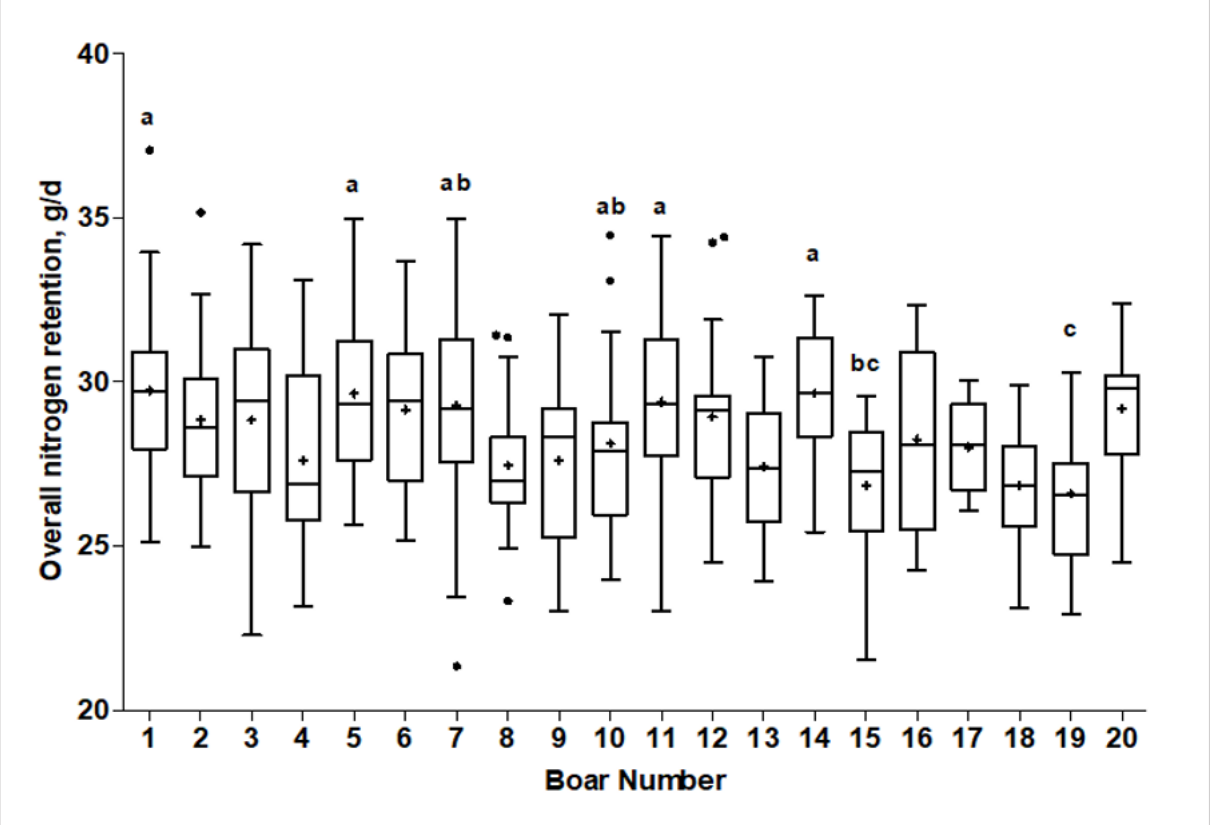

Figure 15. Distribution of the overall nitrogen retention of the offspring of the boars (no. 1 to 20) within the 10-week experimental period. Overall nitrogen retention was calculated based on carcass data. Observations of 445 animals were available of which 250 were gilts and 195 barrows. The number of observations available per boar varied between 10 (boar no. 13) and 33 (boar no. 11) and was 22 on average. Different superscript letters above the boxes indicate significant differences between the group of offspring of the respective boar ($p \leq 0.05$).

Regarding ONUE, the values varied between 30.1 and 54.6% (Figure 16), but differences between the groups of offspring of the boars were less pronounced than for ONR. Significant differences only existed for the offspring of boar no. 14, no. 17, and no. 20. The offspring of boar no. 14 (44.0% ONUE) and no. 20 (44.4% ONUE) utilized dietary N more efficiently than the offspring of boar no. 17 (40.5% ONUE). Nevertheless, no observed value differed significantly from the overall mean of 42.8% ONUE. However, a considerable variation within the offspring group of the respective boars existed. With values ranging from 37.4 to 50.5%, the largest variation was observed among the offspring of boar no. 20. Minimum ONUE for the offspring of boar no. 17 was 37.5% and maximum ONUE was 43.8%, thus this group showed the smallest variation in ONU

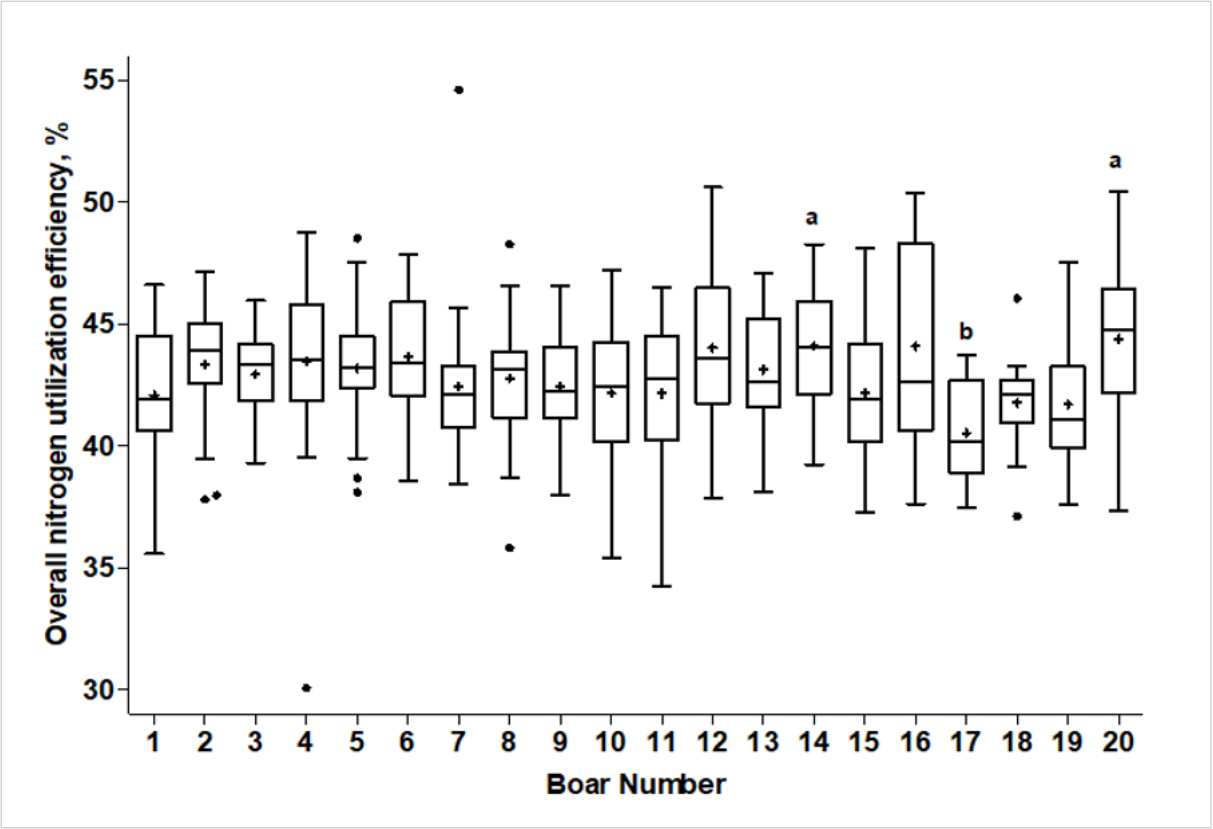

Figure 16. Distribution of the overall nitrogen utilization efficiency of the offspring of the boars (no. 1 to 20) within the 10-week experimental period. Overall nitrogen utilization efficiency was calculated based on carcass data and weekly nitrogen intake. Observations of 445 animals were available of which 250 were gilts and 195 barrows. The number of observations available per boar varied between 10 (boar no. 13) and 33 (boar no. 11) and was 22 on average. Different superscript letters above the boxes indicate significant differences between the group of offspring of the respective boar ($p \leq 0.05$).

5 DISCUSSION

Improving the NUE of growing pigs is necessary to reduce the negative impact of N excretions on the environment. Since current feeding practices achieve utilization efficiencies of only 30 – 45%, alternative strategies must be found to improve the sustainability of pork production. It is well known that the NUE can vary between individual animals of the same population. For example, in the study of Kasper et al. (2020), environmental conditions explained up to 60% of the variation in NUE between individuals, and thus up to 40% of the variation could be explained by genotype. However, selection for efficiently N utilizing pigs requires accurate determination of the protein retention of all animals in a population from which to select. Therefore, methods are needed for phenotyping large numbers of individuals with acceptable effort. In this context, the objectives of the present study were:

a) to accurately quantify the NR and NUE of a subsample of pigs housed in metabolism crates in two different growth stages and to determine the impact of body protein turnover on these variables.
b) to assess the suitability of serum hormone and BUN concentrations to establish equations for the estimation of NR and NUE as easy to collect alternatives for phenotyping large numbers of animals with acceptable effort.
c) to evaluate the individual variation of NR and NUE between animals of a F1 crossbred population fed diets scarce in lysine supply during the fattening period as a basis for estimating genetic parameters in further steps.

5.1 Problems and deviations from the work plan

5.1.1 Crude protein digestibility

In the present experiment, the CP ATTD for the complete data set should be determined via the indicator method (see chapter 3.3.2). However, as can be seen in Table 32, the TiO_2 recovery in the quantitative fecal samples of the N balance pigs was very low, at 80.4% on average, for all balance periods and, moreover, highly variable at 59.8 – 110%.

The reasons for low recoveries of indigestible markers are diverse. Possible sources of error are inhomogeneous mixing of the marker into the feed, adhesion of the TiO_2 to feeding equipment and thus incomplete intake by the animals, non-representative sampling, incomplete total collection, and inaccuracies in sample preparation, and these individual errors can accumulate to the point of analysis (Kavanagh et al., 2001; Le Goff et al., 2002). The diet composition, and thus digestibility, may also influence the recovery rate.

For example, Li et al. (2016) observed that for highly digestible corn and soybean meal-based diets, the calculated recovery rate can reach 111%. For barley and rapeseed meal-based diets with high fiber content and therefore lower digestibility, using the time-based collection method, with 94% the TiO_2 recovery rate was noticeably decreased.

Table 32. Variation of the titanium dioxide recovery rate (%) in the quantitative feces samples of the nitrogen (N) balance pigs in two sampling periods (SP)

N balance	1		2		3		4		5		6		7	
SP	2	1	2	1	1	2	1	2	1	2	1	2	1	2
MEAN	98.5	72.2	80.0	66.4	81.2	87.1	70.6	78.9	88.6	83.4	71.9	86.4
SD	16.2	5.53	6.22	3.88	8.28	5.21	7.62	10.3	5.73	3.30	8.60	4.87
%CV	16.5	7.66	7.77	5.84	10.2	5.99	10.8	13.0	6.46	3.95	12.0	5.64
MIN	63.1	63.3	68.7	59.8	69.5	80.1	62.4	64.2	79.1	78.6	61.3	78.9
MAX	110	81.0	87.0	70.2	93.4	96.0	83.2	93.0	95.0	89.2	83.0	91.8

SD = standard deviation; CV = coefficient of variation; MIN = minimum value; MAX = maximum value.

Kavanagh et al. (2001) also observed a fecal TiO_2 recovery rate of only 93% for wheat, barley, and soybean meal-based diets, as used in the present experiment. The authors discussed the possibility of TiO_2 accumulation in the cecum, as reported for rats (Lloyd et al., 1955), which may result from increased microbial fermentation due to the fiber content of the diet and thus incorporation of TiO_2 into bacterial cells. Nevertheless, this would not explain the reduced recovery rate of titanium by another average of 13 percentage points and the high variability in the present experiment.

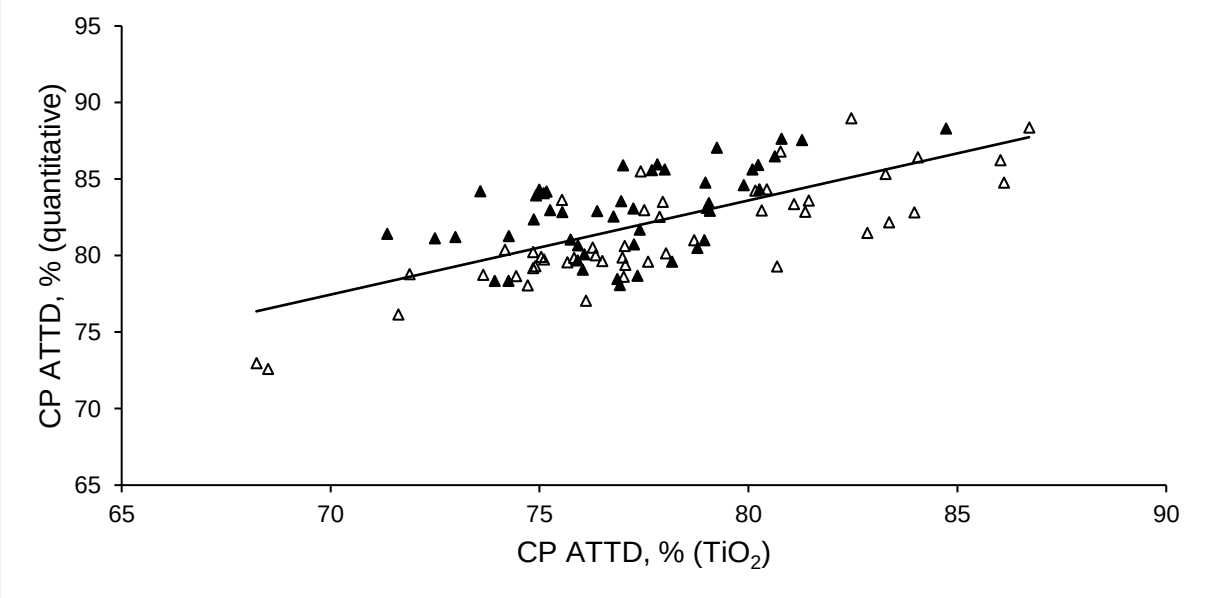

Figure 17. Apparent total tract crude protein digestibility (CP ATTD) obtained by four-day quantitative collection as a function of CP ATTD obtained by indicator method using titanium dioxide, (TiO_2; R^2 = 0.46, RMSE = 2.34). Data result from the extended data-collection subsample housed in metabolism crates in two sampling periods (filled symbols = sampling period 1; open symbols = sampling period 2).

When the N balance data set was used as a validating data set for the ATTD values obtained by fecal TiO_2 concentrations, the regression of CP ATTD obtained by the indicator method and by quantitative collection showed only a moderate relationship (R^2 = 0.46; Figure 17) and no directed deviation of the measured values was observed. Furthermore, the analysis of single feed samples of the animals of the N balance data set in comparison to the bulk feed samples indicated inhomogeneous inclusion of the marker into the diets. Therefore, it was decided not to use CP ATTD values obtained by the indicator method for the estimation of NR and NUE of the complete data set, as the additional error would be greater than the benefit.

5.1.2 Body composition

In the present experiment, it was intended to obtain additional data on protein retention and body composition by use of the D_2O dilution method. One task was to prove whether differences in NUE lead to differences in the protein content of the empty bodies and, the other task was to determine the protein retention for the period between the two SP. However, this method raised some difficulties, and the data from the individual animals could not be used conclusively. For example, oral administration of the marker was difficult to perform without losses, and individual animals regurgitated large amounts of the administered D_2O solution. Analysis of the D_2O solution further showed an intraassay CV of 13.5% and an interassay CV of 17.3%, indicating that even if the animals had reliably swallowed the D_2O solution, the amount of D_2O consumed could differ substantially from that calculated. Furthermore, the amounts of blood collected were sometimes too small to obtain sufficient replicated measures from the individual animals. These circumstances led to the fact that the results of the body water determination were highly variable and could not be tested for validity due to missing comparison values. Furthermore, no independent regression equations based on comparative carcass analyses were generated in the present experiment, but the equations determined by Landgraf et al. (2006) were used. By using these equations, nearly constant EBP contents of 16.6% in SP1 and 16.5% in SP2 were determined, regardless of the measured body-water content. Since the method was thus not suitable for revealing differences in the protein content between individual animals due to a large number of possible errors, it was decided not to use these data for further analyses.

5.1.3 Voluntary feed intake

As described in chapter 4.2.1, SP2 was forwarded by two weeks because the animals grew faster than assumed in the planning phase. The diets were formulated to supply sufficient ME to allow an ADG of 800 g in both the starter phase and the grower phase.

An ADFI of 1.7 kg as fed (corresponding to about 1.5 kg DM) in the starter phase and 2.3 kg as fed (corresponding to about 2.0 kg DM) in the grower phase was assumed. These values were based on data from practical feeding recommendations for fattening pigs (Staudacher et al., 2014). However, as can be seen in Figure 18, the ADFI was already at a high level from the beginning of the trial and increased almost linearly during the fattening period. Thus, the animals consumed on average 0.36 kg/d in SP1 and 0.51 kg/d in SP2 more feed than calculated.

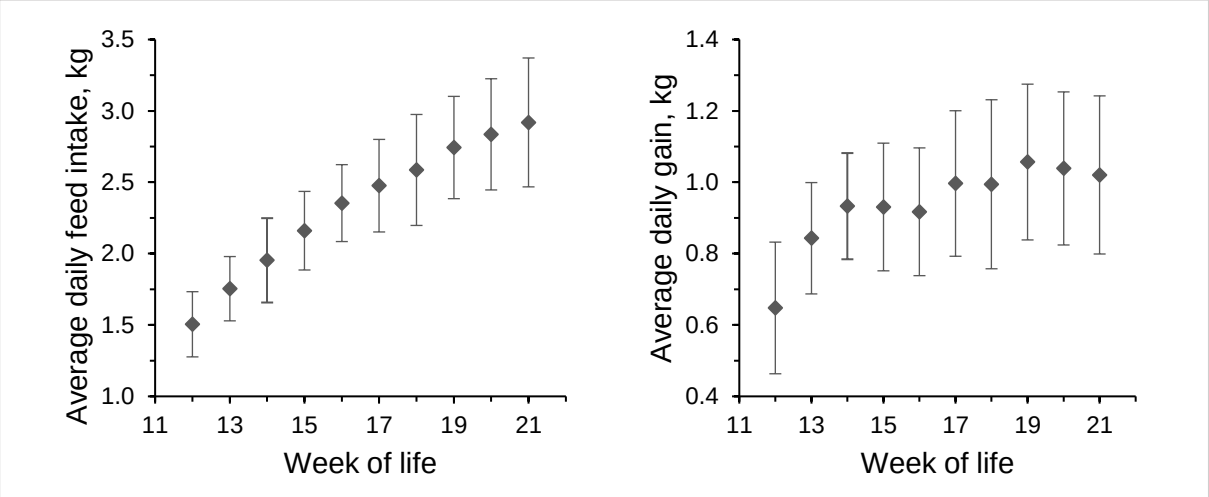

Figure 18. Weakly development of average daily feed intake and average daily gain of the pigs of the complete data set during the 10-week experimental period (n = 507). Data are means ± standard deviation.

This discrepancy between the observed and calculated values can be explained mainly by the housing conditions of the animals. Under practical housing conditions, a variety of factors can limit the pigs' voluntary feed intake (Ellis and Augspurger, 2001; Tokach et al., 2012), which can be excluded to a large extent under controlled, experimental conditions. Because of higher feed intake, the growth performance of pigs housed under controlled conditions is usually higher, even if the pigs are housed under practice-like conditions. Godinho et al. (2018) published the results of the fattening performance data of 2,230 crossbred pigs in 29 successive batches housed under near commercial conditions at an experimental station. The average ADFI of the pigs in the starter phase (22 – 45 kg BW) was 1.38 kg, and thus 0.5 kg/d lower than in SP1 of the present study, but the animals were also about 10 kg lighter on average. At maximum, the animals consumed up to 2.17 kg of feed per day, which is very close to the observed maximum values in the present study. In the grower phase (45 – 84 kg BW), the average ADFI was 2.20 kg, which is about 0.30 kg lower than in the present study. However, individual animals consumed up to 3.42 kg of feed per day at maximum, which is well within the range of the observed values of the present study.

Furthermore, when pigs are housed individually instead of groups with ad libitum access to feed, even higher feed intake values were reported. Quiniou et al. (1996) observed an ADFI of 2.56 kg in Large White barrows in the grower-finisher phase (45 – 100 kg BW). Colpoys et al. (2016) reported an average ADFI of 3.04 kg in crossbred growing-finishing gilts (56 – 112 kg BW). Kil et al. (2011) observed an average ADFI of 1.93 kg in crossbred starter barrows (22 – 50 kg BW) and an average ADFI of 3.57 kg in finishing barrows (85 – 130 kg BW). The main reason for the higher feed intake in individual housing compared to group housing is that animals can spend more time eating because of lower between-animal interactions. Lower-ranking animals are especially less hindered in feed intake (Hansen et al., 1982). Haer and Vries (1993) observed that individually housed pigs spent 20 min more time eating each day and thus consumed on average 150 g more feed per day during the 30 – 100 kg fattening period.

In addition to the experimental individual housing, marginal dietary lysine supply may have been another possible explanation for the high ADFI, causing the animals to consume more feed to meet the daily lysine requirements for their intrinsic growth potential. When feeding diets containing 80% the lysine content of the control diet in the grower and finisher phase, a 2.7 – 6.1% increase in feed intake to maintain similar growth rates was reported for barrows of different genotypes (Fabian et al., 2002; Ruiz-Ascacibar et al., 2017; Yang et al., 2008), whereas boars and gilts did not increase their voluntary feed intake (Ruiz-Ascacibar et al., 2017).

The influence of age and sex on ADFI is well known. Up to about 40 – 50 kg BW, the size of the gastrointestinal tract limits the voluntary feed intake and pigs cannot respond to changes in energy and nutrient concentrations by altering feed intake (Bikker, 1994; Li and Patience, 2017). At this early stage of growth, the effect is independent of sex (Quiniou et al., 2010). With increasing age and pubertal development, barrows consume more feed than boars and gilts (Ruiz-Ascacibar et al., 2017; Quiniou et al., 2010; Quiniou et al., 1996), which is due to the appetite inhibiting effect of the androgenic hormones testosterone and estradiol circulating in the bloodstream (Claus and Weiler, 1994; Weiler et al., 1998). Significant differences ($p < 0.001$) in the ADFI between the sexes could also be observed in the present experiment even in SP1. Until slaughter, the feed intake of castrates was consistently higher than that of gilts. Simultaneously, in both SP, the mean serum IGF-I concentration of barrows was significantly ($p < 0.001$) lower than that of gilts, indicating the higher estradiol secretion of gilts. Nevertheless, no significant or relevant correlations between serum IGF-I concentrations and ADFI were observed in either SP (Appendix 12).

Altogether, the feed intake potential of the pigs of the present experiment, both for the two SP and the complete fattening period, was in a similar range as described by literature data and should be considered for possible subsequent experiments.

5.2 Performance traits

5.2.1 Growth performance

The BW development until slaughter was influenced by the pigs' IBW. However, the average ADG was independent of the BW of the animals (Appendix 12). Since the ADG increases linearly with increasing energy intake (Quiniou et al., 1996), the higher ADG of the pigs in the present experiment than what was assumed in the planning phase was a consequence of the high ADFI. A pronounced linear relationship between ADFI and ADG throughout the complete fattening period was illustrated by the strong coefficient of correlation (r = 0.79; Appendix 12). Hence, an extra ADG of 0.32 kg was realized per additional kg of DM feed intake, regardless of the sex of the animals. Assuming the calculated dietary ME concentration of 14.9 MJ/kg DM (Table 1), an extra ADG of 22 g was achieved per additional MJ ME consumed. This value was lower than described for comparable fattening pigs. For example, Quiniou et al. (1996) observed an extra ADG of 27 g per additionally ingested MJ ME for castrates, and Bikker (1994) observed an extra ADG of 35 g per additionally ingested MJ ME for gilts. In the requirement recommendations, a mean extra ADG of 29 g per ingested MJ ME is assumed during the fattening period of 30 – 100 kg BW, which is slightly reduced for castrates (28.5 g/MJ ME) and slightly higher for gilts (30 g/MJ ME; GfE, 2006).

An apparent explanation for these lower ADG relative to energy intake and the non-existent sex effect in the present study is the marginal lysine supply. Growth is primarily driven by energy intake, but if insufficient amounts of nutrients are available intermediary, the animals' inherent growth potential cannot be realized regardless of sex. Since lysine, as the first-limiting AA, limits the protein retention of the pigs and the growth potential is largely determined by the protein deposition capacity (de Lange et al., 2012), a marginal lysine supply inevitably leads to a decreased growth rate. Several studies have shown that lowering the dietary lysine concentration to 80% of the recommendations significantly reduces the ADG of growing pigs, regardless of the sex and genotype of the animals (e.g., Cloutier et al., 2015; Ruiz-Ascacibar et al., 2017; Zhang et al., 2012). However, when dietary CP and lysine content were lowered to 90% of the supply recommendations, as in the present experiment, Millet et al. (2010) did not observe a reduction in ADG in crossbred gilts during the different fattening phases and within the overall fattening period of 20 – 106 kg BW.

The authors discussed an overestimation of requirements and the pigs' adaptation to marginal supply conditions as possible reasons for their observation.

This could also explain the ADG in the present study. Considering the two SP, the ADG values were higher than the dietary LI would suggest. Assuming a mean pc lysine digestibility of 80% for the given diet composition (Centraal Veevoeder Bureau (CVB), 2018; GfE, 2006; NRC, 2012), a mean pc LI of 15.8 g/d in SP1 and 15.9 g/d in SP2 was achieved. This would be sufficient to meet the requirements for 0.80 kg ADG in both SP (GfE, 2006), but not for the observed mean ADG of 0.90 kg in SP1 and mean ADG of 0.97 kg in SP2. Assuming a pc lysine digestibility of 85%, the animals in SP1 would have ingested 16.8 g pc digestible lysine/d and 16.9 g in SP2. This would be almost sufficient to cover the requirement for 0.90 kg ADG (about 17.0 g pc digestible lysine/d), but it cannot explain the ADG in SP2. Therefore, because of the marginal supply, it appears that the animals utilized the ingested lysine more efficiently for BW gain than assumed for requirement recommendations (GfE, 2006; NRC, 2012). This is consistent with the results for LUE, which will be discussed in chapter 5.3.2.

However, the ADG in the two SP could also have been slightly overestimated. As indicated by weaker correlations between ADFI and ADG during both SP ($r = 0.54$ for SP1 and $r = 0.57$ for SP2; Appendix 12), both traits did not show this pronounced linear relationship during the SP, as reported for the overall fattening period ($r = 0.79$). One possible explanation can be the duration of the SP. For accurate determination of ADG, sufficient long periods are recommended to minimize daily variation in BW (NRC, 2012), otherwise under or overestimation of ADG may occur.

Nevertheless, the efficient utilization of lysine for BW gain is also evident in the evaluation of the performance data of the overall fattening period. The dietary lysine concentration was analyzed only during the SP and not during the overall fattening period, but since the CV for the lysine concentration of the analyzed samples was < 5%, the mean lysine concentrations can be assumed for all diets fed during the fattening period. Thus, a mean pc LI of 15.2 g/d during the overall fattening period was achieved, which is sufficient to cover the requirement for a mean ADG of 0.80 kg (GfE, 2006). However, the mean ADG of the pigs was 0.91 kg. Even assuming a mean pc lysine digestibility of 85%, the mean pc LI would be 16.2 g/d and would not meet the requirement for an average ADG of 0.90 kg. Thus, the animals utilized dietary lysine more efficiently not only during the SP but also throughout the overall fattening period.

The efficiency of the utilization of lysine for ADG differed between the sexes neither in the different SP nor during the overall fattening period. The results of feed and LI indicate that the energy supply did not limit growth, but it was limited by LI.

Furthermore, the pigs were able to adapt to the marginal feeding conditions by utilizing dietary lysine more efficiently for growth as expected.

5.2.2 Feed efficiency

The efficient utilization of feed for growth is underlined by the high G:F ratio, which was on average 0.49 in SP1 and 0.38 in SP2. Thus, the FE was in the same range as described for growing pigs fed diets that meet or exceed the nutrient requirements at the respective growth stage. Millet et al. (2010) also did not observe a deterioration in FE when the lysine supply was lowered to 90% of the requirement. A decrease in FE as a result of lowered lysine concentrations in the starter and grower period, as in the present experiment, is to be expected only after a lysine deficiency of 15 – 20% of the requirement recommendations (Cloutier et al., 2015; Fabian et al., 2002; Millet et al., 2010; Ruiz-Ascacibar et al., 2017; Yang et al., 2008).

The G:F ratio was not significantly different between the sexes in both SP. Differences in the FE between the sexes were only reported from the finisher period onwards but can affect the overall FE of the fattening period because of the high feed intake during this period (Quiniou et al., 2010; Ruiz-Ascacibar et al., 2017). Generally, barrows have a lower FE than gilts, since due to the higher feed intake, the energy and nutrients consumed above the protein retention capacity are used for the energetically more inefficient lipid retention (GfE, 2006; NRC, 2012). However, in the present experiment, the G:F ratio of barrows (0.41) was slightly, although significantly ($p < 0.001$), higher than that of gilts (0.40). One possible explanation might be that, as indicated earlier and discussed in more detail in chapter 5.3.2, LI was the limiting factor for protein retention rather than energy intake. The protein retention was linearly dependent on LI and no plateau could be detected in this regard. Thus, the pigs did not reach their maximum protein retention regardless of sex. Hence, each additional unit of feed intake (and thus LI) resulted in an additional unit of protein retention. Due to the high growth performance of the barrows because of the high ADFI, the proportion of unproductive maintenance requirement was most likely lower, resulting in the higher G:F ratio compared to the gilts.

However, when RFI was considered as a measure of FE, the situation was exactly reversed and, consequently, gilts would utilize feed significantly ($p = 0.035$) more efficiently than barrows. RFI reflects the difference between the observed and the expected feed intake based on growth performance (Young and Dekkers, 2012). In its theory, it is intended to represent differences in maintenance energy requirements between animals. Because of the method of calculation, pigs with lower ADFI tend to have lower RFI, as shown by the correlation between these variables (r = 0.49; Appendix 12). It was also shown that selection for RFI results in pigs with lower feed intake but maintaining similar ADG (Gilbert et al., 2017; Saintilan et al., 2015).

Since, as explained before, due to hormonal influence, gilts have a lower voluntary feed intake than barrows; this should inevitably result in a lower RFI. Another problem with using RFI to assess FE is that animal-individual differences in nutrient utilization for retention cannot be represented, but average nutrient utilization efficiency for all animals is assumed. It has even been shown that low-RFI pigs utilize dietary lysine less efficiently (Hewitt et al., 2020) and respond to lysine restriction with a greater decrease in growth performance than high-RFI pigs (Gilbert et al., 2017). Hence, low-RFI pigs have a higher requirement for dietary lysine than high-RFI pigs, which, together with the lower LUE, results in reduced ADG when feeding diets deficient in lysine. However, since the G:F ratio is mainly influenced by the level of ADG (Saintilan et al., 2015), this may be a possible explanation for the discrepancies regarding RFI and G:F ratio between the sexes in the present experiment. Nevertheless, there was a pronounced negative correlation between both traits (r = - 0.83; Appendix 12), which means that animals with high G:F ratios also have low RFI and vice versa.

5.2.3 Comparison between the nitrogen balance data set and the complete data set

A prerequisite for the application of estimation equations is that the data set from which the equations are derived is representative of the data set to which the equations are to be applied. Looking at the BW of the animals, in SP1 both the mean and the variation were almost the same for both data sets. In SP2 on the other hand, the mean and CV were higher for the complete data set because the maximum BW was larger. This was mainly due to the results of the first cohort, which was sampled in the 18th week of life and not in the 16th week of life. Excluding the results of cohort 1, the mean BW in SP2 was 59.5 kg, the maximum value was 78.0 kg and the CV decreased from 11.7 to 10.3%. Therefore, the mean and variance of the complete data set also closely matched the N balance data set in SP2. The ADFI of the N balance pigs, on the other hand, was on average 170 g/d lower in SP1 and 300 g/d lower in SP2 than that of the complete data set. One explanation is that the animals were housed in metabolism crates. It is well known that a change in the housing environment with the accompanying restriction of animal behaviors, social isolation, and increased or unfamiliar contact with humans can affect animal activity, causes stress, and results in diarrhea and decreased nutrient digestibility (Borges et al., 2020; Gerrits et al., 2015; Oliveira et al., 2016; Petherick, 2007). In the present study, the results of the first balance cohort had to be discarded, because the animals showed stress-related diarrhea due to the missing adaption period. This was accompanied by an increased UCE. On the first day of urine collection, a mean of 0.46 mg cortisol per pig was excreted (data not shown).

During the balance period, the excretion decreased to 0.33 mg/d, but the mean UCE was about twice as high as in the following balance cohorts, in which the animals were allowed a two-day adaption period to the metabolism crates. Similar results were observed by Oliveira et al. (2016) in collared peccaries, a porcine species. After re-housing in metabolism crates, the authors noted a doubling of fecal glucocorticoid concentrations compared to the original individual housing. However, this was not accompanied by a decrease in feed intake, as is known for individual housing in metabolism crates.

Cole et al. (1967) already observed a reduced voluntary feed intake of growing Large White pigs compared to their individually housed counterparts. This effect was observed independently of the diet and the difference between the two housing types even increased over the fattening period of 38 – 105 kg. Furthermore, the decrease in feed intake was greater than the reduced energy requirement explained by decreased activity. These findings were confirmed by Quiniou et al. (1996). They observed a 15% lower ADFI of the pigs housed in metabolism crates compared to the pigs in individual floor pens, which could not be explained by the lower activity and thus lower energy requirement alone but seems to be a specific effect of the housing conditions. In addition, this effect was independent of the genotype of the pigs. Both authors further observed a lower growth rate because of the reduced feed intake. Similar results were observed in the present work. The mean ADG of the N balance pigs was on average 0.13 kg lower in SP1 and 0.11 kg lower in SP2 than the mean ADG of the complete data set. In addition, the maximum ADG was not achieved by the pigs housed in the metabolism crates. Due to both lower ADFI and ADG, there was no difference in the G:F ratio between the two data sets, neither in mean nor in variation. In addition, the correlations between the two SP were almost identical for both data sets. Altogether, based on the performance traits, it can be assumed that the N balance data set was representative of the complete data set, despite the unavoidable limitations regarding ADFI and ADG.

5.3 Nitrogen metabolism

5.3.1 Nitrogen retention

Because of the high voluntary feed intake, the N, and thus lysine, intake of the animals was also at a high level, which in turn led to a high NR due to a linear relationship ($R^2 = 0.60$; Figure 6). According to the correlation, within the observed LI range of 10 – 22 g, for every additional gram of LI, 1.54 g N or 9.6 g protein were retained, on average, by the pigs. This value is slightly higher as reported by Susenbeth (1995) in a review of N balance data of growing pigs fed diets where the marginal lysine supply was the limiting factor for protein retention.

Within a LI range of 5 – 25 g/d, for each additional gram of lysine ingested, 9.0 g protein retention was achieved on an average of 21 different trials. However, the observed slope of the present study is within the reported SD (1.08 g/d) of the meta-analysis, indicating that the effect of LI on protein retention was not different from the reported values. This confirms the independence of the effect of LI on protein retention from animal age, BW, and energy intake, and indicates that the marginal lysine supply was the limiting factor for protein retention in the present experiment. Moreover, it can be concluded that the relationship is also independent of the protein retention capacity of the animals and that breeding measures do not change this fundamental relationship. Even though it is reported that breeding progress increases the lean meat percentage by 0.5% each year (Shirali et al., 2012), and thus also the protein retention capacity of the animals, there is no difference in the pigs' NR response to LI, although there are about 30 years of breeding progress between the observed results.

With an average of 27.9 g/d in SP1 and 26.7 g/d in SP2, the values of NR were comparable to the results of other authors who fed conventional wheat, barley and soybean meal-based diets under ad libitum feeding conditions to growing barrows of similar genotypes and measured NR via the N balance method. These range from 27.4 to 32.7 g/d for animals of comparable BW to those in the two SP of the present experiment, and in these studies, there was also no evidence of significant differences in the NR between the two growth stages (Barea et al., 2010; Hansen et al., 2006; Le Bellego et al., 2001; Noblet et al., 2001; Quiniou et al., 1995b; Renaudeau et al., 2013; Zervas and Zijlstra, 2002a). When corn and soybean meal-based diets with similar energy and CP contents are fed, crossbred barrows at similar BW under ad libitum feeding conditions were reported to have an even higher NR than the pigs in the present experiment that reached the maximum. For example, Chen et al. (2017) observed a mean NR of 42.2 g/d between the BW range of 40.0 to 63.9 kg, which was achieved with a mean DM feed intake of 2.35 kg/d and a NI of 62.8 g/d. Since the NR to DM feed intake ratio was in a similar range as in the present experiment, the higher NR could be explained by the higher dietary lysine concentrations and thus a more adequate supply. In addition, the authors reported a CP ATTD of 90.4%, which is about eight percentage points higher than the CP ATTD of the animals in the present experiment, which leads to a higher intermediary N availability that can be utilized for NR. In the present experiment, with a moderate correlation of r = 0.40 (Appendix 6), an effect of CP ATTD on NR was observed only in SP1. This indicates that especially when the digestive capacity of the animals is still limiting the level of feed and thus N intake, differences in the digestibility of the CP and thus in the intermediary availability of N and AA affect the level of NR. This seems not the case when the maximum feed intake capacity of the animals is reached.

Zhao et al. (2019) also observed a high mean NR of 40.6 g/d in the starter phase (25 – 50 kg BW) and of even 49.9 g/d in the grower phase (50 – 75 kg BW), which is about 20% higher than the maximum in the present experiment. The authors reported a similar DM and thus N intake as of the pigs in the present study, and with on average about 84%, the CP ATTD was not significantly higher, which can hardly explain the observed large differences. However, the high NR reported in the study of Zhao et al. (2019) should be regarded with caution. Regarding the level of N intake, the published values for UNE are extremely low at 10.2 g/d for the starter phase and 14.4 g/d for the grower phase and are about the same as the daily FNE. In addition, because the authors did not report measures taken to prevent N losses with urine during collection, the UNE in their experiment was most likely underestimated and thus the NR was overestimated. It is well known that due to bacterial fermentation, the urinary urea is rapidly converted to ammonia and lost through volatilization. For example, van Kempen et al. (2003) reported that even minor fecal contamination of the urine collection container degrades 68% of the urea N within 24 h if the urine is not acidified.

Converting the NR to protein retention yields a mean protein retention of 174 g/d in SP1 and 167 g/d in SP2, respectively. The variation in the observed protein retention of 93 – 238 g/d is also within the range reported in the literature for growing pigs under practical feeding conditions, although using different methods to determine protein retention. For example, Remus et al. (2021) observed a variation in protein retention of crossbred barrows (mean BW of 46 kg), measured by body composition using dual X-ray absorptiometry, of 145 – 230 g/d during a 21-day experimental period. Godinho et al. (2018) reported a variation in protein retention of 77 – 212 g/d during the starter phase (22 – 45 kg BW) and 68 – 204 g/d during the grower phase (45 – 84 kg BW) when feeding a wheat and barley-based diet to crossbred entire males and gilts. The authors estimated the protein retention by measuring the pigs' back fat thickness and BW development at the respective growth stages.

However, compared to the protein retention measured via the comparative slaughter technique, used by Campbell et al. (1984), Landgraf et al. (2006), or Quiniou et al. (1995a), the observed values of the present study are slightly higher. Growth curves, which are the basis of requirement recommendations, indicate a daily protein retention of 140 – 150 g/d for barrows at comparable BW stages as in the present trial (NRC, 2012). This value was recently confirmed by a serial slaughter study, where Ruiz-Ascacibar et al. (2017) observed average protein retention of 139 g/d for barrows in the 40 – 60 kg BW interval. The reasons for the discrepancy between the values for protein retention measured by N balance or the slaughter method are diverse but well known and can result in a potential overestimation of the protein retention measured by N balance of up to 20% (Rao and McCracken, 1990).

The most probable reason is that the N balance is carried out over a short period and therefore it is a momentary observation that is representative only for a narrow BW interval (GfE, 2006). Furthermore, in this short period, NR can occur, which is not protein retention (Figueroa et al., 2002). In addition, due to the behavioral change of the animals caused by the housing in the metabolism crates and the associated reduction in activity, there may be a reduced N excretion with the feces and urine, but also a reduced voluntary feed intake (Cole et al., 1967; Quiniou et al., 1996). This may also be the reason why the NR between the SP was not correlated.

Since the application of the estimation equations for NR can be performed independently of the two SP, equation No. 3 (Table 14) was used to compare the mean protein retention of the two SP with the protein retention of the period between the SP. The calculated mean protein retention rate averaged 146 g/d and varied from 112 – 183 g/d for the 3 weeks. Thus, the values are in a comparable range as reported for comparative slaughter studies and about 20% lower than the mean of the two SP. Since the estimation equation only includes the two variables BW and NI, this may indicate that the mean NI, and thus the mean feed intake, was overestimated during the two SP due to daily variation.

5.3.2 Nitrogen utilization efficiency

The efficiency of dietary protein utilization in pig fattening depends on several factors besides feeding, such as management, animal health, and animal genetics, and therefore the maximum NUE for fattening is assumed to be around 50 – 60% under practical conditions (Millet et al., 2018b; Rotz, 2004). Explicitly considering feeding, in addition to digestibility, the NUE depends on matching the AA supply to the animals' requirements (van Milgen and Dourmad, 2015), which is mainly determined by the daily protein retention, which varies over time and between individuals (de Lange et al., 2012; Gaillard et al., 2020). Therefore, to improve the NUE in growing pigs, an optimal AA pattern for protein retention is aimed for by supplementation of essential AA, to simultaneously lower the dietary CP content without compromising animal performance. Achieving an ideal AA pattern improves the intermediary utilization of AA, reduces the need to break down excess AA to urea, and reduces urinary N excretion by about 8 – 10% for every 1% reduction in dietary CP content (Kerr et al., 2003; Noblet et al., 2001; Zervas and Zijlstra, 2002a). When adjusting for an optimal AA pattern, the NUE reflects the efficiency of utilization of the first limiting AA for NR (Moughan, 1991), which is reported for lysine to be about 65% on average (GfE, 2006; NRC 2012). Therefore, under experimental conditions of feeding low CP diets with simultaneous supplementation of essential AA, NUE values of 60 – 70% are not unusual (Chen et al., 2017; Kim et al., 2020; O'Connell et al., 2006; Saggau et al., 2000; van der Peet-Schwering et al., 2021; Windisch et al., 2000).

With a severely deficient supply of essential AA and simultaneously scarce supply of non-essential AA, even so, an NUE of 82.6% was achieved (Roth et al., 1999), which is close to the digestive capacity, which represents the natural theoretical limit for the level of dietary N utilization.

In the present experiment, the dietary AA pattern was intentionally not set according to the optimal AA pattern to create a marginal lysine supply, which limits growth, so that the animals can express their full genetic potential of utilizing dietary lysine for NR. Therefore, surpluses in the other essential AA were targeted when formulating the diets, averaging about 20% over the requirements, so that the level of lysine supply alone would limit NR and it would not be limited by deficiencies in other essential AA. In addition, because the diet was composed only of plant feedstuffs and was not supplemented with synthetic AA, the CP, and thus the non-essential AA concentration was relatively high. This was reflected by the ratio of lysine to CP in the diet, which was 0.05 in SP1 and 0.045 in SP2. According to Millet et al. (2018b), the optimal ratio of lysine to CP in the diet to achieve maximum NUE is 0.07. If this ratio becomes narrower, a deficiency of non-amino N can affect the synthesis of non-essential AA and thus performance; if this becomes wider, amino N is in excess and must be deaminated, resulting in increased BUN concentrations (Millet et al., 2018a).

The observed results of NUE were at a similar level as reported in the literature when feeding wheat, barley, and soybean meal-based diets for ad libitum intake, which range from 44.9 – 51.4% (Barea et al., 2010; Hansen et al., 2006; Le Bellego et al., 2001; Noblet et al., 2001; Quiniou et al., 1995b; Renaudeau et al., 2013; Zervas and Zijlstra, 2002a). However, in these studies, the diets were supplemented with free AA to achieve a lysine supply sufficient to meet requirements and/or to establish an ideal AA pattern relative to lysine. Nevertheless, in these studies, the ratio of lysine to CP in the diet was also about 0.05. Therefore, as in the present experiment, the level of NUE is significantly dependent on the supply of non-essential AA and non-amino N above requirements. Assuming empirical values for pc AA digestibility (CVB, 2018; GfE, 2006) and that pc digestible AA, which according to the ideal AA pattern are present in excess compared to lysine, cannot be utilized by the animals and must be deaminated and excreted as urea with the urine, the diet composition of the present experiment yields an imputed maximum NUE of 67% in SP1 and 63% in SP2. However, this would imply an intermediary AA utilization of 100%, which means that no catabolism would occur. Assuming an intermediary lysine utilization, which limits the amount of total intermediary AA utilization under marginal supply conditions, of 75% for high-performing animals, 65% for average-performing animals, and 55% for low-performing animals (NRC, 2012), the theoretical NUE varies from 37 – 50% in SP1 and from 34 – 47% in SP2 between individual animals.

The theoretical mean NUE would be 43.5% in SP1 and 40.5% in SP2. This agrees well with the observed results, which varied from 34.5 to 56.5%, but it indicates that the maximum intermediary utilization of dietary AA for protein retention exceeds 75%. With the implemented approach, the intermediary efficiency of dietary AA could not be measured, but the observed values of LUE varied from 57 – 89%. Assuming the average pc lysine digestibility as already discussed, the mean intermediary lysine utilization for protein retention would be 83% in SP1 and 81% in SP2, and the observed values would range from 67 – 105%. Thus, the values would be in a range reported when feeding diets with a severe lysine deficit, which is known to trigger metabolic mechanisms to increase the recycling of AA (Mnilk et al., 1996). Since the observed values are at an extremely high level, and a maximum of 105% can be considered unrealistic, this could indicate an increased pc lysine digestibility under the circumstances of marginal lysine supply. For example, assuming a pc lysine digestibility of 90%, the values for LUE would range from 63 – 99% with means of 78% in SP1 and 77% in SP2. However, the calculation of the LR is based on an average lysine concentration in the retained protein of 7.2% (GfE, 2006). If the retained protein consists of different proportions of lysine, this can also lead to an overestimation of the efficiency. For example, if the lysine concentration is reduced by 0.5 percentage points, this leads to a reduction in LR of about one gram per day. Furthermore, differences between individual animals in the AA pattern of the retained protein are also conceivable, which could misestimate the efficiency. Regardless of these considerations, the results of LUE illustrate the high efficiency that can be achieved at this growth performance under the conditions of marginal lysine supply.

The dependence of the intermediary N utilization on lysine utilization under conditions of marginal lysine supply was confirmed by the strong linear relationship between the DNUE and LUE across both SP ($r = 0.75$; Appendix 5). Furthermore, the LUE was positively correlated with NR ($r = 0.60$; Appendix 5) implying that the LUE improved with increasing protein retention. The results are similar to the observations of Remus et al. (2021), who reported, that 80% of the variation in LUE could be explained by the protein retention potential of the animals. This seems obvious, since the rate of inevitable lysine catabolism decreases by 0.2% per each additional gram of protein retention (Moehn et al., 2004; Moehn et al., 2000) and the level of LUE is almost completely dependent on the amount of protein retention because no significant amounts of lysine are required for the synthesis of non-protein compounds (Moehn et al., 2003). Nevertheless, a correlation of $r = 0.60$ indicates that 40% of the variation in LUE cannot be explained by the increase in protein retention but is most likely due to differences in the intermediary lysine utilization or different lysine requirements between individual animals.

These different requirements probably result from differences in the inevitable lysine catabolism between individuals since preferential catabolism should be negligible under marginal feeding conditions, and minimum catabolism is not expected to have a large impact on the total NUE. Nevertheless, the maintenance requirement is approximately 10% of the total lysine requirement (0.71 mg/kg MBW; NRC, 2012). At an average feed intake of 2 kg DM, the maintenance requirement is approximately 1.3 g lysine/d for a 50-kg pig. In the present experiment, an intercept of 1.77 was observed from the regression of LI on NR, which can be interpreted as the maintenance requirement in g/d but was not significant because of the small number of observations. However, if this maintenance requirement was reduced, for example by half, this would result in an increase in NR of 1.4 g/d or an average of 5% given the relationship between LI and NR. Since the lysine maintenance requirement is essentially due to the endogenous losses associated with digestion, a higher pc lysine digestibility could lead to a higher LUE because of reduced maintenance requirement. As explained, the pc lysine digestibility was not measured in the present experiment, but the moderate correlation of about 0.4 between CP ATTD and LUE across both SP and SP1 indicates this relationship.

Although the differences in LUE likely result from differences in inevitable catabolism, no pronounced correlation between LUE and protein degradation was observed in the present experiment. In only SP1, there was a moderate, negative correlation between the two traits (r = - 0.47), suggesting the presumed relationship. Because the absolute level of protein degradation partly depends on the body protein mass, which in turn is dependent on the animal's BW (Waterlow, 2006b), the characteristics of body protein turnover were also expressed in fractional rates to account for the variation in BW. Nevertheless, no relevant correlation between FDR and LUE could be observed. Furthermore, a cluster analysis was performed, i.e., the animals were grouped into low, medium, and high efficiency based on NUE, and the group means were analyzed for significant differences. However, there was no evidence of lower protein degradation or turnover in the highly efficient animals (Appendix 13). Hewitt et al. (2020) also did not observe a difference in body protein turnover when comparing animals of different RFI lines, although the LUE of the low-RFI animals was 13% lower on average.

One possible explanation is that by using the end-product method with the administration of a single oral dose of ^{15}N glycine, accurate estimates of whole-body N flux and thus protein turnover are obtained (Duggleby and Waterlow, 2005), but the contribution of individual AA pools cannot be quantified (Waterlow, 2006a). As explained earlier, due to diet composition, an excess intake of CP and AA occurred, which enters the same free AA pool as the ^{15}N glycine.

Although the high CP and AA supply stimulate the synthesis of body protein (Roth et al., 1999), not all absorbed AA can be used for synthesis because of the limiting marginal lysine supply. The AA not used for synthesis are oxidized in the same way as the AA degraded from body protein and excreted via urine. Thus, it is also possible that the complete ^{15}N glycine was not used for protein synthesis but rather part of it was directly oxidized and excreted. However, this cannot be differentiated using the end-product method, and the total ^{15}N excreted with urine is used for the calculation of N flux, regardless of whether the ^{15}N was previously incorporated in the body protein or not. Therefore, differences between individual animals in the degradation of body protein as a result of inevitable or minimum AA catabolism may have been masked by the excretion of ^{15}N, which did not contribute to the synthesis of body protein and thus by a high preferential AA catabolism. This is indicated by the individual ^{15}N excretion patterns of the first two N balance periods. Within the first 12-h collection interval, 48 and 42% of the total ^{15}N excretion had already occurred in SP1 and SP2, respectively. Considering the half-lives of the different body proteins (Stangl, 2010), this would not suggest the incorporation of the ^{15}N glycine, which was excreted during this interval. Furthermore, the end-product method cannot quantify the contribution of individual tissues and thus individual AA to protein turnover, but only the average of all body proteins (Therkildsen and Oksbjerg, 2009). Thus, because of the different AA compositions of proteins, it is likely that body protein turnover and LUE are not necessarily related. However, in the present study, no relevant correlation with protein turnover was found for NUE either, which again can also be explained by the excesses of CP and AA. Thus, under the given feeding conditions of the experiment, the differences in inevitable catabolism between individual animals cannot be determined by using the end-product method. Therefore, for revealing differences in basal turnover, it would be appropriate to feed diets with a low excess of AA to minimize preferential catabolism, even if there is a risk of limiting the protein synthesis capacity of the animals.

5.4 Blood metabolites

The blood metabolites BUN, SC, and IGF-I, which are related to protein turnover, were of particular interest in the present experiment since it was assumed that their determination could be an alternative to N balance data to accurately phenotype large numbers of animals with as little effort as possible. The BUN concentration was especially considered of special interest since urea is the major degradation product of AA metabolism and thus an indicator of the intermediary utilization of the ingested AA.

Several studies have demonstrated the general dependence of the BUN concentration on protein utilization efficiency (e.g., Berschauer, 1977; Chen et al., 1999; Whang and Easter, 2000), and it has been shown that urinary N excretion can be estimated from BUN concentration (Kohn et al., 2005). Moreover, BUN concentration is considered a suitable response criterion for empirical determination of AA requirements (e.g., Borgesa et al., 2013; Coma et al., 1995; Millet et al., 2018a). Additionally, because BUN concentration can serve to reveal differences in protein utilization between different genotypes and sexes (Chen et al., 1995; Fabian et al., 2003; Whang and Easter, 2000), it has been hypothesized that it can also serve to reveal differences in NUE between individuals of the same genotype. Since lower intermediate N or lysine utilization is associated with higher inevitable AA catabolism, this should be associated with higher BUN concentrations.

In the present experiment, the mean BUN concentration of 5.94 mmol/L in SP1 was slightly, although significantly (p = 0.022), higher than in SP2 (5.70 mmol/L) and varied from 4.15 to 7.72 mmol/L between individual animals. In contrast to the highly significant correlation (r = - 0.92) between BUN concentration and protein utilization efficiency of growing pigs fed diets differing in CP and energy contents over a large number of trials reported by Berschauer (1977), only a weak correlation between BUN concentration and NUE was observed in the present experiment. This was r = - 0.31 in SP1 and r = 0.29 in SP2, indicating that BUN concentration tended to increase with increasing NUE in SP2. Due to the oppositely directed correlations in the respective SP, no significant relationship between BUN concentration and NUE was observed across both SP.

It is known that a positive correlation between the level of CP intake and BUN concentration exists when diets differing in CP concentration are fed (e.g., Chen et al., 1999; Lopez et al., 1994; Zervas and Zijlstra, 2002b). The BUN concentration is not only dependent on the absolute level of NI but also on the AA pattern of the ingested CP. If this corresponds to the concept of ideal protein through supplementation of free AA, the BUN concentration decreases, depending on the LI level, to about 30 – 80% of the BUN concentration of diets that were not optimized according to their AA pattern and consisted only of natural components (Lopez et al., 1994). The dietary AA supplied above the requirement are oxidized in the liver to urea, which is then transported via the bloodstream to the kidney, increasing the BUN concentration.

The non-significant correlation between the BUN concentration and the NUE is consistent with the linear relationship between the N or lysine intake and the NR. This means that for all animals a relatively constant proportion of the ingested AA could be transferred into body protein, regardless of the absolute level of CP intake.

Thus, the proportion of ingested AA that had to be oxidized to urea was also relatively constant and independent of the level of CP intake. This is, therefore, fundamentally different from the studies that have investigated the change in BUN concentration when the dietary CP concentration is altered, since all additional AA supplied in excess of the requirement must be degraded, thereby increasing the BUN concentration. Due to the diet composition in the present experiment, there was an oversupply of all AA except lysine. Therefore, it is likely that the differences in LUE, and thus NUE, between individual animals resulting from differences in inevitable catabolism were masked by the degradation of dietary AA present in excess and, therefore, no relationship was observed between NUE and BUN concentration. This assumption could be examined in a similar experimental approach with marginal lysine supply by adjusting the dietary AA in the ratio of the ideal protein so that preferential catabolism is excluded as far as possible. However, this carries the risk that other essential AA besides lysine will limit protein retention and thus NUE.

With 3.9 to 8.2 mmol/L, Zervas and Zijlstra (2002a) reported a similar extent of variation in BUN concentration in crossbred barrows fed wheat, barley, and soybean meal-based diets with a CP content of 19.5% twice daily and blood sampling 4 h after morning feeding. In a companion experiment feeding similar diets under ad libitum conditions, the same authors (Zervas and Zijlstra, 2002b) detected a mean BUN concentration of 6.8 mmol/L (SD = 0.5 mmol/L) 4 h after feed administration. Thus, the observed values of the present experiment are in a comparable range under similar feeding conditions. Furthermore, the authors observed a correlation between BUN concentration and UNE of r = 0.81, which corresponds exactly to the correlation observed for SP1 in the present experiment. This correlation decreased to r = 0.55 in SP2, resulting in an overall correlation of r = 0.50 across both SP. Because of this only moderate correlation, estimation of UNE by BUN concentration was possible only with a large RMSE of 3.34 g/d or 15% of the mean UNE of 22.4 g/d. Assuming that under ad libitum feeding conditions the BUN concentration is constant throughout the day (Zervas and Zijlstra, 2002b) and the urea has to be excreted via urine (Patience, 2012), better accuracy of the estimation of UNE should be achievable. For example, Zervas and Zijlstra (2002a) reported an R^2 value of 0.71 for the prediction of UNE via BUN concentrations obtained by blood sampling 4 h after morning feeding. However, treatments with different CP concentrations were used to set up the regression equation, which must inevitably lead to a more pronounced correlation due to the afore mentioned relationships between dietary N or AA intake, BUN, and UNE.

Nevertheless, with 2.30, the slope of the regression equation was similar to the observed slope in the present experiment (2.66), indicating that the principal relationship between BUN concentration and UNE exists even when all animals are fed the same diet.

Also, the RMSE of 2.38 g/d in the study of Zervas and Zijlstra (2002a), is comparable to the RMSE of the equation of the present study when expressed as a percentage of the mean UNE and the range of variation in UNE was similar for both studies. Nevertheless, Zervas and Zijlstra (2002a) concluded that due to the high RMSE, the prediction of UNE via BUN concentration is too inaccurate to conclude the NUE of the animals.

To obtain a more accurate estimate of UNE, multiple regression of the traits that correlated with the animal's UNE was performed in the present experiment. The variables used for the model with the best goodness of fit (adj. R^2 = 0.77, RMSE = 1.88 g/d) were IBW, BW, NI, and the concentrations of the blood metabolites BUN, SC, and IGF-I. Thus, the estimation accuracy was doubled compared with using the BUN concentration alone. This allows the UNE to be estimated with sufficient accuracy under the given feeding conditions.

In addition to the intermediary N utilization, the level of UNE and the ratio of urinary N to fecal N in the TNE is affected by the level of protein supply and the digestibility of the diet. Hence, UNE decreases by 10% when the CP content of the diet is lowered by one percentage point (Noblet et al., 2001; Zervas and Zijlstra, 2002a), and a higher CP ATTD results in a higher UNE if the additionally ingested N cannot be used for NR (Windisch et al., 2000). Therefore, when feeding diets with lower CP ATTD due to higher fiber content, the ratio between UNE and FNE within TNE is closer than when feeding highly digestible diets with low fiber content (Canh et al., 1997; Jha and Berrocoso, 2016). As a result, the UUE decreases relative to TNE, and less ammonia is released. In the present experiment, an almost constant ratio of UNE to FNE was observed over both periods; two-thirds of TNE were UNE and one-third was FNE. Furthermore, about one-third of the ingested N was excreted again via urine. These values agree with the observations of other N balance studies under ad libitum feeding conditions of conventional wheat, barley, and soybean meal-based diets with similar composition (Hansen et al., 2006; Zervas and Zijlstra, 2002a). Feeding the same diets, differences in N excretion patterns may indicate different utilization of ingested N between individual animals. Thus, animals with a lower proportion of UNE within TNE should generally have a better intermediary N utilization. Looking at the individual results, the proportion of UNE in TNE varied from 54 – 78% between the pigs, and the results correlated with DNUE (r = - 0.38 in SP1 and r = - 0.41 in SP2), although only moderately.

Nonetheless, no significant correlation between the UNE:TNE ratio and NUE or LUE was observed, suggesting that in addition to the intermediary N utilization, the CP ATTD had a significant effect on the total NUE of the pigs. With a reliable estimation of the UNE and the determination of the FNE, the NUE of the animals could be predicted with sufficient accuracy, which was attempted in the present experiment.

Because of the low goodness of fit for the direct estimation of the NUE (adj. $R^2 = 0.41$) and the high RMSE (3.44), the NR was estimated in the first step. Subsequently, the NUE was calculated, since the goodness of fit for the estimation of NR was higher (adj. $R^2 = 0.72$) and the RMSE smaller (1.98). However, the chosen model for estimating NR was not the best model of the multiple regression. The model with the lowest AIC (model no. 1; Table 14) contained CP ATTD as a variable, but this model had to be rejected, as the values for CP ATTD could not be utilized for all animals.

Comparing the best linear models with each other (Model 1 and Model 2; Table 14), using CP ATTD data results in an improvement in estimation accuracy and reduction in RMSE of about 10%. However, by including interactions in the estimation, the same goodness of fit could be achieved as with the linear equation containing the CP ATTD as a variable. Furthermore, for the application of the estimation equation for phenotyping large numbers of animals, it might even be advantageous to omit CP ATTD data, since the feces collection represents a non-negligible experimental and analytical effort (Déru et al., 2021). Moreover, the estimation allowed for dividing the animals into low, medium, and high performers according to their NR, which may be sufficiently accurate, especially for genetic evaluations. However, according to the 95% confidence interval, the estimated values for NR can range from ± 4.3 g/d, which would mean a deviation of about 15% for the mean NR of 27.2 g/d. Furthermore, the model used in the present study has the disadvantage that it contains the IBW as a variable, and the use of the equation is thus limited to an application with a similar experimental approach (start of the experiment in the 11^{th} week of life). Furthermore, due to the data structure with the repeated measurements on the same animals, an independent validation of the equation was not possible; otherwise, the number of animals and thus observations would have been too small for reliable estimates. Therefore, the capacity of extrapolation of the model is also limited (Bastianelli et al., 2015), resulting in an estimation and not a prediction for the values obtained via the model. Also, no internal validation data set could be generated via the bootstrapping procedure, since for this method, 50 independent observations are considered the minimum number to obtain reliable prediction results (Wright et al., 2011). Furthermore, a leave-one-out cross-validation did not lead to a better fit of the model either. For the application of the equation outside of this data set, a prior validation on an independent test data set would be recommended (Esbensen, 2012).

5.5 Conclusions

In conclusion, under the present conditions of a marginal lysine supply, the NR of growing pigs could be estimated based on N balance data and postprandial concentrations of the blood metabolites BUN, SC, and IGF-I with sufficient accuracy to classify the pigs into low, medium, and high retainers. This approach may provide comparatively rapid and reliable alternatives to carcass or N balance data for phenotyping large numbers of animals as needed for genetic evaluations. Another application of estimating NR is the real-time determination of AA requirements of individual animals as required for the concept of precision feeding. In this context, the application of the estimation equation is independent of the two growth stages as sampled in the present experiment and can be applied in the starter/grower phase over a BW range of about 30 – 70 kg. However, it would be advisable to validate the estimation equation on an independent test data set to verify its suitability for application outside the present data set. Furthermore, the availability of reliable data on CP ATTD would improve the goodness of fit of the estimation of NR by about 10%. However, because the accurate collection of digestibility data is costly and carries the risk of additional error, it may even be advantageous to have a sufficiently accurate estimate without the use of digestibility data. Especially concerning phenotyping under practical conditions in the field, the reduced experimental effort is advantageous. Since the BUN concentration depends on several dietary factors, standardization of feeding is essential for the use of BUN as an indicator of protein utilization. Diet formulation based on the concept of the ideal protein with also the least possible excess of nonessential AA could result in differences in NUE between individual animals becoming more apparent and could help increase the accuracy of the estimation.

Phenotyping revealed a large variation in NUE between individual animals in both SP, which is an important prerequisite for possible breeding strategies on NUE in pig fattening. Furthermore, the significant differences in the N metabolism traits between the offspring groups of the used boars under the standardized conditions indicate a genetic component of NUE. As expected, NUE decreased on average across both SP, but differences between individual animals also existed, with NUE unchanged or even increased so that no correlation of NUE between the SP could be observed. This illustrates that phenotyping within a short period represents snapshots, and repeated observations are necessary for reliable conclusions about the protein utilization potential of individual animals. With this experimental approach, the level of NUE was independent of the level of N or lysine intake, and approximately 50% of the variation in NUE could be explained by variation in NR between the individuals. Therefore, pigs with a higher protein retention capacity principally utilize dietary N more efficiently.

Despite the linear dependence of NR on the level of LI due to the marginal lysine supply across both SP, only about 70% of the variation in NR could be explained by variation in LI. Because no relationship was observed between body protein turnover and the utilization of N and lysine for retention, differences in maintenance requirements are apparently too small to affect the overall utilization efficiency. Therefore, the remaining part of the variation can be attributed to differences in the intermediary utilization of lysine for protein retention or to differences in the lysine content of the retained body protein between the pigs. However, how and whether differences in protein utilization between individual animals affect body composition could not be clarified in this experiment and could be the subject of investigation in subsequent studies.

6 SUMMARY

Efficient utilization of dietary nitrogen (N) in pork production is of increasing concern. Previous studies revealed that a genetic basis for N utilization efficiency (NUE) might exist, but to assess the potential of breeding for improved NUE, the between-animal variation of a large number of animals needs to be known. The standard method to determine N retention (NR) is laborious and not feasible for the required numbers of animals. However, correlations between protein utilization and blood urea nitrogen (BUN) concentration have been shown to exist and body protein turnover is subject to hormonal control. Hence, the objective of the present thesis was to quantify NR of growing pigs at two different growth stages by N balance and to determine the impact of body protein turnover on NUE. In addition, equations for the estimation of NR were established, using performance data and blood metabolite concentrations, which were applied to evaluate the variation in NUE of a F1 crossbred population.

A total of 508 crossbred pigs (German Landrace x Pietrain), half gilts and half barrows, from 20 different boars was investigated in the course of 2.5 years. The experimental period started at the beginning of the 11th week of life and lasted until slaughter 73 days later. The pigs were housed individually throughout the experimental period and were weighed initially and in weekly intervals to calculate the average daily gain (ADG). A two-phase fattening was performed and the transition from starter phase to grower phase took place in the 14th week of life. All animals received the same diet for ad libitum intake which was formulated to contain 90% of the recommended lysine concentration so that marginal lysine supply was the limiting factor for protein retention and pigs were allowed to express their full genetic potential of NUE. In both fattening phases, a five-day sampling period (SP) was conducted. SP1 was carried out in the starter phase in the 13th week of life and SP2 in the grower phase in the 16th week of life. During the SP, daily feed intake was recorded for each animal. Blood samples were taken from the jugular vein at around 13:00 h on three consecutive days, for determination of BUN, cortisol, and insulin-like growth factor 1 (IGF-I) concentration. In both SP, N balance was performed on a randomly selected subsample of 56 barrows. The barrows were housed in metabolism crates for six days, two days for adaption and four days for quantitative collection of feces and urine. Simultaneously, their body protein turnover was determined by the end-product method after administration of a single oral dose of ^{15}N-labeled glycine. Based on the N balance results, models for estimation of NR were obtained by multiple regression of performance data and blood metabolite concentrations. The significance of the variables was validated using a bootstrapping method to avoid overfitting the models to the observed data. The goodness of fit of the equations was assessed using the coefficient of determination (R^2) and the root mean square error (RMSE).

The pigs' mean body weight (60.2 kg), average daily feed intake (ADFI; 2.51 kg), and ADG (0.97 kg) were all significantly higher in SP2 than in SP1 (40.5 kg, 1.86 kg, and 0.90 kg, respectively). Across both SP, the values ranged from 27.5 – 87.0 kg for body weight, 1.20 – 3.56 kg for ADFI, and 0.45 – 1.38 kg for ADG. The N balance results showed that the mean daily NR did not differ between the SP (27.9 g/d in SP1 vs. 26.7 g/d in SP2; p = 0.114). Individual NR varied from 14.8 – 38.0 g/d and strongly correlated with N and lysine intake (r = 0.70 and r = 0.73, respectively). The values for NUE ranged from 34.5 – 56.5% and were moderately correlated with NR (r = 0.54). However, the mean NUE was higher in SP1 (47.4%) than in SP2 (43.0%; $p < 0.001$). The mean body protein turnover did not differ between the SP (669 g/d in SP1 vs. 668 g/d in SP2; p = 0.581) and no correlation with NUE existed. In estimating NR, the model with the best goodness of fit included the variables initial body weight, ADG, ADFI, N intake, BUN, cortisol, and IGF-I concentration and provided an adjusted R^2 of 0.72 and a RMSE of 1.98 g/d or 7.25%. The application of the estimation equation for all pigs resulted in a mean NR of 31.1 g/d in SP1 and 31.7 g/d in SP2 and the individual values varied from 13.6 – 46.7 g/d. The high NR resulted in a high mean NUE of 48.4% in SP1 and 44.6% in SP2, with values ranging from 35.2 – 57.9%. When NR was described as a linear function of lysine intake across both SP, the animals retained an average of 1.48 g N, or the equivalent of 9.25 g protein, with each additional gram of lysine ingested. This corresponded to an average marginal efficiency of lysine utilization for protein retention of 67%. Despite a wide variation in NUE within the offspring of the same boars, significant differences were found between the offspring groups of the boars. The relative difference in NUE between the offspring of the most efficient and the most inefficient boar was 4.3 percentage points (49.3% vs. 45.0%).

Under the prevailing circumstances of marginal lysine supply, the NR of fattening pigs could be estimated from performance data and blood metabolite concentrations with satisfying accuracy. This provides a fast and reliable alternative to performing N balance studies, reducing the experimental effort considerably in studies with large numbers of animals. Although lysine supply was the limiting factor for protein retention, only about 70% of the variation in NR could be explained by the level of lysine intake. The remaining part of the variation was likely caused by differences in the intermediary lysine utilization or differences in the lysine content of the retained body protein between individuals. Despite the marginal lysine supply, the pigs' growth performance was high overall, but phenotyping revealed large differences in NUE among individuals. About 50% of the variation in NUE could be explained by differences in the level of NR, implying that pigs with higher protein retention potential utilized dietary N more efficiently. However, this was not accompanied by differences in body protein turnover. The large variation in the observed data and the significant boar effect indicate the possibility of improving NUE through breeding measures.

7 ZUSAMMENFASSUNG

Die effiziente Verwertung des Futter-Stickstoffs (N) wird in der Schweineerzeugung zunehmend bedeutender. Frühere Studien haben gezeigt, dass eine genetische Grundlage der N-Nutzungseffizienz (NNE) zu bestehen scheint, aber um das Potential von Züchtungsmaßnahmen zur Verbesserung der NNE beurteilen zu können, muss die individuelle Variation einer ausreichend großen Tierzahl erfasst werden. Die Standardmethode zur Bestimmung des N Ansatz (NA) ist aufwändig und kaum mit den benötigten Tierzahlen durchführbar. Allerdings wurden signifikante Korrelationen zwischen der Proteinverwertung und der Blutharnstoff-Konzentration (BHK) nachgewiesen und der Körperproteinumsatz unterliegt hormonellen Einflüssen. Ziel der vorliegenden Arbeit war es daher, den NA von Mastschweinen in zwei verschiedenen Wachstumsabschnitten durch N-Bilanzierung zu quantifizieren und den Einfluss des Körperproteinumsatzes auf die NNE zu bestimmen. Darüber hinaus wurden Gleichungen für die Schätzung des NA anhand von Leistungsdaten und Blutmetabolit-Konzentrationen aufgestellt, welche im Anschluss zur Beschreibung der Variation der NNE einer F1-Kreuzungspopulation verwendet wurden.

Insgesamt wurden 508 Kreuzungstiere (Deutsche Landrasse x Pietrain) die von 20 verschiedenen Ebern abstammten, jeweils zur Hälfte Jungsauen und Börge, verteilt über 2,5 Jahre, untersucht. Der Versuchszeitraum startete mit dem Beginn der 11. Lebenswoche und dauerte bis zur Schlachtung nach 73 Tagen. Während des gesamten Versuchszeitraums waren die Schweine in Einzelhaltung untergebracht und wurden zum Einstallen sowie wöchentlich gewogen, um die durchschnittliche tägliche Lebendmassezunahme (LMZ) zu berechnen. Es wurde eine zweiphasige Mast durchgeführt und der Wechsel von der Vor- zur Mittelmastphase erfolgte in der 14. Lebenswoche. Allen Tieren wurde das gleiche Futter zur *ad libitum* Aufnahme vorgelegt, welches lediglich 90% der empfohlenen Lysinkonzentration enthielt, so dass die marginale Lysinversorgung der begrenzende Faktor für den Proteinansatz war und die Schweine ihr volles genetisches Potenzial der effizienten N-Nutzung entfalten konnten. In beiden Mastphasen wurden in einem fünftägigem Probenahmezeitraum (PZ) jeweils die gleichen Merkmale erfasst. PZ1 erfolgte in der Vormastphase in der 13. Lebenswoche und PZ2 in der Mittelmastphase in der 16. Lebenswoche. Während der PZ wurde die tägliche Futteraufnahme (FA) der Tiere erfasst, und an drei aufeinanderfolgenden Tagen gegen 13:00 Uhr Blutproben aus der Jugularvene entnommen, um die BHK und die Konzentrationen von Cortisol und dem insulinähnlichen Wachstumsfaktor 1 (IGF-I) zu bestimmen. In beiden PZ wurde in demselben Versuchsstall zusätzlich eine N-Bilanz an einer zufällig ausgewählten Stichprobe von 56 Börgen durchgeführt. Dafür wurden die Börge für sechs Tage in Stoffwechselkäfigen gehalten.

Einer zweitägigen Eingewöhnungsphase folgte eine viertägige quantitative Kot- und Harnsammlung. Zeitgleich wurde deren Körperproteinumsatz mittels Endprodukt-Methode nach einmaliger, oraler Verabreichung von ^{15}N-markiertem Glycin bestimmt. Auf der Grundlage der N-Bilanzergebnisse wurden durch multiple Regression der Leistungsdaten und der Blutmetabolit-Konzentrationen Modelle zur Schätzung des NA erstellt. Die Signifikanz der Modellvariablen wurde durch ein Bootstrapping-Verfahren überprüft, um eine Überanpassung der Modelle an die beobachteten Daten zu vermeiden. Die Anpassungsgüte der abgeleiteten Gleichungen wurde anhand des Bestimmtheitsmaßes (R^2) und der Wurzel der mittleren Fehlerquadratsumme (RMSE) bewertet.

Das durchschnittliche Körpergewicht der Schweine (60,2 kg), die durchschnittliche tägliche FA (2,51 kg) und die LMZ (0,97 kg) waren in PZ2 signifikant höher als in PZ1 (40,5 kg, 1,86 kg bzw. 0,90 kg). In beiden PZ variierten die Einzelwerte von 27,5 – 87,0 kg für das Körpergewicht, 1,20 – 3,56 kg für die FA und 0,45 – 1,38 kg für die LMZ. Die Ergebnisse der N-Bilanz zeigten, dass sich der mittlere tägliche NA zwischen den PZ nicht unterschied (27,9 g/d in PZ1 gegenüber 26,7 g/d in PZ2; p = 0,114). Der individuelle NA variierte zwischen 14,8 und 38,0 g/d und war stark mit der N- und Lysinaufnahme korreliert (r = 0,70 bzw. r = 0,73). Es wurde eine mäßige Korrelation zwischen dem NA und der NNE beobachtet (r = 0,54), und die Werte der NNE variierten von 34,5 bis 56,5%. Allerdings war die mittlere NNE in PZ1 (47,4%) höher als in PZ2 (43,0%; p < 0,001). Der mittlere Körperproteinumsatz unterschied sich nicht zwischen den PZ (669 g/d in PZ1 gegenüber 668 g/d in PZ2; p = 0,581) und es wurde keine Korrelation mit der NNE beobachtet. Das Modell für die Schätzung des NA mit der besten Anpassungsgüte beinhaltete die Variablen Einstallgewicht, LMZ, FA, N-Aufnahme, BUN, Cortisol und IGF-I-Konzentration. Damit konnte der NA mit einem korrigiertem R^2 von 0,72 und einem RMSE von 1,98 g/d geschätzt werden, was einem mittleren Schätzfehler von 7,25% entsprach. Die Anwendung der Schätzgleichung für alle Schweine ergab einen mittleren NA von 31,1 g/d in PZ1 und 31,7 g/d in PZ2, wobei die Einzelwerte zwischen 13,6 und 46,7 g/d variierten. Der hohe NA führte zu einer hohen mittleren NNE von 48,4% in PZ1 und 44,6% in PZ2, wobei die Einzelwerte zwischen 35,2 und 57,9% lagen. Wurde der NA als lineare Funktion der Lysinaufnahme beschrieben, setzen die Tiere im Mittel beider PZ mit jedem zusätzlich aufgenommenen Gramm Lysin durchschnittlich 1,48 g N oder das Äquivalent von 9,25 g Protein an. Dies entsprach einer durchschnittlichen marginalen Effizienz der Lysinverwertung für den Proteinansatz von 67%. Trotz großer Unterschiede in der NNE innerhalb der Nachkommen der jeweiligen Eber konnten signifikante Unterschiede zwischen den Nachkommengruppen der Eber festgestellt werden. Der relative Unterschied in der NNE zwischen den Nachkommen des effizientesten und des ineffizientesten Ebers betrug 4,3 Prozentpunkte (49,3 % gegenüber 45,0 %).

Unter den vorliegenden Bedingungen einer marginalen Lysinversorgung konnte der NA von Mastschweinen anhand von Leistungsdaten und Blutmetabolit-Konzentrationen mit zufriedenstellender Genauigkeit geschätzt werden. Dies stellt eine schnelle und zuverlässige Alternative zur Durchführung von N-Bilanzstudien dar und reduziert damit den Versuchsaufwand erheblich, insbesondere wenn eine große Anzahl von Tieren beprobt werden muss. Obwohl die Lysinversorgung der begrenzende Faktor für den Proteinansatz war, konnten nur etwa 70% der Variation des NA durch die Höhe der Lysinaufnahme erklärt werden. Der verbleibende Teil der Variation wurde wahrscheinlich durch Unterschiede in der intermediären Lysinverwertung oder Unterschiede im Lysingehalt des angesetzten Körperproteins zwischen den Individuen verursacht. Trotz der marginalen Lysinversorgung war die Mastleistung der Schweine insgesamt hoch, aber die Phänotypisierung ergab große Unterschiede in der NNE zwischen den Individuen. Etwa 50% der Variation in der NNE konnten durch Unterschiede in der Höhe des NA erklärt werden, was darauf hindeutet, dass Schweine mit einem höheren Proteinansatzpotenzial den mit dem Futter aufgenommenen N effizienter verwerteten. Dies ging jedoch nicht mit Unterschieden im Körperproteinumsatz einher. Die große Variation in den beobachteten Daten und der signifikante Eber-Effekt deuten die Möglichkeit an, die NNE durch züchterische Maßnahmen verbessern zu können.

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9 APPENDIX

Appendix 1 (1/3). Schedule of the experiment from October 2018 to April 2021

Date	Trial week	Row 1	Row 2	Row 3
22.10.-28.10.2018	1	**C 1**		
29.10.-04.11.2018	2			
05.11.-11.11.2018	3	**SP 1**		
12.11.-18.11.2018	4		**C 2**	
19.11.-25.11.2018	5			
26.11.-02.12.2018	6	**FT**	**SP 1**	
03.12.-09.12.2018	7		**FT**	
10.12.-16.12.2018	8	**SP 2**		
17.12.-23.12.2018	9		**SP 2**	
24.12.-30.12.2018	10			
31.12.-06.01.2019	11	**Slaughter**		
07.01.-13.01.2019	12			
14.01.-20.01.2019	13		**Slaughter**	
21.01.-27.01.2019	14			
28.01.-03.02.2019	15			
04.02.-10.02.2019	16			**C 3**
11.02.-17.02.2019	17			
18.02.-24.02.2019	18			**SP 1**
25.02.-03.03.2019	19			**FT**
04.03.-10.03.2019	20	**C 4**		
11.03.-17.03.2019	21			**SP 2**
18.03.-24.03.2019	22	**SP 1**		
25.03.-31.03.2019	23	**FT**		
01.04.-07.04.2019	24		**C 5**	
08.04.-14.04.2019	25	**SP 2**		
15.04.-21.04.2019	26		**SP 1**	**Slaughter**
22.04.-28.04.2019	27		**FT**	
29.04.-05.05.2019	28			**C 6**
06.05.-12.05.2019	29		**SP 2**	
13.05.-19.05.2019	30	**Slaughter**		**NBP 1**
20.05.-26.05.2019	31			**FT**
27.05.-02.06.2019	32	**C 7**		
03.06.-09.06.2019	33			**NBP 2**
10.06.-16.06.2019	34	**SP 1**	**Slaughter**	
17.06.-23.06.2019	35	**FT**		
24.06.-30.06.2019	36			
01.07.-07.07.2019	37	**SP 2**	**C 8**	
08.07.-14.07.2019	38			
15.07.-21.07.2019	39		**SP 1**	**Slaughter + DS**
22.07.-28.07.2019	40		**FT**	
29.07.-04.08.2019	41			
05.08.-11.08.2019	42	**Slaughter**	**SP 2**	**C 9**
12.08.-18.08.2019	43			

The experimental barn was equipped with three rows of individual pens, hence three cohorts could be kept simultaneously. C = Start of the respective cohort; SP = sampling period; NBP = nitrogen balance period; FT = feed transition from starter to grower diet; DS = digesta sampling

Appendix 1 (2/3). Schedule of the experiment from October 2018 to April 2021

Date	Trial week	Row 1	Row 2	Row 3
19.08.-25.08.2019	44			**NBP 1**
26.08.-01.09.2019	45			**FT**
02.09.-08.09.2019	46	**C 10**		
09.09.-15.09.2019	47		**Slaughter**	**NBP 2**
16.09.-22.09.2019	48	**SP 1**		
23.09.-29.09.2019	49	**FT**		
30.09.-06.10.2019	50		**C 11**	
07.10.-13.10.2019	51	**SP 2**		
14.10.-20.10.2019	52		**SP 1**	**Slaughter + DS**
21.10.-27.10.2019	53		**FT**	
28.10.-03.11.2019	54			
04.11.-10.11.2019	55		**SP 2**	
11.11.-17.11.2019	56	**Slaughter**		
18.11.-24.11.2019	57			
25.11.-01.12.2019	58			
02.12.-08.12.2019	59			
09.12.-15.12.2019	60		**Slaughter**	
16.12.-22.12.2019	61			
23.12.-29.12.2019	62			
30.12.-05.01.2020	64			
06.01.-12.01.2020	65			
13.01.-19.01.2020	66	**C 12**		
20.01.-26.01.2020	67			
27.01.-02.02.2020	68			
03.02.-09.02.2020	69	**SP 1**		
10.02.-16.02.2020	70	**FT**		
17.02.-23.02.2020	71		**C 13**	
24.02.-01.03.2020	72	**SP 2**		
02.03.-08.03.2020	73		**NBP 1**	
09.03.-15.03.2020	74		**FT**	
16.03.-22.03.2020	75			
23.03.-29.03.2020	76	**Slaughter**		
30.03.-05.04.2020	77			
06.04.-12.04.2020	78			
13.04.-19.04.2020	79			
20.04.-26.04.2020	80	**C 14**		
27.04.-03.05.2020	81		**Slaughter**	
04.05.-10.05.2020	82	**NBP 1**		
11.05.-17.05.2020	83	**FT**		
18.05.-24.05.2020	84			
25.05.-31.05.2020	85	**NBP 2**	**C 15**	
01.06.-07.06.2020	86			
08.06.-14.06.2020	87		**NBP 1**	

The experimental barn was equipped with three rows of individual pens, hence three cohorts could be kept simultaneously. C = Start of the respective cohort; SP = sampling period; NBP = nitrogen balance period; FT = feed transition from starter to grower diet; DS = digesta sampling

Appendix 1 (3/3). Schedule of the experiment from October 2018 to April 2021

Date	Trial week	Row 1	Row 2	Row 3
15.06.-21.06.2020	88		**FT**	
22.06.-28.06.2020	89			
29.06.-05.07.2020	90	**Slaughter + DS**	**NBP 2**	
06.07.-12.07.2020	91			**C 16**
13.07.-19.07.2020	92			
20.07.-26.07.2020	93			**SP 1**
27.07.-02.08.2020	94		**Slaughter + DS**	**FT**
03.08.-09.08.2020	95			
10.08.-16.08.2020	96	**C 17**		**SP 2**
17.08.-23.08.2020	97			
24.08.-30.08.2020	98	**SP 1**		
31.08.-06-09.2020	99	**FT**		
07.09.-13.09.2020	100		**C 18**	
14.09.-20.09.2020	101	**SP 2**		**Slaughter**
21.09.-27.09.2020	102			
28.09.-04.10.2020	103		**SP 1**	
05.10.-11.10.2020	104		**FT**	
12.10.-18.10.2020	105			
19.10.-25.10.2020	106	**Slaughter + DS**	**SP 2**	
26.10.-01.11.2020	107			**C 19**
02.11.-08.11.2020	108			
09.11.-15.11.2020	109			**NBP 1**
16.11.-22.11.2020	110			**FT**
23.11.-29.11.2020	111		**Slaughter + DS**	
30.11.-06.12.2020	112			**NBP 2**
07.12.-13.12.2020	113			
14.12.-20.12.2020	114			
21.12.-27.12.2020	115			
28.12.-03.01.2021	116			
04.01.-10.01.2021	117	**C 20**		**Slaughter**
11.01.-17.01.2021	118			
18.01.-24.01.2021	119	**SP 1**		
25.01.-31.01.2021	120	**FT**		
01.02.-07.02.2021	121		**C 21**	
08.02.-14.02.2021	122	**SP 2**		
15.02.-21.02.2021	123		**NBP 1**	
22.02.-28.02.2021	124		**FT**	
01.03.-07.03.2021	125			
08.03.-14.03.2021	126		**NBP 2**	
15.03.-21.03.2021	127	**Slaughter**		
22.03.-28.03.2021	128			
29.03.-04.04.2021	129			
05.04.-11.04.2021	130			
12.04.-18.04.2021	131		**Slaughter**	

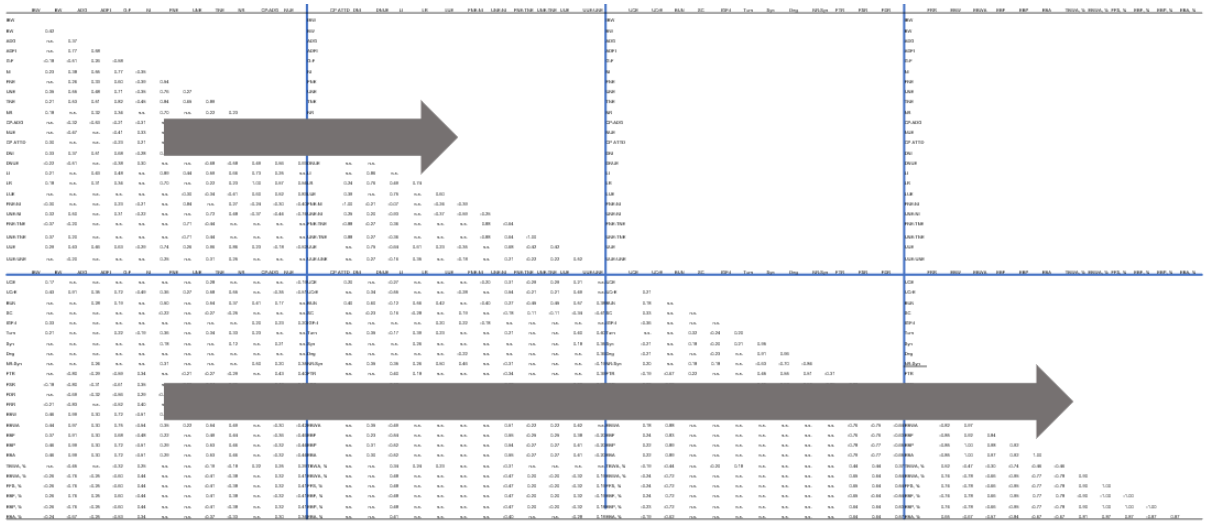
The experimental barn was equipped with three rows of individual pens, hence three cohorts could be kept simultaneously. C = Start of the respective cohort; SP = sampling period; NBP = nitrogen balance period; FT = feed transition from starter to grower diet; DS = digesta sampling

Appendix 2. Variation of the analyzed nutrient concentrations of the experimental diets (values per kg dry matter) fed throughout the sampling periods (SP)

	SP1					SP2				
	MEAN	SD	%CV	MIN	MAX	MEAN	SD	%CV	MIN	MAX
CP	215	6.1	2.8	204	224	177	6.3	3.6	165	185
Lys	10.6	0.5	4.5	9.6	11.5	7.9	0.3	4.2	7.3	8.4
Met+Cys	7.0	0.3	3.8	6.6	7.5	6.1	0.3	4.5	5.7	6.6
Thr	8.0	0.1	4.5	3.1	3.6	6.4	0.2	3.7	6.0	6.8
Val	10.0	0.4	3.5	9.3	10.8	8.3	0.4	4.5	7.7	9.0
Leu	16.1	0.6	3.9	15.2	17.4	13.0	0.6	4.3	11.9	14.1
Ile	8.8	0.4	4.1	8.1	9.5	6.9	0.3	4.7	6.4	7.6
His	5.8	0.2	3.9	5.5	6.3	4.7	0.2	4.6	4.3	5.0
Phe+Tyr	17.7	0.7	4.0	16.5	19.0	14.4	0.7	5.2	13.1	15.7
TiO_2	5.4	0.4	6.9	4.8	6.1	5.1	0.3	6.5	4.4	5.6

For every cohort, a new diet was mixed for feeding in the SP, hence, 21 diets were analyzed in SP1, and 19 diets were analyzed in SP2. SD = standard deviation; %CV = percent coefficient of variation; MIN = minimum value; MAX = maximum value; CP = crude protein, Lys = lysine; Met = methionine; Cys = cysteine; Thr = threonine; Val = valine; Leu = leucine; Ile = isoleucine; His = histidine; Phe = phenylalanine; Tyr = tyrosine; TiO_2 = titanium dioxide.

Appendix 3. Scheme of the correlation matrices displayed in Appendices 5 – 7 and 12



Appendix 4. Abbreviations used for the correlation matrices in Appendices 5 – 7 and 12 in alphabetical order

Abbreviation	Variable	Abbreviation	Variable
ADFI	Average daily feed intake, kg dry matter	G:F	Gain to feed ratio, kg/kg
ADG	Average daily gain, kg	IBW	Initial body weight, kg
BUN	Blood urea nitrogen, mmol/L	IGF-I	Insulin-like growth factor 1
BW	Body weight, kg	LI	Lysine intake, g/d
CP ATTD	Crude protein digestibility, %	LR	Lysine retention, g/d
Deg	whole-body protein degradation, g/d	LUE	Lysine utilization efficiency, %
DNI	Digestible nitrogen intake, g/d	NI	Nitrogen intake, g/d
DNUE	Digestible nitrogen utilization efficiency, %	NR	Nitrogen retention, g/d
EBA	Empty body ash, kg	NR:Syn	Nitrogen retention/ protein synthesis, %
EBA, %	Empty body ash, %	NUE	Nitrogen utilization efficiency, %
EBF	Empty body fat, kg	OEP	Overall experimental period
EBF, %	Empty body fat, %	PR:ADG	Protein retention/ average daily gain, %
EBP	Empty body protein, kg	RFI	Residual feed intake, kg/d
EBP, %	Empty body protein, %	SC	Serum cortisol, ng/mL
EBW	Empty body weight, kg	SP1	Sampling period 1
EBWA	Empty body water, kg	SP2	Sampling period 2
EBWA, %	Empty body water, %	Syn	whole-body protein synthesis, g/d
FBW	Final body weight, kg	TBWA, %	Total body water, %
FDR	Fractional degradation rate, %	TNE	Total nitrogen excretion, g/d
FFS, %	Fat free substance, %	Turn	whole-body protein turnover, g/d
FNE	Fecal nitrogen excretion, g/d	UCE	Urinary cortisol excretion, mg/d
FNE:NI	Fecal nitrogen excretion/ nitrogen intake, g/g	UCrE	Urinary creatinine excretion, g/d
FNE:TNE	Fecal nitrogen excretion/ total nitrogen excretion, g/g	UNE	Urinary nitrogen excretion, g/d
FRR	Fractional retention rate, %	UNE:NI	Urinary nitrogen excretion/ nitrogen intake, g/g
FSR	Fractional synthesis rate, %	UNE:TNE	Urinary nitrogen excretion/ total nitrogen excretion, g/g
FTR	Fractional turnover rate, %	UUE	Urinary urea excretion, g/d
		UUE:UNE	Urinary urea excretion/ urinary nitrogen excretion, %

Appendix 5 (1/6). Pearson correlations between the measured traits of the nitrogen balance dataset across both sampling periods (see Appendix 4 for the abbreviation code)

	IBW	BW	ADG	ADFI	G:F	NI	FNE	UNE	TNE	NR	PR:ADG	NUE
IBW												
BW	0.42											
ADG	n.s.	0.37										
ADFI	n.s.	0.77	0.58									
G:F	-0.18	-0.51	0.26	-0.58								
NI	0.23	0.38	0.55	0.77	-0.35							
FNE	n.s.	0.26	0.33	0.60	-0.39	0.54						
UNE	0.35	0.55	0.48	0.71	-0.35	0.76	0.27					
TNE	0.21	0.53	0.51	0.82	-0.45	0.84	0.65	0.89				
NR	0.18	n.s.	0.32	0.34	n.s.	0.70	n.s.	0.22	0.23			
PR:ADG	n.s.	-0.32	-0.53	-0.21	-0.31	n.s.	-0.18	-0.21	-0.24	0.56		
NUE	n.s.	-0.47	n.s.	-0.41	0.33	n.s.	-0.40	-0.55	-0.61	0.54	0.62	
CP ATTD	0.30	n.s.	n.s.	-0.23	0.21	n.s.	-0.84	n.s.	-0.27	0.24	0.30	0.40
DNI	0.33	0.37	0.51	0.68	-0.28	0.95	0.29	0.77	0.73	0.76	0.21	n.s.
DNUE	-0.22	-0.51	n.s.	-0.38	0.30	n.s.	n.s.	-0.68	-0.58	0.49	0.56	0.93
LI	0.21	n.s.	0.43	0.48	n.s.	0.89	0.44	0.59	0.66	0.73	0.25	n.s.
LR	0.18	n.s.	0.31	0.34	n.s.	0.70	n.s.	0.22	0.23	1.00	0.57	0.54
LUE	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.30	-0.34	-0.41	0.60	0.52	0.83
FNE:NI	-0.30	n.s.	n.s.	0.23	-0.21	n.s.	0.84	n.s.	0.27	-0.24	-0.30	-0.40
UNE:NI	0.32	0.50	n.s.	0.31	-0.22	n.s.	n.s.	0.72	0.48	-0.37	-0.44	-0.74
FNE:TNE	-0.37	-0.20	n.s.	n.s.	n.s.	n.s.	0.71	-0.44	n.s.	n.s.	n.s.	n.s.
UNE:TNE	0.37	0.20	n.s.	n.s.	n.s.	n.s.	-0.71	0.44	n.s.	n.s.	n.s.	n.s.
UUE	0.29	0.43	0.46	0.63	-0.29	0.74	0.26	0.96	0.86	0.23	-0.18	-0.52
UUE:UNE	n.s.	-0.20	n.s.	n.s.	n.s.	0.25	n.s.	0.31	0.25	n.s.	n.s.	n.s.

Correlations with |R| ≥ 0.20 are significant at $p \leq 0.05$
Correlations with |R| ≥ 0.17 are significant at $p \leq 0.10$
n.s. = not significant

Appendix 5 (2/6). Pearson correlations between the measured traits of the nitrogen balance dataset across both sampling periods (see Appendix 4 for the abbreviation code)

	CP ATTD	DNI	DNUE	LI	LR	LUE	FNE:NI	UNE:NI	FNE:TNE	UNE:TNE	UUE	UUE:UNE
IBW												
BW												
ADG												
ADFI												
G:F												
NI												
FNE												
UNE												
TNE												
NR												
PR:ADG												
NUE												
CP ATTD												
DNI	0.21											
DNUE	n.s.	n.s.										
LI	n.s.	0.86	n.s.									
LR	0.24	0.76	0.49	0.74								
LUE	0.39	n.s.	0.75	n.s.	0.60							
FNE:NI	-1.00	-0.21	-0.07	n.s.	-0.24	-0.39						
UNE:NI	0.25	0.20	-0.93	n.s.	-0.37	-0.59	-0.25					
FNE:TNE	-0.88	-0.27	0.36	n.s.	n.s.	n.s.	0.88	-0.64				
UNE:TNE	0.88	0.27	-0.36	n.s.	n.s.	n.s.	-0.88	0.64	-1.00			
UUE	n.s.	0.75	-0.64	0.61	0.23	-0.35	n.s.	0.68	-0.42	0.42		
UUE:UNE	n.s.	0.27	-0.16	0.35	n.s.	-0.18	n.s.	0.21	-0.22	0.22	0.52	

Correlations with |R| ≥ 0.20 are significant at $p \leq 0.05$
Correlations with |R| ≥ 0.17 are significant at $p \leq 0.10$
n.s. = not significant

Appendix 5 (3/6). Pearson correlations between the measured traits of the nitrogen balance dataset across both sampling periods (see Appendix 4 for the abbreviation code)

	IBW	BW	ADG	ADFI	G:F	NI	FNE	UNE	TNE	NR	PR:ADG	NUE
UCE	0.17	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.28	n.s.	n.s.	n.s.	-0.19
UCrE	0.43	0.91	0.35	0.72	-0.49	0.36	0.27	0.58	0.55	n.s.	-0.35	-0.51
BUN	n.s.	n.s.	0.28	0.19	n.s.	0.50	n.s.	0.54	0.37	0.41	0.17	n.s.
SC	n.s.	n.s.	n.s.	n.s.	n.s.	-0.22	n.s.	-0.27	-0.25	n.s.	n.s.	n.s.
IGF-I	0.33	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.20	0.23	0.20
Turn	0.21	n.s.	n.s.	0.22	-0.19	0.36	n.s.	0.34	0.33	0.23	n.s.	n.s.
Syn	n.s.	n.s.	n.s.	n.s.	n.s.	0.18	n.s.	n.s.	0.12	n.s.	0.21	n.s.
Deg	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
NR:Syn	n.s.	n.s.	0.24	n.s.	n.s.	0.31	n.s.	n.s.	n.s.	0.50	0.20	0.35
FTR	n.s.	-0.80	-0.29	-0.59	0.34	n.s.	-0.21	-0.27	-0.29	n.s.	0.43	0.40
FSR	-0.18	-0.80	-0.31	-0.61	0.35	n.s.	-0.22	-0.34	-0.35	n.s.	0.43	0.43
FDR	n.s.	-0.69	-0.32	-0.56	0.29	-0.21	-0.21	-0.28	-0.30	n.s.	0.33	0.28
FRR	-0.21	-0.83	n.s.	-0.52	0.40	n.s.	n.s.	-0.33	-0.32	0.48	0.56	0.68
EBW	0.46	0.99	0.30	0.72	-0.51	0.29	n.s.	0.53	0.46	n.s.	-0.33	-0.44
EBWA	0.44	0.97	0.30	0.75	-0.54	0.35	0.22	0.54	0.49	n.s.	-0.30	-0.42
EBF	0.37	0.91	0.30	0.68	-0.48	0.22	n.s.	0.49	0.44	n.s.	-0.36	-0.46
EBP	0.46	0.99	0.30	0.72	-0.51	0.29	n.s.	0.53	0.46	n.s.	-0.32	-0.44
EBA	0.46	0.99	0.30	0.72	-0.51	0.29	n.s.	0.53	0.46	n.s.	-0.32	-0.44
TBWA, %	n.s.	-0.45	n.s.	-0.32	0.25	n.s.	n.s.	-0.19	-0.18	0.22	0.25	0.29
EBWA, %	-0.26	-0.76	-0.25	-0.60	0.44	n.s.	n.s.	-0.41	-0.38	n.s.	0.32	0.41
FFS, %	-0.26	-0.76	-0.25	-0.60	0.44	n.s.	n.s.	-0.41	-0.38	n.s.	0.32	0.41
EBF, %	0.26	0.76	0.25	0.60	-0.44	n.s.	n.s.	0.41	0.38	n.s.	-0.32	-0.41
EBP, %	-0.26	-0.76	-0.25	-0.60	0.44	n.s.	n.s.	-0.41	-0.38	n.s.	0.32	0.41
EBA, %	-0.24	-0.67	-0.25	-0.53	0.34	n.s.	n.s.	-0.37	-0.33	n.s.	0.30	0.34

Correlations with $|R| \geq 0.20$ are significant at $p \leq 0.05$
Correlations with $|R| \geq 0.17$ are significant at $p \leq 0.10$
n.s. = not significant

Appendix 5 (4/6). Pearson correlations between the measured traits of the nitrogen balance dataset across both sampling periods (see Appendix 4 for the abbreviation code)

	CP ATTD	DNI	DNUE	LI	LR	LUE	FNE:NI	UNE:NI	FNE:TNE	UNE:TNE	UUE	UUE:UNE
UCE	0.20	n.s.	-0.27	n.s.	n.s.	n.s.	-0.20	0.31	-0.29	0.29	0.21	n.s.
UCrE	n.s.	0.34	-0.55	n.s.	n.s.	-0.28	n.s.	0.54	-0.21	0.21	0.49	n.s.
BUN	0.40	0.60	-0.12	0.56	0.42	n.s.	-0.40	0.27	-0.45	0.45	0.57	0.38
SC	n.s.	-0.23	0.16	-0.28	n.s.	0.19	n.s.	-0.18	0.11	-0.11	-0.34	-0.41
IGF-I	n.s.	n.s.	n.s.	n.s.	0.20	0.22	-0.18	n.s.	n.s.	n.s.	n.s.	n.s.
Turn	n.s.	0.35	-0.17	0.39	0.23	n.s.	n.s.	0.21	n.s.	n.s.	0.40	0.40
Syn	n.s.	n.s.	n.s.	0.26	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.18	0.35
Deg	n.s.	n.s.	n.s.	n.s.	n.s.	-0.22	n.s.	n.s.	n.s.	n.s.	n.s.	0.36
NR:Syn	n.s.	0.35	0.35	0.26	0.50	0.46	n.s.	-0.31	n.s.	n.s.	n.s.	-0.19
FTR	n.s.	n.s.	0.40	0.18	n.s.	n.s.	n.s.	-0.34	n.s.	n.s.	n.s.	0.39
FSR	n.s.	n.s.	0.43	n.s.	n.s.	n.s.	n.s.	-0.38	n.s.	n.s.	-0.20	0.37
FDR	n.s.	-0.18	0.27	n.s.	n.s.	n.s.	n.s.	-0.23	n.s.	n.s.	n.s.	0.39
FRR	0.19	n.s.	0.71	0.30	0.48	0.44	-0.19	-0.64	n.s.	n.s.	-0.23	0.25
EBW	n.s.	0.30	-0.52	n.s.	n.s.	n.s.	n.s.	0.55	-0.27	0.27	0.41	-0.20
EBWA	n.s.	0.35	-0.49	n.s.	n.s.	n.s.	n.s.	0.51	-0.22	0.22	0.42	n.s.
EBF	n.s.	0.23	-0.54	n.s.	n.s.	n.s.	n.s.	0.55	-0.25	0.25	0.38	-0.20
EBP	n.s.	0.31	-0.52	n.s.	n.s.	n.s.	n.s.	0.54	-0.27	0.27	0.41	-0.20
EBA	n.s.	0.30	-0.52	n.s.	n.s.	n.s.	n.s.	0.55	-0.27	0.27	0.41	-0.20
TBWA, %	n.s.	n.s.	0.34	0.24	0.23	n.s.	n.s.	-0.31	n.s.	n.s.	n.s.	n.s.
EBWA, %	n.s.	n.s.	0.48	n.s.	n.s.	n.s.	n.s.	-0.47	0.20	-0.20	-0.32	0.19
FFS, %	n.s.	n.s.	0.48	n.s.	n.s.	n.s.	n.s.	-0.47	0.20	-0.20	-0.32	0.19
EBF, %	n.s.	n.s.	-0.48	n.s.	n.s.	n.s.	n.s.	0.47	-0.20	0.20	0.32	-0.19
EBP, %	n.s.	n.s.	0.48	n.s.	n.s.	n.s.	n.s.	-0.47	0.20	-0.20	-0.32	0.18
EBA, %	n.s.	n.s.	0.41	n.s.	n.s.	n.s.	n.s.	-0.40	n.s.	n.s.	-0.28	0.18

Correlations with |R| ≥ 0.20 are significant at $p \leq 0.05$
Correlations with |R| ≥ 0.17 are significant at $p \leq 0.10$
n.s. = not significant

Appendix 5 (5/6). Pearson correlations between the measured traits of the nitrogen balance dataset across both sampling periods (see Appendix 4 for the abbreviation code)

	UCE	UCrE	BUN	SC	IGF-I	Turn	Syn	Deg	NR:Syn	FTR	FSR	FDR
UCE												
UCrE	0.21											
BUN	0.18	n.s.										
SC	0.33	n.s.	n.s.									
IGF-I	-0.36	n.s.	n.s.	n.s.								
Turn	n.s.	n.s.	0.32	-0.24	0.20							
Syn	-0.21	n.s.	0.18	-0.20	0.21	0.95						
Deg	-0.21	n.s.	n.s.	-0.23	n.s.	0.91	0.96					
NR:Syn	0.20	n.s.	0.18	0.18	n.s.	-0.63	-0.70	-0.84				
FTR	-0.19	-0.67	0.22	n.s.	n.s.	0.45	0.55	0.51	-0.31			
FSR	-0.22	-0.66	n.s.	n.s.	n.s.	0.45	0.57	0.54	-0.35	0.99		
FDR	-0.21	-0.54	n.s.	n.s.	n.s.	0.57	0.69	0.70	-0.55	0.95	0.96	
FRR	n.s.	-0.77	0.20	n.s.	n.s.	n.s.	n.s.	n.s.	0.30	0.76	0.74	0.57
EBW	0.23	0.89	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.79	-0.77	-0.65
EBWA	0.18	0.88	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.76	-0.75	-0.64
EBF	0.24	0.83	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.76	-0.76	-0.63
EBP	0.22	0.89	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.78	-0.77	-0.65
EBA	0.22	0.89	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.79	-0.77	-0.65
TBWA, %	-0.19	-0.44	n.s.	-0.20	0.18	n.s.	n.s.	n.s.	n.s.	0.44	0.44	0.37
EBWA, %	-0.24	-0.72	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.65	0.64	0.54
FFS, %	-0.24	-0.72	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.65	0.64	0.54
EBF, %	0.24	0.72	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.65	-0.64	-0.54
EBP, %	-0.23	-0.72	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.64	0.64	0.53
EBA, %	-0.19	-0.62	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.64	0.64	0.57

Correlations with |R| ≥ 0.20 are significant at $p \leq 0.05$
Correlations with |R| ≥ 0.17 are significant at $p \leq 0.10$
n.s. = not significant

Appendix 5 (6/6). Pearson correlations between the measured traits of the nitrogen balance dataset across both sampling periods (see Appendix 4 for the abbreviation code)

	FRR	EBW	EBWA	EBF	EBP	EBA	TBWA, %	EBWA, %	FFS, %	EBF, %	EBP, %	EBA, %
UCE												
UCrE												
BUN												
SC												
IGF-I												
Turn												
Syn												
Deg												
NR:Syn												
FTR												
FSR												
FDR												
FRR												
EBW	-0.85											
EBWA	-0.82	0.97										
EBF	-0.85	0.92	0.84									
EBP	-0.85	1.00	0.98	0.92								
EBA	-0.85	1.00	0.97	0.92	1.00							
TBWA, %	0.52	-0.47	-0.30	-0.74	-0.46	-0.46						
EBWA, %	0.74	-0.78	-0.66	-0.95	-0.77	-0.78	0.90					
FFS, %	0.74	-0.78	-0.66	-0.95	-0.77	-0.78	0.90	1.00				
EBF, %	-0.74	0.78	0.66	0.95	0.77	0.78	-0.90	-1.00	-1.00			
EBP, %	0.74	-0.78	-0.66	-0.95	-0.77	-0.78	0.90	1.00	1.00	-1.00		
EBA, %	0.65	-0.67	-0.57	-0.84	-0.67	-0.67	0.81	0.87	0.87	-0.87	0.87	

Correlations with |R| ≥ 0.20 are significant at $p \leq 0.05$
Correlations with |R| ≥ 0.17 are significant at $p \leq 0.10$
n.s. = not significant

Appendix 6 (1/6). Pearson correlations between the measured traits of the nitrogen balance dataset in sampling period 1 (see Appendix 4 for the abbreviation code)

	IBW	BW	ADG	ADFI	G:F	NI	FNE	UNE	TNE	NR	PR:ADG	NUE
IBW												
BW	0.91											
ADG	n.s.	n.s.										
ADFI	0.44	0.58	0.44									
G:F	n.s.	n.s.	0.73	-0.18								
NI	0.48	0.61	0.35	0.97	-0.27							
FNE	n.s.	n.s.	0.25	0.46	n.s.	0.42						
UNE	0.47	0.56	0.28	0.79	-0.26	0.80	n.s.					
TNE	0.31	0.45	0.31	0.84	-0.27	0.82	0.58	0.88				
NR	0.45	0.49	n.s.	0.66	n.s.	0.75	n.s.	0.37	0.27			
PR:ADG	n.s.	n.s.	-0.67	n.s.	-0.81	0.24	n.s.	n.s.	n.s.	0.50		
NUE	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.42	-0.47	-0.61	0.51	0.41	
CP ATTD	0.43	0.37	n.s.	n.s.	n.s.	n.s.	-0.79	0.32	n.s.	0.40	0.32	0.38
DNI	0.57	0.67	0.32	0.93	-0.25	0.96	n.s.	0.81	0.72	0.79	0.27	-0.02
DNUE	n.s.	-0.27	n.s.	n.s.	n.s.	n.s.	n.s.	-0.63	-0.61	0.40	0.31	0.92
LI	0.48	0.60	0.28	0.92	-0.30	0.96	0.43	0.73	0.76	0.75	0.28	-0.08
LR	0.45	0.49	n.s.	0.66	n.s.	0.75	n.s.	0.36	0.27	1.00	0.50	0.51
LUE	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.41	-0.32	-0.48	0.53	0.39	0.91
FNE:NI	-0.43	-0.37	n.s.	n.s.	n.s.	n.s.	0.79	-0.32	n.s.	-0.40	-0.32	-0.38
UNE:NI	0.37	0.42	n.s.	0.25	n.s.	0.24	n.s.	0.73	0.56	n.s.	n.s.	-0.74
FNE:TNE	-0.49	-0.45	n.s.	n.s.	n.s.	-0.24	0.64	-0.58	n.s.	n.s.	n.s.	0.05
UNE:TNE	0.49	0.45	n.s.	n.s.	n.s.	0.24	-0.64	0.58	n.s.	0.21	n.s.	-0.05
UUE	0.38	0.48	n.s.	0.70	-0.26	0.72	n.s.	0.96	0.85	0.28	n.s.	-0.52
UUE:UNE	n.s.	n.s.	n.s.	n.s.	-0.26	0.27	n.s.	0.52	0.46	n.s.	n.s.	-0.48

Correlations with |R| ≥ 0.29 are significant at $p \leq 0.05$
Correlations with |R| ≥ 0.24 are significant at $p \leq 0.10$
n.s. = not significant

Appendix 6 (2/6). Pearson correlations between the measured traits of the nitrogen balance dataset in sampling period 1 (see Appendix 4 for the abbreviation code)

	CP ATTD	DNI	DNUE	LI	LR	LUE	FNE:NI	UNE:NI	FNE:TNE	UNE:TNE	UUE	UUE:UNE
IBW												
BW												
ADG												
ADFI												
G:F												
NI												
FNE												
UNE												
TNE												
NR												
PR:ADG												
NUE												
CP ATTD												
DNI	0.41											
DNUE	n.s.	n.s.										
LI	n.s.	0.91	n.s.									
LR	0.40	0.79	0.41	0.75								
LUE	0.43	n.s.	0.79	n.s.	0.53							
FNE:NI	-1.00	-0.41	n.s.	n.s.	-0.40	-0.43						
UNE:NI	0.26	0.30	-0.93	n.s.	n.s.	-0.60	-0.26					
FNE:TNE	-0.88	-0.45	0.38	n.s.	n.s.	-0.04	0.88	-0.64				
UNE:TNE	0.88	0.45	-0.38	n.s.	n.s.	-0.04	-0.88	0.64	-1.00			
UUE	0.27	0.73	-0.67	0.66	0.28	-0.38	-0.27	0.76	-0.56	0.56		
UUE:UNE	n.s.	0.27	-0.53	0.25	n.s.	-0.41	-0.04	0.55	-0.30	0.30	0.70	

Correlations with |R| ≥ 0.29 are significant at $p \leq 0.05$
Correlations with |R| ≥ 0.24 are significant at $p \leq 0.10$
n.s. = not significant

Appendix 6 (3/6). Pearson correlations between the measured traits of the nitrogen balance dataset in sampling period 1 (see Appendix 4 for the abbreviation code)

	IBW	BW	ADG	ADFI	G:F	NI	FNE	UNE	TNE	NR	PR:ADG	NUE
UCE	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.32	0.26	n.s.	n.s.	n.s.
UCrE	0.78	0.76	n.s.	0.35	n.s.	0.41	n.s.	0.40	0.30	0.34	n.s.	n.s.
BUN	0.24	0.32	0.25	0.58	n.s.	0.61	n.s.	0.81	0.63	0.30	n.s.	-0.31
SC	n.s.	-0.31	n.s.	n.s.	n.s.	-0.28	n.s.	-0.36	-0.34	n.s.	n.s.	0.26
IGF-I	0.39	0.43	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.35	n.s.	0.27
Turn	n.s.	0.31	n.s.	0.36	n.s.	0.39	n.s.	0.40	0.39	n.s.	n.s.	-0.32
Syn	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Deg	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.35
NR:Syn	n.s.	n.s.	n.s.	n.s.	n.s.	0.26	n.s.	n.s.	n.s.	0.50	0.29	0.51
FTR	-0.46	-0.40	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
FSR	-0.48	-0.44	n.s.	-0.24	n.s.	n.s.	n.s.	-0.26	n.s.	n.s.	n.s.	n.s.
FDR	-0.36	-0.31	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.28	n.s.	n.s.
FRR	-0.35	-0.36	n.s.	n.s.	n.s.	n.s.	n.s.	-0.26	n.s.	0.57	0.34	0.74
EBW	0.91	0.96	n.s.	0.43	n.s.	0.46	n.s.	0.56	0.40	0.31	n.s.	-0.25
EBWA	0.83	0.93	n.s.	0.54	n.s.	0.58	n.s.	0.59	0.46	0.45	n.s.	n.s.
EBF	0.68	0.57	n.s.	n.s.	n.s.	n.s.	n.s.	0.48	0.37	n.s.	n.s.	-0.44
EBP	0.91	0.96	n.s.	0.42	n.s.	0.46	n.s.	0.54	0.39	0.32	n.s.	n.s.
EBA	0.91	0.96	n.s.	0.43	n.s.	0.46	n.s.	0.55	0.39	0.31	n.s.	n.s.
TBWA, %	n.s.	n.s.	n.s.	n.s.	n.s.	0.33	n.s.	n.s.	n.s.	0.45	0.34	n.s.
EBWA, %	-0.30	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.33
FFS, %	-0.30	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.32
EBF, %	0.30	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.32
EBP, %	-0.29	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.32
EBA, %	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Correlations with |R| ≥ 0.29 are significant at $p \leq 0.05$
Correlations with |R| ≥ 0.24 are significant at $p \leq 0.10$
n.s. = not significant

Appendix 6 (4/6). Pearson correlations between the measured traits of the nitrogen balance dataset in sampling period 1 (see Appendix 4 for the abbreviation code)

	CP ATTD	DNI	DNUE	LI	LR	LUE	FNE:NI	UNE:NI	FNE:TNE	UNE:TNE	UUE	UUE:UNE
UCE	n.s.	n.s.	-0.27	n.s.	n.s.	n.s.	n.s.	0.30	-0.29	0.29	0.30	n.s.
UCrE	0.29	0.45	n.s.	0.47	0.33	n.s.	-0.29	0.36	-0.40	0.40	0.40	n.s.
BUN	0.37	0.65	-0.48	0.58	0.31	n.s.	-0.37	0.60	-0.55	0.55	0.80	0.46
SC	n.s.	-0.25	0.32	-0.26	n.s.	n.s.	n.s.	-0.34	n.s.	n.s.	-0.39	-0.34
IGF-I	0.28	0.26	n.s.	n.s.	0.34	0.30	-0.28	n.s.	n.s.	n.s.	n.s.	n.s.
Turn	n.s.	0.36	-0.32	0.46	n.s.	-0.36	n.s.	0.32	n.s.	n.s.	0.41	0.38
Syn	n.s.	n.s.	n.s.	0.32	n.s.	-0.30	n.s.	n.s.	n.s.	n.s.	n.s.	0.25
Deg	n.s.	n.s.	-0.29	n.s.	n.s.	-0.47	n.s.	n.s.	n.s.	n.s.	n.s.	0.31
NR:Syn	0.28	0.31	0.40	n.s.	0.50	0.63	-0.28	-0.28	n.s.	n.s.	n.s.	-0.28
FTR	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
FSR	-0.25	-0.28	n.s.	n.s.	n.s.	n.s.	0.25	n.s.	0.25	-0.25	n.s.	n.s.
FDR	-0.28	-0.28	n.s.	n.s.	-0.29	-0.37	0.28	n.s.	n.s.	n.s.	n.s.	n.s.
FRR	n.s.	n.s.	0.77	n.s.	0.57	0.72	n.s.	-0.71	0.28	-0.28	-0.29	-0.30
EBW	0.40	0.53	-0.41	0.45	0.31	n.s.	-0.40	0.56	-0.58	0.58	0.53	0.30
EBWA	0.36	0.63	-0.33	0.59	0.45	n.s.	-0.36	0.47	-0.50	0.50	0.58	0.35
EBF	0.29	0.29	-0.59	n.s.	n.s.	-0.31	-0.29	0.67	-0.57	0.57	0.40	n.s.
EBP	0.40	0.52	-0.40	0.45	0.32	n.s.	-0.40	0.55	-0.58	0.58	0.52	0.30
EBA	0.40	0.52	-0.41	0.45	0.31	n.s.	-0.40	0.56	-0.58	0.58	0.52	0.30
TBWA, %	n.s.	0.30	n.s.	0.39	0.45	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
EBWA, %	n.s.	n.s.	0.43	n.s.	n.s.	n.s.	n.s.	-0.43	0.40	-0.40	n.s.	n.s.
FFS, %	n.s.	n.s.	0.42	n.s.	n.s.	n.s.	n.s.	-0.43	0.41	-0.41	n.s.	n.s.
EBF, %	n.s.	n.s.	-0.42	n.s.	n.s.	n.s.	n.s.	0.43	-0.41	0.41	n.s.	n.s.
EBP, %	n.s.	n.s.	0.42	n.s.	n.s.	n.s.	n.s.	-0.43	0.40	-0.40	n.s.	n.s.
EBA, %	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Correlations with |R| ≥ 0.29 are significant at $p \leq 0.05$
Correlations with |R| ≥ 0.24 are significant at $p \leq 0.10$
n.s. = not significant

Appendix 6 (5/6). Pearson correlations between the measured traits of the nitrogen balance dataset in sampling period 1 (see Appendix 4 for the abbreviation code)

	UCE	UCrE	BUN	SC	IGF-I	Turn	Syn	Deg	NR:Syn	FTR	FSR	FDR
UCE												
UCrE	n.s.											
BUN	0.25	n.s.										
SC	n.s.	-0.35	n.s.									
IGF-I	-0.35	0.34	n.s.	n.s.								
Turn	n.s.	0.34	0.31	-0.28	n.s.							
Syn	n.s.	0.32	n.s.	n.s.	n.s.	0.94						
Deg	n.s.	0.27	n.s.	-0.24	n.s.	0.88	0.94					
NR:Syn	n.s.	n.s.	n.s.	n.s.	n.s.	-0.65	-0.74	-0.89				
FTR	n.s.	n.s.	n.s.	n.s.	n.s.	0.66	0.72	0.75	-0.70			
FSR	n.s.	n.s.	n.s.	n.s.	n.s.	0.59	0.70	0.73	-0.68	0.98		
FDR	n.s.	n.s.	n.s.	n.s.	n.s.	0.69	0.80	0.87	-0.87	0.94	0.94	
FRR	n.s.	-0.35	n.s.	n.s.	n.s.	n.s.	n.s.	-0.31	0.53	n.s.	n.s.	n.s.
EBW	n.s.	0.67	0.35	n.s.	0.27	0.47	0.37	0.35	n.s.	-0.26	-0.29	n.s.
EBWA	n.s.	0.68	0.43	-0.29	n.s.	0.43	0.34	0.30	n.s.	-0.28	-0.31	n.s.
EBF	0.35	0.41	n.s.	n.s.	n.s.	0.33	n.s.	n.s.	-0.28	n.s.	n.s.	n.s.
EBP	n.s.	0.68	0.35	n.s.	0.28	0.46	0.36	0.35	n.s.	-0.27	-0.30	n.s.
EBA	n.s.	0.67	0.35	n.s.	0.28	0.47	0.37	0.35	n.s.	-0.27	-0.29	n.s.
TBWA, %	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
EBWA, %	-0.31	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
FFS, %	-0.31	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
EBF, %	0.31	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
EBP, %	-0.29	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
EBA, %	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Correlations with $|R| \geq 0.29$ are significant at $p \leq 0.05$
Correlations with $|R| \geq 0.24$ are significant at $p \leq 0.10$
n.s. = not significant

Appendix 6 (6/6). Pearson correlations between the measured traits of the nitrogen balance dataset in sampling period 1 (see Appendix 4 for the abbreviation code)

	FRR	EBW	EBWA	EBF	EBP	EBA	TBWA, %	EBWA, %	FFS, %	EBF, %	EBP, %	EBA, %
UCE												
UCrE												
BUN												
SC												
IGF-I												
Turn												
Syn												
Deg												
NR:Syn												
FTR												
FSR												
FDR												
FRR												
EBW	-0.52											
EBWA	-0.39	0.94										
EBF	-0.58	0.63	0.40									
EBP	-0.51	1.00	0.95	0.61								
EBA	-0.52	1.00	0.94	0.62	1.00							
TBWA, %	n.s.	n.s.	0.44	-0.55	n.s.	n.s.						
EBWA, %	0.39	n.s.	n.s.	-0.84	n.s.	n.s.	0.89					
FFS, %	0.39	n.s.	n.s.	-0.84	n.s.	n.s.	0.89	1.00				
EBF, %	-0.39	n.s.	n.s.	0.84	n.s.	n.s.	-0.89	-1.00	-1.00			
EBP, %	0.38	n.s.	n.s.	-0.84	n.s.	n.s.	0.89	1.00	1.00	-1.00		
EBA, %	n.s.	n.s.	n.s.	-0.51	n.s.	n.s.	0.65	0.65	0.65	-0.65	0.65	

Correlations with |R| ≥ 0.29 are significant at $p \leq 0.05$
Correlations with |R| ≥ 0.24 are significant at $p \leq 0.10$
n.s. = not significant

Appendix 7 (1/6). Pearson correlations between the measured traits of the nitrogen balance dataset in sampling period 2 (see Appendix 4 for the abbreviation code)

	IBW	BW	ADG	ADFI	G:F	NI	FNE	UNE	TNE	NR	PR:ADG	NUE
IBW												
BW	0.80											
ADG	n.s.	n.s.										
ADFI	n.s.	n.s.	0.61									
G:F	-0.29	n.s.	0.42	-0.38								
NI	n.s.	n.s.	0.66	0.97	-0.29							
FNE	n.s.	n.s.	0.24	0.61	-0.45	0.55						
UNE	0.27	0.38	0.54	0.64	n.s.	0.72	n.s.					
TNE	n.s.	n.s.	0.59	0.84	-0.27	0.88	0.57	0.85				
NR	n.s.	n.s.	0.52	0.77	n.s.	0.78	0.32	0.32	0.44			
PR:ADG	n.s.	n.s.	-0.24	0.34	-0.66	0.31	n.s.	n.s.	n.s.	0.62		
NUE	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.29	-0.31	0.64	0.58	
CP ATTD	n.s.	0.26	n.s.	n.s.	0.33	n.s.	-0.85	0.25	n.s.	n.s.	n.s.	0.34
DNI	n.s.	0.24	0.64	0.90	n.s.	0.96	0.33	0.77	0.80	0.81	0.34	n.s.
DNUE	-0.32	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.49	-0.31	0.54	0.60	0.84
LI	n.s.	n.s.	0.69	0.91	n.s.	0.95	0.54	0.67	0.83	0.72	0.24	n.s.
LR	n.s.	n.s.	0.52	0.76	n.s.	0.78	0.32	0.33	0.44	1.00	0.62	0.64
LUE	-0.24	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.26	-0.27	0.63	0.60	0.88
FNE:NI	n.s.	-0.26	n.s.	n.s.	-0.33	n.s.	0.85	-0.25	n.s.	n.s.	n.s.	-0.34
UNE:NI	0.34	0.27	n.s.	n.s.	n.s.	n.s.	-0.52	0.54	n.s.	-0.41	-0.48	-0.53
FNE:TNE	-0.26	-0.35	n.s.	n.s.	-0.35	n.s.	0.82	-0.40	n.s.	n.s.	n.s.	n.s.
UNE:TNE	0.26	0.35	n.s.	n.s.	0.35	n.s.	-0.82	0.40	n.s.	n.s.	n.s.	n.s.
UUE	n.s.	0.27	0.55	0.66	n.s.	0.73	n.s.	0.96	0.84	0.35	n.s.	-0.24
UUE:UNE	n.s.	n.s.	0.29	0.37	n.s.	0.41	n.s.	0.41	0.39	0.26	n.s.	n.s.

Correlations with |R| ≥ 0.28 are significant at $p \leq 0.05$
Correlations with |R| ≥ 0.24 are significant at $p \leq 0.10$
n.s. = not significant

Appendix 7 (2/6). Pearson correlations between the measured traits of the nitrogen balance dataset in sampling period 2 (see Appendix 4 for the abbreviation code)

	CP ATTD	DNI	DNUE	LI	LR	LUE	FNE:NI	UNE:NI	FNE:TNE	UNE:TNE	UUE	UUE:UNE
IBW												
BW												
ADG												
ADFI												
G:F												
NI												
FNE												
UNE												
TNE												
NR												
PR:ADG												
NUE												
CP ATTD												
DNI	n.s.											
DNUE	n.s.	n.s.										
LI	n.s.	0.89	n.s.									
LR	n.s.	0.81	0.54	0.72								
LUE	0.24	0.24	0.81	n.s.	0.64							
FNE:NI	-1.00	n.s.	n.s.	n.s.	n.s.	-0.24						
UNE:NI	0.50	n.s.	-0.87	n.s.	-0.40	-0.56	-0.50					
FNE:TNE	-0.91	n.s.	0.41	n.s.	n.s.	n.s.	0.91	-0.75				
UNE:TNE	0.91	n.s.	-0.41	n.s.	n.s.	n.s.	-0.91	0.75	-1.00			
UUE	n.s.	0.77	-0.45	0.69	0.35	n.s.	n.s.	0.50	-0.36	0.36		
UUE:UNE	n.s.	0.42	n.s.	0.42	0.26	n.s.	n.s.	n.s.	n.s.	n.s.	0.61	

Correlations with |R| ≥ 0.28 are significant at $p \leq 0.05$
Correlations with |R| ≥ 0.24 are significant at $p \leq 0.10$
n.s. = not significant

Appendix 7 (3/6). Pearson correlations between the measured traits of the nitrogen balance dataset in sampling period 2 (see Appendix 4 for the abbreviation code)

	IBW	BW	ADG	ADFI	G:F	NI	FNE	UNE	TNE	NR	CP:ADG	NUE
UCE	n.s.	0.31	n.s.	n.s.	0.26	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
UCrE	0.68	0.72	n.s.	n.s.	n.s.	n.s.	n.s.	0.45	0.30	n.s.	n.s.	-0.29
BUN	n.s.	0.26	0.45	0.45	n.s.	0.55	n.s.	0.55	0.42	0.51	n.s.	0.29
SC	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.26	-0.26	n.s.	n.s.	n.s.
IGF-I	0.29	n.s.	-0.29	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Turn	n.s.	n.s.	n.s.	0.37	-0.36	0.35	n.s.	0.40	0.30	0.24	0.33	n.s.
Syn	n.s.	n.s.	n.s.	n.s.	-0.36	n.s.	n.s.	n.s.	n.s.	n.s.	0.36	n.s.
Deg	n.s.	n.s.	n.s.	n.s.	-0.33	n.s.	n.s.	n.s.	n.s.	n.s.	0.25	n.s.
NR:Syn	n.s.	n.s.	0.47	0.34	0.20	0.39	0.28	n.s.	0.25	0.51	n.s.	n.s.
FTR	-0.28	-0.39	n.s.	0.24	-0.28	n.s.	n.s.	n.s.	n.s.	n.s.	0.27	n.s.
FSR	-0.27	-0.41	n.s.	n.s.	-0.24	n.s.	n.s.	n.s.	n.s.	n.s.	0.25	n.s.
FDR	n.s.	-0.30	-0.25	n.s.	-0.26	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
FRR	-0.51	-0.45	0.42	0.60	n.s.	0.56	0.38	n.s.	0.32	0.70	0.36	0.41
EBW	0.74	0.94	n.s.	n.s.	n.s.	n.s.	n.s.	0.27	n.s.	n.s.	n.s.	n.s.
EBWA	0.74	0.84	n.s.	n.s.	n.s.	n.s.	n.s.	0.30	n.s.	n.s.	n.s.	n.s.
EBF	0.56	0.69	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
EBP	0.75	0.94	n.s.	n.s.	n.s.	n.s.	n.s.	0.28	n.s.	n.s.	n.s.	n.s.
EBA	0.74	0.94	n.s.	n.s.	n.s.	n.s.	n.s.	0.27	n.s.	n.s.	n.s.	n.s.
TBWA, %	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
EBWA, %	-0.39	-0.46	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
FFS, %	-0.39	-0.46	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
EBF, %	0.39	0.46	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
EBP, %	-0.39	-0.46	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
EBA, %	-0.42	-0.42	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Correlations with |R| ≥ 0.28 are significant at $p \leq 0.05$
Correlations with |R| ≥ 0.24 are significant at $p \leq 0.10$
n.s. = not significant

Appendix 7 (4/6). Pearson correlations between the measured traits of the nitrogen balance dataset in sampling period 2 (see Appendix 4 for the abbreviation code)

	CP ATTD	DNI	DNUE	LI	LR	LUE	FNE:NI	UNE:NI	FNE:TNE	UNE:TNE	UUE	UUE:UNE
UCE	0.24	n.s.	n.s.	n.s.	n.s.	n.s.	-0.24	n.s.	-0.30	0.30	n.s.	-0.25
UCrE	n.s.	n.s.	-0.42	0.24	n.s.	-0.47	n.s.	0.46	-0.33	0.33	0.39	n.s.
BUN	0.38	0.64	n.s.	0.56	0.51	0.27	-0.38	n.s.	-0.36	0.36	0.53	0.33
SC	n.s.	-0.24	n.s.	-0.30	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.32	-0.44
IGF-I	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Turn	n.s.	0.37	n.s.	0.35	0.24	n.s.	n.s.	n.s.	n.s.	n.s.	0.43	0.48
Syn	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.26	0.43
Deg	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.41
NR:Syn	n.s.	0.39	0.36	0.32	0.52	0.29	n.s.	-0.35	n.s.	n.s.	n.s.	n.s.
FTR	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.41
FSR	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.38
FDR	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.32
FRR	n.s.	0.52	0.51	0.50	0.69	0.49	n.s.	-0.49	0.33	-0.33	0.26	0.30
EBW	0.40	n.s.	n.s.	n.s.	n.s.	n.s.	-0.40	n.s.	-0.45	0.45	n.s.	n.s.
EBWA	0.32	n.s.	n.s.	n.s.	n.s.	n.s.	-0.32	n.s.	-0.32	0.32	n.s.	n.s.
EBF	0.34	n.s.	n.s.	n.s.	n.s.	n.s.	-0.34	n.s.	-0.39	0.39	n.s.	n.s.
EBP	0.40	n.s.	n.s.	n.s.	n.s.	n.s.	-0.40	n.s.	-0.44	0.44	n.s.	n.s.
EBA	0.40	n.s.	n.s.	n.s.	n.s.	n.s.	-0.40	n.s.	-0.44	0.44	n.s.	n.s.
TBWA, %	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
EBWA, %	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.28	-0.28	n.s.	n.s.
FFS, %	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.28	-0.28	n.s.	n.s.
EBF, %	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.28	0.28	n.s.	n.s.
EBP, %	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.28	-0.28	n.s.	n.s.
EBA, %	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Correlations with |R| ≥ 0.28 are significant at $p \leq 0.05$
Correlations with |R| ≥ 0.24 are significant at $p \leq 0.10$
n.s. = not significant

Appendix 7 (5/6). Pearson correlations between the measured traits of the nitrogen balance dataset in sampling period 2 (see Appendix 4 for the abbreviation code)

	UCE	UCrE	BUN	SC	IGF-I	Turn	Syn	Deg	NR:Syn	FTR	FSR	FDR
UCE												
UCrE	0.33											
BUN	n.s.	n.s.										
SC	0.46	n.s.	n.s.									
IGF-I	-0.39	n.s.	n.s.	n.s.								
Turn	-0.24	n.s.	0.35	n.s.	0.32							
Syn	-0.31	n.s.	n.s.	n.s.	0.35	0.97						
Deg	-0.36	n.s.	n.s.	n.s.	0.35	0.92	0.97					
NR:Syn	0.38	n.s.	n.s.	n.s.	-0.36	-0.61	-0.69	-0.82				
FTR	-0.37	n.s.	n.s.	n.s.	n.s.	0.79	0.83	0.80	-0.56			
FSR	-0.41	n.s.	n.s.	n.s.	n.s.	0.77	0.84	0.84	-0.62	0.98		
FDR	-0.43	n.s.	n.s.	n.s.	0.29	0.78	0.87	0.91	-0.82	0.90	0.94	
FRR	n.s.	-0.39	0.26	n.s.	n.s.	n.s.	n.s.	n.s.	0.37	0.49	0.43	n.s.
EBW	0.54	0.70	0.28	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.41	-0.41	-0.34
EBWA	0.39	0.68	0.27	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.29	-0.30	n.s.
EBF	0.44	0.44	n.s.	0.32	n.s.	n.s.	n.s.	n.s.	n.s.	-0.37	-0.38	-0.32
EBP	0.53	0.70	0.29	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.39	-0.40	-0.33
EBA	0.52	0.70	0.29	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.40	-0.41	-0.34
TBWA, %	n.s.	n.s.	n.s.	-0.28	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.27	n.s.
EBWA, %	-0.30	-0.27	n.s.	-0.29	n.s.	n.s.	n.s.	n.s.	n.s.	0.29	0.29	n.s.
FFS, %	-0.30	-0.27	n.s.	-0.29	n.s.	n.s.	n.s.	n.s.	n.s.	0.29	0.29	n.s.
EBF, %	0.30	0.27	n.s.	0.29	n.s.	n.s.	n.s.	n.s.	n.s.	-0.29	-0.29	n.s.
EBP, %	-0.31	-0.27	n.s.	-0.32	n.s.	n.s.	n.s.	n.s.	n.s.	0.28	0.29	n.s.
EBA, %	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.39	0.40	0.33

Correlations with |R| ≥ 0.28 are significant at $p \leq 0.05$
Correlations with |R| ≥ 0.24 are significant at $p \leq 0.10$
n.s. = not significant

Appendix 7 (6/6). Pearson correlations between the measured traits of the nitrogen balance dataset in sampling period 2 (see Appendix 4 for the abbreviation code)

	FRR	EBW	EBWA	EBF	EBP	EBA	TBWA, %	EBWA, %	FFS, %	EBF, %	EBP, %	EBA, %
UCE												
UCrE												
BUN												
SC												
IGF-I												
Turn												
Syn												
Deg												
NR:Syn												
FTR												
FSR												
FDR												
FRR												
EBW	-0.42											
EBWA	-0.32	0.85										
EBF	-0.37	0.75	0.38									
EBP	-0.40	1.00	0.87	0.72								
EBA	-0.41	1.00	0.86	0.74	1.00							
TBWA, %	n.s.	n.s.	n.s.	-0.79	n.s.	n.s.						
EBWA, %	0.28	-0.50	n.s.	-0.93	-0.47	-0.49	0.94					
FFS, %	0.28	-0.50	n.s.	-0.93	-0.47	-0.49	0.94	1.00				
EBF, %	-0.28	0.50	n.s.	0.93	0.47	0.49	-0.94	-1.00	-1.00			
EBP, %	0.28	-0.50	n.s.	-0.93	-0.47	-0.49	0.94	1.00	1.00	-1.00		
EBA, %	0.36	-0.42	n.s.	-0.84	-0.39	-0.41	0.85	0.88	0.88	-0.88	0.88	

Correlations with |R| ≥ 0.28 are significant at $p \leq 0.05$
Correlations with |R| ≥ 0.24 are significant at $p \leq 0.10$
n.s. = not significant

Appendix 8. Equations for estimating lysine utilization efficiency (%) of growing pigs fed diets with marginal lysine supply in two sampling periods (SP) based on nitrogen balance data and blood metabolite concentrations

Model	Parameter	b	BCI_b	SE_b	*P*-value	AIC	adj. R^2	RMSE
14						587	0.45	4.74
	Intercept	-41.29	[-71.29; -10.29]	15.14	0.008			
	BW	-0.569	[-0.785; -0.345]	0.093	<0.001			
	ADFI	20.93	[13.27; 28.83]	3.543	<0.001			
	CP ATTD	1.474	[1.042; 1.920]	0.194	<0.001			
	LI	-0.983	[-1.830; -0.204]	0.381	0.012			
	BUN	-2.858	[-4.760; -0.999]	0.812	<0.001			
	SC	0.198	[0.092; 0.299]	0.052	<0.001			
	IGF-I	0.035	[0.004; 0.059]	0.013	0.010			
15						625	0.17	5.89
	Intercept	64.72	[49.27; 80.57]	6.904	<0.001			
	BW	-0.256	[-0.422; -0.069]	0.078	0.001			
	NI	0.804	[0.245; 1.374]	0.271	0.004			
	LI	-2.484	[-4.562; -0.614]	0.878	0.006			
	SC	0.154	[0.026; 0.273]	0.063	0.017			
	IGF-I	0.047	[0.017; 0.072]	0.016	0.004			
16						606	0.32	5.27
	BUN	25.19	[22.87; 27.41]	1.049	<0.001			
	BW x BUN	0.192	[0.111; 0.265]	0.044	<0.001			
	BW x IGF-I	-0.007	[-0.009; -0.005]	0.001	<0.001			
	NI x LI	0.058	[0.050; 0.068]	0.005	<0.001			
	NI x BUN	-0.578	[-0.664; -0.497]	0.046	<0.001			
	NI x IGF-I	0.017	[0.013; 0.021]	0.002	<0.001			
	LI x IGF-I	-0.037	[-0.046; -0.027]	0.001	<0.001			
17						624	0.17	5.82
	ADFI	-90.49	[-151.1; -24.06]	27.02	0.001			
	NI	5.802	[3.379; 8.393]	1.010	<0.001			
	LI	-3.221	[-5.757; -1.276]	1.039	0.003			
	SC	0.155	[0.036; 0.283]	0.063	0.015			
	IGF-I	0.049	[0.019; 0.075]	0.016	0.003			
	ADFI x ADFI	19.87	[3.495; 34.65]	6.636	0.004			
	NI x NI	-0.038	[-0.054; -0.018]	0.007	<0.001			

In total 96 observations of 56 animals in two SP were used for estimations, resulting in 56 independent and 40 repeated measures, evenly distributed over both SP. Mean lysine utilization efficiency was 69.6%. Equations are equally valid for both SP. SP1 = 13th week of life; SP2 = 16th week of life.
Regression coefficients (b) and standard errors (SE_b) were obtained using multiple regression. Confidence intervals of the regression coefficients (BCIb) were generated by 1,000 bootstrap data sets. AIC = Akaike information criterion; RMSE = root mean square error; BW = bodyweight, kg; ADFI = average daily feed intake, kg dry matter; NI = nitrogen intake, g/d; LI = lysine intake, g/d; CP ATTD = apparent total tract crude protein digestibility, %; BUN = blood urea nitrogen, mmol/L; SC = serum cortisol, ng/mL; IGF-I = insulin-like growth factor 1, ng/mL.

Appendix 9. Means of the measured and estimated traits of the offspring of the used boars (no. 1 to 20) across both sampling periods (SP)

Boar	BW	ADG	ADFI	G:F	NI	LI	UNE	UUE	BUN	SC	IGF-I
1	54.7	0.95^{abc}	2.37^{ab}	0.42^{ab}	74.2^{a}	20.1^{ab}	26.6^{a}	21.8^{a}	6.63^{a}	23.8^{abcd}	190^{fgh}
2	53.5	0.91^{abc}	2.18^{abc}	0.43^{ab}	68.2^{abc}	18.7^{abcd}	23.9^{abc}	19.3^{abc}	5.79^{bc}	24.6^{abc}	218^{bcde}
3	51.9	0.90^{bc}	2.12^{abc}	0.44^{ab}	65.4^{cd}	17.8^{de}	23.4^{bc}	19.0^{bc}	5.77^{bcd}	22.7^{bcd}	215^{bcdef}
4	49.7	0.95^{abc}	2.11^{abc}	0.46^{ab}	65.4^{bcd}	18.1^{bcde}	22.5^{bc}	18.3^{bc}	5.44	19.0^{cd}	191^{efgh}
5	50.7	0.95^{abc}	2.16^{abc}	0.43^{ab}	66.3^{bcd}	18.7^{abcd}	22.1^{bc}	17.8^{bc}	5.23^{def}	23.2^{abcd}	225^{bcd}
6	50.1	1.01^{a}	2.21^{abc}	0.47^{a}	66.6^{abcd}	18.7^{abcd}	22.9^{bc}	18.6^{bc}	5.73^{bcd}	24.0^{abc}	208^{bcdefg}
7	50.7	0.99^{ab}	2.29^{abc}	0.42^{ab}	69.6^{abc}	19.4^{abcd}	23.6^{bc}	18.7^{bc}	5.26^{cde}	20.8^{bcd}	196^{efgh}
8	50.0	0.91^{abc}	2.04^{bc}	0.45^{ab}	65.1^{cd}	17.8^{cde}	23.4^{abc}	19.1^{abc}	6.25^{ab}	22.9^{abcd}	199^{defgh}
9	51.9	0.89^{bc}	2.12^{abc}	0.42^{ab}	66.4^{abcd}	18.0^{cde}	23.1^{bc}	18.5^{bc}	5.65^{cde}	28.8^{a}	228^{abc}
10	46.0	0.84^{c}	1.92^{c}	0.44^{ab}	58.9^{d}	16.4^{e}	20.5^{c}	16.6^{c}	5.26^{cde}	23.5^{abcd}	208^{bcdefgh}
11	49.8	0.97^{ab}	2.33^{ab}	0.42^{ab}	71.6^{abc}	20.2^{ab}	24.3^{ab}	19.9^{ab}	5.84^{bc}	22.4^{bcd}	202^{cdefgh}
12	50.0	0.93^{abc}	2.21^{abc}	0.43^{ab}	67.5^{abc}	19.2^{abcd}	22.5^{bc}	18.2^{bc}	5.26^{def}	21.8^{bcd}	212^{bcdef}
13	46.8	0.87^{bc}	2.16^{abc}	0.42^{ab}	66.5^{abcd}	18.9^{abcd}	22.2^{bc}	18.1^{bc}	5.66^{bcde}	23.2^{abc}	206^{bcdefg}
14	49.9	0.90^{bc}	2.18^{abc}	0.42^{ab}	67.0^{abc}	19.5^{abc}	22.3^{bc}	18.1^{bc}	5.08^{f}	20.1^{cd}	202^{bcdefg}
15	47.2	0.92^{abc}	2.12^{abc}	0.44^{ab}	64.7^{cd}	18.1^{bcde}	22.1^{bc}	17.8^{bc}	5.82^{bc}	29.1^{a}	183^{gh}
16	51.3	0.97^{ab}	2.24^{abc}	0.44^{ab}	68.2^{abc}	19.5^{abcd}	23.6^{ab}	19.3^{ab}	5.57^{cdef}	17.9^{d}	232^{ab}
17	50.9	0.95^{abc}	2.43^{a}	0.40^{b}	74.1^{ab}	20.7^{a}	24.4^{ab}	19.8^{ab}	5.27^{cdef}	22.1^{abcd}	206^{bcdefg}
18	50.1	0.91^{abc}	2.24^{abc}	0.42^{ab}	67.9^{abc}	18.9^{abcd}	21.6^{bc}	17.4^{bc}	5.12^{ef}	26.8^{ab}	258^{a}
19	48.3	0.90^{abc}	2.12^{abc}	0.43^{ab}	66.7^{abcd}	17.7^{cde}	23.7^{abc}	19.3^{ab}	5.88^{bc}	21.1^{bcd}	177^{h}
20	49.4	0.97^{ab}	2.27^{abc}	0.44^{ab}	71.3^{abc}	18.9^{abcd}	23.5^{ab}	19.1^{abc}	5.58^{cdef}	22.5^{bcd}	221^{bcde}
pooled SEM	11.2	0.14	0.43	0.07	9.77	2.69	4.04	3.40	0.75	7.92	36.6
p-Value	0.243	<0.001	<0.001	0.020	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Observations of 508 animals were available in SP1 and observations of 458 animals were available in SP2. SP1 = 13th week of life; SP2 = 16th week of life. Different superscript letters within a column indicate significant differences between the group of offspring of the respective boar ($p < 0.05$).
BW = body weight, kg; ADG = average daily gain, kg; ADFI = average daily feed intake, kg dry matter, G:F = gain to feed ratio, kg/kg; NI = nitrogen intake, g/d; LI = lysine intake, g/d; UNE = urinary nitrogen excretion, g/d; UUE = urinary urea nitrogen excretion, g/d; BUN = blood urea nitrogen, mmol/L; SC = serum cortisol, ng/mL; IGF-I = insulin-like growth factor 1; SEM = standard error of the mean.

Appendix 10. Means of the measured and estimated traits of the offspring of the used boars (no. 1 to 20) in sampling period 1 (n = 508 pigs)

Boar	BW	ADG	ADFI	G:F	NI	LI	UNE	UUE	BUN	SC	IGF-I
1	42.6^{a}	0.96	1.88^{ab}	0.52^{ab}	64.5^{ab}	19.8^{abc}	23.6^{a}	19.5^{a}	6.57^{a}	23.9^{ab}	186^{def}
2	41.2^{ab}	0.89	1.80^{ab}	0.50^{abcd}	61.7^{ab}	18.4^{bc}	20.5^{abc}	16.7^{abc}	5.71^{bcde}	26.7^{ab}	220^{abcd}
3	42.3^{ab}	0.88	1.77^{b}	0.50^{abcd}	60.9^{b}	18.3^{c}	21.3^{abc}	17.4^{abc}	5.95^{abc}	23.6^{ab}	222^{abcd}
4	41.2^{ab}	0.96	1.80^{ab}	0.54^{a}	60.9^{ab}	19.2^{abc}	21.1^{abc}	17.3^{abc}	5.64^{bcde}	20.5^{b}	188^{cdef}
5	41.0^{ab}	0.90	1.86^{ab}	0.49^{abcd}	62.2^{ab}	19.4^{abc}	19.5^{c}	15.8^{c}	5.03^{e}	25.0^{ab}	222^{abcd}
6	40.1^{ab}	0.96	1.91^{ab}	0.51^{abcd}	63.8^{ab}	19.6^{abc}	21.2^{abc}	17.3^{abc}	5.87^{abc}	25.4^{ab}	202^{bcdef}
7	40.3^{ab}	0.93	1.91^{ab}	0.49^{abcd}	64.6^{ab}	20.1^{abc}	20.8^{abc}	16.9^{abc}	5.19^{cde}	23.0^{ab}	197^{cdef}
8	40.4^{ab}	0.91	1.78^{b}	0.51^{abc}	62.9^{ab}	19.4^{abc}	21.6^{abc}	17.8^{abc}	6.18^{ab}	24.0^{ab}	202^{bcde}
9	42.6^{a}	0.85	1.87^{ab}	0.46^{cd}	65.5^{ab}	19.7^{abc}	21.6^{abc}	17.5^{abc}	5.74^{bcde}	30.4^{a}	235^{ab}
10	38.9^{ab}	0.84	1.76^{b}	0.48^{abcd}	61.6^{ab}	18.5^{bc}	20.2^{bc}	16.6^{bc}	5.68^{bcde}	22.5^{ab}	219^{abcde}
11	40.6^{ab}	0.90	2.00^{ab}	0.45^{d}	69.4^{a}	21.1^{ab}	23.1^{ab}	19.1^{ab}	6.07^{ab}	21.8^{b}	202^{bcde}
12	40.3^{ab}	0.86	1.90^{ab}	0.46^{cd}	64.7^{ab}	20.4^{abc}	20.5^{abc}	16.7^{abc}	5.24^{cde}	23.4^{ab}	210^{abcde}
13	37.5^{b}	0.86	1.82^{ab}	0.47^{abcd}	63.9^{ab}	20.1^{abc}	20.3^{abc}	16.5^{abc}	5.72^{abcde}	27.2^{ab}	206^{abcde}
14	40.4^{ab}	0.86	1.86^{ab}	0.46^{cd}	64.8^{ab}	20.7^{ab}	20.7^{abc}	16.9^{abc}	5.24^{cde}	22.6^{ab}	202^{bcde}
15	38.1^{b}	0.86	1.81^{ab}	0.48^{abcd}	61.4^{ab}	19.1^{bc}	20.2^{bc}	16.3^{bc}	5.92^{abc}	31.6^{a}	182^{ef}
16	41.4^{ab}	0.94	1.93^{ab}	0.49^{abcd}	66.6^{ab}	20.9^{ab}	22.2^{abc}	18.3^{abc}	5.80^{abcd}	20.8^{b}	229^{abc}
17	40.8^{ab}	0.95	2.05^{a}	0.46^{bcd}	70.2^{a}	22.1^{a}	21.8^{abc}	17.8^{abc}	5.21^{cde}	25.1^{ab}	209^{abcde}
18	40.6^{ab}	0.88	1.89^{ab}	0.47^{bcd}	64.0^{ab}	20.1^{abc}	19.1^{c}	15.3^{c}	5.05^{de}	31.1^{a}	258^{a}
19	38.8^{ab}	0.87	1.80^{ab}	0.48^{abcd}	62.1^{ab}	18.5^{bc}	21.5^{abc}	17.6^{abc}	5.92^{abc}	23.8^{ab}	165^{f}
20	39.2^{ab}	0.97	1.91^{ab}	0.51^{abcd}	65.7^{ab}	19.6^{abc}	20.9^{abc}	17.1^{abc}	5.55^{bcde}	25.7^{ab}	204^{bcde}
pooled SEM	4.23	0.11	0.23	0.06	7.73	2.43	3.03	2.64	0.71	7.53	35.3
p-Value	0.003	<0.001	0.002	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Different superscript letters within a column indicate significant differences between the group of offspring of the respective boar ($p < 0.05$).
BW = body weight, kg; ADG = average daily gain, kg; ADFI = average daily feed intake, kg dry matter, G:F = gain to feed ratio, kg/kg; NI = nitrogen intake, g/d; LI = lysine intake, g/d; UNE = urinary nitrogen excretion, g/d; UUE = urinary urea nitrogen excretion, g/d; BUN = blood urea nitrogen, mmol/L; SC = serum cortisol, ng/mL; IGF-I = insulin-like growth factor 1; SEM = standard error of the mean.

Appendix 11. Means of the measured and estimated traits of the offspring of the used boars (no. 1 to 20) in sampling period 2 (n = 458 pigs)

Boar	BW	ADG	ADFI	G:F	NI	LI	UNE	UUE	BUN	SC	IGF-I
1	67.7^{a}	0.97	2.82^{a}	0.35^{bc}	82.4^{a}	20.7^{a}	30.8^{a}	25.1^{a}	6.72^{a}	23.5^{ab}	187^{d}
2	65.9^{ab}	0.93	2.57^{abcd}	0.37^{bc}	74.6^{ab}	19.0^{abc}	27.2^{ab}	21.9^{ab}	5.87^{abc}	22.4^{ab}	215^{abcd}
3	61.5^{abc}	0.92	2.46^{abcd}	0.38^{abc}	69.9^{bc}	17.3^{bcde}	25.5^{bc}	20.5^{bc}	5.58^{bcd}	21.8^{abc}	207^{abcd}
4	59.1^{bc}	0.95	2.38^{bcd}	0.40^{abc}	69.0^{bc}	17.3^{bcde}	24.5^{bc}	19.8^{bc}	5.37^{cd}	18.0^{bc}	195^{bcd}
5	60.3^{abc}	1.01	2.47^{abcd}	0.37^{bc}	70.4^{abc}	17.9^{abcde}	24.6^{bc}	19.9^{bc}	5.42^{cd}	21.5^{ab}	228^{abc}
6	60.1^{abc}	1.05	2.50^{abcd}	0.43^{a}	69.4^{bc}	17.8^{abcde}	24.7^{bc}	19.8^{bc}	5.60^{bcd}	22.7^{ab}	214^{abcd}
7	60.6^{abc}	1.05	2.63^{abcd}	0.36^{bc}	73.0^{ab}	18.4^{abcd}	25.7^{b}	20.7^{b}	5.31^{cd}	19.1^{abc}	195^{cd}
8	59.5^{bc}	0.91	2.28^{cd}	0.39^{abc}	66.4^{bc}	16.3^{cde}	25.4^{b}	20.6^{b}	6.28^{ab}	21.6^{abc}	196^{bcd}
9	61.1^{abc}	0.94	2.37^{cd}	0.39^{abc}	67.3^{bc}	16.3^{de}	24.6^{bc}	19.6^{bc}	5.55^{cd}	27.2^{a}	222^{abc}
10	54.9^{c}	0.88	2.19^{d}	0.40^{abc}	60.1^{c}	15.1^{e}	21.0^{c}	16.8^{c}	5.02^{cd}	21.4^{abc}	211^{abcd}
11	59.9^{bc}	1.03	2.65^{abc}	0.40^{abc}	74.3^{ab}	19.0^{abc}	26.2^{b}	21.2^{ab}	5.82^{abc}	21.3^{abc}	204^{bcd}
12	59.8^{abc}	0.99	2.52^{abcd}	0.40^{abc}	70.3^{abc}	18.1^{abcd}	24.5^{bc}	19.8^{bc}	5.27^{cd}	20.2^{abc}	214^{abcd}
13	56.1^{c}	0.89	2.50^{abcd}	0.36^{bc}	69.1^{abc}	17.7^{abcde}	24.2^{bc}	19.6^{bc}	5.60^{bcd}	19.3^{abc}	207^{abcd}
14	59.5^{bc}	0.93	2.49^{abcd}	0.37^{abc}	69.1^{abc}	18.2^{abcd}	24.1^{bc}	19.5^{bc}	4.96^{d}	16.6^{bc}	202^{bcd}
15	56.4^{c}	0.98	2.44^{abcd}	0.40^{ab}	68.1^{bc}	17.2^{bcde}	24.0^{bc}	19.3^{bc}	5.73^{bc}	26.6^{a}	185^{d}
16	61.3^{abc}	1.01	2.56^{abcd}	0.39^{abc}	69.7^{abc}	18.0^{abcd}	25.0^{bc}	20.4^{bc}	5.33^{cd}	14.9^{c}	236^{ab}
17	61.1^{abc}	0.96	2.80^{ab}	0.34^{c}	78.0^{ab}	19.4^{ab}	26.9^{ab}	21.8^{ab}	5.34^{cd}	19.1^{abc}	203^{bcd}
18	59.7^{abc}	0.95	2.59^{abcd}	0.37^{bc}	71.7^{abc}	17.8^{abcde}	24.1^{bc}	19.4^{bc}	5.19^{cd}	22.4^{ab}	259^{a}
19	57.8^{c}	0.93	2.45^{abcd}	0.38^{abc}	71.3^{abc}	17.0^{bcde}	25.9^{b}	21.1^{ab}	5.84^{abc}	18.4^{bc}	190^{cd}
20	59.6^{abc}	0.96	2.63^{abcd}	0.37^{abc}	76.8^{ab}	18.2^{abcd}	26.0^{ab}	21.2^{ab}	5.61^{bcd}	19.3^{abc}	238^{ab}
pooled SEM	6.41	0.15	0.34	0.05	10.10	2.45	3.75	3.23	0.74	7.26	35.0
p-Value	<0.001	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Different superscript letters within a column indicate significant differences between the group of offspring of the respective boar ($p < 0.05$).
BW = body weight, kg; ADG = average daily gain, kg; ADFI = average daily feed intake, kg dry matter, G:F = gain to feed ratio, kg/kg; NI = nitrogen intake, g/d; LI = lysine intake, g/d; UNE = urinary nitrogen excretion, g/d; UUE = urinary urea nitrogen excretion, g/d; BUN = blood urea nitrogen, mmol/L; SC = serum cortisol, ng/mL; IGF-I = insulin-like growth factor 1; SEM = standard error of the mean.

Appendix 12 (1/3). Pearson correlations between the measured and estimated traits of the complete balance dataset across both sampling periods and the overall experimental period (see Appendix 4 for the abbreviation code)

	IBW	BW SP1	BW SP2	FBW	ADG SP1	ADG SP2	ADG OEP	ADFI SP1	ADFI SP2	ADFI OEP	G:F SP1	G:F SP2
IBW												
BW SP1	0.90											
BW SP2	0.79	0.92										
FBW	0.56	0.67	0.77									
ADG SP1	0.26	0.34	0.48	0.43								
ADG SP2	n.s.	0.15	0.25	0.43	0.39							
ADG OEP	0.20	0.39	0.55	0.83	0.43	0.57						
ADFI SP1	0.40	0.55	0.58	0.60	0.54	0.33	0.51					
ADFI SP2	0.15	0.28	0.48	0.57	0.46	0.57	0.67	0.57				
ADFI OEP	0.38	0.46	0.58	0.81	0.45	0.45	0.79	0.59	0.72			
G:F SP1	-0.14	-0.20	-0.10	-0.18	0.47	0.11	n.s.	-0.41	-0.09	-0.14		
G:F SP2	n.s.	-0.11	-0.19	n.s.	n.s.	0.54	n.s.	-0.13	-0.31	-0.18	0.19	
G:F OEP	-0.24	n.s.	n.s.	0.14	n.s.	0.23	0.38	n.s.	n.s.	-0.21	0.13	0.22
RFI	n.s.	n.s.	-0.09	n.s.	0.11	n.s.	n.s.	0.17	0.26	0.49	n.s.	-0.27
NI SP1	0.39	0.54	0.57	0.58	0.53	0.29	0.48	0.98	0.56	0.56	-0.40	-0.16
NI SP2	0.18	0.32	0.51	0.58	0.47	0.57	0.62	0.55	0.97	0.66	n.s.	-0.30
NI OEP	0.44	0.54	0.68	0.80	0.47	0.45	0.78	0.58	0.70	0.94	-0.11	-0.15
NR SP1	0.41	0.52	0.55	0.54	0.46	0.23	0.42	0.89	0.49	0.51	-0.39	-0.16
NR SP2	n.s.	0.18	0.37	0.45	0.40	0.45	0.52	0.46	0.88	0.54	n.s.	-0.35
NR OEP	0.40	0.56	0.68	0.76	0.49	0.46	0.73	0.52	0.60	0.67	n.s.	n.s.
NUE SP1	0.15	0.10	n.s.	n.s.	n.s.	-0.12	n.s.	n.s.	n.s.	n.s.	-0.11	n.s.
NUE SP2	-0.35	-0.33	-0.30	-0.24	-0.10	-0.17	-0.17	-0.15	n.s.	-0.23	n.s.	-0.16
NUE OEP	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.16	-0.36	0.10	0.11
BUN SP1	0.12	0.19	0.23	0.19	0.20	0.17	0.20	0.21	0.17	0.15	n.s.	n.s.
BUN SP2	n.s.	n.s.	0.21	0.24	0.14	0.29	0.33	0.11	0.34	0.22	n.s.	n.s.
SC SP1	n.s.	-0.09	n.s.	n.s.	n.s.	n.s.	n.s.	0.09	n.s.	n.s.	n.s.	n.s.
SC SP2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
IGF SP1	0.36	0.38	0.34	0.15	n.s.	n.s.	n.s.	0.15	n.s.	n.s.	-0.10	-0.09
IGF SP2	0.32	0.27	0.21	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Correlations with $|R| \geq 0.09$ are significant at $p \leq 0.05$; n.s. = not significant

Appendix 12 (2/3). Pearson correlations between the measured and estimated traits of the complete balance dataset across both sampling periods and the overall experimental period (see Appendix 4 for the abbreviation code)

	G:F OEP	RFI	NI SP1	NI SP2	NI OEP	NR SP1	NR SP2	NR OEP	NUE SP1	NUE SP2	NUE OEP	BUN SP1
IBW												
BW SP1												
BW SP2												
FBW												
ADG SP1												
ADG SP2												
ADG OEP												
ADFI SP1												
ADFI SP2												
ADFI OEP												
G:F SP1												
G:F SP2												
G:F OEP												
RFI	-0.83											
NI SP1	n.s.	0.17										
NI SP2	n.s.	0.19	0.55									
NI OEP	-0.15	0.39	0.56	0.67								
NR SP1	n.s.	0.14	0.92	0.48	0.50							
NR SP2	n.s.	0.17	0.46	0.94	0.54	0.44						
NR OEP	0.17	n.s.	0.51	0.62	0.74	0.47	0.53					
NUE SP1	n.s.	n.s.	n.s.	n.s.	n.s.	0.38	n.s.	n.s.				
NUE SP2	n.s.	n.s.	-0.14	n.s.	-0.25	n.s.	0.34	-0.16	0.33			
NUE OEP	0.49	-0.59	n.s.	-0.10	-0.35	n.s.	n.s.	0.32	n.s.	0.15		
BUN SP1	n.s.	n.s.	0.27	0.22	0.23	n.s.	0.11	0.17	-0.50	-0.23	n.s.	
BUN SP2	0.22	n.s.	0.14	0.42	0.27	n.s.	0.32	0.23	-0.40	-0.15	n.s.	0.74
SC SP1	-0.10	0.15	n.s.	n.s.	n.s.	0.20	n.s.	n.s.	0.35	0.12	-0.11	n.s.
SC SP2	-0.12	0.15	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.10	n.s.	-0.20	n.s.
IGF SP1	n.s.	-0.13	0.15	n.s.	n.s.	0.36	n.s.	0.16	0.63	0.17	0.13	-0.14
IGF SP2	n.s.	-0.19	n.s.	n.s.	n.s.	0.24	0.10	n.s.	0.47	0.36	0.21	-0.12

Correlations with $|R| \geq 0.09$ are significant at $p \leq 0.05$; n.s. = not significant

Appendix 12 (3/3). Pearson correlations between the measured and estimated traits of the complete balance dataset across both sampling periods and the overall experimental period (see Appendix 4 for the abbreviation code)

	BUN SP2	SC SP1	SC SP2	IGF SP1	IGF SP2
IBW					
BW SP1					
BW SP2					
FBW					
ADG SP1					
ADG SP2					
ADG OEP					
ADFI SP1					
ADFI SP2					
ADFI OEP					
G:F SP1					
G:F SP2					
G:F OEP					
RFI					
NI SP1					
NI SP2					
NI OEP					
NR SP1					
NR SP2					
NR OEP					
NUE SP1					
NUE SP2					
NUE OEP					
BUN SP1					
BUN SP2					
SC SP1	n.s.				
SC SP2	n.s.	0.54			
IGF SP1	-0.11	n.s.	n.s.		
IGF SP2	-0.16	n.s.	-0.11	0.75	

Correlations with |R| ≥ 0.09 are significant at $p \leq 0.05$; n.s. = not significant

Appendix 13 (1/2). Differences between the measured traits of the nitrogen balance dataset across both sampling periods, when pigs were clustered in low, medium, and high nitrogen utilization efficiency (see Appendix 4 for the abbreviation code)

Cluster	low NUE		medium NUE		high NUE		pooled	*p*-	low NUE	medium NUE	high NUE	pooled	*p*-
SP	1	2	1	2	1	2	SEM	Value				SEM	Value
BW	41.0^{b}	59.9^{a}	40.1^{b}	58.6^{a}	38.9^{b}	60.3^{a}	3.16	<0.001	50.8	49.2	49.6	3.52	0.833
ADG	0.77	0.84	0.78	0.87	0.75	0.85	0.08	0.021	0.80	0.82	0.80	0.04	0.696
ADFI	1.72^{b}	2.14^{a}	1.70^{b}	2.26^{a}	1.66^{b}	2.19^{a}	0.15	<0.001	1.94	1.97	1.92	0.11	0.854
G:F	0.45^{abc}	0.40^{bc}	0.46^{a}	0.39^{c}	0.46^{ab}	0.39^{bc}	0.04	<0.001	0.42	0.42	0.42	0.02	0.969
NI	59.3	60.4	59.3	63.1	57.9	61.8	4.70	0.348	59.9	61.2	59.8	4.70	0.689
FNE	10.8^{ab}	12.2^{a}	10.4^{ab}	11.9^{a}	8.87^{b}	10.3^{ab}	1.38	<0.001	11.5^{a}	11.1^{a}	9.60^{b}	1.38	0.008
UNE	23.5^{ab}	24.7^{a}	21.1^{bc}	24.4^{a}	18.4^{c}	21.4^{abc}	2.24	<0.001	24.1^{a}	22.7^{a}	19.9^{b}	2.24	<0.001
TNE	34.2^{ab}	36.9^{a}	31.4^{bc}	36.3^{a}	27.3^{c}	31.7^{bc}	2.75	<0.001	35.6^{a}	33.8^{a}	29.5^{b}	2.75	<0.001
NR	25.1^{cd}	23.5^{d}	27.9^{abc}	26.8^{bcd}	30.6^{a}	30.0^{ab}	2.12	<0.001	24.3^{c}	27.4^{b}	30.3^{a}	2.12	<0.001
PR:ADG	20.7^{bcd}	17.6^{d}	22.9^{ab}	19.3^{cd}	26.5^{a}	22.2^{abc}	1.16	<0.001	19.1^{b}	21.1^{b}	24.4^{a}	1.28	<0.001
NUE	42.4^{c}	38.8^{d}	47.1^{b}	42.4^{c}	53.0^{a}	48.7^{b}	1.10	<0.001	40.5^{c}	44.8^{b}	50.8^{a}	0.88	<0.001
CP ATTD	81.7^{abc}	79.7^{c}	82.5^{abc}	81.1^{bc}	84.7^{a}	83.4^{ab}	1.86	<0.001	80.7^{b}	81.8^{b}	84.1^{a}	0.95	<0.001
DNI	48.6	48.2	49.0	51.2	49.0	51.4	4.03	0.582	48.3	50.0	50.2	2.01	0.479
DNUE	51.9^{c}	48.7^{d}	57.1^{b}	52.3^{c}	62.6^{a}	58.5^{b}	1.74	<0.001	50.3^{c}	54.8^{b}	60.5^{a}	1.10	<0.001
LI	17.8	16.7	18.1	17.8	17.5	17.3	1.36	0.531	17.3	18.0	17.4	0.68	0.331
LUE	63.3^{c}	63.0^{c}	69.3^{b}	67.6^{b}	79.0^{a}	78.2^{a}	2.21	<0.001	63.2^{c}	68.5^{b}	78.6^{a}	1.11	<0.001
FNE:NI	18.3^{abc}	20.3^{a}	17.5^{abc}	18.9^{ab}	15.3^{c}	16.6^{bc}	1.86	<0.001	19.3^{a}	18.2^{a}	16.0^{b}	0.95	<0.001
UNE:NI	39.4^{a}	40.9^{a}	35.4^{b}	38.7^{a}	31.7^{c}	34.8^{bc}	2.06	<0.001	40.2^{a}	37.0^{b}	33.2^{c}	1.12	<0.001
FNE:TNE	31.8	33.2	33.0	32.7	32.6	32.4	3.41	0.987	32.5	32.9	32.5	1.68	0.934
UNE:TNE	68.2	66.8	67.0	67.3	67.4	67.6	3.41	0.987	67.5	67.1	67.5	1.68	0.934
UUE	19.5^{a}	19.3^{a}	17.3^{ab}	19.7^{a}	14.5^{b}	16.8^{ab}	2.07	<0.001	19.4^{a}	18.4^{a}	15.6^{b}	1.07	<0.001
UUE:UNE	82.9^{a}	77.9^{c}	81.8^{ab}	80.5^{abc}	78.6^{bc}	78.1^{c}	2.29	<0.001	80.3^{ab}	81.2^{a}	78.3^{b}	1.21	0.015

Observations of 48 animals in each SP were used for calculations except for whole-body protein turnover data in SP2, where 42 observations were available. SP1 = 13th week of life; SP2 = 16th week of life. Different superscript letters indicate significant differences in trait means between the clusters in the respective SP (left side of the table) or between the clusters across both SP (right side of the table; $p < 0.05$). SEM = standard error of the mean.

Appendix 13 (2/2). Differences between the measured traits of the nitrogen balance dataset across both sampling periods, when pigs were clustered in low, medium, and high nitrogen utilization efficiency (see Appendix 4 for the abbreviation code)

Cluster	low NUE		medium NUE		high NUE		pooled	*p*-	low NUE	medium NUE	high NUE	pooled	*p*-
SP	1	2	1	2	1	2	SEM	Value				SEM	Value
BUN	6.33	5.49	5.91	5.72	5.59	5.91	0.52	0.132	5.90	5.82	5.75	0.27	0.827
SC	21.1	23.3	20.0	20.9	24.9	26.4	6.32	0.429	22.2	20.5	25.6	3.12	0.111
IGF-I	182	190	205	194	223	207	23.7	0.081	186^{b}	200^{ab}	215^{a}	11.8	0.024
Turn	693	593	679	687	625	700	75.4	0.187	648	683	659	38.4	0.480
Syn	547	448	548	535	508	563	71.2	0.181	502	542	533	36.2	0.390
Deg	390	302	373	369	319	387	70.4	0.273	350	371	350	35.7	0.659
NR:Syn	29.4^{b}	32.5^{ab}	32.6^{ab}	32.1^{ab}	38.2^{a}	34.4^{ab}	4.14	0.038	30.8^{b}	32.4^{b}	36.5^{a}	2.07	0.012
FTR	11.0^{a}	6.25^{b}	11.0^{a}	7.44^{b}	10.5^{a}	7.28^{b}	0.92	<0.001	8.85	9.30	9.03	0.77	0.755
FSR	8.73^{a}	4.73^{b}	8.89^{a}	5.80^{b}	8.54^{a}	5.87^{b}	0.91	<0.001	6.91	7.41	7.33	0.69	0.667
FDR	6.23^{a}	3.20^{c}	6.05^{a}	3.99^{bc}	5.36^{ab}	4.04^{bc}	0.90	<0.001	4.85	5.07	4.76	0.57	0.773
FRR	2.50^{c}	1.53^{d}	2.84^{b}	1.80^{d}	3.18^{a}	1.84^{d}	0.17	<0.001	2.06^{b}	2.34^{ab}	2.57^{a}	0.20	0.027

Observations of 48 animals in each SP were used for calculations except for whole-body protein turnover data in SP2, where 42 observations were available. SP1 = 13th week of life; SP2 = 16th week of life. Different superscript letters indicate significant differences in trait means between the clusters in the respective SP (left side of the table) or between the clusters across both SP (right side of the table; $p < 0.05$). SEM = standard error of the mean.

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CURRICULUM VITAE

Personal Data

Name:	Daniel Berghaus
Date of birth:	03.11.1989
Place of birth:	München, Germany

Academic Career

Since 11/2018	Research for PhD, Institute of Animal Science, Department of Animal Nutrition, University of Hohenheim, Stuttgart, Germany
10/2016 – 10/2018	Undergraduate studies of Agricultural Science with specialization in Animal Sciences, University of Hohenheim, Stuttgart, Germany Qualification gained: Master of Science
10/2012 – 09/2016	Undergraduate studies of Agriculture with specialization in Animal Production, University of Applied Sciences Weihenstephan-Triesdorf, Triesdorf, Germany Qualification gained: Bachelor of Science
09/2000 – 07/2008	Gymnasium Neubiberg, Neubiberg, Germany Qualification gained: Abitur

Professional Career

Since 02/2022	Employee for Research and Development at ISF GmbH, Pinneberg, Germany
11/2016 – 09/2018	Student assistant at the Central Experimental Animal Husbandry, University of Hohenheim, Stuttgart, Germany
08/2014 – 09/2016	Internship and subsequent part-time employment at Voglers Bauernhof GbR, Neuwirtshaus, Germany
04/2010 – 08/2012	Agricultural apprenticeship in sheep husbandry, Schafzucht Schleich, Kirchstockach, Germany and Schäferei Scherpf, Hammelburg, Germany

Pinneberg, 05.04.2022

Daniel Berghaus

DECLARATION IN LIEU OF AN OATH ON INDEPENDENT WORK

according to Sec. 18(3) sentence 5 of the University of Hohenheim's Doctoral Regulations for the Faculties of Agricultural Sciences, Natural Sciences, and Business, Economics and Social Sciences

1. The dissertation submitted on the topic:

Variation and estimation of nitrogen utilization efficiency in a crossbred pig population

is work done independently by me.

2. I only used the sources and aids listed and did not make use of any impermissible assistance from third parties. In particular, I marked all content taken word-for-word or paraphrased from other works.

3. I did not use the assistance of a commercial doctoral placement or advising agency.

4. I am aware of the importance of the declaration in lieu of oath and the criminal consequences of false or incomplete declarations in lieu of oath.

I confirm that the declaration above is correct. I declare in lieu of oath that I have declared only the truth to the best of my knowledge and have not omitted anything.

Pinneberg, 05.04.2022

Daniel Berghaus