The influence of dietary concentration of nitrogen and phosphorus on performance and nutrient excretion of growing-finishing pigs, potential genotype by diet interactions and the role of gut microbiome

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Abstract

Rising feed costs and a high environmental burden from nutrient excretion need a re-evaluation of feeding and breeding strategies in pig production. Approaches focus on minimizing nitrogen and phosphorus (N/P) excretion, with dietary N/P restriction being well-established in animal nutrition. Studies have shown genetically determined variations in adaptability to such restriction, termed genotype×diet interactions (G×D). The background of these variations is largely unexplored, possibly influenced by the pigs' gut microbiome. This study examined the effects of N/P restriction on performance and N/P-related traits in growing-finishing pigs, while investigating heritability (h^2), G×D, and the microbiome's influence characterized by the microbiability (m^2) on these traits.

To achieve this, a 3-phase fattening trial was conducted under farm conditions, including 103 Piétrain×German Landrace pigs, originating from 20 artificial insemination boars (AI-boars). Half of the pigs were fed extremely N/P-reduced diets according to the German Agricultural Society (DLG), while the other half were fed strongly N/P-reduced diets. The animals did not show any impairments in performance under the extreme N/P restriction. Only under an N/P restriction stronger than originally intended a reduction in N/P intake and excretion was observed. Estimated h^2 varied depending on the trait and fattening period (h^2: 0.00-0.77). The values of the AI-boar×feeding group interaction and the correlation coefficients between the AI-boars and feeding groups indicate a potential G×D for N/P intake and faecal N/P excretion.

The gut microbiome was examined in 52 of the 103 pigs across four different gut sections (Jejunum, Ileum, Caecum, Colon). The N/P restriction showed only a limited effect on the microbiome composition. A clear distinction in gut microbiome composition was observed between the small (jejunum, ileum) and large intestine (caecum, colon). This difference in gut section microbiomes was also reflected by marked m^2 estimates for relevant traits. Specifically, the microbiome of the large intestine affected feed efficiency traits (m^2: 0.26-0.61), whereas the microbiome of the small intestine influenced N/P digestibility (m^2: 0.22-0.27).

The estimated h^2 indicate a genetic foundation for most N/P-related traits, showing potential for genetic progress. Genotypes react differently to feed types, and the existence of G×D allows for selecting animals that maintain performance under strong dietary N/P restriction, enhancing robustness. The m^2 estimates show a clear influence of the gut microbiome on target traits, suggesting microbial information can help elucidate inter-animal variability and help in ranking breeding animals. Study results induce further interdisciplinary research in animal nutrition, breeding, and microbiology to enhance the efficiency of growing-finishing pigs.

Kurzfassung

Steigende Futterkosten und hohe Umweltbelastung durch Stickstoff- und Phosphor- (N/P)-Ausscheidungen erfordern eine Neubewertung von Fütterungs- und Zuchtstrategien in der Schweineproduktion. Ziel ist es, die N/P-Ausscheidungen zu minimieren. Eine bereits etablierte Strategie ist die N/P-restriktive Fütterung. Studien zeigten zudem genetische Variationen in der Anpassungsfähigkeit an die N/P-Restriktion, bekannt als Genotyp×Futter-Interaktionen (G×D), möglicherweise beeinflusst durch das Darmmikrobiom. Ziel der Arbeit war, die Auswirkungen einer N/P-Restriktion auf die Leistung und N/P-relevante Merkmale sowie deren Erblichkeit (h^2), mögliche G×D und den Einfluss des Mikrobioms (Mikrobiabilität, m^2) zu untersuchen.

Hierzu wurde ein 3-Phasen-Mastversuch unter Produktionsbedingungen mit 103 Piétrain×Deutsche Landrasse Schweinen, abstammend von 20 Piétrain Besamungsebern (KB-Eber), durchgeführt. Die Hälfte der Tiere erhielt gemäß dem Leitfaden der Deutschen Landwirtschaftsgesellschaft (DLG) ein sehr stark N/P-reduziertes Futter, die andere Hälfte ein stark N/P-reduziertes Futter. Die Auswertung zeigte keine signifikanten Leistungseinbußen durch die sehr starke N/P-Restriktion. Nur eine stärker als geplante N/P-Restriktion führte zu einer Minderung der N/P-Aufnahme und -Ausscheidung. Die h^2 variierten je nach Merkmal und Mastphase (h^2: 0,00-0,77). Die Werte für die KB-Eber×Fütterungsgruppe und die Korrelationskoeffizienten zwischen den KB-Ebern in den Fütterungsgruppen deuten auf eine mögliche G×D für die N/P-Aufnahme und -Ausscheidung hin.

Das Darmmikrobiom wurde anhand von 52 der 103 Schweine in vier unterschiedlichen Darmabschnitten untersucht (Jejunum, Ileum, Caecum, Colon). Die N/P-Restriktion zeigte nur einen begrenzten Effekt auf die Mikrobiomzusammensetzung. Es gab einen klaren Unterschied in der Zusammensetzung, sowie in den m^2 für relevante Merkmale zwischen Dünn- und Dickdarmabschnitten. Das Dickdarmmikrobiom beeinflusste Futtereffizienzmerkmale (m^2: 0,26-0,61), das Dünndarmmikrobiom die N/P-Verdaulichkeit (m^2: 0,22-0,27).

Die geschätzten h^2 weisen auf die genetische Fundierung der meisten N/P-relevanten Merkmale hin und bieten Potential für Zuchtfortschritt. Genotypen reagieren je nach Fütterung unterschiedlich. Diese G×D erlaubt die gezielte Auswahl von Tieren, die auch unter sehr starker N/P-Restriktion hohe Leistungen erreichen, was die Robustheit verbessert. Die m^2 zeigen einen Einfluss des Mikrobioms auf die Zielmerkmale. Mikrobielle Informationen könnten den Hintergrund der Variabilität zwischen den Tieren weiter aufklären und bei der Rangierung von Zuchttieren helfen. Die Ergebnisse der Arbeit regen zu weiterer interdisziplinärer Forschung in Tierernährung, Zucht und Mikrobiologie an.

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List of abbreviations

AA	Amino acid
ATP	Adenosine triphosphate
ADF	Acid detergent fibre
ADFC	Average daily feed consumption
ADFI	Average daily feed intake
$ADFI_{est}$	Estimated average daily gain
$ADFI_{obs}$	Observed average daily gain
ADG	Average daily gain
ADN	Nitrogen digestibility
ADP	Phosphorus digestibility
AIA	Acid insoluble ash
AI-boar	Artificial insemination boar
AMS	Average metabolic body size
ASV	Amplicon sequence variant
BFT	Back fat thickness
BW	Body weight
BRS	German Livestock Association
BUN	Blood urea nitrogen
CON	Control group
Ca	Calcium
CA	Crude ash
CF	Crude fibre
color	Meat color
CP	Crude protein
CR	Clearance rate of urea
DLG	German Agricultural Society
DM	Dry matter
DNA	Deoxyribonucleic acid
DP	Dressing percentage
EAA	Essential amino acid
EE	Ether extract
FA	Fatty acids
FCR	Feed conversion ratio

FFW	Final fattening weight
G×D	Genotype×diet interaction
G×E	Genotype×environment interaction
GfE	Society of Nutrition Physiology
gi^2	Variance ratio of artificial insemination boar×group and phenotype
GWAS	Genome-wide association analysis
h^2	Heritability
IMF	Intramuscular fat content
KB-Eber	Besamungseber
LMC	Lean meat content
LP	Group with dietary nitrogen and phosphorus restriction
M	Timepoint of measurement
m^2	Microbiability
ME	Metabolisable energy
M-matrices	Microbiome variance-covariance matrices
MUFA	Monounsaturated fatty acids
MWAS	Microbiome-wide association study
N	Nitrogen
N/P	Nitrogen and phosphorus
NDF	Neutral detergent fibre
NEAA	Non-essential amino acid
NEXf	Faecal nitrogen excretion
NEXu	Urinary nitrogen excretion
NIN	Nitrogen intake
NSP	Non-starch polysaccharides
NUE	Nitrogen utilisation efficiency
OTU	Operational taxonomic unit
P	Phosphorus
PD	Predicted progeny differences
PEX	Faecal phosphorus excretion
Pi	Piétrain
PIN	Phosphorus intake
PUFA	Polyunsaturated fatty acids
QTL	Quantitative trait loci

RFI	Residual feed intake
r_g	Genetic correlation
RNA	Ribonucleic acid
r_{PD}	Correlation coefficient of the predicted progeny differences
SCFA	Short-chain fatty acids
SE	Standard error
SFA	Saturated fatty acids
SNP	Single nucleotide polymorphism
SW	Slaughter weigt

Chapter 1. General introduction

1.1 Sustainable development of pig production

Sustainability, which includes social, economic, and ecologic dimensions, has become crucial across all fields of the agricultural sector, including pig production (Enquete Commission of the German Bundestag, 1998). Particular in industrial countries, the issue of animal welfare has gained rising importance. In a pan-European study, more than 80% of surveyed citizens recognize the necessity to enhance the well-being of farm animals (Christoph-Schulz and Rovers, 2018).

At the same time, pig farmers are facing rising costs (AMI, 2023), with the largest portion attributed to feed costs (Rohlmann et al. 2022). Sustainable resource management is not only motivated by these rising costs but also by environmental considerations. Notably, approximately 95% of ammonia emissions derive from animal husbandry and the associated storage and application of manure (Taube et al., 2020). Moreover, agriculture is responsible for 90% of nitrate contamination in groundwater (Bach et al., 2020). These issues have adverse effects on both human health, and on soil and water eutrophication. Phosphate pollution, particularly prominent in many German regions, contributes significantly to these problems (Taube et al., 2020). Additionally, the EU lists phosphorus as a critical raw material (European Commission, 2020). Alongside performance and animal welfare, feed and nutrient utilisation efficiency (defined by Berghaus et al. (2023) as nutrient retention in body relative to nutrient intake), have thus gained increased attention. As a result, numerous pig breeding and feeding organizations are re-evaluating breeding goals and feeding strategies.

Animal nutrition strategies aim at feeding pigs with nutrients close to their requirements, and thereby preventing both under- and over-supply, with their detrimental effects on animals and the environment. Key milestones in this evolution include the implementation of phase feeding, the use of feed additives such as free amino acids and phytase, and the implementation of nitrogen- and phosphorus (N/P)-restricted rations (Yin and Tan, 2010). Animal breeding approaches are based on findings from studies that demonstrate the heritability (h^2) of feed efficiency traits (feed conversion ratio and residual feed intake) and N/P-related traits (intake, digestibility and excretion). Estimates of heritability of those traits vary depending on breed and ration but generally fall within moderate ranges (Satintilan et al., 2013, Déru et al., 2021).

However, it is essential to recognize that the approaches in animal breeding and nutrition are not mutually exclusive but interrelated. For instance, Déru et al. (2021) revealed that h^2 estimates are influenced by dietary fibre content. Additionally, studies have shown variability in adaptability to dietary nutrient restriction. Approximately 30% of purebred Swiss Large

White pigs did not show any performance or health impairments in a study involving a substantial restriction in crude protein (CP) and essential amino acids (EAA) (Ruiz-Ascacibar et al., 2017). It is presumed that the variation in adaptability to dietary N/P restriction is partly genetically determined (Kasper et al., 2020). The dependencies between genetics and nutrition can be characterised as genotype by diet interactions (G×D), which become evident in the re-ranking of genotypes under altered dietary conditions. While G×D can be seen as a confounding factor in breeding (Falconer and Mackay, 1995), it nevertheless provides the opportunity to determine the environmental sensitivity of animals (Knap and Su, 2008). Consequently, it becomes feasible to identify exceptionally robust pigs that can cope with limited nutrient resources.

The underlying mechanisms of the genetically based differences in adaptability remain largely unresolved. Recent studies have indicated that, besides genetic and environmental factors, there is a microbial component influencing the variation in target traits in pig production, referred to as "microbiability" (m^2) (Difford et al., 2016, Camarinha-Silva et al., 2017, Aliakbari et al., 2022). More precisely, this pertains to the influence of the gut microbiome, which affects the host's performance and immune system through microbial metabolites (Mowat and Agace, 2014). Research in this direction is still in its early stages; however, there is promising potential that microbiome information may help to more efficiently predict the expression of target traits in pig production, thereby enhancing the ranking of breeding animals (Verschuren et al., 2020, Aliakbari et al., 2022).

An interdisciplinary approach, merging animal nutrition, breeding and genetics as well as gut microbiome research, could provide a framework for incorporating feed efficiency and N/P-related traits into pig breeding programmes. This could lay the foundation for a pig production system, which aligns with social acceptability, cost-effectiveness, resource efficiency, and environmental sensitivity.

1.2 Aim of this thesis

This thesis aims to comprehensively investigate N/P-related traits in pigs. Further, feeding and breeding strategies for enhancing efficiency are assessed while emphasising its relevance in the context of a sustainable development. An important aspect of this research is a pig fattening trial conducted under German farm conditions, which includes supplying N/P-restricted rations. This study deliberately excludes the possibility of nutrient deficiency for animal welfare considerations. Special focus is placed on examining the influence of artificial insemination boars (AI-boars) and exploring G×D. Additionally, the thesis includes an investigation of the gut microbiome, a critical factor impacting host's performance and health.

Chapter 2 provides a literature review on the importance of N/P in pigs, their dietary supply recommendations, and the impact of N/P concentrations on performance, health, behaviour, and excretion. It presents nutritional and genetic approaches to reduce N/P excretion, including their interaction (G×D). The chapter ends with an overview of the gut microbiome's significance for growing-finishing pigs.

Chapter 3 demonstrates the effects of N/P-restricted rations on pig performance, feed efficiency, and N/P excretion and the significance of G×D in relation to relevant traits. This is illustrated by a fattening trial under farm conditions, which involved 103 Piétrain×German Landrace growing-finishing pigs. The chapter includes the estimation of variance components for target traits including G×D effects and rank correlations for AI-boars with respect to their progeny performance within the different feeding groups.

Chapter 4 is about the description and analysis of the gut microbiome in four gut sections of 52 of these 103 growing-finishing pigs. This chapter provides an overview of the microbiome composition and assesses microbiability (m^2) of fattening performance, feed efficiency and N/P-related traits with a subsequent microbiome-wide association analysis (MWAS).

Chapter 5 offers a general discussion, starting with an overview of DLG leaflet 418 (2019) on strongly reduced N/P feeding programmes and legal tolerances. It finally explores trait characterisation and challenges in integrating N/P-related traits into pig feeding programmes, highlighting the benefits of G×D and gut microbiome information.

Chapter 2. Literature review

2.1 Importance and impacts of nitrogen and phosphorus in growing-finishing pigs

2.1.1 Essential roles of nitrogen and phosphorus in pig physiology

Nitrogen (N) and phosphorus (P) are essential elements for all forms of life, including livestock. They constitute integral components of crucial molecules such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA), proteins and adenosine triphosphate (ATP). Nitrogen and P play a critical role in maintenance, encompassing health and behaviour, as well as in performance, including growth. The full performance potential in farm animals can only be realised when the nutritional requirements for maintenance and performance are covered. Both, under- and over-supply of N/P are undesirable for animal welfare, economics, and ecology.

Nitrogen is provided to organisms mainly through dietary protein, which typically contains about 16% N (Maclean et al., 2003). Proteins, which are categorised into various functional classes being essential for metabolism, are composed of amino acids (AA). Amino acids can be classified into non-essential and essential amino acids (EAA) (GfE, 2008).

Phosphorus, a crucial macronutrient, primarily functions as a structural and regulatory substance in organisms. It is essential for bone and teeth development, forming hydroxyapatite with calcium (Ca). It also plays a critical role in energy metabolism being a key component of ATP, nucleotides, and phospholipids (Lautrou et al., 2021, Lautrou et al., 2022).

2.1.2 Dietary requirements for amino acids and phosphorus

German recommendations for energy and nutrient supply in pigs are published and regularly updated by the Committee for Requirement Standards of the Society of Nutrient Physiology (GfE), most recently in 2008 (GfE, 2008). The nutritional requirements depend on factors such as growth, body weight, gender, and genetics. They can be divided into maintenance and performance requirements. The overall objective is to provide a nutrient supply that closely matches the pig's requirements throughout their entire life.

The pig as a non-ruminant does not have a general, non-specific requirement for N or protein, but for AA and specifically for EAA. Daily requirements are given in form of "precaecally digestible AA" to characterise supply to the animal. This unit is chosen, because AA are mainly absorbed from the small intestine, before reaching the caecum. In general, digestibility refers to the amount of a nutrient that disappears from the digestive tract relative to the amount of intake, i.e. the quantity of an ingested nutrient that is not found in faeces. Regarding N, both endogenous nutrient quantities and undigested dietary protein can be excreted via faeces or

converted into microbial protein in the large intestine before being excreted. Consequently, measurements of "total tract digestibility" and "precaecal digestibility," although more precise, may diverge due to their varying methodologies, with the latter being notably more complex to assess (GfE, 2005).

Given that sufficient data for deriving supply recommendations is currently only available for the first-limiting essential amino acid, lysine, the daily requirements for precaecal digestible lysine are provided, ranging from 9.6 to 20.3 g/day depending on body weight (BW) and daily BW gain (GfE, 2008). Requirements for all other EAA are expressed in relation to lysine using the "ideal protein" concept (van Milgen and Dourmad, 2015), which describes the ratio of EAA at which optimal dietary protein efficiency is achieved. Supply recommendations for precaecally digestible CP range between 143 and 296 g/day (GfE, 2008), covering the needs for non-essential AA as well.

Phosphorus requirements are based on "digestible phosphorus", reflecting the amount of P usable by animals (GfE, 2008). Phosphorus digestibility depends on its chemical form and feed components. For example, P is often present as phytate, which is not readily absorbable without phytase (Eeckhout and de Paepe, 1994). Besides environmental and dietary factors, P digestibility also depends on animal's gender, age, gut microbiome, and genetics (Saintilan et al., 2013, Ruiz-Ascacibar et al., 2017, Déru et al., 2022). The GfE provides recommendations for digestible P supply based on body weight and daily gain, ranging from 3.0 to 6.5 g/day. Considering P, it is essential to also account for calcium (Ca) requirements, as their digestive and metabolic processes are closely connected (Létourneau-Montminy et al., 2012). According to Schlegel and Gutzwiller (2020), the Ca to digestible P ratio should not fall below 2.5:1 to maximize dietary P utilisation.

2.1.3 Impact of dietary N/P concentration on performance, health and behaviour, and N/P excretion

As already described, N/P are essential elements for the maintenance and performance of growing-finishing pigs, fulfilling various metabolic functions. Thus, dietary N/P concentration, and consequently N/P restriction, can influence performance, health, behaviour, and N/P excretion.

Impact on performance

Fattening and slaughter performance, as well as meat quality, are the most important parameters for evaluating the profitability of a pig production system (Wang et al., 2018). An under-supply of N (in form of AA and CP) or P will impair the performance of growing-finishing pigs, leading to reduced growth rates and compromised carcass composition and meat quality (Harper et al., 1997, Ruiz-Ascacibar et al., 2017, Sørensen et al., 2018, Li et al., 2018). Moreover, there are indications of an increase in daily feed intake, as suggested by the "protein leverage model", which proposes higher consumption of low-protein diets to regulate protein intake (Carcó et al., 2018, Raubenheimer and Simpson, 2019). However, the magnitude and duration of these impacts may vary, dependent on the extent of nitrogen/phosphorus restriction and potential concurrent decrease in EAA levels.

Impact on health and behaviour

Nitrogen and P are not only essential for performance but also for maintenance, including bodily functions and normal behaviour, aligning with the animal welfare principles ("five freedoms"; FAWC, 1993).

Amino acids are essential for synthesizing proteins, cytokines, and antibodies, which are crucial for immune responses against pathogens. They also regulate metabolic pathways, immune cell activation, and cellular functions like ATP generation and gene expression (Li et al., 2007, Kelly and Pearce, 2020). Given its critical role in both energy metabolism and immune cell function, phosphorus is indispensable for animal health. Moreover, it is vital for maintaining bone mineral content and, as a result, skeletal stability (Gutierrez et al., 2015). Deficient dietary phosphorus can lead to bone density issues and potential skeletal deformities (Doige et al., 1975, Harper et al., 1997, Rieger, 2017).

Furthermore, low crude protein content is linked to increased aggression, such as tail biting, possibly due to its association with neurotransmitters (van der Meer et al., 2017). The protein leverage model suggests that pigs may exhibit heightened exploratory behaviour to regulate protein intake, potentially resulting in aggression towards pen mates if feed is unavailable (Studnitz et al., 2007). However, it is important to note that the extent of health impairments and behavioural disorders resulting from dietary N/P restriction may vary based on the strength and duration of the N/P restriction.

Impact on excretion

Dietary N/P concentrations above the required levels can lead to increased N/P excretion, which can be classified as environmental pollution. Recently, this tissue has gained further attention alongside animal performance and health concerns due to the necessity for sustainable development of pig production. To prevent performance and health impairments, many pig ration formulations include safety margins beyond actual nutrient requirements (Misiura et al., 2020, Krieg et al., 2023). Specifically for P, these margins address uncertainties in P intake, bone mineralization, muscle tissue interaction, and resource allocation (Misiura et al., 2020). However, supplying N/P beyond maintenance and performance requirements generally increases their excretion.

Besides dietary N/P content, N/P availability is crucial when considering the amount of N/P excretion. Therefore, recommendations are provided as precaecal digestible EAA and CP, and digestible P (GfE, 2008). For example, if P is bound in a non-utilisable form, it is excreted undigested (Eeckhout and de Paepe, 1994). It should be noted that digestibility, utilisation efficiency, and the subsequent excretion of N/P depend not only on environmental and dietary factors but also on the animal's gender, age, gut microbiome, and genetics (Saintilan et al., 2013, Ruiz-Ascacibar et al., 2017, Déru et al., 2022). These influencing factors suggest that there are also opportunities to improve N/P utilisation efficiency and reduce N/P excretion in non-nutritional fields.

2.2 Approaches to reducing N and P excretion

Approaches to reducing N/P excretion of growing-finishing pigs can be categorised into the fields of animal nutrition and genetics. These approaches aim to minimize the environmental impact while maintaining performance and animal welfare.

2.2.1 Animal nutrition

In animal nutrition, various strategies have been established in recent years with the objective of consistently satisfying the individual nutritional requirements of each growing-finishing pig at each stage of its growth. This approach tries to prevent both, an over- and undersupply with N/P, leading to reduced N/P excretion (Aarnink and Verstegen, 2007).

One of the strategies is phase feeding. Its underlying principle is that an increased number of feeding phases enhances precision, enabling closer alignment with the specific nutritional requirements in different growth stages, as depicted in Figure 1. For instance, in a study by Pomar et al. (2014), increasing the number of feeding phases from a 3-phase to daily-phase feeding strategy reduced excretion by 12.6% and 6.6% for N and P, respectively. Feed costs were also slightly reduced by 1%.

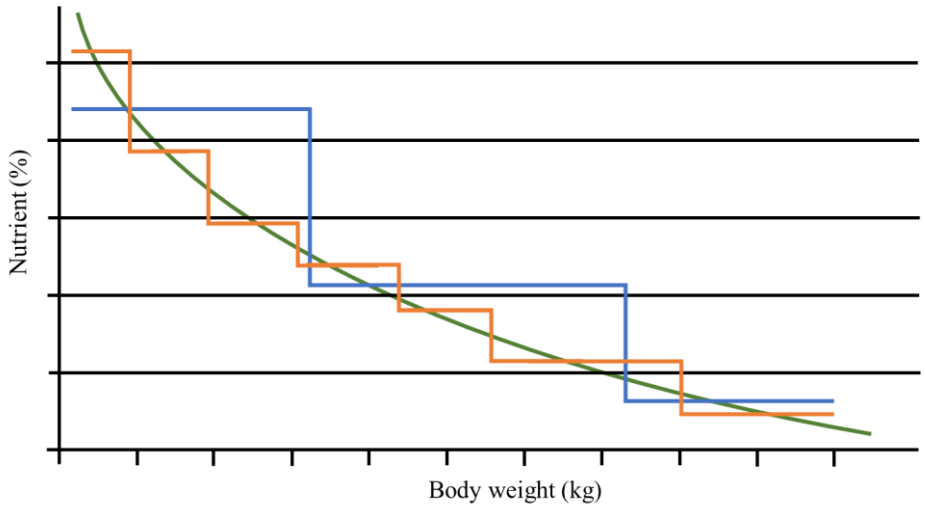

Figure 1: Phase feeding vs. daily requirements for amino acids and phosphorus of growing-finishing pigs (Lautrou et al., 2022)
Note: green, nutrient requirement; blue, 3-phase feeding; orange, multi-phase feeding.

Moreover, dietary N/P restriction is a commonly employed strategy to reduce the N/P excretion (Aarnink and Verstegen, 2007, Pomar et al., 2021). In this context, the DLG (2019) has developed a guideline aimed at facilitating the practical implementation of low N/P diets in

growing-finishing pigs in Germany, which is detailed in the general discussion of this thesis (Chapter 5.1.1). This approach requires a high availability of AA and P in feed components. Thus, diets are supplemented with free AA (Aarnink and Verstegen, 2007), aligning with the ideal protein concept. Wang et al. (2018) demonstrated that reducing CP by one percentage unit, alongside supplementation with free AA, led to a notable reduction of 8-10 % N excretion. Additionally, the supplementation of phytase to enhance P digestibility is widely adopted in practice (Yin and Tan, 2010, Rosenfelder-Kuon et al., 2020), which has been proven to significantly reduce P excretion (Harper et al., 1997).

A perspective strategy in animal nutrition, targeting the minimization of N/P excretion, is the concept of individualised daily-phase feeding, also known as individual precision feeding (Pomar et al., 2021). As the name suggests, this approach considers not only the fluctuating nutrient requirements during growth, but also the previously mentioned individual variations in requirements between pigs. The so-called individual real-time requirement is calculated using a model that incorporates data on body weight, feed intake, and weight gain (Pomar et al., 2021). Based on this, an individual pig can be supplied with an adequate daily ration. According to Andretta et al. (2016), precision feeding can reduce N/P excretion by almost 40%, and feed costs by more than 8%. Implementation in practice requires a corresponding technical system, which currently needs further research. Individual precision feeding could advance sustainability of future pig production.

As mentioned above, the described nutritional strategies focus on providing growing-finishing pigs with AA and P close to their individual requirements (Aarnink and Verstegen, 2007). In addition to the individual requirements, the used feedstuffs also play an important role. The basis for ration planning and an appropriate AA and P supply in pigs is the assessment of the availability of nutrients. To evaluate the protein value of a feedstuff, the standardised precaecally digestible CP and AA are determined. Usually, this is done *in vivo* using an invasive method, a fistula at the terminal ileum (GfE, 2008). In a recent approach, Schumacher et al. (2022) developed a simple laboratory method to estimate the standardised precaecally digestibile CP and AA in feedstuffs. Such a method may help to assess the protein value of a feedstuff in a quick and safe way, without affecting the animal. It may therefore contribute not only to animal welfare, but also to ensuring an adequate CP and AA supply, thereby reducing N excretion.

2.2.2 Animal breeding and genetics

The nutritional requirements for N/P in pigs are determined not only by environmental factors but also by the pigs' genetic background (NRC, 2012). Research indicates that variations in N/P utilisation efficiency and N/P excretion can, to some extent, be attributed to genetic aspects (Kasper et al., 2020, Berghaus, 2022). Therefore, investigating genetic approaches to maximise N/P utilisation efficiency and minimise excretion is both meaningful and worthwhile.

In contrast to approaches in animal nutrition, those in animal breeding and genetics have not yet been fully integrated into practical applications. Instead, emphasis has primarily been placed on indirect selection through feed efficiency traits such as feed conversion ratio (FCR) and residual feed intake (RFI). Feed conversion ratio characterises the ratio between feed intake and body weight gain, while RFI defines the difference between expected and observed feed intake (Saintilan et al., 2013). Gilbert et al. (2017) demonstrated that nutrient utilisation efficiency can be improved through reduced feed intake and changes in carcass composition, enabling indirect selection for N/P utilisation efficiency via direct selction for improved feed efficiency. Thus, recent breeding efforts focusing on feed efficiency (Patience et al., 2015) have indirectly led to improvements in N/P utilisation efficiency and a consequent reduction in N/P excretion (Saintilan et al., 2013, Shirali et al., 2012, Shirali et al., 2013). This improvement is based on the notably high genetic and phenotypic correlations between FCR, RFI, and excretion traits, as evidenced by observations on over 21,000 animals (Table 1) (Saintilan et al., 2013).

Table 1: Genetic and phenotypic correlations of feed conversion ratio and residual feed intake with total N and P excretion (modified from Saintilan et al., 2013)

	Feed conversion ratio	**Residual feed intake**
	Genetic correlation	
Total N excretion	0.99	0.46-0.84*
Total P excretion	0.99	0.52-0.85*
	Phenotypic correlation	
Total N excretion	0.99	0.67-0.79*
Total P excretion	0.99	0.71-0.80*

Note: *range provides estimated values of four different breeds (French Landrace dam breed, Large White dam breed, Large White sire breed, Piétrain sire breed).

One reason for the limited attention paid to directly selection for N/P-related traits in pig breeding could be the high labor and time demands associated with their assessment. For example, direct assessment of faecal and urinary N/P excretion via balance trials is an accurate

but labour-intensive method. Pigs are individually kept in metabolic cages and faeces and urine are collected quantitatively to measure N/P excretion. The retention of N/P can be determined from N/P intake and excretion (Berghaus, 2022). A less labour intensive, but also less accurate alternative is the estimation of faecal N/P excretion via digestibility using a non-digestible marker. In this method, individual faeces samples are collected over several days. Thus, animals are often also individually penned (Adeola, 2001).

However, recent research has focused on identifying which N/P traits may be most suitable for genetic improvement, particularly those that are easily phenotyped on a large scale. For example, blood urea nitrogen (BUN) has emerged as a potential marker for nitrogen utilization efficiency (Berghaus et al., 2023), based on an estimation formula proposed by Kohn et al. (2005) to approximate urinary N excretion using BUN, urea clearance rate, and body weight.

Several studies have already been conducted on the genetic parameters of different N/P-related traits. The literature reports h^2 estimates ranging from nearly zero (for P utilisation efficiency) to 0.56 (digestibility of N), consistently observing that h^2 values for P traits fall below those for N traits (Table 2). These estimates and the observed variability in traits serve as a basis for potential breeding activities (Sainitilan et al., 2013, Godhino et al., 2018, Berghaus, 2022).

Table 2: Heritability of N/P-related and feed efficiency traits

Trait	**h^2**	**Study**
total N excretion	0.31-0.40	Saintilan et al. (2013)
total P excretion	0.29-0.40	Saintilan et al. (2013)
N digestibility	0.27-0.56	Dèru et al. (2021)
N utilisation efficiency	0.54	Ewaoluwagbemiga et al. (2023)
	0.40	Kasper et al. (2020)
P utilisation efficiency	0.27	Ewaoluwagbemiga et al. (2023)
	0.003	Kasper et al. (2020)
Feed conversion ratio	0.30-0.40	Saintilan et al. (2013)
	0.39	Ewaoluwagbemiga et al. (2023)
	0.10	Kasper et al. (2020)
Residual feed intake	0.21-0.33	Saintilan et al. (2013)

The genetic component underlying N/P-related traits may be rooted in biological and physiological mechanisms: Variations in DNA can result in differences in transcription and translation products, ultimately influencing protein synthesis. It is conceivable that, due to their

genetic background, certain animals exhibit more efficient metabolic pathways than others do. In their review on feed efficiency, Gilbert et al. (2017) illustrated differences in basal metabolism, muscle energy metabolism, and nutritional AA requirements between efficient and less efficient pigs. Despite these clear differences, they emphasised that the precise underlying physiological mechanisms remain unclear, necessitating further research. However, distinctions between feed efficient and less feed efficient pigs have been evident in variations in gene expression, e.g. of genes associated with mitochondrial metabolism (Vincent et al., 2015) and those linked to immune functions (Grubbs et al., 2014). Furthermore, genome-wide association studies (GWAS) targeting feed efficiency-related traits have identified single nucleotide polymorphisms (SNPs) and quantitative trait loci (QTL) linked to these characteristics. The identified gene loci have been implicated in digestive and metabolic processes, fat synthesis and lipid transport, as well as olfactory transduction and the insulin pathway (e.g. Do et al., 2014, Fu et al., 2020, Li et al., 2022). However, to our knowledge, there is currently a lack of studies or data regarding GWAS for N/P-related traits across different breeds and under varying environmental conditions including feeding. Further investigations are therefore necessary to comprehensively understand the genetic basis of N/P-related traits and their interaction with environmental factors, particularly feeding practices.

2.3 Role of genotype by diet interactions

Several studies have investigated the potential effects of N/P restricted diets on pig performance. Ruiz-Ascacibar et al. (2017) aimed to restrict dietary CP to a minimum of 10.2%, while simultaneously reducing EAA in purebred Large White pigs. The results showed that approximately 30% of pigs showed no impairments under these extreme dietary conditions, which was attributed to an increased efficiency in resource utilisation. Subsequent research by Kasper et al. (2020) explored the genetic factors contributing to the variation in adaptability to the altered diets. They found that up to 40% of the variability could be explained by genetic factors. These findings indicate a potential re-ranking of genotypes under N/P restricted diets, highlighting the important role of G×D.

2.3.1 Definition of genotype by environment interactions and their importance in pig breeding

Genotype×environment interactions (G×E), the overarching term of G×D, exist in many forms. Haldane (1946) defined the occurrence of G×E as the influence of the production environment on the performance of animals within that environment. Different environments (Falconer, 1952) affect different genotypes unequally, potentially leading to a re-ranking based on their performance (Cameron, 1993). This relationship can be expressed by the following equation, breaking down the phenotype into its components (Tiezzi and Maltecca, 2022):

Phenotype = Genotype + Environment + Genotype×Environment

The component “Genotype” reflects the breeding value, representing the marginal deviation of an individual from the mean genetic value of the population, independent of environmental conditions. The “Environment” component is the deviation caused by the environment, independent of genotype. The “Genotype×Environment” component signifies the extent to which genotypes differ in their phenotype in response to the environment (Tiezzi and Maltecca, 2022).

Genotype by environment interactions can be classified in several ways. One way is based on the strength of differences between environments. If the environmental differences are large and controllable (e.g. the dietary nutrient content), it is called a macro-environmental component. If the differences are small and uncontrollable (e.g. social effects in a group), it is called a micro-environmental component (James, 2009, Tiezzi and Maltecca, 2022). Statistical models express the interaction of genotype and macro-environmental component through an

interaction term, while the interaction with the micro-environmental component is reflected in the random residual (Tiezzi and Maltecca, 2022). Furthermore, G×E can be distinguished into rank-type and scale-type interactions. In a scale-type interaction, a genotype selected as the best in environment 1 is also the best in environment 2, with a different degree of superiority over other genotypes. In a rank-type interaction, the magnitude of the differences may remain constant, but the sign changes: The best genotype in environment 1 is not the best in environment 2 (James, 2009).

In summary, the extent of G×E expression varies across environments, traits, and species. Figure 2 illustrates different states of G×E, ranging from its absence (a) to a weak (b) and stronger influence (c) on the phenotype, up to the strongest influence (d) resulting in a complete re-ranking of all genotypes. In the absence of G×E, the environment has no effect on the phenotypic expression of a genotype's trait. The greater the magnitude of G×E is, the stronger is the environmental influence on the phenotype.

Due to the potentially significant effect of G×E on the phenotype, its existence should be carefully considered in the selection of breeding animals to avoid potential confounding. To maintain the efficiency of a breeding programme, animal selection should take place in the environment in which the animals will later perform (Cameron, 1993). In the pig sector, where a typical breeding programme follows a pyramidal structure with nucleus, multiplication, and commercial levels, ignoring existing G×E could lead to challenges. Pigs are selected at the nucleus level under specific test conditions, while the breeding goal must be defined at the commercial level. Test and commercial environments may differ, e.g. in group size per pen, feeding practices, and medical care (Brascamp et al., 1985). Thus, in the presence of G×E, as shown in Figure 2 (d), it is possible that the best-selected pig (Genotype 1) at nucleus level (Environment 1) is not the best performing pig at commercial level (Environment 2).

While G×E is commonly perceived as a confounding factor in breeding programmes, there is an opportunity to turn it into positive asset. This potential lies not only in breeding lines that are well adapted to particular environmental conditions (Tiezzi and Maltecca, 2022) but also in identifying robust genotypes. Robust individuals are characterised by consistent, preferably high performance across diverse environments despite the presence of G×E (Li and Hermesch, 2016).

In this context, the term "environmental sensitivity" is often used, defined as a measure of the magnitude of change in performance and physiology that an organism exhibits in response to an environmental change (Li and Hermesch, 2016). According to Falconer (1952), the different

sensitivities of genotypes to environmental conditions are the primary drivers of G×E. In general, selection results in highly sensitive genotypes under favourable environmental conditions, whereas it results in less sensitive, more robust genotypes under unfavourable environmental conditions (Falconer, 1952).

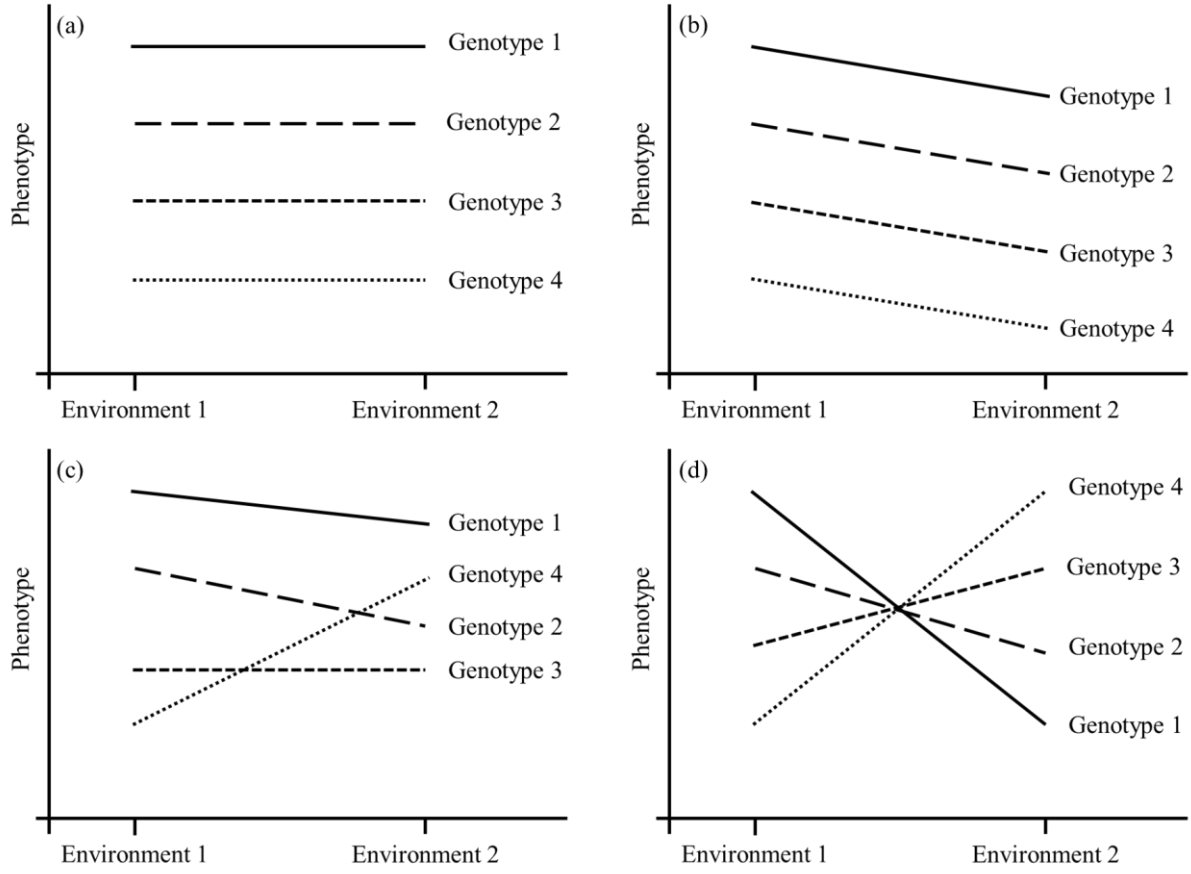

Figure 2: Comparison of the phenotype of five genotypes in two environments under the influence of varying extent of genotype by environment interactions (G×E) (modified from Lautrou et al., 2022)

Note: (a), non-existing G×E; (b), weak G×E; (c), stronger G×E; (d), strongest G×E.

In recent decades, there has been a notable emphasis on selection criteria that have led to the development of efficient pig breeds. However, these breeds are characterised by heightened sensitivity to environmental changes and by having an increased risk of behavioural problems (Rauw and Gomez-Raya, 2015). As a result, the importance of breeding for robustness is increasingly recognised, especially in the context of a sustainable development of pig production mentioned at the beginning of this thesis. The desired outcome is the identification of genotypes capable of achieving high overall performance while maintaining environmental stability. This includes ensuring superior production and resilience to the effects of environmental conditions such as changes in dietary protein sources (Powell et al., 2012).

Therefore, the consideration of G×E may play a crucial role in shaping future pig breeding and nutritional strategies.

This thesis primarily focuses on a specific type of G×E, namely G×D, where the altered environment is equated with the modification of the diet. This modification involves a restriction of dietary N/P.

2.3.2 Estimation of genotype by environment interactions

There are different approaches to assessing G×E. Two commonly used approaches are the interaction model and the reaction norm model (Brade, 2020). According to Brade (2020), the interaction model is an extension of the traditional genetic model by incorporating the interaction between genotype and environment. The model can be expressed as follows:

$$P_{ij} = \mu + g_i + e_j + (g \times e)_{ij}$$

where P_{ij} represents the phenotype of genotype i in environment j; μ is the mean of population; g_i represents the genotype i; e_j is the environment j; $(g \times e)_{ij}$ is the interaction of genotype i in environment j.

By conducting a corresponding analysis of variance, the influence each factor on a trait (P) can be determined, thereby assessing the extent of G×E (Brade, 2020). In addition, the genetic correlation (r_g) of a trait measured in two environments can be derived from the estimated variance components (Brascamp et al., 1985), which describes G×E on a mathematical level (Falconer, 1952). According to Falconer (1952), r_g can be calculated as follows:

$$r_g = \frac{cov_{XY}}{\sqrt{var_X \times var_Y}}$$

where r_g represents the genetic correlation; cov_{XY} represents the genetic covariance between the trait measured in environment 1 (X) and in environment 2 (Y); var_X is the genetic variance of the trait in environment 1 and var_Y is the genetic variance of the trait in environment 2.

This concept considers the trait measured in two different environments as two different traits. The physiological mechanisms underlying these two traits may be partly different, and consequently the genes involved may also be different. For example, the growth rate of a pig under a restricted feeding regime is mainly influenced by nutrient utilisation efficiency, whereas under an *ad libitum* feeding regime, it is more influenced by appetite (Falconer, 1952). As a result, the additive genetic variance influencing a trait can vary in two different environments,

leading to variations in h^2. The genetic correlation coefficient can range from -1.00 to 1.00. A r_g of 1.00 indicates no interaction (see Figure 2, (a)). The smaller the r_g, the stronger is the interaction. Robertson (1959) classified a G×E as biologically important when $r_g<0.80$.

A reaction norm model is a second possible approach to quantifying G×E. A reaction norm describes the phenotypes that a genotype can manifest in different environments (Woltereck, 1909). The idea is that the same genotypes can show different expressions in their phenotypes due to their alleles (Brade, 2020). Reaction norm models are primarily used to capture G×E when the distribution of the environment is continuous. They express phenotypes based on a function of environmental values, assuming that the coefficients are under genetic influence (Brade, 2020, de Jong, 1995). An example of a visual illustration of such reaction norms has already been presented in Figure 2. The intercept of a reaction norm represents the general production level, while the slope indicates the environmental sensitivity (de Jong, 1995). In the reaction norm model, the estimated breeding value depends on the environment in which the animals perform. It can be calculated using intercept and slope (Brade, 2020).

2.3.3 Genotype by environment interactions in growing-finishing pigs

Research in growing-finishing pigs has identified several instances of G×E, including various traits and environmental conditions. Studies have investigated G×E between purebred and crossbred pigs, and between *ad libitum* and restricted feeding regimes (Hermesch et al., 2015). While initial studies focused primarily on performance traits, the increasing importance of animal welfare, environmental sustainability, and resource conservation led to the inclusion of traits such as feed efficiency, nutrient excretion, and functional health traits. In addition, studies have addressed comparisons between modern and indigenous breeds (Brandt et al., 2010), the use of different feed ingredients (Godinho et al., 2018), and variations in dietary nutrient content (Déru et al., 2021).

Genotype by environment interactions have been identified for fattening performance, slaughter performance, and meat quality traits (Merks, 1986, Brandt et al., 2010, Li and Hermesch, 2012, Gourdine et al., 2019). As early as 1986, Merks highlighted the essential consideration of G×E in the pyramidal system of pig production, emphasising the importance of selecting the right breeding animals at the nucleus, multiplication and commercial levels. Although G×E may not have been consistently considered in every breeding programme at every level in the past, correct decisions have been made that led to breeding progress, as demonstrated by Brandt et al. (2010). They compared the performance of modern and indigenous breeds under

conventional and organic production conditions. Despite the common perception that indigenous breeds are often more robust, modern lines are superior in both production systems (Brandt et al, 2010).

However, this should not be a reason to ignore existing G×E. It remains crucial to use them positively to maintain and improve the efficiency of breeding programmes and to select for robust animals. This is particularly evident in research that focuses on feeding practices and diet design, addressing not only performance parameters but also feed and nutrient efficiency. For example, a G×E in the rate of gain was found when the feeding regime was changed from *ad libitum* to restricted feeding (Fowler and Ensminger, 1960). Examining the diet as environmental factor has provided evidence to support further research. Studies comparing diets based on soybean meal or grain revealed G×D in feed efficiency traits such as lipid deposition and residual energy intake (Knap and Wang, 2012). When investigating feed efficiency and growth performance under diets with high (maize-soybean meal) and low (wheat-barley and protein-rich coproducts) input, Godinho et al. (2018) observed similar results. While no G×D was found for protein deposition, ADFI, and FCR, it was evident for lipid deposition, residual energy intake, and RFI. These results highlight the importance of considering G×D in breeding for feed and nutrient utilisation efficiency, especially during changes in dietary protein sources. In addition, the increase of dietary fibre content, driven by animal welfare concerns, the use of local dietary protein sources, and an increased dietary grain content through N/P restriction, has shown the impact of G×D. For example, Déru et al. (2021) demonstrated the strong influence of dietary fibre concentration on N digestibility, proving a G×D.

In the context of N-restricted diets in pigs and related G×D, an initial study in Switzerland provided interesting insights (Ruiz-Ascacibar et al., 2017). As already mentioned regarding N/P-restricted diets and genetics, approx. 30% of individuals of the pigs, did not show any impairments in their performance under a strongly CP- and EAA-restricted feeding regime. These results led to the conclusion that these animals had a higher N utilisation efficiency than the average of the population. A subsequent study by Kasper et al. (2020) investigated whether genotypes differ in their ability to cope with N-restricted diets, i.e., the presence of G×D. Genetic factors explained about 32% of the variation in N utilisation efficiency, while environmental effects accounted for the remaining 68%. Although no pronounced trade-offs with other economically relevant traits were found except for fattening duration, which was higher under dietary CP restriction, potential negative effects on meat quality, behaviour, and health remained unclear.

The results by Ruiz-Ascacibar et al. (2017) provide an important basis for further research on G×D in pig production, particularly with the aim of investigating whether G×D affects N/P-related traits under dietary N/P restriction. To the best of our knowledge, there is currently a lack of studies specifically addressing P in this context. The interaction between genotype and diet, exemplifying the collaboration between animal breeding and nutrition, could be a crucial factor in breeding robust animals for a sustainable development of pig production.

2.4 Role of gut microbiome

Despite notable advancements and promising nutritional and genetic strategies to improve feed efficiency and to reduce nutrient excretion in recent years, the phenotypic characterisation of these traits on a large scale remains challenging, in terms of both cost and time. Current research suggests that, in addition to genetic and environmental factors, a microbial component influences the hosts' phenotypic variation in target traits, such as feed efficiency and nutrient excretion, in pig production (Camarinha-Silva et al., 2017, Aliakbari et al., 2022). This phenomenon, termed "microbiability" (m^2) (Difford et al., 2016), highlights the important role of the microbiome in shaping key aspects of pig physiology.

The pig's phenotype is influenced by its genotype and the environment. At the same time, phenotype, genotype and environment are in a complex relationship with a diverse community of microbes inhabiting the pig's gut, known as the microbiome. As represented in Figure 3, all factors in this network are influencing each other. The composition of the microbiome is shaped by both environmental factors and host genome, making it partly heritable (Benson et al., 2010).

Exploring the complex relationship between the host genome, phenotype, environment, and gut microbiome could identify potential drivers for the differential genetic potential in adaption to dietary N/P restriction. In addition, specific microbial species, primarily in faeces, could potentially serve as biomarkers for efficiency and excretion traits.

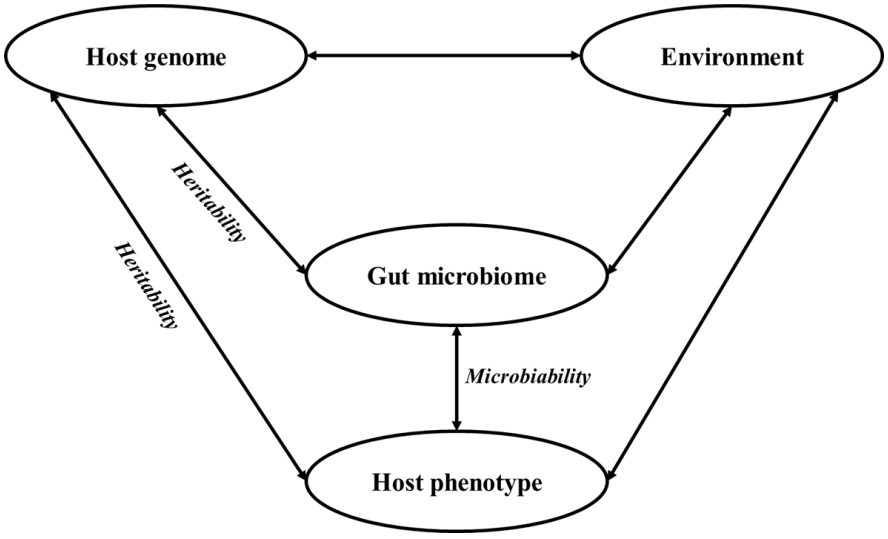

Figure 3: Complex relationship of host genome, host phenotype, gut microbiome and environment

2.4.1 Foundation and structure of microbiome analysis

Before providing an overview of the importance and functions of the gut and the gut microbiome in pigs, the following section will briefly introduce the foundation and structure of microbiome analysis.

To determine the gut microbiome composition, 16S rRNA gene sequencing is commonly used nowadays (Goodrich et al., 2014), which has its roots in studies by Woese (1987). The 16S rRNA gene encodes the RNA component of the 30S subunit of a prokaryotic ribosome and is known for its ubiquity in all microorganisms. The gene consists of approx. 1550 base pairs and contains eight highly conserved and nine hypervariable regions, termed V1 to V9. These regions allow the identification and taxonomic assignment of bacteria by comparing sequences with databases of known organisms (Weinroth et al., 2022). 16S sequencing data can be processed and quality checked using software such as DADA2 in QIIME2 or R-Studio (Boylen et al., 2019) to identify amplicon sequence variants (ASV), which are taxonomically classified with a database, e.g. SILVA database (Yarza et al., 2014). The classification is based on the following taxonomic ranks as presented in Figure 4:

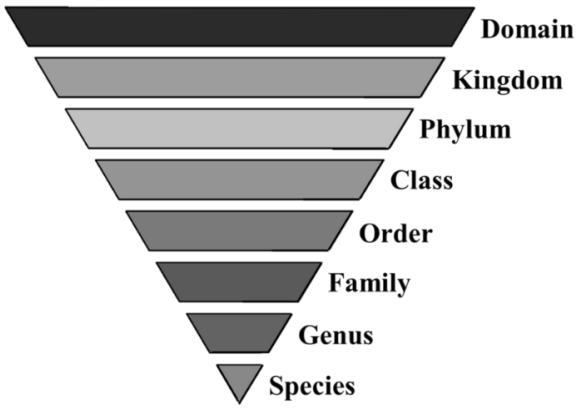

Figure 4: Major taxonomic ranks (Samal et al., 2019)

Amplicon sequence variants (ASVs), a relatively new alternative to traditional clustering of 16S sequences into operational taxonomic units (OTUs), represent unique biological sequencing variations and distinguish them from sequencing errors (Callahan et al., 2018). Further statistical analysis of microbiome data can be performed using additional software packages. One popular example is the phyloseq package in R-Studio, developed by McMurdie and Holmes (2013). This package allows for the analysis of information obtained from the DADA2 pipeline, including an ASV abundance table of the analysed samples, an ASV taxonomy table, a phylogenetic tree, and a corresponding metadata table with information about

the analysed samples. In addition to absolute and relative abundances of individual taxonomic ranks for groups of analysed samples, alpha and beta diversity metrics can be determined.

Alpha diversity is a measure of the diversity or richness of species within a single ecological community, e.g. within a gut sample (Beule, 2018). The quantification of the alpha diversity can be based on species richness (number of different species) and the distribution of species in a sample. There are various indices that combine these two metrics in different ways (Beule, 2018). Examples include "observed richness" (number of different species) and "Shannon entropy" (number of species and their distribution) (Shannon, 1948), which have been chosen in Chapter 4 to measure the alpha diversity.

In contrast to the alpha diversity, which describes species diversity within one environment (e.g. gut samples), the beta diversity is a measure of differences in species composition between two environments (Beule, 2018). Thus, the beta diversity can characterise differences in species composition between e.g. different gut sections, feeding groups, or sexes. Similar to the alpha diversity, the beta diversity can be measured using different indices that consider the occurrence, the abundance, and/or the phylogenetic relatedness of species between samples. In Chapter 4 of this thesis, the beta diversity has been determined using "Bray-Curtis" and "UniFrac" distance measures. While Bray-Curtis focuses on differences in the relative abundance of species between two samples (Bray and Curtis, 1957), UniFrac also incorporates the phylogenetic relatedness of species into the distance calculation (Lozupone and Knight, 2005).

2.4.2 Anatomy, physiology and functions of different gut sections

The intestine is divided into small and large intestine, which differ in function, anatomy, and physiology, as described by DeRouchey et al. (2009). The small intestine consists of duodenum, jejunum, and ileum (Figure 5). It serves as the primary site for nutrient digestion and absorption. In the duodenum, digestion, initiated in the stomach, continues with pancreatic enzymes, while sodium bicarbonate from the pancreas increases the pH of the chyme to prevent cell damage during its passage through the gut. Bile salts help in the digestion of fats and fat-soluble vitamins. Further breakdown and initial absorption of nutrients take place in the jejunum, the largest part of the small intestine, followed by further absorption in the ileum. The intestinal mucosa of the jejunum and ileum is characterised by folds and villi, which increase the surface for nutrient absorption (DeRouchey et al., 2009).

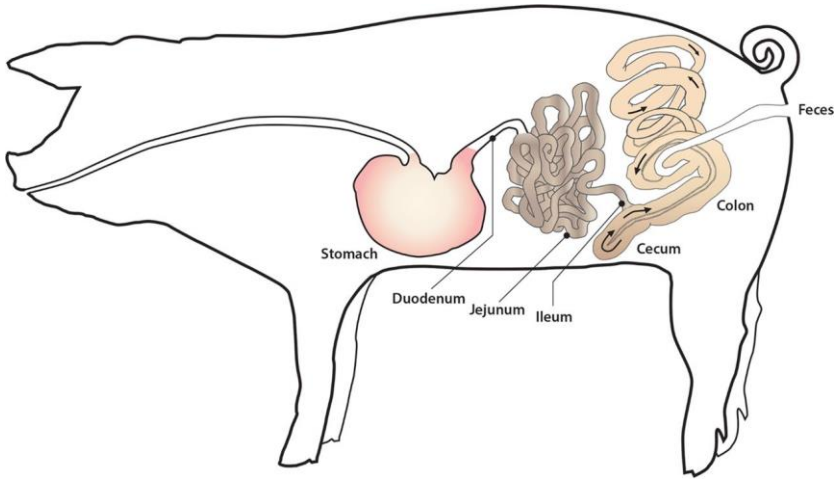

Figure 5: Major sections of the pig's gastrointestinal tract (Holman et al., 2017)

The large intestine, which includes caecum and colon (Figure 5), primarily absorbs water and macro minerals, facilitated by a high water exchange capacity of the epithelium. Although digestion by host enzymes does not occur in the large intestine, microbial enzyme activity degrades poly-and monomers and, eventually, produces short-chain fatty acids (SCFA), which can serve as an energy source for epithelial cells. Limited vitamin B synthesis also takes place (DeRouchey et al., 2009). Additionally, besides water absorption, the large intestine plays a crucial role in the immune system and the maintenance of pig health (Szabó et al., 2023).

In addition to the enumerated differences between the small and large intestine, it is also evident that the small intestine is characterised by higher oxygen levels (He et al., 1999), a faster transit time of chyme (Schwarz et al., 2002), and the presence of microbial peptides compared to the large intestine (Bevins and Salzman 2011).

2.4.3 Pig gut as habitat for microbes

The mammalian gastrointestinal tract is often referred to as the "forgotten organ". It harbours approximately 10^{14} microbes (O'Hara and Shanahan 2006). Mowat and Agace (2014) titled the intestine as an environment for microbes and their digestive and immune functions. Through various environmental factors and host genetics, a complex gut microbiome develops, primarily with beneficial functions for the host in nutrient digestion, maintenance of health, and immune defense and tolerance (Mowat and Agace, 2014). Disruption of this symbiosis through changes in the gut microbiome can lead to diseases such as diarrhoea, inflammation, and allergic reactions, resulting in reduced productivity, feed efficiency, increased mortality, and economic losses (Carding et al., 2015).

Composition and diversity of the gut microbiome in pigs

The composition of the pig gut microbiome has been the subject of numerous studies (e.g. Looft et al., 2014, Yang et al., 2016, Holman et al., 2017, Chen et al., 2021), including investigations of both the faecal and gut microbiomes. According to Chen et al. (2021), the gut microbiome comprises a diverse community of microorganisms that covers over 30 different phyla, more than 100 genera, and over 1,000 annotated species. The so-called core microbiome, defined as the microbial taxa present in >90% of gastrointestinal tract samples of pigs, consists of 19 phyla, 234 genera, and 254 species. However, caution should be exercised when using the term "core microbiome" in pigs, as demonstrated by Holman et al. (2017) in their meta-analysis: The factors "study" and sample origin (faecal sample or gut section sample) are the most important determinants of the microbiome composition. Nevertheless, certain bacterial taxa appear to form a core microbiome in pigs, independent of these determining factors (Holman et al., 2017). The predominant phyla in the gut are Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria, with Firmicutes being the largest proportion (Chen et al., 2021). Genera present in >90% of all samples in the above-mentioned meta-analysis include *Clostridium*, *Blautia*, *Lactobacillus*, *Prevotella*, *Ruminococcus*, *Roseburia*, the *RC9 gut group*, and *Subdokigranulum*, with *Prevotella* being the most abundant genus (Holman et al., 2017).

Significant variations in microbiome composition and diversity are observed across different segments of the gastrointestinal tract, which may be attributed to the anatomical, physiological, and functional differences between these gut sections, as described in Chapter 2.4.1. Alpha diversity is generally higher in the large than in the small intestine, presumably due to the slower passage rate of chyme (Schwarz et al., 2002), lower levels of antimicrobial factors (Bevins and Salzmann, 2011), and increased presence of unabsorbed fermentable substrates (Kelly et al., 2017). Beta diversity between samples from different gut sections reveals marked differences in microbiome composition between the small and large intestine. Certain species are predominantly or exclusively localised in one of the two gut sections. While the phylum Proteobacteria is predominant in the small intestine, Bacteroidetes is predominant in the large intestine. Correspondingly, there are genera classified within Proteobacteria that are found exclusively in the small intestine (e.g. *Escherichia-Shigella*), and conversely, there are genera exclusively present in the large intestine (e.g. *Prevotella*). In this example, different oxygen requirements of the species could explain the different occurrence (Kersters et al., 2006, Spence et al., 2006).

Factors influencing microbiome composition

The composition of the gut microbiome is influenced by several factors, which can be divided into environmental and genetic factors. The early life stages of pigs are of particular importance: Microbes enter the organism of piglets through the contact with the maternal birth canal during birth and through breast milk during lactation. During pregnancy, the placenta acts as an immunological interface and enables the exchange of nutrients between mother and foetus. For a long time, it has been assumed that maternal microbes cannot traverse the placental barrier, so the foetus develops in a sterile environment, except for potential infection (Kai-Larsen et al., 2014). However, recent evidence from next-generation sequencing has shown that microbes can also be detected in a normal, healthy placenta, amniotic fluid, and umbilical cord blood, although the underlying mechanisms remain unclear. Primarily probiotic genera, such as *Lactobacillus*, *Bifidobacterium*, and *Streptococcus*, have been found in meconium (the first stool of a newborn) (Oktaviyani et al., 2021).

The gastrointestinal tract of the piglet is significantly microbially colonised after birth, facilitated primarily by exposure to maternal sources such as the birth canal, maternal faeces, udder skin, and breast milk (Liu et al., 2019). Of these, breast milk emerges as a key contributor. It mirrors the microbial composition found in the small intestine, particularly noteworthy during the initial 35 days of life. In these days, it significantly influences the mucosa-associated microbial populations in the small intestine, whereas breast milk plays a minor role in the colonisation of the large intestine. In this regard, the maternal faeces are of paramount importance (Liu et al., 2019). After weaning, environmental factors become most important in shaping the gut microbiome composition, with dietary changes playing a crucial role. During the transition to solid feed, the gut microbiome undergoes a shift towards a more stable composition (Frese et al., 2015, Saladrigas-García et al., 2022).

Sex also has an influence on the gut microbiome. Observed differences between uncastrated boars, castrates and gilts may be attributed to hormonal disparities, particularly in androgens (testosterone). Studies have shown that the faecal microbiome of uncastrated boars is enriched in species associated with carbohydrate metabolism (e.g. *Roseburia, Bulleidia, Escherichia*), whereas that of gilts and castrated boars is dominated by species associated with obesity and energy harvest (e.g. *Treponema, Bacteroides*) (He et al., 2019, Albuquerque et al., 2023).

Throughout the life of a pig, a dynamic equilibrium characterises the gut microbiome. The microbial abundance fluctuates both within and been individuals. The equilibrium is influenced by several factors, including age-related physiological changes, housing conditions, feed

composition and quality, health status, and interventions such as the use of antibiotics or probiotics (Upadhaya and Kim, 2022, Huang and Chen, 2023).

Regarding nutrition, there is an increasing focus on dietary fibre and its effect on the gut microbiome in the large intestine. This has received considerable attention in numerous research studies, highlighting its importance in understanding gut health (e.g. Yang and Zhao, 2021, Niu et al., 2022, Albuquerque et al., 2023). High-fibre diets contribute to the enrichment of complex carbohydrate-fermenting microbial genera, such as *Ruminococcus* and *Sphaerochaeta* (Yang et al., 2021, Albuquerque et al., 2023). In addition, Niu et al. (2022) have identified further differentiation of species associated with the digestibility of different fibre components, namely acid detergent fibre (e.g. *Ruminococcaceae_UCG_002*) and neutral detergent fibre (e.g. *Ruminococcaceae_UCG_005*). The microbial composition changes depending on the type and amount of substrate available for microbial fermentation. Overall, dietary fibre appears to play a crucial role in maintaining microbial diversity, which can serve as an indicator of the host's health status (Upadhaya and Kim, 2022).

In addition, feed additives such as pre- and probiotics can be used to target the composition of the gut microbiome, as highlighted by Upadhaya and Kim (2022) in their review. Prebiotics, particularly indigestible fibre that can only be degraded by microbial enzymes in the large intestine, may serve as a substrate for beneficial microbes in the gut, indirectly promote the maintenance and improvement of gut health by enhancing microbial activity and growth. Probiotics represent directly administered, living organisms that may also have a positive effect on gut health. They are used, e.g. as an alternative to banned antibiotic feed additives against post-weaning diarrhoea (Upadhaya and Kim, 2022).

Host genotype and heritability of gut microbiome

In addition to the several environmental factors that influence the composition of the gut microbiome, another crucial factor is the host genotype (e.g. Benson et al., 2010, Camarinha-Silva et al., 2017), highlighting the complex relationship between the microbiome and the host genome (Figure 3).

The influence of the host genotype on microbiome composition has been shown in studies comparing different pig breeds, such as Landrace and Large Black, as well as Yorkshire and Tibetian pigs. Notably, the microbiome of Landrace and Tibetian pigs showed a higher Firmicutes/Bacteroidetes ratio compared to that of other breeds (Upadhaya and Kim, 2022). Furthermore, the influence of host genotype has been shown not only in comparisons between

breeds but also within a breed. Regarding this, certain porcine microbes have been identified (Camarinha-Silva et al., 2017, Crespo-Piazuelo et al., 2019). While the underlying mechanism remains largely unknown, it has been suggested that host genetics indirectly influence microbiome composition through differences in the secretion of immunoglobulins and antibacterial molecules in the gut. These differences may be due to individual differences in mucosal gut structure (Camarinha-Silva et al., 2017). Additionally, the impact of host genetics on intestinal motility, the modification of intestinal epithelial cells, and the internal environment of the host (biochemical and physical factors, e.g. pH, permeability, and metabolite concentration) may also play an indirect role (Davenport et al., 2017).

Some studies have investigated the h^2 of microbiome composition. Camarinha-Silva et al. (2017) examined the h^2 in colon samples from 207 Piétrain sows and reported values ranging from 0.32 to 0.57 for eight of 49 investigated genera, including *Alloprevotella, Blautia, Catenibacterium, Lactobacillus,* Uncultured *Spirochaetales,* Uncultured *Spirochaetes,* Uncultured *Succinivibrionaceae*, and Uncultured *Veillonellaceae*. In addition, a study of 256 caecum and 244 faecal samples from gilts and castrated boars of two Chinese pig breeds revealed that sample origin also influenced h^2 values. Heritability estimates >0.15 were found for 81 microbial taxa in faecal samples, and 67 taxa in caecum samples. In 31 cases, the taxa from the two sample origins overlapped (Chen et al., 2018). Besides sample origin, the host age also affects h^2 of microbial taxa. Studies by Lu et al. (2018) and Bergamaschi et al. (2020b) analysed faecal samples from approx. 1,000 crossbred pigs at three time points of life: one day, 15 weeks, and 22 weeks post-weaning. They demonstrated an increase in h^2 values with increasing age. This indicates that the microbiome composition becomes less dependent on the environment with increasing age (Bergamaschi et al., 2020b).

To further investigate the genetic background, a GWAS of the gut microbiome composition can be performed, considering it as a complex quantitative trait of the host (Benson et al., 2010). The primary objective of GWAS for microbiome composition is to derive new hypotheses about the genetic mechanisms underlying the h^2 of microbiome composition (Camarinha-Silva et al., 2017). Some studies have already explored GWAS of the gut microbiome. For example, Chen et al. (2018) discovered 40 significant associations between SNPs and the abundance of microbial taxa in caecum samples. They particularly highlighted the families Christensenellaceae and Peptococcaceae in these samples. Additionally, they found 34 significant associations in faecal samples, emphasising the order Acholeplasmatales and the species *Prevotella copri*. Host candidate genes were functionally associated with host metabolism, immune functions and response, and signal transduction (Chen et al., 2018).

Crespo-Piazuelo et al. (2019) identified, in their GWAS involving 288 Duroc×Iberian pigs, 52 SNPs across 17 regions of the porcine genome associated with six genera of microbial taxa (*Akkermansia*, *CF231*, *Phascolarctobacterium*, *Prevotella*, *SMB53*, *Streptococcus*). The corresponding host candidate genes are associated with functions related to immune barrier and the metabolism of mucopolysaccharides and bile acids. Similarly, in a study of approx. 1,000 crossbred pigs, Bergamaschi et al. (2020b) identified several SNPs that were significantly associated with microbial taxa. These SNPs were located in genomic regions including 68 genes associated with growth and fatness.

In summary, it is evident that the host genotype has become an increasingly important factor in microbiome research in recent years. The genotype plays a crucial role in shaping the composition of the gut microbiome. However, the underlying mechanisms remain largely unexplored. Further investigation offers the opportunity to develop a deeper understanding of how host genotype influences microbiome composition, thereby uncovering another field of the complex relationship between the host and its microbiome (Figure 3).

2.4.4 Functions and contributions of the gut microbiome in pigs

As repeatedly mentioned, the relationship between host and gut microbiome is complex. There exists a continuous interchange between the host's gastrointestinal tract and the microbiome (Wolowczuk et al., 2008). Interactions of host and microbiome can be commensal, pathogenic, or mutualistic. In this context, commensal denotes that the host benefits from the microbes, while the microbes remain unaffected. In pathogenic interactions, the microbes harm the host, whereas in mutualistic interactions, both the microbiome and the host benefit from each other (Crespo-Piazuelo et al., 2019). Commensal and mutualistic gut microbes provide benefical functions to the host that the host cannot perform alone. Examples for such functions are the digestion of luminal components, synthesis of useful host nutrients, stimulation of the immune system, and prevention of colonization by pathogens (Wolowczuk et al., 2008).

Among the luminal components that resist host digestion is dietary fibre, specifically non-starch polysaccharides (NSP). As non-ruminants, pigs lack the endogenous enzymes necessary for the digestion of NSP. So, NSP enter the large intestine undigested, where they serve as substrates for microbial degradation and anaerobic fermentation. As a result, dietary fibre, influences the composition of the gut microbiome, as mentioned previously (Upadhaya and Kim, 2022). The predominant products of bacterial fermentation are SCFA such as acetate, propionate, and butyrate. They are absorbed and can serve as an energy source for colonocytes or be used for

lipogenesis or gluconeogenesis (Zhang et al., 2018). Additionally, SCFA can regulate and lower intestinal pH, thereby suppressing the growth of pathogenic microbes (Annison et al., 2003, Duncan et al., 2009). Short chain fatty acids are also involved in key regulatory mechanisms of the immune response and maintenance of health, including lymphocyte development and tissue barrier function (Kim, 2023). Besides SCFA, gut microbes synthesise B vitamins (e.g. thiamine, riboflavin, niacin, vitamin B6, B12, folates) and vitamin K, which are important not only for microbial metabolism but also for host metabolism (Hill, 1997, Maynard and Weinkove, 2020). Furthermore, gut microbes regulate endogenously produced bile acids, thereby intervening in immune homeostasis (Dossa et al., 2016). Finally, gut microbes are involved in the synthesis of AA: In the colon, unabsorbed ammonium can be used to synthesise microbial proteins, which are then excreted via faeces rather than re-absorbed as ammonium or ammonia, used for ures synthesis and finally excreted via urine. This may have environmental benefits by reducing ammonia emissions (Morgan and Whittemore, 1998, Schmitz et al. 2024).

The aforementioned microbial metabolites, including SCFA, vitamins B and K, and microbiota-regulated bile acids, play a crucial role in the host's immune system, particularly at the epigenetic level. Epigenetics includes changes in gene expression or activity that are not due to changes in DNA sequence but are induced by environmental stimuli and can be inherited (Waddington, 1956). Microbial metabolites can act as such environmental stimuli and induce DNA methylation or histone modification (Oster et al., 2016, Pan et al., 2018).

Methylation of DNA means the addition of methyl groups to specific positions of DNA, resulting in changes in gene expression. Microbial folate, vitamin B2, B6, and B12 are involved in carbohydrate metabolism and synthesise the methyl donor S-adenosylmethionine that indirectly participates in DNA methylation (Oster et al., 2016). Moreover, methylation can be induced by butyrate and acetate (Pan et al., 2018). Histone modification involves the transfer of an acetyl group from acetyl-CoA to a lysine residue catalysed by histone acetyltransferases. Simultaneously, CoA is produced (Paul et al., 2015). There is a dynamic balance between histone acetylation and deacetylation, which is regulated by the enzymes histone acetyltransferases and histone deacetyltransferases (Peserico and Simone, 2011). In addition, microbial SCFA play a crucial role: Butyrate and propionate have a role in the inhibition of histone deacetyltransferases (Mowat and Agace, 2014).

The epigenetic effects induced by microbial metabolites influence the regulation of the immune system by modulating the gene expression in immune cells. They contribute to the regulation of host immune functions and thus play an important role in maintaining immune homeostasis (Oster et al., 2016).

2.4.5 Microbiability and microbiome-wide association studies

Given the complex relationship between the pig and its gut microbiome, the microbiome may account for some of the phenotypic variation in target traits in pig production, alongside genotype and environment (Camarinha-Silva et al., 2017). This hypothesis is supported by studies by McCormack et al. (2017) and Metzler-Zebeli et al. (2018), which showed a correlation between feed efficiency and microbiome composition. From the perspective of quantitative genetics, the effect of the microbiome can be described by estimating the so-called "microbiability" (m^2). Difford et al. (2016), who first defined this concept, described the m^2 of a trait as the proportion of phenotypic variance explained by variance in the gut microbiome, akin to the concept of "heritability" for genetic effects (Bergamaschi et al., 2020b).

The effect of m^2 on target traits in pig production and thus, the microbiome effect of an animal can be quantified using mixed linear models that include a random microbial effect (Camarinha-Silva et al., 2017, Aliakbari et al., 2022). Accurate estimation of these effects requires a large number of animals with information on genotype, phenotype, and microbial composition.

A limited number of studies have addressed the estimation of m^2 (e.g. Camarinha-Silva et al., 2017, Verschuren et al., 2020, Khanal et al., 2021, Aliakbari et al., 2022). Verschuren et al. (2020) investigated the influence of the faecal microbiome of 160 three-way crossbred pigs on nutrient digestibility. They estimated m^2 values ranging from 0.37 to 0.93 for the digestibility of dry matter, organic matter, crude protein, crude fibre, NSP, and crude fat. Although they noted relatively low prediction accuracies, they described the values as promising for ranking animals in a genetic selection context. In a study by Khanal et al. (2021), m^2 values ranging from zero to 0.29 were observed for carcass composition and meat quality traits in approx. 1,100 three-way crossbred pigs at three growth stages. The strength of the microbiome effect increased for most traits from weaning to slaughter, with m^2 values for carcass composition exceeding those for meat quality traits (Khanal et al., 2021). Camarinha-Silva et al. (2017) and Aliakbari et al. (2022) investigated the impact of the microbiome on various growth performance and feed efficiency traits in 207 Piétrain sows and 604 pigs from two experimental French Large White pig lines, respectively. While the microbiome's influence in the first study was similar for all investigated traits (0.16 to 0.28, Camarinha-Silva et al., 2017), the second study found higher m^2 values for feed efficiency traits (0.11 to 0.20) compared to those for fattening performance (0.03 to 0.04, Aliakbari et al., 2022).

Similar to the genetic background and the impact of h^2 discussed above, the microbial background can be further investigated by conducting a microbiome-wide association analysis

(MWAS), which is comparable to GWAS in genetics. The aim of MWAS is to detect microbial taxa that may be significantly associated with target traits in pig production (Difford et al., 2018). Several research groups, e.g. Camarinha-Silva et al. (2017), Bergamaschi et al. (2020b), and Aliakbari et al. (2022), have performed a MWAS of relevant pig production traits. Camarinha-Silva et al. (2017) identified six significant associations between OTUs and key production traits, such as daily gain, daily feed intake, or feed conversion ratio. These OTUs were predominantly assigned to the orders Veillonellaceae and Prevotellaceae, the classes Bacteroidales and Clostridiales, or to the phylum Proteobacteria. The effect estimates of the individual taxa ranged were in low ranges (-0.009 to 0.008). The study by Bergamashi et al. (2020b) cited above showed associations between 245 OTUs and daily gain, 115 OTUs and backfat thickness, and 26 OTUs and loin depth. Notably, an almost equal number of positive and negative associations were observed between taxa and their corresponding traits. In addition, taxa were identified that were associated with both daily gain and backfat thickness. Aliakbari et al. (2022) reported eight significant or suggestive significant associations of OTUs with residual feed intake, feed conversion ratio, daily feed intake, or backfat thickness. The majority of these significant OTUs were classified in the order Clostridiales, followed by the Bacteroidales and Lactobacillales. The estimated regression coefficients for these associations ranged from -0.09 to 4.46.

Based on the findings on microbiability and MWAS for target traits in pig production, it can be hypothesised that microbiome information may help predict the expression of such traits and thereby improve selection in animal breeding (Aliakbari et al., 2022). From a breeding perspective, although the available research is currently limited, promising approaches have been identified to enhance the ranking of breeding animals (Verschuren et al., 2020).

2.5 Consequences for the improvement of N/P-related traits at the genetic level

To effectively improve N/P-related traits at the genetic level, such as N/P excretion, it is necessary to investigate the genetic foundation in detail. This includes not only studying h^2 and G×D but also the underlying mechanisms. To achieve this, this thesis was part of a project entitled “Nitrogen-reduced nutrition of growing pigs: a contribution to conserving resources and improving robustness”, which aimed to evaluate the effects of dietary N/P restriction on nutrient excretion, animal performance, health, and welfare, and to explore potential G×D and the role of the pig’s gut microbiome. The results of the project should serve as basis for possible follow-up projects with much larger sample sizes to provide directions for future research. The interdisciplinary approach of nutrition, genetics and the microbiome represents both, a challenge and perhaps the greatest opportunity. Possible gaps that need to be addressed before breeding for N/P-related traits can be put into practice are considered in the general discussion of this thesis.

Chapter 3. Evaluation of the effect of nitrogen- and phosphorus restricted diets on the performance of commercial growing-finishing pigs and the importance of genotype×diet interactions

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3.1 Abstract

Reduction of nitrogen (N) and phosphorus (P) excretion is crucial for sustainable pig production, achievable through animal nutrition and genetics. This study examined the performance and N/P excretion of 103 Pietrain×Landrace pigs from 20 artificial insemination boars under farm conditions. The goal was to assess the impact of dietary N/P reduction on performance, feed efficiency, N/P traits, and genotype×diet-interactions (G×D) during a 3-phase fattening. Due to practical conditions, N/P restriction varied and was inconsistently implemented. Despite this variability, pig performance was not negatively affected. Dietary N/P reduction positively influenced the fatty acid profile in meat and significantly affected N/P intake, digestibility, and calculated excretion. Heritability (h^2) for these traits ranged from low to moderate (0.00-0.39), with estimated urinary N excretion showing the highest values (0.57-0.77). Indications of G×D were evident through AI-boar×group interactions and correlation coefficients of progeny differences between feeding groups (0.30-0.80), particularly concerning N/P intake and faecal excretion. These findings highlight the influence of genetics and G×D on N/P traits, suggesting their potential inclusion in pig breeding programmes. Incorporating N/P-related traits into breeding programmes presents a promising approach to improving sustainability in pig production, addressing both environmental and economic aspects effectively.

Key words: fattening pigs, dietary protein, N and P emissions, genotype nutrition interaction, environmental protection

3.2 Introduction

About 95% of ammonia emissions in Germany derive from animal husbandry and the associated storage and spreading of manure (Taube et al., 2020, Federal Environment Agency, 2023). In this context, 90% of the release of nitrate into groundwater can be traced back to agriculture (Bach et al., 2020) resulting in eutrophication of soil and water. In addition to nitrate, the release of phosphate also plays a crucial role, representing the greatest cause of pollution in many German regions (Taube et al., 2020) and being on the EU-list for critical raw materials (European Commission, 2020). Due to this, relevance of an eco-friendly, cost-efficient and resource-saving production of pork increased over the last years. Consequently, breeding objectives as well as feeding strategies are reconsidered by many pig breeding organizations and livestock feed industries.

To avoid an oversupply of animals with N and P (N/P) and thus to reduce N/P excretion, diets restricted in N/P have been established in the recent years (Pomar et al., 2021). In this context, the German Agricultural Society (DLG) developed a leaflet for low N/P feeding strategies for growing-finishing pigs (DLG, 2019). The basis of such feeding strategies is a high bioavailability of N/P in feed components, as well as a high N/P utilisation efficiency of the individual pig. Otherwise, the restriction may have negative effects on performance, health, and welfare (Ruiz-Ascacibar et al., 2017).

In order to verify the consequences of an N-restricted feeding situation, Ruiz-Ascacibar et al. (2017) fed a diet containing 10.2% CP with additional reduction of essential amino acids (EAA) to purebred Swiss Large White pigs. In this study about 30% of animals did not show any impairment under these strongly N reduced diets, which was explained by a higher potential of the animals to use the resources more efficiently (Ruiz-Ascacibar et al., 2017), with genetic factors explaining up to 40% of the variation in adaptability to the changed diet (Kasper et al., 2020). Based on such results, a re-ranking of genotypes under diets low in N/P can be expected, so that there is a high probability of relevant genotype by environmental interactions (G×E).

The impact of G×E in pig production has been reviewed in the context of robustness by Rauw and Gomez-Raya (2015) and Rauw et al. (2020): Robustness is defined as an animal's ability to maximize its performance under varying and suboptimal environmental conditions while maintaining good health. In light of climate change and the use of alternative protein sources in animal feed, the consideration of a G×E could help to select environmentally robust animals. A limited number of studies investigated genotype by feed interactions (G×D) in pigs. Brandt et al. (2010) observed that G×D affect the performance in a comparison of modern and traditional

pig breeds in conventional and organic production systems and fed a diet with a less-balanced protein to energy ratio. Godinho et al. (2018) described changes in the ranking of genotypes for the traits lipid deposition, and residual energy and feed intake.

The objective of this study was on the one hand to assess the effects of N/P-restricted diets on individual faecal N/P excretion and urinary N excretion including phenotyping of commercial crossbred growing-finishing pigs and on the other hand to investigate the existence of G×D by taking paternal origin into account.

3.3 Material and methods

Animals, experimental design and diet composition

The experiment was conducted with 103 German Piétrain × Landrace crossbred pigs, 45 entire males and 58 females at Campus Frankenforst, University of Bonn (Germany) (50°42'51.9"N, 7°12'25.1"E). The animals originated from 20 litters, whose sire were 20 different artificial insemination (AI) boars (Figure 6), covering the current genetic performance potential of the breed Piétrain in Germany. Landrace sows belong to the Frankenforst herd. During the entire experiment, the animals showed no symptoms of illness or other abnormalities.

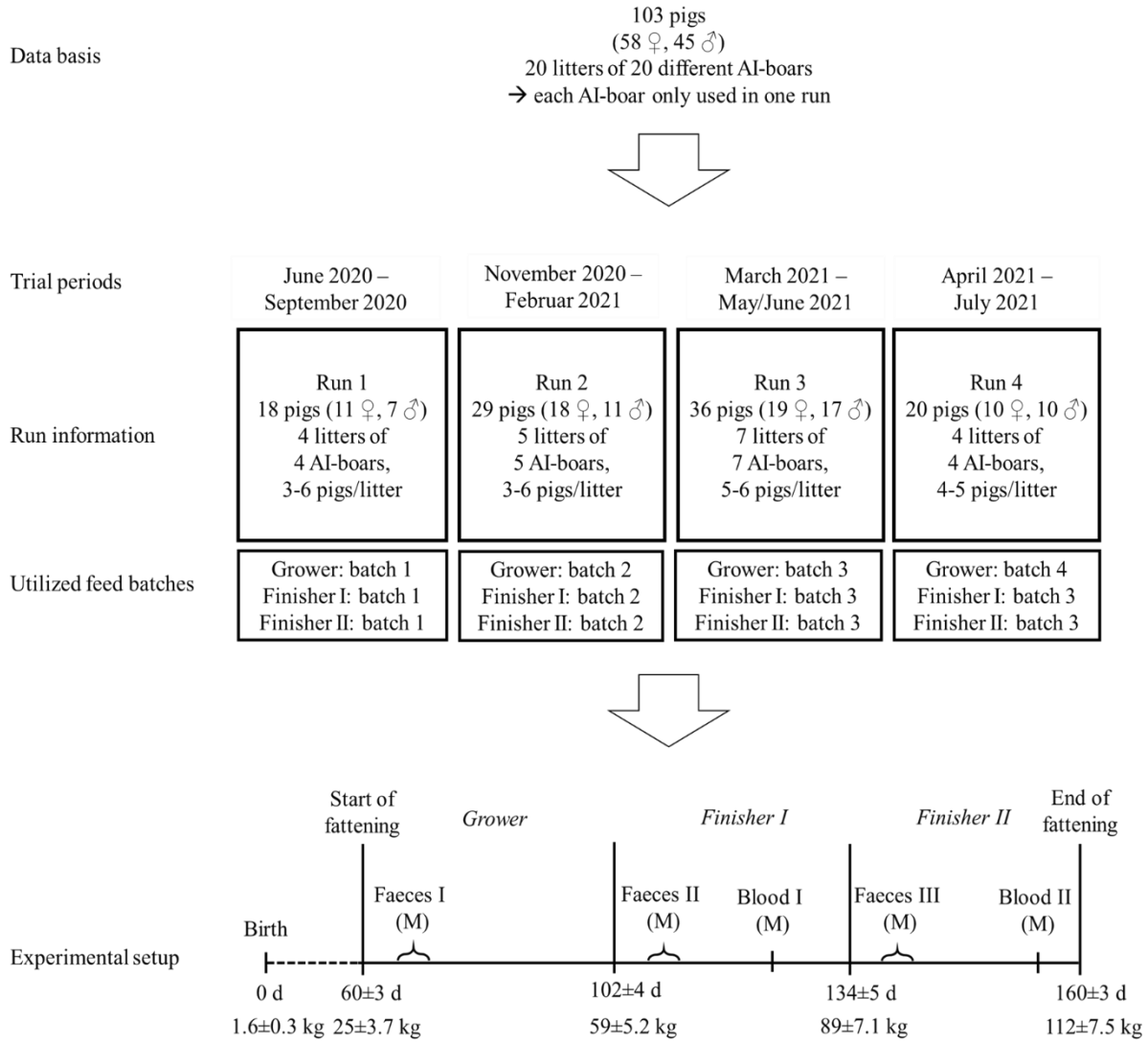

Figure 6: Data basis, trial periods, run information, number of utilized feed batch per run, and experimental setup and body weights (kg±SD) and age (d±SD) of pigs.
Note: Blood I, II, timepoint of measurement (M) for blood sampling, Faeces I, II, III, timepoint of measurement (M) for faeces sampling.

The whole experiment was conducted between June 2020 and July 2021 in four consecutive runs (Figure 6). From weaning to start of fattening (approx. 30 kg body weight (BW)), all pigs received the same diet according to the recommendations for growing pigs in Germany (GfE, 2008). After this, they were randomly assigned to control group (CON) or group with restricted dietary N/P, called low protein group (LP), considering a balanced allocation of gender and paternal origin. All pigs were individually housed (pen size 1.00×2.50 m²) which allowed a comprehensive individual phenotyping including individual registration of feed consumption and collecting individual faecal spot samples. In each run, pigs were fed for ad libtium consumption up to a final BW of approx. 115 kg. The 3-phase fattening period was categorised into grower (30 to 60 kg BW), finisher I (60 to 90 kg BW) and finisher II (90 to 115 kg BW). Pigs were fed according to the guidelines outlined in leaflet 418 of the DLG (2019). As per this leaflet, diets of CON were assigned to a "strongly N/P-reduced feeding programme", diets of LP to an "extremely N/P-reduced feeding programme".

Diets were based on wheat, barley, and triticale grains and the protein sources rapeseed meal and sunflower meal in various proportions and were each phytase supplemented (Table 3).

Table 3: Average content of main ingredients of the control and N/P-restricted grower, finisher I and finisher II diets

	Grower diet		**Finisher diet I**		**Finisher diet II**	
	CON	**LP**	**CON**	**LP**	**CON**	**LP**
Wheat (%)	40.4	44.1	39.9	43.3	40.1	38.8
Barley (%)	20.0	22.3	21.8	21.7	21.7	25.0
Triticale (%)	9.3	9.5	14.7	14.7	16.0	16.7
Rapeseed meal (%)	10.7	6.6	11.2	4.6	9.4	5.7
Sunflower meal (%)	4.1	4.0	3.7	3.7	3.0	3.0
Vegetable fatty acids (%)	3.0	2.1	1.1	0.7	0.8	0.7

Note: average content is the average value of main ingredient content, calculated from values of three or four different batches. Further ingredients: maize, wheat semolina bran, potato protein, calcium carbonate, sodium chloride, monocalcium phosphate, palm oil, 500 FYT 6-phytase/kg diet (DSM Nutritional Products, Grenzach) (Declaration, Deutsche Tiernahrung Cremer).

Abbreviations: N/P, N and P; CON, control diets; LP, N and P-restricted diets; CP; crude protein.

To ensure a high feed quality during the entire duration of the experiment diets were produced in 20 batches delivered by a commercial feed company (Deutsche Tiernahrung Cremer, Düsseldorf, Germany) on demand (Figure 6). The diets were pelleted, so a segregation of components was rather unlikely. The ratio of EAA as well as the energy content did not differ between C and LP diets. The formulation of the EAA was adjusted in relation to precaecal digestible lysine (% of precaecal digestible lysine) as follows in all periods: methionine = 30%, methionine + cysteine = 61%, and tryptophan = 18%, respectively, and separated according to fattening period: threonine = 65% (grower), 60% (finisher I) and 61% (finisher II), respectively.

Recording of growth performance, carcass composition and meat quality

During fattening, feed consumption was recorded for every individual pig over the grower, finisher I and finisher II period by weighing the feed before manual feeding. Pigs were weighed fortnightly. Start of fattening and the change between fattening periods, along with corresponding diets, were determined by the mean body weight of all pigs in a run, occurring at approx. 30 kg, 60 kg, and 90 kg BW, as shown in Figure 6.

Calculation of average daily feed consumption (ADFC), average daily gain (ADG) and feed conversion ratio (FCR) was conducted according to the guideline for station testing for fattening performance, carcass value and meat quality in pigs (BRS, 2019) as follows:

$$\text{ADFC (kg/d)} = \frac{\text{total consumed feed (kg)}}{\text{days of fattening (d)}}$$

$$\text{ADG (g/d)} = \frac{\text{total gain (g)}}{\text{days of fattening (d)}}$$

$$\text{FCR (kg/kg)} = \frac{\text{daily consumed feed (kg)}}{\text{daily gain (kg)}}$$

Additionally, residual feed intake (RFI) was estimated according to Saintilan et al. (2013).

$$\text{RFI (kg/d)} = \text{ADFC (kg/d)} - \text{ADFI (kg/d)}$$

where ADFI represents the estimated average daily feed intake; ADFC represents the registered ADFC.

ADFI was estimated by a multiple linear regression of ADFC on ADG to account for growth, and on back fat thickness (BFT), lean meat content (LMC), and dressing percentage (DP) to account for composition of body weight gain, and on average metabolic body size to account for maintenance requirements (according to Saintilan et al., 2013). Using the formula of Noblet

et al. (1999), average metabolic body size was calculated from body weight at the start (body weight1) and end of fattening (body weight2):

$$\text{Average metabolic body size } \left(\text{kg}^{0.60}\right) = \frac{\text{body weight (kg)}_2^{1.6}\text{-body weight (kg)}_1^{1.6}}{1.6 \cdot (\text{body weight (kg)}_2\text{- body weight (kg)}_1)}$$

All pigs in each run were slaughtered at reaching a mean body weight of 112 (± 7.5) kg within one (run 1, 2, 4) or two (run 3) days. Mean fattening duration was 99 (± 3) days. Slaughtering took place at Landesanstalt für Schweinezucht Boxberg (Baden-Württemberg, Germany). Pigs were stunned in pairs for 140 s with CO2 (90%), weighed and then exsanguinated and weighed again. Carcasses were de-bristled, scalded and eviscerated. Carcasses were longitudinally split into two halves and chilled to 1-3°C. Determination of carcass value and meat quality was carried out in accordance with the BRS (2019) guidelines using Fat-O-Meter II classification in Boxberg. For each carcass the following parameters were recorded: slaughter weight (SW, kg), dressing percentage (%), lean meat content (%) and BFT at the 13/14th rib (cm), pH in loin 1 hour after slaughter (pH1), pH in loin 24 hours after slaughter (pH²4), meat color (color), drip loss (%), intramuscular fat content (IMF, %), polyunsaturated fatty acids (PUFA, % of total fatty acids), monounsaturated fatty acids (MUFA, % of total fatty acids), and saturated fatty acids (SFA, % of total fatty acids).

Sample collection

Samples of each delivered diet batch were collected during the four runs of the experiment and the chemical composition was analysed at the end of the experiment. To determine the faecal N/P excretion (NEXf, PEX), within each fattening period faecal spot samples of each pig were collected twice daily for 5 days after a 7-day feed adaptation period (Figure 6). Samples were stored at -20°C until chemical analyses.

The NEXf and PEX was calculated for each pig and fattening period from N/P intake (NIN, PIN) and N/P digestibility (ADN, ADP), which was determined using acid insoluble ash as inert marker, which has been widely investigated and is known as well applicable (McCarthy et al., 1974). Digestibility of N and P was calculated according to Adeola (2001):

$$AD\ (\%) = 100 - \left[100 \cdot \left(\frac{marker_{feed}\ (g/kg\ DM) \cdot component_{faeces}\ (g/kg\ DM)}{marker_{faeces}\ (g/kg\ DM) \cdot component_{feed}\ (g/kg\ DM)}\right)\right]$$

where AD represents the apparent digestibility; $marker_{feed}$ and $marker_{faeces}$ are the marker concentrations in feed and faeces, respectively; $component_{feed}$ and $component_{faeces}$ are the concentrations of components in feed and faeces, respectively.

Since samples of urine could not be collected due to the experimental design, urinary N excretion (NEXu) was estimated using blood urea content. For this purpose, blood was taken from the vena jugularis externa in the middle of fattening (approx. 70 kg BW) and one week before slaughtering (approx. 110 kg BW) (Figure 6). Blood serum samples were analysed on the day of collection. Urinary N excretion was estimated according to Kohn et al. (2005):

$$NEXu\ (g/d) = CR\ (L\ of\ cleared\ blood/d \cdot kg\ BW) \cdot BUN\ (g/L) \cdot BW\ (kg)$$

where NEXu represents the urinary N excretion; CR represents the clearance rate of urea; BUN represents the blood urea N; BW is the body weight.

Animal-specific clearance rate could not be determined due to the experimental design, therefore a constant CR of 3.1 L cleared blood/(d · kg BW) was used (Kohn et al., 2005).

Laboratory analyses

Each diet of the 20 batches as well as the three faecal samples per animal were analysed in duplicate according VDLUFA (2012). The following analyses were carried out: dry matter (DM, method 3.1), crude ash (CA, method 8.1), N was analysed and crude protein calculated (N · 6.25) (CP, method 4.1.1, according to Kjeldahl), ether extract (EE, method 5.1.1, with HCl digestion), crude fibre (CF, method 6.1), neutral detergent fibre amylase treated expressed exclusive residual ash (aNDFom, method 6.5.1), acid detergent fibre expressed exclusive residual ash (ADFom, method 6.5.2), P (method 10.6.1) and acid insoluble ash (method 8.5). In addition, starch (method 7.2.1, polarimetric) was determined at AGROLAB LUFA GmbH Kiel (Germany). For faeces, analyses of DM, N, P and acid insoluble ash were carried out according to the scheme described above (VDLUFA, 2012).

Blood serum samples were analysed photometrically for blood urea N at Synlab Vet Laboratory (Leverkusen, Germany).

Statistical analysis

Statistical analysis was conducted using a linear mixed model realised by the R software package lme4 (Bates et al., 2023). An overview of all investigated traits is given in Supplementary Table S1, including the information whether the trait was directly measured or estimated and which model was used for analysis. Possible repeated measurements of traits were considered as such in the model. In total, there were three models:

Model 1: Linear mixed model with single measurement:

$$y_{ijkl} = \mu + b_1 \left(weight_{ijkl}\right) + gender_i + run_j + group_k + s_l + (s\times group)_{lk} + e_{ijkl}$$

where y_{ijkl} represents the observation; μ represents the common constant of y-values; gender (i = 1,2), run (j = 1, 2, 3, 4), group (k = 1, 2) represent fixed class effects; $b_1(weight_{ijkl})$ represents the linear covariate of weight (body or slaughter weight); s_l represents the random effect of the AI-boar (l = 1 to 20) of the investigated family; e_{ijkl} represents the error associated with each observation.

In model 2, a linear mixed model was set up comprising the repeated measurements of the three fattening periods:

$$y_{ijklmn} = \mu + b_{1n} \left(weight_{ijklmn}\right) + gender_i + run_j + group_k + M_n + (M\times run)_{nj} + (M\times group)_{nk} + (run\times group)_{jk} + s_l + pe_m + (s\times group)_{lk} + e_{ijklmn}$$

where M represents the time of measurement (n = 1, 2, 3); $b_{1n}(weight_{ijklmn})$ is the linear covariate of weight (body or slaughter weight) nested within the n^{th} M class. In addition, the individual permanent effect of the animal (pe_m) is included as random.

In order to clarify further the effects among the different runs, model 2 was extended by the interaction among measurement (M)×run×group resulting in model 3:

$$y_{ijklmn} = \mu + b_{1n} \left(weight_{ijklmn}\right) + gender_i + run_j + group_k + M_n + (M\times run)_{nj} + (M\times group)_{nk} + (run\times group)_{jk} + (M\times run\times group)_{njk} + s_l + pe_m + (s\times group)_{lk} + e_{ijklmn}$$

Whereas all main effects remain in the final models, fixed interactions, which were non-significant for all corresponding traits, were generally excluded.

To determine the influence of fixed and random effects, an ANOVA with corresponding F-Test or a Likelihood Ratio Test was performed, respectively. In addition, least squared means

(LSMeans) and related contrasts between feeding groups were determined using the R package emmeans (Lenth et al., 2023). A significance level of $p \leq 0.05$ was chosen.

The relevance of the AI-boar for the repeatedly measured traits was quantified by estimating the variance ratios heritability (h^2) and interaction of AI-boar (s)×group (gi^2). Variance components of the random effects were estimated according to model 1, 2, and 3 and h^2 were calculated as

$h^2 = \frac{2\sigma_s^2}{o_p^2}$ (Falconer and Mackay, 1995)

where h^2 is the heritability; σ_s^2 represents the variance of AI-boar; σ_p^2 represents the phenotypic variance ($\sigma_s^2 + \sigma_{s\times group}^2 + \sigma_{pe}^2 + \sigma_e^2$) (for single measured traits without σ_{pe}^2); $\sigma_{s\times group}^2$ represents the variance of AI-boar×group effect; σ_{pe}^2 is the variance of the permanent effect of the individual pig; σ_e^2 represents the variance of residual error. This formula given by Falconer and Mackay (1995) reflects the full sib structure of the data set.

Possible G×D were quantified by the AI-boar×group variance ratio:

$gi^2 = \frac{\sigma_{s\times group}^2}{o_p^2}$

where gi^2 represents the interaction of AI-boar×group; $\sigma_{s\times group}^2$ is the variance of AI-boar×group; σ_p^2 represents the phenotypic variance ($\sigma_s^2 + \sigma_{s\times group}^2 + \sigma_{pe}^2 + \sigma_e^2$) (for single measured traits without σ_{pe}^2); σ_s^2 represents the variance of AI-boar; σ_{pe}^2 represents the variance of the permanent effect of the individual pig; σ_e^2 is the variance of residual error.

In addition, gi^2 interactions were estimated within the fattening periods. For this purpose, the M, M comprising interaction terms and the random individual permanent animal effect were removed from models 2 and 3.

To further investigate G×D, the predicted progeny differences of each AI-boar within each feeding group were derived from model 2 and 3. Subsequently, spearman correlation coefficients of the predicted progeny differences (r_{PD}) between CON and LP were estimated.

3.4 Results

Diet composition

The experiment was carried out under farm conditions, which means the feed company composed diets according to DLG (2019) guidelines and resulting nutrient contents were declared on the label. At the end of the experiment, the diets were analysed. Depending on batch and diet, analysed CP ranged from 188 g/kg DM to 151 g/kg DM and P ranged from 5.7 g/kg DM to 3.9 g/kg DM (Table 4). Tolerances for the analytical constituents of compound feed are ±12.5% (relative) for CP and ±3 g/kg (absolute) for P (EU, 2017). All analysed values were within this range.

Table 4: Analysed crude protein and phosphorus of the control and N/P-restricted grower, finisher I and finisher II diets per run and in the average in comparison to the target

	Run 1		Run 2		Run 3		Run 4		Target	
	CON	**LP**	**CON**	**LP**	**CON**	**LP**	**CON**	**LP**	**CON**	**LP**
					Grower period					
CP (g/kg DM)	169	158	182	172	179	170	175	178	188	176
P (g/kg DM)	5.1	4.5	5.7	4.9	4.9	5.6	4.7	4.5	5.3	5.2
					Finisher I period					
CP (g/kg DM)	157	151	173	172	188	154	188	154	176	165
P (g/kg DM)	4.7	4.6	4.8	4.5	5.2	4.1	5.2	4.1	5	4.8
					Finisher II period					
CP (g/kg DM)	159	169	159	159	163	157	163	157	165	153
P (g/kg DM)	4.2	3.9	4.5	4.7	4.6	5.1	4.6	5.1	4.8	4.4

Note: Highlighted in green, intended CP/P reduction was realised; highlighted in red, intended CP/P reduction was not realised; highlighted in blue, intended CP/P reduction was overfulfilled.

Abbreviations: N/P, N and P; CON, control diets; LP, N and P-restricted diets; CP, crude protein; P, phosphorus, DM, dry matter.

However, across all runs, the intended dietary N/P restriction in LP compared to CON was realised in grower and finisher I diets, but not in finisher II diet (Table 5). These observations became even more apparent within the runs (Table 4). Regarding CP, the intended restriction in LP was not observed in run 1 finisher II, in run 2 finisher I and II, and in the grower period of run 4. Concerning P, no restriction in LP was achieved in finisher II period in run 2 and run 4, as well as in run 3, grower and finisher II. In all other runs and fattening periods, the intended restrictions were achieved or overfulfilled.

Table 5: Analysed composition of the control and N/P-restricted grower, finisher I and finisher II diets

	Grower diet		**Finisher diet I**		**Finisher diet II**	
	CON	**LP**	**CON**	**LP**	**CON**	**LP**
Dry matter (g/kg)	903	905	904	905	904	903
Ash (g/kg DM)	47.8	46.2	44.9	46.2	47.8	45.1
CP (g/kg DM)	176	169	173	159	160	160
Crude Fibre (g/kg DM)	48.6	45.2	51.8	45.8	48.5	45.6
ADFom (g/kg DM)	84.9	69.8	75.0	64.4	74.2	66.5
aNDFom (g/kg DM)	219	221	234	227	240	228
Ether extract (g/kg DM)	57.8	45.7	37.3	33.6	36.1	32.9
Phosphorus (g/kg DM)	5.09	4.88	4.88	4.40	4.42	4.56
AIA (g/kg DM)	2.44	2.13	2.34	2.01	1.90	2.05
ME (MJ/kg DM)	14.1	14.1	13.7	13.8	13.7	13.8

Note: Grower, finisher I and finisher II diets for ad libitum consumption from 20 to 60 kg BW, from 60 to 90 kg BW and from 90 to 120 kg BW, respectively. Listed nutrient content represent average values of individual batches.

Abbreviations: N/P, N and P; CON, control diets; LP, N and P-restricted diets; CP, crude protein; ME, metabolizable energy; DM, dry matter; ADFom, acid detergent fibre; aNDFom, neutral detergent fibre; BW, body weight.

Impact of the feeding group and its interactions with performance traits

A general overview of significance levels of all environmental effects and their interactions on all performance traits is given in Table 6.

Growth performance

The evaluation of the fattening performance traits average daily feed consumption, average daily gain, final fattening weight (FFW) and feed efficiency traits feed conversion ratio and residual feed intake utilised models 1 or 2 (Supplementary Table S1). The model decision was based on whether measurements were recorded only once at the experiments end or within each fattening period, respectively. Significant effects were found for the factor measurement, gender, run, measurement×run and for the particular weight parameter. Notably, there was no significant impact of the feeding group or group-comprising interaction terms except for average daily gain (M×group), which was significantly higher in CON in finisher I period (Table 7). Consequently, the reduction in CP or P content did not influence most classical fattening and feed efficiency traits in the present study.

As expected, gender had a significant effect on average daily gain, feed conversion ratio and final fattening weight, with male pigs having higher gain and final weight, and an improved feed conversion ratio (Supplementary Table S2). The factor run was significant for residual feed intake, and, as part of the interaction M×run for average daily feed consumption, average daily gain, and feed conversion ratio. LSMeans of residual feed intake were close to zero, and slightly negative in run 1 and 3, and positive in run 2 and 4. LSMeans of runs for average daily feed consumption were partly different (grower, finisher II). For feed conversion ratio no significant differences were observed. Average daily gain differences among LSMeans in fattening periods varied within runs, with no consistent direction observed. In the finisher II period, distinct differences between runs up to 276 g/d (run 1 vs. run 3) were found (Table 8).

Table 6: Impact of fixed environmental effects on performance and N/P related traits (results of ANOVA)

	Model	M	gender	run	group	b(W)	M×run	M×group	run×group	M×run×group	MSE	CV
ADFC	2	<0.001	0.31	0.02	0.47	<0.001	<0.001	0.46	0.61		0.03	0.08
ADG	2	<0.001	<0.001	0.10	0.36	0.36	<0.001	0.03	0.37		0.01	0.11
FCR	2	<0.001	<0.001	0.15	0.28	0.15	<0.001	0.13	0.18		0.08	0.12
FFW	1*		<0.001	0.92	0.75				0.44		38.8	0.06
RFI	1		0.76	0.002	0.57	0.48			0.11		0.01	0.00
Slaughter weight	1*		0.06	0.90	0.87						27.6	0.06
DP	1		<0.001	0.93	0.85	0.01					0.55	0.01
LMC	1		0.004	0.37	0.65	<0.001					1.88	0.02
BFT	1		0.01	0.02	0.90	<0.001					0.03	0.14
pH1 loin	1		0.00	0.22	0.54	0.10					0.03	0.03
pH²4 loin	1		0.14	0.01	0.53	0.21					0.01	0.02
Meat color	1		0.06	0.01	0.31	0.65					29.6	0.08
Drip loss	1		0.002	0.05	0.73	0.70					1.23	0.42
IMF	1		<0.001	0.46	0.37	0.79					0.04	0.17
PUFA	1		<0.001	0.003	0.01	0.32					1.81	0.08
MUFA	1		<0.001	<0.001	0.15	0.53					237	0.31
SFA	1		0.50	0.06	0.03	0.01					1.30	0.03

	Model	M	gender	run	group	b(W)	M×run	M×group	run×group	M×run×group	MSE	CV
ADN	3	<0.001	0.35	<0.001	0.02	0.82	<0.001	<0.001	0.23	0.001	7.32	0.03
NIN	3	<0.001	0.67	<0.001	0.26	<0.001	<0.001	<0.001	0.05	<0.001	19.7	0.08
NEXf	3	<0.001	0.56	<0.001	0.35	0.456	<0.001	<0.001	0.09	<0.001	4.18	0.15
NEXu	3	<0.001	0.03	0.03	0.17	<0.001	0.071	0.071	0.27	0.02	13.1	0.18
ADP	3	0.68	0.27	<0.001	0.02	0.46	<0.001	<0.001	0.01	<0.001	27.8	0.11
PIN	3	<0.001	0.58	0.03	0.04	<0.001	<0.001	<0.001	0.14	<0.001	0.60	0.08
PEX	3	<0.001	0.28	<0.001	0.26	0.001	<0.001	<0.001	0.02	<0.001	0.50	0.14

Note: *model 1 without linear covariate weight.

Abbreviations: N/P, N and P; M, time of measurement; b(W), regression coefficient of body weight at time of measurement or in model 1 carcass weight; MSE, mean squared error; CV, coefficient of variation; ADFC, average daily feed consumption; ADG, average daily gain; FCR, feed conversion ratio; FFW, final fattening weight; RFI, residual feed intake; DP, dressing percentage; LMC, lean meat content; BFT, back fat thickness; pH1 loin, pH in loin 1 hour after slaughter; pH²4 loin, pH in loin 24 hours after slaughter; IMF, intramuscular fat content; PUFA, polyunsaturated fatty acids; MUFA, mono unsaturated fatty acids; SFA, saturated fatty acids; N, nitrogen; P, phosphorus; AD, apparent digestibility; IN, intake; NEXf, faecal N excretion; NEXu, urinary N excretion, PEX, faecal P excretion.

Table 7: LSMeans (±SE) of CON and LP group for fattening performance in different fattening periods

	Grower period		**Finisher I period**		**Finisher II period**	
	CON	**LP**	**CON**	**LP**	**CON**	**LP**
ADFC (kg/d)	1.78±0.027[a]	1.79±0.026[a]	2.32±0.033[a]	2.28±0.033[a]	2.85±0.043[a]	2.79±0.046[a]
ADG (g/d)	816±15.2[a]	810±14.8[a]	964±17.4[a]	912±16.9[b]	910±18.3[a]	944±17.4[a]
FCR (kg/kg)	2.19±0.044[a]	2.24±0.04[a]	2.42±0.05[a]	2.55±0.05[a]	3.15±0.074[a]	3.10±0.072[a]
	Overall periods					
FFW (kg)	112±1.1[a]	112±1.1[a]				
RFI (kg/d)	0.00±0.021[a]	0.01±0.019[a]				

Note: different letters within a row in a fattening period indicate statistically significant differences ($p \leq 0.05$); *model 1 without linear covariate weight. Abbreviations: CON, control group; LP, group with restricted N and P; LSMeans, least squared means; SE, standard error; ADFC, average daily feed consumption; ADG, average daily gain; FCR, feed conversion ratio; FFW, final fattening weight; RFI, residual feed intake.

Table 8: LSMeans (±SE) for fattening performance in different fattening periods of different runs

	Grower period				Finisher I period				Finisher II period			
	Run 1	**Run 2**	**Run 3**	**Run 4**	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 1**	**Run 2**	**Run 3**	**Run 4**
ADFC (kg/d)	1.59±0.045^{a}	1.89±0.040^{b}	1.70±0.035^{a}	1.95±0.043^{b}	2.19±0.057^{a}	2.33±0.046^{a}	2.38±0.042^{a}	2.30±0.056^{a}	3.03±0.074^{a}	2.65±0.057^{b}	2.61±0.052^{b}	2.99±0.080^{a}
ADG (g/d)	716±27.3^{a}	864±23.4^{b}	816±21^{b}	856±26^{b}	867±29.9^{a}	956±23.6^{ab}	1028±21.3^{b}	902±28.5^{a}	1088±30.0^{a}	822±23.6^{b}	812±20.4^{b}	985±29.3^{a}
FCR (kg/kg)	2.25±0.073^{a}	2.21±0.063^{a}	2.10±0.056^{a}	2.31±0.070^{a}	2.55±0.085^{a}	2.49±0.067^{a}	2.33±0.061^{a}	2.57±0.081^{a}	2.81±0.140^{a}	3.25±0.113^{a}	3.25±0.101^{a}	3.20±0.136^{a}
	Overall periods											
FFW (kg)	112±1.1^{a}	111±2.0^{a}	112±1.2^{a}	113±1.9^{a}								
RFI (kg/d)	-0.06±0.031^{a}	0.03±0.031^{b}	-0.06±0.019^{c}	0.11±0.028^{d}								

Note: different letters within a row in a fattening period indicate statistically significant differences ($p \leq 0.05$).

Abbreviations: LSMeans, least squared means; SE, standard error; ADFC, average daily feed consumption; ADG, average daily gain; FCR, feed conversion ratio; FFW, final fattening weight; RFI, residual feed intake

Slaughter performance and meat quality

Carcass composition and meat quality traits were analysed using model 1 (Supplementary Table S1). All main effects were significant at least for one trait, with no significant interaction effects (Table 6). The impact of the group factor was only significant for PUFA and SFA, reflecting the meat's fatty acid composition. Meat of LP pigs had a reduced concentration of PUFA with simultaneously higher concentrations of SFA (Table 9).

Table 9: LSMeans (±SE) of CON and LP for slaughter performance and meat quality traits

	CON	**LP**
Slaughter weight (kg)	89.4±0.89[a]	89.2±0.83[a]
DP (%)	79.7±0.16[a]	79.7±0.16[a]
LMC (%)	59.7±0.33[a]	59.5±0.33[a]
BFT (cm)	1.21±0.039[a]	1.20±0.039[a]
pH1 loin	6.34±0.030[a]	6.37±0.030[a]
pH24 loin	5.41±0.015[a]	5.40±0.015[a]
Meat color	69.1±1.11[a]	67.7±1.12[a]
Drip loss (%)	2.68±0.233[a]	2.78±0.234[a]
IMF (%)	1.26±0.055[a]	1.20±0.055[a]
PUFA (% of total FA)	17.4±0.35[a]	16.4±0.35[b]
MUFA (% of total FA)	46.4±2.93[a]	51.2±2.95[a]
SFA (% of total FA)	34.4±0.26[a]	35.4±0.26[b]

Note: different letters within a row indicate statistically significant differences ($p \leq 0.05$).
Abbreviations: CON, control group; LP, group with restricted N and P; LSMeans, least squared means; SE, standard error; DP, dressing percentage; LMC, lean meat content; BFT, back fat thickness; pH1 loin, pH in loin 1 hour after slaughter; pH24 loin, pH in loin 24 hours after slaughter; IMF, intramuscular fat content; FA, fatty acids; PUFA, polyunsaturated fatty acids; FA, mono unsaturated fatty acids; SFA, saturated fatty acids.

Gender influenced all slaughter performance and meat quality traits listed in Table 6, except slaughter weight, pH²4, meat color and SFA. Male pigs had a lower dressing percentage, lean meat content, intramuscular fat content and MUFA, while gilts had a lower PUFA concentration (Supplementary Table S3). Gender×group interaction as well as other interactions were not significant, indicating that the N/P restriction did not differently influence slaughter and meat quality performance traits of males or gilts. The traits back fat thickness, pH24, drip loss, PUFA and MUFA were significantly influenced by model factor run (Supplementary Table S4).

Faecal N and P excretion and urinary N excretion

Model 3 was used to assess the data on the N/P-related traits, recorded during every fattening period. Gender significantly affected urinary N excretion, with boars excreting 5.6 g/d less N via urine than gilts in the average of finisher I and II period (Supplementary Table S5). The run had a significant effect on every trait. Time of measurement influenced every trait with exception of P digestibility (Table 6).

Concerning the interaction terms, almost all combinations of measurement, run and feeding group were significant for all traits, particularly in two-factorial (M×group) and three-factorial (M×run×group) interactions ($p \leq 0.001$ to $p=0.016$). LSMeans of the M×group-interaction are presented in Table 10. In the grower period and across all runs, LSMeans of feeding groups did not significantly deviate for any traits. In finisher I period, LSMeans of the LP were lower for all N/P-related traits compared to CON, with the exception of faecal N/P excretion being not influenced by the feeding group. In finisher II period, lower values for N/P digestibility were found in LP, while faecal N/P excretion increased in the LP by 2.0 g/d and 0.8 g/d, respectively. The other N/P-related traits were not influenced by the feeding group.

As elucidated in a previous section, the intended N/P restriction in the LP diet was only partially realised. The measurement (M)×run×group interaction is crucial in this context. LSMeans of this interaction are ordered by fattening periods presented in the following subsections and Table 11. In the **grower period,** CP and P was unexpectedly higher in LP diets in run 3 (P) and run 4 (CP) (Table 4). Despite the unexpected CP composition in run 4, no significant LSMeans deviations between CON and LP were detected for the N-related traits. In contrast to this, the higher P content in the LP diet of run 3 led to a higher ADP in LP by 8.2%. Phosphorus intake and excretion were not affected. In almost all other runs dietary P restriction was as intended or overfulfilled. Only in that case (run 2), in which it was overfulfilled, intake and excretion of P was significantly lower in LP up to a maximum of 1.5 g/d (PIN) and 1.4 g/d (PEX) (run 2) (Table 11).

In **finisher I** period, CP did not differ between the feeding groups in run 1 and 2. In contrast, the dietary CP restriction in run 3 and 4 was overfulfilled, with the CP difference between both diets being 34 g/kg DM (intended 11 g/kg DM) (Table 4). This strong reduction resulted in a significant lower N intake in run 3 and 4. Irrespective of the strength of dietary CP restriction, all other N-related traits did not differ between feeding groups (Table 11). Regarding dietary P content, the intended differences between CON and LP diets were realised in run 1 and 2 and were overfulfilled in run 3 and 4, with a difference of 1.1 g/kg DM (intended 0.2 g/kg DM)

(Table 4). Similar to the N-related traits, only in the case of such strong reduction, the intake of P was significantly reduced in LP. The other P-related traits were not influenced by the dietary P restriction.

In **finisher II** period, the intended restriction of 12 g/kg DM in CP content was realised in run 3 and 4, whereas CP was higher or not different in LP in run 1 and 2, respectively (Table 4). In those cases, in which the CP restriction was realised, N digestibility was significantly reduced in LP and faecal N excretion was higher in LP in run 4. All other N-related traits were not influence by the feeding group (Table 11). Concerning dietary P, only in run 1, the intended restriction was realised, whereas in all other runs, the CON diet had a 0.2 to 0.7 g/kg DM lower P content (Table 4). Despite realising the intended P-restriction in run 1, there was no significant difference in P-related traits between feeding groups. Although, the P-restriction was not realised, digestibility of P was reduced in run 3, while faecal P excretion increased in run 3 and 4 in LP (Table 11).

Table 10: LSMeans (±SE) of CON and LP for N/P related traits in different fattening periods

	Grower period		Finisher I period		Finisher II period	
	CON	**LP**	**CON**	**LP**	**CON**	**LP**
ADN (%)	72.3±0.81^{a}	74.2±0.79^{a}	79.9±0.55^{a}	77.1±0.53^{b}	78.2±0.60^{a}	75.1±0.59^{b}
NIN (g/d)	45.3±0.68^{a}	43.7±0.66^{a}	58.9±0.84^{a}	52.2±0.82^{b}	66.1±1.00^{a}	66.2±0.97^{a}
NEXf (g/d)	12.4±0.41^{a}	11.4±0.40^{a}	12.1±0.40^{a}	12.1±0.39^{a}	14.3±0.50^{a}	16.3±0.50^{b}
NEXu (g/d)	/	/	18.1±0.663^{a}	15.3±0.660^{b}	29.3±1.402^{a}	28.6±1.382^{a}
ADP (%)	47.7±1.00^{a}	50.6±0.97^{a}	53.0±0.99^{a}	44.8±0.97^{b}	51.3±1.14^{a}	47.6±1.12^{b}
PIN (g/d)	8.16±0.124^{a}	7.85±0.120^{a}	10.4±0.19^{a}	8.92±0.144^{b}	11.5±0.19^{a}	12.0±0.20^{a}
PEX (g/d)	4.23±0.100^{a}	3.89±0.097^{b}	4.91±0.134^{a}	4.94±0.131^{a}	5.57±0.167^{a}	6.39±0.165^{b}

Note: different letters within a row in a fattening period indicate statistically significant differences ($p \leq 0.05$).

Abbreviations: N/P, N and P; CON, control group; LP, group with restricted N and P; LSMeans, least squared means; SE, standard error; N, nitrogen; P, phosphorus; AD, apparent digestibility; IN, intake; NEXf, faecal N excretion; NEXu, urinary N excretion; PEX, faecal P excretion.

Table 11: LSMeans (±SE) of CON and LP for N/P related traits in different runs and fattening periods

	Run 1 (n=19)		**Run 2 (n=29)**		**Run 3 (n=36)**		**Run 4 (n=20)**	
	CON	**LP**	**CON**	**LP**	**CON**	**LP**	**CON**	**LP**
				Grower period (30 to 60 kg BW)				
ADN (%)	74.9±1.44^{a}	74.6±1.33^{a}	74.5±1.13^{a}	77.7±1.15^{a}	65.7±1.08^{a}	68.5±1.01^{a}	74.3±1.32^{a}	75.1±1.32^{a}
NIN (g/d)	37.8±1.85^{a}	37.2±1.67^{a}	50.7±1.45^{a}	46.0±1.48^{a}	43.4±1.36^{a}	41.8±1.23^{a}	49.4±1.65^{a}	50.1±1.66^{a}
NEX (g/d)	9.23±0.990^{a}	9.56±0.91^{a}	13.0±0.78^{a}	10.4±0.79^{a}	14.9±0.73^{a}	13.4±0.68^{a}	12.8±0.91^{a}	12.5±0.91^{a}
ADP (%)	50.2±2.23^{a}	52.8±2.06^{a}	48.8±1.78^{a}	56.7±1.82^{a}	37.7±1.67^{a}	45.9±1.52^{b}	54.3±2.04^{a}	46.9±2.05^{a}
PIN (g/d)	7.09±0.337^{a}	6.66±0.304^{a}	9.85±0.263^{a}	8.31±0.270^{b}	7.49±0.246^{a}	8.51±0.223^{a}	8.24±0.300^{a}	7.92±0.303^{a}
PEX (g/d)	3.52±0.300^{a}	3.15±0.272^{a}	5.00±0.235^{a}	3.58±0.240^{b}	4.63±0.221^{a}	4.62±0.201^{a}	3.78±0.270^{a}	4.19±0.271^{a}
				Finisher I period (60 to 90 kg BW)				
ADN (%)	85.8±1.43^{a}	84.8±1.33^{a}	76.3±1.08^{a}	73.3±1.11^{a}	79.4±1.02^{a}	78.2±0.97^{a}	77.6±1.33^{a}	72.5±1.32^{a}
NIN (g/d)	49.4±1.9^{a}	46.9±1.7^{a}	59.8±1.35^{a}	56.2±1.40^{a}	64.5±1.29^{a}	52.5±1.20^{b}	61.4±1.66^{a}	52.7±1.66^{b}
NEXf (g/d)	7.00±0.988^{a}	7.32±0.913^{a}	14.3±0.74^{a}	15.1±0.76^{a}	13.4±0.70^{a}	11.5±0.67^{a}	13.8±0.91^{a}	14.5±0.91^{a}
NEXu (g/d)	14.6±2.52^{a}	11.7±2.46^{a}	17.2±1.92^{a}	17.1±1.95^{a}	18.1±1.87^{a}	14.5±1.78^{a}	22.3±2.34^{a}	16.8±2.35^{a}
	Run 1 (n=19)		Run 2 (n=29)		Run 3 (n=36)		Run 4 (n=20)	
	CON	LP	CON	LP	CON	LP	CON	LP
ADP (%)	67.1±2.3^{a}	64.1±2.1^{a}	40.8±1.67^{a}	28.5±1.73^{b}	53.3±1.59^{a}	49.2±1.49^{a}	50.9±2.05^{a}	37.1±2.05^{b}
PIN (g/d)	9.21±0.336^{a}	8.93±0.304^{a}	10.3±0.25^{a}	9.07±0.256^{a}	11.2±0.23^{a}	8.78±0.219^{b}	10.6±0.30^{a}	8.85±0.302^{b}

	Run 1 (n=19)		Run 2 (n=29)		Run 3 (n=36)		Run 4 (n=20)	
	CON	**LP**	**CON**	**LP**	**CON**	**LP**	**CON**	**LP**
PEX (g/d)	3.01±0.300[a]	3.20±0.272[a]	6.13±0.221[a]	6.50±0.228[a]	5.23±0.210[a]	4.46±0.197[a]	5.28±0.271[a]	5.59±0.270[a]
				Finisher II period (90 to 115 kg BW)				
ADN (%)	86.6±1.43[a]	84.8±1.36[a]	72.1±1.08[a]	74.1±1.10[a]	76.2±1.01[a]	70.9±1.00[b]	78.5±1.35[a]	71.1±1.32[b]
NIN (g/d)	68.5±1.84[a]	73.3±1.80[a]	51.3±1.35[a]	58.8±1.40[a]	62.8±1.27[a]	57.7±1.20[a]	71.9±1.76[a]	74.9±1.66[a]
NEXf (g/d)	9.13±0.987[a]	11.9±0.91[a]	17.2±0.74[a]	15.2±0.76[a]	15.0±0.69[a]	16.6±0.68[a]	15.6±0.92[a]	21.1±0.94[b]
NEXu (g/d)	29.7±2.50[a]	34.0±2.39[a]	23.3±1.91[a]	19.9±1.95[a]	29.3±1.78[a]	31.5±1.75[a]	35.3±2.35[a]	29.9±2.35[a]
ADP (%)	67.1±2.27[a]	66.8±2.11[a]	38.8±1.67[a]	43.0±1.72[a]	46.9±1.57[a]	35.9±1.53[b]	52.9±2.09[a]	45.3±2.05[a]
PIN (g/d)	11.2±0.335[a]	11.1±0.31[a]	10.9±0.25[a]	11.1±0.25[a]	11.0±0.23[a]	11.6±0.22[a]	12.8±0.31[a]	14.0±0.38[a]
PEX (g/d)	3.71±0.300[a]	3.78±0.278[a]	6.69±0.221[a]	6.34±0.228[a]	5.86±0.207[a]	7.38±0.202[b]	6.01±0.276[a]	8.05±0.284[b]

Note: different letters within a row in a run indicate statistically significant differences ($p \leq 0.05$); Highlighted in green, intended CP/P reduction was realised; highlighted in red, intended CP/P reduction was not realised; highlighted in blue, intended CP/P reduction was overfulfilled.

Abbreviations: N/P, N and P; n, number of animals in a run; CON, control group; LP, group with restricted N and P; LSMeans, least squared means; SE, standard error; N, nitrogen; P, phosphorus; AD, apparent digestibility; IN, intake; NEXf, faecal N excretion; NEXu, urinary N excretion; PEX, faecal P excretion.

Estimation of heritability and variance components

Results of h^2 and gi^2 estimation are presented in Table 12, both within and across the fattening periods. In general, the h^2 and gi^2 variance ratios within the fattening periods were considerably higher than those estimated across all fattening periods.

Estimates of moderate heritability (h^2) for the fattening traits ADFC, ADG, and FCR ranged from 0.07 to 0.48, regardless of the fattening period, with notable genetic interaction (gi^2) estimates only for ADFC. Across periods, higher h^2 was observed only for FCR, with gi^2 values near zero. A similar trend was seen in nitrogen (N) intake and faecal excretion, with moderate h^2 (0.14-0.27) in grower and finisher I periods but lower values in finisher II and overall analyses. Notable gi^2 (>0.1) were found for N intake in finisher I and faecal N excretion in finisher II and overall datasets. The highest h^2 (0.15-0.35) and gi^2 (0.08-0.28) were reported for N digestibility, and urinary N excretion had h^2 of 0.51-0.77 across periods, with gi^2 close to zero. For phosphorus (P) traits, moderate h^2 (0.15-0.29) was seen for P intake across all periods, while P excretion and digestibility were notably heritable (0.26 and 0.39) only in finisher II. Sporadic gi^2 values (>0.1) were found in finisher I and II. Permanent environmental effects were close to zero for all traits except urinary N excretion (pe=0.16).

As an alternative to the gi^2 ratio, correlation coefficients of the predicted progeny (PD) differences (r_{PD}) of AI-boars between feeding groups can serve as an indicator of the existence of G×D interactions. Estimations of PD values were based on the genetic parameters given in Table 12. Correlations close to unity were expected when gi^2 were close to zero. In case of gi^2, values being higher than the corresponding h^2, r_{PD} either were close to zero or negative. Such low (<0.30) or negative r_{PD} were identified for faecal N excretion (r_{PD} =-0.10, h^2=0.00, gi^2=0.24) in finisher II and for N digestibility (r_{PD} =-0.16, h^2=0.00, gi^2=0.21) in finisher I period. In case of h^2>0.10 and gi^2 >0.10 the r_{PD} were in a range of 0.30-0.90. With respect to a possible G×D, r_{PD} values well below 0.80 were of particular importance (Robertson, 1959). Such correlations were found for N digestibility (all periods), ADFC and N intake in finisher I and II, faecal N excretion and P intake in finisher II and faecal P excretion in finisher I period.

Additionally, indications of G×D were found for the carcass and meat quality traits BFT (r_{PD} =0.81, h^2=0.39, gi^2=0.20), IMF (r_{PD} =0.62, h^2=0.40, gi^2=0.19), and drip loss (r_{PD} =0.68, h^2=0.23, gi^2=0.11). For all other carcass and meat quality traits, gi^2 was close to zero or r_{PD} close to 1.00 (results not shown).

Table 12: Heritability, proportionate variance of AI-boar×group, permanent environmental effects, and spearman rank correlations of boars between C and LP based on predicted values

	Grower period			**Finisher I period**			**Finisher II period**			**Overall periods**			
	h^2	**gi^2**	**r_{PD}**	**h^2**	**gi^2**	**r_{PD}**	**h^2**	**gi^2**	**r_{PD}**	**h^2**	**gi^2**	**pe**	**r_{PD}**
ADFC	0.13	0.03	0.92	0.18	0.12	0.66	0.16	0.09	0.64	0.00	0.01	0.10	0.01
ADG	0.25	0.00	1.00	0.13	0.00	1.00	0.07	0.00	1.00	0.04	0.00	0.00	1.00
FCR	0.28	0.00	1.00	0.17	0.00	1.00	0.48	0.00	1.00	0.17	0.00	0.00	1.00
ADN	0.35	0.17	0.61	0.11	0.19	0.33	0.15	0.28	0.34	0.24	0.08	0.00	0.58
NIN	0.14	0.03	0.92	0.27	0.10	0.80	0.09	0.04	0.76	0.00	0.02	0.06	-0.12
NEXf	0.24	0.08	0.79	0.22	0.09	0.79	0.00	0.24	-0.10	0.08	0.12’	0.01	0.26
NEXu	/	/	/	0.51	0.00	1.00	0.77**	0.00	1.00	0.57**	0.00	0.16*	1.00
ADP	0.00	0.02	-0.36	0.00	0.19	-0.16	0.39	0.15	0.84	0.05	0.03	0.00	0.60
PIN	0.15	0.02	0.97	0.29	0.07	0.89	0.18	0.11	0.66	0.01	0.02	0.08	0.36
PEX	0.04	0.00	0.97	0.06	0.21	0.15	0.26	0.02	0.98	0.00	0.05	0.00	0.04

Note: significance thresholds: ‘ = p≤0.1, *= p≤0.05, ** = p≤0.01, *** = p≤0.001.

Abbreviations: h^2, heritability; gi^2, variance of AI-boar×group; pe = permantent environmental effect; r_{PD} = rank correlations of boars between CON and LP; CON, control group; LP, group with restricted N and P; ADFC = average daily feed consumption, ADG, average daily gain; FCR, feed conversion ratio; N, nitrogen; P, phosphorus; AD, apparent digestibility; IN, intake; NEXf, faecal N excretion; NEXu, urinary N excretion; PEX, faecal P excretion.

3.5 Discussion

Due to the high degree of relevance of N/P-restricted diets in growing-finishing pigs on an ecological and economic level, many studies have been carried out on this topic in recent years. Beside feeding trials, focusing exclusively on phenotypic performance, trait expression and N/P excretion of pigs (e.g. Carpenter et al., 2004, Ruiz-Ascacibar et al., 2017), the focus shifted to the aspect of genetics. Comparisons and studies with different breeds, estimates of genetic parameters like h^2 for feed and nutrient utilisation efficiency have been addressed in further studies (Shirali et al., 2012, Saintilan et al., 2013, Kasper et al., 2020). Differences in the adaptability to N/P-restricted diets do not only exist between different breeds, they also were found between individuals of the same breed living in the same environment (Ruiz-Ascacibar et al., 2017). This led to the question of the existence of biologically relevant G×D.

Growth performance

Irrespective of some differences in the feed composition of the experimental diets and levels of growth performance and feed efficiency, the results of the present study were within the ranges reported earlier (e.g. Ruiz-Ascacibar et al., 2017). An important result of our study was that the LSMeans of average daily feed consumption, feed conversion ratio and residual feed intake remained unaffected among the groups. These results were as expected: According to GfE (2008), the N/P content of the LP diets was not below animals' maintenance and performance requirements. Furthermore, regarding N, it should be emphasised that CP was restricted, while the concentration of EAA was equalized between feeding groups. With respect to P, a growth restriction was not expected either. A nationwide phosphorus ringtest (Germany) showed, that a significantly stronger P restriction (3.3 g/kg DM) in finisher II period (90 to 120 kg BW) than in the present study is feasible without impairments (Krieg et al., 2023). Despite these facts, average daily gain was significantly lower in finisher I in the LP of the present study. These results confirm findings from other studies. For example, Ruiz-Ascacibar et al. (2017) did not report differences in feed intake and feed conversion ratio, but found differences in average daily gain when investigating the impact of an N/P-restricted nutrition. The differences between feeding groups in this study were even more pronounced than in our study. This could be related to the fact that not only a more substantial restriction in CP (down to 10.2% in finisher II) but also a decrease in EAA among feeding groups was implemented by Ruiz-Ascacibar et al. (2017).

The significant impact of gender on average daily gain has already been described by several studies (e.g. Matthes et al., 2014), which may be due to the growth stimulating effects of male sex hormones. In the present study, the higher average daily gain of boars resulted in both, a higher final fattening weight for the same fattening duration, and a lower feed conversion ratio for the same average daily feed consumption.

Slaughter performance and meat quality

As expected, there were no differences in slaughter performance between feeding groups in the present study, probably due to the N/P supply above requirements (GfE 2008), as already discussed above. The same applies to most meat quality traits with the exception of fatty acid pattern in meat. The lower concentration of PUFA in meat of LP pigs and higher concentration of SFA may be due to the differences in fatty acid pattern between CON and LP diets. The LP diets had a lower proportion of rapeseed meal throughout all fattening periods and a reduced proportion of vegetable fatty acids in the grower and finisher I period, resulting in a relatively lower dietary content of PUFA. It is known that in non-ruminants the fatty acids from feed are incorporated into body fat without alteration, if not used as source of energy (Wood, 1984, Nürnberg et al., 1998). Thus, the fatty acid pattern of the feed is mirrored in animal's fatty acid pattern.

Nürnberg et al. (1998) demonstrated that fatty acid pattern in pork depends not only on the composition of feed and other factors, but also on sex. The difference between boars and gilts in the present study may be due to the male sex hormones stimulating body metabolism and growth. The same reason may apply to differences in dressing percentage, lean meat content, back fat thickness and intramuscular fat content between boars and gilts. Our results confirm the findings of Nürnberg and Ender (1989) and Matthes et al. (2014).

Faecal N and P excretion and urinary N excretion

In relation to the initial plan of the experiment, the intended N/P restriction was only partially realised in fattening periods and individual runs (Table 4). However, all observed deviations between intended and analysed CP and P content were within the range of tolerance of the legal declaration (CP ±12.5% (relative), P ±0.3 g/kg (absolute)) (EU, 2017). Given these ranges, the intended difference in N/P content between C and LP diets is comparatively small. In light of this, a higher N/P content, as well as a much lower N/P content than intended in LP diets is

explainable. When interpreting the study results regarding N/P related traits, it is important to consider the actual realised differences in dietary CP and P among C and LP diets. Additionally, it should be noted that results might not precisely reflect those of an adequate comprehensive balance experiment, given the calculation or estimation rather than direct measurement for nutrient excretion. However, the chosen methods were the best possible given the number of animals and available resources in the present study.

Distinctions in average daily feed consumption between CON and LP, along with all corresponding interaction terms, were not observed. This implies that variations in N/P intake are intrinsically connected to differences in N/P content of the diets. Consequently, the significance of the measurement (M)×group interaction was limited. Therefore, the subsequent focus was directed towards the 3-way M×run×group. In case of the realisation of the CP restriction (8 out of 12 M×run combinations, Table 4) a significant lower N intake was observed if the CP restriction was overfulfilled (34 g/kg DM) as was the case in run 3 and 4 in the finisher I period (Table 11). Consequently, it is evident that the intended dietary CP restriction was insufficient to exhibit significant differences in N intake.

No clear tendency between realised dietary CP restriction and group differences in N digestibility can be derived from the present study's results. Even in the cases of extreme CP restriction (run 3, 4, finisher I), there was no difference between the feeding groups. However, the only significant differences in N digestibility between CON and LP were observed in run 3 and 4 in finisher II period. The effects of restricting dietary CP on N digestibility are likely multifactorial: Digestibility of N is mainly influenced by feed components in the diets. In the present study, the restriction of CP was fulfilled by decreasing rapeseed meal, where CP is partially bound as tannin CP and phytate CP, with the additional presence of Maillard products, making N less absorbable (Bell, 1993; Cheng et al., 2022). Consequently, reducing rapeseed meal in LP diets could potentially enhance ADN. Additionally, the supplementation of free AA in LP diets to compensate for missing EAA resulting from CP restriction could have a digestibility-increasing effect as they do not require digestion and can be absorbed more easily and completely (Weijzen et al., 2022; Eugenio et al., 2013). However, this increase in N digestibility in LP may be countered by a potential decrease arising from a higher cereal content (wheat and barley), containing components such as ß-glucan and pentosanes. These elevate digesta viscosity and subsequently may impair nutrient digestibility (Agyekum and Nyachoti, 2017). Furthermore, wheat and barley possess a relatively high proportion of non-starch polysaccharides. This indicates a greater presence of soluble and fermentable fibre, quantified as 19.1 g/kg DM for wheat and 50.6 g/kg DM for barley by Rodehutscord et al. (2016).

Intestinal microbes in large intestine utilise soluble fibre, undigested in small intestine, as energy source and synthesize microbial protein using ammonia. This microbial protein may be excreted via faeces (Mosenthin et al., 1992). Hence, the N digestibility, representing the amount of ingested N not recovered in faeces, is reduced. In addition to the various factors influencing N digestibility, it is important that, while it is considerably less resource-intensive to assess, it does not accurately reflect N balance, as total N excretion is primarily regulated via urine. Digestibility of N is mainly affected by the N intake; a higher intake leads to an increasing digestibility by more absorbed N, which is potentially not utilised and excreted via urine (Carpenter et al., 2004). This may lead to an overvaluation of N efficiency in CON of the present study. Due to the lack of further differentiation, whether N in faeces was of dietary or microbial origin (Schmitz et al., 2024), it remains unclear why N digestibility remains unchanged between feeding groups in most cases.

Faecal N excretion is directly linked to the intake and digestibility of N as it was calculated by these parameters. By theory, group differences in faecal N excretion are constant if intake and digestibility remain constant or digestibility effects compensate the effects of N intake. Group differences in faecal N excretion can be expected if N intake and digestibility differences have the same sign or overcompensate each other. In most cases, there were no feeding group differences in faecal N excretion. This was expected, as the digestibility and the intake of N were also not different. However, faecal N excretion remained unchanged in finisher I period in the runs 3 and 4, possibly attributed to the compensatory effect of a slightly, but not significantly decreased digestibility of N on the significantly reduced N intake in the LP. Only in run 4, finisher II period, an undesirable higher faecal N excretion in LP was observed. This is mainly due to a reduction in N digestibility. At this point, it is noteworthy to remind that N digestibility and, consequently faecal N excretion provide less information than an N balance.

As already mentioned, N balance is mainly influenced by urinary N excretion. Absorbed N is partly converted to urea in the liver and excreted via urine. Urinary N excretion was estimated by the formula of Kohn et al. (2005) by means of body weight and blood urea nitrogen. This indicator trait is directly linked to N intake because it is related to the in small intestine absorbed N. The high correlation between urinary N excretion and intake of approx. 0.67 between feeding groups underlines this statement. Reductions in N intake in LP generally resulted in decreased urinary N excretion. When N intake remained unchanged between CON and LP, urinary N excretion typically remained unchanged as well. A deviation was observed in run 4, finisher II period: the urinary N excretion significantly decreased in the LP despite the N intake remaining unchanged. The increased proportion of soluble fibre in LP diets could explain this, as this also

was associated with the above-mentioned increase in faecal N excretion in this period. Ammonium, which can be utilised as a substrate by microbes in large intestine, is no longer absorbed and excreted via urine. Thus, an increase in fermentable fibre at the same N intake shifts N excretion from urine to faeces. This shift leads to a reduction of ammonia emissions, yielding favorable environmental implications (Morgan and Whittemore, 1988, Schmitz et al., 2024).

Similar to the dietary N, the P restriction was realised in 8 out of 12 M×run combinations (Table 4). In these, the P intake was only in runs with the at least realised P restriction of more than 0.8 g/kg DM significant different between feeding groups (Table 11). From this, we conclude that the P restriction was insufficient to yield a relevant difference in P intake. Digestibility of P was significantly different between CON and LP in four cases, but these deviations had positive and negative signs and became apparent in runs with and without realised feed P restriction. Such differences were unexpected, given the inclusion of 500 FYT 6-phytase/kg diet to enhance the ADP in rapeseed meal, containing approx. 12 g/kg DM P with a digestibility of only 30% (DLG, 2014). The potential impairment of phytase efficiency due to the previously mentioned higher viscosity in fibre-rich diets (Schmitz et al., 2024) could be a plausible explanation. However, no clear interpretation can be found for the distinctions in P digestibility. The possibility that P digestibility overvalues P efficiency can be largely dismissed, as the P absorption is hormonally regulated, and P is excreted through urine only in the presence of a large over-supply (Schröder et al., 1996).

Intake and digestibility of P directly influence faecal P excretion. Most differences in P excretion between feeding groups were not significant. However, in run 2, grower period faecal P excretion was reduced in the LP due to a decrease in P intake. This result might be related to the realised distinct P restriction in this group. Moreover, in run 3 and 4, finisher II period, faecal P excretion of LP increased undesirably due to decreasing P digestibility and increasing intake. Notably, this was observed in runs where the realised dietary P content in the LP was higher than in the CON.

Estimation of heritability and variance components

Heritability estimates in this study were in accordance with previous findings: Saintilan et al. (2013) reported breed-dependent values for FCR, ADFI, and ADG in the range of 0.35-0.40, 0.21-0.48, and 0.05-0.48, respectively. Total N/P excretion showed h^2 of 0.31-0.37 and 0.29-0.40 in their study. In another study, Déru et al. (2021) reported h^2 values of 0.27 and 0.56 for ADN, depending on dietary fibre content.

In addition to estimating h^2 across all fattening periods, our study focused on specific fattening periods. Notably, h^2 for individual periods were generally higher than those for the entire period, except for NEXu. Heritability estimates for NEXf were particularly striking. In grower and finisher I period they were approx. 0.20, whereas in finisher II a h^2 of zero was estimated. For the P related traits, a reverse order was found. Heritability estimates for ADP and PEX were close to zero in grower and finisher I period, whereas it was remarkably higher in finisher II period (0.39 and 0.26). The variation in h^2 across fattening periods may be attributed to physiological mechanisms. Pigs' changing energy and nutrient requirements during different growth stages, influenced by maintenance and performance intensity, could contribute to this variation. The genetically determined limit for daily protein deposition defines the AA requirements of a pig (Campbell and Taverner, 1988), at first increasing with age and decreasing around 120 days (gilts) or 150 days (entire males) (Danfær and Strathe, 2020). Consequently, less protein is deposited, and less N is required during finisher II compared to finisher I period. Lipid deposition, influenced by genotype, energy intake, and protein deposition, becomes a major component in weight gain when protein deposition decreases (Danfær and Strathe, 2020). This dynamic results in increased fat accumulation in finisher II.

Similar to h^2, gi^2 estimates were more pronounced within individual fattening periods, particularly in finisher I and II. In order to verify these gi^2 results, r_{PD} of the AI-boars in CON and LP were estimated for relevant traits. Low gi^2 estimates resulted in higher r_{PD} values up to an extreme of 1.00. Only when both, h^2 and gi^2 >0.10, the r_{PD} correlation coefficients fell below 0.80. This value could be considered as a threshold for relevant G×D, which might be related to physiological processes and growth stage changes (Robertson, 1959). Based on this assessment G×D was relevant for ADFC (finisher I period), NIN (finisher I period), and PIN (finisher II period). Moreover, particularly striking were NEXf (grower period) and ADN (all fattening periods), confirming literature findings (Geiscnek-Koltay et al. 2022). Variation in ADN among genotypes may be associated with different digesta passage rates or altered activity of microbes and digestive enzymes, as observed in comparisons between indigenous, obese and contemporary, lean pig breeds (e.g. Varel et al., 1988, Déru et al., 2022). Comparing these two

breeds, other studies have identified G×D that are relevant for N retention, feed efficiency, and lysine utilisation (Fabian et al., 2002, Barea et al., 2011).

From a physiological perspective, differences in the genetic potential to utilise dietary N/P efficiently might be the cause of G×D (Ruiz-Ascacibar et al., 2017). This involves genetic variations in lean growth potential with effects on AA requirements (Schinkel and de Lange, 1996). Underlying mechanisms might be evident through variations in the expression of genes associated with mitochondrial metabolism or specific enzymes, observed in comparisons of animals with low and high feed efficiency (Gilbert et al., 2017). In terms of anatomy, Barea et al. (2011) identified structural changes in the small intestine linked to absorption capacity, when comparing efficient and less efficient pigs. In this context, individual variability in the gut microbiome (Aliakbari et al., 2022) could also play an important role in explaining the variations in adaptability to N/P-restricted diets. However, a re-ranking of genotypes under N/P-restricted diets and the associated G×D could not be proven in every study. Thus, comparisons of two genotypes under a digestible versus a less digestible diet did not result in any differences in growth potential (Schiavon et al., 2019, Godhino et al., 2018). The authors attributed this to the distinction between limited and non-limited N/P supply.

Regarding the estimated r_{PD}, h^2 and gi^2 in the present study, it is crucial to note that these estimations relied on a relatively small dataset, leading to notable uncertainties. Further validation with a more extensive dataset is essential. Nevertheless, clear indications of a genetic influence, variable between fattening periods, and of a G×D were observed.

3.6 Conclusion

In the present study, the intended dietary N/P restriction was only partially realised, posing challenges to result interpretation. The subtle differences between "strongly N/P-reduced" and "extremely N/P-reduced" diets (DLG, 2019) make it difficult to observe considerable changes in performance and N/P excretion under practical conditions. Nevertheless, distinct differences in dietary N/P content, occurring sporadically in some fattening periods and runs, demonstrate a significant impact on N/P intake, digestibility, and excretion. From a breeding perspective, moderate h^2 suggest the possibility of breeding for these traits. However, it is essential to consider the existing G×D, offering the opportunity to select robust animals. This study highlights the potential to reduce N/P excretion without compromising animal performance. It provides a foundation for future research on N/P related traits and G×D and their incorporation in pig breeding.

Chapter 4. Microbiability and microbiome-wide association analysis for feed efficiency and nutrient excretion in different gut sections of growing-finishing pigs

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(submitted)

4.1 Abstract

Background

Approaches to enhance sustainability in pig production, including the reduction of nitrogen and phosphorus (N/P) excretion, entail strategies such as dietary N/P restriction and the integration of N/P efficiency traits into breeding programmes. Beyond that, the relevance of the gut microbiome gained more attention, not only by physiologists but also by animal breeders. However, the investigation of microbiability (m^2), which represents the microbial variance influencing target traits, remains in its early stages, particularly concerning data within individual gut sections. The present study focused on the gut microbiome across four gut sections (jejunum, ileum, caecum, and colon) in 52 German Piétrain (Pi) × Landrace pigs originating from 19 Pi artificial insemination boars, intended to mirror the genetic performance potential of Pi. Half the pigs were fed N/P-restricted diets. The aim was to explore the gut microbiome composition and m^2 for fattening performance, feed efficiency and N/P related traits.

Results

One notably result from our investigation was that moderate N/P restriction had a relatively small effect on microbial diversity. However, significant differences were observed between small (jejunum, ileum) and large intestine (caecum, colon) in terms of alpha- and beta-diversity. High m^2 values for feed efficiency traits feed conversion ratio and residual feed intake were observed in large intestine, primarily the caecum (0.61 and 0.26, respectively), contrasting with moderate values for N/P digestibility in small intestine, specifically the jejunum (0.27 and 0.22, respectively).

Conclusion

These findings highlight the substantial microbial impact on these traits. Furthermore, microbiome-wide association analysis revealed that numerous species with small effect sizes influence target traits, particularly N/P digestibility, faecal P, and urinary N excretion. These results indicate the potential utility of microbiome data in future rankings of breeding animals.

Key words: gut microbiome, microbial variance, N and P emissions, sustainability, small and large intestine

4.2 Introduction

Modern pig farming is confronted with a multitude of challenges, demanding comprehensive and sustainable approaches. Efficiency in feed and nutrient utilisation is a crucial objective for ensuring efficient and sustainable production. To achieve this, various strategies have been implemented, including dietary approaches involving the restriction of nutrients, notably nitrogen (N) and phosphorus (P), in pig diets (Pomar et al., 2020). Additionally, there are ongoing endeavors to integrate efficiency related traits into breeding programmes (Kasper et al., 2020). In this context, the gut microbiome has become increasingly important due to its impact on the host's performance and immune system through microbial metabolites like short chain fatty acids (SCFA) and vitamins (Mowat and Agace, 2014).

Several studies have shown, that both environmental influence the composition of the gut microbiome and host genetics, which means it has a heritable component (e.g. Benson et al., 2010, Camarinha-Silva et al., 2017, Bergamaschi et al., 2020b). In addition, the authors concluded that the variation in the gut microbiome composition among individual pigs could account for some of the variation seen in the phenotypic expression of target traits (Camarinha-Silva et al., 2017, Aliakbari et al., 2022). This influence is referred to as "microbiability" (m^2), akin to "heritability" for genetic effects (Difford et al., 2016). One promising option is to identify microbial species as biomarkers for efficiency traits, which evaluation has been cost- and time-consuming until now (Difford et al., 2018). The initial studies on m^2 focused on colon or faecal samples (Camarinha-Silva et al., 2017, Aliakbari et al., 2022). Considering the recognized functional and microbial distinctions between the small and large intestine (Szabó et al., 2013, Looft et al., 2014), investigations into m^2 specific to individual gut sections could potentially offer further insights into the foundational aspects of this complex subject.

The objective of this study was to conduct a comprehensive analysis of the microbiome in the small (jejunum and ileum) and large intestine (caecum and colon) of fattening pigs. This encompassed a detailed examination of gut microbiome composition, along with the evaluation of alpha and beta diversity metrics, serving as parameters to describe species diversity in samples and the similarity between samples. Various influencing factors were considered, including the implementation of dietary N and P (N/P) restriction, gender, and genetic effects (sire of pigs). Additionally, particular attention was paid to m^2 and microbiome-wide association studies (MWAS) for traits associated with fattening performance, feed efficiency, and N/P-related traits to quantify the impact of the gut microbiome in individual gut sections. The results of the present study are expected to contribute to a better understanding of the complex relationship between microbiome, host genetics, and environmental factors.

4.3 Material and methods

Animals, feed and experimental design

The experiment was conducted involving 52 German Piétrain (Pi) × Landrace crossbred pigs, 26 entire males and 26 females at Campus Frankenforst, University of Bonn (Germany) (50°42'51.9"N, 7°12'25.1"E). Pigs originated from 19 litters, each from different artificial insemination boars (AI-boars) which should represent the contemporary genetic performance potential of the Pi breed in Germany. Landrace sows belonged to the Frankenforst herd. Throughout the experiment, the animals showed no signs of illness or abnormalities.

The entire experiment took place from June 2020 to July 2021, organized in four consecutive runs. From weaning to start of fattening (approx. 30 kg body weight (BW)), all pigs were fed the same diet according to the recommendations for growing pigs in Germany (GfE, 2008). Subsequently, they were randomly assigned to control group (CON) or group with restricted dietary N/P, called low protein group (LP), ensuring an even distribution of gender and paternal origin. All pigs were housed individually (pen size 1.00×2.50 m²), allowing for comprehensive individual phenotyping including monitoring of feed consumption and collection of individual faecal spot samples. In each run, pigs were fed for *ad libitum* consumption until they reached a final BW of approx. 115 kg. The 3-phase fattening period was categorized by BW into grower (30 to 60 kg BW), finisher I (60 to 90 kg BW) and finisher II (90 to 115 kg BW). Feeding followed the guidelines outlined in leaflet 418 of the DLG (2019), with diets of CON following a "strongly N/P-reduced feeding programme" and diets of LP following an "extremely N/P-reduced feeding programme". Diets were based on the protein sources rapeseed meal and sunflower meal, and on wheat, barley, and triticale grains in various proportions and were each phytase supplemented (Table 3).

To maintain consistent feed quality throughout the experiment, diets were produced in 20 batches as needed by a commercial feed company (Deutsche Tiernahrung Cremer, Düsseldorf, Germany). The diets were pelleted, so a segregation of components was rather unlikely. The essential amino acid (EAA) ratio and energy content were consistent between CON and LP diets. The formulation of the EAA was adjusted in relation to precaecal digestible lysine (pcDLys) (% of pcDLys) as follows in all periods: methionine = 30%, methionine + cysteine = 61%, and tryptophan = 18%, respectively, and separated according to fattening period: threonine = 65% (grower), 60% (finisher I) and 61% (finisher II), respectively.

Recording of growth performance, feed efficiency and N/P excretion

During fattening, feed consumption of each individual pig was documented, with pigs being weighed every two weeks. Start of fattening and the change between fattening periods, along with respective diets, were determined based on average body weight of all pigs within a batch, occurring at approx. 30 kg, 60 kg, and 90 kg BW.

Calculation of average daily feed consumption (ADFC), average daily gain (ADG) and feed conversion ratio (FCR) was conducted according to the guideline for station testing for fattening performance, carcass value and meat quality in pigs for the entire fattening period (BRS, 2019). Residual feed intake (RFI) was estimated as follows (Saintilan et al., 2013):

RFI(kg/d) = ADFC (kg/d) - ADFI (kg/d)

where ADFI represents the estimated average daily feed intake; ADFC represents the registered ADFC.

ADFI was estimated by a multiple linear regression of ADFC on ADG to account for growth, and on back fat thickness (BFT), lean meat content (LMC), and dressing percentage (DP) to account for composition of body weight gain, and on average metabolic body size (AMS) to account for maintenance requirements (according to Saintilan et al., 2013). Using the formula of Noblet et al. (1999), AMS was calculated from BW at the start (body weight$_1$) and end of fattening (body weight$_2$):

$$\text{AMS}\left(\text{kg}^{0.60}\right) = \frac{\text{body weight (kg)}_2^{1.6} - \text{body weight (kg)}_1^{1.6}}{1.6 \cdot (\text{body weight (kg)}_2 - \text{body weight (kg)}_1)}$$

All pigs of a run were slaughtered at reaching a mean BW of 112 (± 7.5) kg within one (run 1, 2, 4) or two (run 3) days. The average fattening period lasted (± 3) days. Slaughtering was conducted at Landesanstalt für Schweinezucht (LSZ) Boxberg (Baden-Württemberg, Germany). Pigs were stunned in pairs for 140 s with CO_2 (90%), weighed, exsanguinated, and weighed again. Carcasses were de-bristled, scalded and eviscerated, after which they were longitudinally split into halves and chilled to 1-3°C.

Sample collection

Samples of each diet batch delivered throughout the four runs were collected, and their chemical composition was analysed at the end of the experiment. To assess faecal N/P excretion (NEXf, PEX), within each fattening period faecal spot samples of each pig were collected twice daily for 5 days after a 7-day feed adaptation period (Kim et al., 2020). Samples were stored at -20°C until chemical analyses.

Faecal N/P excretion for each pig and fattening period was assessed based on N/P intake (NIN, PIN) and N/P digestibility (ADN, ADP). Digestibility of N/P was determined using acid insoluble ash (AIA) as an inert marker according to Adeola et al. (2001):

$$AD(\%) = 100 - \left[100 \cdot \left(\frac{marker_{feed}\ (g/kg\ DM) \cdot component_{faeces}\ (g/kg\ DM)}{marker_{faeces}\ (g/kg\ DM) \cdot component_{feed}\ (g/kg\ DM)}\right)\right]$$

where AD represents the apparent digestibility; $marker_{feed}$ and $marker_{faeces}$ are the marker concentrations in feed and faeces, respectively; $component_{feed}$ and $component_{faeces}$ are the concentrations of components in feed and faeces, respectively.

Due to limitations in the experimental design, urine samples could not be collected. Therefore, urinary N excretion (NEXu) was estimated using blood urea content. Blood was taken from the vena jugularis externa in the middle of fattening (approx. 70 kg BW) and one week before slaughter (approx. 110 kg BW). Blood serum samples were analysed on the day of collection. Following the method outlined by Kohn et al. (2005), BUN was estimated:

$$NEXu\ (g/d) = CR\ (L\ of\ cleared\ blood/(d \cdot kg\ BW)) \cdot BUN\ (g/L) \cdot BW\ (kg)$$

where NEXu represents the urinary N excretion; CR represents the clearance rate of urea; BUN represents the blood urea N; BW is the body weight.

Animal-specific clearance rate could not be determined due to the experimental design. Therefore a constant CR of 3.1 L cleared blood/(d · kg BW) was used (Kohn et al., 2005). Despite N/P-related traits were assessed throughout all individual fattening periods, only data from the finisher II period were considered for microbiome analysis. It was anticipated that traits measured during this period would show the best correlation with the gut microbiome at the end of fattening.

At the slaughterhouse, the complete gastrointestinal tract of each animal was collected. Following this, tissue samples (approx. 2×2 cm) and intestinal content (as much as possible) from the jejunum, ileum, caecum and colon were collected and transferred to a 50 ml reaction

tube and topped up with a buffer of NaCl+Tween80 as required. Sample transfer was performed on dry ice. All 208 samples were subsequently frozen at -80 °C until microbiome analysis.

Analyses of feed, faeces, and blood serum

Each diet of the 20 batches as well as the three faecal samples per animal were analysed in duplicate according VDLUFA (2012). The following analyses were carried out: dry matter (DM, method 3.1), crude ash (CA, method 8.1), N was analysed and crude protein calculated (N · 6.25) (CP, method 4.1.1, according to Kjeldahl), ether extract (EE, method 5.1.1, with HCl digestion), crude fibre (CF, method 6.1), neutral detergent fibre amylase treated expressed exclusive residual ash (aNDFom, method 6.5.1), acid detergent fibre expressed exclusive residual ash (ADFom, method 6.5.2), P (method 10.6.1) and acid insoluble ash (AIA, method 8.5). In addition, starch (method 7.2.1, polarimetric) was determined at AGROLAB LUFA GmbH Kiel (Germany). For faeces, analyses of DM, N, P and AIA were carried out according to the scheme described above (VDLUFA, 2012).

Microbiome analysis

Extraction of total genomic DNA was performed from 120 mg of appropriate material using the ZR BashingBead lysis tubes (0.1 and 0.5 mm, Zymo Research, Freiburg, Germany) in combination with the chemagic DNA Stool Kit (Perkin Elmer, Rodgau, Germany) according to the manufacturer's instructions (12-14). After addition of the lysis buffer, a mechanical lysis step was performed using the Precellys 24 Tissue Homogenizer (Bertin Instruments, Frankfurt am Main, Germany). After extraction, the DNA was stored at -20 °C until further analysis.

Library Preparation

Amplicon sequencing of the gut microbiome was performed at Life & Brain GmbH (Bonn). Here, the V3V4 region of the 16S rRNA gene was amplified in a first PCR step with the primers Bakt_341F (5'-CCTACGGGNGGCWGCAG-3') and Bakt_805R (5'-GACTACHVGGTATCTAATCC-3') in a 25 µL PCR reaction, the 2.5 µL template (5 ng/µL), 12.5 µL 2 × KAPA HiFi HotStart ReadyMix (Roche, Mannheim, Germany) and 5 µL of the corresponding primers (1 µM). PCR was performed in a thermal cycler as described below: first denaturation step at 95 °C for 3 min, followed by 25 cycles of denaturation (30 s at 95 °C), annealing (30 s at 55 °C), elongation (30 s at 72 °C) and a final elongation step at 72 °C for 5 min. In a second PCR step, dual indexes and an Illumina sequencing adapter were added using

the Nextera XT v2 Index Kit (Illumina, San Diego, CA, USA). A total volume of 50 µL of 25 µL 2× KAPA HiFi HotStart ReadyMix (Roche, Mannheim, Germany), 5 µL of the corresponding Nextera XT Index primer and 10 µL PCR quality water were used per sample for the second PCR reaction. Following cycling conditions were used: initial denaturation at 95 °C for 3 min, followed by 8 cycles of denaturation (30 s at 95 °C), annealing (30 s at 55 °C), elongation (30 s at 72 °C) and a final elongation step at 72 °C for 5 min. At the end of each PCR step, amplicon libraries were randomly sampled on an Agilent TapeStation 4200 with D1000 ScreenTape (Santa Clara, CA, USA) and purified with AMPure XP beads (Beckman Coulter, Krefeld, Germany). Samples were normalised to 4 nM and pooled equimolarly.

16S rRNA Sequencing

The final pool was quantitated by Qubit dsDNA HS Assay Kit from Thermo Fisher Scientific (Waltham, MA, USA) and fragment size was determined on a D1000 ScreenTape. Sequencing was carried out on a MiSeq system from Illumina using MiSeq Reagent Kit v3 with 2 × 300 cycles. Clustering was conducted at 8 pM with a 20% spike-in of PhiX. Demultiplexing was carried out on the MiSeq system.

Bioinformatics and statistical analysis

Characterisation of microbial community

16S rRNA gene sequencing data were processed using DADA2 in QIIME2 (version 2022.8) (Boylen et al., 2019). Sequence quality control and denoising were performed, including filtering of chimeric sequences. The sequences obtained after denoising were classified using SILVA databases to identify amplicon sequence variants (ASVs) for sequences with >99% sequence similarity. A rarefied abundance table with a sampling depth of 13.000 sequences was used for calculation of alpha and beta diversity metrics.

Further statistical analysis of microbiome data was conducted using the phyloseq package (McMurdie and Holmes, 2013) in RStudio (version 4.3.0). To characterise the microbial community, the relative abundances of the most abundant phyla, families and genera were calculated across all samples and (due to discovered differences) within the individual gut sections. The species diversity in the individual samples was described by alpha diversity, more precisely by observed richness and Shannon entropy (Shannon, 1948). A linear mixed model

using the lme4 package (Bates et al., 2023) in R, which was as follows, investigated the importance of different factors for alpha diversity:

$$y_{ijklmno} = \mu + b_1(FFW_{ijklmno}) + \text{gut section}_j + \text{gender}_k + \text{run}_l + \text{group}_m + s_n + i_o + (s\times\text{group})_{nm} + e_{ijklmno} \text{ (model 1)}$$

where $y_{ijklmno}$ represents the alpha diversity; μ represents the common constant of y-values; $b_1(FFW_{ijklmno})$ is the fixed linear covariate final fattening weight; gut section$_i$ (j = jejunum, ileum, caecum, colon), gender$_k$ (k = 1, 2), run$_l$ (l = 1, 2, 3, 4), and group$_m$ (m = 1, 2) are the fixed class effects; s_n represents the random effect of the AI-boar of the investigated family; i_o represents the random individual permanent effect of the host ID, and $e_{ijklmno}$ represents the effect of residual error associated with each observation.

Fixed effects influencing alpha diversity first were examined across all samples and afterwards for each of the four gut sections, performing an ANOVA and estimating least square means (package emmeans (Lenth et al., 2023)). Heritability (h^2) of alpha diversity was estimated according to the following formula from the random AI-boar effect:

$$h^2 = \frac{2\sigma_s^2}{o_p^2} \text{ (Falconer and Mackay, 1995)}$$

where h^2 is the heritability; σ_s^2 is the variance of AI-boar; σ_p^2 represents the phenotypic variance ($\sigma_s^2 + \sigma_{s\times group}^2 + \sigma_i^2 + \sigma_e^2$); $\sigma_{s\times t}^2$ represents the variance of AI-boar×group effect; σ_i^2 is the variance of the permanent effect of the individual pig; σ_e^2 represents the variance of residual error. This formula given by Falconer and Mackay (1995) reflects the full sib structure of the data set.

Possible genotype×feed-interactions (G×F) were quantified by the AI-boar×group variance ratio

$$gi^2 = \frac{\sigma_{s\times group}^2}{o_p^2}$$

where gi^2 is the interaction of AI-boar×group; $\sigma_{s\times group}^2$ is the variance of AI-boar×group; σ_p^2 is the phenotypic variance ($\sigma_s^2 + \sigma_{s\times group}^2 + \sigma_i^2 + \sigma_e^2$) (for single measured traits without σ_i^2); σ_s^2 is the variance of AI-boar; σ_i^2 is the variance of the permanent effect of the individual pig; σ_e^2 is the variance of residual error.

Differences in the composition of microbial communities between samples were described using beta diversity metrics, more precisely by Bray-Curtis dissimilarity (Bray and Curtis,

1957) and the (weighted) UniFrac (Lozupone and Knight, 2005), which additionally includes phylogenetic information, through calculating distances. These distances were presented via classical multidimensional scaling (MDS (=PCoA, principal coordinates analysis)). In addition, a restricted PerMANOVA with 999 permutations was performed to quantify the influence of final fattening weight (FFW), gender, run, group, run×group (all other interactions were removed from model due to lack of significance) and gut section (vegan package (Oksanen et al., 2022). PerMANOVA was initially conducted for all 208 samples, and subsequently, based on the results obtained from this analysis for the individual runs. Model factors were adjusted accordingly. A significance level of $p \leq 0.05$ was chosen. Additionally, a $p \leq 0.1$ was considered as trend.

Estimation of variance components and microbiome wide association study (MWAS)

To characterise the impact of the microbial community on fattening performance traits, feed efficiency and N/P excretion, variance components were estimated using two univariate models according to the procedure described by Aliakbari et al. (2022):

$$y = Xb + Z_2m + e \qquad \text{(model 2)}$$

$$y = Xb + Z_1a + Z_2m + e \qquad \text{(model 3)}$$

where y represents the vector of observations of considered traits; X, Z_1 and Z_2 are the design matrices for b, a and m, respectively; b is the vector of fixed effects; a represents the vector of random breeding values (random effect of AI-boar); m represents the vector of random microbial values, and e is vector of random residuals; with distributions $a \sim N(0, A\sigma^2_a)$, $m \sim N(0, M\sigma^2_m)$ and $e \sim N(0, I\sigma^2_e)$ (σ^2_a is the genetic variance; σ^2_m is the microbial variance; σ^2_e is the residual variance). While I represents the identity matrix, A represents the pedigree relationship matrix of hosts based on paternal origin, and M represents the microbial relationship matrix that was structured as follows (Aliakbari et al., 2022):

$$M = \frac{Z_3 \times Z_3'}{k}$$

where k is the number of ASVs and Z_3 is a matrix with dimension n × k (n is the number of hosts; k is the number of ASVs), representing the standardized individual abundance of each ASV j for host i (= z_{3ij}). This standardised individual abundance was defined as (Aliakbari et al., 2022):

$$z_{3ij} = \frac{\log(P_{ij}) - \overline{\log(P_{ij})}_j}{sd\left(\log(P_{ij})\right)_j}$$

where P_{ij} represents the relative abundance of ASV j for host i; sd is the standard deviation.

As a fixed effect, a combination of gender, group and run (16 levels) was chosen, accounting for the environmental context (Aliakbari et al., 2022). Variance components of the random effects above were estimated by REML using package sommer (Covarrubias-Pazaran, 2016). Heritability (h^2) and m^2 were estimated as follows:

$$h^2 = \frac{2\sigma_g^2}{\sigma_g^2 + \sigma_m^2 + \sigma_e^2}$$

$$m^2 = \frac{\sigma_m^2}{\sigma_g^2 + \sigma_m^2 + \sigma_e^2}$$

where h^2 is the heritability; m^2 is the microbiability; σ_g^2 represents the variance of the AI-boar; σ_m^2 represents the microbial variance; σ_e^2 represents the residual variance.

In addition to m^2, the effect of each ASV on performance traits, feed efficiency and N/P excretion was estimated via back-solving BLUP solutions as described by Aliakbari et al., (2022). Therefore, the following equation was applied:

$$E(ASV) = \widehat{ASV} = \frac{1}{k} \times Z_3' \times M^{-1} \times \hat{m}$$

where E(ASV) and $\widehat{ASV}$ represent the effect of each ASV; $\hat{m}$ is the in model 2 or 3 predicted microbial value; and k, Z_3 and M are as described above.

To calculate a standardized Z-score for each ASV, the variance of ASV estimates was determined according to Aliakbari et al. (2022):

$$var\left(\widehat{ASV}\right) = \frac{1}{k} \times Z_3' \times M^{-1} \times var(\hat{m}) \times M^{-1} \times Z_3 \times \frac{1}{k}$$

where $var(\widehat{ASV})$ represents the variance of ASV estimates; and k, Z_3, M, $\hat{m}$ are as described above.

E(ASV) and the diagonal elements of var(ASV) resulted in the $Z\text{-score}_j$ for each ASV j:

$Z\text{-score}_j = \frac{\widehat{ASV}_j}{\sqrt{var\left(\widehat{ASV}_j\right)}}$ (Aliakbari et al., 2022).

Corresponding p-values were calculated using a Chi-square test (1 degree of freedom) (R Core Team, 2012).

Significance thresholds for the MWAS were adjusted to control the error rate and to take account of multiple testing. For this purpose, a principal component analysis was applied to the correlation matrix of the ASVs (Z_3'Z_3) of each gut section (Gao et al., 2008; Aliakbari et al, 2022). The results showed that 47, 46, 50 and 50 eigenvalues accounted for 99.5% of variability in the correlation matrix of jejunum, ileum, caecum and colon, respectively. This led to a threshold for significance at 5% error rate of -log10(0.05/x) and for suggestive significance at 10% error rate of -log(0.10/x), with x being the number of eigenvalues accounting for 99.5% of variability.

4.4 Results

Realisation of dietary N/P restriction

Given that the trial was conducted under farm conditions, the precise composition of rations varied to some extent from one run to another, as extensively detailed in the previous publication (Große-Brinkhaus et al., 2023) (Table 4). It is important to consider this variability when interpreting the results concerning a comparison of the feeding groups. In total, 11 target traits were investigated related to the microbial community. These were the fattening and feed efficiency traits average daily feed consumption (ADFC), average daily gain (ADG), feed conversion ratio (FCR), residual feed intake (RFI), N/P digestibility (ADN, ADP), N/P intake (NIN, PIN), faecal N/P excretion (NEXf, PEX), and urinary N excretion (NEXu).

Characterisation of microbial community

The characterisation of the microbial community showed, as expected, a clear differentiation between the small (jejunum and ileum) and the large intestine (caecum and colon) at the level of phylum, family and genus. Figure 7 and Table 13 illustrate the distribution of the most abundant microbes.

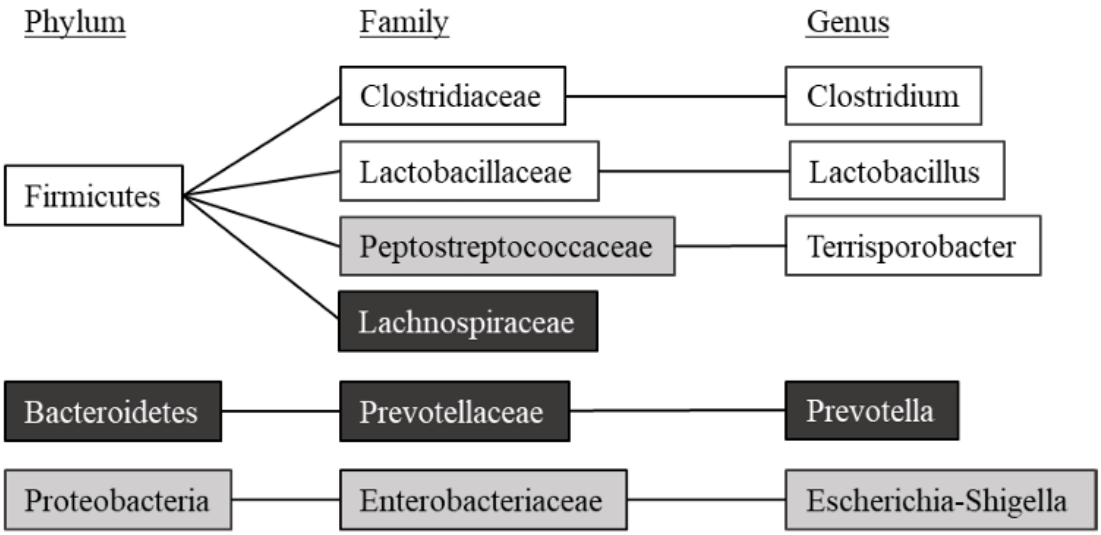


Figure 7: Most abundant phyla, families and genera and their phylogenetic relationship
Note: white background, present in small and large intestine; light grey background, primary present in small intestine; dark grey background, primary present in large intestine.

With regard to all 208 samples, the most common phyla were Firmicutes (70 %), Bacteroidetes (15 %), Proteobacteria (13 %) and Campilobacteroidetes (1 %). Except for the ileum, where it was Proteobacteria, the phylum Firmicutes was also by far the most frequent phylum in the individual gut sections. While Bacteroidetes rarely occurred in the small intestine (jejunum and

ileum), but often in large intestine (caecum and colon), it is worth noting that Proteobacteria were found in small, but not in large intestine (Figure 7, Table 13).

In terms of families, Clostridiaceae was the most abundant (12 %), closely followed by Peptostreptococcaceae (11 %), Lactobacillaceae and Lachnospiraceae (10 %). In the large intestine, Prevotellaceae and Lachnospiraceae were most abundant, which did not occur in remarkable amounts in small intestine. The same applies to Peptostreptococcaceae and Enterobacteriaceae being common in small, but not in large intestine.

Table 13: Relative abundances of most abundant phyla, families and genera across all samples and in gut sections jejunum, ileum, caecum and colon

	Relative abundance (%)				
	all samples (n = 208)	**Jejunum (n = 52)**	**Ileum (n = 52)**	**Caecum (n= 52)**	**Colon (n= 52)**
Phylum					
Firmicutes	68.6	79.3	31.1	67.3	66.0
Bacteroidetes	14.9	n.p.	3.4	27.7	24.2
Proteobacteria	13.2	20.0	32.0	3.3	2.9
Campilobacterodetes	1.2	n.p.	n.p.	n.p.	2.9
Family					
Clostridiaceae	11.7	19.3	16.2	9.0	8.9
Peptostreptococcaceae	11.4	18.0	15.1	n.p.	n.p.
Lactobacillaceae	10.3	35.5	17.0	12.6	8.9
Lachnospiraceae	10.1	n.p.	n.p.	14.3	11.3
Enterobacteriaceae	7.8	19.3	28.0	n.p.	n.p.
Prevotellaceae	2.4	n.p.	n.p.	23.1	16.9
Genus					
Lactobacillus	18.5	35.5	17.0	13.0	9.1
Clostridium_sensu_stricto1	12.4	18.0	15.0	8.9	8.5
Escherichia-Shigella	11.9	19.3	28.1	n.p.	n.p.
Terrisporobacter	7.2	13.8	9.1	3.6	3.4
Prevotella	6.9	n.p.	n.p.	13.0	10.8

Abbreviations: n.p., phylum/family/genus is not present in gut section.

The most abundant genera found in all samples were *Lactobacillus* (19 %), followed by *Clostridium_sensu_stricto1*, *Escherichia-Shigella* (both 12 %), *Terrisporobacter* and *Prevotella* (both 7 %). It is noticeable that the abundance of *Lactobacillus*, *Clostridium* and *Terrisporobacter* decreased from gut section to gut section: their quantity was greatest in jejunum and lowest in colon. *Escherichia-Shigella* only appeared in the small intestine in noteworthy amount, *Prevotella* only in large intestine (Figure 7, Table 13).

Alpha diversity

Alpha diversity metrics were estimated using "observed richness", which is the number of different species in a sample (direct counting), and "Shannon entropy", which considers both the species richness and evenness in a sample (Shannon, 1948). Due to rarefaction (section "Characterisation of microbial community"), five samples from jejunum and eleven samples from ileum were removed before estimating diversity metrics.

The impact of host's final fattening weight, gender, feeding group, run and gut section on alpha diversity metrics is shown in Table 14. The fixed effect gut section was significant for alpha diversity metrics. As expected, alpha diversity increased steadily from jejunum to ileum, to caecum, and up to colon, whereby almost all differences were statistically proven. In general, the large intestine sections were characterised by a significantly higher alpha diversity than the small intestine sections, indicating a higher species richness in caecum and colon (Table 15). The remaining fixed effects, including the feeding group (Table 16), did not have significant influence on the alpha diversity, regardless of considering all samples or the individual gut sections (Table 14).

Table 14: Influence of final fattening weight, gender, group, run and gut section on alpha diversity (observed richness and Shannon entropy) across all samples and for gut sections jejunum, ileum, caecum and colon (results of ANOVA)

	FFW	**Gender**	**Group**	**Run**	**Gut section**	**MSE**	**CV**
All samples (n= 192)							
Observed richness	0.563	0.148	0.603	0.234	≤0.001	8198.38	0.30
Shannon entropy	0.517	0.374	0.408	0.517	≤0.001	0.474	0.19
Jejunum (n = 47)							
Observed richness	0.831	0.200	0.278	0.792	/	450.29	0.32
Shannon entropy	0.622	0.372	0.827	0.802	/	2.21	0.41
Ileum (n = 41)							
Observed richness	0.645	0.603	0.726	0.260	/	12763.18	0.63
Shannon entropy	0.953	0.630	0.745	0.701	/	0.82	0.35
Caecum (n = 52)							
Observed richness	0.947	0.247	0.917	0.864	/	11521.69	0.24
Shannon entropy	0.394	0.966	0.306	0.945	/	0.05	0.05
Colon (n = 52)							
Observed richness	0.303	0.463	0.561	0.463	/	9092.83	0.18
Shannon entropy	0.183	0.600	0.355	0.786	/	0.23	0.09

Abbreviations: FFW, final fattening weight.

Table 15: LSMeans (±SE) of jejunum, ileum, caecum and colon for alpha diversity (observed richness and Shannon entropy)

	Jejunum	**Ileum**	**Caecum**	**Colon**
Observed richness	70.8 ± 16.9^{a}	139.0 ± 17.8^{b}	447.0 ± 15.8^{c}	517.4 ± 15.8^{d}
Shannon entropy	1.91 ± 0.12^{a}	2.66 ± 0.12^{b}	4.76 ± 0.11^{c}	4.89 ± 0.11^{c}

Note: different letters indicate statistically significant differences ($p \leq 0.05$).

Table 16: LSMeans (±SE) of CON and LP group for alpha diversity (observed richness and Shannon) entropy across all samples and in jejunum, ileum, caecum and colon

	CON	**LP**
All samples (n = 192)		
Observed richness	298±12.5[a]	289±12.8[a]
Shannon entropy	3.59±0.08[a]	3.49±0.09[a]
Jejunum (n = 47)		
Observed richness	71.2±7.04[a]	60.0±7.53[a]
Shannon entropy	3.67±0.16[a]	3.62±0.17[a]
Ileum (n = 41)		
Observed richness	137±29.8[a]	123±31.4[a]
Shannon entropy	2.67±0.34[a]	2.58±0.25[a]
Caeum (n = 52)		
Observed richness	447±24.3[a]	4451±24.3[a]
Shannon entropy	4.83±0.16[a]	4.59±0.16[a]
Colon (n =52)		
Observed richness	537±25.8[a]	515±25.8[a]
Shannon entropy	4.98±0.17[a]	4.75±0.17[a]

Note: different letters indicate statistically significant differences ($p \leq 0.05$).

Abbreviations: CON, control group; LP, group with dietary N/P restriction.

Results of the estimated random effects of model 1 are shown in Table 17. Estimates of h^2 were close to zero or zero, regardless of the used method (observed richness vs. Shannon) and gut section. The interaction component gi^2 was zero in the analysis of all samples, of ileum and caecum (observed richness) and jejunum (Shannon entropy). Higher gi^2 values between 0.15 and 0.73 were found for colon, jejunum (obeserved richness) and caecum (Shannon entropy). Due to the low sample size, only the highest value in the caecum (gi^2=0.73) deviates significantly (p=0.047) from zero.

Table 17: Heritability, permanent environmental effect, and proportionate variance of AI-boar×group for alpha diversity (observed richness and Shannon entropy) across all samples and for jejunum, ileum, caecum, and colon

	h^2	**pe**	**gi^2**
All samples (n = 192)			
Observed richness	0.00	0.02	0.00
Shannon entropy	0.01	0.00	0.00
Jejunum (n = 47)			
Observed richness	0.00	/	0.18
Shannon entropy	0.00	/	0.00
Ileum (n = 41)			
Observed richness	0.00	/	0.00
Shannon entropy	0.00	/	0.00
Caecum (n = 52)			
Observed richness	0.00	/	0.00
Shannon entropy	0.00	/	0.73*
Colon (n = 52)			
Observed richness	0.00	/	0.15
Shannon entropy	0.00	/	0.34

Note: * = $p \leq 0.05$.

Abbreviations: h^2, heritability; pe, permanent environmental effect; gi^2, proportionate variance of AI-boar×group.

Beta diversity

Regarding beta diversity, PerMANOVA results confirmed significant differences ($p<0.05$) in species composition between the gut sections and the four experimental runs. As expected, these findings hold for both measurement methods (Table 18). Using the quantitative measurement method (Bray-Curtis), differences in species composition between CON and LP were observed, as well as in the run×group interaction. However, using the phylogeny-incorporating measurement method (UniFrac), these differences were less pronounced. Additionally, a significant deviation in beta diversity was shown between male and female pigs.

Table 18: Influence of final fattening weight, gut section, run, group, gender and run×group on beta diversity (Bray-Curtis and UniFrac) across all samples, in jejunum, ileum, caecum, colon, and in run 1, 2, 3, and 4 (results of PerMANOVA)

	FFW	**Gut section**	**Run**	**Group**	**Gender**	**Run×Group**
All samples (n = 192)						
Bray-Curtis	0.909	0.001	0.001	0.048	0.059	0.005
UniFrac	0.183	0.001	0.001	0.097	0.031	0.217
Runs						
Run 1 (n=31)						
Bray-Curtis	0.377	0.001	/	0.020	0.004	/
UniFrac	0.284	0.001	/	0.173	0.137	/
Run 2 (n=50)						
Bray-Curtis	0.523	0.001	/	0.567	0.292	/
UniFrac	0.006	0.001	/	0.164	0.092	/
Run 3 (n=49)						
Bray-Curtis	0.613	0.001	/	0.065	0.376	/
UniFrac	0.025	0.001	/	0.099	0.098	/
Run 4 (n=61)						
Bray-Curtis	0.153	0.001	/	0.011	0.028	/
UniFrac	0.039	0.001	/	0.189	0.019	/

Abbreviations: FFW, final fattening weight.

Principal coordinates analysis, based on Bray-Curtis dissimilarity, further elucidated the difference in species composition, visually demonstrating dissimilarities between samples (Figure 8). Applied on UniFrac, PCoA yielded almost identical patterns, so they are not presented. Distinct differences were observed in the comparison of the different gut sections; microbial compositions in the small intestine sections (jejunum and ileum) were markedly different from those in large intestine (caecum and colon). Additionally, greater variation was observed in ileum and jejunum samples than in caecum and colon samples. Although visual differences between samples of feeding groups were not as prominent, considering the variation in the diet composition, distinct patterns were observed for individual runs (Figure 8). Especially, run 2 differed unexpectedly from the others, while samples from run 1 and 4 were very similar in their species composition (Table 18).

The significant run×group effect was evident in the analysis across all 208 samples. However, direct estimation of least squared means (LSMeans) of run×group was not feasible. Therefore, as an alternative strategy, the analysis was performed separately within each run. Within the individual runs, the gut section significantly influenced species composition, mirroring the findings across all runs. Results based on Bray-Curtis showed significant differences in beta diversity between CON and LP for all runs except run 2, as well as between male and female pigs for run 1 and 4. Results based on UniFrac indicated a significant influence of final fattening weight on beta diversity in runs 2, 3 and 4 (Table 18).

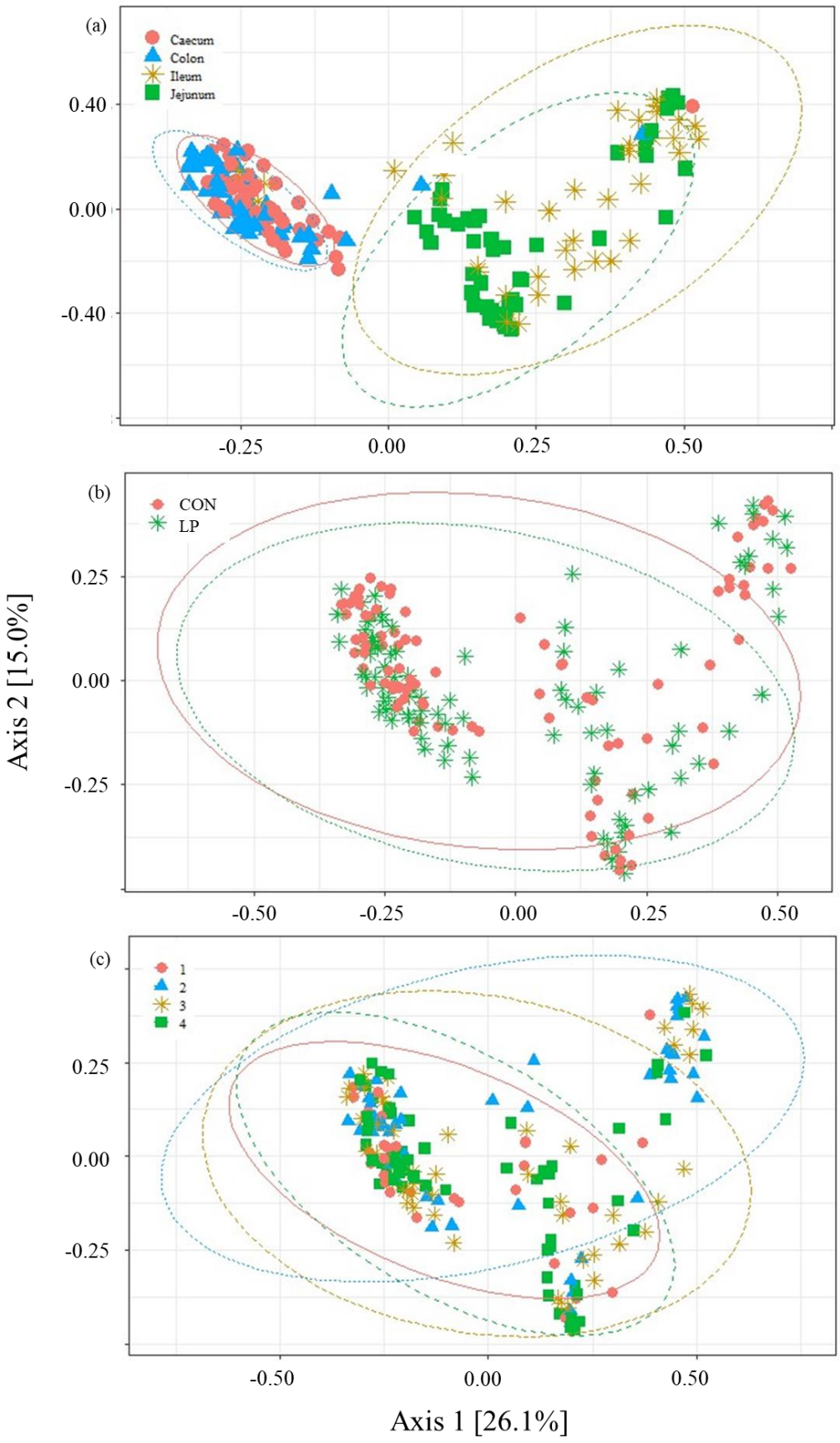


Figure 8: Beta diversity (Bray-Curtis) of the intestinal microbiome

Note: principal coordinate analysis in (a), gut sections (jejunum, ileum, caecum, colon); (b), control group; (CON), group with dietary N/P restriction (LP); (c), in runs (1,2,3,4).

Estimation of variance components

Estimates of h^2 and m^2 are derived from the variance component estimation based on model 3, which included the AI-boar as random effect, considering the host genetics aspect. In contrast to model 3, model 2, which did not include the AI-boar effect, provided nearly identical m^2 estimates. As such, these results are not explicitly presented (Table 19).

For most traits medium to high h^2 were estimated ranging between 0.32 and 0.79, which underlines the distinct additive genetic background of these traits with the highest values for feed efficiency traits feed conversion ratio and residual feed intake, as well as urinary N excretion and P digestibility (0.59-0.79). The only exceptions were the h^2 values for average daily gain, faecal N and P excretion being below 0.10 (Table 19). All variance ratios were estimated separately by using gut section specific microbiome variance-covariance matrices (M). However, there were only small differences between h^2 estimates for each trait using these different M-matrices. The largest difference between gut section and trait specific h^2 estimates was 0.07, but most of these deviations were close to zero. From this, it can be concluded that h^2 estimates are relative robust to the choice of M-matrix.

Microbiability values, derived from the gut section specific M-matrix, were generally lower compared to h^2 values with one exception (average daily gain in colon). Only eight of 44 m^2 values exceeding 0.10. Among these, four were found within the colon microbiome; two were estimated within the caecum and jejunum, respectively, while none within the ileum. The microbiome of the caecum and colon significantly influenced the feed efficiency traits feed conversion ratio and residual feed intake with m^2 ranging from 0.04 to 0.61. Noteworthy moderate m^2 for digestibility of N (0.27) and P (0.22) were found in the jejunum, and for average daily gain (0.17) and P digestibility (0.18) in colon. Moreover, it appears that the microbiome revealed a weak influence on urinary N excretion in ileum, caecum, and colon (Table 19).

Table 19: Additive genetic variance, heritability, microbial variance and microbiability for performance, feed and N/P excretion traits for jejunum, ileum, caecum and colon

		Jejunum	**Ileum**	**Caecum**	**Colon**
ADG	h^2	0.07±0.39	0.07±0.37	0.07±0.39	0.00±0.37
	m^2	0.00±0.22	0.00±0.15	0.00±0.44	0.17±0.66
ADFC	h^2	0.32±0.42	0.32±0.42	0.32±0.42	0.32±0.42
	m^2	0.00±0.23	0.00±0.15	0.00±0.42	0.00±0.62
FCR	h^2	0.77±0.40	0.77±0.40	0.77±0.37	0.79±0.41
	m^2	0.00±0.19	0.00±0.13	0.61±0.43	0.27±0.51
RFI	h^2	0.67±0.41	0.67±0.41	0.72±0.40	0.67±0.41
	m^2	0.00±0.20	0.00±0.13	0.26±0.43	0.04±0.57
ADN	h^2	0.23±0.39	0.15±0.40	0.15±0.40	0.15±0.40
	m^2	0.27±0.31	0.004±0.15	0.00±0.44	0.00±0.63
NIN	h^2	0.39±0.42	0.38±0.42	0.38±0.42	0.38±0.41
	m^2	0.00±0.22	0.00±0.14	0.00±0.42	0.00±0.61
NEXu	h^2	0.00±0.35	0.00±0.38	0.00±0.38	0.00±0.38
	m^2	0.00±0.23	0.00±0.15	0.00±0.44	0.00±0.63
NEXf	h^2	0.59±0.07	0.67±0.13	0.67±0.14	0.68±0.13
	m^2	0.00±0.12	0.06±0.12	0.03±0.22	0.10±0.35
ADP	h^2	0.63±0.40	0.67±0.41	0.68±0.41	0.68±0.41
	m^2	0.22±0.27	0.00±0.13	0.00±0.37	0.18±0.59
PIN	h^2	0.35±0.42	0.35±0.42	0.34±0.42	0.34±0.42
	m^2	0.00±0.22	0.00±0.14	0.00±0.42	0.00±0.61

		Jejunum	Ileum	Caecum	Colon
PEX	h^2	0.01±0.38	0.02±0.38	0.02±0.39	0.02±0.38
	m^2	0.001±0.23	0.00±0.15	0.00±0.44	0.00±0.63

Abbreviations: h^2, heritability; m^2, microbiability; ADG, average daily gain; ADFC, average daily feed consumption; FCR, feed conversion ratio; RFI, residual feed intake; N, nitrogen; P, phosphorus; AD, apparent digestibility; IN, intake; NEXf, faecal N excretion; NEXu, urinary N excretion; PEX, faecal P excretion.

Microbiome wide association analysis (MWAS)

The basis for determining the effect of each ASV were BLUP predicted individual microbial effects estimated with model 3. First, it is important to mention that upon adjusting the significance thresholds for MWAS, no associations between ASVs and target traits reached levels of significance ($p \leq 0.001$) or tendentially significance ($p \leq 0.002$), as illustrated in Figure 9.

Nevertheless, 497 different ASVs were detected, which were nominally significant ($p \leq 0.05$) or nominally tendentially significant ($p \leq 0.1$) in their associations with one of five target traits. Specifically, 58 ASVs in jejunum were associated with faecal P excretion, 384 ASVs in ileum were associated with N digestibility, 6 ASVs in ileum and 15 ASVs in caecum were associated with urinary N excretion, and 20 ASVs and 15 ASVs in colon were associated with average daily gain and P digestibility, respectively. Table 20 lists the ASVs with the lowest p-values for each trait and gut section, with a maximum of five ASVs per trait and gut section. All corresponding regression coefficients were less pronounced and had positive signs (Table 20). This indicates that the greater the presence of the corresponding ASV in the respective gut sections, the higher the phenotypic expression of the associated trait. It is worth noting, that higher phenotypic values are desirable for average daily gain, and N and P digestibility, while lower ones are preferred for N/P excretion traits.

Twenty-one of the 26 ASVs, listed in Table 20, belonged to the phylum Firmicutes, while the remaining were classified as Bacteroidetes and Desulfobacterota. In the jejunum, ASVs associated with faecal P excretion belonged to the classes of Bacteroidia, Negativicutes and Clostridia, and to the order of Bacteroidales, Veillonellales-Selemonadales and Oscillospirales, respectively. In the ileum, ASVs associated with N digestibility, belonged to Bacteroidia and Clostridia, and to the order of Bacteroidales, Oscillospirales, Peptococcales, and Lachnospirales. Regardless of the gut section ileum or caecum, all ASVs found in association

with urinary N excretion belonged to the phylum Firmicutes, the class Clostridia, and the order Oscillospirales. They were further subdivided into different families and genera. The three ASVs found in colon for average daily gain, were not only Clostridia, but also Bacilli, and did not belong to the order of Oscillospirales, as the previously mentioned ones, but to the Christensenellales, Lactobacillales and Clostridiales. The ASVs in colon, associated with P digestibility, were predominantly assigned to the Clostridia class. However, one ASV also belonged to Bacteroidia, and another to Desulfuromonadia, and the order Bradymonadales.

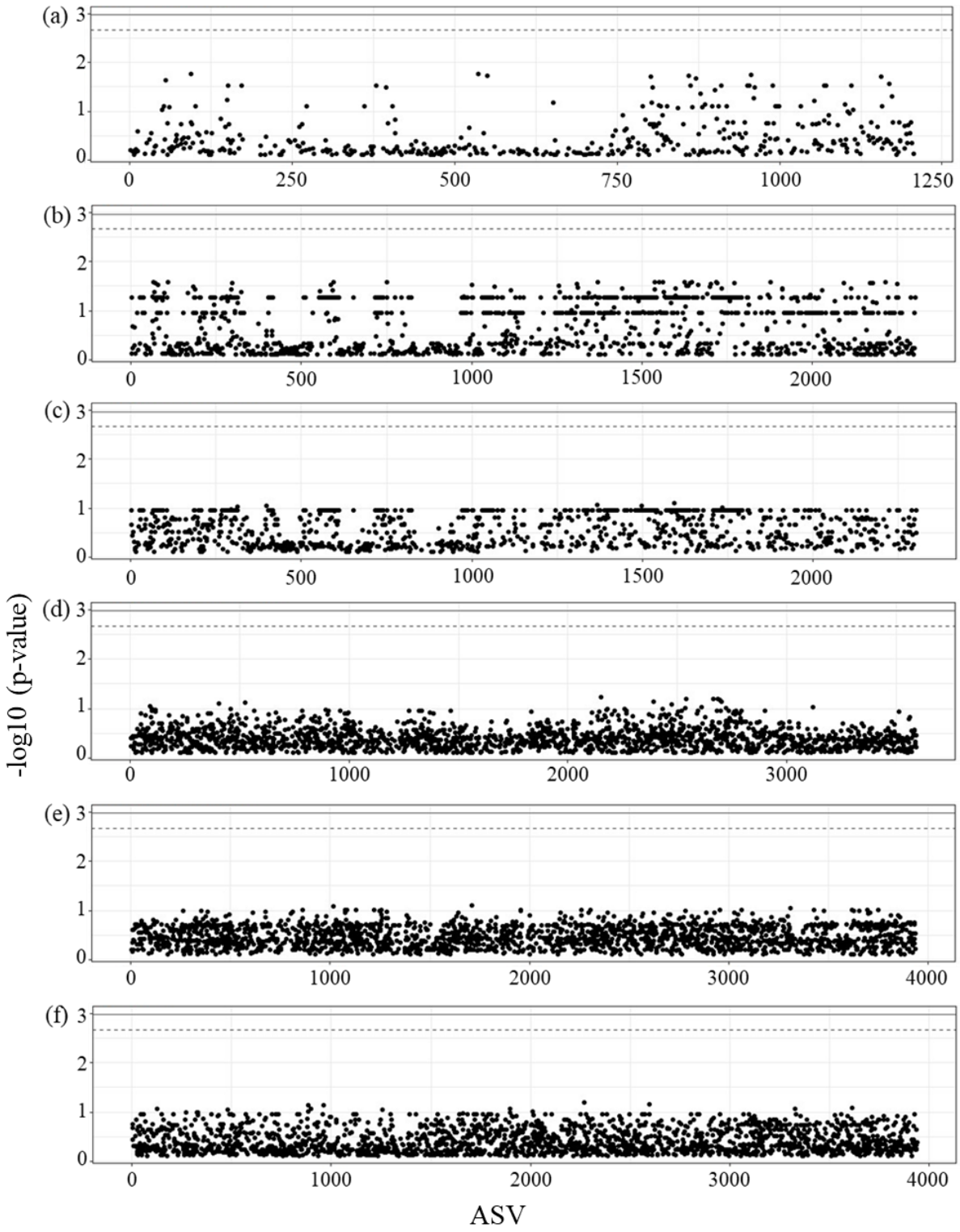


Figure 9: Results of microbiome wide association analysis (MWAS) of amplicon sequence variants (ASVs)

Note: (a), in jejunum for faecal P excretion; (b), in ileum for digestibility of N; (c), in ileum for urinary N excretion; (d), in caecum for urinary N excretion; (e), in colon for average daily gain; (f), for digestibility of P; solid and dashed lines represent significant and suggestively significant thresholds at 5% and 10% error rates, respectivel

Table 20: Taxonomy and regression coefficient estimates from back solving BLUP solutions for the ASVs with nominal significant ($p \leq 0.05$) or nominal suggestive significant ($p \leq 0.1$) associations with performance and N/P-related traits

Gut section	Associated trait	ASV	p-value	Phylum	Class	Order	Family	Genus	Regression coefficient
Jejunum	PEX	ASV0505	0.017	Bacteroidetes	Bacteroidia	Bacteroidales	Muribaculaceae	*undefined*	0.00002
		ASV2178	0.017	Firmicutes	Negativicutes	Selenomonadales	Selenomonadaceae	*undefined*	0.00002
		ASV2285	0.019	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	*Megasphaera*	0.00002
		ASV3534	0.019	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Alloprevotella*	0.00002
		ASV4383	0.018	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*undefined*	0.00002
Ileum	ADN	ASV4523	0.026	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00004
		ASV0398	0.026	Bacteroidetes	Bacteroidia	Bacteroidales	Muribaculaceae	*undefined*	0.00004
		ASV2100	0.026	Firmicutes	Clostridia	Peptococcales	Peptococcaceae	*undefined*	0.00004
		ASV4528	0.026	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00004
		ASV5370	0.026	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Moryella*	0.00004
Ileum	NEXu	ASV3837	0.085	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*undefined*	0.00264
		ASV4148	0.088	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*undefined*	0.00269
		ASV4385	0.078	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*undefined*	0.00148
Caecum	NEXu	ASV3869	0.058	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*undefined*	0.00152
		ASV4461	0.063	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00141
		ASV4672	0.065	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00129
		ASV4716	0.065	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00128
		ASV4727	0.067	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00123

Gut section	Associated trait	ASV	p-value	Phylum	Class	Order	Family	Genus	Regression coefficient
Colon	ADG	ASV1703	0.083	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.05490
		ASV2896	0.080	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	*RF39*	0.05018
		ASV5086	0.088	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridium*	0.04733
Colon	ADP	ASV3691	0.064	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotellaceae_UCG-003*	0.00667
		ASV4157	0.067	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*undefined*	0.00495
		ASV1642	0.071	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia vadinBB60 group*	0.00497
		ASV1554	0.072	Desulfobacterota	Desulfuromonadia	Bradymonadales	Bradymonadaceae	*undefined*	0.00574
		ASV5633	0.084	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Eubacterium ruminantum group*	0.00454

Abbreviations: ADG, average daily gain; AND, N digestibility; ADP, P digestibility; PEX, faecal P excretion; NEXu, urinary N excretion.

4.5 Discussion

Several studies have investigated the composition of the gut microbiome in various gut sections (e.g. Yang et al., 2016, Szabó et al., 2023). However, up to now the influence of the microbiome on the phenotypic expression of hosts' target traits, i.e., m^2, has been investigated only in colon and faecal samples (Camarinha-Silva et al., 2017, Aliakbari et al., 2022). Thus, the novel objective of this study was to comprehensively investigate the microbial community, as well as h^2 and m^2 related to fattening performance and N/P excretion, across different sections of the intestine.

In general, small and large intestine differ in function, anatomy and intestinal environment. The small intestine serves as the primary site for nutrient digestion and absorption, while the large intestine is important for host's immune system and maintenance of health (Yang et al., 2016, Szabó et al., 2023). Studies in humans and mice have shown, that compared to the large intestine, the small is characterised by a higher oxygen level (He et al., 1999), a faster transit time of chymus (Schwarz et al., 2002), and the presence of antimicrobial peptides (Bevins and Salzman 2011). These physiological differences result in variations in the composition and diversity of microbial community.

Characterisation of microbial community

The three most prevalent phyla observed in the present study, namely Firmicutes, Bacteroidetes, and Proteobacteria, align with findings from previous studies (Looft et al., 2014, Holman et al., 2017). These phyla collectively form to the "core microbiome" in pigs, characterised by their continuous abundance in >90% of all gastrointestinal tract samples (Holman et al., 2017). The most prevalent families in the present study can be attributed to the above-mentioned phyla, with the majority belonging to *Firmicutes*. The most common genera, namely *Clostridium*, *Lactobacillus*, *Terrisporobacter*, *Prevotella*, and *Escherichia-Shigella*, can in turn be assigned to these families. This pattern is generally consistent with findings in literature (Looft et al., 2014, Yang et al., 2016). An exception is *Terrisporobacter*, which was relatively high abundant in the present study, potentially influenced by factors like sample size, host's breed and age, and environmental conditions, including feed diets (Patil et al., 2020).

In our study, significant differences between the small and large intestine in the microbial community composition were observed. Consistent with previous research (Looft et al., 2014, Yang et al., 2016, Holman et al., 2017), Proteobacteria were enriched in small intestine, Bacteroidetes in large intestine. This corresponds to the exclusive presence of *Escherichia-*

Shigella (phylum Proteobacteria) in small intestine, and of *Prevotella* (phylum Bacteroidetes) in large intestine. These distinctions likely stem from their oxygen-related characteristics: Proteobacteria include aerobic, anaerobic and facultative anaerobic species (Kersters et al., 2006); whereas Bacteroidetes are obligate anaerobes (Spence et al., 2006).

Alpha diversity

A clear distinction between small and large intestine was also evident in alpha diversity. Regardless of the used metric (observed richness or Shannon entropy), ANOVA results revealed a significant influence of the gut section on species diversity. Alpha diversity increased along the course of the intestine with greater species diversity observed in caecum and colon compared to jejunum and ileum. This consistency with previous studies (Looft et al., 2014, Yang et al., 2016, Holman et al., 2017) may attributed to the lower passage rate of chyme in large intestine (Schwarz et al., 2002), reduced antimicrobial factors (Bevins and Salzman 2011), as well as an increased supply of unabsorbed fermentable substrates, with particular emphasis on soluble fibre (Kelly et al., 2017). The remaining fixed effects (model 1), including feeding group, were not significant for microbial species diversity. This could potentially be due to the limited magnitude of changes in dietary N/P content and proportion of feed components in CON and LP.

Estimates of the permanent environmental effect for alpha diversity were close or zero, suggesting that alpha diversities in the four gut sections can be considered as independent traits. Heritability estimates for all samples and for each gut section were nearly negligible. This is in contrast with the findings of Lu et al. (2018), who reported h^2 values up to 0.20. Possible reasons for these discrepancies could be attributed to differences in breeds (Piétrain sires (present study) vs. Duroc sires (Lu et al., 2018)) and the number of observations per sire (52 pigs from 19 sires (present study) vs. approx.1000 pigs from 28 sires (Lu et al., 2018)). The low h^2 values for alpha diversity in the present study complicate the interpretation of the interaction component gi^2 related to relevant rank shifts of AI-boars within the feeding groups. However, this gi^2 values provide a (weak) evidence for AI-boar×group playing a role in microbial species diversity, similar to what has been demonstrated for N/P intake and faecal excretion in our previous study (Große-Brinkhaus et al., 2023). Further investigation in microbiome composition may elucidate the biological background underlying N/P intake and excretion.

Beta diversity

In the present study, beta diversity exhibited distinct patterns across various gut sections. Consistent with the observations of Yang et al. (2016), a clustering of the microbiome composition was noted, with distinct groupings observed between the jejunum and ileum on one side, and the caecum and colon on the other. This clear demarcation can be attributed to the anatomical, functional, and physiological differences between the small and large intestine.

Along with the influences of the factor gut section, the interaction feeding group×run, significantly influenced Bray-Curtis dissimilarity. Distinctions between feeding groups were particularly pronounced in run 1 and 4, and to a lesser tendentially extent in run 3. In run 2, the group factor was not significant, possibly due to the non-realised N/P reduction (Table 4). The variations observed between CON and LP in the Bray-Curtis dissimilarity in run 1, 4, and 3 may stem from differences in crude protein (CP) and fibre content. Zhang et al. (2020) reported that high-protein diets could lead to a higher amount of undigested protein in large intestine, serving as food source for (primarily pathogenic) bacteria. The reduction in CP content resulting from the decrease in rapeseed meal in the present study was associated with an increase in the proportion of grains, implicating dietary fibre as well. Fibre, particularly in its soluble form, can be a substrate for (beneficial) microbes contributing to intestinal health (Szabó et al., 2023). Consequently, lowering CP content while simultaneously increasing fibre content appears to have a favorable impact on the development of the bacterial community and host's intestinal health (Szabó et al., 2023).

In the present study, differences in the composition of the gut microbiome between genders were observed in some instances, particularly obvious in the caecum. These disparities in the microbiome of entire male and female pigs are consistent with results of previous studies; e.g. He et al. (2019) reported that the faecal microbiome of females was dominated by species related to obesity and energy harvest, whereas the microbiome of boars was dominated by species associated with carbohydrate metabolism. Furthermore, a significant interaction between gut microbiome and sex hormone metabolism has been described (He et al., 2019).

In run 2, 3 and 4, final fattening weight was significantly related to the phylogeny incorporating UniFrac distance. This could be attributed to the association of different phyla with distinct metabolism pathways. It is well known that the Firmicutes to Bacteroidetes ratio is linked to obesity occurrence, with Firmicutes playing a crucial role in the regulation of hunger and satiety, while Bacteroidetes are associated with immune modulation (Stojanov et al., 2020). Consistent with this, Pedersen et al. (2013) demonstrated a positive correlation between the

weight of finisher pigs and the proportion of Firmicutes, while the correlation between weight and Bacteroidetes was negative.

Estimation of variance components: heritability and microbiability

After characterising the bacterial community, the next step was the estimation of the variance components for target traits including h^2 and m^2, and conducting MWAS. Heritability values derived from model 3 exceeded the m^2 estimates, with one exception (average daily gain in colon). This suggests that the host genetics usually had a greater impact on the target traits than the variation within the microbiome. Alikabari et al. (2022) also reported $h^2 > m^2$ results for traits related to growth performance and feed efficiency. However, Camarinha-Silva et al. (2017) could confirm this for average daily gain, but not for average daily feed consumption and feed conversion ratio. Regarding N/P excretion traits, to our knowledge, there is a lack of available data presenting m^2 estimates with the exception of estimations by Verschuren et al. (2020) for digestibility of CP.

In a previous study by Große-Brinkhaus et al. (2023), an extended data set (N=103) was analysed, which partly overlaps with the data used in the present study, but without microbiome information. Comparing the estimated h^2 values, minor differences were found between these surveys, in general. However, it is noteworthy that the h^2 of P digestibility was substantially higher in the present study (0.63-0.68 vs. 0.00-0.39). Differences in sample size and the abandonment of microbiome information may account for this disparity.

As expected, the estimated h^2 values in the present study differ only negligibly between gut sections. Heritability for average daily gain was lower than reported in literature (0.00-0.07 vs. 0.42-0.47). Conversely, for feed conversion ratio and residual feed intake, h^2 estimates were higher than in literature (FCR: 0.77-0.79; RFI: 0.67-0.72 vs. FCR: 0.19-0.35; RFI: 0.30-0.32) (Camarinha-Silva et al., 2017, Aliakbari et al., 2022). These discrepancies could be due to differences between studies in sample size, gut section, host gender, and breed. Regarding breed and sex differences, in the present study, intestinal samples originated from 26 gilts and 26 entire boars of type German Pietrain×German Landrace. In contrast, in the studies of Camarinha-Silva et al. (2017) and Aliakbari et al. (2022) faecal samples from 207 female Pietrain pigs and 604 female and castrated male Large White pigs, respectively, were analysed. Heritability estimates for faecal N and P excretion were much lower than values reported by the study of Saintilan et al. (2013), being 0.31-0.37 and 0.29-0.40 for total N- and P-excretion,

respectively. However, the h^2 of N digestibility was consistent with the findings of Déru et al. (2021).

In contrast to h^2 estimates, significantly more pronounced m^2 values were found in the across gut sections analysis. Regarding the impact of the microbiome in the small intestine (jejunum and ileum) only negligible m^2 values of most target traits were estimated. Exceptions were observed for microbial influence on N and P digestibility in jejunum: Given the jejunum's role as the primary site for nutrient absorption (Szabó et al., 2023), the distinct m^2 values of 0.27 and 0.22 may be explained by the known impact of the microbiome in the host's metabolism (Wang et al., 2020).

The m^2 for average daily feed consumption in the whole large intestine and average daily gain in the caecum were zero, which aligns with the findings of Aliakbari et al. (2022). The moderate m^2 of 0.17 for average daily gain in colon suggests a microbial influence on this trait and falls within the range of m^2 reported in literature (Camarinha-Silva et al., 2017, Aliakbari et al., 2022). However, high m^2 values were observed for the feed efficiency traits feed conversion ratio and residual feed intake in large intestine, particularly in caecum. This emphasises the significant role of the microbiome modulating feed efficiency, consistent with findings by Bergamaschi et al. (2020a), who identified genera (e.g. Lactobacillus) associated with these traits. Physiologically, this aligns with the large intestine's function as the primary site for microbial fermentation, conferring various benefits to the host (Yang et al., 2016, Szabó et al., 2023).

Regarding N/P-related traits, m^2 estimates in the large intestine, particularly in colon, indicated microbial influence on P digestibility and urinary N excretion, but not on N and P intake, and faecal N and P excretion. Surprisingly, there was also no discernible influence of the microbiome on N digestibility. This result contrasts with the findings of Verschuren et al. (2020), who reported an m^2 of 0.93 for the digestibility of CP, considering CP being in a constant ratio to N. Additionally, the observed lack of microbial influence on N digestibility contradicts previous studies (Mosenthin et al., 1992, Morgan and Whittemore, 1998), which highlighted the microbial synthesis of protein in the large intestine.

In general, the small sample size and the corresponding high standard errors of the m^2 values (Tab. 9) limit the explanatory power of our work. However, the results of the present study are at least suggestive indicators that the microbiome influences important target traits in pig production, including feed efficiency and N/P digestibility.

Microbiome wide association analysis

Microbiome wide association analyses found in literature have been based on colon or faecal samples and solely focused on traits related to fattening performance (Camarinha-Silva et al., 2017, Aliakbari et al., 2022). The present study provides MWAS results for individual gut sections and investigates not only fattening performance but also N/P excretion related traits.

As a first result, we did not find any ASV, which was significantly associated ($p \leq 0.001$) with any target trait. This disappointing result can be explained by the limited sample size. Moreover, the applied conservative method according to Aliakbari et al. (2022) to address the problem of multiple testing is questionable. Against this background, ASVs with p-values below the nominal ($p \leq 0.05$) or nominal suggestive significance ($p \leq 0.01$) thresholds in different parts of the intestine are encouraging candidates which are worthwhile to discuss, but should be verified in further studies.

Despite the clear differences observed in microbial colonization between the small and large intestine, a similar number of nominal or suggestive nominal ASVs was found in both. Possible reasons explaining this remain unclear, given the limited research base. ASVs were detected for the traits faecal P excretion in the jejunum, N digestibility and urinary N excretion in the ileum, urinary N excretion in the caecum, and average daily gain and P digestibility in the colon. The corresponding regression coefficients fell within low ranges, indicating that traits are influenced by numerous ASVs, each with a small effect. Furthermore, former studies identified species associated with feed intake and feed efficiency traits feed conversion ratio and residual feed intake (Camarinha-Silva et al., 2017, Aliakbari et al., 2022). However, these associations were not observed in the present study.

The mechanisms by which different microbial species in various gut sections influence examined traits remain unclear (Zheng et al., 2017). Microbes interact indirectly with their host through products from microbial fermentation, such as short chain fatty acids (SCFA) (Stojanov et al., 2020), which may in turn influence target traits. Of particular importance are the SCFA acetate, propionate, lactate, and butyrate, produced by different microbial communities:

Among the ASVs identified in the MWAS, the families Muribaculaceae, Prevotellaceae, and Christensensellaceae are acetate producers (Orrmerod et al., 2016, Amat et al., 2020). These microbes not only exhibit protective properties against oxidative stress but also play a role in immunomodulation. Moreover, acetate can serve as a substrate for butyrate synthesis of other microbial species (Amat et al., 2020). In addition to acetate, Muribaculaceae also produce propionate (Ormerod et al., 2016). Propionate has been shown to have performance-enhancing

effects and influence lipid metabolism, serving as a substrate for the production of butyrate (Scheiman et al., 2019). Veillonellaceae, identified as the second propionate producer in our study, also contribute to this beneficial effect. The family Enterococcaceae, in addition to producing lactate, acts as a substrate for propionate production and produces bactericides against pathogens (Scheiman et al., 2019, Krawczyk et al., 2021). However, the majority of detected ASVs are butyrate producers, including families such as Oscillospiraceae, Ruminococcaceae, and Clostridiales, all belonging to the order Clostridia (Wong et al., 2006, Konikoff and Gophna, 2016, Guo et al., 2020). Butyrate serves as the primary energy and carbon source for intestinal epithelial cells, fortifying the intestinal barrier and maintaining intestinal homeostasis, leading to a reduced risk of diarrhoea and gut cancer (Wong et al., 2006, Luo et al., 2018, Gu et al., 2022).

Interestingly, ASVs associated with average daily gain, N and P digestibility, and faecal P excretion represent producers of acetate, propionate, lactate, and butyrate. Conversely, ASVs associated with urinary N excretion all belong to the group of butyrate producers. However, explaining the association of specific ASVs with target traits remains challenging. The complex interplay between the host, its gut microbiome, and the environment is not yet fully understood and requires further in-depth investigations. Despite the limited sample size in our study, the results of the MWAS could serve as a starting point for further investigation into the function of single ASVs for target traits in individual gut sections.

4.6 Conclusion

In the present study, moderate dietary N/P restriction resulted in slight changes in the gut microbiome composition. Distinct differences in species diversity and microbiome composition were evident between the small (jejunum and ileum) and large (caecum and colon) intestines, reflecting their anatomical and physiological distinctions. From a breeding perspective, it is noteworthy that feed efficiency and N/P-related traits are influenced not only by genetics but also by the gut microbiome, particularly that of the large intestine. Moderate to high microbiability values supports this thesis. The underlying mechanisms by which the microbiome influences target traits potentially involve differential production of short-chain fatty acids. These findings provide valuable insights for future research, suggesting the potential utility of microbiome data in the ranking of breeding animals.

Chapter 5. General discussion

The need for environmentally friendly pig production systems, driven by social, economic, and ecological considerations, results in a re-evaluation of both feeding and breeding strategies, with a particular focus on minimizing N/P emissions. To implement dietary N/P restriction in pig production, valuable guidance can be found in the recommendations of the GfE (2008) and the DLG leaflet 418 (2019) titled “Leitfaden zur nachvollziehbaren Umsetzung stark N-/P-reduzierter Fütterungsverfahren bei Schweinen” ("Guideline for implementing transparent approaches to significantly reduce nitrogen and phosphorus in pig feeding practices”).

From a breeding perspective, h^2 values hold promise for genetic progress in N/P-related traits. If G×D exist, they should be considered in pig breeding programmes. At the same time, G×D provide opportunities for the identification of robust animals better adapted to dietary N/P restriction. Nonetheless, the measurement of efficiency traits on a large scale, essential for sophisticated genetic evaluations, including variance component estimation, remains challenging. Furthermore, the underlying biological and genetic mechanisms governing efficiency and adaptability remain largely unexplored.

Besides direct genetic effects, the gut microbiome, which has garnered increased interest in recent years, presents a potential approach for elucidating these genetic underpinnings, given its close relationship with the host and the influence on target traits. Thus, the incorporation of microbial information into pig breeding programmes holds promise for enhancing the ranking of breeding animals in the future.

Given the different topics of this thesis, the subsequent discussion will focus on the challenges and perspectives within the fields of animal nutrition, genetics, and gut microbiome, concerning the improvement of feed efficiency and the reduction of N/P excretion. In doing so, the findings of the current study will be examined and placed into practical context of animal breeding and nutrition.

5.1 Dietary N/P restriction to reduce N/P excretion: DLG leaflet 418

The realisation of intended nutrient contents in our fattening trial is most important regarding the interpretation of the results presented in Chapters 3 and 4 of this thesis. As already mentioned, the diets of the CON group and of the LP group were assigned to the “strongly” and “extremely N/P reduced” feeding programmes of the DLG leaflet 418 (2019), respectively. Comparatively large variations in the N/P content of the used diets led to intensive discussion of the study results (see Chapters 3.4 and 3.5). Against this background, in the following section, the consequences of standards presented in DLG leaflet 418 along with the legal nutrient tolerances in feed production will be discussed in more detail.

DLG leaflet 418

The DLG provides farmers and advisors the guideline 418 (2019) to simplify the implementation of dietary N/P restriction and to estimate corresponding N/P emissions. The guideline introduces "strongly N/P-reduced" and "extremely N/P-reduced" feeding programmes, which were utilized in the fattening trial of this thesis. The leaflet provides recommendations for a clear documentation of the feeding programmes and establishes the required average CP and P contents in rations for fattening pigs, which are 153.5 g/kg CP and 4.3 g/kg P for the strongly reduced programme, and 144 g/kg CP and 4.1 g/kg P for the extremely reduced programme. Additionally, the guideline briefly outlines a model for N/P balance calculation (DLG, 2019):

Nutrient excretion (g) = Nutrient intake via feed (g) - nutrient deposition in product (g)

with defined equations:

Nutrient intake via feed (g) = feed intake (kg) · dietary nutrient concentration (g/kg)

Nutrient deposition in product (g) = weight gain (kg) · concentration in product (g/kg)

where the product represents meat in growing-finishing pigs.

The DLG (2019) provides exemplary dietary N/P concentrations for the above-mentioned feeding programmes for a 4-phase fattening period, as shown in Table 21.

Table 21: Dietary crude protein and phosphorus concentrations in a strongly or extremely N/P-reduced feeding programme (88% dry matter) (adapted according to DLG, 2019)

		Body weight			
		28-40 kg	**>40-65 kg**	**>65-90 kg**	**>90-118 kg**
Crude Protein (g/kg)	strongly reduced	175	165	155	140
	extremely reduced	165	155	140	135
Phosphorus (g/kg)	strongly reduced	4.70	4.50	4.20	4.20
	extremly reduced	4.40	4.20	4.00	4.00

The nutrient concentration in the product is set at 25.6 g/kg gain for N and 5.1 g/kg gain for P. Utilising these values and the outlined equations, the DLG offers exemplary values for nutrient excretion in pig fattening for both strongly and extremely reduced N/P feeding programmes. These values also depend on performance level and the number of cycles per year. For instance, considering a performance level of 850 g daily BW gain and a total BW gain of 90 kg, it is demonstrated that N excretion per growing-finishing pig is reduced by 380 g in the extremely reduced feeding programme in comparison to the strongly reduced feeding programme. This results in approximately 1 kg less N per fattening place (with 2.73 cycles/year), and 4.2 g less N per kg weight gain. In terms of P reduction, an extremely reduced feeding programme can lead to a decrease of 32 g per pig, 161 g per fattening place, and 0.7 g per kg gain compared to a strongly reduced feeding programme.

In the context of implementing N/P-reduced feeding programmes in pigs, it is crucial to clarify the term "reduced." Here, "reduced" does not imply providing animals with dietary N/P concentrations below their estimated requirements (see GfE, 2008). On the contrary, the DLG (2019) explicitly emphasises the importance of meeting the pig’s requirements for EAA and digestible P to prevent performance losses and health issues. Similarly, attention must be paid to maintaining the crucial Ca to P ratio when reducing the dietary P concentration. The DLG (2019) states that additional safety margins beyond those recommended by the GfE (2008) are unnecessary, a point also supported by Krieg et al. (2023) from results of a ring test conducted in Germany.

Legal tolerances for analytical constituents

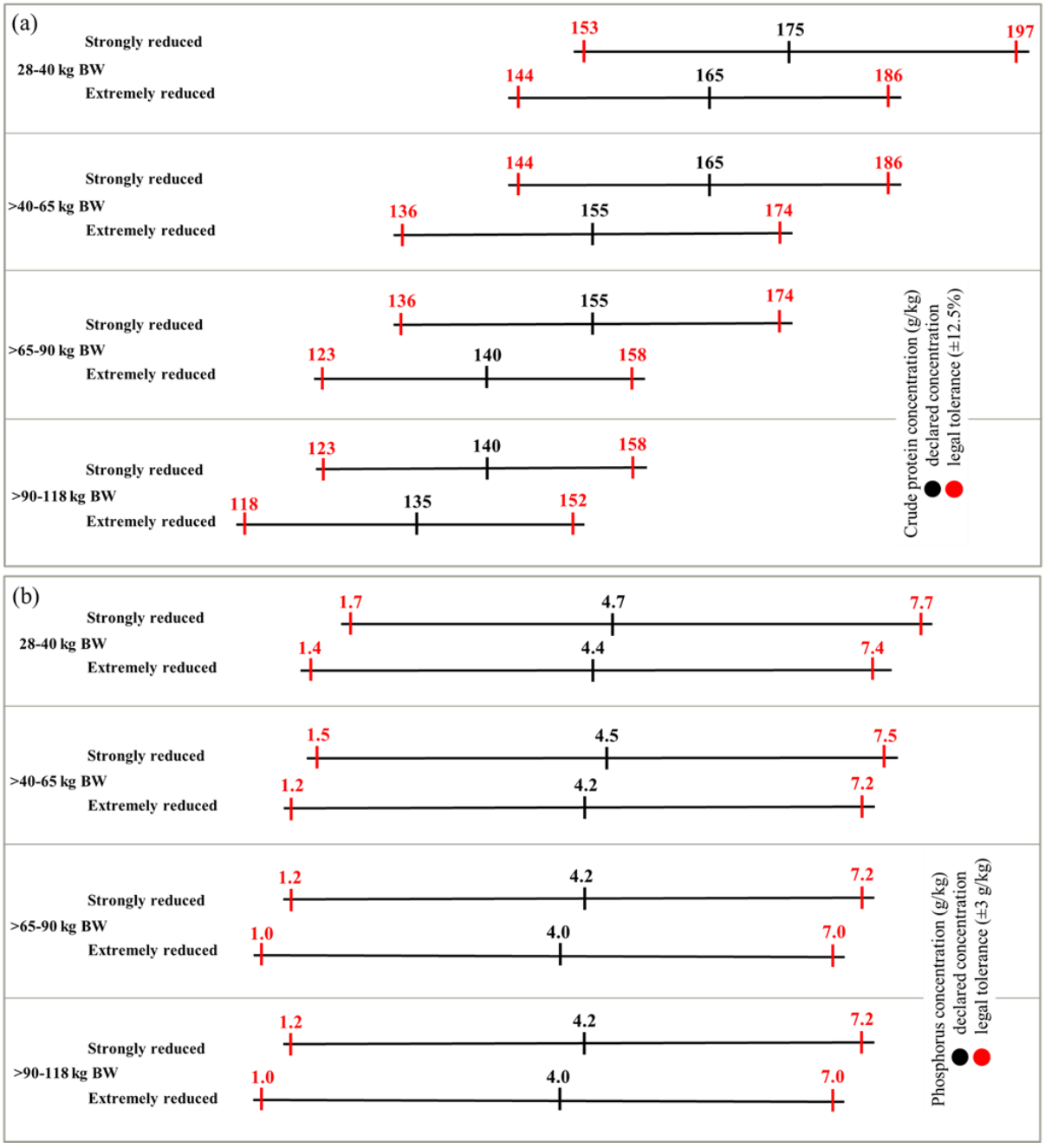
The feeding programmes shown above are typical feeding programmes in German pig production. Feed companies and farmers can use the given nutrient concentrations as a guide. However, under practical conditions, achieving the exact declared levels of dietary nutrient contents is hardly possible for feed companies. Hence, specific tolerances have been established in the Official Journal of the European Union, L328/3, for the production of compound and complete feedingstuffs. For dietary CP concentrations ranging from 8 – 24%, which includes the concentration in feed for growing-finishing pigs, the tolerance is ±12.5% (relative). For dietary P concentration of <1%, it is ±0.3 (absolute) (EU, 2017).

Applying these tolerance ranges to the exemplary dietary CP and P concentrations in strongly and extremely reduced feeding programmes (Table 21), it is evident that the differences between these programmes are relatively small, while the legal tolerances appear relatively high (Figure 10). This raises questions about the practical distinction between strong and extreme reduction, including the provided calculation examples for N/P excretion. There may be limited scope for adjustment at the declaration limits, allowing feed companies to effectively operate in practice.

In the fattening trial presented in Chapters 3 and 4, the analysed values consistently fell within the declared range of tolerance. Similar outcomes can be expected in real-world practice. While the estimations of N/P excretion by the DLG (2019) seem logical, the presented considerations raise questions about whether the excretions between feeding programmes truly differ significantly in every case.

Figure 10: Example of declared (a) dietary crude protein and (b) phosphorus concentrations (g/kg feed at 88% dry matter) and legal tolerances

Note: black, declared concentration; red, legal tolerance; tolerance for P ±3 g/kg feed, ±0.3%; declared concentrations from fattening trial in Chapters 3 and 4.



DLG leaflet 418 and legal tolerances in the present fattening trial

In the animal trial detailed in Chapters 3 and 4, the feeding regimen falls under N/P-reduced feeding programmes, with the C group following a strongly N/P-reduction and the LP group following an extreme N/P reduction according to DLG (2019) guidelines. It is important to note that this trial was conducted under farm conditions, differing from conventional feeding trials where feed analysis precedes to ensure planned nutrient concentrations align with actual ones (e.g. Krieg et al., 2023).

As noted in Chapter 3.4 and shown in Table 4, significant variations in dietary N/P concentrations were observed between the individual runs of the presented fattening trial. These variations were within the expected ranges of tolerance. In most cases, the N/P restriction was realised as intended, or even overfulfilled, albeit with a few exceptions. These variations posed challenges in result interpretation (see "three-factorial interaction"). Interestingly, statistically significant differences in N/P intake were detected only with overfulfilled dietary N/P restriction, not with the planned restriction (Chapter 3.4). This raises questions about the theoretical estimation of N/P excretion according to DLG (2019) (Chapter 5.1.1) when applied in practice with the possible variations shown above (e.g. Figure 10).

In summary, DLG leaflet 418 (2019) provides farmers and advisors with a clear guideline for implementing dietary N/P restriction in pig fattening and aids in estimating N/P excretions for strongly and extremely N/P reduced feeding programmes. Given the relatively high legal tolerances compared to the relatively small differences between these programmes, along with results from the presented fattening trial, it is worth considering whether and how feeding programmes could be improved based on dietary N/P concentrations.

5.2 Recording of feed efficiency and N/P related traits

In animal science, including animal nutrition and genetics, the accurate measurement of target traits, particularly those related to feed efficiency and N/P, is crucial. However, achieving precise measurements often entails a considerable investment of resources, and discrepancies between the effort expended and the accuracy achieved. Additionally, many traits cannot be directly measured but instead necessitate calculation or estimation, introducing the possibility of cumulative inaccuracies. This section aims to discuss the measurement techniques of feed efficiency and N/P-related traits from the fattening trial presented in Chapters 3 and 4.

Evaluation of measurement techniques in the present fattening trial

Feed efficiency traits, such as feed conversion ratio (FCR) or residual feed intake (RFI), as introduced in Chapter 3.3, were calculated or estimated, respectively. Feed conversion ratio, calculated derived from feed consumption and BW gain, provides valuable insights into feed efficiency. However, uncertainties arise due to potential individual feed losses, necessitating careful interpretation of FCR results. Residual feed intake estimation, following the approach of Saintilan et al. (2013) involves several intermediate steps, which introduce possible cumulative errors. Therefore, when comparing results across studies, methodological differences are of particular importance. Nevertheless, a direct comparison within the presented trial remains meaningful as the measurements and estimates were carried out uniformly.

Accurate assessment of N/P-related traits is a challenging task. The intake of N/P can be relatively easily determined by the individual feed consumption and dietary N/P concentration. Therefore, a feed intake recording equipment or the permission to keep the animals in single pens is required. In contrast, the assessment of N/P excretion or retention requires more complex methods. In the presented trial, faecal N/P excretion of individual penned growing-finishing pigs was determined via marker method, while urinary N excretion was estimated from established equations (Kohn et al., 2005). These published estimation equations can be regarded as a well-accepted indirect method for estimating urinary N excretion, as confirmed by Berghaus et al. (2023).

However, the accuracy of these variable is frequently discussed and partly critisised, as partially discussed in Chapter 3.5. As has been shown previously, the digestibility of N/P is influenced by several factors, including the content of soluble fibre, the utilisation of free AA, and the effect of phytase supplementation (Mosenthin et al., 1992, Weijzen et al., 2022, Rosenfelder-Kuon et al., 2020). Furthermore, it is important to recognise that N digestibility tends to increase

with higher nitrogen intake, potentially leading to overestimation, as total N excretion is predominantly regulated via urine (Carpenter et al., 2004). Therefore, determining faecal N excretion via N digestibility may provide less comprehensive information than that obtained from a balance study, where urinary N excretion is measured directly.

Regarding the estimation of urinary nitrogen excretion via blood urinary nitrogen (BUN), it would be advisable in a potential follow-up study to ensure better synchronization between the timing of blood and faecal sampling. This adjustment could potentially facilitate a rough estimation of N retention and total N excretion. Disregarding urinary N excretion is inadvisable, as approximately two-thirds of N is excreted via urine, while only one-third is excreted via faeces (Berghaus, 2022). In situations where urine collection is impractical due to experimental constraints, as was the case in the presented fattening trial, determining urinary N excretion via BUN serves as a viable alternative. This is supported by the observed linear relationship between these parameters (Kohn et al., 2005).

As has been described in detail in Chapter 3.3, urinary N excretion was estimated using the formula proposed by Kohn et al. (2005), which includes BUN, the pig's body weight, and the N clearance rate. The authors reported a coefficient of variation of 0.52 for their formula regarding the true urinary N excretion. Due to experimental limitations, it was not possible to determine the N clearance rate in our fattening trial. Therefore, a constant derived from a regression analysis, also according to Kohn et al. (2005), was chosen. Such a constant may have reduced the accuracy of urinary N excretion estimation, as N clearance rate may be variable to some extent in experiments. Nevertheless, the applied method is still valuable for the assessment urinary N excretion in the present fattening trial.

Alternative measurement approaches

There are several alternatives for assessing N/P excretion, each with its own trade-offs between effort and accuracy. As already described in Chapter 2.2, a quite accurate, but labor-intensive method involves determining N/P excretion directly through a technically sophisticated balance trial. In such balance trials, animals are individually housed in metabolic cages, and faeces and urine are quantitatively collected. From the results, retention and N/P utilisation efficiency can be determined (Berghaus, 2022). For experimental reasons, male pigs are preferred for balance trials. Moreover, an animal experiment application is required.

Another option is the calculation of N/P excretion using N/P intake and retention. Retention, in turn, can be indirectly determined through multiple regression formulas including N/P contents

in the body at the start and end of a test period, as exemplified by Saintilan et al. (2013). Alternatively, a more accurate determination of retention is feasible through the quantification of different body composition tissues (e.g. bones, fascia, fat, lean). This can be achieved either through analysis of individual biological fractions of slaughtered animals or in live animals through techniques such as computer tomography or through the deuterium oxide dilution method (Berghaus, 2022).

Berghaus et al. (2023) investigated the use of BUN as a biomarker for N retention, building upon the work of Kohn et al. (2005). Berghaus et al. (2023) developed a regression formula incorporating BUN, together with body weight gain, body weight, dry matter intake, N intake, serum cortisol, and insulin like growth factor. The authors concluded that under conditions of marginal lysine supply, BUN could serve as a valuable tool for phenotyping nitrogen utilisation efficiency (NUE) on a large scale (Berghaus et al., 2023).

In summary, the methods used to assess traits are critical when comparing results across studies. In the present study, the principles of trait recording were the same in both feeding groups. Under these conditions, group differences were indeed interpretable but may have been confounded by measurement errors, particular in the recording of urinary N excretion. The aim of the trial was to carry out basic research on N/P related traits. Our results thus provide a solid foundation for future research.

5.3 Genetic approaches to reduce N/P excretion

In addition to nutritional approaches, there has been an increased focus on breeding strategies to improve N/P utilisation efficiency in recent years. Initial investigations in this field seek to establish fundamental knowledge and explore the potential for genetic improvement of N/P utilisation efficiency, as outlined by Kasper et al. (2020). This aligns with the objectives of the fattening trial presented in Chapter 3. Therefore, this section aims to discuss the results in a more general way and highlight potential challenges and perspectives for integrating N/P-related traits into pig breeding programmes.

The trial results highlighted the influence of genetics on several traits, including digestibility and urinary excretion of N, as well as intake of P. In particular, the h^2 estimates varied across different fattening periods, suggesting that performance traits recorded at different growth stages are partly influenced by different sets of genes. To our knowledge, genetic parameter estimates for N/P-related traits recorded at different growth stages have not previously been published. So, our results represent an initial exploration into this field and may provide insights into the underlying biological mechanisms.

In our study G×D were sporadically identified for some N/P-related traits as indicated by gi^2 estimates significantly higher than zero. Correlation coefficients of predicted progeny differences between feeding groups further underlined the existence of G×D for traits such as N digestibility, intake and faecal excretion, and P intake and faecal excretion. These results support the hypothesis that the adaptability to dietary N/P restriction is partially under genetic control.

However, it should be noted that the relatively small sample size of 103 animals limits the statistical power to estimate reliable variance components with low standard errors. Nevertheless, the estimated h^2 (>0.15), gi^2 (>0.10), and correlation coefficients (<0.80) provide evidence of genetic influence and the existence of G×D, underscoring the need for future research efforts.

Future directions and integration of N/P-related traits in breeding programmes

Future studies could benefit from animal genotyping to highlight possible genetic differences between individuals, potentially using the animal model for increased prediction accuracy (Sun et al., 2009). Moreover, genotyping lays the groundwork for GWAS for N/P-related traits, aiming to explore the genetic architecture of these traits and enhance breeding program efficiency (Schmid and Bennewitz, 2017).

To integrate N/P utilisation efficiency into future breeding programs, further investigations are needed. A primary objective should entail defining accurate recording methods for N/P-related traits, as outlined in Chapter 5.2, which can be applied under practical conditions on a large scale. Besides the validation of the genetic variation of these traits, the potential existence of G×D should be approved. This should ensure that G×D interactions do not compromise the breeding programme's efficiency. In case of the existence of such interaction, robust individuals can be detected by incorporating G×D into the model equations for breeding values. Different statistical methods like genetic correlations or reaction norm approaches are conceivable and applicable (Brascamp et al., 1985, de Jong, 1995). Furthermore, replicating production-level environmental conditions, such as dietary N/P-restriction, at the nucleus level could improve the efficiency of a pig breeding programme, as suggested by Hofer et al. (2018). Finally, providing farmers with a breeding value that describes N/P utilisation efficiency and the magnitude of a pig’s N/P excretion would be benificial regarding avoidance of unnecessary environmental pollution.

5.4 Complex relationship of gut microbiome, host and N/P-related traits

The gut microbiome is closely linked to the host and the environment and as such an essential aspect of pig health and productivity. Details of these interdependencies have been described in Chapter 2.4 and illustrated in Figure 3. The following section aims to discuss the results of Chapter 4 regarding the potential use of microbiome data in pig breeding, emphasising both challenges and perspectives.

The pig's gut microbiome is shaped by environmental factors, such as nutrition, as well as by genetics (Bergamaschi et al., 2020b). In turn, the microbiome also affects host traits like feed efficiency and N/P-related traits through microbial metabolites (e.g. SCFA). The results of m^2 and MWAS, as discussed in Chapter 4.5, emphasised the importance of the gut microbiome in the explanation of the variation of N/P-related traits.

However, the analysis was limited by a small sample size, as intestinal samples were available from 52 growing-finishing pigs only. Due to this limited sample size, determining the accuracy of the microbial predictions is presumably low. Moreover, verifying the accuracy by standard methods like cross-validation was regarded as not useful.

Nevertheless, to the best of our knowledge, the study in Chapter 4 represents one of the first efforts in the field of microbiability (m^2) estimations and MWAS, which specifically investigate m^2 variations across different gut sections. The observed differences in m^2 estimates between the small and large intestine highlight the distinct importance and functions of these gut sections for the host, as extensively described in Chapter 2.4. Despite the relatively small sample size, this study provides innovative insights into microbiome research in growing-finishing pigs and motivates further research in this area.

Future directions and utilisation of microbiome data for breeding purposes

There are two primary approaches to integrating microbiome data into pig breeding programmes. First, breeding for a "beneficial" gut microbiome composition presents an opportunity, as the microbiome is known to be heritable (Benson et al., 2010). This strategy includes enhancing beneficial gut microbes while reducing pathogenic ones through selection, similar to practices in animal nutrition using pro- and prebiotics (Upadhaya and Kim, 2022).

Second, it may be possible to use the gut microbiome itself in predicting target traits, with MWAS uncover the biological mechanisms underlying adaptability to dietary N/P restriction. For example, the microbiome could potentially be used to rank breeding animals (Verschuren

et al., 2020, Aliakbari et al., 2022). Microbiome information could be another option, in addition to nutrition and genetics, to improve the feed and nutrient utilisation efficiency of growing-finishing pigs.

Achieving practical use of microbiome information for breeding requires addressing several critical gaps. Robust evaluations and estimations rely on large, accurately recorded, and standardised datasets to ensure reproducibility and comparability across studies (Bharti and Grimm, 2021). Factors that influence microbiome composition, such as pig age, gender, housing conditions, gut sections, and sample collection methods, must be carefully considered (Bharti and Grimm, 2021).

In addition, standardisation of sampling and sequence data analysis is of vital importance to achieve comparable and reproducible results. This includes the choice of the hypervariable region of the 16S rRNA gene and the conduction of quality control and data normalisation (McKnight et al., 2019, Forcina et al., 2022). Furthermore, it is essential, to clarify whether to assign sequences to OTUs or ASVs (Callahan et al., 2018). The choice of ASVs as the standard unit in microbiome analysis may facilitate meaningful further research and data collection due to their advantages in reproducibility and comprehensiveness (Callahan et al., 2018). This standardisation may lay the foundation for the advancement of microbiome-related breeding programmes in the future.

Despite persistent challenges, significant progress has been made in microbiome research in recent years, primarily attributed to the introduction of high-throughput DNA/RNA sequencing techniques. These innovations have enabled a more comprehensive understanding of microbiome composition and function. At the same time, the development of bioinformatic tools has improved the ability of researchers to predict microbiome functions with increasing precision (Forcina et al., 2022).

The findings from Chapter 4 suggest that there is a foundation for potential future breeding applications based on microbiome data. Although there are remaining gaps and hurdles to overcome, there have already been notable success and promising prospects. Ideally, certain gut microbiome species, especially those that are easily identifiable in faecal samples, could serve as biomarkers for specific traits of interest. Leveraging this knowledge has the potential to drive the development of more efficient growing-finishing pigs in the years ahead.

5.5 Importance and impact on animal welfare in the present fattening trial

The presented fattening trial (Chapters 3 and 4) focused on production and efficiency traits linked to possible avoidance of environmental pollution. Although part of the underlying project, animal welfare issues received only cursory attention in the literature review (Chapter 2.1.3) but was not extensively covered in the main part of the thesis. However, the aspect of animal welfare, encompassing animal health and behaviour, was considered in the trial as well. For completeness, a brief overview of the results analysed using descriptive statistics will be provided.

The health status of the growing-finishing pigs in the fattening trial was monitored at various intervals. This included assessments of necrosis scores and parameters from whole blood analysis to evaluate overall health. Salivary cortisol levels were measured as an indicator of stress, and the composition and density of the third and fourth metacarpals of the right front trotter were analysed to assess bone health. Additionally, the behaviour of the animals was evaluated using two different tests: The so-called Test S (modified from Scott et al. (2009)) was used to assess behaviour towards humans, while the Novel Object Test (modified from Mieloch et al. (2020)) was employed to evaluate behaviour towards unfamiliar objects.

Overall, the pigs showed no signs of health impairment or abnormal behaviour, with no differences observed between the two feeding groups regarding the investigated parameters. This suggests that the dietary N/P restriction within the range of the extremely reduced N/P feeding programme (DLG, 2019) did not negatively affect animal welfare. In contrast to a study by van der Meer et al. (2017), who reported some impairments, the presented fattening trial only reduced CP concentrations but not EAA. Furthermore, P was not reduced as significantly as in the German nationwide ring test, where no health impairments were found either (Krieg et al., 2023). Krieg et al. (2023) concluded that bone fractures are not associated with typical German feeding programmes.

Therefore, with adequate supply with EAA and digestible P, an extremely reduced N/P feeding programme (DLG, 2019) can be implemented while ensuring animal welfare. However, it is still essential to consider animal health and behaviour in future nutritional and genetic approaches aimed at enhancing the efficiency of growing-finishing pigs.

Chapter 6. Conclusion

Reducing N/P excretion and improving the nutrient utilisation efficiency of growing-finishing pigs are crucial for a sustainable pig production. This thesis, comprising a literature review, fattening trial results, and a general discussion, has revealed that achieving these objectives is possible through nutritional and genetic strategies. Underlying biological mechanisms of these strategies are interconnected, with the gut microbiome emerging as an important factor.

Applying the extremely N/P-reduced feeding programme, as devised by DLG (2019) guideline, did not negatively affect fattening and slaughter performance compared to the application of a strongly N/P-reduced feeding programme. Thus, the use in practice seems feasible without impairments in all relevant performance traits. Notably, N/P excretions are significantly influenced by dietary N/P concentration. However, achieving the reduction in N/P excretions as suggested by DLG (2019) may not always be possible due to high legal tolerance ranges. Thus, reconsidering the definition of DLG feeding programmes may be necessary.

Approaches in animal breeding to reduce N/P excretions are still in their early stages. However, study results clearly indicate that the genetic foundation, i.e., h^2 of feed efficiency and N/P-related traits, provides opportunities for genetic selection. Within such an approach, G×D, observed for some traits, expand complexity to breeding efforts. Nevertheless, these interactions offer the possibility to identify robust animals capable of thriving on temporary or permanent limited nutrient resources without impairments. Furthermore, to the best of our knowledge, our study is the first, which dealt with genetic parameters of N/P-related traits recorded at different growth stages. Observed variations in h^2 across fattening periods suggest the involvement of distinct sets of genes depending on the pig's age, potentially contributing to N/P utilisation efficiency. In this context, the gut microbiome could provide further insights. Results of m^2 estimates and MWAS have shown that the gut microbiome influences feed efficiency and N/P-related traits. Of particular interest are the distinctions between the small and large intestine microbiomes, each influencing different target traits. Such distinctions were derived from gut section-specific m^2 estimations, which to our knowledge are the first gut-specific m^2 results in this research field. From these results, we conclude that microbiome information could improve the understanding in the regulation of N/P efficiency and increase the reliability of breeding values used for the selection of robust breeding animals for the next generation.

Accurately measuring of target traits on a large scale and defining the optimal traits for selection remains challenging, but initial established approaches are further developed. An example is the use of markers for nutrient utilisation efficiency, such as BUN for N utilisation efficiency.

To summarise, the cooperation between animal nutrition and animal breeding and genetics should be further intensified in the future in order to minimise environmental pollution of pig production.

Chapter 7. Summary

Sustainable pig production systems need to address concerns related to environmental pollution and economy of all participants along the pig meat production chain. Because of rising feed costs and an improved appreciation of environment protection, feed efficiency and particular N/P utilisation and N/P excretions were brought into the focus of pig nutritionists and pig breeders. Integrating research across animal nutrition, genetics, and gut microbiome studies offers a promising approach for improving these target traits. In terms of nutrition, implementing a dietary N/P restriction is a well-established method for reducing N/P excretions. Research has shown variability in adaptability to these restrictions among pigs, even within the same breed, with genetics playing a significant role. This serves as an indicator for G×D, which can be useful in future breeding programmes aiming to improve efficiency and identify robust individuals. The gut microbiome appears to play a critical role in pigs' varying adaptability to dietary N/P restriction, suggesting that microbiome information may support in effectively ranking breeding animals.

The aim of this thesis was to investigate feed efficiency and N/P-related traits in pig production as well as highlighting their significance within the context of a sustainable pig production. Therefore, a 3-phase fattening trial was conducted, involving 103 Piétrain×German Landrace growing finishing-pigs, originating from 20 AI-boars, under typical German farm conditions. There were four consecutive runs, in which half of the pigs (C group) were fed following a strongly N/P reduced feeding programme, and the other half (LP group) following an extremely reduced N/P feeding programme, based on DLG (2019) guidelines. Intended dietary N/P restriction between C and LP group was only partially realised due to relatively high legal EU (2017) tolerance ranges in the production of rations, resulting in variations in the extent of N/P restriction across batches of the diet. The fattening trial prioritized animal welfare by ensuring the absence of nutrient deficiency. Emphasis was placed on the possible impact of N/P restriction on performance parameters and on exploring the genetic background of feed efficiency, N/P-related traits, and potential G×D in different growth stages. Additionally, the thesis focused on the role of the gut microbiome in four different gut sections, recognising its significant influence on the performance and health of pigs as their hosts.

In the first study (Chapter 3), the effect of dietary N/P restriction on fattening and slaughter performance, feed efficiency, and N/P-related traits of the 103 growing-finishing pigs was investigated. Furthermore, the genetic background and potential G×D for feed efficiency and N/P-related traits were explored. For this analysis, a mixed linear model with repeated measurements was used, incorporating the AI-boar and AI-boar×feeding group as a random effect. The LSMeans of the feeding groups for examined traits were calculated. Moreover, h^2

and gi^2 were estimated for relevant traits across each fattening period as well as collectively across all periods. As an alternative to gi^2, serving as indicator for G×D, correlation coefficients of the predicted progeny differences of AI-boars between feeding groups were computed. Results showed that pigs were not adversely affected in their performance by dietary N/P restriction. The fatty acid pattern in meat was influenced by dietary N/P restriction and associated changes in feed composition with a higher proportion of saturated fatty acids. This is considered favourable regarding the processing of the meat. As expected, dietary N/P concentrations significantly influenced feed efficiency and N/P-related traits. Those showed low to moderate h^2, varying across fattening periods, suggesting the involvement of a distinct set of genes depending on the pig's age. Indications of G×D were observed for some traits, particularly for N/P intake and faecal excretion. Despite a relatively small sample size being the basis of estimations, these findings indicate a genetic foundation of N/P-related traits and their potential for selection.

In the second study (Chapter 4), gut samples from four different gut sections (jejunum, ileum, caecum, colon) of 52 out of the 103 growing-finishing pigs were analysed for gut microbiome composition using 16S rRNA analysis. Additionally, a univariate model including the fixed effect of environment and the random effects of AI-boar and the microbial component was used to estimate m^2, describing the microbial effect on feed efficiency and N/P-related traits. Subsequently, a MWAS was conducted to investigate the significance of single microbial species on target traits. Despite dietary N/P restriction having a limited effect on microbial diversity, significant differences were observed between the small (jejunum, ileum) and large intestine (caecum, colon) in terms of alpha- and beta-diversity, consistent with clear anatomical and functional distinctions. Such differences across gut sections were also shown in terms of m^2. The m^2 estimates for feed efficiency traits were higher in the large intestine than in small intestine, while m^2 for N/P digestibility in the small intestine exceeded that of the large intestine. Overall, m^2 estimates underscored the significant microbial influence on feed efficiency and N/P-related traits, with the MWAS results revealing numerous species with small effect sizes influencing these target traits. These findings motivate to verify and validate the use of microbial information in future pig breeding programmes.

The general discussion addressed various points, one of which indicated the potential need for re-evaluating strongly and extremely N/P reduced feeding programmes, defined by DLG (2019) guidelines, regarding their specified dietary N/P concentrations. Particularly, differences among the N/P concentrations across these feeding programmes are relatively small, especially when considering the relatively high legal tolerances. However, the feeding programmes can

be applied in practice without anticipated impairments to performance and animal welfare. Genetic and microbial approaches to further minimise N/P excretion show promising prospects. Observed G×D can help in identifying robust individuals. However, there is still a need to develop cost-efficient and precise trait assessment on a large scale, and to uncover the biological background to successfully incorporate N/P-related traits into pig breeding programmes. As has been demonstrated in this thesis, that an interdisciplinary approach including nutrition, breeding and genetics, and gut microbiome is promising regarding the avoidance of unnecessary N/P excretion.

Chapter 8. References

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Chapter 9. Appendix

Table S 1: Recorded traits with abbreviations and units

Trait	Abbreviation	Unit	Type of recording	Used model*
Fattening performance and feed efficiency				
Average daily gain	ADG	g/d	estimated	2
Average daily feed consumption	ADFC	kg/d	measured	2
Feed conversion ratio	FCR	kg/kg	estimated	2
Final fattening weight	FFW	kg	measured	1
Residual feed intake	RFI	kg/d	estimated	1
Slaughter performance and meat quality				
Back fat thickness	BFT	cm	measured	1
Meat color			measured	1
Dressing percentage	DP	%	estimated	1
Drip loss		%	measured	1
Intramuscular fat content	IMF	%	measured	1
Lean meat content	LMC	%	measured	1
Mono unsaturated fatty acids	MUFA	% of total fatty acids	measured	1
pH1 loin (13th/14th rib)	pH1		measured	1
pH24 loin (13th/14th rib)	pH24		measured	1
Polyunsaturated fatty acids	PUFA	% of total fatty acids	measured	1
Saturated fatty acids	SFA	% of total fatty acids	measured	1
Slaughter weight	SW	kg	measured	1
Nitrogen and phosphorus related traits				
Digestibility of Nitrogen	ADN	%	estimated	3
Digestibility of Phosphorus	ADP	%	estimated	3
Faecal Nitrogen excretion	NEXf	g/d	estimated	3
Faecal phosphorus excretion	PEX	g/d	estimated	3
Nitrogen intake	NIN	g/d	estimated	3
Phosphorus intake	PIN	g/d	estimated	3
Urinary nitrogen excretion	NEXu	g/d	estimated	3

Note: *used model refers to the models illustrated in Chapter 3.3.

Table S 2: LSMeans (±SE) of male and female pigs

	Male	**Female**
ADFC (kg/d)	2.29±0.021[a]	2.31±0.018[a]
ADG (g/d)	922±10.4[a]	864±9.1[b]
FCR (kg/kg)	2.50±0.037[a]	2.71±0.034[b]
FFW (kg)	115±1.2[a]	109±1.0[b]
RFI (kg/d)	0.002±0.0198[a]	0.009±0.0196[a]

Note: different letters within a row indicate statistically significant differences ($p \leq 0.05$).

Abbreviations: LSMeans, least squared means; SE, standard error; ADFC, average daily feed consumption; ADG, average daily gain; FCR, feed conversion ratio; FFW, final fattening weight; RFI, residual feed intake.

Table S 3: LSMeans (±SE) of male and female pigs for slaughter performance and meat quality traits

	Male	**Female**
SW (kg)	90.4±0.95[a]	88.2±0.85[a]
DP (%)	78.8±0.17[a]	80.6±0.16[b]
LMC (%)	59.1±0.35[a]	61.5±0.70[b]
BFT (cm)	1.14±0.042[a]	1.26±0.038[b]
pH1 loin	6.28±0.032[a]	6.41±0.028[b]
pH²4 loin	5.38±0.016[a]	5.42±0.014[a]
Meat color	66.7±1.16[a]	69.4±1.04[b]
Drip loss (%)	3.19±0.250[a]	2.30±0.222[b]
IMF (%)	1.12±0.055[a]	1.31±0.051[b]
PUFA (% of total FA)	17.8±0.37[a]	16.2±0.35[b]
MUFA (% of total FA)	35.1±3.22[a]	62.7±2.91[b]
SFA (% of total FA)	35.1±0.28[a]	34.9±0.26[a]

Note: different letters within a row indicate statistically significant differences ($p \leq 0.05$).

Abbreviations: LSM, least squared means; SE, standard error; SW, slaughter weight; DP, dressing percentage; LMC, lean meat content; BFT, back fat thickness; pH1 loin, pH in loin 1 hour after slaughter; pH²4 loin, pH in loin 24 hours after slaughter; IMF, intramuscular fat content; FA, fatty acids; PUFA, polyunsaturated fatty acids; MUFA, mono unsaturated fatty acids; SFA, saturated fatty acids.

Table S 4: LSMeans (±SE) of run 1 to 4 up for slaughter performance and meat quality traits

	Run 1	**Run 2**	**Run 3**	**Run 4**
SW (kg)	89.1±1.58[a]	88.9±1.25[a]	88.9±1.13[a]	90.2±1.51[a]
DP (%)	79.7±0.30[a]	79.6±0.24[a]	79.6±0.22[a]	79.8±0.30[a]
LMC (%)	58.7±0.66[a]	60.2±0.53[a]	59.8±0.48[a]	59.6±0.64[a]
BFT (cm)	1.19±0.075[ab]	1.03±0.060[b]	1.28±0.055[a]	1.32±0.073[07a]
pH1 loin	6.41±0.051[a]	6.31±0.040[a]	6.36±0.036[a]	6.30±0.049[a]
pH²4 loin	5.47±0.026[a]	5.34±0.021[b]	5.38±0.019[b]	5.40±0.025[ab]
Meat color	73.8±2.02[a]	66.4±1.61[b]	64.5±1.46[b]	67.6±1.95[ab]
Drip loss (%)	2.16±0.424[a]	3.16±0.338[a]	2.24±0.309[a]	3.42±0.421[a]
IMF (%)	1.17±0.102[a]	1.12±0.082[a]	1.29±0.075[a]	1.28±0.100[a]
PUFA (% of total FA)	14.8±0.72[a]	18.4±0.58[b]	16.8±0.53[ab]	17.9±0.70[b]
MUFA (% of total FA)	78.1±5.63[a]	48.4±4.50[b]	40.0±4.08[b]	30.6±5.46[b]
SFA (% of total FA)	35.2±0.50[a]	34.0±0.42[a]	35.5±0.39[a]	35.4±0.51[a]

Note: different letters within a row indicate statistically significant differences ($p \leq 0.05$).

Abbreviations: LSM, least squared means; SE, standard error; SW, Slaughter weight; DP, dressing percentage; LMC, lean meat content; BFT, back fat thickness; pH1 loin, pH in loin 1 hour after slaughter; pH²4 loin, pH in loin 24 hours after slaughter; IMF, intramuscular fat content; FA, fatty acids; PUFA, polyunsaturated fatty acids; MUFA, mono unsaturated fatty acids; SFA, saturated fatty acids.

Table S 5: LSMeans (±SE) of male and female pigs for N/P related traits

	Male	**Female**
ADN (%)	76.2±0.45[a]	76.1±0.42[a]
NIN (g/d)	55.2±0.50[a]	55.6±0.45[a]
NEXf (g/d)	13.1±0.29[a]	13.1±0.27[a]
NEXu (g/d)	15.5±0.85[a]	21.1±0.79[b]
ADP (%)	49.9±0.62[a]	48.5±0.56[a]
PIN (g/d)	9.74±0.095[a]	9.83±0.084[a]
PEX (g/d)	4.91±0.079[a]	5.07±0.071[a]

Note: different letters within a row indicate statistically significant differences ($p \leq 0.05$).

Abbreviations: N/P, N and P; LSMeans, least squared means; SE, standard error; N, nitrogen; P, phosphorus; AD, apparent digestibility; IN, intake; NEXf, faecal N excretion; NEXu, urinary N excretion; PEX, faecal P excretion.

Table S 6: Mean, standard deviation (SD), minimum (Min), and maximum (Max) for all investigated traits (raw data)

	N	Mean	SD	Min	Max
ADFC (kg/d)	103	2.22	0.18	1.79	2.66
ADFC grower (kg/d)	103	1.79	0.23	1.26	2.52
ADFC finisher I (kg/d)	103	2.31	0.21	1.74	2.91
ADFC finisher II (kg/d)	103	2.82	0.39	1.82	3.89
ADG (g/d)	103	875	74.2	746	1082
ADG grower (g/d)	103	817	109	512	1133
ADG finisher I (g/d)	103	952	128	516	1233
ADG finisher II (g/d)	103	895	180	511	1329
FCR (kg/kg gain)	103	2.55	0.21	2.07	3.18
FCR grower (kg/kg gain)	103	2.21	0.27	1.54	3.21
FCR finisher I (kg/kg gain)	103	2.46	0.33	1.90	4.30
FCR finisher II (kg/kg gain)	103	3.23	0.55	2.20	5.59
RFI (kg/d)	103	0.00	0.13	-0.37	0.31
Final fattening weight (kg)	103	112	7.33	96.0	132
Slaughter weight (kg)	103	89.0	5.75	76.7	105
DP (%)	103	79.8	1.30	77.0	82.0
LMC (%)	101	59.8	1.99	54.3	63.7
BFT (cm)	102	1.21	0.27	0.51	1.76
pH1	103	6.35	0.21	5.87	6.82
pH24	103	5.39	0.10	5.20	5.65
Brightness	103	67.4	7.35	55.5	87.2
Drip loss (%)	100	2.65	1.53	0.42	6.61
IMF (%)	102	1.23	0.32	0.61	2.00
PUFA (%)	103	17.0	2.53	12.7	22.9
MUFA (%)	103	49.5	27.8	2.00	97.0
SFA (%)	101	34.9	1.67	31.0	38.8
ADN grower (%)	103	72.3	6.41	54.6	84.4
ADN finisher I (%)	103	78.1	5.11	66.6	89.8
ADN finisher II (%)	103	75.7	6.22	62.2	90.3
NIN grower (g/d)	103	44.7	6.62	31.3	66.0

	N	Mean	SD	Min	Max
NIN finisher I (g/d)	103	56.2	7.39	37.9	78.6
NIN finisher II (g/d)	103	65.0	9.47	41.0	94.5
NEXf grower (g/d)	103	12.3	2.96	5.89	20.2
NEXf finisher I (g/d)	103	12.4	3.59	4.32	24.8
NEXf finisher II (g/d)	103	15.7	4.35	5.99	28.0
NEXu finisher I (g/d)	102	17.0	5.62	6.80	33.5
NEXu finisher II (g/d)	103	29.2	10.2	7.99	50.8
ADP grower (%)	103	48.3	8.82	23.3	63.4
ADP finisher I (%)	103	47.5	12.9	22.3	71.7
ADP finisher II (%)	103	46.8	12.4	16.4	72.3
PIN grower (g/d)	103	8.14	1.26	5.59	12.8
PIN finisher I (g/d)	103	9.68	1.24	7.22	13.6
PIN finisher II (g/d)	103	11.7	1.77	8.26	17.3
PEX grower (g/d)	103	4.19	0.88	2.62	6.93
PEX finisher I (g/d)	103	5.07	1.36	2.53	8.29
PEX finisher II (g/d)	103	6.26	1.84	2.52	12.2

Abbreviations: N, number of observations; ADFC, average daily feed consumption; ADG, average daily gain; FCR, feed conversion ratio; FFW, final fattening weight; RFI, residual feed intake; DP, dressing percentage; LMC, lean meat content; BFT, back fat thickness; pH1 loin, pH in loin 1 hour after slaughter; pH²4 loin, pH in loin 24 hours after slaughter; IMF, intramuscular fat content; PUFA, polyunsaturated fatty acids; MUFA, mono unsaturated fatty acids; SFA, saturated fatty acids; N, nitrogen; P, phosphorus; AD, apparent digestibility; IN, intake; NEXf, faecal N excretion; NEXu, urinary N excretion, PEX, faecal P excretion.

Table S 7: Mean data and standard deviation (SD) for fattening performance in CON and LP group (raw data)

		CON	**SD**	**LP**	**SD**
Grower period	ADFC (kg/d)	1.79	±0.25	1.79	±0.21
(30 - 60 kg BW)	ADG (g/d)	819.04	±90.17	814.88	±125.37
	FCR (kg/kg gain)	2.19	±0.22	2.22	±0.31
Finisher I period	ADFC (kg/d)	2.33	±0.23	2.29	±0.19
(60 - 90 kg BW)	ADG (g/d)	979.06	±114.59	926.03	±136.76
	FCR (kg/kg gain)	2.40	±0.25	2.52	±0.38
Finisher II period	ADFC (kg/d)	2.82	±0.30	2.82	±0.46
(90 - 115 kg BW)	ADG (g/d)	887.10	±183.30	902.52	±178.65
	FCR (kg/kg gain)	3.26	±0.52	3.19	±0.59
Overall	ADFC (kg/d)	2.23	±0.17	2.21	±0.18
(30 - 115 kg BW)	ADG (g/kg gain)	884.09	±78.70	867.19	±69.37
	FCR (kg/kg gain)	2.53	±0.20	2.56	±0.21
	FFW (kg)	111.95	±8.00	111.17	±6.70
	RFI (kg/d)	0.00	±0.12	0.00	±0.15

Abbreviations: CON, control group; LP, group with dietary N/P-restriction; SD, standard deviation, ADFC, average daily feed consumption; ADG, average daily gain; FCR, feed conversion ratio; FFW, final fattening weight; RFI, residual feed intake.

Table S 8: Mean and standard deviation (SD) for slaughter performance and meat quality in CON and LP group (raw data)

	CON	**SD**	**LP**	**SD**
Slaughter weight (kg)	89.22	±6.39	88.83	±5.13
DP (%)	79.72	±1.21	79.90	±1.32
LMC (%)	59.82	±2.19	59.77	±1.81
BFT (cm)	1.21	±0.25	1.20	±0.28
pH1	6.33	±0.21	6.38	±0.20
pH24	5.39	±0.10	5.39	±0.11
Brightness	67.67	±7.09	67.05	±7.65
Drip loss (%)	2.70	±1.53	2.61	±1.54
IMF (%)	1.24	±0.32	1.22	±0.33
PUFA (%)	17.65	±2.60	16.36	±2.31
MUFA (%)	45.54	±26.85	53.13	±28.39
SFA (%)	34.57	±1.61	35.25	±1.67

Abbreviations: CON, control group; LP, group with dietary N/P-restriction; SD, standard deviation; DP, dressing percentage; LMC, lean meat content; BFT, back fat thickness; IMF, intramuscular fat content; PUFA, poly unsaturated fatty acids; MUFA, mono unsaturated fatty acids; SFA, saturated fatty acids.

Table S 9: Mean and standard deviation (SD) for digestibility, intake and excretion of nitrogen and phosphorus in CON and LP group (raw data)

		CON	SD	LP	SD
Grower period	ADN (%)	71.36	±6.68	73.15	±6.08
(30 - 60 kg BW)	NIN (g/d)	45.81	±7.01	43.71	±6.13
	NEXf (g/d)	12.95	±2.83	11.71	±2.98
	NEXu (g/d)	/	/	/	/
	ADP (%)	46.26	±9.29	50.16	±7.99
	PIN (g/d)	8.27	±1.48	8.02	±1.00
	PEX (g/d)	4.39	±0.82	4.01	±0.90
Finisher I period	ADN (%)	79.17	±4.44	77.09	±5.51
(60 - 90 kg BW)	NIN (g/d)	60.00	±7.49	52.59	±5.21
	NEXf (g/d)	12.65	±3.65	12.20	±3.54
	NEXu (g/d)	18.53	±5.48	15.50	±5.39
	ADP (%)	51.16	±10.23	44.13	±14.17
	PIN (g/d)	10.48	±1.17	8.92	±0.72
	PEX (g/d)	5.15	±1.33	5.00	±1.39
Finisher II period	ADN (%)	77.16	±5.79	74.28	±6.35
(90 - 115 kg BW)	NIN (g/d)	65.48	±7.11	64.47	±11.15
	NEXf (g/d)	14.83	±3.65	16.46	±4.82
	NEXu (g/d)	28.99	±10.14	29.30	±10.34
	ADP (%)	48.78	±10.85	45.00	±13.57
	PIN (g/d)	11.40	±1.22	12.02	±2.14
	PEX (g/d)	5.83	±1.32	6.67	±2.16

Abbreviations: CON, control group; LP, group with dietary N/P-restriction; SD, standard deviation; N, nitrogen; P, phosphorus; AD, apparent digestibility; NIN/PIN, N/P intake; NEX/PEX, faecal N/P excretion; NEXu, urinary N excretion.

Table S 10: Taxonomy and regression coefficient estimates from back solving BLUP solutions for all 497 ASVs with nominal significant (p≤0.05) or nominal suggestive significant (p≤0.1) associations with performance and N/P-related traits

Gut section	Associated trait	ASV	p-value	Phylum	Class	Order	Family	Genus	Regression coefficient
Jejunum	PEX	ASV0261	0.093	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	*Treponema*	0.00002
Jejunum	PEX	ASV0283	0.080	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	*Treponema*	0.00002
Jejunum	PEX	ASV0307	0.024	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	*Treponema*	0.00002
Jejunum	PEX	ASV0365	0.081	Bacteroidetes	Bacteroidia	Bacteroidales	Muribaculaceae	*undefined*	0.00002
Jejunum	PEX	ASV0505	0.017	Bacteroidetes	Bacteroidia	Bacteroidales	Muribaculaceae	*undefined*	0.00002
Jejunum	PEX	ASV0555	0.078	Bacteroidetes	Bacteroidia	Bacteroidales	Muribaculaceae	*undefined*	0.00002
Jejunum	PEX	ASV0806	0.059	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00002
Jejunum	PEX	ASV0823	0.029	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00002
Jejunum	PEX	ASV0970	0.029	Actinobacteriota	Coriobacteriia	Coriobacteriales	undefined	*undefined*	0.00002
Jejunum	PEX	ASV1197	0.078	Campilobacterota	Campylobacteria	Campylobacterales	Campylobacteraceae	*Campylobacter*	0.00002
Jejunum	PEX	ASV1404	0.078	Proteobacteria	Gammaproteobacteria	Burkholderiales	Sutterellaceae	*Parasutterella*	0.00002
Jejunum	PEX	ASV1487	0.029	Proteobacteria	Alphaproteobacteria	Rhodospirillales	undefined	*undefined*	0.00002
Jejunum	PEX	ASV1558	0.032	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_vadinBB60_group*	0.00002
Jejunum	PEX	ASV1754	0.078	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.00002
Jejunum	PEX	ASV2178	0.017	Firmicutes	Negativicutes	Veillonellales-	Selenomodaceae	*undefined*	0.00002
Jejunum	PEX	ASV2285	0.019	Firmicutes	Negativicutes	Veillonellales-	Veillonellaceae	*Megasphaera*	0.00002
Jejunum	PEX	ASV2459	0.066	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	*Enterococcus*	0.00002
Jejunum	PEX	ASV3006	0.080	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotella*	0.00002
Jejunum	PEX	ASV3192	0.019	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotellaceae_NK3B31_group*	0.00002
Jejunum	PEX	ASV3194	0.066	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotellaceae_NK3B31_group*	0.00002
Jejunum	PEX	ASV3198	0.032	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotellaceae_NK3B31_group*	0.00002
Jejunum	PEX	ASV3294	0.076	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotella*	0.00002
Jejunum	PEX	ASV3305	0.078	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*undefined*	0.00002
Jejunum	PEX	ASV3367	0.080	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotella*	0.00002
Jejunum	PEX	ASV3463	0.087	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	*Bacteroides*	0.00002
Jejunum	PEX	ASV3534	0.019	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Alloprevotella*	0.00002
Jejunum	PEX	ASV3576	0.029	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Alloprevotella*	0.00002
Jejunum	PEX	ASV3616	0.081	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Alloprevotella*	0.00002
Jejunum	PEX	ASV3655	0.021	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Alloprevotella*	0.00002
Jejunum	PEX	ASV3696	0.044	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotellaceae_UCG-004*	0.00002
Jejunum	PEX	ASV3728	0.081	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*Ruminococcus*	0.00002
Jejunum	PEX	ASV3799	0.071	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*Ruminococcus*	0.00001
Jejunum	PEX	ASV3880	0.078	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*[Clostridium]_methylpentosum_group*	0.00002
Jejunum	PEX	ASV3928	0.036	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*Eubacterium_coprostanoligenes_group*	0.00002
Jejunum	PEX	ASV3969	0.080	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*Faecalibacterium*	0.00002

Gut section	Associated trait	ASV	p-value	Phylum	Class	Order	Family	Genus	Regression coefficient
Jejunum	PEX	ASV4013	0.078	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*Pygmaiobacter*	0.00002
Jejunum	PEX	ASV4320	0.029	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*Oscillospira*	0.00002
Jejunum	PEX	ASV4363	0.029	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-002*	0.00002
Jejunum	PEX	ASV4383	0.018	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*NK4A214_group*	0.00002
Jejunum	PEX	ASV4481	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00002
Jejunum	PEX	ASV4487	0.032	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00002
Jejunum	PEX	ASV4668	0.029	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00002
Jejunum	PEX	ASV4771	0.078	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00002
Jejunum	PEX	ASV4976	0.078	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00002
Jejunum	PEX	ASV5006	0.080	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00002
Jejunum	PEX	ASV5368	0.078	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Lachnospiraceae_UCG-001*	0.00002
Jejunum	PEX	ASV5426	0.061	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Lachnospiraceae_UCG-001*	0.00002
Jejunum	PEX	ASV5586	0.030	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Shuttleworthia*	0.00002
Jejunum	PEX	ASV5596	0.029	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Eubacterium_eligens_group*	0.00002
Jejunum	PEX	ASV5764	0.071	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Lachnoclostridium*	0.00002
Jejunum	PEX	ASV5773	0.099	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Lachnoclostridium*	0.00002
Jejunum	PEX	ASV5814	0.029	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Blautia*	0.00002
Jejunum	PEX	ASV5823	0.093	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Blautia*	0.00002
Jejunum	PEX	ASV6037	0.019	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Lachnospiraceae_NK4A136_group*	0.00002
Jejunum	PEX	ASV6040	0.079	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Lachnospiraceae_NK4A136_group*	0.00002
Jejunum	PEX	ASV6076	0.027	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*undefined*	0.00002
Jejunum	PEX	ASV6105	0.050	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Lachnospiraceae_ND3007_group*	0.00002
Ileum	ADN	ASV4523	0.026	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00004
Ileum	ADN	ASV0398	0.026	Bacteroidetes	Bacteroidia	Bacteroidales	Muribaculaceae	*undefined*	0.00004
Ileum	ADN	ASV2100	0.026	Firmicutes	Clostridia	Peptococcales	Peptococcaceae	*undefined*	0.00004
Ileum	ADN	ASV4528	0.026	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00004
Ileum	ADN	ASV5370	0.026	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Moryella*	0.00004
Ileum	ADN	ASV4475	0.026	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00004
Ileum	ADN	ASV1780	0.026	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.00004
Ileum	ADN	ASV4292	0.027	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*undefined*	0.00004
Ileum	ADN	ASV4259	0.027	Firmicutes	Clostridia	Oscillospirales	Butyricicoccaceae	*UCG-009*	0.00004
Ileum	ADN	ASV4309	0.027	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*Oscillibacter*	0.00004
Ileum	ADN	ASV0259	0.027	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	*Treponema*	0.00004
Ileum	ADN	ASV4698	0.027	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00004
Ileum	ADN	ASV3847	0.027	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*undefined*	0.00004
Ileum	ADN	ASV6090	0.027	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Lachnospiraceae_FCS020_group*	0.00004

Gut section	Associated trait	ASV	p-value	Phylum	Class	Order	Family	Genus	Regression coefficient
Ileum	ADN	ASV6015	0.027	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Lachnoclostridium*	0.00004
Ileum	ADN	ASV4972	0.027	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00004
Ileum	ADN	ASV6026	0.027	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*undefined*	0.00004
Ileum	ADN	ASV4507	0.027	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00004
Ileum	ADN	ASV4715	0.027	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00004
Ileum	ADN	ASV0269	0.027	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	*Treponema*	0.00004
Ileum	ADN	ASV0871	0.027	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00004
Ileum	ADN	ASV5847	0.028	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Blautia*	0.00004
Ileum	ADN	ASV4513	0.028	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00004
Ileum	ADN	ASV4638	0.028	Firmicutes	Clostridia	Oscillospirales	Butyricicoccaceae	*Butyricicoccus*	0.00004
Ileum	ADN	ASV6165	0.028	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*undefined*	0.00004
Ileum	ADN	ASV3689	0.029	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotellaceae_UCG-003*	0.00004
Ileum	ADN	ASV4572	0.029	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*undefined*	0.00004
Ileum	ADN	ASV1763	0.030	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.00004
Ileum	ADN	ASV0293	0.030	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	*Treponema*	0.00004
Ileum	ADN	ASV2744	0.030	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	*undefined*	0.00004
Ileum	ADN	ASV4482	0.030	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00004
Ileum	ADN	ASV3543	0.031	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Alloprevotella*	0.00004
Ileum	ADN	ASV4496	0.031	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00004
Ileum	ADN	ASV4637	0.032	Firmicutes	Clostridia	Oscillospirales	Butyricicoccaceae	*UCG-008*	0.00004
Ileum	ADN	ASV2992	0.032	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotella*	0.00004
Ileum	ADN	ASV0516	0.032	Bacteroidetes	Bacteroidia	Bacteroidales	Muribaculaceae	*Muribaculaceae*	0.00004
Ileum	ADN	ASV3696	0.034	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotellaceae_UCG-004*	0.00004
Ileum	ADN	ASV4777	0.035	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00004
Ileum	ADN	ASV4009	0.035	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*undefined*	0.00004
Ileum	ADN	ASV5454	0.035	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*undefined*	0.00004
Ileum	ADN	ASV4393	0.035	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*NK4A214_group*	0.00004
Ileum	ADN	ASV5901	0.036	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*undefined*	0.00004
Ileum	ADN	ASV0868	0.036	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00004
Ileum	ADN	ASV4257	0.036	Firmicutes	Clostridia	Oscillospirales	Butyricicoccaceae	*UCG-009*	0.00004
Ileum	ADN	ASV5462	0.037	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*undefined*	0.00004
Ileum	ADN	ASV0559	0.037	Bacteroidetes	Bacteroidia	Bacteroidales	Muribaculaceae	*undefined*	0.00004
Ileum	ADN	ASV3164	0.038	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotellaceae_NK3B31_group*	0.00004
Ileum	ADN	ASV5713	0.038	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Frisingicoccus*	0.00004
Ileum	ADN	ASV3166	0.038	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotellaceae_NK3B31_group*	0.00004
Ileum	ADN	ASV1772	0.039	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.00004
Ileum	ADN	ASV1552	0.041	Proteobacteria	Deltaproteobacteria	Bradymonadales	Bradymonadaceae	*undefined*	0.00004

Gut section	Associated trait	ASV	p-value	Phylum	Class	Order	Family	Genus	Regression coefficient
Ileum	ADN	ASV3553	0.041	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Alloprevotella*	0.00004
Ileum	ADN	ASV0970	0.041	Actinobacteriota	Coriobacteriia	Coriobacteriales	undefined	*undefined*	0.00004
Ileum	ADN	ASV0263	0.042	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	*Treponema*	0.00004
Ileum	ADN	ASV4314	0.042	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*Oscillospira*	0.00004
Ileum	ADN	ASV3781	0.043	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*Ruminococcus*	0.00004
Ileum	ADN	ASV0493	0.044	Bacteroidetes	Bacteroidia	Bacteroidales	Muribaculaceae	*undefined*	0.00004
Ileum	ADN	ASV0368	0.044	Bacteroidetes	Bacteroidia	Bacteroidales	Muribaculaceae	*undefined*	0.00004
Ileum	ADN	ASV4625	0.045	Firmicutes	Clostridia	Oscillospirales	Butyricicoccaceae	*UCG-008*	0.00004
Ileum	ADN	ASV5639	0.048	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Eubacterium_rumiundefinedntium_group*	0.00004
Ileum	ADN	ASV5340	0.048	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*undefined*	0.00004
Ileum	ADN	ASV4395	0.050	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*NK4A214_group*	0.00003
Ileum	ADN	ASV4320	0.051	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*Oscillospira*	0.00004
Ileum	ADN	ASV3821	0.052	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*Ruminococcus*	0.00004
Ileum	ADN	ASV0250	0.052	Fibrobacterota	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	*Fibrobacter*	0.00003
Ileum	ADN	ASV0859	0.052	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00004
Ileum	ADN	ASV2647	0.052	Firmicutes	Bacilli	Erysipelotrichales	Erysipelatoclostridiaceae	*UCG-004*	0.00004
Ileum	ADN	ASV0838	0.052	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00004
Ileum	ADN	ASV2937	0.053	Firmicutes	Bacilli	Acholeplasmatales	Acholeplasmataceae	*Aundefinederoplasma*	0.00003
Ileum	ADN	ASV4536	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00003
Ileum	ADN	ASV0747	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	*F082*	0.00003
Ileum	ADN	ASV0855	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00003
Ileum	ADN	ASV4157	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*undefined*	0.00003
Ileum	ADN	ASV0886	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00003
Ileum	ADN	ASV2058	0.053	Firmicutes	Clostridia	Peptostreptococcales	undefined	*undefined*	0.00003
Ileum	ADN	ASV5177	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00003
Ileum	ADN	ASV5536	0.053	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Roseburia*	0.00003
Ileum	ADN	ASV0678	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Tannerellaceae	*Parabacteroides*	0.00003
Ileum	ADN	ASV0875	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00003
Ileum	ADN	ASV2232	0.053	Firmicutes	Negativicutes	Veillonellales	Selenomonadaceae	*undefined*	0.00003
Ileum	ADN	ASV4499	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00003
Ileum	ADN	ASV4849	0.053	Firmicutes	Clostridia	Monoglobales	Monoglobaceae	*Monoglobus*	0.00003
Ileum	ADN	ASV6164	0.053	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Aundefinederostipes*	0.00003
Ileum	ADN	ASV4111	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*Eubacterium_siraeum_group*	0.00003
Ileum	ADN	ASV1762	0.053	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.00003
Ileum	ADN	ASV2150	0.053	Firmicutes	Negativicutes	Veillonellales	Selenomonadaceae	*Mitsuokella*	0.00003
Ileum	ADN	ASV3587	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Alloprevotella*	0.00003
Ileum	ADN	ASV3627	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Alloprevotella*	0.00003

Gut section	Associated trait	ASV	p-value	Phylum	Class	Order	Family	Genus	Regression coefficient
Ileum	ADN	ASV4925	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00003
Ileum	ADN	ASV0138	0.053	Verrucomicrobiota	Lentisphaeria	Oligosphaerales	Oligosphaeraceae	*Z20*	0.00003
Ileum	ADN	ASV1766	0.053	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.00003
Ileum	ADN	ASV3584	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Alloprevotella*	0.00003
Ileum	ADN	ASV4466	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00003
Ileum	ADN	ASV4725	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00003
Ileum	ADN	ASV5668	0.053	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*undefined*	0.00003
Ileum	ADN	ASV2792	0.053	Firmicutes	Bacilli	undefined	undefined	*undefined*	0.00003
Ileum	ADN	ASV2961	0.053	Firmicutes	Clostridia	Peptococcales	Peptococcaceae	*undefined*	0.00003
Ileum	ADN	ASV3886	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*Candidatus_Soleaferrea*	0.00003
Ileum	ADN	ASV4117	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*Eubacterium_siraeum_group*	0.00003
Ileum	ADN	ASV4138	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*undefined*	0.00003
Ileum	ADN	ASV4169	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*undefined*	0.00003
Ileum	ADN	ASV4237	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*CAG-352*	0.00003
Ileum	ADN	ASV4355	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*Oscillibacter*	0.00003
Ileum	ADN	ASV4551	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-007*	0.00003
Ileum	ADN	ASV4729	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00003
Ileum	ADN	ASV4855	0.053	Firmicutes	Clostridia	Monoglobales	Monoglobaceae	*Monoglobus*	0.00003
Ileum	ADN	ASV4904	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00003
Ileum	ADN	ASV5063	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00003
Ileum	ADN	ASV5897	0.053	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Marvinbryantia*	0.00003
Ileum	ADN	ASV6109	0.053	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*undefined*	0.00003
Ileum	ADN	ASV2299	0.053	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	*Dialister*	0.00003
Ileum	ADN	ASV4215	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*undefined*	0.00003
Ileum	ADN	ASV4321	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*Oscillospira*	0.00003
Ileum	ADN	ASV5007	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00003
Ileum	ADN	ASV0854	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00003
Ileum	ADN	ASV1582	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_vadinBB60_group*	0.00003
Ileum	ADN	ASV2664	0.053	Firmicutes	Bacilli	Erysipelotrichales	Erysipelatoclostridiaceae	*UCG-004*	0.00003
Ileum	ADN	ASV4356	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*Oscillibacter*	0.00003
Ileum	ADN	ASV4439	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00003
Ileum	ADN	ASV4655	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00003
Ileum	ADN	ASV4908	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00003
Ileum	ADN	ASV4956	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00003
Ileum	ADN	ASV5635	0.053	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Eubacterium_rumiundefinedntium_group*	0.00003
Ileum	ADN	ASV5950	0.053	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Lachnospiraceae_NK4A136_group*	0.00003
Ileum	ADN	ASV0195	0.053	Kritimatiellota	Kiritimatiellia	Kritimatiellales	Kritimatiellaceae	*WCHB1-41*	0.00003

Gut section	Associated trait	ASV	p-value	Phylum	Class	Order	Family	Genus	Regression coefficient
Ileum	ADN	ASV0332	0.053	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	*Sphaerochaeta*	0.00003
Ileum	ADN	ASV0365	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Muribaculaceae	*undefined*	0.00003
Ileum	ADN	ASV0391	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Muribaculaceae	*undefined*	0.00003
Ileum	ADN	ASV1531	0.053	Proteobacteria	Alphaproteobacteria	undefined	undefined	*undefined*	0.00003
Ileum	ADN	ASV1644	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_vadinBB60_group*	0.00003
Ileum	ADN	ASV1658	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_vadinBB60_group*	0.00003
Ileum	ADN	ASV1728	0.053	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.00003
Ileum	ADN	ASV1753	0.053	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.00003
Ileum	ADN	ASV2811	0.053	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	*RF39*	0.00003
Ileum	ADN	ASV2878	0.053	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	*RF39*	0.00003
Ileum	ADN	ASV2923	0.053	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	*RF39*	0.00003
Ileum	ADN	ASV4377	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*NK4A214_group*	0.00003
Ileum	ADN	ASV4736	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00003
Ileum	ADN	ASV4802	0.053	Firmicutes	Clostridia	Monoglobales	Monoglobaceae	*Monoglobus*	0.00003
Ileum	ADN	ASV4921	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00003
Ileum	ADN	ASV0819	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00003
Ileum	ADN	ASV0831	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00003
Ileum	ADN	ASV3590	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Alloprevotella*	0.00003
Ileum	ADN	ASV4027	0.053	Firmicutes	Clostridia	Oscillospirales	Eubacteriaceae	*Eubacterium_coprostanoligenes_group*	0.00003
Ileum	ADN	ASV1199	0.053	Campilobacterota	Campylobacteria	Campylobacterales	Campylobacteraceae	*Campylobacter*	0.00003
Ileum	ADN	ASV0265	0.053	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	*Treponema*	0.00003
Ileum	ADN	ASV4067	0.053	Firmicutes	Clostridia	Oscillospirales	Eubacterium_coprostanoligenes_group	*Eubacterium_coprostanoligenes_group*	0.00003
Ileum	ADN	ASV4381	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*NK4A214_group*	0.00003
Ileum	ADN	ASV0300	0.053	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	*Treponema*	0.00003
Ileum	ADN	ASV4371	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-002*	0.00003
Ileum	ADN	ASV4396	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*NK4A214_group*	0.00003
Ileum	ADN	ASV0702	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Tannerellaceae	*Parabacteroides*	0.00003
Ileum	ADN	ASV0837	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00003
Ileum	ADN	ASV0882	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00003
Ileum	ADN	ASV4378	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*NK4A214_group*	0.00003
Ileum	ADN	ASV5083	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00003
Ileum	ADN	ASV5620	0.053	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Coprococcus*	0.00003
Ileum	ADN	ASV5688	0.053	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Coprococcus*	0.00003
Ileum	ADN	ASV6228	0.053	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Lachnospiraceae_NK4A136_group*	0.00003
Ileum	ADN	ASV1213	0.053	Campilobacterota	Campylobacteria	Campylobacterales	Campylobacteraceae	*Campylobacter*	0.00003
Ileum	ADN	ASV2050	0.053	Firmicutes	Clostridia	Peptostreptococcales	undefined	*undefined*	0.00003
Ileum	ADN	ASV3854	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*undefined*	0.00003

Gut section	Associated trait	ASV	p-value	Phylum	Class	Order	Family	Genus	Regression coefficient
Ileum	ADN	ASV4195	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00003
Ileum	ADN	ASV4409	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*NK4A214_group*	0.00003
Ileum	ADN	ASV4885	0.053	Firmicutes	Clostridia	Monoglobales	Monoglobaceae	*Monoglobus*	0.00003
Ileum	ADN	ASV4909	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00003
Ileum	ADN	ASV0172	0.053	Kritimatiellota	Kiritimatiellia	Kritimatiellales	Kritimatiellaceae	*WCHB1-41*	0.00003
Ileum	ADN	ASV0857	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00003
Ileum	ADN	ASV1769	0.053	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.00003
Ileum	ADN	ASV3692	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotellaceae_UCG-003*	0.00003
Ileum	ADN	ASV4407	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*undefined*	0.00003
Ileum	ADN	ASV4468	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00003
Ileum	ADN	ASV4653	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00003
Ileum	ADN	ASV6253	0.053	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Oribacterium*	0.00003
Ileum	ADN	ASV4441	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00003
Ileum	ADN	ASV0806	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00003
Ileum	ADN	ASV1783	0.053	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.00003
Ileum	ADN	ASV2029	0.053	Firmicutes	Clostridia	Peptostreptococcales	Eubacteriaceae	*Eubacterium_nodatum_group*	0.00003
Ileum	ADN	ASV3068	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotellaceae_NK3B31_group*	0.00003
Ileum	ADN	ASV3808	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*Ruminococcus*	0.00003
Ileum	ADN	ASV4267	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-002*	0.00003
Ileum	ADN	ASV4662	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00003
Ileum	ADN	ASV5279	0.053	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*undefined*	0.00003
Ileum	ADN	ASV4033	0.053	Firmicutes	Clostridia	Oscillospirales	Eubacteriaceae	*Eubacterium_coprostanoligenes_group*	0.00003
Ileum	ADN	ASV2011	0.053	Firmicutes	Clostridia	Peptostreptococcales	undefined	*undefined*	0.00003
Ileum	ADN	ASV4041	0.053	Firmicutes	Clostridia	Oscillospirales	Eubacteriaceae	*Eubacterium_coprostanoligenes_group*	0.00003
Ileum	ADN	ASV4430	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00003
Ileum	ADN	ASV5987	0.053	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Lachnospiraceae_NK4A136_group*	0.00003
Ileum	ADN	ASV0561	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Muribaculaceae	*Muribaculaceae*	0.00003
Ileum	ADN	ASV1800	0.053	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.00003
Ileum	ADN	ASV3759	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*Ruminococcus*	0.00003
Ileum	ADN	ASV4334	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*Oscillibacter*	0.00003
Ileum	ADN	ASV4478	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00003
Ileum	ADN	ASV0186	0.053	Kritimatiellota	Kiritimatiellia	Kritimatiellales	Kritimatiellaceae	*WCHB1-41*	0.00003
Ileum	ADN	ASV2022	0.053	Firmicutes	Clostridia	Peptostreptococcales	undefined	*undefined*	0.00003
Ileum	ADN	ASV3217	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotellaceae_NK3B31_group*	0.00003
Ileum	ADN	ASV4421	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00003
Ileum	ADN	ASV4472	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00003
Ileum	ADN	ASV3927	0.053	Firmicutes	Clostridia	Oscillospirales	Eubacteriaceae	*Eubacterium_coprostanoligenes_group*	0.00003

Gut section	Associated trait	ASV	p-value	Phylum	Class	Order	Family	Genus	Regression coefficient
Ileum	ADN	ASV4262	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-002*	0.00003
Ileum	ADN	ASV1805	0.053	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.00003
Ileum	ADN	ASV1908	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridium_sensu_stricto_1*	0.00003
Ileum	ADN	ASV3975	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*Faecalibacterium*	0.00003
Ileum	ADN	ASV0589	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Muribaculaceae	*Muribaculaceae*	0.00003
Ileum	ADN	ASV1822	0.053	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.00003
Ileum	ADN	ASV1824	0.053	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.00003
Ileum	ADN	ASV2797	0.053	Firmicutes	Bacilli	undefined	undefined	*undefined*	0.00003
Ileum	ADN	ASV4366	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-002*	0.00003
Ileum	ADN	ASV0550	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Muribaculaceae	*Muribaculaceae*	0.00003
Ileum	ADN	ASV4031	0.053	Firmicutes	Clostridia	Oscillospirales	Eubacteriaceae	*Eubacterium_coprostanoligenes_group*	0.00003
Ileum	ADN	ASV4244	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*undefined*	0.00003
Ileum	ADN	ASV4380	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*NK4A214_group*	0.00003
Ileum	ADN	ASV5071	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00003
Ileum	ADN	ASV4392	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*NK4A214_group*	0.00003
Ileum	ADN	ASV0302	0.053	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	*Treponema*	0.00003
Ileum	ADN	ASV0822	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00003
Ileum	ADN	ASV4733	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00003
Ileum	ADN	ASV5183	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00003
Ileum	ADN	ASV4502	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00003
Ileum	ADN	ASV0938	0.053	Planctomycetota	Planctomycetes	Pirellulales	Pirellulaceae	*p-1088-a5_gut_group*	0.00003
Ileum	ADN	ASV0805	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00003
Ileum	ADN	ASV0595	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Muribaculaceae	*Muribaculaceae*	0.00003
Ileum	ADN	ASV4398	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*NK4A214_group*	0.00003
Ileum	ADN	ASV0937	0.053	Planctomycetota	Planctomycetes	Pirellulales	Pirellulaceae	*p-1088-a5_gut_group*	0.00003
Ileum	ADN	ASV1831	0.053	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.00003
Ileum	ADN	ASV4542	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00003
Ileum	ADN	ASV5031	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00003
Ileum	ADN	ASV1537	0.053	Proteobacteria	Deltaproteobacteria	Bradymonadales	Bradymonadaceae	*undefined*	0.00003
Ileum	ADN	ASV1381	0.053	Proteobacteria	Gammaproteobacteria	Burkholderiales	undefined	*undefined*	0.00003
Ileum	ADN	ASV4414	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00003
Ileum	ADN	ASV4156	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*undefined*	0.00003
Ileum	ADN	ASV4539	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00003
Ileum	ADN	ASV0689	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Tannerellaceae	*Parabacteroides*	0.00003
Ileum	ADN	ASV1680	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_vadinBB60_group*	0.00003
Ileum	ADN	ASV1718	0.053	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.00003
Ileum	ADN	ASV2049	0.053	Firmicutes	Clostridia	Peptostreptococcales	undefined	*undefined*	0.00003

Gut section	Associated trait	ASV	p-value	Phylum	Class	Order	Family	Genus	Regression coefficient
Ileum	ADN	ASV2906	0.053	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	*RF39*	0.00003
Ileum	ADN	ASV3073	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotellaceae_NK3B31_group*	0.00003
Ileum	ADN	ASV4214	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*undefined*	0.00003
Ileum	ADN	ASV4652	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00003
Ileum	ADN	ASV5597	0.053	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Eubacterium_eligens_group*	0.00003
Ileum	ADN	ASV0887	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00003
Ileum	ADN	ASV5552	0.053	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*undefined*	0.00003
Ileum	ADN	ASV0012	0.053	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	*Methanosphaera*	0.00003
Ileum	ADN	ASV0925	0.053	Elusimicrobiota	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae	*Elusimicrobium*	0.00003
Ileum	ADN	ASV1564	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_vadinBB60_group*	0.00003
Ileum	ADN	ASV1842	0.053	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*undefined*	0.00003
Ileum	ADN	ASV2787	0.053	Firmicutes	Bacilli	Izemoplasmatales	Izemoplasmatales	*Izemoplasmatales*	0.00003
Ileum	ADN	ASV2911	0.053	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	*RF39*	0.00003
Ileum	ADN	ASV3916	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*Eubacterium_coprostanoligenes_group*	0.00003
Ileum	ADN	ASV4763	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00003
Ileum	ADN	ASV4903	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00003
Ileum	ADN	ASV0337	0.053	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	*Sphaerochaeta*	0.00003
Ileum	ADN	ASV0931	0.053	Elusimicrobiota	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae	*Elusimicrobium*	0.00003
Ileum	ADN	ASV1633	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_vadinBB60_group*	0.00003
Ileum	ADN	ASV4364	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-002*	0.00003
Ileum	ADN	ASV4279	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-002*	0.00003
Ileum	ADN	ASV0334	0.053	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	*Sphaerochaeta*	0.00003
Ileum	ADN	ASV4602	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*Colidextribacter*	0.00003
Ileum	ADN	ASV0884	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00003
Ileum	ADN	ASV1681	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_vadinBB60_group*	0.00003
Ileum	ADN	ASV3917	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*Eubacterium_coprostanoligenes_group*	0.00003
Ileum	ADN	ASV3938	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*Eubacterium_coprostanoligenes_group*	0.00003
Ileum	ADN	ASV4326	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*Oscillibacter*	0.00003
Ileum	ADN	ASV4872	0.053	Firmicutes	Clostridia	Monoglobales	Monoglobaceae	*Monoglobus*	0.00003
Ileum	ADN	ASV0341	0.053	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	*Sphaerochaeta*	0.00003
Ileum	ADN	ASV2741	0.053	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	*undefined*	0.00003
Ileum	ADN	ASV2745	0.053	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	*undefined*	0.00003
Ileum	ADN	ASV2953	0.053	Firmicutes	Clostridia	Peptococcales	Peptococcaceae	*undefined*	0.00003
Ileum	ADN	ASV3620	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Alloprevotella*	0.00003
Ileum	ADN	ASV3812	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*Ruminococcus*	0.00003
Ileum	ADN	ASV3944	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*Eubacterium_coprostanoligenes_group*	0.00003
Ileum	ADN	ASV4145	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*undefined*	0.00003

Gut section	Associated trait	ASV	p-value	Phylum	Class	Order	Family	Genus	Regression coefficient
Ileum	ADN	ASV4435	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00003
Ileum	ADN	ASV4535	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00003
Ileum	ADN	ASV4683	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00003
Ileum	ADN	ASV4798	0.053	Firmicutes	Clostridia	Monoglobales	Monoglobaceae	*Monoglobus*	0.00003
Ileum	ADN	ASV4823	0.053	Firmicutes	Clostridia	Monoglobales	Monoglobaceae	*Monoglobus*	0.00003
Ileum	ADN	ASV5084	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00003
Ileum	ADN	ASV5350	0.053	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*undefined*	0.00003
Ileum	ADN	ASV1703	0.053	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.00003
Ileum	ADN	ASV1823	0.053	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.00003
Ileum	ADN	ASV1834	0.053	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.00003
Ileum	ADN	ASV2697	0.053	Firmicutes	Bacilli	Erysipelotrichales	Erysipelatoclostridiaceae	*UCG-004*	0.00003
Ileum	ADN	ASV2839	0.053	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	*RF39*	0.00003
Ileum	ADN	ASV2943	0.053	Firmicutes	Bacilli	Mycoplasmatales	Mycoplasmataceae	*Mycoplasma*	0.00003
Ileum	ADN	ASV3749	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*Ruminococcus*	0.00003
Ileum	ADN	ASV3839	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*Candidatus_Soleaferrea*	0.00003
Ileum	ADN	ASV3880	0.053	Firmicutes	Clostridia	Oscillospirales	Clostridiaceae	*Clostridium_methylpentosum_group*	0.00003
Ileum	ADN	ASV4246	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*undefined*	0.00003
Ileum	ADN	ASV4567	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*undefined*	0.00003
Ileum	ADN	ASV4693	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00003
Ileum	ADN	ASV4727	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00003
Ileum	ADN	ASV4808	0.053	Firmicutes	Clostridia	Monoglobales	Monoglobaceae	*Monoglobus*	0.00003
Ileum	ADN	ASV4845	0.053	Firmicutes	Clostridia	uncultured	uncultured	*undefined*	0.00003
Ileum	ADN	ASV5048	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00003
Ileum	ADN	ASV5281	0.053	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Tuzzerella*	0.00003
Ileum	ADN	ASV5560	0.053	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Roseburia*	0.00003
Ileum	ADN	ASV1819	0.053	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.00003
Ileum	ADN	ASV2785	0.053	Firmicutes	Bacilli	undefined	undefined	*undefined*	0.00003
Ileum	ADN	ASV2802	0.053	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	*RF39*	0.00003
Ileum	ADN	ASV3899	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*Eubacterium_coprostanoligenes_group*	0.00003
Ileum	ADN	ASV2627	0.053	Firmicutes	Bacilli	Erysipelotrichales	Erysipelatoclostridiaceae	*Erysipelotrichaceae_UCG-003*	0.00003
Ileum	ADN	ASV3904	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*Eubacterium_coprostanoligenes_group*	0.00003
Ileum	ADN	ASV4738	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00003
Ileum	ADN	ASV4756	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00003
Ileum	ADN	ASV0692	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Tannerellaceae	*Parabacteroides*	0.00003
Ileum	ADN	ASV1648	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_vadinBB60_group*	0.00003
Ileum	ADN	ASV3033	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotellaceae_NK3B31_group*	0.00003
Ileum	ADN	ASV4024	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*Eubacterium_coprostanoligenes_group*	0.00003

Gut section	Associated trait	ASV	p-value	Phylum	Class	Order	Family	Genus	Regression coefficient
Ileum	ADN	ASV4098	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*Eubacterium_coprostanoligenes_group*	0.00003
Ileum	ADN	ASV4189	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*undefined*	0.00003
Ileum	ADN	ASV4200	0.053	Firmicutes	Clostridia	Oscillospirales	Butyricicoccaceae	*UCG-009*	0.00003
Ileum	ADN	ASV4453	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00003
Ileum	ADN	ASV4768	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00003
Ileum	ADN	ASV4933	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00003
Ileum	ADN	ASV5046	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00003
Ileum	ADN	ASV5444	0.053	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Lachnospiraceae_NK4B4_group*	0.00003
Ileum	ADN	ASV3244	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*undefined*	0.00004
Ileum	ADN	ASV6113	0.053	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*[Bacteroides]_pectinophilus_group*	0.00004
Ileum	ADN	ASV1555	0.053	Proteobacteria	Deltaproteobacteria	Bradymonadales	Bradymonadaceae	*undefined*	0.00003
Ileum	ADN	ASV1722	0.053	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.00003
Ileum	ADN	ASV2711	0.053	Firmicutes	Bacilli	Erysipelotrichales	Erysipelatoclostridiaceae	*UCG-004*	0.00003
Ileum	ADN	ASV2934	0.053	Firmicutes	Bacilli	Acholeplasmatales	Acholeplasmataceae	*T2WK15B57*	0.00003
Ileum	ADN	ASV3842	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*Candidatus_Soleaferrea*	0.00003
Ileum	ADN	ASV4141	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*undefined*	0.00003
Ileum	ADN	ASV4197	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00003
Ileum	ADN	ASV4225	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*Ruminococcus*	0.00003
Ileum	ADN	ASV4532	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00003
Ileum	ADN	ASV5034	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00003
Ileum	ADN	ASV2053	0.053	Firmicutes	Clostridia	Peptostreptococcales	undefined	*undefined*	0.00004
Ileum	ADN	ASV0123	0.053	Cyanobacteria	Vampirivibrionia	undefined	undefined	*undefined*	0.00003
Ileum	ADN	ASV1573	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_vadinBB60_group*	0.00003
Ileum	ADN	ASV1710	0.053	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.00003
Ileum	ADN	ASV2103	0.053	Firmicutes	Clostridia	Peptococcales	Peptococcaceae	*undefined*	0.00003
Ileum	ADN	ASV2959	0.053	Firmicutes	Clostridia	Peptococcales	Peptococcaceae	*undefined*	0.00003
Ileum	ADN	ASV3289	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotella*	0.00003
Ileum	ADN	ASV3500	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotellaceae_UCG-004*	0.00003
Ileum	ADN	ASV3965	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*undefined*	0.00003
Ileum	ADN	ASV4660	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00003
Ileum	ADN	ASV4686	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00003
Ileum	ADN	ASV4720	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00003
Ileum	ADN	ASV4851	0.053	Firmicutes	Clostridia	Monoglobales	Monoglobaceae	*Monoglobus*	0.00003
Ileum	ADN	ASV4859	0.053	Firmicutes	Clostridia	Monoglobales	Monoglobaceae	*Monoglobus*	0.00003
Ileum	ADN	ASV4975	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00003
Ileum	ADN	ASV6169	0.053	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*undefined*	0.00003
Ileum	ADN	ASV2309	0.053	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	*Veillonellaceae*	0.00003

Gut section	Associated trait	ASV	p-value	Phylum	Class	Order	Family	Genus	Regression coefficient
Ileum	ADN	ASV2673	0.053	Firmicutes	Bacilli	Erysipelotrichales	Erysipelatoclostridiaceae	*UCG-004*	0.00003
Ileum	ADN	ASV2687	0.053	Firmicutes	Bacilli	Erysipelotrichales	Erysipelatoclostridiaceae	*UCG-004*	0.00003
Ileum	ADN	ASV2875	0.053	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	*RF39*	0.00003
Ileum	ADN	ASV3777	0.053	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*Ruminococcus*	0.00003
Ileum	ADN	ASV4354	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*Oscillibacter*	0.00003
Ileum	ADN	ASV4970	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00003
Ileum	ADN	ASV0253	0.053	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	*Treponema*	0.00003
Ileum	ADN	ASV1177	0.053	Desulfobacterota	Desulfovibrionia	Desulfovibrionales	Desulfohalobiaceae	*undefined*	0.00003
Ileum	ADN	ASV1371	0.053	Proteobacteria	Gammaproteobacteria	Burkholderiales	Alcaligenaece	*undefined*	0.00003
Ileum	ADN	ASV1704	0.053	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_vadinBB60_group*	0.00003
Ileum	ADN	ASV3417	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	*Bacteroides*	0.00003
Ileum	ADN	ASV4384	0.053	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*NK4A214_group*	0.00003
Ileum	ADN	ASV5860	0.053	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*undefined*	0.00003
Ileum	ADN	ASV0864	0.054	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00003
Ileum	ADN	ASV3931	0.054	Firmicutes	Clostridia	Oscillospirales	Eubacteriaceae	*Eubacterium_coprostanoligenes_group*	0.00004
Ileum	ADN	ASV5521	0.055	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*undefined*	0.00004
Ileum	ADN	ASV0803	0.056	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00003
Ileum	ADN	ASV5680	0.056	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Lachnospiraceae_UCG-009*	0.00004
Ileum	ADN	ASV0285	0.061	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	*Treponema*	0.00003
Ileum	ADN	ASV0357	0.061	Bacteroidetes	Bacteroidia	Bacteroidales	Muribaculaceae	*Muribaculaceae*	0.00003
Ileum	ADN	ASV0842	0.063	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	*Rikenellaceae_RC9_gut_group*	0.00005
Ileum	ADN	ASV5565	0.063	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Roseburia*	0.00003
Ileum	ADN	ASV4906	0.063	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00004
Ileum	ADN	ASV4128	0.063	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*undefined*	0.00004
Ileum	ADN	ASV5807	0.064	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Lachnoclostridium*	0.00003
Ileum	ADN	ASV6030	0.067	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*undefined*	0.00004
Ileum	ADN	ASV0939	0.068	Planctomycetota	Planctomycetes	Pirellulales	Pirellulaceae	*p-1088-a5_gut_group*	0.00003
Ileum	ADN	ASV6043	0.069	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Lachnospiraceae_AC2044_group*	0.00004
Ileum	ADN	ASV4368	0.071	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-002*	0.00003
Ileum	ADN	ASV3832	0.072	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*Ruminococcus*	0.00004
Ileum	ADN	ASV5215	0.075	Firmicutes	Clostridia	Clostridiidiales	Clostridiaceae	*Clostridia_UCG-014*	0.00003
Ileum	ADN	ASV0740	0.076	Bacteroidetes	Bacteroidia	undefined	undefined	*undefined*	0.00004
Ileum	ADN	ASV4477	0.082	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00004
Ileum	ADN	ASV3686	0.083	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotellaceae_UCG-003*	0.00005
Ileum	ADN	ASV3962	0.084	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*Eubacterium_coprostanoligenes_group*	0.00004
Ileum	ADN	ASV5973	0.091	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Lachnospiraceae_NK4A136_group*	0.00004
Ileum	ADN	ASV3641	0.099	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Alloprevotella*	0.00004

Gut section	Associated trait	ASV	p-value	Phylum	Class	Order	Family	Genus	Regression coefficient
Ileum	NEXU	ASV4385	0.078	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*NK4A214_group*	0.00202
Ileum	NEXU	ASV3837	0.085	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*undefined*	0.00269
Ileum	NEXU	ASV4148	0.088	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*undefined*	0.00264
Ileum	NEXU	ASV1150	0.090	Desulfobacterota	Desulfovibrionia	Desulfovibrionales	Desulfohalobiaceae	*undefined*	0.00148
Ileum	NEXU	ASV0939	0.094	Planctomycetota	Planctomycetes	Pirellulales	Pirellulaceae	*p-1088-a5_gut_group*	0.00123
Ileum	NEXU	ASV4771	0.097	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00096
Caecum	NEXU	ASV3869	0.058	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*undefined*	0.00575
Caecum	NEXU	ASV4461	0.063	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00587
Caecum	NEXU	ASV4672	0.065	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00582
Caecum	NEXU	ASV4716	0.065	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00667
Caecum	NEXU	ASV4727	0.067	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00495
Caecum	NEXU	ASV4736	0.072	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00459
Caecum	NEXU	ASV4200	0.073	Firmicutes	Clostridia	Oscillospirales	Butyricicoccaceae	*UCG-009*	0.00421
Caecum	NEXU	ASV0936	0.075	Planctomycetota	Planctomycetes	Pirellulales	Pirellulaceae	*p-1088-a5_gut_group*	0.00572
Caecum	NEXU	ASV0747	0.081	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	*F082*	0.00492
Caecum	NEXU	ASV4339	0.084	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*Oscillibacter*	0.00489
Caecum	NEXU	ASV0168	0.088	Kritimatiellota	Kiritimatiellia	Kritimatiellales	Kritimatiellaceae	*WCHB1-41*	0.00375
Caecum	NEXU	ASV4568	0.092	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*undefined*	0.00593
Caecum	NEXU	ASV4449	0.096	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-005*	0.00498
Caecum	NEXU	ASV4771	0.098	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.00454
Caecum	NEXU	ASV5474	0.092	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Roseburia*	0.00468
Colon	ADG	ASV2896	0.080	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	*RF39*	0.05018
Colon	ADG	ASV1703	0.083	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.05489
Colon	ADG	ASV5086	0.088	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridium*	0.04733
Colon	ADG	ASV1783	0.096	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.04444
Colon	ADG	ASV2013	0.097	Firmicutes	Clostridia	Peptostreptococcales	undefined	*undefined*	0.02048
Colon	ADG	ASV2052	0.097	Firmicutes	Clostridia	Peptostreptococcales	undefined	*undefined*	0.02065
Colon	ADG	ASV1526	0.097	Proteobacteria	Alphaproteobacteria	Rickettsiales	uncultured	*undefined*	0.05445
Colon	ADG	ASV4637	0.097	Firmicutes	Clostridia	Oscillospirales	Butyricicoccaceae	*UCG-008*	0.02443
Colon	ADG	ASV5289	0.098	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Lachnospiraceae_UCG-010*	0.02091
Colon	ADG	ASV3232	0.098	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotella*	0.01992
Colon	ADG	ASV3231	0.098	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotella*	0.01984
Colon	ADG	ASV1776	0.098	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.02097
Colon	ADG	ASV5869	0.098	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*undefined*	0.01981

Gut section	Associated trait	ASV	p-value	Phylum	Class	Order	Family	Genus	Regression coefficient
Colon	ADG	ASV1838	0.098	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	*Christensenellaceae_R-7_group*	0.02555
Colon	ADG	ASV3672	0.099	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotellaceae_UCG-003*	0.01961
Colon	ADG	ASV3755	0.099	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*Ruminococcus*	0.01966
Colon	ADG	ASV5783	0.099	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*undefined*	0.02128
Colon	ADG	ASV4772	0.099	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	*UCG-010*	0.02128
Colon	ADG	ASV5644	0.099	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Eubacterium_rumiundefinedntium_group*	0.02131
Colon	ADG	ASV5389	0.100	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*undefined*	0.01936
Colon	ADP	ASV3691	0.064	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotellaceae_UCG-003*	0.00667
Colon	ADP	ASV4157	0.069	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	*undefined*	0.00496
Colon	ADP	ASV1642	0.072	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_vadinBB60_group*	0.00498
Colon	ADP	ASV1554	0.072	Proteobacteria	Deltaproteobacteria	Bradymonadales	Bradymonadaceae	*undefined*	0.00575
Colon	ADP	ASV5633	0.084	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	*Eubacterium_rumiundefinedntium_group*	0.00454
Colon	ADP	ASV3155	0.085	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotellaceae_NK3B31_group*	0.00593
Colon	ADP	ASV5118	0.086	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridia_UCG-014*	0.00459
Colon	ADP	ASV1571	0.086	Firmicutes	Clostridia	Clostritdiales	Clostridiaceae	*Clostridia_vadinBB60_group*	0.00488
Colon	ADP	ASV0178	0.087	Kritimatiellota	Kiritimatiellia	Kritimatiellales	Kritimatiellaceae	*WCHB1-41*	0.00375
Colon	ADP	ASV0786	0.090	Bacteroidetes	Bacteroidia	Bacteroidales	Muribaculaceae	*undefined*	0.00572
Colon	ADP	ASV2053	0.091	Firmicutes	Clostridia	Peptostreptococcales	Acidaminobacteraceae	*undefined*	0.00587
Colon	ADP	ASV3297	0.098	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	*Prevotellaceae_NK3B31_group*	0.00582
Colon	ADP	ASV1557	0.099	Proteobacteria	Deltaproteobacteria	Bradymonadales	Bradymonadaceae	*undefined*	0.00421
Colon	ADP	ASV0561	0.100	Bacteroidetes	Bacteroidia	Bacteroidales	Muribaculaceae	*undefined*	0.00492

Abbreviations: ADG, average daily gain; AND, N digestibility; ADP, P digestibility; PEX, faecal P excretion; NEXu, urinary N excretion.

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Publications and presentations

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