Giannuzzi, D., Toscano, A., Don, G., Tono, V., Pegolo, S., Cecchinato, A., Schiavon, S., Gallo, L. (2026) Unveiling region-specific gut transcriptomic impact in pigs fed increasing levels of *Arthrospira platensis* using RNAseq, *Proceedings 13th World Congress on Genetics Applied to Livestock Production* 23720: https://doi.org/10.31274/wcgalp.23720 ©2026 Giannuzzi, et al. CC BY-NC-ND

Unveiling region-specific gut transcriptomic impact in pigs fed increasing levels of *Arthrospira platensis* using RNAseq

D. Giannuzzi[1*], A. Toscano[1], G. Don[1], V. Tono[1], S. Pegolo[1], A. Cecchinato[1], S. Schiavon[1], and L. Gallo[1]

[1]*University of Padova, Padova, Italy;* [*]*Corresponding author: diana.giannuzzi@unipd.it*

Introduction

Soybean meal (SBM) is currently the primary protein source in pig nutrition, yet its use is increasingly questioned due to the environmental impacts of its cultivation and global supply chain, including land use change, extensive land occupation, and high energy and water consumptions (Mungkung *et al.,* 2013). *Arthrospira platensis* (AP), a cyanobacterium commonly known as microalga spirulina, is a promising alternative protein source containing 43–63% crude protein (on dry matter) and valuable bioactive compounds, and its water-based cultivation allows efficient processing into a feed-ready powder (Altmann and Rosenau, 2022).

When used as supplement in weanling and weaned pigs, AP reduced diarrhoea, improved jejunal morphology, and enhanced nutrient digestibility, because of up-regulation of genes involved in antioxidant activity and tight junction integrity, along with down-regulation of genes linked to intestinal inflammation (Furbeyre *et al.,* 2017; Alagbe *et al.,* 2025).

To date, only a few studies have assessed the effects of high dosage of AP until a complete replacement of conventional protein sources on the performance of fattening pigs (Don *et al*., 2025), whereas its transcriptomic effects on the intestine remain largely unexplored. Here, we investigated how partial to total substitution of SBM with AP influences gene expression profile of the ileum and the colon by means of RNA-Sequencing (RNAseq).

Materials & Methods

Animals, experimental design, and sample collection. Samples for this study originated from a feeding trial fully described by Don *et al*. (2025), involving 88 pigs (37 gilts, 51 barrows) of a genetic line intended for dry-cured ham production (Goland-C21×Camborough-43). All experimental procedures were approved by the animal ethics committee of the University of Padova ("Organismo preposto per il Benessere Animale", approval #37/2023).

Briefly, at 52.4 ± 4.2 kg body weight (BW) pigs were allocated to 8 pens equipped with single-space electronic feeders (Compident MLP 2-SMARTCON, Schauer Agrotronic) and assigned to four dietary treatments (2 pens per treatment): a conventional control diet (CTRL) based on cereals and SBM; and three experimental diets, where SBM was progressively replaced with a nucleus based on spray-dried AP powder (Aim Grow Biotech Co., Ltd., Port Coquitlam, BC, Canada) at 33, 66, and 100% inclusion (AP33, AP66, and AP100, respectively). The AP nucleus was formulated to match the nutrient profile of SBM, obtaining the formulation of isoenergetic, isoproteic, and isoaminoacidic diets. Pigs were fed according to a mild restricted feeding curve adjusted every 2 weeks, providing 1.90 to 3.20 kg/d from the first to the last week on feed, and were slaughtered in a commercial slaughterhouse at the age of 238 days at 174.9 ± 6.4 kg BW.

At slaughter, intestinal tissue samples were collected from each animal. One ileal segment (60 cm proximal to the ileocecal junction) and one segment from the proximal colon (20 cm aboral to the ileocecal junction) were excised, immediately immersed in RNA-later within 2

mL cryovials, and stored at -20°C until analysis. From the full set of samples, the total 60 animals were randomly selected, yielding 120 samples for whole-transcriptome analysis (15 pigs per diet per tissue).

RNA extraction, sequencing, and data mining. RNA was extracted using RNeasy Mini Kit (Qiagen) and evaluated using RNA was determined by A260/280 absorbance with Nanodrop One (ThermoFisher) and Qubit 4 (ThermoFisher) fluorometry. Libraries were prepared using Truseq Stranded mRNA library kit (Illumina) and inspected on a TapeStation D1000. Sequencing on an Illumina Hiseq X platform generated 100 PE reads. Raw reads were quality checked with FASTQC and trimmed using Trimmomatic. Clean reads were aligned to the Sscrofa11.1 reference genome using STAR and uniquely mapped reads were quantified with HTSeq based on Ensembl release 113 annotations. Differential expression analysis was performed in edgeR. Differentially expressed (DE) genes were identified using pairwise comparison between each AP diet to CTRL for the two tissues and considering only genes with $|\log_2$ Fold Change (FC)$| > 0.5$ and false discovery rate (FDR) < 0.05. Functional Gene Ontology (GO) categories (i.e. molecular function and biological process) of DE genes were explored and identified through gene-set overrepresentation analysis using PANTHER, and gene functional annotation by DAVID.

Results & Discussion

In this study, gut transcriptome regulation was investigated in growing-finishing pigs fed diets in which AP partially to fully replaced SBM, to identify molecular responses and evaluate the feasibility of AP as a primary protein source. Results from the feeding trial evidenced that the replacement of SBM with AP, even at full substitution, did not negatively affect the growth and carcass traits of growing pigs and the main attributes of meat (Don *et al.,* 2025). Similarly, systemic and local health of pigs, assessed through blood biochemical analyses and histological examinations of ileum and colon, were fully comparable between SBM and AP dietary groups (Giannuzzi *et al.*, 2026).

From RNAseq, we obtained an average of 51M raw reads per sample, with 98% retained after trimming. Mapping to Sscrofa11.1 with STAR yielded 83% uniquely mapped reads (range: 61-96%). These moderate-to-high mapping rates consistent with previous studies, given the complex, multi-lineage transcriptome of the intestinal tract (Tan *et al.,* 2017).

Comparing the traditional dry-cured ham diet with AP100 revealed 20 DE genes in the ileum and 296 in the colon, with no other contrasts showing significant changes. These findings indicate that full dietary substitution with AP induced intestinal molecular shifts, marked by a markedly stronger, tissue-specific transcriptional response in the colon. Expression patterns also diverged: 19 of 20 ileal DE genes were downregulated, whereas 272 of 296 colonic DE genes were upregulated. The main significant downregulated DE genes in the ileum under the AP100 diet included *SPRED1*, *P2RY12* and *MARCKS* ($\log_2$FC = −0.614, −0.895 and −0.529, respectively), together with *YOD1* and *TBL1XR1* ($\log_2$FC = −0.603 and −0.650, respectively) and *NAA30* and *ARL5B* ($\log_2$FC= −0.562 and −0.650, respectively) (Figure 1A). In contrast, the colon showed significant upregulation of *NFKBID*, *GNLY*, *CIAO3* and *TNFRSF25* ($\log_2$FC = +0.679, +1.458, +0.601 and +0.768, respectively), along with *NUDT22*, *SLC14A1,* and *PIP5KL1* ($\log_2$FC = +0.837, +3.319, and +0.927 respectively) (Figure 1B).

Gene functional annotation analysis using PANTHER and DAVID, integrating GO categories with metabolic pathways using both KEGG and REACTOME databases, supported these outcomes. In the ileum the AP100 diet DE genes were associated with the down-representation of pathways related to immune signalling, inflammatory activation, protein degradation, cellular stress responses, and vesicle-trafficking functions.

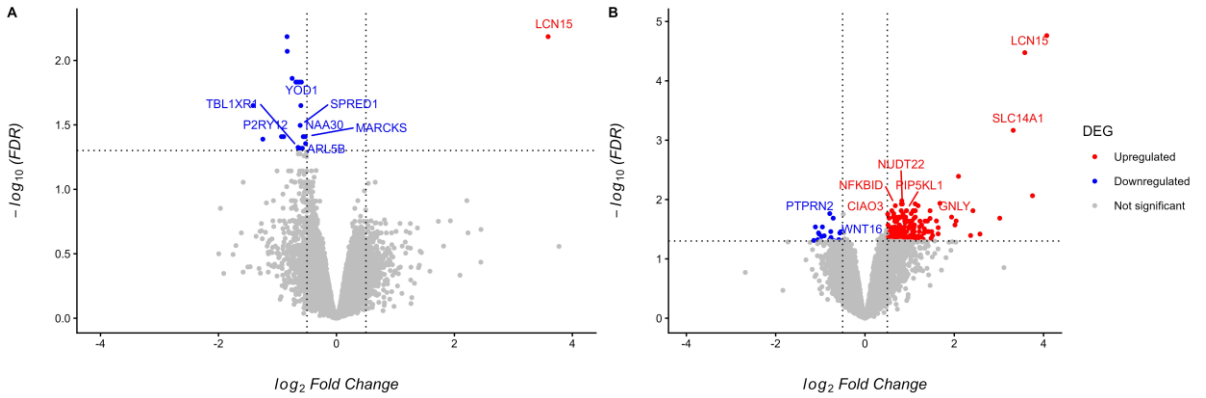

Figure 1. Volcano plots depicting differential expressed genes (DEG) in control and AP100 dietary treatment in the ileum (A) and colon (B). Red dots represent genes expressed at higher levels in AP100 diet, blue dots represent genes with lower expression in AP100 diet, and grey dots non-significant genes. Y-axis denotes $-\log_{10}$ false discovery rate (FDR) values while X-axis shows $\log_2$ fold change values. The DEGs were defined as genes with $|\log_2$ Fold Change$| > 0.5$ (vertical dashed lines) and an FDR < 0.05 (horizontal dashed line).

In contrast, the colon displayed an overrepresentation of pathways involved in mucosal immune readiness, epithelial stress adaptation, metabolic reprogramming, lipid transportation, and immune-related signalling cascades (Figure 2).

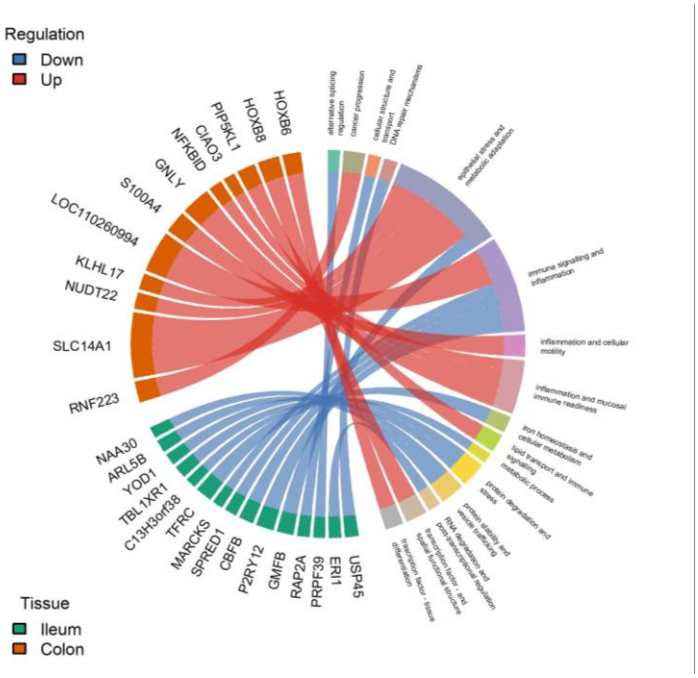

Figure 2. Functional categories associated with differential expressed (DE) genes in ileum and colon. Most relevant DE genes are grouped by tissue (ileum = green; colon = orange), with chords linking each gene to its functional category. Chord thickness reflects the magnitude of DE (|log2FC|), and chord colour indicates whether genes are up- (red) or down-(blue) regulated.

This pattern is consistent with the distinct physiological roles of the two gut segments in pigs: the ileum is primarily responsible for the active uptake of amino acids and glucose, whereas the colon serves as the main site of microbial fermentation (Jing *et al.,* 2025), with both regions contributing to mucosal immunity through the presence of gut-associated lymphoid

tissues (Jorgensen *et al.,* 2022). On the one hand, AP has been repeatedly shown to alleviate inflammation and oxidative stress, probably through phycocyanin, in the small intestine by downregulating MAPK and NF-κB signalling cascades in cell-culture systems animal models, and even in weanling pigs (Wu *et al.,* 2024; Alagbe *et al.,* 2025). On the other hand, as AP components are known to be only partially digested in the swine upper gut, indigestible residues and bioactive polysaccharides reach the distal intestine (Spinola *et al.,* 2022), where they undergo further degradation and are detected by mucosal immune cells. Polysaccharides from AP can activate immune responses in intestinal epithelial and immune cells by promoting interleukin production and IgA synthesis in pigs and other monogastrics (Balachandran *et al.,* 2006; Furbeyre *et al.,* 2017). Specifically, high-molecular-weight polysaccharide fractions can stimulate monocytes via toll-like receptor 2-dependent activation of NF-κB (Balachandran *et al.,* 2006), supporting that AP can modulate inflammatory signalling, exerting both stimulatory and regulatory mechanisms.

Conclusions

These findings advance understanding of how the use of AP as a feed ingredient modulates the gut transcriptome in growing–finishing pigs, revealing region-specific effects with a pronounced impact on the proximal colon. Overall, AP appeared to drive DE genes toward reduced immune–stress activity in the ileum and toward enhanced mucosal readiness and epithelial renewal in colon.

Acknowledgements

This study was carried out within the Agritech National Research Center, funded by the European Union (EU) Next Generation EU (PNRR – MISSIONE 4 COMPONENTE 2, CUP C93C22002790001). Additional funding was provided by the University of Padova (2024DAFNAE1SIDPROGETTI-00004) and the European Union. Views and opinions expressed are those of the authors only and do not necessarily reflect those of the EU or REA. Neither the EU nor REA can be held responsible for them.

References

Alagbe E.O., Ajuwon K.M., Schulze H., *et al*. (2025) J. Anim. Sci. 103:skaf338. https://doi.org/10.1093/jas/skaf338

Altmann B.A. and Rosenau S. (2022) Foods. 11:965. https://doi.org/10.3390/foods11070965

Balachandran P., Pugh N.D., Ma G., *et al*. (2006) Int. Immunopharmacol. 6:1808-14. https://doi.org/10.1016/j.intimp.2006.08.001

Don G., Giannuzzi D., Toscano A., *et al*. (2025) J. Anim. Sci. Biotechnol. 16:77. https://doi.org/10.1186/s40104-025-01197-7

Giannuzzi D., Toscano A., Don G., *et al*. (2026) Front. Vet. Sci. 13:1728650. https://doi.org/10.3389/fvets.2026.1728650

Furbeyre H., van Milgen J.J., Mener T., *et al*. (2017) Animal 11:183-192. https://doi.org/10.1017/S1751731116001543

Jing W., Tiejin T., Changqing Y., *et al*. (2025) Front. Vet. Sci. 12–2025. https://doi.org/10.3389/fvets.2025.1564519

Jorgensen P.B., Eriksen L.L., Fenton T.M., *et al*. (2022) Dev. Comp. Immunol. 131:104375. https://doi.org/10.1016/j.dci.2022.104375

Mungkung R., Aubin J, Prihadi T.H., *et al*. (2013) J. Clean Prod. 57:249-256. https://doi.org/10.1016/j.jclepro.2013.05.029

Spínola M.P., Costa M.M., and Prates J.A.M. (2022) Foods 11:2984. https://doi.org/10.3390/foods11192984

Tan Z., Wang Y., Yang T., *et al*. (2017) Sci Rep. 7:14886. https://doi.org/10.1038/s41598-017-14568-6

Wu Q., Liu L., Miron A., *et al*. (2016) Arch. Toxicol. 90:1817–1840. https://doi.org/10.1007/s00204-016-1744-5