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A comparative study of the effects of crude chicory and inulin on gut health in weaning piglets

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ABSTRACT

Dysfunction of the host-microbial balance and an impaired intestinal barrier can trigger inflammation and increase the antigen penetration. Inulin, commonly extracted from chicory root, is a prebiotic beneficial to gut health. The objective of this study was to compare the effect of chicory flour to inulin on gut health, few weeks after weaning. Two dose-dependent experiments (E1 and E2) were performed sequentially, each consisting of 80 castrated male piglets, weaned at day 21 and subsequently divided in 3 groups with *ad libitum* feed: control (Ctrl), inulin (INU) and crude chicory flour (CHI). For INU and CHI groups, a daily supplementation with the equated 'inulin content' increasing weekly was done by oral force-feeding, while the Ctrl groups received an isotonic sucrose solution. For E1, these doses were 1.5 g/day, 2 g/day and 2.5 g/day in W1, W2 and W3, respectively. For E2, these doses were 3 g/day, 4 g/day and 5 g/day in W1, W2 and W3, respectively. For each experiment at W0, W1 and W3, eight piglets per group were euthanized to assess gut structural and functional parameters. In E1, the CHI had lower average daily calorie intake (kcal/day) only at W3, while in E2 it was consistently lower than Ctrl and INU. In W3 of E2, CHI showed improved villi-to-crypt ratio and lower diarrhea occurrence than INU and Ctrl. Both supplemented groups in E2 showed higher butyrate production and lower D-xylose permeability (W3), compared to Ctrl. Interestingly, in E2, CHI had a more dominant effect on increasing the abundance of health promoting genera like *Catenisphaera*, *Butyricicoccus*, etc. and decreasing harmful genera like *Erysipelotrichaceae_UCG-002* and *Turicibacter*. In E2, on W3 several inflammatory target genes (CXCL10, IL18, TNFα) and inflammation signalling genes (MyD88, NFκB1) were downregulated in ileum of INU and CHI. In colon, both chicory and inulin, proved to be beneficial, as the inflammation signalling and inflammatory targets genes NFκB1, DEFβ4A, TLR2 and IFNα were significantly downregulated. Therefore, crude chicory flour might also be a promising cost-effective alternative supplement to improve gut health in weaned piglets.

1. Introduction

The weaning period, a critical transition phase, presents significant challenges to gut health in both humans and animals (Campbell et al., 2013; Pluske et al., 2018; Pohl et al., 2015). Detrimental changes occur during this phase, encompassing disruptions in gut morphology, physiology, immunology, and function, such as changes in intestinal barrier integrity and gut microbiota composition, mucosal inflammation, villus atrophy, and crypt hyperplasia (Awad et al., 2013; Bailey et al., 2005; Boudry et al., 2004; Lallès et al., 2007; Moeser et al., 2017). Zinc oxide, previously used as a potential solution for weaning-related issues, was banned in Europe due to concerns over environmental

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impact and the development of antimicrobial resistance (European Commission, 2017; Pejsak et al., 2023). Considering these restrictions, prebiotics, such as inulin, emerge as a promising alternative to replace banned feed additives and promote gut health.

Inulin, a fructan-based prebiotic composed of fructose units linked *via* β-(2-1)-d-fructosyl fructose linkages terminated with a glucose molecule linked through an α-(1-2) bond, has demonstrated various beneficial effects on gut health. It has been shown to reduce pro-inflammatory processes, enhance intestinal integrity, maintain gut morphology, and promote the composition of beneficial intestinal microbiota in different species including rodents, humans, and pigs (Apolinário et al., 2014; Cani et al., 2009; Chen et al., 2017; T.-W. Liu et al., 2016; Mensink et al., 2015). Crude chicory roots, which are recognized for their substantial inulin content, serve as the primary source for commercial extraction of inulin. Inulin consists of a blend of linear oligomers and polymers with chain lengths ranging from 2 to 60 units. Oligofructose, with a Degree of Polymerization (DP) < 10, makes up for approximately 10 % of total inulin, while 90 % are long length inulin fractions (DP = 11 to 60), with an average DP of around 25 (Niness, 1999). Studies have shown that both chain length and dietary concentration of inulin play a crucial role in its prebiotic effect (Herosimczyk et al., 2020). Inulin production from chicory involves two stages: extraction and initial purification of raw syrup, followed by refinement for commercialization (Shoaib et al., 2016; Redondo-Cuenca et al., 2021). The classical purification process involves multiple steps at high temperatures (80–90 °C), causing inulin hydrolysis and degrading other bioactives (Franck & Leenheer, 2005). Although advanced extraction methods (Lingyun et al., 2007; Loginova et al., 2010) and membrane-based filtration technologies (Cho, 2009) offer potential for obtaining high-quality long-chain inulin, increasing production costs with an impact on inulin recovery and quality (Khuenpet et al., 2017; Rubel et al., 2015; Ruiz-Aceituno et al., 2016).

In addition to inulin and oligofructose, crude chicory boasts a diverse range of bioactive compounds, including phenolic compounds, sesquiterpene lactones (such as lactucin, lactucopicrin, and 8-deoxy lactucin), guaianolid glycosides, caffeic acid derivatives (like chicoric acid, chlorogenic acid, isochlorogenic acid, and dicaffeoyl tartaric acid), hydroxycoumarins, flavonoids, alkaloids, steroids, terpenoids, volatile compounds, vitamins, β-carotene, zeaxanthin, etc. These bioactives have been associated with various beneficial effects, including reducing inflammation, modulating lipid metabolism, inhibiting the growth of certain pathogens, enhancing growth performance, antioxidant activity or anticancer properties (Ferioli & D'Antuono, 2012; Karioti et al., 2008; Kocsis et al., 2003; Pouille et al., 2022; San Andres et al., 2019; Schumacher et al., 2011).

It is now recognized that there exists a complex signalling interplay among the epithelium, mucosal immune system, and gut microbial ecosystem, which plays a critical role in preserving intestinal epithelial cell homeostasis, consequently influencing health and performance (Fouhse et al., 2016). Thus, studying these three parameters concurrently serves a dual purpose, allowing for a comprehensive evaluation of the effects of crude chicory supplementation by shedding light on the intricate interactions within the gastrointestinal tract. Considering the potential benefits of crude chicory's bioactive components, the aim of this study was to compare the effects of inulin (positive control) and crude chicory flour on intestinal health by equating the inulin content to explore the potential contributions of other bioactive components of crude chicory on gut health during the weaning period.

2. Material and methods

2.1. Experimental setup, animals and diets

The animal experiments, conducted in accordance with European (EU Directive 2010/63/EU) and Belgian regulations regarding the use and care of animals for scientific purposes, received approval from the ethical committee of the University of Liège (License- 21-2385). Two *in vivo* experiments (E1 and E2) were performed, each consisting of 80 castrated male piglets (Pietrain X Landrace), weaned at day 21 (D21) and with an average weight of 5.48 ± 0.5 kg (E1) and 5.36 ± 0.2 (E2), obtained from Walloon Agricultural Centre, Gembloux, Belgium. They were then distributed into 24 pens with three animals per pen, divided into three groups: control (Ctrl), INU, and CHI. In the IN and CHI, daily supplementation was administered through oral force-feeding, with the amount of actual 'inulin content' increasing weekly (W) (W1: 1.5 g/day, W2: 2 g/day, W3: 2.5 g/day) in experiment 1 (E1), and the dosage doubled in experiment 2 (E2) and administered in two parts (morning and evening). The inulin content for both groups was equated to ensure equivalence for the inulin related effect. The dosage was decided based on previous studies (Xu et al., 2005; Estrada et al., 2001; Li et al., 2018). Inulin (Orafti®SIPX) was sourced from Beneo, Belgium, while crude chicory flour (*Cichorium intybus*) came from Leroux-Waast Mill, France with a degree of polymerization (DP) range of 2–60 for both (Table 1). The Ctrl group received an isotonic sucrose solution (dosage dependent on average solute concentration of inulin and crude chicory flour at respective timepoints) to mimic the stress induced by force feeding. Throughout the experiments, the piglets had *ad libitum* access to water and Baby-mix© feed from Quartes (Supplementary Table 1). The temperature inside the room was kept at a constant 25 °C, and the lighting schedule followed a pattern of 12 h of light followed by 12 h of darkness. At W0, W1 and W3, eight piglets per treatment were euthanized using Anoxia® Pallet Box v.1.1, employing inert nitrogen gas encapsulated in foam to rapidly reduce the atmospheric oxygen from 20.9 % to 2 % or less. The W0 group consisted of untreated and just weaned piglets (Day 1- post-weaning). The gastrointestinal tract was then removed, and tissues and contents from the jejunum, ileum, caecum, colon, and rectum were collected from each piglet. The collected samples were immediately snap-frozen in liquid nitrogen and stored at −80 °C for further

Table 1
Nutrient Composition of Inulin and Chicory Flour.

Nutrients% (DM)	INU	CHI
(DM) (g/kg)	940	930
Protein	3.85	4.4
Fat	1.3	1.2
Ash	max 9	max 9
Sugars	8.6	4.3
NDF	0.56	4.47
ADF	0.73	3.63
Fructan	76.2	57.1
Calcium	–	–
Phosphorus	–	0.169
Sodium	–	0.83
Monosaccharides (% of DM)		
Rhamnose	0.09	0.38
Arabinose	0.05	2.12
Xylose	0.08	0.46
Mannose	9.52	3.87
Glucose	15.82	10.51
Galactose	0.29	2.25
Fructose	8.57	10.9
Molecular weight Distribution		
M_w	1800	2000
M_n	1400	1500
PDI	1.28	1.33
DP_w	9.99	10.9
DP_n	7.41	8.2

Abbreviations: DM: Dry Matter; NDF: Neutral Detergent Fibre (Hemicelluloses + Cellulose + Lignin); ADF: Acid Detergent Fibre (Cellulose + Lignin); M_w: Weight-Average Molecular Weight; M_n: Number-Average Molecular Weight; PDI: Poly Dispersity Index; DP_w: Weight-Average Degree of Polymerization; DP_n: Number – Average Degree of Polymerization.

analysis. Additionally, 5 cm long segments from duodenum, jejunum and ileum were collected in formaldehyde for histomorphological measurements.

2.2. Chemical analyses

The nutrient composition of the experimental diets was assessed in accordance with the protocols outlined by the Association of Official Analytical Chemists (Horwitz, 2010). To determine the Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) components, the Foss Fibrecap system was employed, following the methodology described by (Van Soest et al., 1991). For the analysis of monosaccharide composition (including rhamnose, arabinose, xylose, mannose, glucose, and galactose), samples underwent initial hydrolysis using H_2SO_4 (1 M) for 3 h at 100 °C. The resulting sugars were then derivatized and analysed using an HP Agilent 6890 series gas chromatograph (Agilent Technologies, Santa Clara, CA), equipped with a high-performance capillary column, HP1-methylsiloxane (Scientific Glass Engineering, Melbourne, Australia), and utilizing 2-deoxyglucose (Sigma-Aldrich Co., St Louis, MO) as an internal standard (Aguedo et al., 2014; Englyst & Cummings, 1984).

Molecular weight distributions were analysed via size-exclusion high-performance liquid chromatography (HPSEC) using a Waters 2690 Alliance chromatograph and a refractometer Waters 2410, with a TSKGEL G3000 PWXL column (7 μm 7.8*300 mm) (Tosoh, Tokyo, Japan). The eluent, consisting of $NaNO_3$ / NaN_3 at ambient temperature, was delivered at a flow rate of 0.7 mL/min (Aguedo et al., 2014). Peak molecular masses were determined using dextran standards from Sigma-Aldrich (Bornem, Belgium), which were utilized to construct a calibration curve. Additionally, the polydispersity index (PDI) was calculated by dividing the weight-average molecular weight (M_w) by the number-average molecular weight (M_n) (Aguedo et al., 2014).

2.3. Zootechnical performance metrices

During the experimental period, daily feed intake and weekly body weight gain were assessed for each treatment group, with a total of 8 pens per treatment and the occurrences were tallied on a weekly basis. The diarrhoea status of the piglets was visually evaluated by a single observer daily. Fecal scoring was performed using a scale ranging from 0 to 3, where 0 represented normal feces, 1 indicated soft feces, 2 denoted mild diarrhoea, and 3 indicated severe diarrhoea.

2.4. Histomorphological analysis

Duodenum, jejunum, and ileum tissues were cleaned using PBS, cut into 5 cm-long segments, and placed in formaldehyde on the day of dissection. Subsequently after 24 h, they were transferred to a 70 % ethanol solution for long-term storage. To prepare the tissue samples for analysis, they were embedded in paraffin and sliced using a microtome with sharp blades (5 μm thickness). Hematoxylin-eosin coloration was applied to the tissue sections. Several parameters were observed, including villi height, villi width, crypt depth, and the size of the muscularis layer, including the *lamina propria*. Measurements were conducted using the Toupview® application and a Toupcam® camera (UA510CA) mounted on an Olympus BX51 microscope at a magnification of 10x.

2.5. In vivo permeability (lactulose, mannitol and D-xylose)

In vivo intestinal permeability testing was conducted on the last day of W1 and W3 by orally force-feeding a cocktail of marker probes to overnight fasted piglets. The cocktail comprised D-xylose (100 mg/kg BW; VWR International, Belgium), mannitol (100 mg/kg BW; Sigma Aldrich, Belgium), and lactulose (500 mg/kg BW; Sigma Aldrich, Belgium). Blood samples were collected from the external jugular vein, one-hour post-administration, in gel and clot activator vacuum tubes (VWR International, Belgium) and subsequently centrifuged at 2000*g* for 10 min at 4 °C. The resulting serum samples were stored at −80 °C until analysis. For lactulose and mannitol estimation, serum samples were filtered through a 0.25 μm membrane and analysed utilizing High Performance Anion Exchange Chromatography with a Dionex CarboPac™ MA1 (Thermo Scientific, USA) column and a Pulsed Amperometric Detector (Dionex Pvt. Ltd.). The eluent comprised 612 mM NaOH with a flow rate of 0.4 mL/min. Chromatograms were interpreted using Chromeleon Client software version 7.3. Regarding D-xylose analysis, standard solutions were prepared by dissolving D-xylose in saturated benzoic acid to achieve concentrations ranging from 0 to 800 mg/L. A phloroglucinol colour reagent solution, sourced from Sigma-Aldrich Co. (St Louis, MO, USA), was prepared using 0.5 g of phloroglucinol, 100 mL of glacial acetic acid, and 10 mL of concentrated hydrochloric acid. Standard and serum samples (50 μL) were mixed with 5 mL of the phloroglucinol colour reagent solution, heated at 100 °C for 4 min, and then cooled in a water bath at room temperature. Absorbance was measured at 570 nm utilizing a spectrophotometer (VICTOR plate reader, PerkinElmer, Waltham, MA, USA). The blank utilized a 0 mg/L D-xylose standard solution, and D-xylose-free serum was employed for determining net absorptive concentrations (Uerlings et al., 2021).

2.6. Short chain fatty acids (SCFAs) composition

Samples of colon and cecum contents underwent analysis using an isocratic High-Performance Liquid Chromatography (HPLC) system featuring a Waters E2695 Alliance HPLC machine. The HPLC consisted of an Aminex HPX-87H ion exclusion column from BioRad (Hercules, CA, USA) and a UV detector set at 210 nm. The eluent flow rate was maintained at 0.6 ml/min of H_2SO_4 (mM) at 60 °C. Prior to analysis, the contents were centrifuged at 13,000 rpm for 15 min, and 1.6 mL of the supernatant was collected and acidified with H_2SO_4 (1 M) to achieve a pH range of 2 to 3. Subsequently, the samples underwent double filtration using Chromafil AO-45/25 and Chromafil AO-20/25 filters. Peak integration was executed utilizing the Empower 3 software from Waters Corporation (Milford, MA, USA), with manual verification for accuracy. Quantification was conducted using an external standard calibration method. The expression of intermediate metabolites and the sum of SCFAs was reported in mg/g of fresh content, while the quantities of acetate, propionate, butyrate, and branched-chain fatty acids (BCFAs) were expressed as a percentage ratio of the sum of SCFAs (Leblois et al., 2017).

2.7. Microbiota analysis

DNA was extracted from the colonic contents using the QIAamp PowerFaecal Pro DNA kit, Qiagen (Hilden, Germany) as per manufacturer's instruction. The DNA concentration and quality were determined using Nanodrop from Thermo Fisher Scientific (Waltham, MA, USA). For 16S rRNA gene sequencing, the V3-V4 region was amplified using Illumina MiSeq at GIGA (Genomics platform, ULiege, Belgium). The Amplicon Sequence Variant (ASV) determination was carried out with the Quantitative Insights into Microbial Ecology II 1.9.0 QIIME (Bolyen et al., 2019) software using the DADA2 plugin, as it models and rectifies errors inherent in amplicon sequencing conducted with Illumina technology (Callahan et al., 2016). Taxonomic classification utilized the SILVA database (version 138). Subsequent data visualization and statistical analyses were conducted in R Studio. A single ASV of the genus *Pseudomonas* was excluded from the analysis as it was identified as a contaminant in the negative control. Microbial alpha diversity (Chao1, Shannon indexes, and Simpson index) and beta diversity (PERMANONVA), were computed using the phyloseq package in R studio (Version 4.3.2). Microbiota results were analysed per time point using a Kruskal-Wallis test, with the treatment as a fixed factor. The Bonferroni Correction was applied to calculate the adjusted p-values and control the

family-wise error rate (FWER) for microbiota and gene expression data sets.

2.8. Gene expression

Total RNA was extracted from ileum and colonic tissues using the ReliaPrep RNA Tissue Miniprep System Kit (Promega Corporation, Madison, WI). RNA concentration of 60 ng, determined by Nanodrop (Thermo Fisher Scientific, Waltham, MA), was converted into cDNA using the Reverse Transcription Master Mix (Fluidigm Corporation, South San Francisco, CA). High-throughput quantitative PCR (qPCR) was conducted, utilizing intron-spanning primer pairs (Table 2) designed via Primer-BLAST (NCBI), as outlined by (Uerlings et al., 2021). These primer pairs were validated through agarose gel electrophoresis and melting curves. The qPCR assays were performed in 48 × 48 dynamic array-integrated fluidic circuits (Standard BioTools Inc., South San Francisco, CA, USA) with the following protocol: 60 s at 95 °C, followed by 30 cycles (5 s at 96 °C and 20 s at 60 °C). Quantification cycles (Cq) were obtained using the Fluidigm real-time PCR analysis software 3.0.2 (Standard BioTools Inc., South San Francisco, CA, USA). Housekeeping genes were assessed, and the three most stable genes between treatments were determined using NormFinder (Andersen et al., 2004). Three reference genes were selected separately for colon and ileum tissues, as well as for different experimental conditions (E1 and E2). Relative gene expression levels were calculated utilizing the Pfaffl method (Pfaffl, 2001), and the geometric mean of the relative expression of the three most stable housekeeping genes was employed to normalize all samples.

Table 2
Primer sequences to investigate gene expression of ileum and colonic tissues.

Gene		Sequence		Accession no
		F (5′-3′)	R (5′-3′)	
Housekeeping genes	ACTB	*CTACGTCGCCCTGGACTTC*	*GCAGCTCGTAGCTCTTCTCC*	XM_003124280.5
	GAPDH	*GATGGTGAAGGTCGGAGTGAA*	*GTGGAGGTCAATGAAGGGGT*	XM_021091114.1
	HPRT1	*AATTCTTTGCTGACCTGCTGGA*	*TCCACCAATTACTTTTATATCGCCC*	XM_021079503.1
	PPIA	*GGGACCTGGAAACCAAGAAGTG*	*ACTTTGTCTGCAAACAGCTCCAATC*	XM_013985800.2
	RPL13a	*ATTGTGGCCAAGCAGGTACT*	*AATTGCCAGAAATGTTGATGC*	XM_013998640.2
	RPL32	*GCTTGAAGTGCTGCTAATGTG*	*GGATTGGTGACCCTGATGGC*	XM_021068582.1
	RPL4	*GAGAAACCGTCGCCGAATCC*	*CCCACCAGGAGCAAGTTTCAA*	XM_005659862.3
	TBP	*CGGACCACCGCACTGATATT*	*TTCTTCACTCTTGGCTCCCG*	XM_021085483.1
	YWHAZ	*TTGTAGGAGCCCGTAGGTCA*	*AGCACCTTCCGTCTTTTGCT*	NM_001315726.1
Apoptosis related genes	CASP3	*AAGCAAATCAATGGACTCTGGAA*	*TTGCAGCATCCACATCTGTACC*	NM_214131.1
	BAX	*CCCAGAGGCGGGGTTTCAT*	*CAATGCGCTTGAGACACTCG*	XM_013998624.2
	CASP1	*GTTATTCGGAAAGGGCCCCA*	*CACCGCCTGGGATTCTTGTA*	NM_214162.1
	JUN	*CTTTCCTCCTTCACGGTCCC*	*CACTTCACGTGGGGTGAGTT*	NM_213880.1
Barrier Integrity genes	CDH1	*AGCCCTGCAATCCTGGCTTT*	*AGAAACATAGACCGTCCTTGGC*	NM_001163060.1
	Claudin-1	*GGTGACAACATTGTGACGGC*	*TACCATCAAGGCACGGGTTG*	NM_001244539.1
	Claudin-3	*TATCACAGCGCGGATCACC*	*CTCTGCACCACGCAGTTCAT*	NM_001160075.1
	Claudin-4	*CTTCATCGGCAGCAACATCG*	*CGAGTCGTACACCTTGCACT*	XM_013995522.2
	MARVELD2	*CTCAGCCCCGCCATTACCTG*	*TAGAGGTGATGTGCTGTTGCC*	NM_001243948.1
	MUC1	*GGATTTCTGAATTGTTTTTGCAG*	*ACTGTCTTGGAAGGCCAGAA*	XM_021089728.1
	Occludin	*AACGTATTTATGACGAGCAGCCC*	*CACTTTCCCGTTGGACGAGTA*	NM_001163647.2
	ZO-1	*AAGGTCTGCCGAGACAACAG*	*TCACAGTGTGGTAAGCGCAG*	XM_021098827.1
Inflammation signaling pathway genes	AKT1	*CTAAGCCCAAACACCGCGT*	*TCAGGATCTTCATGGCGTAGT*	XM_021081499.1
	MAPK14	*TACCCGAGCGTTACCAGAAC*	*TTCACTGCAACACGTAACCCA*	XM_001929490.6
	MyD88	*GCATCACCATTCGAGATGACC*	*TCCTGCACAAACTGGGTATCG*	NM_001099923.1
	NF-κB1	*AAGAAGTCCTACCCTCAGGTCA*	*CAGTGACAGTGCAGATCCCA*	NM_001048232.1
	NF-κBIα	*GAGGATGAGCTGCCCTATGAC*	*CCATGGTCTTTTAGACACTTTCC*	NM_001005150.1
	NOD1	*GTCGTCAACACCGATCCAGT*	*CCTCCTTCTGGGCATAGCAC*	NM_001114277.1
	PPARγ	*ACAGCGACCTGGCGATATTTA*	*GAGGACTCTGGGTGGTTCAA*	XM_005669784.3
	TLR2	*GTTTTACGGAAATTGTGAAACTG*	*TCCACATTACCGAGGGATTT*	XM_005653576.3
	TLR4	*ATGATTCCTCGCATCCGCCT*	*AATTCAGCTCCATGCATTGGTAA*	NM_001113039.2
Inflammatory Target genes	CXCL10	*CCCACATGTTGAGATCATTGC*	*GCTTCTCTCTGTGTTCGAGGA*	NM_001008691.1
	DEFβ4a	*CAGGATTGAAGGGACCTGTT*	*CTTCACTTGGCCTGTGTGTC*	AY506573.1
	IFNβ	*TTCGAGGTCCCTGAGGAGATT*	*GCTGGAGCATCTCGTGGATAA*	NM_001003923.1
	IL1β	*CCAAAGAGGGACATGGAGAA*	*GGGCTTTTGTTCTGCTTGAG*	XM_021085847.1
	IL18	*CTGAAAACGATGAAGACCTGGA*	*CCTCAAACACGGCTTGATGTC*	XM_005667326.2
	IL6	*TGGGTTCAATCAGGAGACCT*	*CAGCCTCGACATTTCCCTTA*	NM_001252429.1
	IL8	*GACTTCCAAACTGGCTGTTGC*	*ATTTGGGGTGGAAAGGTGTG*	JF906514.1
	ILRN1	*TGCCTGTCCTGTGTCAAGTC*	*GTCCTGCTCGCTGTTCTTTC*	NM_214262.1
	MCP1	*CTCACTGCAGCCACCTTCT*	*CACTTGCTGCTGGTGACTCT*	NM_214214.1
	TNFα	*TCTGCCTACTGCACTTCGAG*	*GTTGATGCTCAAGGGGCCA*	NM_214022.1

Abbreviations: ACTB – actin beta; GAPDH – glyceraldehyde-3-phosphate dehydrogenase; HPRT1 – hypoxanthine phosphoribosyltransferase 1; PPIA – peptidylprolyl isomerase A; RPL13a – ribosomal protein L13a; RPL32 – ribosomal protein L32; RPL4 – ribosomal protein L4; TBP – TATA box binding protein; YWHAZ – tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta; CASP3 – caspase 3; BAX – BCL2-associated X protein; CASP1 – caspase 1; JUN – Jun proto-oncogene; CDH1 – E-cadherin; Claudin-1 – claudin-1; Claudin-3 – claudin-3; Claudin-4 – claudin-4; MARVELD2 – tricellulin; MUC1 – mucin 1; Occludin – occludin; ZO-1 – zonula occludens-1; AKT1 – serine/threonine-protein kinase 1; MAPK14 – mitogen-activated protein kinase 14; MyD88 – myeloid differentiation primary response 88; NF-κB1 – nuclear factor-kappa B; NF-κBIα – nuclear factor-kappa B inhibitor alpha; NOD1 – nucleotide-binding oligomerisation domain-containing protein 1; PPARγ – peroxisome proliferator-activated receptor gamma; TLR2 – toll-like receptor 2; TLR4 – toll-like receptor 4; CXCL10 – C-X-C motif chemokine 10; DEFβ4a – defensin beta 4a; IFNβ – interferon beta; IL1β – interleukin 1 beta; IL18 – interleukin 18; IL6 – interleukin 6; IL8 – interleukin 8; ILRN1 – interleukin-1 receptor antagonist; MCP1 – monocyte chemoattractant protein 1; TNFα – tumour necrosis factor alpha.

2.9. Statistical analyses

In the present study, all the variables were analyzed using the general linear model (GLM) implemented in SAS Enterprise Guide 7.4 (SAS Institute Inc, Cary, NC) followed by Tukey's multiple range test was employed to discern significant differences among treatment means, with significance levels established at $p < 0.05$ (significant), $p < 0.01$ (highly significant), and $p < 0.0001$ (very highly significant). For microbiota analyses at each time point, the Kruskal-Wallis test followed by Dunn's test was performed. Amplicon Sequence Variant (ASV.) determination, taxonomic classification using the SILVA database (version 138), data visualization, and subsequent statistical analyses were conducted using R Studio. The p values were adjusted using Bonferroni correction to control the family-wise error rate (FWER). Integration analysis was performed across multiple datasets to uncover potential relationships using supervised Regularized Generalized Canonical Correlation Analysis (RGCCA) (Tenenhaus & Tenenhaus, 2011) following treatment groups. Datasets variables have been grouped in 4 blocks following data types: "Clinical" (ADWG, ADCI, Length, Diarrhoea Incidences), "Gut" (Histomorphology, Permeability, SCFAs), "Gene Expression", and "Microbiota". RGCCA *tau* value was fixed at is optimal value with a *factorial scheme*. P-values of Pearson correlation coefficients (r) were adjusted for multiple testing using Bonferroni method. All analyses were performed using R version 4.3.1 and the following packages: RGCCA (v3.0.3), factoextra (v1.0.7), FactoMineR (v2.9), ggplot2 (v3.5.0), and corrplot (0.92).

3. Results

3.1. Growth performances & diarrhoea incidences

As shown in Fig. 1B, in E1, the group fed with crude CHI exhibited significantly lower ADCI (Average Daily Calorie Intake, kcal/day) only during W3, while in E2, it was lower for all three weeks when compared to the INU and Ctrl groups. Interestingly, this did not cause any significant difference in ADWG (Average Daily Weight Gain) (g/day) on W2 and W3 of both E1 and E2 (Fig. 1A). During W1 of E2, there was significant weight loss in the CHI group piglets compared to the INU group ($p < 0.05$). In E1, INU and CHI treatments had no effect on the occurrences of diarrhoea (Fig. 1C). However, in E2, the occurrence of severe diarrhoea (S3) was significantly lower for CHI than IN and Ctrl in W3 (Fig. 1D). In E2, during W2, the mild diarrhoea scores (S2) were lower for both the CHI and INU groups compared to Ctrl ($p < 0.001$).

3.2. Duodenum, jejunum, and ileum histomorphology

In W1, CHI and INU supplementation had no significant effect on villus height, width, or V:C ratio compared to the Ctrl group, neither in E1 nor in E2. However, after W3 of supplementation in E1, the INU group exhibited significantly higher villi height ($p < 0.01$), width ($p < 0.05$) in the ileum and a higher V:C ratio ($p < 0.0001$) than both CHI and Ctrl in both jejunum and ileum. Interestingly, CHI supplementation led to a significant reduction in crypt depth in the duodenum (W3, $p < 0.0001$) and jejunum (W1, $p < 0.01$) (Supplementary Table 2).

Doubling the dose in E2 did not result in any significant effect on the histomorphological parameters of the duodenum. However, it did yield

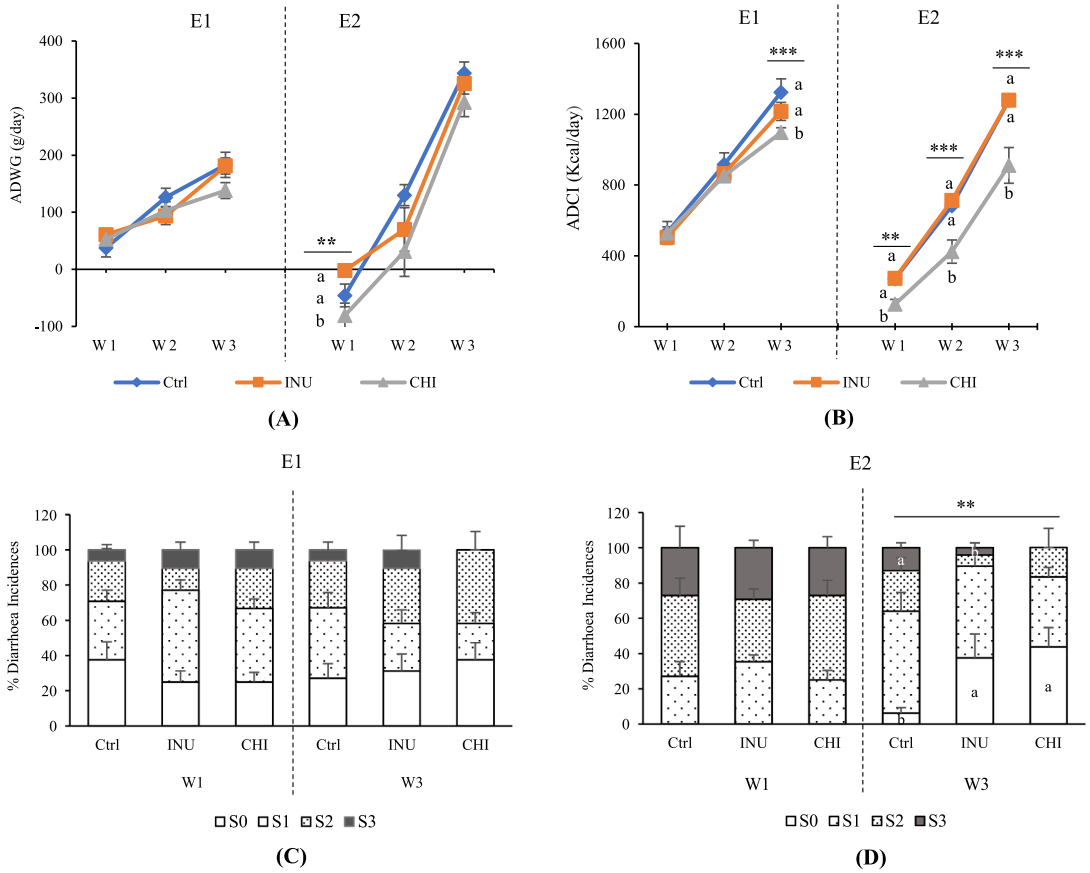


Fig. 1. Effect of INU and CHI supplementation on weaning piglet at W1 &W3 in E1 & E2 on (A) Average Daily Weight Gain (ADWG) (g/day) (B) Average Daily Calorie Intake (ADCI) (C) E1 (D) E2 % Diarrhea Incidences. Values are means (n = 8 piglets) ± SEM. Statistical analysis ANOVA + Tukey's test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Abbreviations: S0: Normal feces; S1: Soft feces, S2: Mild diarrheas; S3: Severe diarrhea.

some interesting effect on the jejunum and ileum (Table 3). In E2 (W3), chicory supplementation showed significantly higher villus height ($p < 0.01$) and V:C ratio ($p < 0.01$) than the Ctrl group piglets. Furthermore, CHI had even higher villus height than INU in the ileum in E2 (W3). Throughout both experiments, chicory and inulin supplementation did not influence the thickness of the duodenal, jejunum, or ileum's *muscularis mucosae* and *tela submucosa* or *tunica muscularis* layers (Table 3).

3.3. Intestinal permeability of lactulose, mannitol and D-xylose

The serum concentrations of lactulose and mannitol in the blood exhibited no statistically significant differences across the Ctrl, INU, and CHI groups, in both experimental phases E1 and E2. However, in E2, on W3, although not statistically significant ($p = 0.057$), the CHI group had the lowest L:M (Lactulose: Mannitol) ratio and a 50 % reduction in plasma lactulose concentration relative to both the Ctrl and INU groups (Fig. 2A). However, at W3, INU and CHI group had decreased levels of D-xylose ($p < 0.05$) compared to the Ctrl group in E1 ($p < 0.05$) and E2 ($p < 0.01$) (Fig. 2B).

3.4. Cecal and colonic microbial metabolites

In Supplementary Fig. 1, the analysis of cecal and colonic contents at W1 and W3 indicated that CHI and INU treatments in E1 did not elicit significant alterations in short-chain fatty acid (SCFA) profiles. In E2 (Fig. 3) at W3, both the INU and CHI groups exhibited higher lactate levels ($p < 0.01$) and lower propionate levels ($p < 0.001$) in cecal content compared to Ctrl group. Conversely, during W1 of E2, significantly elevated lactate ($p < 0.001$) and butyrate ($p < 0.001$) levels were detected in the colonic content of the INU and CHI groups compared to the Ctrl group. Moreover, solely the INU group demonstrated a significantly heightened concentration of total SCFAs ($p < 0.001$) in W1. Subsequently, by W3, both the INU and CHI groups demonstrated significantly low acetate levels ($p < 0.001$) and exhibited the highest levels of butyrate ($p < 0.001$) in comparison to the Ctrl group.

3.5. Microbiota diversity and abundance

The taxonomic diversity was evaluated and compared utilizing various indices, including Chao1, Shannon, and Simpson. Alpha diversity analysis revealed no significant differences between treatments across both the experiments, E1 and E2 (Fig. 4 & Supplementary Fig. 2), indicating a stabe diversity across both groups. To further elucidate the dissimilarities between samples, Principal Coordinates Analysis (PCoA) was conducted employing the Bray-Curtis dissimilarity index. Regarding beta diversity at the ASV level, the impact of inulin and crude chicory was explored. Notably, statistically significant differences were observed at W3 of E1 (PERMANOVA, adj.$p < 0.05$) and W1 of E2 (PERMANOVA, adj.$p < 0.01$), suggesting distinct community compositions influenced by these treatments at specific timepoints. Conversely, no statistically significant differences were detected among the treatments at other timepoints.

Among the 24 phyla identified through sequencing, Firmicutes and Bacteroidota emerged as the predominant phyla W1 and W3 timepoints in both E1 (Supplementary Fig. 2) and E2 (Fig. 4). Specifically, in W1 of E1, Bacteroidota exhibited significantly higher abundance in the INU and CHI group compared to the Ctrl group (adj.$p < 0.01$), but this difference was not evident at W3. Similarly, in E2, the relative abundance of Firmicutes was notably elevated in the INU and CHI group compared to the Ctrl group at W1 (adj.$p < 0.05$), yet no significant disparities were observed at W3. Other notable phyla present at levels exceeding 1 % included Proteobacteria, Spirochaetota, and Actinobacteriota.

The alterations observed in the colonic microbiota at the genus level,

Table 3
Effect of CHI and INU supplementation on histomorphological parameters of Duodenum, Jejunum, and Ileum in E2 at W0, W1 and W3 post-weaning.

Intestinal Part	Week	Treat-ments	Villus Height (μm)	Villus Width (μm)	Crypt Depth (μm)	V:C	Muscularis Mucosae + Tela submucosa (μm)	Tunica Muscularis (μm)
Duodenum	**W0**	**Ctrl**	257 ± 5	106 ± 3	215 ± 15	1.26 ± 0.08	55 ± 4	214 ± 6
	W1	**Ctrl**	238 ± 13	102 ± 4	205 ± 9	1.19 ± 0.101	47.7 ± 1.61	231 ± 19
		INU	261 ± 9	111 ± 5	237 ± 12	1.19 ± 0.09	47 ± 1.7	235 ± 16
		CHI	245 ± 16	97.3 ± 4	212 ± 8	1.14 ± 0.06	48.3 ± 2.14	207 ± 10
		p value	0.523	0.413	0.371	0.633	0.488	0.219
	W3	**Ctrl**	327 ± 15.7	116 ± 6	229 ± 15	1.46 ± 0.07	44 ± 2.78	226 ± 11
		INU	338 ± 24.4	112 ± 6	233 ± 14	1.46 ± 0.08	42 ± 2.93	243 ± 20
		CHI	370 ± 12.9	118 ± 6	236 ± 11	1.6 ± 0.13	41 ± 2.36	205 ± 10
		p value	0.55	0.3535	0.441	0.315	0.467	0.214
Jejunum	**W0**	**Ctrl**	264 ± 12.5	102 ± 5	222.94 ± 16	1.24 ± 0.11	80.9 ± 45.5	270 ± 14
	W1	**Ctrl**	331 ± 9.14	133 ± 15.9	237 ± 18^{b}	1.35 ± 0.09	45.2 ± 1.49	259 ± 17.1
		INU	306 ± 15.2	118 ± 5.45	238 ± 6.76^{b}	1.31 ± 0.08	50.1 ± 2.8	250 ± 9.23
		CHI	340 ± 12.7	117 ± 4.34	298 ± 7.53^{a}	1.17 ± 0.04	46.3 ± 1.32	233 ± 6.32
		p value	0.421	0.601	0.01	0.217	0.487	0.515
	W3	**Ctrl**	339 ± 5.25^{b}	139 ± 6.68	255 ± 18.5	1.27 ± 0.03^{b}	51 ± 3.19	247 ± 6.72
		INU	369 ± 26.2^{ab}	127 ± 8.4	260 ± 5.98	1.35 ± 0.2^{ab}	52.5 ± 2.96	253 ± 8.03
		CHI	420 ± 20.3^{a}	139 ± 4.11	236 ± 8.69	1.79 ± 0.13^{a}	58.5 ± 1.64	266 ± 20.6
		p value	**0.033**	0.353	0.633	**0.03**	0.421	0.221
Ileum	**W0**	**Ctrl**	262 ± 6	122 ± 5	166 ± 3.53	1.59 ± 0.06	28 ± 2	243 ± 4
	W1	**Ctrl**	247 ± 11.8	94 ± 4.72	215 ± 8.2	1.17 ± 0.07	58 ± 3.2	232 ± 10.7
		INU	245 ± 11.4	108 ± 6.94	224 ± 7.34	1.11 ± 0.07	53.9 ± 3.89	256 ± 7.1
		CHI	239 ± 7.91	110 ± 5.46	200 ± 10.2	1.22 ± 0.07	49.3 ± 2.13	246 ± 7.19
		p value	0.438	0.122	0.3625	0.396	0.438	0.072
	W3	**Ctrl**	338 ± 14.2^{b}	133 ± 2.54^{b}	206 ± 8.33	1.41 ± 0.17^{b}	44.6 ± 1.41	274 ± 10.9
		INU	323 ± 21.7^{b}	155 ± 9.26^{a}	243 ± 19.9	1.67 ± 0.1^{ab}	43.8 ± 1.77	288 ± 16.7
		CHI	440 ± 28^{a}	155 ± 2.57^{a}	214 ± 8.9	2.1 ± 0.15^{a}	44.4 ± 2.07	285 ± 7.65
		p value	**0.002**	**0.015**	0.427	**0.008**	0.746	0.341

Values are means ± SEM, n = 8 per treatment group. Means in a column with timepoint without a common superscript letter differ ($p < 0.05$) as analysed by one-way ANOVA and the Tukey test. Abbreviations: V:C = Villi height: Crypt depth).

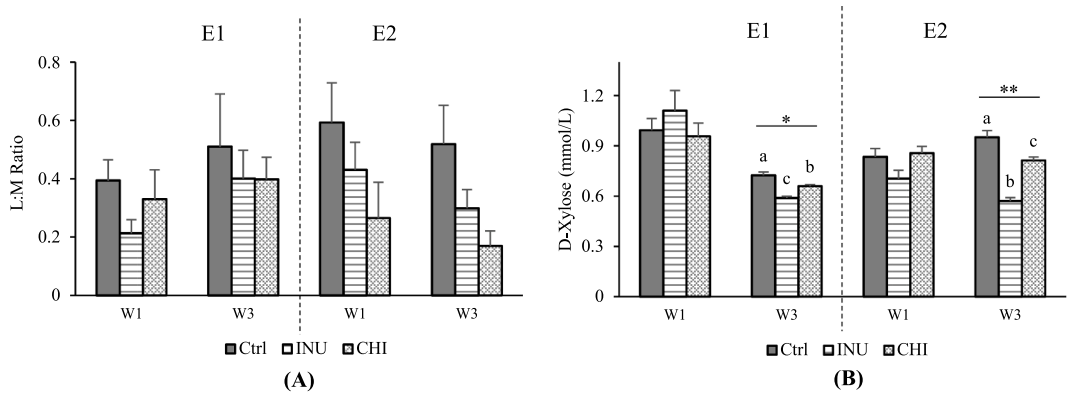


Fig. 2. Effect of INU and CHI supplementation on weaning piglet at W1 &W3 in E1 & E2 on permeability of (A) Lactulose: Mannitol (L:M) Ratio and (B) D-Xylose concentration, measured in serum samples 1 h after dosage. Values are means (n = 8 piglets) ± SEM. Statistical analysis ANOVA: Tukey's test, Bars with different letters are significantly different (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

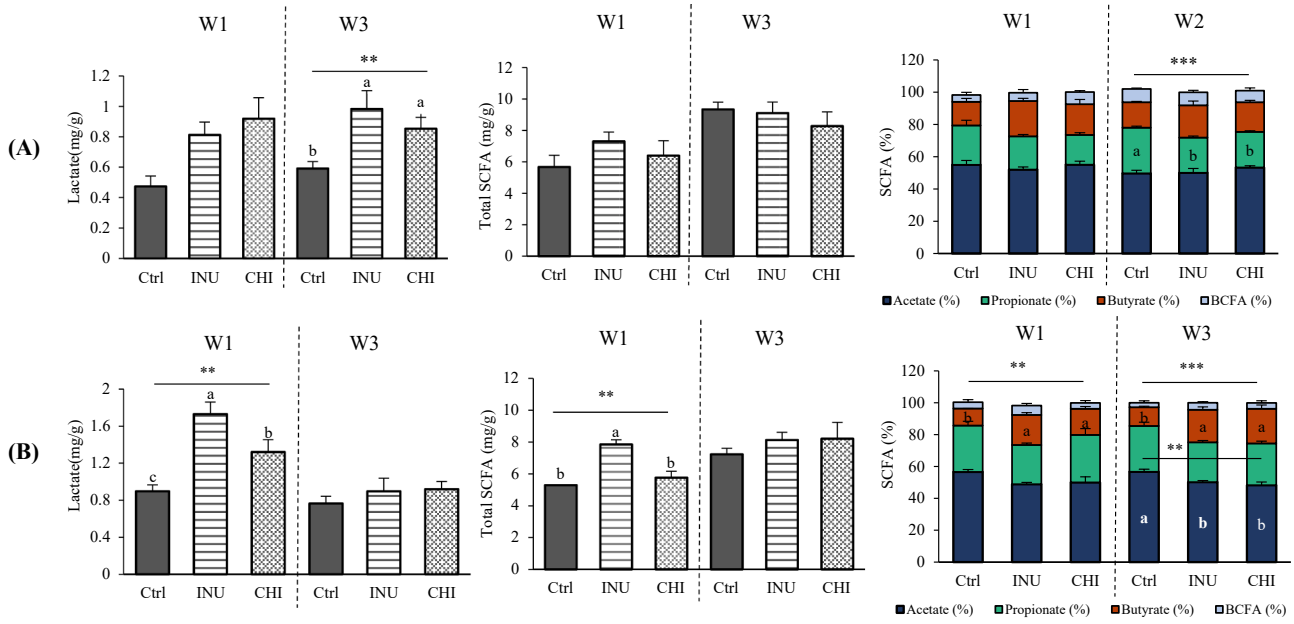


Fig. 3. Effect of INU and CHI supplementation in E2 on Lactate, Total Short Chain Fatty Acids (SCFA) and % composition of Acetate, Propionate, Butyrate and Branch Chain Fatty Acids in (A) Cecal & (B) Colonic content. Values are means (n = 8 piglets) ± SEM. Statistics: ANOVA: Tukey's test. Bars with different letters are significantly different (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

predominantly consisted, *Prevotella*, *Lactobacillus*, *Lachnospiraceae*, and *Megasphaera* emerged as the most abundant genera across both experiments. In E1 (Supplementary Table 3) at W1, a notable increase in the relative abundance of *Prevotella* was discerned in the INU and CHI group (adj. $p < 0.05$). Furthermore, ASVs affiliated with *Succinivibrio* exhibited significantly higher abundance in the INU group compared to CHI and Ctrl (adj.$p < 0.05$). However, at W3, the relative abundance of *Megasphaera* (adj.$p < 0.05$) and *Dialister* (adj.$p < 0.05$) was significantly elevated in the INU group compared to both CHI and Ctrl groups.

At W1 of the E2 (Table 4), there was a dramatic increase in the abundance of *Catenibacterium*, with a sevenfold higher population observed in the crude CHI-fed (CHI) group (7.86 ± 1.63) and a fourfold increase in the INU-fed (INU) group (4.53 ± 2.1) compared to the control (Ctrl) group (0.67 ± 0.18), as evidenced by statistical significance (adj. $p < 0.01$). Additionally, the abundance of *Megasphaera* was notably elevated in the INU and CHI groups compared to the Ctrl group, although this difference was not statistically significant (adj.$p = 0.051$). Upon expanding the analysis to include genera with less than 1 % relative abundance (data not shown), seven genera exhibited significant differences among the treatment groups. Piglets fed crude chicory exhibited a substantially higher abundance of *Catemisphaera* ($p < 0.01$), a member of the *Ruminococcaceae* family, compared to those fed with INU. Interestingly, Ctrl group showed even lower levels of *Catemisphaera*. Furthermore, the *Collinsella* genus was significantly more abundant ($p < 0.05$) in the CHI group compared to the Ctrl and INU groups.

As shown in the Table 4 the microbiota composition of W3 of E2, there were 4 genera with significant differences (relative abundance higher than 1 %). The abundance of *Unclassified Lachnospiraceae* was found to be significantly high (adj.$p < 0.01$) for the Ctrl compared to the INU and CHI group colonic content. Interestingly the *Streptococcus* abundance was significantly altered (adj.$p < 0.05$) by the addition of CHI (0.41 ± 0.11) and then INU (1.67 ± 0.55) when compared to Ctrl (4.03 ± 1.8). The inclusion of the Crude CHI and INU increased ($p = 0.05$) the population of *Ruminococcus* genus than Ctrl. On the contrary, CHI inclusion caused significant reduction ($p = 0.01$) in the abundance of *Erysipelotrichaceae_UCG-002* than Ctrl and INU. When broadening the analysis in W3-E2 to encompass genera with relative

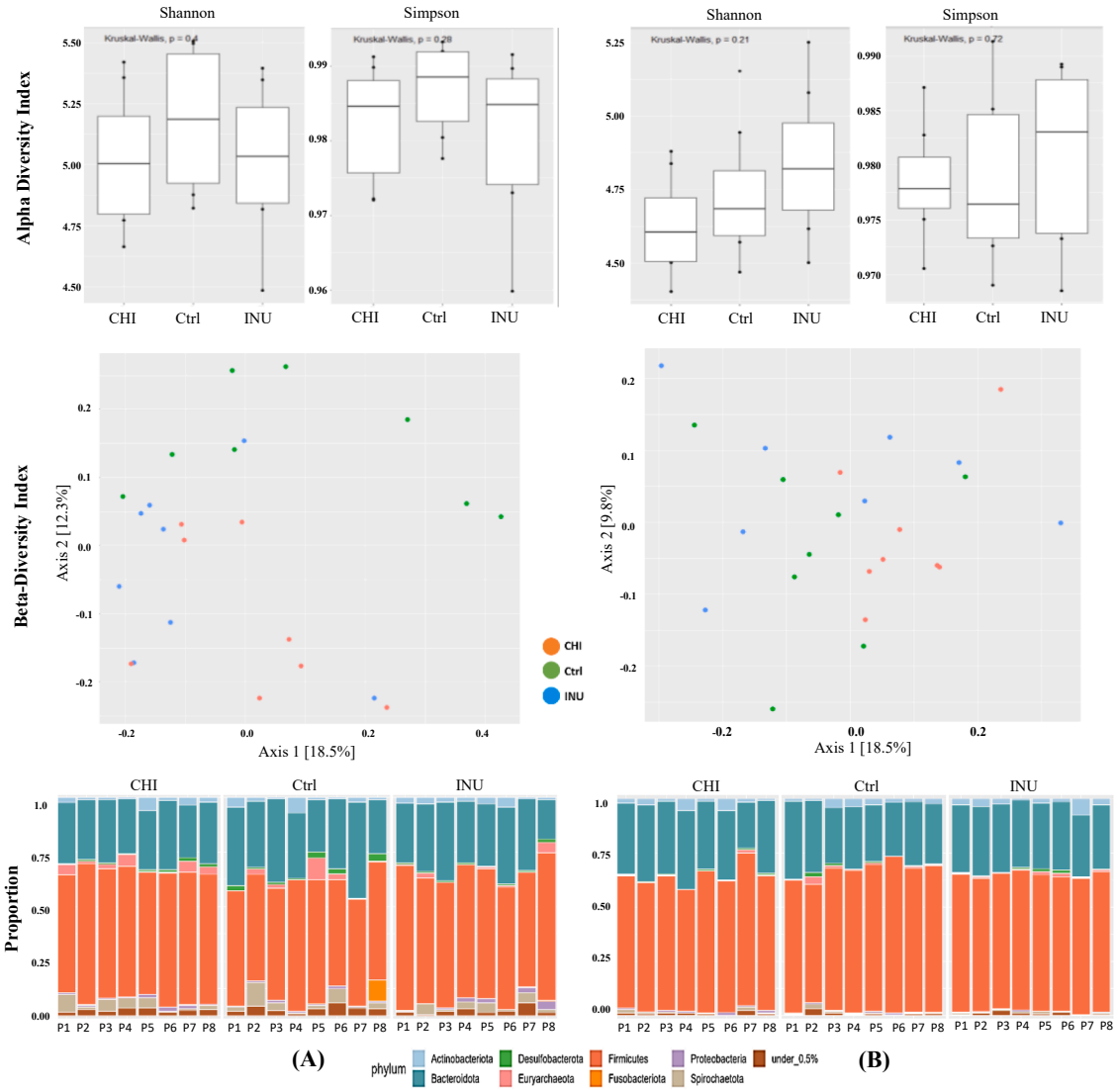


Fig. 4. Effect of INU and CHI in E2 on Shannon and Simpson Index in Alpha (α) diversity & PCoA plot analysis (Bray-Curtis) for Beta (β) diversity and abundance at phylum level composition of microbiota at (A) W1 (B) W3. The β-diversity PERMANOVA p values were 0.002 (A) and 0.535 (B). Values are means ± SEM, n = 8 per treatment except for phylum abundance as values are shown for each piglet per treatment group.

abundances below 1 % (Data not shown) it was found that, *Butyricicoccus* showed significant reduction in relative abundance across the INU fed, and CHI-fed groups (adj.p < 0.05) as compared to Ctrl. *Colidextribacter*, on the other hand exhibited significant increase (adj. p < 0.05) in relative abundance among the INU and CHI groups contrast with the Ctrl group.

3.6. Relative gene expression in ileum and colonic tissues

The results pertaining to Barrier Integrity Genes showed a mixed pattern of results in both, E1 (data not shown) and E2 (Fig. 5). On one hand, in the colon, an upregulation of ZO-1 was observed at W1 of E1 in the CHI-fed piglets (adj.p < 0.05) but on the other hand it was found to be downregulated (adj.p < 0.05) in W3 of E2 in CHI group. In the ileum at W1 in E1, INU and crude CHI-supplemented piglets showed an upregulation of MUC1 (adj.p < 0.05) and Occludin (p $= 0.018$). While in E2, MUC1 (p $= 0.04$) was upregulated in the colon at W3 in CHI group. Additionally, the apoptosis related gene CASP3 was downregulated in INU fed piglets in both E1 (adj.p < 0.01) and E2 (adj. p < 0.05) at W3 in the ileum.

The analysis of gene expression in colon tissue following CHI and INU supplementation demonstrated limited overall impact on inflammatory target and signalling pathway target genes. This indicates that crude CHI or INU at this dosage had a minor effect in reducing inflammation. However, in E2, when the dosage was doubled, an effect was seen on some genes in the ileum as well as the in colon. In the ileum, at W3, three pro-inflammatory genes (CXCL10, IL18, TNFα) and inflammation signalling genes (NFκB1 (adj.p < 0.05) were downregulated in INU and CHI groups. In the case of colonic tissue, a significant (adj. p < 0.05) downregulation of inflammation signalling pathway genes like TANK and TLR2 as well as inflammatory target genes like CXCL10 and INFγ genes were observed at W1. Similarly, at W3, both CHI and INU proved to be beneficial at this higher dosage level in colon tissue, as inflammation signalling (NFκB1 (adj.p < 0.01), TLR2 (adj.p < 0.05) and inflammatory target genes (DEFβ4A (adj.p < 0.05), and INFα (adj. p < 0.05)) were significantly downregulated compared to the Ctrl group.

3.7. Integrative analysis

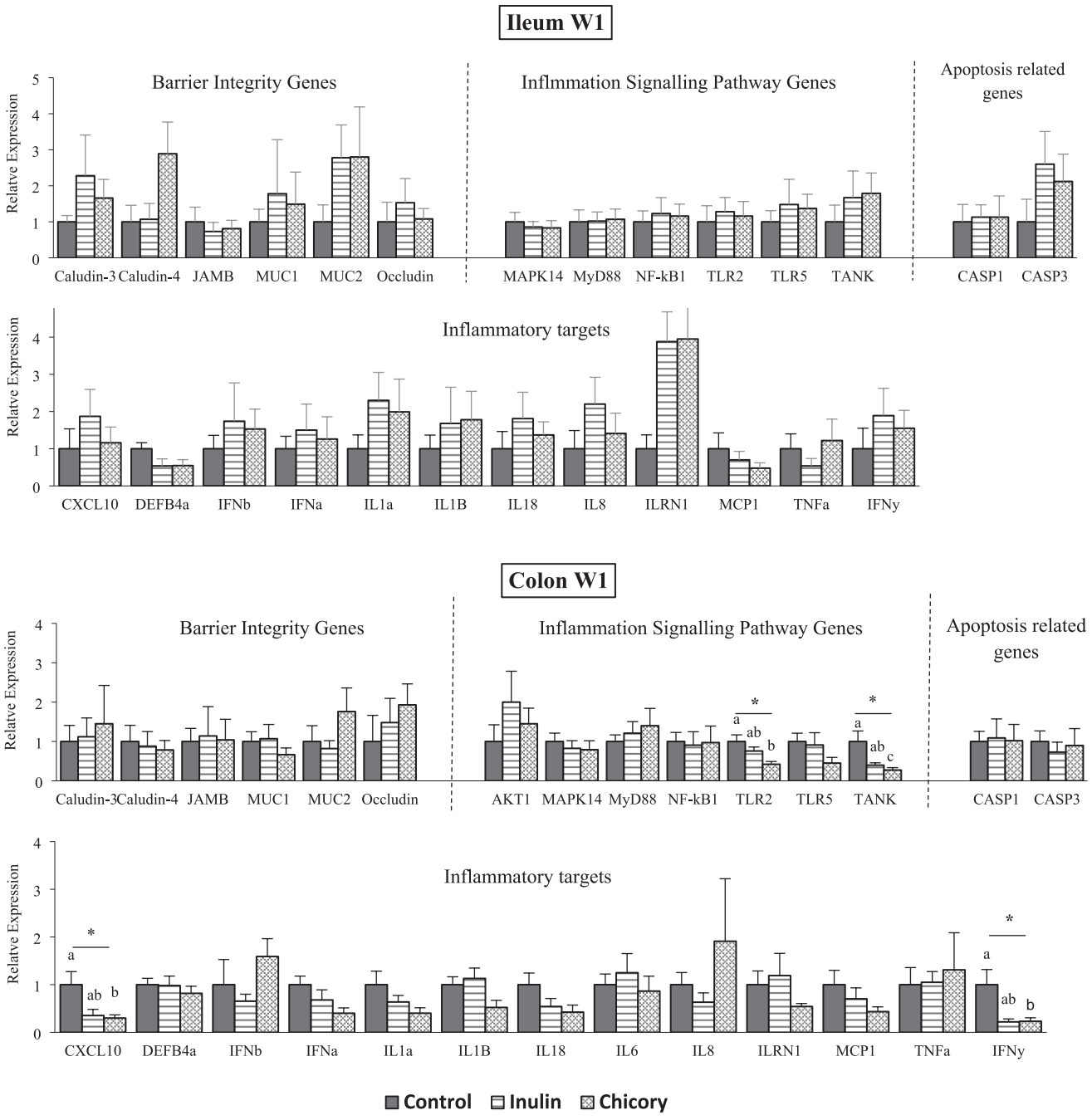
As seen in Fig. 6A of PCA and correlation plots in E2, at both W1, and W3, there was a significant (adj.p < 0.05) positive correlation of ADWG (r $= 0.887, 0.729$) and ADCI (r $= 0.0.837, 0.662$) to dimension 1. The

Table 4
Relative abundance (≥1%) at genera level in E2 at W1 & W3 after supplementation of INU and CHI.

	Genus	Control	Inulin	Chicory	p values
Week 1 (W1)	*Unclassified Lachnospiraceae*	13.05 ± 1.97	13.76 ± 1.21	10.91 ± 1.06	0.3936
	Prevotella	6.21 ± 2.13	12.02 ± 2.06	9.27 ± 1.82	0.0961
	Lactobacillus	4.39 ± 1.74	7.06 ± 2.23	1.75 ± 0.66	0.0961
	Catenibacterium	0.67 ± 0.18^{c}	4.53 ± 2.1^{b}	7.86 ± 1.63^{a}	0.0015
	Unclassified Prevotellaceae	7.89 ± 1.74	4.32 ± 0.66	4.6 ± 0.52	0.1624
	Megasphaera	1.13 ± 0.56	4.23 ± 0.9	2.81 ± 0.9	0.0510
	Unclassified Bacteroidales	2.91 ± 0.69	3.41 ± 0.55	2.94 ± 0.63	0.6521
	Muribaculaceae	2.83 ± 0.52	3.31 ± 0.66	2.24 ± 0.48	0.4317
	Treponema	3.02 ± 1.2	2.42 ± 0.68	2.92 ± 0.91	0.9704
	Christensenellaceae_R-7_group	2.99 ± 0.88	2.01 ± 0.31	4.54 ± 1.39	0.2491
	Blautia	1.44 ± 0.27	1.95 ± 0.3	1.52 ± 0.29	0.3439
	Alloprevotella	3.53 ± 0.83	1.87 ± 0.78	1.44 ± 0.26	0.1145
	Rikenellaceae_RC9_gut_group	2.58 ± 0.53	1.86 ± 0.2	2.9 ± 0.65	0.3936
	Unclassified Oscillospiraceae	2.71 ± 0.91	1.55 ± 0.24	1.78 ± 0.29	0.8981
	Faecalibacterium	0.68 ± 0.27	1.5 ± 0.35	2.16 ± 1.02	0.3387
	Bifidobacterium	1.77 ± 0.82	1.48 ± 0.45	1.12 ± 0.66	0.3538
	Phascolarctobacterium	1.27 ± 0.12	1.4 ± 0.2	1.63 ± 0.23	0.3320
	Ruminococcus	2.09 ± 0.29	1.37 ± 0.21	1.32 ± 0.2	0.1297
	Subdoligranulum	1.36 ± 0.47	1.22 ± 0.25	1.11 ± 0.27	0.9116
	[Eubacterium]_coprostanoligenes	1.73 ± 0.29	1.19 ± 0.11	1.57 ± 0.28	0.3679
	Methanobrevibacter	2.11 ± 1.15	1.18 ± 0.59	2.73 ± 0.74	0.2299
	UCG-005	1.26 ± 0.39	1.14 ± 0.14	0.88 ± 0.08	0.3858
	Mitsuokella	0.27 ± 0.17	0.97 ± 0.5	1.68 ± 0.87	0.0778
	Catenisphaera	0.21 ± 0.09^{c}	0.95 ± 0.26^{b}	1.7 ± 0.35^{a}	0.0012
	Clostridium_sensu_stricto_1	1.83 ± 0.63	0.94 ± 0.56	0.59 ± 0.17	0.2645
	Holdemanella	0.51 ± 0.1	0.91 ± 0.16	1.19 ± 0.31	0.1005
	Unclassified Peptostreptococcaceae	1.89 ± 0.67^{a}	0.78 ± 0.38^{b}	0.46 ± 0.15^{c}	0.0438
	Anaerovibrio	1 ± 0.29	0.57 ± 0.11	0.42 ± 0.16	0.2328
	NK4A214_group	1.04 ± 0.19	0.75 ± 0.08	0.94 ± 0.11	0.4437
Week 3 (W3)	*Prevotella*	20.16 ± 1.53	18.54 ± 2.8	19.13 ± 1.53	0.859
	Unclassified Lachnospiraceae	15.33 ± 0.22^{a}	13.69 ± 0.68^{b}	12.71 ± 0.7^{b}	0.004
	Lactobacillus	11.84 ± 1.2	10.08 ± 2.25	15.08 ± 2.37	0.178
	Megasphaera	4.46 ± 0.28	4.21 ± 0.21	4.87 ± 0.81	0.605
	Unclassified Prevotellaceae	2.98 ± 0.7	4.08 ± 0.59	4.07 ± 0.64	0.134
	Blautia	3.13 ± 0.32	3.5 ± 0.56	3.17 ± 0.3	0.462
	Catenibacterium	1.55 ± 0.29	2.96 ± 0.95	2.18 ± 0.58	0.573
	Muribaculaceae	1.32 ± 0.17	2.33 ± 0.68	2.18 ± 0.56	0.581
	Mitsuokella	1.45 ± 0.28	1.96 ± 0.52	0.86 ± 0.33	0.174
	Streptococcus	4.03 ± 1.8^{a}	1.67 ± 0.55^{b}	0.41 ± 0.11^{c}	0.037
	Dialister	1.96 ± 0.27	1.63 ± 0.29	1.46 ± 0.25	0.310
	uncultured	1.49 ± 0.19	1.57 ± 0.13	1.18 ± 0.14	0.174
	Ruminococcus	0.94 ± 0.08^{b}	1.55 ± 0.29^{a}	1.42 ± 0.25^{a}	0.050
	Unclassified Bacteroidales	1.19 ± 0.24	1.5 ± 0.43	1.56 ± 0.35	0.785
	Faecalibacterium	1.28 ± 0.21	1.41 ± 0.31	1.26 ± 0.24	0.949
	Rikenellaceae_RC9_gut_group	1.04 ± 0.1	1.34 ± 0.3	1.36 ± 0.29	0.761
	Bifidobacterium	1.11 ± 0.37	1.33 ± 0.51	1.45 ± 0.76	0.860
	Alloprevotella	1.09 ± 0.12	1.19 ± 0.34	0.97 ± 0.22	0.739
	Erysipelotrichaceae_UCG-002	1.48 ± 0.91^{a}	1.14 ± 0.77^{a}	0.04 ± 0.02^{b}	0.010
	Unclassified Atopobiaceae	0.72 ± 0.2	1.13 ± 0.41	0.41 ± 0.09	0.247
	Subdoligranulum	1.7 ± 0.35	1.12 ± 0.19	1.45 ± 0.28	0.442
	Christensenellaceae_R-7_group	0.54 ± 0.12	1.11 ± 0.4	1.08 ± 0.59	0.838
	Clostridia_UCG-014	1.15 ± 0.15	0.97 ± 0.16	1.01 ± 0.17	0.613

Values are means ± SEM, n = 8 per treatment group. Means in a column with timepoint without a common superscript letter differ, (Kruskal–Wallis, followed by Dunn's test adj. ($p < 0.05$). The composition expressed in % is only for genera with a relative abundance ≥ 1 % for either of the group.

supervised RGCCA plots for the Gut block (Fig. 7A) show that, at W3, component 1 separates the Ctrl samples from CHI and INU samples. The INU and CHI samples were separated by component 2. Pearson correlation analysis demonstrated significant positive correlation ($r = 0.671$, adj.$p < 0.05$) between ileum villus width and component 1 while a strong significant negative correlation between ileum V:C ratio and component 2 ($r = -0.83$, adj.$p < 0.05$)) as represented in variables plots. Similar observations were made in case of jejunum and duodenum crypt depth in W1 (data not shown here). RGCCA also shows a positive correlation between cecum butyrate ($r = 0.411$, $p > 0.05$) and colon butyrate ($r = 0.817$, adj.$p < 0.05$) in W3 (as well in W1). Along the component 1, Ctrl samples are distinct from INU and CHI and D-Xylose ($r = -0.565$, $p < 0.05$) as well as L:M ratio ($r = -0.558$, $p < 0.05$) were negatively correlated to this component. In the similar supervised RGCCA (W1) for the Microbiota block (figure not shown), variable plot shows a significant negative correlation between component 1 and some microbes like *Megasphaera* ($r = -0.755$, adj.$p < 0.05$) and *Prevotella* ($r = -0.737$, adj.$p < 0.05$) thus separating the clusters of Ctrl from CHI and INU. CHI and INU samples were separated on the component 2 as expected across the different blocks. While in W3 (Fig. 7C), *Ruminococcus* ($r = 0.794$) tend to have significant positive correlation while *Butyricoccus* ($r = -0.589$), *Lachanospiraceae* ($r = -0.628$), *Slackia* ($r = -0.557$), *Prevotella* ($r = -0.509$) and *Streptococcus* ($r = -0.473$) significant negative correlation with component 1 ($p < 0.05$). A significant positive correlation by supervised RGCCA ($r = 0.817$) of butyrate with component 1 was found (Fig. 7A). Also, negative correlation with component 1 of several colonic (NFκB1 ($r = -0.837$), CASP-1 ($r = -0.744$), MAPK14 ($r = -0.696$) as well as Ileum (Il18 ($r = -0.835$), CASP3 ($r = -0.79$), CXCL10 ($r = -0.782$), TANK ($r = -0.758$), MyD88 ($r = -0.696$) genes (Fig. 7B).



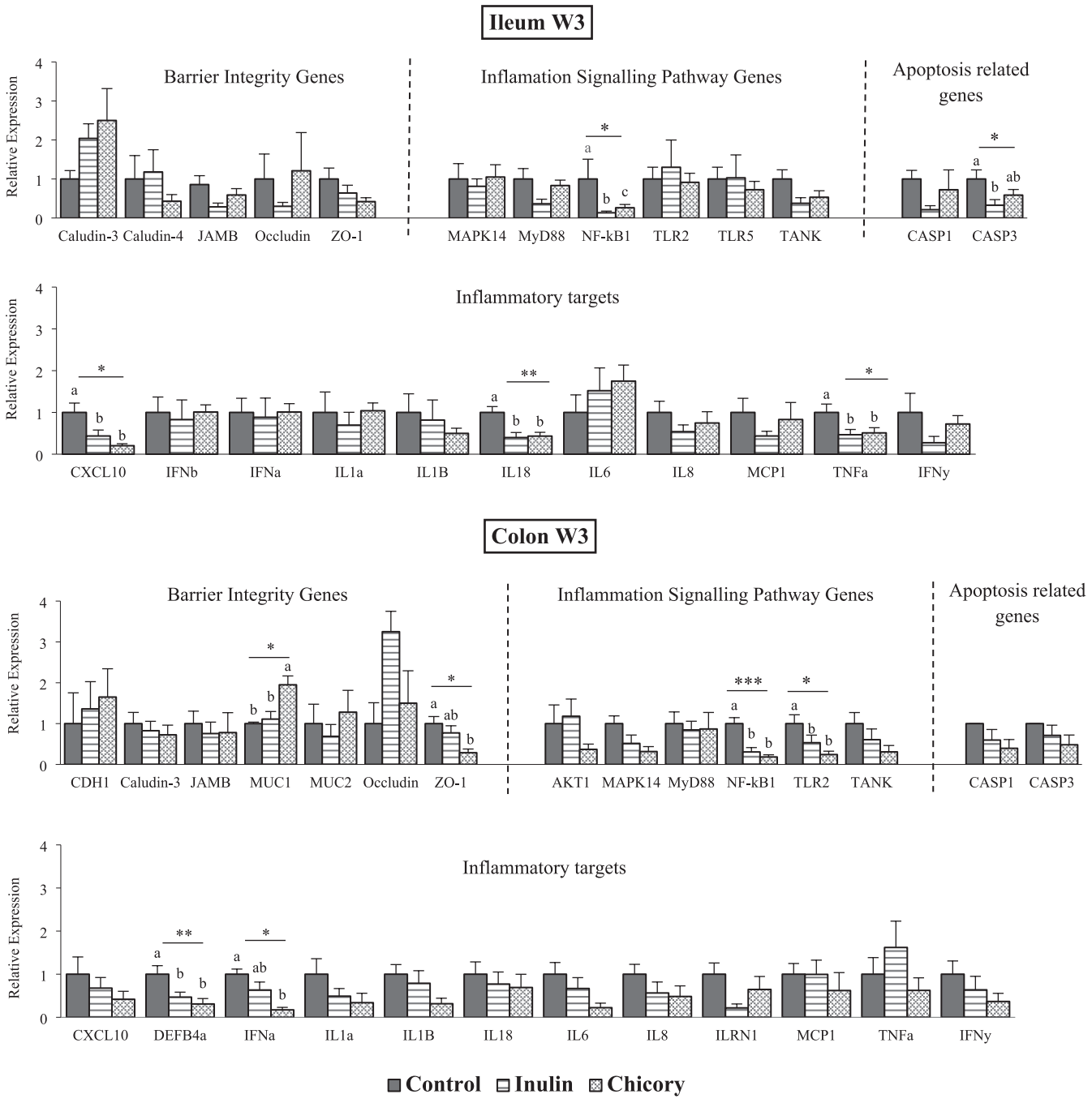
(A)

Fig. 5. Effect of INU and CHI in E2 on expression of barrier integrity, inflammation signalling pathway and apoptosis related genes in Ileum and Colon at (A) W1 and (B) W3. Values are means (n = 8 piglets) ± SEM. Statistical analysis was performed using one way ANOVA and Tukey's test. Bars with different letters are significantly different (*p < 0.05, **p < 0.01, ***p < 0.001).

4. Discussion

The gastrointestinal tract's intricate layers, encompassing the physical, biochemical, and immunological realms, serve as barriers against the infiltration of harmful microorganisms, toxins, and antigens (Barreau & Hugot, 2014; Mu et al., 2017). To address the variability in inulin dosages documented in prior research on weaned piglets, we conducted two distinct animal experiments, with force-feeding, which unlike most previous studies, sought to monitor the exact amount of supplementation (Apper et al., 2016; Ayuso et al., 2020; Xu et al., 2005; Sabater-Molina et al., 2011; Trevisi et al., 2008; Y. Wu et al., 2020).

The chemical analysis of crude chicory flour (see Table 1) reveals high levels of Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) compared to pure inulin. This composition (high cellulose and hemicellulose content) likely contributes to slower digestion rates and reduced palatability compared to pure inulin (Weimer, 1996), which may explain the lower Average Daily Calorie Intake (ADCI) (kcal/day) in the CHI group during W3 of E1 and throughout E2. Furthermore, the



(B)

Fig. 5. (*continued*).

reduced ADCI might be influenced by intestinal hormones such as cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1), pivotal in the gut-brain axis, regulating appetite and food intake (Caron et al., 2017; Morton et al., 2006; Wren & Bloom, 2007). Notably, (Fouré et al., 2018) demonstrated increased secretion of CCK and GLP-1 following crude chicory supplementation, and this was elevated even more with sesquiterpene lactones. Additionally, the presence of short-chain fatty acids (SCFAs) in the colonic lumen might augment GLP-1 levels and diminish ghrelin levels (Cho, 2009; Tarini & Wolever, 2010). Notably, lack of impact on weight gain or average calorie intake, consistent with various studies (Barszcz et al., 2016, 2018, 2020; Li et al., 2018; H. Liu et al., 2012; Uerlings et al., 2021; Wang et al., 2020).

Stress-induced diarrhoea poses a significant threat to the gut health of weaned piglets, rendering them more susceptible to intestinal inflammation and post-weaning diarrhoea due to the rapid proliferation of pathogenic bacteria and a decline in microbial diversity (de Lange et al., 2010; Pluske, 2016; Qiu et al., 2021; Winter et al., 2013). Lower incidences of mild diarrhoea in INU and CHI in E2, exhibited prebiotic effects. It is well-established that oligosaccharides possess anti-inflammatory properties and anti-adhesive activity, inhibiting pathogen binding (Quintero et al., 2011; Zenhom et al., 2011). Remarkably, at W3 in E2, supplementation of chicory flour led to fewer instances of severe diarrhoea (S3), surpassing the effect of inulin.

Doubling the dose in E2 led to some enhancements in the jejunum

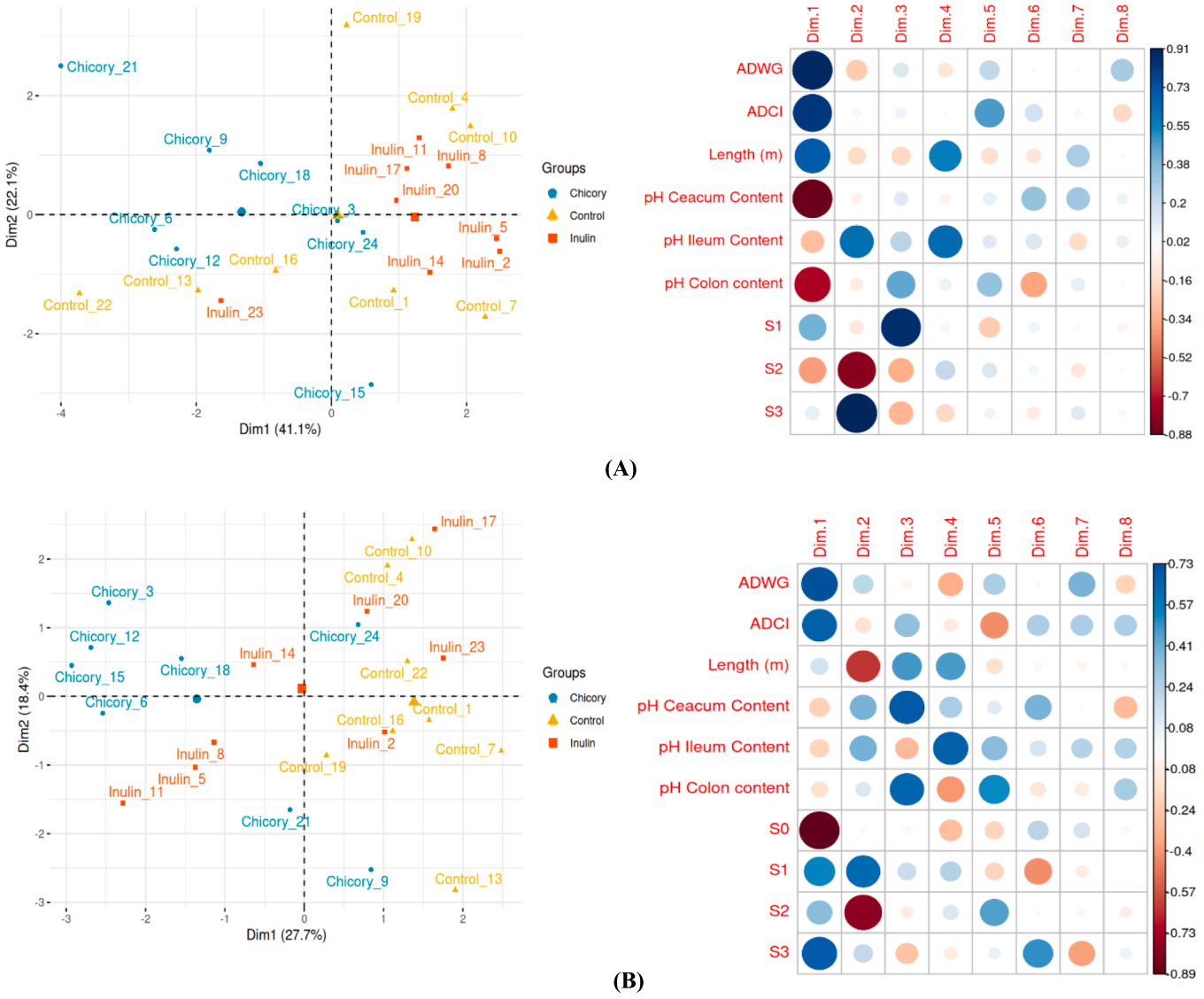


Fig. 6. Principal Component Analysis (PCA) plots (Dim 1 & Dim 2) and correlation plots for (A) W1 and (B) W3 of E2 considering only the variables of the 'Clinic' block. Samples in the PCA plot are coloured by group. Colour and size of circles in the correlation plot represent Pearson correlation level (from negative correlations in red to positive correlations in blue and larger circle for higher absolute correlation value) of each 'Clinic' variable with each of the first 8 components of the PCA (range –0.89 to 0.91). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and ileum of CHI-fed piglets as CHI exhibited greater average villus height than INU in the ileum. This finding aligns with (H. Liu et al., 2012; Uerlings et al., 2021), who also reported increased villus height in piglets fed with a crude chicory root diet compared to controls. Similarly, Wan et al., 2020 associated these improvements in with elevated serum IGF-1 concentration, considering its critical role in organ development and growth (Walton et al., 1995). This histomorphological improvements could also potentially be associated with the higher butyrate levels observed in both the INU and CHI groups in our study (E2). Bawish et al., 2023 similarly observed a significant rise in IGF-1 levels after supplementing with sodium butyrate for 3 to 5 weeks. The supervised RGCCA (Fig. 7A) confirmed a significant positive correlation of ileum villus width ($r = 0.761$) with colon butyrate ($r = 0.817$) produced at W3 thus indicating an increasing absorptive surface area during the recovery period of weaning stress.

Recognizing the potential of prebiotics like inulin to preserve intestinal mucosal barrier function (Uerlings et al., 2020; Wan et al., 2020), our study sought to determine if crude CHI could offer comparable or superior effects. Given that a single blood measurement may be influenced by premucosal and postmucosal factors, we opted to employ a '3 sugar cocktail' (S. X. Hu et al., 2023; Ortega & Szabó, 2021) to study the barrier integrity. While weaning compromises paracellular barrier function (Kim et al., 2019), CHI showed a trend, although statistically insignificant, towards reducing the L:M ratio (adj.$p = 0.057$), suggesting reduced permeability. Interestingly, D-Xylose exhibited a reduced permeability in the INU group compared to CHI, aligning with those of Uerlings et al. (2021). The supervised RGCCA (Fig. 7A) shows that butyrate may contribute to maintaining gut barrier function and reducing intestinal permeability as D-xylose ($r = -0.565$) and L:M ($r = -0.558$) were negatively correlated with colon butyrate ($r = 0.871$) and villus width ($r = 0.671$) in W3.

The decreased permeability in INU and CHI groups may underlie the lower diarrhoea incidence in these groups because a good mucosal barrier prevents harmful substance translocation and maintains fluid balance (Arrieta, 2006; Groschwitz & Hogan, 2009; Wijtten et al., 2011). While our study did not find consistent differences in tight junction gene expression, the downregulation of leucocyte elastase inhibitor (SERPINB1) observed in crude CHI-fed pigs suggests a potential mechanism for intestinal barrier modulation (Herosimczyk et al., 2018; Uchiyama et al., 2012). To obtain a clearer understanding of the effect of chicory

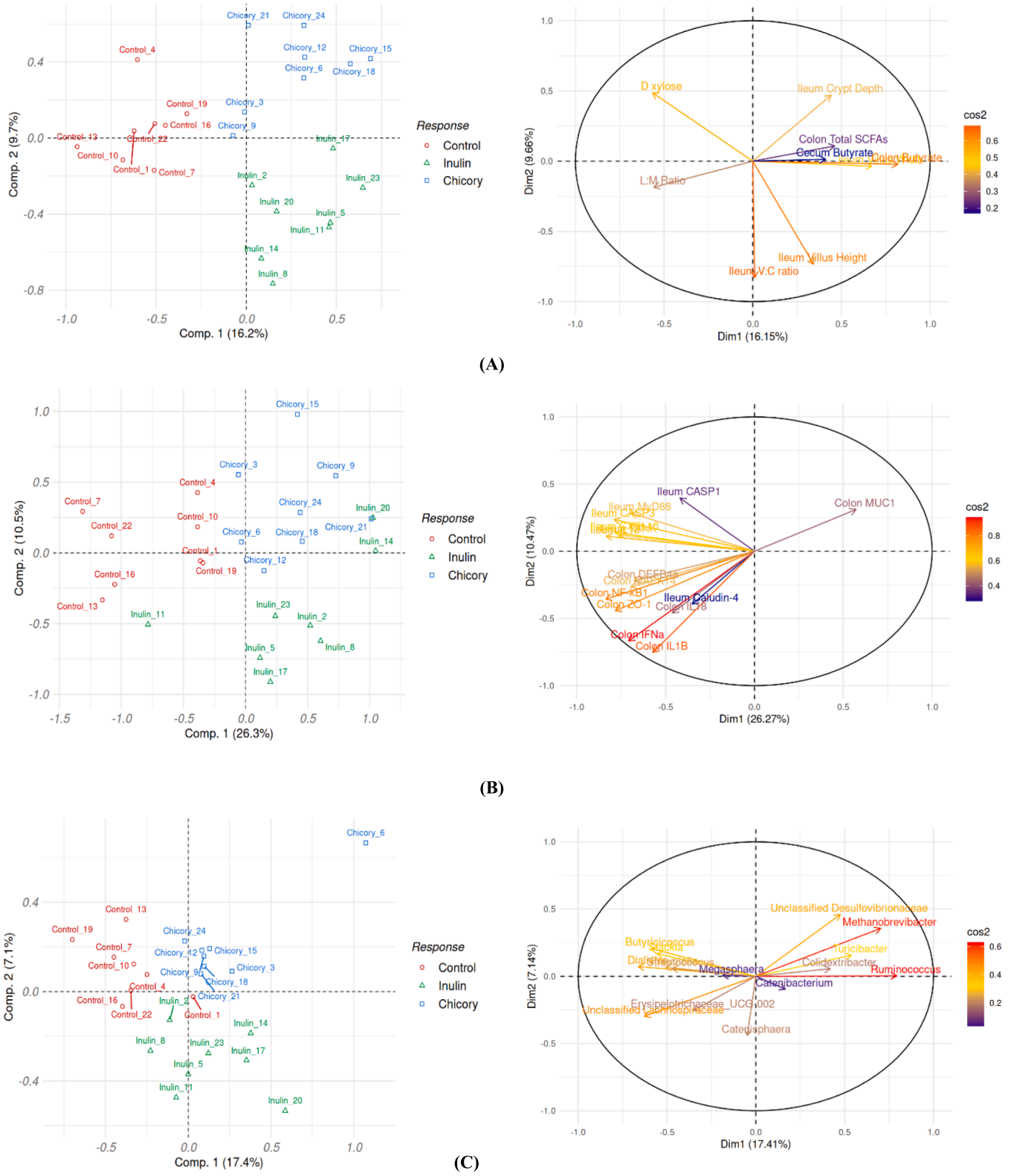


Fig. 7. Individuals (left) and variables (right) plots from supervised RGCCA displayed for components 1 and 2 considering the variables from (A) Gut, (B) Gene Expression and (C) Microbiota blocks at W3 in E2. Samples in the individual plots are coloured by group similarly as PCA. Arrows colour and length in the variable plot are based on square cosine (cos2) indicating the quality of the representation of the variables by the two first components of RGCCA for a given block (higher cos2 value, represented by long red arrow, means a better representation of the variable by the two first components). Direction of arrows indicates which components represent the corresponding variables, horizontal for component 1, vertical for component 2 and diagonal for both components. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

on barrier junction network it would be recommended to determine changes at the protein level.

Despite the absence of a significant impact on the total SCFA production in E2, both chicory and inulin supplementation led to changes in SCFA composition. Although literature reports disparities in SCFA levels post-INU supplementation (Eberhard et al., 2007; Halas et al., 2009), our findings of E2 are consistent with previous studies by H. Liu et al., 2012; Loh et al., 2006; Mair et al., 2010; Mikkelsen & Jensen, 2004. The reduced acetic acid concentration in colonic content may result from butyrate-producing bacteria utilizing acetate, with some acting as net consumers (Barcenilla et al., 2000), highlighting acetic acid's role in cross-feeding interactions among colonic bacteria (De Vuyst et al., 2014; De Vuyst & Leroy, 2011; Moens et al., 2017). This effect of CHI and INU could also be supported by supervised RGCCA as it shows a significant negative correlation of butyrate (r = 0.817) with acetate (r = −0.796) in colon at W3.

The increase in butyrate production, consistent with few other findings from previous inulin supplementation studies (Le Bastard et al., 2020; Uerlings et al., 2019; Wang et al., 2020), underscores its beneficial impact on gut health. Butyrate is known for its ability to improve intestinal barrier function and possesses anti-inflammatory, anti-cancer, and antioxidant properties (Corrêa-Oliveira et al., 2016; Cui et al., 2019; Hamer et al., 2008; Hodgkinson et al., 2023; Le Bastard et al., 2020; Millard et al., 2002; Rivière et al., 2016) Additionally, the reduction in mild and severe diarrhoea incidences during W1 and W3 respectively, of E2 following crude chicory and inulin supplementation may also be linked to increased butyrate production in the colon. Xiong et al., (2019) demonstrated that dietary supplementation of 0.1 % sodium butyrate reduced diarrhoea and improved gut integrity in weaned pigs. Butyrate production is not solely dependent on *Lactobacillus* and *Bifidobacterium*, but also involves numerous other commensals of the microbiota that contribute to similar effects (Bian et al., 2016; Levine et al., 2013). Our study revealed an increase in the abundance of several genera known for butyrate production, suggesting their contribution to butyrate synthesis. Therefore, the observed outcomes at the end of W3 in the CHI and INU groups, such as improved permeability, reduced expression of pro-inflammatory genes, decreased diarrhoea, and enhanced histomorphology, may be attributed to increased butyrate production.

Early-life microbial colonisation has a substantial impact on the development of the intestinal and immune systems, with long-term benefits for immunological function (Laforest-Lapointe & Arrieta, 2017). The increased abundance of Firmicutes and Bacteroidetes, the predominant bacterial phyla, following supplementation with inulin and chicory in both experiments, corresponds with findings by Juhász et al., 2022. The higher total SCFA concentration observed in the CHI and INU groups in W1 of E2 may be attributed to many bacteria within these phyla, with hydrolytic enzymes capable of degrading non-starch carbohydrates (Dodd et al., 2011; He et al., 2021). Our study also revealed disparate microbial responses between experiments despite similar nutrient interventions but at different dosages, underscoring the challenges in replicating experiments related to microbiota composition.

The 2–3 times higher abundance of *Prevotella* after W1 in E1 (Supplementary Table 3), along with *Megaspheara*, *Dialister*, and *Succinovibrio*, is associated with improved intestinal health and has been previously observed in pigs fed with inulin (Dou et al., 2017; Nielsen et al., 2016; Van den Abbeele et al., 2018; W. Wu et al., 2020). The prevalence of the harmful microbe, *Turicibacter,* linked to steroid and lipid metabolism, was significantly reduced in both the INU and CHI groups. This bacterium is negatively correlated with protein and energy digestibility in low-fiber fed growing-finishing pigs (Le Sciellour et al., 2018).

Interestingly, in E2, likely attributable to the higher supplementation dose, a significant increase in beneficial microbiota and a reduction in harmful one was observed in the CHI group compared to both the INU groups. Major abundant (>1%) genus affected by chicory or inulin were *Catenibacterium*, *Megasphaera*, *Catenispaera*, *Peptostreptococcaecea*, *Lachanospiraceae*, *Streptococcus*, *Ruminococus*, *Erysipelotrichaceae*. Minor (<1%) abundance genera were also found to be altered like *unclassified Desulfovibrionaceae*, *Butyricoccus*, *Collinsela* and *Colidextribacter*. *Megasphaera* (r = −0.755) and *Prevotella* (r = −0.737) were negatively correlated with *Colidextribacter* (r = 0.733) and *Cornybacterium* (r = 0.695). *Catenibacterium* was the fourth most abundant genus, with significantly greater levels in both CHI and INU groups during W1 of E2. While inulin-type fructans have been shown to increase *Catenibacterium* levels (Neyrinck et al., 2021; Rodriguez et al., 2022; Sarmiento-Andrade et al., 2022), it was surprising to find a seven-fold increase in CHI compared to the Ctrl, whereas the increase with INU was only 1.5-fold, indicating additional positive effects from other bioactives in CHI. According to Ju{\acute{s}}kiewicz et al., 2011, crude chicory roots contain phenolic chemicals such as Monocaffeoyllquinic acids (MCQA), dicaffeoylquinic acids (DCQA), and chicoric acid. Silva et al., 2022 experienced a comparable rise (13.4-fold) with polyphenol-enriched supplements, as did Peron et al., 2022.

Although bacteria of the genus *Megasphaera* are nonfructan-degrader, increase in its abundance after W1 (E2) of supplementation was observed in the colon of piglets fed with chicory and inulin (adj. p = 0.051) which aligns with other studies (Barszcz et al., 2016; Halas et al., 2009). *Megasphaera* can produce SCFAs, vitamins and essential amino acids (Shetty et al., 2013). Similarly, *Catenisphaera* showed significant enrichment in CHI (1.7 % ± 0.35) compared to INU (0.95 % ± 0.26), while the Ctrl group had very low abundance (0.21 % ± 0.09). Furthermore, the supervised RGCCA (Fig. 7C) shows the negative correlation of *Catenisphaera* (r = −0.713) with *Ruminicoccus*. Yang et al., 2022 also observed increased *Catenisphaera* abundance in pigs fed a fiber-rich diet (inulin and cellulose). This genus has been associated with mitigating inflammation, *E. coli* infection, weaning diarrhoea, and various diseases in pigs across multiple studies (Hankel et al., 2022; Hou et al., 2020; Smith et al., 2020; Su et al., 2021).

The reduction in the relative abundance of *unclassified Peptostreptococcaecea* upon prebiotics supplementation has also been documented by Wu et al., 2020 and H. Wu et al., 2023. While some members of this family are considered opportunistic pathogens, others are commensal bacteria vital for gut health. Thus, drawing definitive conclusions regarding the role of *unclassified Peptostreptococcaceae* in crude CHI or INU is challenging. Sarmiento-Andrade et al., 2022 noted inulin concentration positively correlating with butyrate and negatively correlating with *Desulfovibrionaceae* abundance, consistent with our findings of increased butyrate and a four-fold reduction in *Desulfovibrionaceae* genus abundance in the INU and CHI groups. RGCCA shows that *Desulfovibrionaceae* (r = 0.816) was negatively correlated to *Prevotella* (r = −0.735). The genus *Collinsella*, although relatively low in abundance (<1%), exhibited a threefold increase in prevalence among piglets in the CHI group. This finding is consistent with the study by Carlson et al., 2017, which reported heightened levels of *Collinsella* following both INU and WholeFiber supplementation. Notably, clinical investigations have highlighted the capacity of INU-type fructans to significantly foster the growth of *Collinsella*, correlating with elevated urinary hippurate levels (Dewulf et al., 2013) which is associated with reduced inflammation (Calvani et al., 2010; Kassinen et al., 2007; Salek et al., 2007; Waldram et al., 2009). The rise in *Ruminococcaceae* genera in CHI is beneficial due to their capacity to hydrolyse starch and other sugars, generating butyrate and other SCFAs (Biddle et al., 2013; Wong et al., 2014).

Mizutani et al., 2021 identified increased *Erysipelotrichaceae* prevalence alongside significant decreases in *Ruminococcaceae* and *Dialister* in patients with diarrhoea. However, our findings indicate that crude chicory treatment resulted in a substantial decrease in *Erysipelotrichaceae* abundance, coupled with increased *Ruminococcaceae* and *Dialister* than INU, suggesting additional bioactive effects of chicory. Furthermore, Kaakoush, 2015 showed a statistical decrease in one *Erysipelotrichaceae* species within the inulin group, correlating with reduced calprotectin levels and diminished gastrointestinal tract inflammation in

patients with colorectal cancer or inflammatory bowel disease. Alterations in the gastrointestinal microbiota can influence immune cell markers, with an uptick in beneficial bacteria competing for binding sites, consequently diminishing the attachment of pathogenic bacteria (Pahumunto et al., 2023).

Streptococcus ($r = -0.473$) was found to be negatively correlated to *Ruminococcaceae* ($r = 0.794$) (Without Boneferroni correction) suggesting a competitive interaction between these genera, resulting in reduction of *Streptococcus*. *Streptococcus* strains encompass both commensal and pathogenic species implicated in inflammatory diseases, particularly in the gastrointestinal tract mucosa (Dinis et al., 2014; Herrera et al., 2009; Rooks et al., 2014). Our findings suggest that the reduction in *Streptococcus* abundance in CHI may be associated with downregulation of pro-inflammatory genes (CXCL10, IL-18, TNFα), as evidenced by lower expression levels in the CHI group compared to INU, potentially mediated through Toll-Like Receptor 2 (TLR2) modulation (Frolova et al., 2008). This added effect of CHI supplementation could be attributed to bioactives like caffeic acid, known for its antimicrobial and anti-inflammatory effects in macrophages by inhibiting nitrite, tumour necrosis factor alpha (TNFα) and prostaglandin E2 (PGE2) production by the NF-kB dependent pathway. (Sorgi et al., 2021).

Weaning induces inflammatory responses in the gut, characterized by increased production of pro-inflammatory cytokines such as TNFα, IL6, and INFγ, as well as the upregulation of pathways like MAPK and Ras signalling (Bomba et al., 2014; C. H. Hu et al., 2013). The intestinal mucosa, harbouring the largest reservoir of macrophages in the body, plays an essential role in managing gut inflammation and maintaining immunological balance (Elshahed et al., 2021). In E1, inulin and chicory supplementation yielded fewer changes in inflammatory and inflammation signalling pathway genes. The lack of additional gene expression effects may be due to lower microbial load or the low doses of inulin and chicory. Herosimczyk et al., 2020 noted an upregulation of intestinal proliferation genes only at 3 % INU, while Mair et al., 2010 found minimal impact with 0.4 % supplementation.

In E2 (W1), the downregulation of the proinflammatory genes (CXCL10 and INFγ) and inflammation signalling pathway genes (TLR2 and TANK) in the colon of INU and CHI groups may be attributed to higher butyrate, which has been shown to significantly inhibit the production of proinflammatory cytokines like INFγ and IL-2 (Cavaglieri et al., 2009). In W3, the RGCCA shows that many genes in the colon (NFκB1, CASP-1, MAPK14) as well as in the ileum (Il-18, CASP-3, CXCL10, TANK, MyD88) were found to be altered in CHI or INU groups. Furthermore, distinct gene expression patterns and differences between these two groups of samples were observed. These observations may reflect underlying biological differences, responses, or effects associated with the inulin or chicory treatment or conditions compared to the Ctrl group. TNFα, a transmembrane protein pivotal in systemic inflammation, can induce apoptosis by activating caspases, thereby enhancing apoptotic activity in the colon mucosa (EL Andaloussi et al., 2013). Our findings align with studies by (Uerlings et al., 2019), and Wang et al., 2020, showing significant downregulation of both TNFα and CASP3 (caspase-3) in the ileum tissues of piglets of INU and CHI group at W3. This suggests a positive correlation between TNFα and CASP3 in ileal mucosa, potentially indicating a beneficial effect of crude chicory in suppressing mucosal inflammation. Additionally, chicoric acid, a bioactive found in chicory, has been shown to significantly decrease caspase3 and TNFα levels Alharthi, 2023. Increased butyrate production in W3 could activate the NLRP3 inflammasome via GPR43 & GPR109A or intrinsic inhibition of histone deacetylases (HDACs), thus reducing TNFα expression (MacDonald & Howe, 2009; Macia et al., 2015; Zimmerman et al., 2012). The higher permeability observed in the Ctrl group may be related to elevated TNFα levels, as TNF-α can increase intestinal permeability by up-regulating myosin light chain kinase (MLCK) protein expression, leading to tight junction disintegration and intestinal barrier dysfunction (Al-Sadi et al., 2016; Berkes, 2003; Kucharzik et al., 2006). Therefore, the reduced expression of TNFα by CHI could decrease the influx of toxins and pathogens into the intestine.

Like our findings in the INU and crude CHI groups, Vogt et al., 2015 also observed an inulin mediated effect involving the suppression of NF-κB1 and the JNK pathway. Butyrate has been shown in other studies to inhibit NF-κB1 activation and upregulate other cytoplasmic inhibitors of NF-κB1, thereby reducing inflammation (Segain, 2000; Tedelind et al., 2007). Chicoric acid has been demonstrated the ability to ameliorate lipopolysaccharide (LPS)-induced inflammation both *in vitro* and *in vivo* by downregulating NFκB and TNF-α genes (Kassab et al., 2022; Q. Liu et al., 2017; Zheng et al., 2021). However, *Echinacea* extracts containing chicoric acid were reported to upregulate LPS-induced TNFα in rat alveolar macrophages (Goel et al., 2002). Chicoric acid has been shown to downregulate several proinflammatory factors, including NO synthase, COX-2, prostaglandin E2, IL1β, IL12, and IL18 (Kour et al., 2016; Matthias et al., 2007). Other colonic genes downregulated by CHI and INU treatment included DEFβ4a, IFNα, MAPK14, and TLR2. MAPKs have been implicated in the regulation of pro-inflammatory cytokine release, including TNFα, IL1β, and IL6 (Jia et al., 2020; Qian et al., 2015). Chicoric acid supplementation decreased the expression of nitric oxide synthase and MAPK14 at the molecular level (Alharthi, 2023). Pouille et al., 2022 proved that fructose, chlorogenic acids and sesquiterpene lactones contained in chicory flour have an anti-inflammatory effect on TNF-α, IL-1β and IL-8. The results presented above indicate that crude CHI significantly contributed to reducing inflammation, equally or sometimes effectively than INU.

5. Conclusion

This study represents the first comparative analysis conducted in piglets to underscore the dual beneficial effects of bioactives and inulin from crude chicory for enhancing gut health. Notably, our experiments, meticulously designed to monitor the dosages through force-feeding, provided insights into the nuanced effects of chicory compared to previous studies. The comprehensive analysis of our research data illuminates that chicory either exhibited similar or better effects than inulin for most of parameters tested. Chicory like inulin, promoted beneficial changes in gut microbiota composition, and stimulating butyrate production, a critical factor in maintaining gut homeostasis. Notably, chicory supplementation significantly reduced the occurrence of severe diarrhoea. Histomorphological analyses revealed improvements in villus height and villus: crypt ratio, particularly evident with higher doses (1.7–2 %) of chicory, signifying enhanced intestinal architecture. Moreover, the downregulation of pro-inflammatory genes and modulation of inflammatory pathways in the colon and ileum further support the anti-inflammatory properties of both chicory and inulin, with chicory showing particularly promising results in attenuating inflammatory responses. Although inulin has been extensively known for its prebiotic properties, our findings suggest that crude chicory, with its diverse nutritional profile and plethora of bioactive compounds (chicoric acid and polyphenols), can be a more cost-effective superior intervention. Further research is warranted to unravel the specific mechanisms underlying the observed effects and optimize dietary formulations for possible maximal efficacy in animal and human application.

Ethical Statement

The authors would like to confirm that all animal experiments conducted for this study were performed in full compliance with European (EU Directive 2010/63/EU) and Belgian regulations regarding the use and care of animals for scientific purposes. The experimental procedures were approved by the Ethical Committee of the University of Liège (License No. 21-2385).

The study involved two in vivo experiments (E1 and E2), each with 80 castrated male piglets (Pietrain X Landrace), weaned at day 21 (D21), with an average weight of 5.48 ± 0.5 kg in E1 and 5.36 ± 0.2 kg in E2.

The animals were sourced from the Walloon Agricultural Centre in Gembloux, Belgium. Throughout the experiments, all efforts were made to ensure the animals' welfare and minimize any potential distress.

CRediT authorship contribution statement

Tushar Kulkarni: Investigation, Formal analysis, Data curation, Writing – review & editing. **Pawel Siegien:** Writing – review & editing, Investigation. **Luke Comer:** Formal analysis, Data curation. **Jimmy Vandel:** Visualization, Formal analysis, Data curation. **Gabrielle Chataigne:** Investigation. **Aurore Richel:** Investigation. **José Wavreille:** Resources. **Benoit Cudennec:** Writing – review & editing, Supervision, Methodology. **Anca Lucau:** Resources. **Nadia Everaert:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Rozenn Ravallec:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Martine Schroyen:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2024.106578.

Data availability

Data will be made available on request.

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