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Microbial and molecular signatures for enhanced gut health in pigs fed a diet with spray-dried plasma as functional protein source

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This study investigates the effects of dietary spray-dried plasma (SDP) supplementation on the gut and health of growing pigs using an advanced “FeedOmics” approach. This integrative methodology employs a range of omics-based techniques to analyze microbial and molecular “signatures” associated with the dietary impact of SDP. Sixteen male growing pigs (34.9 ± 3.4 kg, 10–11 week old; n = 8/treatment group) were randomly assigned to receive one of two experimental diets for three weeks. These diets were prepared with one of the following protein sources containing (as fed basis): soybean meal (SBM, 343 g/kg), or SDP protein (SDPP, 196 g/kg). At the end of the trial, pigs were euthanized to collect jejunal and ileal mucosal tissues for gene expression analysis, jejunal and ileal digesta for microbiota profiling, blood plasma for systemic metabolomic assessment, and serum for cytokine and chemokine quantification. The SBM-based diet group was used as the reference. Findings indicate that SDPP influences the jejunum more than the ileum, where it causes a significant reduction in bacterial alpha diversity (P < 0.05) compared to SBM. We report a significant (P < 0.05) decrease in the relative abundance of the *Sarcina* genera in the small intestine, an effect comparable to pharmaceutical concentrations of zinc oxide (ZnO). Transcriptomic analysis identified 319 genes with altered expression in the jejunal mucosal tissue. Notably, SDPP upregulated tight junction receptors in the jejunum, which suggests it improves intestinal integrity by strengthening the gut barrier. We measured metabolites like threonine, taurine, and glutamine/glutamate in the blood of SDPP-fed pigs. These metabolites act as distinctive “fingerprints,” contributing to gut health by providing cellular energy, restoring tight junctions, and orchestrating immune responses in the small intestine mucosa. Overall, our results highlight the nutritional and functional value of SDP as functional dietary protein source.

KEYWORDS

feedomics, gut health, immune system, microbiome, pig, spray dried plasma protein, transcriptome

1 Introduction

The increasing global demand for sustainable and efficient animal protein production necessitates the exploration of alternative protein sources. Traditional protein sources, such as soybean meal (SBM) and fishmeal, face challenges related to environmental impact, supply chain volatility, and potential human health concerns. To address these issues, researchers are investigating novel protein sources, including plant-based, insect-based, and microbial proteins (Kar, 2017; Kar et al., 2020, Kar et al., 2021a; Roques et al., 2022). Among these alternatives, spray-dried plasma (SDP) has emerged as a promising functional protein source for pig nutrition (Perez-Bosque et al., 2016; Roques et al., 2022). Spray dried plasma, derived from porcine or bovine blood plasma, is a rich source of high-quality protein, essential amino acids, growth factors, and immunoglobulins. By SDP in pig diets, it is possible to enhance growth performance, intestinal health, and immune function. Furthermore, the production of SDP aligns with the principles of a circular economy, as it utilizes a valuable byproduct from the slaughterhouse, thereby reducing waste and promoting sustainability.

Dietary SDP is widely used in nursery pig diet due to its nutritional value (high protein content with a high digestibility and a balanced amino acid profile) and its functional properties in relation to gut health. Improved growth performance was recorded as a response of weaned piglets to dietary SDP (Bikker et al., 2004; Kim et al., 2022). Additional research findings indicate that the inclusion of SDP enhances the health and growth performance of piglets, safeguarding the histological integrity and functionality of the small intestine (Moreto and Perez-Bosque, 2009; Gao et al., 2011).

From previous research, it is known that dietary protein sources can have profound effects on host-microbe interactions, both local (small intestine) and systemic (blood) levels (Kar et al., 2017a, Kar et al., 2017b). Spray dried plasma may support gut health and productivity in young pigs due to its functional value, enhanced by bioactive proteins like immunoglobulins (Balan et al., 2021) and bioactive peptides (Kar et al., 2016), which regulate factors affecting intestinal microbiota and mucosal immunity. However, information is missing on understanding the intricate interactions at the microbial and molecular level in the lumen and tissues of the gut that highlights the functional value of SDP. To fully evaluate SDP's functional value, it is essential to understand how dietary protein sources impact digestive physiology through gut microbiota, intestinal mucosal response, and systemic nutrient metabolism in pigs. The functional value of dietary proteins from insects fed to pigs after weaning was determined by a FeedOmics approach (Kar et al., 2021a). Using ~ omics techniques within the FeedOmics approach, relevant data can be generated, allowing to gain a deep and thorough insight into the biological responses to differences in diet composition. However, such an approach has not yet been used in relation to diets containing SDP as a protein source for pigs.

Using a FeedOmics approach, we evaluated the effects of diets formulated with either soybean meal (SBM) as a reference protein source or SDP protein (SDPP) in pigs, focusing on both local (small intestine) and systemic (blood) responses. Data were collected

across multiple biological levels to assess the functional value of SDP as a dietary protein source. Specifically, transcriptomic analyses of intestinal tissues and microbial profiling of the jejunal and ileal digesta were conducted to investigate gut functionality. Systemic effects were evaluated through blood amine profiling, while cytokine and chemokine concentrations in serum were measured to assess the immune and health status of the pigs.

2 Materials and methods

2.1 Protein sources and experimental diets

The following protein sources were evaluated in the experimental diets, 1) soybean meal (SBM), commodity batch obtained via Research Diet Services, Wijk bij Duurstede, the Netherlands, and 2) SDP, obtained from Darling Ingredients B.V., the Netherlands. The evaluated nutrient composition of the SDP batch is as follows- Dry Matter (DM, as in) 915 g/kg; Protein 875 g/kg of DM; Ash content 78 g/kg of DM; Total fat 5 g/kg of DM and Gross energy 22 kJ/g of DM. The SDP sourced from Darling Ingredients is a reliable and high-quality ingredient for animal feed, meeting stringent processing and safety standards in compliance to the 'Good Manufacturing' Practices meeting industrial standards (Blazquez et al., 2020). The experimental diets were formulated to be iso-proteinaceous (CP, 160 g/kg as-fed basis) and included as single protein sources in the two experimental diets. Both diets were produced as mash by Research Diet Services (Wijk bij Duurstede, the Netherlands).

2.2 Animals and housing

All experimental procedures were conducted in compliance with the ARRIVE guidelines and received approval from the Animal Experimentation Board of Wageningen University & Research Centre (approval number 2014099.b). All methods performed here adhered to the relevant guidelines and regulations governing animal research. The full experimental design has already been described (Kar, 2017), a schematic representation can be seen in [Supplementary Figure 1](#). The experimental design and the measured biomolecular parameters have been thoroughly detailed in a previous publication (Kar et al., 2021a). However, the key methodologies are outlined below for clarity. A total of 16 growing pigs (boars) (Topigs 20 × Tempo from Van Beek, Lelystad, the Netherlands) with an average initial body weight of 34.9 ± 3.4 kg (10–11-week-old) on the day (d) of arrival were included in the study. Pigs were blocked on litter and pigs within a block were randomly allocated to one of the experimental diets with eight pigs per experimental diet. The pigs were housed individually in metabolic cages (1.3 x 1.3 m or 2.0 x 1.0 m) with a tender foot floor. The ambient temperature was kept at 24°C on d 1 and 2, at 23°C on d 3, and constant at 22°C from d 4 and onwards. This helps pigs acclimate to the ambient temperature in the experimental unit. During d 1 to 27, the lights were turned on between 5.30 h till 19.00 h. During d 28 to 30, the

lights were turned on between 2.30 h till 19.00 h. Adjustments to feeding strategies on dissection days (detail below), combined with modifications to light timing, ensured synchronized feeding of all experimental pigs. This approach maintained consistent conditions for digestion kinetics and nutrient metabolism, enabling the collection of representative biological samples during dissection. Body weight of animals was measured every week throughout the experimental period.

2.3 Feeding

First an adaptation period of one week was installed, where all pigs were adapted to the experimental diet (Table 1). From week 0 and onwards, pigs were fully on the experimental diets, where these diets were provided in a mash form and mixed with water (ratio 1:2). Water consumption was limited, and an extra 0.3 L of water was provided after each feeding. The feeding level was 2.5 times the net energy (NE) requirement for maintenance (293 kJ NE/kg BW^{0.75}). During week 0 to 3, the daily feed allowance was divided into two equal amounts, fed at 8.00h and 16.00h. During week 3 till the moment of dissection at the end of the experimental period, the daily feed allowance was divided into 6 equal amounts, fed starting at 5.30 h at intervals of 3h.

2.4 Sample collection and dissection procedure

Blood samples were collected via the ear-vein for serum and plasma preparation on d 7 and at dissection days (d28-29) after the morning meal ingestion. For serum, blood samples were collected in sterile Vacuette tubes containing Z-serum separator clot activator (Greiner Bio-One B.V., Alphen aan den Rijn, the Netherlands), tubes were gently inverted and allowed to clot for at least 30 min. All tubes were centrifuged at 2,200x g for 15 min at 20°C and serum was extracted. For plasma, blood samples were collected in sterile Vacuette tubes containing lithium-heparin and immediately centrifuged at 3,000x g for 10 min at 4°C and plasma was extracted. Both serum and plasma were stored at -80°C for further analysis on levels of systemic cytokine and chemokines as well as systemic amine metabolite profiles, respectively.

On dissection days, pigs were euthanized following anesthesia induced with pentobarbital (PentobarsolTM, Dechra, Fort Worth, USA), administered via injection into the ear vein at a dosage recommended by the manufacturer relative to their body weight, before being sacrificed by bleeding. The small intestine was separated from the stomach and the large intestine. Jejunum and ileum were divided into three equal segments and two sub-segments of the same location of each tissue were sampled for intestinal mucosal layer and resident microbiota. For the intestinal mucosal layer, one of the sub-segments was cut open longitudinally along the

lumen and washed with sterile normal saline solution. With a sterile glass slide the mucosal layer was collected, snap frozen in liquid nitrogen and stored at -80°C for further analysis of genome-wide gene expression profiling. From another sub-segment, luminal digesta was collected to perform a community-scale analysis of the gut microbiota.

2.5 Chemical analysis of the diet

Representative samples of dried and ground diets were chemically analyzed for dry matter (DM) (ISO-6496, 1999; by four hours' drying at 104°C); sugar (ISO 15914-1, I, 2004); starch (van Vuuren et al., 1993); ash (ISO-5984, 2002; after three hours' ashing at 550°C); ether extract (ISO-6492, 1999; by extraction with petroleum ether); and nitrogen (N) (ISO-5983-2, 2009; by Kjeldahl method) and crude protein (CP) (calculated as Nx6.25). The results are shown in Table 1.

2.6 Small intestinal genome-wide transcriptome profiling

From each individual pig, jejunal and ileal tissue samples were collected, subsequently total RNA extraction was performed. Total RNA from individual samples from each intestinal tissue was extracted using trizol reagent (Life Technologies, California, United States) as recommended by the manufacturer. Homogenized tissue samples were dissolved in 5ml of trizol reagent (ThermoFisher Scientific, Lelystad, NL). After centrifugation, the supernatant was transferred to a fresh tube. Subsequently a phase separation with chloroform was performed as described by the manufacturer. The RNA was precipitated and dissolved, thereafter quantified by absorbance measurements at 260 nm. Quality check of the RNA samples was performed with the Agilent Bioanalyzer (Agilent, CA, USA). Details of biological replicates per treatment are shown in Supplementary Table 1. One RNA sample from ileum belonging to SDPP group failed to pass the QC performed with the Agilent Bioanalyzer. Downstream handling of the samples, i.e. labeling, hybridization, scanning, feature extraction, and QC by statistical analysis was performed as described previously (Schokker et al., 2014). Briefly, labeling was done as recommended by Agilent Technologies using the One-Color Microarray-Based Gene Expression Analysis Low Input Quick Amp Labelling. The input was 200 ng of total RNA and 600 ng of labelled cRNA was used on the 8-pack array. Hybridization was performed as described in the One-Color Microarray-Based Gene Expression Analysis Low Input Quick Amp Labelling protocol from Agilent in the hybridization oven (G2545A hybridization Oven Agilent Technologies). The hybridization temperature was 65°C with rotation speed 10 rpm for 17 hours. After 17 hours the arrays were washed as described in the One-Color Microarray-Based Gene Expression Analysis Low

TABLE 1 Ingredient and calculated or analyzed¹ nutrient composition of the experimental diets for pigs (as fed basis).

Diet formulation	Diet ²		Composition continued	Diet ²	
	SBM	SDPP		SBM	SDPP
Item	SBM	SDPP	Amino acids, g/kg	AID ⁶	AID
Ingredients, g/kg					
Maize starch	376.2	521.4	Lys	8.7	14.2
Sugar	100	100	Met	2.3	1.2
Dextrose	50	50	Cys	1.9	5.4
Cellulose ³	50	50	Met + Cys	4.2	6.6
Soybean oil	43.3	32.6	Thr	5.1	9
Chalk	14.4	15.7	Trp	1.8	2.7
Mono sodium phosphate	10.1	14.2	Ile	6.3	7
NaCl	4.1	0	Arg	11.1	9.1
Sodium bicarbonate	1.4	0	Phe	7.3	8.9
Calcium carbonate	0	11.2	His	3.8	5.2
Calcium chloride	0	0	Leu	10.5	15
Premix	5	5	Tyr	5.1	8.6
Titanium di-oxide	2.5	2.5	Val	6.4	10.6
DL-Methionine	0.3	1.4			
SDPP	0	196			
Soybean meal	342.7	0			
<i>Total</i>	<i>1000</i>	<i>1000</i>			
Composition ⁴ , g/kg					
Dry matter ¹	904	912			
Crude protein ¹	166	158			
Sugars ¹	175	162			
Starch ¹	296	431			
NSP ⁵	130	64			
Fat ¹	31	20			
Ash ¹	53	57			
Ca	8.2	8.2			
Gross energy, MJ/kg	10.7	10.7			

¹Analyzed nutrient compositions.

²Diet: Diet containing soybean meal (SBM), and diet containing spray dried plasma protein (SDPP).

³Arbocell® (JRS, Germany).

⁴Diet compositions were formulated using data on ingredient nutrient composition and nutrient digestibility coefficients according to the Central Bureau for Livestock Feeding [CVB, Lelystad, the Netherlands (Research, 2020)].

⁵Non-starch polysaccharides.

⁶Apparent ileal digestibility, g/kg.

Input Quick Amp Labelling protocol from Agilent. The porcine Agilent microarray slides, G2519F *Sus scrofa* (035953), harbouring 43,803 probes, were used, and scanned using the DNA microarray scanner with SureScan high resolution Technology (Agilent Technologies). Agilent Scan Control with resolution of 5 µ, 16 bits and PMT of 100%. Feature extraction was performed using protocol 10.7.3.1 (v10.7) for 1 color gene expression.

The files generated by the feature extraction software were loaded in the Gene Expression Omnibus from NCBI with the accession number GSE98261, in which a log₂-transformation and quantile normalization was carried out as described (Schokker et al., 2018), by executing different packages, including LIMMA (Smyth, 2005) within R. Thereafter, principal component analysis (PCA; unsupervised) was performed. In the first approach, transcripts

were defined as significantly differentially expressed when the log fold-change (FC) between the treatment groups for both intestinal locations was > 1 or < -1 and the adjusted P-value (false discovery rate) of LIMMA was < 0.05 . The files generated by the feature extraction software were loaded in the Gene Expression Omnibus from NCBI with the accession number GSE98261, in which a \log_2 -transformation and quantile normalization was carried out as described (Schokker et al., 2018), by executing different packages, including LIMMA (Smyth, 2005) within R. Thereafter, principal component analysis (PCA; unsupervised) was performed. In the first approach, transcripts were defined as significantly differentially expressed when the log fold-change (FC) between the treatment groups for both intestinal locations was > 1 or < -1 and the adjusted P-value (false discovery rate) of LIMMA was < 0.05 .

For a 'data-driven' approach, the differentially expressed genes (DEGs) were used as input to identify enriched pathways, using the Kyoto Encyclopedia of Genes and Genomes (KEGG) collection. In addition, also a 'biology-driven' approach was used, targeting specific pathways involved in gut functionality. To get better insight and proper interpretation of the transcriptomics data, we have superimposed the gene expression values from the genome-wide transcriptomes to KEGG pathways. The selected KEGG pathways encompass the 'Intestinal Immune Network for IgA Production', identified through a data-driven methodology (Table 2), and the 'Tight Junction Pathway,' which serves as an indicator of gut barrier integrity and was chosen based on a biology-driven approach to directly reflect gut health.

2.7 Small intestinal microbiota profiling

2.7.1 Microbial DNA extraction

Details on the number of replicate samples per treatment per type of analysis are shown in Supplementary Table 1. The number of replicates ranged from 5 to 8 animals per treatment group due to non-availability of sufficient luminal digesta in some cases. Luminal digesta of each small intestinal location was mixed 1:1 with Phosphate Buffered Solution and vortexed, spun for 5 min (300x g) at 4°C. DNA was extracted from small intestinal digesta by the repeated bead beating method (Yu and Morrison, 2004) using QIAamp PowerFecal DNA Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. PowerBead solution (750 μ l) was added to the 5ml Eppendorf tube containing small intestinal digesta approximately 200 mg of luminal digesta (wet weight) was used for microbial DNA extraction. The quality and quantity of extracted DNA samples were checked by gel electrophoresis (only representative samples) and Nanodrop (Agilent, CA, USA), respectively.

2.7.2 16S rRNA gene-based amplicon sequencing

Library construction of the V3 hypervariable region (from 16S rRNA gene) followed by sequencing on an Illumina MiSeq platform (paired end reads; 2*300 bp) were performed. Amplicons of the V3 hypervariable region of the 16S rRNA gene, were generated using the primer set V3_F (CCTACGGGAGGCAGCAG) and reverse primer V3_R (ATTACCGCGCTGCTGG). Polymerase chain reaction

TABLE 2 Enriched biological pathways¹ in small intestinal tissue (jejunum and ileum) of pigs fed a SBM or SDPP based diet.

Jejunum	P-value	GenesHit
Intestinal immune network for IgA production	<0.001	TNFRSF17; CCL28;CCR10
Viral protein interaction with cytokine and cytokine receptor	<0.01	CCL19;CCL28;CCR10
Cytokine-cytokine receptor interaction	0.02	TNFRSF17;CCL19; CCL28;CCR10
Chemokine signaling pathway	0.03	CCL19;CCL28;CCR10
Complement and coagulation cascades	0.04	VWF;CFB
PI3K-Akt signaling pathway	0.04	CHRM1;VWF; THBS3;FGFR1
ECM-receptor interaction	0.04	VWF;THBS3
<i>Leukocyte transendothelial migration</i>	0.06	<i>CDH5;CLDN5</i>
<i>Cell adhesion molecules</i>	0.10	<i>CDH5;CLDN5</i>
Ileum	P-value	GenesHit
Steroid biosynthesis	<0.001	SQLE;CYP24A1; CYP51A1;MSMO1
Cholesterol metabolism	<0.01	MYLIP;LDLR
Mineral absorption	<0.01	MT1A;MT1F
Bile secretion	<0.01	NR0B2;LDLR
<i>Bladder cancer</i>	0.06	<i>UPK3A</i>
<i>Intestinal immune network for IgA production</i>	0.07	<i>CCL28</i>
<i>Ovarian steroidogenesis</i>	0.07	<i>LDLR</i>
<i>Glutathione metabolism</i>	0.08	<i>GPX2</i>
<i>Arachidonic acid metabolism</i>	0.08	<i>GPX2</i>
<i>Cortisol synthesis and secretion</i>	0.09	<i>LDLR</i>

¹The Kyoto Encyclopedia of Genes and Genomes (KEGG) was used. Bold indicates pathways with significant enrichment (P<0.05).

(PCR) conditions were as follows: 2 min at 98°C, 15× (10 s at 98°C, 30 s at 55°C, and 10 s at 72°C), and 7 min at 72°C. The PCR products were mixed with the same volume of 1x loading buffer (contained SYBR green) and were detected by electrophoresis on 2% agarose gel. Prior to library preparation, the PCR products were mixed in equimolar ratio and purified using Qiagen Gel Extraction Kit (Qiagen, Hilden, Germany). Samples were sequenced by targeted amplicon 16S sequencing using the MiSeq sequencer (Illumina, San Diego, California, United States). Subsequently, the sample-specific barcodes and primer sequences were trimmed from the Illumina raw reads and the file generated were uploaded in sequence read archive from NCBI with the accession number PRJNA1139701.

2.7.3 16S based amplicon sequencing data analysis

The trimmed paired end reads were imported into the CLC Genomics Workbench version 20.0.4 and were processed using the CLC Microbial Genomics Module version 2.5.1 (CLC bio, Arhus,

Denmark). The paired end reads were merged into one high quality representative sequence using CLC default parameters (Mismatch cost = 1, Minimum score = 40, Gap Cost = 4, Maximum unaligned end mismatches = 5). The sequences were then clustered into operational taxonomic unit (OTUs) at 97% identity threshold, followed by taxonomic annotation using SILVA database v138.1 (Quast et al., 2013). The OTU table is further filtered by removing OTUs with low abundance (Minimum combined count = 10), to get a final abundance table for each sample. Using the relative abundance of matched OTUs at genus level, principle component analysis (PCA; unsupervised) was performed (released on Dec 13, 2017) (Quast et al., 2013). The OTU table is further filtered by removing OTUs with low abundance (Minimum combined count = 10), to get a final abundance table for each sample. Using the relative abundance of matched OTUs at genus level, principle component analysis (PCA; unsupervised) was performed using Canoco 5 (v5.10) according to software developer's instructions (Ter Braak and Smilauer, 2018).

The phylogenetic tree was constructed using Maximum Likelihood Phylogeny tool based on a Multiple Sequence Alignment of the OTU sequences (top 100 most abundant OTUs) generated by MUSCLE (Multiple Sequence Comparison by Log-Expectation) tool (Edgar, 2004) in the workbench. The Maximum Likelihood Phylogeny tool determines the probability of the sequences in the tree, using Neighbour Joining as construction method and Jukes Cantor as Nucleotide substitution model. Abundance analysis, such as alpha- and beta- diversity, PERMANOVA analysis as well as differential abundance analysis (DAA) were performed using CLC- Microbial Genomics Module. Alpha- and beta-diversity measures were calculated with the relative abundance of matched OTU at genus level. To show whether genus abundance profiles of replicate samples for each dietary treatments taken from different locations i.e. jejunum and ileum, varied significantly according to the dietary treatments within a location or not. Comparison of the alpha-diversity measures i.e. Observed species and Shannon indices; considering the number of species and the evenness of the species was performed by a Mann Whitney U-test (non-parametric) or Student's T-test (parametric) in GraphPad Software (8.1.1). Bray-Curtis's measure was used to calculate distance matrices for the beta diversity. To visualize the beta diversity results, Principal Coordinate Analysis (PCoA) was performed on the dissimilarity matrices. Additionally, to measure the effect size and significance on beta-diversity (i.e. Bray Curtis) of the replicate digesta samples for each dietary treatments within a location, permutational multivariate analysis of variance (PERMANOVA) analysis, also known as non-parametric MANOVA (Anderson, 2001), was performed using OTU abundance profiles. Furthermore, we focused on the taxonomic distribution of the abundant bacteria derived from the 16S rRNA gene sequences in jejunal and ileal digesta of pigs fed either SDPP or SBM based diets. Multivariate redundancy analysis (RDA) was employed to identify microbial signatures (with response score $> \pm 0.5$) in different dietary treatment groups within each small intestinal location using CANOCO 5. Additionally, the DAA tool of CLC- Microbial Genomics Module was used to characterize the microbial differences at genus level with False Discovery Rate (FDR)

<0.05 with fold change > 5 , between the digesta samples for the dietary treatment groups collected from each small intestinal location. Heat maps were constructed by hierarchical clustering of microbial families (selected from DAA) in Perseus software (version 1.6.14.0; available at: <http://www.maxquant.org/>), where relative abundance values were normalized by z-score transformation. For hierarchical clustering, Euclidean distance was utilized to measure the distance and clustering was conducted using the average linkage method.

2.8 Systemic metabolomics profiling

2.8.1 Assay description

The amine profiling was performed as described previously (Noga et al., 2012). Briefly, 5 μ L of each sample was spiked with an internal standard solution (Supplementary Table 2), thiol amines are released from proteins and converted to a reduced form using Tris-(2-Carboxyethyl)phosphine (TCEP). Then proteins were precipitated by the addition of MeOH. The supernatant was transferred to an Eppendorf tube (Eppendorf, Hamburg, Germany) and dried in a speedvac (Eppendorf, Hamburg, Germany). The residue was reconstituted in borate buffer (pH 8.5) with AQC reagent (Waters, Etten-Leur, The Netherlands). After reaction, the vials were transferred to an autosampler tray (Waters, Etten-Leur, The Netherlands) and cooled to 10°C prior to injection. For amine metabolite analysis, 1 μ L of the reaction mixture was injected into the UPLC-MS/MS system using an Accq-Tag Ultra column (Waters, Etten-Leur, The Netherlands).

2.8.2 Equipment

We employed an ACQUITY UPLC system with autosampler (Waters, Etten-Leur, The Netherlands) was coupled online with a Xevo Tandem Quadrupole mass spectrometer (Waters, Etten-Leur, The Netherlands) operated using QuanLynx data acquisition software (version 4.1; Waters, Etten-Leur, The Netherlands). The Xevo Tandem Quadrupole was used in the positive-ion electrospray mode and all analytes were monitored in multiple reaction monitoring using nominal mass resolution.

2.8.3 Data processing and quality check of metabolomics data

Acquired data were evaluated using TargetLynx software (Waters, Etten-Leur, The Netherlands), by integration of assigned MRM peaks and normalization using proper internal standards. For analysis of AA, their $^{13}\text{C}^{15}\text{N}$ -labeled analogues were used and for other amines, the closest-eluting internal standard was employed (Supplementary Table 2). Blank samples were used to correct for background, and in-house developed algorithms were applied using the pooled quality check (QC) samples to compensate for shifts in the sensitivity of the mass spectrometer over the batch analysis (van der Kloet et al., 2009). We could detect 58 amines that comply with the acceptance criteria of QC corrections. The data are represented as relative response ratios (amine target area/area of internal standard; unit free) of these metabolites (after QC) are available online: <https://doi.org/10.5281/zenodo.13285418>.

2.8.4 Metabolomics data analysis

To get more insight into the variability in the amine metabolomics data, PCA was performed using the intensities of the identified amine metabolites from plasma sample of pigs fed with either SBM or SDPP-based diets. To assess the contribution of metabolites to the differences observed between dietary treatments, we conducted a Partial Least Squares Discriminant Analysis (PLS-DA), followed by the generation of a Variable Importance in Projection (VIP) plot (Figure 1A). The VIP score quantifies the

significance of each variable in modeling both the predictor (X) and response (Y) variables. In this context, variables with VIP scores greater than 1 were considered highly influential within the PLS-DA model. Employing the statistical module of MetaboAnalyst 4.0 (Chong et al., 2019), the heat map graphic distances were measured using Euclidean distances and the clustering algorithm using a Ward dendrogram. The amine metabolites data was normalized by the pooled sample from group SBM and thereafter log transformation to the data was performed. To retain the most

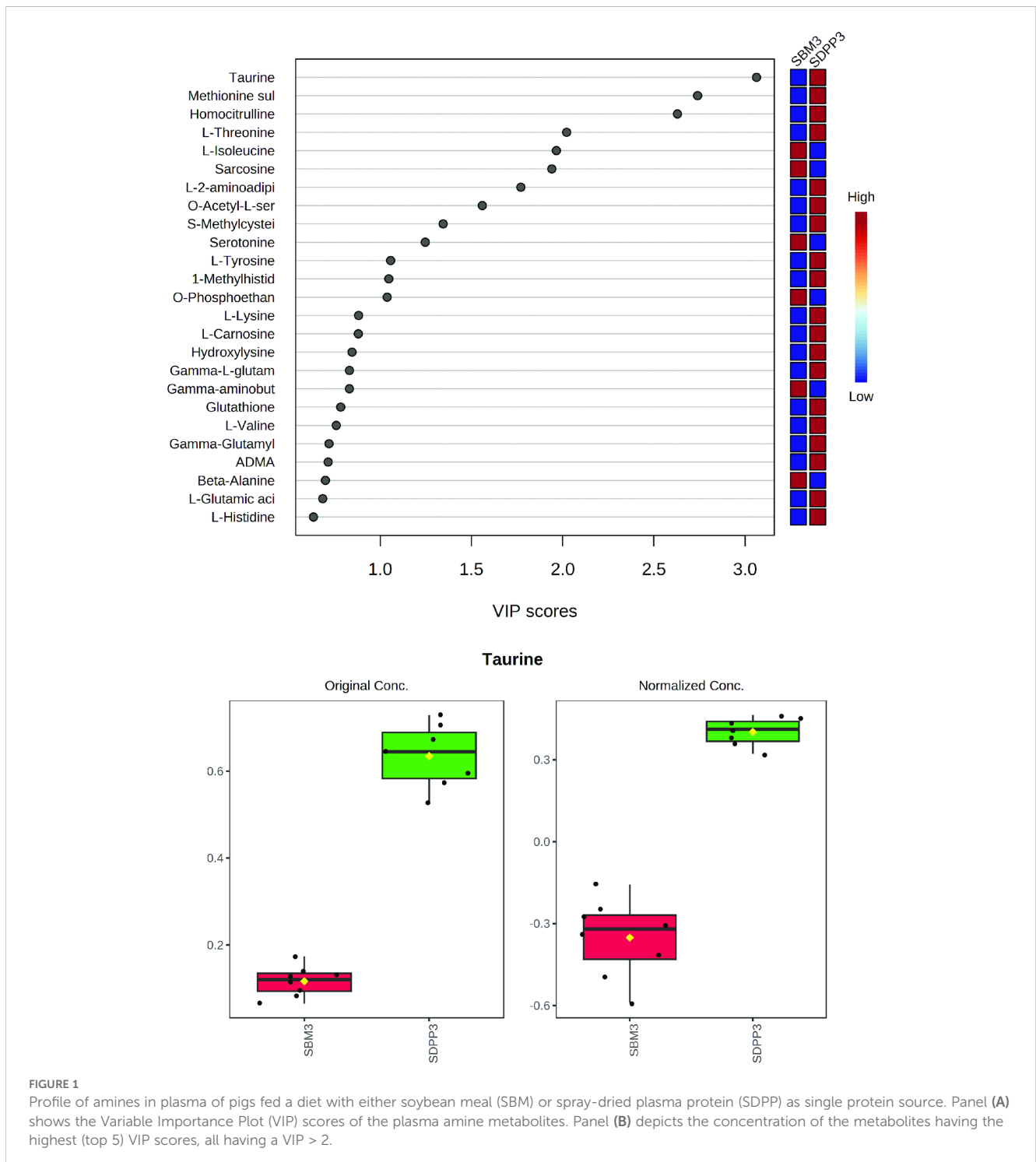


FIGURE 1 Profile of amines in plasma of pigs fed a diet with either soybean meal (SBM) or spray-dried plasma protein (SDPP) as single protein source. Panel (A) shows the Variable Importance Plot (VIP) scores of the plasma amine metabolites. Panel (B) depicts the concentration of the metabolites having the highest (top 5) VIP scores, all having a VIP > 2.

contrasting patterns among the treatment groups, the top 25 metabolites ranked by Student's T-tests were displayed in the heat-map. Additionally, fold changes of the amine metabolites were calculated using the statistic suits of MetaboAnalyst 4.0. Metabolic pathway analysis module of MetaboAnalyst 4.0 (Chong et al., 2019) was employed to determine the amine metabolism that was affected by the SDPP diet compared to the SBM diets. All the compound names of the metabolites were matched with the human metabolome database (HMDB). To normalize the data, a log transformation was performed. Thereafter, the human pathway library was selected, and a reference metabolome based on our technical platform was uploaded. The analysis includes pathway enrichment analysis and topological analysis. Based on the topology analysis, the threshold values were set at 0.4 for pathway impact and 4 for the log(p)-value of pathway enrichment to identify the most relevant metabolic pathways (Kar et al., 2017b).

2.8.5 Systemic inflammatory marker profiling

Serum cytokine and chemokine concentrations (pg/ml) were measured using a ProcartaPlex Porcine kit (Affymetrix, eBioscience, Vienna, Austria). Calibration curves from recombinant cytokine and chemokine standards were prepared for the 8-point standard dilution set with 4-fold dilution steps in sterile PBS. The samples were measured using a Bio-Plex MagPix Multiplex Reader (Bio-Rad Laboratories Inc. by the Luminex Corporation, The Netherlands). The Bio-Plex Manager software's five-parameter logistic curve fitting (5PL) method was used for raw data analysis and calculation of cytokine concentrations. Cytokine concentration levels are presented as means \pm Standard Error of the Mean. Statistical analysis was performed by Student's T-test to calculate P value (two-tailed) using GraphPad prism (v5.03) for Windows Vista (GraphPad Software, San Diego, California, USA). P value <0.05 was considered significant.

3 Results

The diet was not entirely nutritionally balanced as the study was not intended to assess growth performance. Consequently, growth performance is not a primary outcome of this study, therefore no statistical test was performed on BW data. At the start of the experiment (time 0) the pigs weighed approximately 33 kg, whereas at the end, i.e., week 3, the average body weight was increased to approximately 45 kg.

Principle component analysis (PCA) was performed to get more insight into the variability of different responses measured by the -omics based technologies. The plots of microbiota composition and gene expression in the jejunum, along with amine metabolites in the blood, showed distinct clustering of pigs fed either the experimental diet with SBM or SDPP. The first two axes explained 52.9% of the variance in microbiome composition for the jejunum and 51.2% for the ileum (Figure 2A). Furthermore, the genome-wide gene expression responses revealed that the first two axes accounted for 36.4% of the variance for the jejunum and

32.5% for the ileum (Figure 2B). Among the various responses measured by -omics based technologies, the highest proportion of variance explained by dietary treatment was observed for amine metabolites in blood plasma, with the first two axes explaining 52.6% of the variance (Figure 2C).

3.1 Small intestinal microbiota response

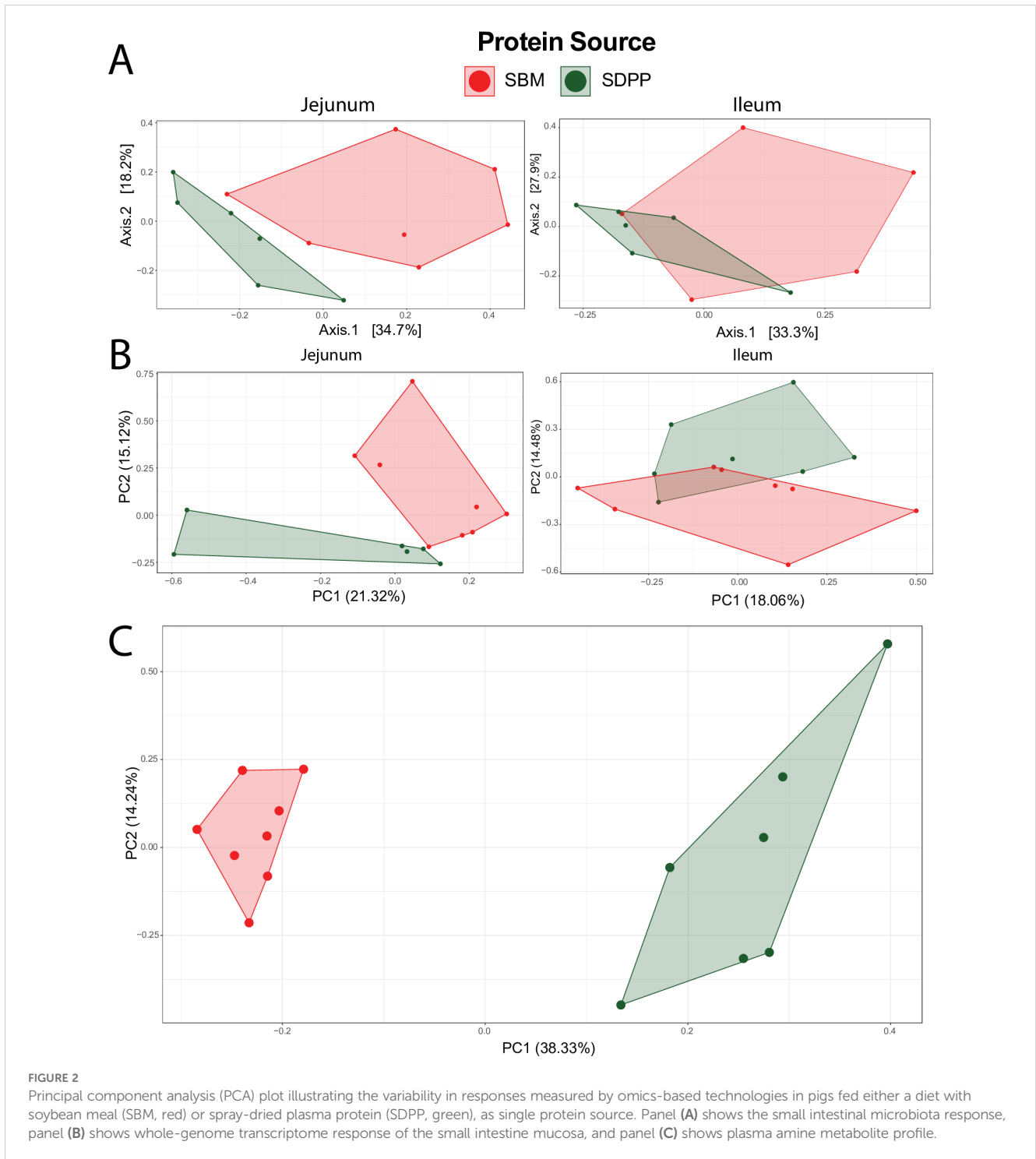
Sequencing of the 16S rRNA (V3 region) amplicons generated on average more than 441,000 high-quality reads that were classified into Operational Taxonomy Units (OTUs) with an average of more than 255,000 reads in OTU for both jejunum and ileum.

The alpha diversity measures calculated were Total number of species (Richness), Chao1, and Shannon entropy (Table 3). For jejunum statistically significant differences were observed for all three measures, where the SDPP group had lower values compared to the SBM group. The experimental diet with SBM included approximately 2,200 species, while the diet with SDPP included around 1,100 species, with Shannon entropy values of 7.8 and 5.6, respectively (Supplementary Figure 2). In the ileum, no significant differences were observed; however, the SDPP group exhibited numerically lower values compared to the SBM group. Beta diversity measures revealed no significant differences in the variability of the microbiome composition in jejunum or ileum digesta (Table 3).

Figure 3 shows the relative abundance of the identified microbiota composition at the genus level (see Supplementary Figure 3 for Phyla and Class). In the jejunum, *Streptococcus*, *Lactobacillus*, and *Veillonella* were the predominant genera, while in the ileum, *Streptococcus*, *Escherichia-Shigella*, *Turicibacter*, and *Romboutsia* were most prevalent. When comparing the experimental diets, a significant shift in the genus *Sarcina* was observed in the jejunum; the average relative contribution in the SBM group was approximately 0.13, which decreased to nearly zero in the SDPP group. A similar pattern was observed in the ileum, where the average relative contribution of *Sarcina* in the SBM diet was about 0.03, decreasing to almost zero in the SDPP fed group.

3.2 Whole-genome transcriptome response of the small intestine mucosa

To gain insight into the biological significance of SDPP-induced changes in the small intestinal transcriptome, we used a data-driven approach to first identify differentially expressed genes (DEGs). Differences between the treatment groups, i.e., SBM and SDPP, were shown to be greater in jejunum compared to ileum as displayed in the PCA (Figure 2B). We observed more DEGs for jejunum, 319 (262 down- and 57 up-regulated) compared to ileum 40 (27 down- and 13 up-regulated) (Table 4). Pathway enrichment analysis was conducted to obtain an unbiased assessment of changes in biological processes associated with these DEGs when comparing



the experimental diets containing SBM and SDPP (Table 2). Results showed seven significant enriched pathways for jejunum, i.e., intestinal immune network for IgA production, viral protein interaction with cytokine and cytokine receptor, cytokine-cytokine receptor interaction, chemokine signaling pathway, complement and coagulation cascades, PI3K-Akt signaling pathway, and ECM-receptor interaction. For ileum four enriched pathways were observed, i.e., steroid biosynthesis, cholesterol metabolism, mineral absorption, and bile secretion.

Overlaying the treatment-induced changes in jejunal and ileal transcriptome responses within KEGG pathways has significantly enhanced the visualization and understanding of the intricate gene expression networks that modulate various biological processes. Specifically, we highlight the ‘Intestinal Immune Network for IgA Production’ as a significantly enriched pathway (Table 2 and Supplementary Figure 4), showcasing its role in immune function. Additionally, the intestinal ‘Tight Junctions’ pathway (Supplementary Figure 5) is emphasized for its critical contribution to the barrier

TABLE 3 Alpha diversity and beta diversity of microbiota in jejunal and ileal digesta of pigs fed diets with soybean meal (SBM) or spray-dried porcine plasma (SDPP) as single protein source.

Indices	Jej_SBM vs Jej_SDPP	Ile_SBM vs Ile_SDPP
Alpha diversity		
Total number of species		
Kruskal-Wallis p-value	0.02	0.51
Chao 1		
Kruskal-Wallis p-value	0.02	0.34
Shannon entropy		
Kruskal-Wallis p-value	0.02	0.34
Beta diversity		
Bray-Curtis		
q-value ¹	0.12	1.00
Jaccard		
q-value	0.13	0.94
Weighted UniFrac		
q-value	0.26	1.00

¹Bonferroni corrected.

Bold indicates significant differences ($P < 0.05$).

function of the small intestine, underscoring its importance in maintaining intestinal integrity. This approach serves as a powerful example of a data-driven methodology for interpreting biological results.

3.3 Post-absorptive plasma amine metabolomic responses

The PCA plot of amine plasma metabolites showed a clear separation of the dietary treatments. Here, we observed 13 metabolites with a VIP score above 1, and for the top 5 metabolites, i.e., taurine, methionine sulphate, homocitrulline, L-threonine, and L-isoleucine, VIP scores were above 2 (Figure 1A). Furthermore, the diet containing SDPP exhibited higher concentrations of taurine (Figure 1B), methionine sulphate, homocitrulline, and L-threonine compared to the diet containing SBM.

Plasma amine metabolites which differed in concentration between SBM and SDPP treatment groups were mapped to the Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathways. This revealed seven metabolic pathways that were modulated by dietary treatment (Table 5). The enriched KEGG pathways were, 1) glycine, serine, and threonine metabolism, 2) taurine and hypotaurine metabolism, 3) arginine and proline metabolism, 4) phenylalanine, tyrosine, and tryptophan biosynthesis, 5) alanine, aspartate, and glutamate metabolism, 6) histidine metabolism, and 7) beta alanine metabolism.

3.4 Systemic inflammatory marker response

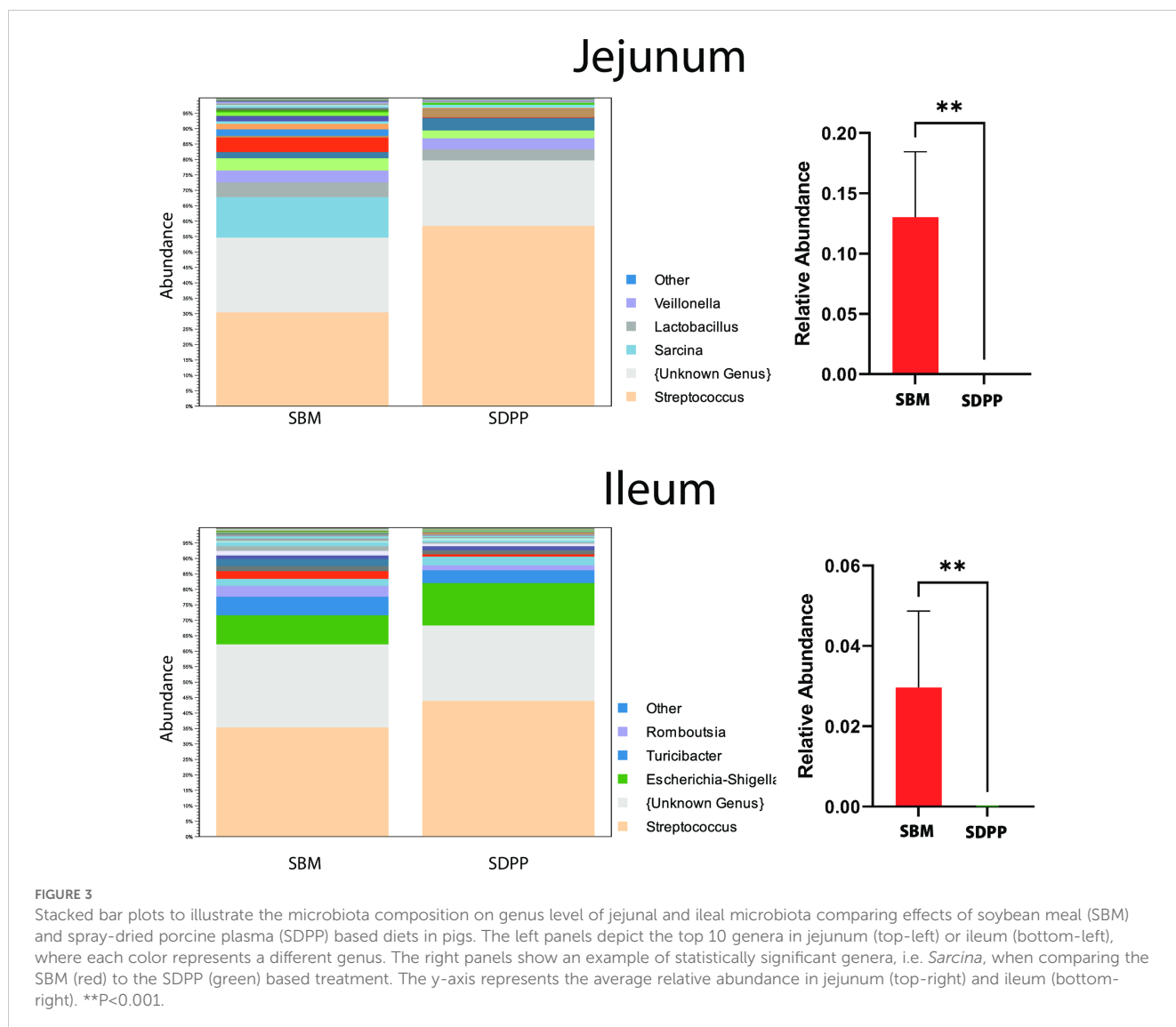
A specific panel of systemic inflammatory markers were measured and did not show significant differences between dietary treatments (see Supplementary Figure 6).

4 Discussion

In pigs, insect proteins are emerging as a potential alternative protein source and show promise for the future among other options (Kar et al., 2021a). While insect proteins are rich in essential amino acids and sustainable, they are not yet as widely studied or utilized as spray dried plasma. Issues such as palatability, variability in composition, potential allergenic compounds, production scalability and broader acceptance need to be addressed before they can match the performance and reliability of spray dried plasma. Spray-dried plasma remains a superior choice for young pigs, particularly during the critical post-weaning period, due to its exceptional nutritional, functional, and health-promoting properties (Kats et al., 1994; Zhang et al., 2015; Kim et al., 2022). In this study, we investigated the bio-functional properties of spray dried plasma as a functional protein source and its role in enhancing gut health in post-weaned pigs. The compositional disparities in the experimental diets, notably in NSP and fat content, induced by the protein sources, likely influenced the intestinal microbiome and host (intestinal) responses. We employed the FeedOmics approach, which combines various -omics-based techniques to evaluate how dietary protein sources impact host physiology (Leibold et al., 2004; Kar, 2017; Kar et al., 2017a). It is important to note that the diets were not fully balanced in terms of essential amino acid concentrations relative to the pig's requirements, as the study was not designed to assess growth performance. Consequently, growth and productive performance metrics are not the primary focus of this study. Instead, we illustrate how the FeedOmics approach offers mechanistic insights into how dietary SDPP, as a functional protein source, affects biological responses and promotes gut health in post-weaned pigs. The following sections discuss the effects of feeding SDPP to young growing pigs, focusing on gut responses and systemic effects.

4.1 Effect of SDPP on small intestinal microbiota

The analysis of the small intestinal microbiome showed that, regardless of the dietary treatment, the most prevalent phyla were Firmicutes, Proteobacteria, and Actinobacteria, with the respective classes Bacilli, Clostridia, and Gammaproteobacteria being dominant in the jejunal and ileal digesta of pigs. This finding aligns with previous research (Zhao et al., 2015; Yang et al., 2016; Li et al., 2017; McCormack et al., 2017; Crespo-Piazuelo et al., 2018; Li et al., 2018; Jin et al., 2019), which also identified Firmicutes and Actinobacteria as



the predominant phyla in pigs, indicating their core role in the porcine intestinal microbiota. Additionally, the ileal microbiome profile is consistent with other studies (Quan et al., 2018), particularly concerning the genera *Escherichia-Shigella* and *Romboutsia*. Our analysis of the taxonomic distribution revealed a significant fraction of bacteria in both intestinal locations classified as ‘unknown taxon or N/A,’ likely due to limitations of the SILVA SSU version 138.1 database (<https://www.arb-silva.de/projects/ssu-ref-nr/>) used for the OTU table. Despite this, we observed similar diet-specific microbial groups in the jejunum and ileum, suggesting that dietary treatment has a stronger influence on microbiota composition than location-specific variations (Holman et al., 2017).

Incorporating SDPP into the diet of post-weaned pigs reduces microbiota richness. The SDPP-based diet significantly altered the small intestinal microbiota compared to the SBM-based diet, leading to a notable reduction in microbial diversity and changes in microbial abundance at both the phylum and genus levels. While some studies report increased gut microbiota diversity with age (Niu et al., 2015; Shao et al., 2021), our findings show reduced diversity with SDPP, consistent with Han et al. (2018) for finishing

pigs. Despite the lower diversity, there was no increase in pathogenic microbes such as *Escherichia-Shigella*, *Clostridium*, or *Corynebacterium* (Supplementary Figure 7), and *Clostridium* levels were significantly lower in SDPP-fed pigs compared to those fed SBM. Thus, while SDPP reduces bacterial diversity, it does not lead to higher pathogenic load, mirroring the microbiota profile observed in finishing pigs (Han et al., 2018).

Inclusion of SDPP in pig diets markedly decreased *Sarcina*, a Firmicutes genus, in the small intestine. *Sarcina*, a Gram-positive anaerobic coccus that thrives in acidic conditions, was more abundant in the jejunum compared to the ileum, indicating its preference for the upper gastrointestinal tract (Lam-Himlin et al., 2011; Savic Vukovic et al., 2021). We hypothesize that the alkaline environment associated with the SDPP diet may have contributed to the reduced *Sarcina* levels, although this remains unverified due to the absence of pH measurements. Conversely, the SBM diet, with its higher content of plant-derived fibers and oligosaccharides (130 g/kg vs. 64 g/kg non-starch polysaccharides; Table 1), likely supported luxuriant *Sarcina* growth, as these microbes rely on carbohydrate fermentation for energy (Lowe et al., 1989; Worrall et al., 2022).

Interestingly, a previous study found that the inclusion of zinc oxide (ZnO) at pharmaceutical levels significantly reduced the abundance of *Sarcina*, suggesting a similar effect of ZnO and SDPP on gut microbiota (Vahjen et al., 2010). Following the European Commission's 2022 ban on ZnO (European Parliament, 2001, European Parliament, 2004) to address antimicrobial resistance and environmental concerns, and the earlier 2006 ban on growth-promoting antibiotics (Millet and Maertens, 2011), SDPP has emerged as a viable alternative. Our study, along with others (Zhe et al., 2021), supports the potential of SDPP to replace ZnO and antibiotics, possibly due to its antimicrobial bioactive peptides. While this hypothesis needs further validation, our results indicate that SDPP promotes a dynamic microbiome with limited potential for pathogenic microbe proliferation is indicative of a healthy, functional microbiome simultaneously offers antimicrobial benefits compared to traditional protein sources like SBM.

4.2 Effect of SDPP on global transcriptome profile of small intestinal mucosa

Our study reveals significant differences in small intestinal transcriptomics between pigs fed SDPP versus SBM, even when using stringent criteria (adjusted P-value < 0.05 and Fold Change |1|). This robust transcriptome response contrasts with the subtle changes often observed using less stringent methods in studies (Kar et al., 2017a) on other sole protein sources like insect proteins (Leibold et al., 2004). The pronounced changes in differentially expressed genes (DEGs) in the jejunum compared to the ileum (Table 4) align with previous findings showing a greater number of DEGs in the jejunum of pigs fed *Cyberlindnera jadinii* yeast (Håkenåsen et al., 2020). This effect may be due to differences in protein digestion kinetics (Chen et al., 2019) and the higher protein and amino acid digestibility of SDPP relative to SBM (Mateo and Stein, 2007; Almeida et al., 2013), suggesting enhanced jejunal functionality.

The SDPP diet appears to induce an “early” release of bio-functional components such as immunoglobulins and bioactive peptides (Kar et al., 2016), which are well-documented for their health benefits (Coffey and Cromwell, 1995; Moreto and Perez-Bosque, 2009; Balan et al., 2021). Gao et al. (2011) further supported this by demonstrating that SDPP enhances antioxidant capacity in pigs, as indicated by improved enzyme activities and reduced malondialdehyde levels. Our transcriptomic analysis corroborates these findings, showing that DEGs in the jejunum of SDPP-fed pigs are associated with immunoregulation and defense pathways.

TABLE 4 Number of differentially expressed genes in jejunum and ileum tissue from pigs fed a SBM or SDPP based diet.

Tissue	Regulation	log ₂ FC 0.58 ^a	log ₂ FC 1 ^b
Jejunum	Down	262	40
	Up	57	10
Ileum	Down	27	14
	Up	13	12

^acorresponds to fold change of 1.5; ^bcorresponds to fold change of 2.

Enrichment in “*complement and coagulation*” pathways underscores SDPP's role in maintaining jejunal homeostasis and supporting local immune responses.

Previous studies have shown reduced immune cell numbers in SDPP-fed piglets, including lymphocytes in mucosa and gut-associated lymphoid tissues such as Peyer's patches and lamina propria (Perez-Bosque et al., 2004, Perez-Bosque et al., 2008). Similarly, a study in mice revealed downregulation of key genes involved in mechanistic target of rapamycin (mTOR) signaling pathways in the ileum when fed SDPP (Kar et al., 2017b). Our findings also showed enrichment of the phosphatidylinositol 3-kinase/Protein Kinase B (PI3K-Akt) pathway in DEGs, which is crucial for cell cycle regulation and immune modulation (Van Laethem et al., 2013; Leopold Wager et al., 2014; Scheeren et al., 2014; Siggs et al., 2015). The parallel results from both mice and pigs suggest that SDPP may modulate immune responses by influencing the PI3K/AKT/mTOR pathway, warranting further investigation using *in vitro* intestinal models and co-culture systems (Kar et al., 2021b; Saleri et al., 2021; Andrani et al., 2023).

Moreover, the enrichment of the “*Intestinal Immune Network for IgA Production*” pathway, with downregulated genes such as CCR10, CXCL12, CCL28, and MadCAM-1, suggests reduced leukocyte trans-endothelial migration in SDPP-fed pigs. This reduction may indicate lower intestinal tissue damage from stressors compared to SBM-fed pigs, supporting SDPP's role in promoting intestinal health. Additionally, higher expression of tight junction receptors in the jejunum of SDPP-fed pigs, with minimal differences in the ileum, suggests improved gut barrier function and overall gut health in SDPP-fed pigs (Bischoff et al., 2014).

4.3 Effect of SDPP on amine metabolites in blood

Dietary protein digestion generates various metabolites that enter the bloodstream and affect different tissues. Utilizing the amine-based ‘endophenotype’ concept, our study explored how different dietary proteins impact host-microbe interactions by analyzing blood plasma amine profiles (Kar et al., 2017b). We identified threonine, taurine, and glutamine/glutamate as key metabolites distinguishing between SDPP and SBM diets.

Threonine, essential for mucosal protein synthesis and energy (Le Floch and Seve, 2005; Wen et al., 2020b), was found at higher plasma levels in SDPP-fed pigs, reflecting its greater dietary content (9 g/kg vs. 5 g/kg). This suggests enhanced absorption and potential benefits for gut barrier function and immune regulation through tight junctions and mTOR signaling (Singh et al., 2023; Wen et al., 2020a). Additionally, threonine modulates lymphocyte activity and IgA secretion, affecting intestinal inflammation (Tang et al., 2021). Taurine, abundant in SDPP, protects the intestinal mucosa by regulating immune responses and maintaining tight junction integrity (Wen et al., 2020a). Its antioxidant properties further mitigate oxidative stress (Hamard et al., 2010; Wang et al., 2010; Koo et al., 2020). Elevated blood glutamate levels in SDPP-fed pigs indicate increased glutamate metabolism, with glutamine

TABLE 5 Metabolic pathways involving amine metabolites in blood plasma differently present in pigs receiving a SBM or SDPP based diet.

Enriched pathways	P value	FDR ¹	Pathway impact ²	Metabolites	Differential abundant in SDPP compared to SBM
Glycine, serine and threonine metabolism	<0.001	<0.001	0.56	Threonine	High
				Cystathionine	High
				Sarcosine	Low
Taurine and hypotaurine metabolism	<0.001	<0.001	0.43	Taurine	High
Arginine and proline metabolism	<0.001	<0.001	0.46	Arginine	Low
				Putrescine	Low
				Glutamate	High
				4-Aminobutanoate	Low
Phenylalanine, tyrosine and tryptophan biosynthesis	<0.001	<0.001	1	Tyrosine	High
Alanine, aspartate and glutamate metabolism	<0.001	<0.001	0.62	Alanine	High
				Glutamate	High
				4-Aminobutanoate	Low
				Glutamine	High
Histidine metabolism	<0.001	<0.001	0.55	Histidine	High
				Carnosine	High
				N(pi)-Methyl-L-histidine	High
				Glutamate	High
beta-Alanine metabolism	<0.001	<0.001	0.46	beta-Alanine	Low
				Carnosine	High
				Histidine	High

¹FDR, False Discovery Rate.

²is a combination of the centrality and pathway enrichment results.

Bold indicates pathways with significant enrichment (P<0.001).

conversion to glutamate supporting gut energy needs (Burrin and Stoll, 2009). Consistent with this, previous research showed that glutamate improves gut health and reduces diarrhea (Rezaei et al., 2013). Overall, SDPP's benefits may stem from its effects on threonine, taurine, and glutamate metabolism, enhancing gut health and functionality.

4.4 Effect of SDPP on health indices in blood

To determine if the SDPP-based diet impacted systemic immunity, we assessed a panel of cytokines and chemokines in pig blood, including pro- and anti-inflammatory markers like IFN α , IFN γ , IL-1 β , IL-10, IL-12p40, IL-4, IL-6, IL-8, and TNF α . Previous research in mice suggests that dietary proteins can influence systemic cytokine levels (Kar et al., 2017b). Our results revealed no significant differences in these cytokines between dietary treatments, consistent with similar findings in pigs fed black soldier fly or SBM (28). We also evaluated the tryptophan

(TRP) and kynurenine (KYN) ratio, which is indicative of systemic immune activity (Scheffold et al., 2009; Harper et al., 2018). Changes in this ratio reflect immune activation, where a higher KYN-TRP ratio suggests increased immune response (Suzuki et al., 2010). Thus, immune activation leads to the formation of KYN and the reduction of tryptophan-TRP, and a higher KYN-TRP ratio indicates a systemic immune response (Suzuki et al., 2010). However, our results showed that the KYN-TRP ratio was stable across diets, aligning with the stable cytokine levels. These findings suggest that the SDPP-based diet does not significantly alter systemic immune responses, likely due to the healthy status of the experimental pigs.

Our findings offer new insights into the role of spray-dried plasma (SDP) as a functional protein source in promoting intestinal functionality and gut health in pigs under non-challenging conditions. The results corroborate the positive effects of SDP on gut health reported in previous studies, even at lower dietary inclusion levels, which were often conducted under the stress conditions of weaning (Torrallardona et al., 2003; Nofrarias et al., 2007; Moreto and Perez-Bosque, 2009; Gao et al., 2011; Zhang et al.,

2015). The FeedOmics approach employed in this study demonstrated the functional effects of SDPP compared to SBM as a dietary protein source, impacting the small intestinal microbiome, gut tissue transcriptome, and amine metabolite profiles in blood plasma. The functional properties of SDP provide added value in relation to supporting gut health and function in pigs and potentially other animal species.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: NCBI Gene Expression Omnibus, accession GSE98261 and Sequence Read Archive, accession PRJNA1139701; and Zenodo, DOI <https://doi.org/10.5281/zenodo.13285418>.

Ethics statement

The animal study was approved by the Animal Experimentation Board of Wageningen University & Research Centre (approval number 2014099.b). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

SK: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. DS: Data curation, Formal analysis, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. CV: Writing – review & editing. HV: Writing – review & editing. AH: Data curation, Methodology, Software, Supervision, Validation, Writing – review & editing. LK: Formal analysis, Investigation, Methodology, Resources, Software, Validation, Writing – review & editing. MS: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing. AJ: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

References

- Almeida, F. N., Htoo, J. K., Thomson, J., and Stein, H. H. (2013). Comparative amino acid digestibility in US blood products fed to weanling pigs. *Anim. Feed Sci. Technol.* 181, 80–86. doi: 10.1016/j.anifeedsci.2013.03.002
- Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* 26, 32–46. doi: 10.1111/j.1442-9993.2001.01070.pp.x
- Andrani, M., Borghetti, P., Ravanetti, F., Cavalli, V., Ferrari, L., De Angelis, E., et al. (2023). Acetate and propionate effects in response to LPS in a porcine intestinal co-culture model. *Porcine Health Manag* 9, 23. doi: 10.1186/s40813-023-00316-y
- Balan, P., Staincliffe, M., and Moughan, P. J. (2021). Effects of spray-dried animal plasma on the growth performance of weaned piglets—A review. *J. Anim. Physiol. Anim. Nutr. (Berl)* 105, 699–714. doi: 10.1111/jpn.13435
- Bikker, P., van Dijk, A. J., Dirkzwager, A., Fledderus, J., Ubbink-Blanksma, M., and Beynen, A. C. (2004). The influence of diet composition and an anti-microbial growth promoter on the growth response of weaned piglets to spray dried animal plasma. *Livestock Production Sci.* 86, 201–208. doi: 10.1016/j.livprodsci.2003.07.003
- Bischoff, S. C., Barbara, G., Buurman, W., Ockhuizen, T., Schulzke, J. D., Serino, M., et al. (2014). Intestinal permeability—a new target for disease prevention and therapy. *BMC Gastroenterol.* 14, 189. doi: 10.1186/s12876-014-0189-7

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fanim.2025.1532914/full#supplementary-material>

- Blazquez, E., Rodriguez, C., Rodenas, J., Segales, J., Pujols, J., and Polo, J. (2020). Biosafety steps in the manufacturing process of spray-dried plasma: a review with emphasis on the use of ultraviolet irradiation as a redundant biosafety procedure. *Porcine Health Manag* 6, 16. doi: 10.1186/s40813-020-00155-1
- Burrin, D. G., and Stoll, B. (2009). Metabolic fate and function of dietary glutamate in the gut. *Am. J. Clin. Nutr.* 90, 850S–856S. doi: 10.3945/ajcn.2009.27462Y
- Chen, M. X., Wang, S. Y., Kuo, C. H., and Tsai, I. L. (2019). Metabolome analysis for investigating host-gut microbiota interactions. *J. Formos Med. Assoc.* 118 Suppl 1, S10–S22. doi: 10.1016/j.jfma.2018.09.007
- Chong, J., Wishart, D. S., and Xia, J. (2019). Using metaboAnalyst 4.0 for comprehensive and integrative metabolomics data analysis. *Curr. Protoc. Bioinf.* 68, e86. doi: 10.1002/cpbi.86
- Coffey, R. D., and Cromwell, G. L. (1995). The impact of environment and antimicrobial agents on the growth response of early-weaned pigs to spray-dried porcine plasma. *J. Anim. Sci.* 73, 2532–2539. doi: 10.2527/1995.7392532x
- Crespo-Piazuelo, D., Estelle, J., Revilla, M., Criado-Mesas, L., Ramayo-Caldas, Y., Ovilo, C., et al. (2018). Characterization of bacterial microbiota compositions along the intestinal tract in pigs and their interactions and functions. *Sci. Rep.* 8, 12727. doi: 10.1038/s41598-018-30932-6
- Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32, 1792–1797. doi: 10.1093/nar/gkh340
- European Parliament (2001). *Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to veterinary medicinal products*. Ed. E. Parliament (Brussels, Belgium: European Parliament).
- European Parliament (2004). *Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Union procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency*. Ed. E. Parliament (Brussels, Belgium: European Parliament).
- Gao, Y. Y., Jiang, Z. Y., Lin, Y. C., Zheng, C. T., Zhou, G. L., and Chen, F. (2011). Effects of spray-dried animal plasma on serous and intestinal redox status and cytokines of neonatal piglets. *J. Anim. Sci.* 89, 150–157. doi: 10.2527/jas.2010-2967
- Håkenåsen, I. M., Øverland, M., Ånestad, R., Åkesson, C. P., Sundaram, A. Y. M., Press, C. M., et al. (2020). Gene expression and gastrointestinal function is altered in piglet small intestine by weaning and inclusion of Cyberlindnera jadinii yeast as a protein source. *J. Funct. Foods* 73, 104118. doi: 10.1016/j.jff.2020.104118
- Hamard, A., Mazurais, D., Boudry, G., Le-Huerou-Luron, I., Seve, B., and Le Floch, N. (2010). A moderate threonine deficiency affects gene expression profile, paracellular permeability and glucose absorption capacity in the ileum of piglets. *J. Nutr. Biochem.* 21, 914–921. doi: 10.1016/j.jnutbio.2009.07.004
- Han, G. G., Lee, J. Y., Jin, G. D., Park, J., Choi, Y. H., Kang, S. K., et al. (2018). Tracing of the fecal microbiota of commercial pigs at five growth stages from birth to shipment. *Sci. Rep.* 8, 6012. doi: 10.1038/s41598-018-24508-7
- Harper, K. M., Mutasa, M., Prendergast, A. J., Humphrey, J., and Manges, A. R. (2018). Environmental enteric dysfunction pathways and child stunting: A systematic review. *PLoS Negl. Trop. Dis.* 12, e0006205. doi: 10.1371/journal.pntd.0006205
- Holman, D. B., Brunelle, B. W., Trachsel, J., and Allen, H. K. (2017). Meta-analysis to define a core microbiota in the swine gut. *mSystems* 2 (3), e00004-17. doi: 10.1128/mSystems.00004-17
- ISO 15914-1, I (2004). *Animal feeding stuffs—Enzymatic determination of total starch content* (Geneva, Switzerland: International Organization for Standardization).
- ISO-5983-2 (2009). *Animal feeding stuffs — Determination of nitrogen content and calculation of crude protein content — Part 2: Block digestion and steam distillation method* (Geneva, Switzerland: International Organization for Standardization).
- ISO-5984 (2002). *Animal feeding stuffs — Determination of crude ash* (Geneva, Switzerland: International Organization for Standardization).
- ISO-6492 (1999). *Animal feeding stuffs — Determination of fat content* (Geneva, Switzerland: International Organization for Standardization).
- ISO-6496 (1999). *Animal feeding stuffs — Determination of moisture and other volatile matter content* (Geneva, Switzerland: International Organization for Standardization).
- Jin, J., Zhang, L., Jia, J., Chen, Q., Yuan, Z., Zhang, X., et al. (2019). Effects of maternal low-protein diet on microbiota structure and function in the jejunum of huzhu bamei suckling piglets. *Animals*. 9 (10), 713. doi: 10.3390/ani9100713
- Kar, S. K. (2017). *FeedOmics. An Approach to Evaluate the Functional Properties of Protein Containing Feed Ingredients*. (Wageningen, The Netherlands: Wageningen University and Research).
- Kar, S. K., Jansman, A. J. M., Benis, N., Ramiro-Garcia, J., Schokker, D., Kruijt, L., et al. (2017a). Dietary protein sources differentially affect microbiota, mTOR activity and transcription of mTOR signaling pathways in the small intestine. *PLoS One* 12, e0188282. doi: 10.1371/journal.pone.0188282
- Kar, S. K., Jansman, A. J. M., Boeren, S., Kruijt, L., and Smits, M. A. (2016). Protein, peptide, amino acid composition, and potential functional properties of existing and novel dietary protein sources for monogastrics1,2. *J. Anim. Sci.* 94, 30–39. doi: 10.2527/jas.2015-9677
- Kar, S. K., Jansman, A. J. M., Schokker, D., Kruijt, L., Harms, A. C., Wells, J. M., et al. (2017b). Amine metabolism is influenced by dietary protein source. *Front. Nutr.* 4. doi: 10.3389/fnut.2017.00041
- Kar, S. K., Schokker, D., Harms, A. C., Kruijt, L., Smits, M. A., and Jansman, A. J. M. (2021a). Local intestinal microbiota response and systemic effects of feeding black soldier fly larvae to replace soybean meal in growing pigs. *Sci. Rep.* 11, 15088. doi: 10.1038/s41598-021-94604-8
- Kar, S. K., van der Hee, B., Loonen, L. M. P., Taverne, N., Taverne-Thiele, J. J., Schokker, D., et al. (2020). Effects of undigested protein-rich ingredients on polarised small intestinal organoid monolayers. *J. Anim. Sci. Biotechnol.* 11, 51. doi: 10.1186/s40104-020-00443-4
- Kar, S. K., Wells, J. M., Ellen, E. D., Te Pas, M. F. W., Madsen, O., Groenen, M. A. M., et al. (2021b). Organoids: a promising new *in vitro* platform in livestock and veterinary research. *Vet. Res.* 52, 43. doi: 10.1186/s13567-021-00904-2
- Kats, L. J., Nelssen, J. L., Tokach, M. D., Goodband, R. D., Hansen, J. A., and Laurin, J. L. (1994). The effect of spray-dried porcine plasma on growth performance in the early-weaned pig. *J. Anim. Sci.* 72, 2075–2081. doi: 10.2527/1994.7282075x
- Kim, H., Lee, S. H., and Kim, B. G. (2022). Effects of dietary spray-dried plasma protein on nutrient digestibility and growth performance in nursery pigs. *J. Anim. Sci.* 100 (1), skab351. doi: 10.1093/jas/skab351
- Koo, B., Choi, J., Yang, C., and Nyachoti, C. M. (2020). Diet complexity and l-threonine supplementation: effects on growth performance, immune response, intestinal barrier function, and microbial metabolites in nursery pigs. *J. Anim. Sci.* 98 (5), skaa125. doi: 10.1093/jas/skaa125
- Lam-Himlin, D., Tsiatis, A. C., Montgomery, E., Pai, R. K., Brown, J. A., Razavi, M., et al. (2011). Sarcina organisms in the gastrointestinal tract: a clinicopathologic and molecular study. *Am. J. Surg. Pathol.* 35, 1700–1705. doi: 10.1097/PAS.0b013e31829211e6
- Le Floch, N., and Seve, B. (2005). Catabolism through the threonine dehydrogenase pathway does not account for the high first-pass extraction rate of dietary threonine by the portal drained viscera in pigs. *Br. J. Nutr.* 93, 447–456. doi: 10.1079/bjn20051375
- Leibold, M. A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J. M., Hoopes, M. F., et al. (2004). The metacommunity concept: a framework for multi-scale community ecology. *Ecol. Lett.* 7, 601–613. doi: 10.1111/j.1461-0248.2004.00608.x
- Leopold Wager, C. M., Hole, C. R., Wozniak, K. L., Olszewski, M. A., and Wormley, F. L. Jr (2014). STAT1 signaling is essential for protection against *Cryptococcus neoformans* infection in mice. *J. Immunol.* 193, 4060–4071. doi: 10.4049/jimmunol.1400318
- Li, K., Xiao, Y., Chen, J., Chen, J., He, X., and Yang, H. (2017). Microbial composition in different gut locations of weaning piglets receiving antibiotics. *Asian-Australas J. Anim. Sci.* 30, 78–84. doi: 10.5713/ajas.16.0285
- Li, L., Chen, S., Li, X., Wan, D., Liu, G., Liu, Y., et al. (2018). Intestinal microbiota in growing pigs: effects of stocking density. *Food Agric. Immunol.* 29, 524–535. doi: 10.1080/09540105.2017.1409195
- Lowe, S. E., Pankratz, H. S., and Zeikus, J. G. (1989). Influence of pH extremes on sporulation and ultrastructure of *Sarcina ventriculi*. *J. Bacteriol.* 171, 3775–3781. doi: 10.1128/jb.171.7.3775-3781.1989
- Mateo, C. D., and Stein, H. H. (2007). Apparent and standardized ileal digestibility of amino acids in yeast extract and spray dried plasma protein by weaning pigs. *Can. J. Anim. Sci.* 87, 381–383. doi: 10.4141/CJAS06011
- McCormack, U. M., Curiao, T., Buzoianu, S. G., Prieto, M. L., Ryan, T., Varley, P., et al. (2017). Exploring a possible link between the intestinal microbiota and feed efficiency in pigs. *Appl. Environ. Microbiol.* 83 (15), e00380-17. doi: 10.1128/AEM.00380-17
- Millet, S., and Maertens, L. (2011). The European ban on antibiotic growth promoters in animal feed: from challenges to opportunities. *Vet. J.* 187, 143–144. doi: 10.1016/j.tvjl.2010.05.001
- Moreto, M., and Perez-Bosque, A. (2009). Dietary plasma proteins, the intestinal immune system, and the barrier functions of the intestinal mucosa. *J. Anim. Sci.* 87, E92–100. doi: 10.2527/jas.2008-1381
- Niu, Q., Li, P., Hao, S., Zhang, Y., Kim, S. W., Li, H., et al. (2015). Dynamic distribution of the gut microbiota and the relationship with apparent crude fiber digestibility and growth stages in pigs. *Sci. Rep.* 5, 9938. doi: 10.1038/srep09938
- Nofrarias, M., Manzanilla, E. G., Pujols, J., Gibert, X., Majó, N., Segalés, J., et al. (2007). Spray-dried porcine plasma affects intestinal morphology and immune cell subsets of weaned pigs. *Livestock Sci.* 108, 299–302. doi: 10.1016/j.livsci.2007.01.103
- Noga, M. J., Dane, A., Shi, S., Attali, A., van Aken, H., Suidgeest, E., et al. (2012). Metabolomics of cerebrospinal fluid reveals changes in the central nervous system metabolism in a rat model of multiple sclerosis. *Metabolomics* 8, 253–263. doi: 10.1007/s11306-011-0306-3
- Perez-Bosque, A., Miro, L., Polo, J., Russell, L., Campbell, J., Weaver, E., et al. (2008). Dietary plasma proteins modulate the immune response of diffuse gut-associated lymphoid tissue in rats challenged with *Staphylococcus aureus* enterotoxin B. *J. Nutr.* 138, 533–537. doi: 10.1093/jn/138.3.533
- Perez-Bosque, A., Pelegri, C., Vicario, M., Castell, M., Russell, L., Campbell, J. M., et al. (2004). Dietary plasma protein affects the immune response of weaned rats challenged with *S. aureus* Superantigen B. *J. Nutr.* 134, 2667–2672. doi: 10.1093/jn/134.10.2667
- Perez-Bosque, A., Polo, J., and Torralardona, D. (2016). Spray dried plasma as an alternative to antibiotics in piglet feeds, mode of action and biosafety. *Porcine Health Manag* 2, 16. doi: 10.1186/s40813-016-0034-1

- Quan, J., Cai, G., Ye, J., Yang, M., Ding, R., Wang, X., et al. (2018). A global comparison of the microbiome compositions of three gut locations in commercial pigs with extreme feed conversion ratios. *Sci. Rep.* 8, 4536. doi: 10.1038/s41598-018-22692-0
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41, D590–D596. doi: 10.1093/nar/gks1219
- Research, W. L. (2020). *Voedernormen varkens en voederwaarden voedermiddelen voor varkens* (Lelystad: Central Bureau for Livestock Feeding (CVB)).
- Rezaei, R., Knabe, D. A., Tekwe, C. D., Dahanayaka, S., Ficken, M. D., Fielder, S. E., et al. (2013). Dietary supplementation with monosodium glutamate is safe and improves growth performance in postweaning pigs. *Amino Acids* 44, 911–923. doi: 10.1007/s00726-012-1420-x
- Roques, S., Koopmans, S. J., Mens, A., van Harn, J., van Krimpen, M., and Kar, S. K. (2022). Effect of Feeding 0.8% Dried Powdered *Chlorella vulgaris* Biomass on Growth Performance, Immune Response, and Intestinal Morphology during Grower Phase in Broiler Chickens. *Anim. (Basel)* 12 (9), 1114. doi: 10.3390/ani12091114
- Saleri, R., Borghetti, P., Ravanetti, F., Andranì, M., Cavalli, V., De Angelis, E., et al. (2021). A co-culture model of IPEC-J2 and swine PBMC to study the responsiveness of intestinal epithelial cells: the regulatory effect of arginine deprivation. *Anim. (Basel)* 11 (9), 2756. doi: 10.3390/ani11092756
- Savic Vukovic, A., Jonjic, N., Bosak Versic, A., Kovac, D., and Radman, M. (2021). Fatal Outcome of Emphysematous Gastritis due to *Sarcina ventriculi* Infection. *Case Rep. Gastroenterol.* 15, 933–938. doi: 10.1159/000518305
- Scheeren, F. A., Kuo, A. H., van Weele, L. J., Cai, S., Glykofridis, I., Sikandar, S. S., et al. (2014). A cell-intrinsic role for TLR2-MYD88 in intestinal and breast epithelia and oncogenesis. *Nat. Cell Biol.* 16, 1238–1248. doi: 10.1038/ncb3058
- Schefold, J. C., Zeden, J. P., Fotopoulou, C., von Haehling, S., Pschowski, R., Hasper, D., et al. (2009). Increased indoleamine 2,3-dioxygenase (IDO) activity and elevated serum levels of tryptophan catabolites in patients with chronic kidney disease: a possible link between chronic inflammation and uraemic symptoms. *Nephrol. Dial. Transplant.* 24, 1901–1908. doi: 10.1093/ndt/gfn739
- Schokker, D., Fledderus, J., Jansen, R., Vastenhouw, S. A., de Bree, F. M., Smits, M. A., et al. (2018). Supplementation of fructooligosaccharides to suckling piglets affects intestinal microbiota colonization and immune development. *J. Anim. Sci.* 96, 2139–2153. doi: 10.1093/jas/sky110
- Schokker, D., Zhang, J., Zhang, L. L., Vastenhouw, S. A., Heilig, H. G., Smidt, H., et al. (2014). Early-life environmental variation affects intestinal microbiota and immune development in new-born piglets. *PLoS One* 9, e100040. doi: 10.1371/journal.pone.0100040
- Shao, M., Wang, Z., He, Y., Tan, Z., and Zhang, J. (2021). Fecal microbial composition and functional diversity of Wuzhishan pigs at different growth stages. *AMB Express* 11, 88. doi: 10.1186/s13568-021-01249-x
- Siggs, O. M., Miosge, L. A., Daley, S. R., Asquith, K., Foster, P. S., Liston, A., et al. (2015). Quantitative reduction of the TCR adapter protein SLP-76 unbalances immunity and immune regulation. *J. Immunol.* 194, 2587–2595. doi: 10.4049/jimmunol.1400326
- Singh, P., Gollapalli, K., Mangiola, S., Schraner, D., Yusuf, M. A., Chamoli, M., et al. (2023). Taurine deficiency as a driver of aging. *Science* 380, eabn9257. doi: 10.1126/science.abn9257
- Smyth, G. K. (2005). “limma: linear models for microarray data,” in *Bioinformatics and Computational Biology Solutions Using R and Bioconductor*. Eds. R. Gentleman, V. J. Carey, W. Huber, R. A. Irizarry and S. Dudoit (Springer New York, New York, NY), 397–420.
- Suzuki, Y., Suda, T., Furuhashi, K., Suzuki, M., Fujie, M., Hahimoto, D., et al. (2010). Increased serum kynurenine/tryptophan ratio correlates with disease progression in lung cancer. *Lung Cancer* 67, 361–365. doi: 10.1016/j.lungcan.2009.05.001
- Tang, Q., Tan, P., Ma, N., and Ma, X. (2021). Physiological functions of threonine in animals: beyond nutrition metabolism. *Nutrients* 13 (8), 2592. doi: 10.3390/nu13082592
- Ter Braak, C. J. F., and Smilauer, P. (2018). *Canoco reference manual and user's guide: Software for ordination, version 5.10* (Ithaca, USA: Microcomputer Power).
- Torrallardona, D., Conde, M. R., Badiola, I., Polo, J., and Brufau, J. (2003). Effect of fishmeal replacement with spray-dried animal plasma and colistin on intestinal structure, intestinal microbiology, and performance of weanling pigs challenged with *Escherichia coli* K99. *J. Anim. Sci.* 81, 1220–1226. doi: 10.2527/2003.8151220x
- Vahjen, W., Pieper, R., and Zentek, J. (2010). Bar-coded pyrosequencing of 16S rRNA gene amplicons reveals changes in ileal porcine bacterial communities due to high dietary zinc intake. *Appl. Environ. Microbiol.* 76, 6689–6691. doi: 10.1128/AEM.03075-09
- van der Kloet, F. M., Bobeldijk, I., Verheij, E. R., and Jellema, R. H. (2009). Analytical error reduction using single point calibration for accurate and precise metabolomic phenotyping. *J. Proteome Res.* 8, 5132–5141. doi: 10.1021/pr900499r
- Van Laethem, F., Tikhonova, A. N., Pobezinsky, L. A., Tai, X., Kimura, M. Y., Le Saout, C., et al. (2013). Lck availability during thymic selection determines the recognition specificity of the T cell repertoire. *Cell* 154, 1326–1341. doi: 10.1016/j.cell.2013.08.009
- van Vuuren, A. M., van der Koelen, C. J., Valk, H., and de Visser, H. (1993). Effects of partial replacement of ryegrass by low protein feeds on rumen fermentation and nitrogen loss by dairy cows. *J. Dairy Sci.* 76, 2982–2993. doi: 10.3168/jds.S0022-0302(93)77637-7
- Wang, W., Zeng, X., Mao, X., Wu, G., and Qiao, S. (2010). Optimal dietary true ileal digestible threonine for supporting the mucosal barrier in small intestine of weanling pigs. *J. Nutr.* 140, 981–986. doi: 10.3945/jn.109.118497
- Wen, C., Guo, Q., Wang, W., Duan, Y., Zhang, L., Li, J., et al. (2020a). Taurine alleviates intestinal injury by mediating tight junction barriers in diquat-challenged piglet models. *Front. Physiol.* 11. doi: 10.3389/fphys.2020.00449
- Wen, C., Li, F., Guo, Q., Zhang, L., Duan, Y., Wang, W., et al. (2020b). Protective effects of taurine against muscle damage induced by diquat in 35 days weaned piglets. *J. Anim. Sci. Biotechnol.* 11, 56. doi: 10.1186/s40104-020-00463-0
- Worrall, E. B., Chhaparia, A., Carpenter, D., and Neuschwander-Tetri, B. A. (2022). A rare cause of upper gastrointestinal bleeding: *sarcina ventriculi*. *ACG Case Rep. J.* 9, e00846. doi: 10.14309/crj.0000000000000846
- Yang, H., Huang, X., Fang, S., Xin, W., Huang, L., and Chen, C. (2016). Uncovering the composition of microbial community structure and metagenomics among three gut locations in pigs with distinct fatness. *Sci. Rep.* 6, 27427. doi: 10.1038/srep27427
- Yu, Z., and Morrison, M. (2004). Improved extraction of PCR-quality community DNA from digesta and fecal samples. *Biotechniques* 36 (5), 808–812. doi: 10.2144/04365ST04
- Zhang, Y., Chen, D. W., Yu, B., He, J., Yu, J., Mao, X. B., et al. (2015). Spray-dried chicken plasma improves intestinal digestive function and regulates intestinal selected microflora in weaning piglets. *J. Anim. Sci.* 93, 2967–2976. doi: 10.2527/jas.2014-8820
- Zhao, W., Wang, Y., Liu, S., Huang, J., Zhai, Z., He, C., et al. (2015). The dynamic distribution of porcine microbiota across different ages and gastrointestinal tract segments. *PLoS One* 10, e0117441. doi: 10.1371/journal.pone.0117441
- Zhe, L., Yang, L., Lin, S., Chen, F., Wang, P., Heres, L., et al. (2021). Differential responses of weaned piglets to supplemental porcine or chicken plasma in diets without inclusion of antibiotics and zinc oxide. *Anim. Nutr.* 7, 1173–1181. doi: 10.1016/j.aninu.2021.05.008