Application Nutritionally enhanced protein may support the integrity of the intestinal barrier, allowing the intestine to maintain its selective permeability. This selectivity is essential for animal health, as it permits the absorption of beneficial compounds such as nutrients while restricting the uptake of harmful substances, including pathogens. Maintaining intestinal barrier function therefore plays a key role in supporting animal performance and overall health.

Introduction A renewed focus has been placed on protein-rich ingredients used in pig nutrition for multiple reasons: a need to reduce nitrogen output for environmental protection, finding more sustainable alternatives than soybean and legislative changes, such as the EU ban on the pharmacological use of zinc oxide. Alternative protein sources must ensure that there are no negative impacts on animal performance and health. Feed materials sourced from plants can contain anti-nutritional factors that can induce inflammation in animals, ultimately leading to reduced cellular viability. Sugar-amino acid complexes (SAC) have been linked to a range of beneficial properties, including antioxidative, anti-inflammatory and anti-bacterial properties. Due to previously reported beneficial properties, this study aimed to assess the effects of SAC on porcine intestinal cell functioning. The study hypothesised that intestinal cell function would be improved when porcine cells are treated with SAC.

Materials and methods Experiments were performed using the IPEC-J2 cell line (ACC 701, DSMZ, Germany), a non-transformed jejunal epithelial cell line derived from an unsuckled newborn piglet. Cells were cultured in uncoated flasks in DMEM supplemented with 10% heat-inactivated foetal bovine serum, penicillin (50 µg/mL), and streptomycin (50 U/mL) at 37 °C in 8% CO_2 and passaged at 80–90% confluency. Cells between passages 45–50 were seeded into 96-well plates at 1×10^5 cells/mL (100 µL/well). Two treatments were used: control (DMEM only) and DMEM supplemented with 0.1% SAC (DevAmine, Devenish Nutrition Ltd, Belfast, UK). Experiments included three biological replicates, each with ten technical replicates. Cells were cultured for 8 days, with SAC treatment applied during the final 48h. Cytotoxicity was assessed using MTT and neutral red assays (Kumar et al., 2018; Repetto et al., 2008), and membrane damage was measured using an LDH assay (CyQUANT LDH Assay, Thermo Fisher Scientific). Optical density readings (Tecan Sapphire 2) were converted to relative percentages of the control. Data were analysed by one-way ANOVA, with $P < 0.05$ considered significant (GraphPad Prism v5.03).

Results There were no significant differences between the control and treatment neutral red or LDH results ($P>0.05$). Supplementation with 0.1% SAC resulted in a significant 11% increase ($P<0.001$) in the metabolic activity of mitochondria compared to the untreated control, as indicated by the increased MTT result (Table 1).

Table 1. The average relative percentage of IPEC-J2 cells treated with control or treatment (0.1% SAC) supplemented media for 48 hours for cytotoxicity assays measured by OD (n=30)

	Control	Treatment[1]	S.E.M.	P-Value
MTT	100	111	2.35	0.001
Neutral Red	100	105	5.20	0.333
LDH	100	90.3	5.09	0.185

Values (mean and standard error of mean (S.E.M.) are presented as a percentage of control non-treated cell responses.
[1]DMEM media supplemented with 10% FBS, penicillin (50µg/ml), streptomycin (50U/ml) and 0.1% SAC

Conclusion and implications The neutral red uptake assay relies on the ability of viable cells to retain dye within lysosomes, with reduced uptake indicating increased cell death (Repetto et al., 2008). The absence of significant differences between treatment groups indicates that SAC supplementation does not negatively affect cell viability. Similarly, unchanged LDH levels suggest that cell membrane integrity was preserved. As overall cell numbers were comparable between groups, the observed increase in MTT activity is likely attributable to enhanced mitochondrial activity rather than increased proliferation. Increased mitochondrial activity may reflect greater mitochondrial abundance through mitochondrial biogenesis, a process that enhances cellular energy production (Quinn, 2025; Rai et al., 2018).

Overall, treatment of porcine intestinal epithelial cells with 0.1% SAC had no detrimental effects on cell viability or membrane integrity and was associated with increased mitochondrial metabolic activity. These findings suggest a potential role for SAC in supporting intestinal energy status and barrier integrity. However, further in-vitro and in-vivo studies are required to confirm these effects and clarify the underlying mechanisms.

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References

KUMAR, P., NAGARAJAN, A. & UCHIL, P. D. 2018. Analysis of cell viability by the MTT assay. *Cold spring harbor protocols,* 2018**,** pdb. prot095505.

POPOV, L.-D. 2020. Mitochondrial biogenesis: An update. *Journal of Cellular and Molecular Medicine,* 24**,** 4892–4899.

QUINN, M., BRYCHOKOVA, G., SCOTT, M., O'CONNELL, M. A comparitive study on the effect of feeding DevAmine technology products and traditional protein sources on the concentration of myo-inositol in weaned pigs. British Society of Animal Science, 2025 Galway.

RAI, Y., PATHAK, R., KUMARI, N., SAH, D. K., PANDEY, S., KALRA, N., SONI, R., DWARAKANATH, B. S. & BHATT, A. N. 2018. Mitochondrial biogenesis and metabolic hyperactivation limits the application of MTT assay in the estimation of radiation induced growth inhibition. *Scientific Reports,* 8**,** 1531.

REPETTO, G., DEL PESO, A. & ZURITA, J. L. 2008. Neutral red uptake assay for the estimation of cell viability/cytotoxicity. *Nature Protocols,* 3**,** 1125–1131.