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Yacon (*Smallanthus sonchifolius*) flour soluble extract improve intestinal bacterial populations, brush border membrane functionality and morphology *in vivo* (*Gallus gallus*)

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ABSTRACT

This study evaluates the effects of intra-amniotic administration of yacon (Smallanthus sonchifolius) flour soluble extracts (YFSE) on intestinal bacterial populations, brush border membrane (BBM) functionality and morphology, by using the *Gallus gallus* model. The YFSE increased ($p < 0.05$) relative abundance of Lactobacillus, Bifidobacterium, Clostridium and *E. coli* compared to 18MΩ H2O. The YFSE had systematic effect on BBM functionality, via the upregulation of zinc (zinc transporters – ZnT1, ZnT7 and ZIP9) and iron (ferroportin, Duodenal cytochrome (DcytB) transporters, sucrose isomaltase (SI), and down regulation of Interleukin 1 beta (IL1β), and hepcidin genes expression when compared to the inulin administered group. The YFSE administration increased glycogen concentrations in pectoral muscle compared to noninjected and 18 Ω H2O groups, however, did not change gene expression of enzymes related to glycolysis (phosphofructokinase) and gluconeogenesis (glucose-6 phosphatase). The YFSE increased the depth of crypts, crypt goblet cell diameter, number and type (acidic), and villi goblet cell diameter and type (acidic) when compared to all other groups. Thus, YFSE demonstrated prebiotic effects resulting in improving intestinal bacterial populations profile, BBM functionality, digestive and absorptive capabilities, intestinal morphology, glycogen status and immune system.

1. Introduction

Yacon (*Smallanthus sonchifolius*) is a herbaceou perennial plant, part of the Asteraceae family, a native to the Andean regions of South America. Yacon yields starchy, fruit-like roots of different shapes and sizes that are usually consumed raw and taste sweet, which resembles a pear or an apple (Caetano et al., 2016). While other roots store energy in the form of starch, yacon mainly stores energy in the form of inulin-type fructans (Ojansivu, Ferreira, & Salminen, 2011). Yacon root contains more than 70% water and the major portion of the dry matter consists on fructooligosacharides (FOS), and small amounts of proteins, vitamins, minerals and phenolic compounds (Caetano et al., 2016). The FOS concentration may range between 0.7% and 13.2% in yacon (fresh weight), and 6.4% and 70% in dry matter, depending on factors such as farming, location, harvest time and post-harvest temperature (Delgado, Teresa, da Silva Cunha Tamashiro, Maróstica Junior, & Pastore, 2013). Dehydrated products, such as yacon flour, guarantees the existence of a natural product with high FOS concentration and stability (Machado, da Silva, Chaves, & Alfenas, 2019).

Fructooligosacharides (FOS) are small soluble dietary fibers fructans, consisting of linear short chains of fructose molecules, non-enzymatic digestible in the upper digestive tract (Corzo, 2015). When FOS reach the colon they undergo intestinal microbial fermentation, thus exhibiting prebiotic activity as a bifidogenic effect stimulating the proliferation of *Bifidobacteria*; increase production of short chain fatty acids (SCFA) which favor the growth of health promoting bacteria and reduce or maintain putrefactive bacteria improving host health and stimulating the immune system in the colonic mucosa; produce low pH and SCFA resulting in hypertrophy of the mucosal cells, enlargement of the intestinal surface, increasing intestinal crypt number, depth, and bifurcations, enhancing solubility of mineral ions (Hou & Tako, 2018). Besides FOS, yacon contains phenolic compounds, which display antioxidant, anti- inflammatory, antimicrobial and anticancer activities as well as antimicrobial effects (de Almeida Paula, Abranches, de Luces Fortes, & Ferreira, 2015).

Lobo, Colli, Alvares, and Filisetti (2007) observed that yacon flour (5

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or 7.5% of FOS) stimulated apparent Ca intestinal absorption as well as Ca and Mg balance, and increased the number and depth of cecal crypts in rats. In pigs, Campos et al. (2012) demonstrated that yacon flour (5% of FOS) promoted the growth of *Bifidobacteria* and *Lactobacillus*, resulting in high levels of SCFA and increased caecum overall cell density and crypt formation. In diabetic rats, Habib, Serra-Barcellona, Honoré, Genta, and Sánchez (2015) showed that yacon flour (340 mg FOS/kg/d) improved antioxidant activity. In preschoolers, (Vaz-Tostes et al., 2014), the intake of yacon flour (0.14 g/FOS/kg/day), increased serum levels of anti-inflammatory cytokine IL-4 and fecal IgA. In overweight adults, Machado et al. (2019) and Machado et al. (2020) observed that daily consumption of energy-restricted diets (−500 kcal/day) and breakfast drink containing 25 g of yacon flour (0.1 g of FOS/kg body weight) decreased body weight, body fat and waist circumference, increased the plasma antioxidant capacity, and decreased oxidative stress. Although, the beneficial effects of yacon flour on the intestinal functions have been investigated in animal models and human, no studies were conducted and aimed to investigate the effects of soluble extract from yacon flour on intestinal functionality, duodenal morphology and microbiota *in vivo*.

The *Gallus gallus* is a fast-growing animal model that presents a complex gut microbiota with high homology of gene sequences at the phylum level to humans, with *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Actinobacteria* representing the four dominant bacterial phyla in both (Yegani & Korver, 2008). Furthermore, the *Gallus gallus* intra amniotic administration method, has been widely used and demonstrated the potential prebiotic effects of raffinose and stachyose (Yegani & Korver, 2008), and soluble fibers extracted from beans (Dias et al., 2019), chickpeas and lentil (Pacifici et al., 2017), chia (Pereira da Silva, Martino, Hart, & Tako, 2019) and wheat (Beasley et al., 2020), on the intestinal functionality, morphology, and microbial populations. Our study is the first to separate the soluble extract from yacon flour in order to evaluate its effects on intestinal bacterial populations, brush border membrane (BBM) functionality, morphology and glycogen concentration, *in vivo* (*Gallus gallus*). Our hypothesis is that yacon flour soluble extracts will increase the abundances of beneficial bacterial population (*Bifidobacterium* and *Lactobacillus)* which will positively affect the immune system, BBM functionality (by upregulate the protein and enzymes involved with digestion and absorption of nutrients), and morphology (villi height and goblet cell diameter).

2. Material and methods

2.1. Sample preparation

The yacon that was used in the current study was grown in the city of Santa Maria de Jetibá, state of Espírito Santo (Brazil). The yacon flour preparation was carried out as was previously described by Vaz-Tostes et al. (2014). The yacon flour was stored in plastic bags, at a temperature of −20 °C until used.

2.2. Polyphenols extraction and analysis

2.2.1. Yacon sample preparation

The polyphenols extraction was conducted as was previously described by Hart, Tako, and Glahn (2017). Briefly, 0.5 g of yacon flour was added with 5 mL of methanol/water (50:50 v/v). The slurry was vortexed for 1 min, incubated in a 24 °C sonicating water bath for 20 min, vortexed again for 1 min. Sample was then placed on a rocker at room temperature for 60 min and centrifuging at 4000 g for 15 min. The supernatant was filtered with a 17 mm PTFE syringe filter (National Scientific) and stored for later use at −20 °C.

2.2.2. LC-MS analysis

Extracts and polyphenol standards of yacon were analyzed by an Agilent 1220 Infinity Liquid Chromatograph (LC; Agilent Technologies, Inc., Santa Clara, CA, USA) coupled to an Advion expressionL® compact mass spectrometer (CMS; Advion Inc., Ithaca, NY, USA), according to previous studies (Pereira da Silva et al., 2019; Dias et al., 2019). Briefly,10 μL samples were injected and passed through an XBridge Shield RP18 3.5 μm 2.1 × 100 mm column (Waters, Milford, MA, USA) at 0.6 mL/minute. The column was temperature-controlled at 40 °C. The mobile phase consisted of ultra-pure water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B). Polyphenols were eluted using linear gradients of 94.0–84.4% A in 1.50 min, 84.4–81.5% A in 2.25 min, 81.5–77.0% A in 6.25 min, 77.0–55.0% in 1.25 min, 55.0–46.0% in 2.25 min, 46.0–94.0% in 2.25 min and hold at 94.0% A for 2.25 min for a total run time of 18 min. From the column, flow was directed into a variable wavelength UV detector set at 265 & 278 nm. Flow was then directed into the source of an Advion expressionL® CMS, and ESI mass spectrometry was performed in negative ionization mode using selected ion monitoring with a scan time of 200 msec. Capillary temperature and voltages were 250 °C and 180 V, respectively. ESI source voltage and gas temperature were 2.5 kV and 250 °C respectively. Desolvation gas flow was 240 L/hour. Advion Mass Express™ software was used to control the LC and CMS instrumentation and data acquisition. Individual polyphenols were identified and confirmed by comparison of *m/z* and LC retention times with authentic standards. Analysis of MS and UV data was performed using Advion Data Express™ software.

2.3. Extraction of yacon flour soluble extract

The extraction of soluble fraction from yacon flour was performed according to Tako, Glahn, Knez, and Stangoulis (2014) and Glahn, Tako, Cichy, and Wiesinger (2016). Briefly, the yacon flour sample were dissolved in distilled water (50 g/L) (60 °C, 60 min) and then centrifuged to remove particulate matter. The supernatant was collected and dialyzed (MWCO 12–14 kDa) exhaustively against distilled water for 72 h. At last, the dialysate was collected and then lyophilized to yield a fine off-white powder.

2.4. Dietary fiber, iron, zinc, phytate, analyses in yacon flour and yacon flour soluble extract

The determination of total fiber in soluble and insoluble fractions was performed by the enzymatic–gravimetric method according to AOAC (AOAC, 2012), using the enzymatic hydrolysis for a heat-resistant amylase, protease and amyloglucosidase (Total dietary fiber assay Kiyonaga, Sigma®, Kawasaki, Japan). Dietary phytic acid (phytate)/total phosphorous was measured as phosphorus released by phytase and alkaline phosphatase, following the kit manufacturer's instructions (n = 5) (K-PHYT 12/12, Megazyme International, Bray, Ireland). Determination of iron and zinc concentration in yacon flour (0.5 g) and flour yacon soluble extract (0.5 g) and, in serum (50 μL) and liver samples (0.5 g) was performed according to Pereira da Silva et al. (2019) and Dias et al. (2018). Briefly, the samples were treated with 3.0 mL of 60:40 HNO_3 and $HClO_4$ mixture and left overnight for incubation, then, the mixture was heated to 120 °C for two hours and 0.25 mL of 40 μg/g Yttrium (sigma Aldrich, St Louis, MO, USA) was added as an internal standard to compensate for any drift during the subsequent inductively coupled plasma atomic emission spectrometer (ICP-MS) analysis.

2.5. The intra amniotic administration procedure (Gallus gallus model)

Cornish-cross fertile broiler eggs (n = 48) were obtained from a commercial hatchery (Moyer's chicks, Quakertown, PA, USA). The eggs were incubated under optimal conditions at the Cornell University Animal Science poultry farm incubator. Yacon flour soluble extract in powder form were diluted in 18 Ω H_2O to determine the concentrations necessary to maintain an osmolarity value (Osm) of less than 320 Osm aimed to ensure that the viable embryos would not be dehydrated upon injection of the tested solution. At 17 day of embryonic incubation, eggs

containing viable embryos were weighed and divided into 4 groups (n = 12). All treatment groups were assigned eggs of similar weight frequency distribution. The groups were assigned as follows: three controls group: (1) non-injected; (2) 18 MΩ H_2O; (3) inulin (50 mg/mL); and; (4) yacon flour soluble extract 1% (10 mg/mL). Each group was injected with the specified solution (1 mL per egg) with a 23–27 gauges needle into the amniotic fluid, which was identified by candling. After administration, the injection holes were sealed with cellophane tape and the eggs placed in hatching baskets such that each treatment was equally represented at each incubator location. On day 21, immediately after hatch, the hatchlings were weighted and euthanized by CO_2 exposure, and proximal small intestine (duodenum), blood, pectoral muscle, cecum and liver samples were collected. The cecum and liver were weighted prior freezing (liquid nitrogen). Cecum weight/body weight ratio and liver weight/body weight ratio were calculated. All animal protocols were approved by Cornell University Institutional Animal Care and Use committee (ethic approval code: 2007–0129).

2.6. Glycogen analysis

The pectoral muscle (20 mg) samples were homogenized in 8% perchloric acid, and glycogen content was determined using modified methods described by Dreiling, Brown, Casale, and Kelly (1987). All samples were read at a wavelength of 450 nm in ELISA reader and the amount of glycogen was calculated according to a standard curve. The amount of glycogen present in pectoral sample was determined by multiplying the weight of the tissue by the amount of glycogen per 1 g of wet tissue.

2.7. Extraction of total RNA from duodenum and liver tissue samples

Total RNA was extracted from 30 mg of the proximal duodenal tissue or liver tissue (n = 8) using Qiagen RNeasy Mini Kit (RNeasy Mini Kit, Qiagen Inc., Valencia, CA, USA) according to the manufacturer's protocol. All steps were carried out under RNase free conditions. Briefly, tissues in buffer RLT®, containing β-mercaptoethanol, were disrupted and homogenized with a rotor–stator homogenizer. The lysate was centrifuged for 3 min at 8000*g* in a micro centrifuge (C2400-R, Labnet International Inc, Edison, NJ USA). The supernatant was transferred to another tube, combined with 70% ethanol and mixed immediately. 700 μL of sample was applied to a RNeasy mini column, centrifuged for 15 s at 8000*g*, and the flow through material was discarded. Next, the RNeasy columns were transferred to new 2-mL collection tubes, and 500 μL of buffer RPE® was pipetted onto the RNeasy column followed by centrifugation for 15 s at 8000*g*. An additional 500 μL of buffer RPE were pipetted onto the RNeasy column and centrifuged for 2 min at 8000*g*. Total RNA was eluted in 50 μL of RNase free water. RNA was quantified by absorbance at A 260/280. Integrity of the 18S ribosomal RNAs was verified by 1.5% agarose gel electrophoresis followed by ethidium bromide staining. DNA contamination was removed using TURBO DNase treatment and removal kit from AMBION (Austin, TX, USA).

2.8. Real time polymerase chain reaction (RT-PCR)

To construct the cDNA, a 20 μL reverse transcriptase (RT) reaction was completed in a BioRad C1000 touch thermocycler using the Improm-II Reverse Transcriptase Kit (Catalog #A1250; Promega, Madison, WI, USA). 1 μg of total RNA template, 10 μMof random hexamer primers, and 2 mM of oligo-dT primers. The RT protocol was to anneal primers to RNA at 94 °C for 5 min, copy the first strand for 60 min at 42 °C (optimum temperature for the enzyme), then heat to inactivate the enzyme at 70 °C for 15 min and hold at 4 °C until ready to analyze by Nanodrop (Waltham, MA, USA). The concentration of cDNA obtained was determined by measuring the absorbance at 260 nm and 280 nm using an extinction coefficient of 33 (for single stranded DNA). Genomic DNA contamination was assessed by a real-time RT-PCR assay for the reference genes samples.

The sequences and the description of the primers used (Table 1) in the real-time qPCR was designed based on gene sequences from Genbank database, using Real-Time Primer Design Tool software (IDT DNA, Coralvilla, IA, USA). The amplicon length was limited to 90 to 150 bp. The length of the primers was 17–25-mer and the GC content was between 41% and 55%. The specificity of the primers was tested by performing a BLAST search against the genomic National Center for Biotechnology Information (NCBI) database. The *Gallus gallus* primer 18S rRNA was designed as a reference gene. Results obtained from the qPCR system were used to normalize those obtained from the specific systems as described below.

cDNA was used for each 10 μL reaction together with 2 × BioRad SSO Advanced Universal SYBR Green Supermix (Cat #1725274, Hercules, CA, USA) which included buffer, Taq DNA polymerase, dNTPs and SYBR green dye (Pereira da Silva et al., 2019). Specific primers (forward and reverse) (Table 1) and cDNA or water (for no template control) were added to each PCR reaction. For each gene, the optimal $MgCl_2$ concentration produced the amplification plot with the lowest cycle product (Cp), the highest fluorescence intensity and the steepest amplification slope. Master mix (8 μL) was pipetted into the 96-well plate and 2 μL cDNA was added as PCR template. Each run contained 7 standard curve points in duplicate. No template control of nuclease-free water was included to exclude DNA contamination in the PCR mix. The double stranded DNA was amplified in the Bio-Rad CFX96 Touch (Hercules, CA, USA) using the following PCR conditions: initial denaturing at 95 °C for 30 s, 40 cycles of denaturing at 95 °C for 15 s, various annealing temperatures according to Integrated DNA Technologies (IDT) for 30 s and elongating at 60 °C for 30 s.

The data on the expression levels of the genes were obtained as Cp values based on the "second derivative maximum" (automated method) as computed by Bio-Rad CFX Maestro 1.1 (Version 4.1.2433.1219, Hercules, CA, USA). For each gene, the reactions were run in duplicate. All assays were quantified by including a standard curve in the real-time qPCR analysis. The next four points of the standard curve were prepared by a 1:10 dilution. Each point of the standard curve was included in duplicate. A graph of Cp vs. log 10 concentrations was produced by the software and the efficiencies were calculated as 10[1/slope]. The specificity of the amplified real-time RT-PCR products were verified by melting curve analysis (60–95 °C) after 40 cycles, which should result in a number of different specific products, each with a specific melting temperature.

2.9. Microbial samples and intestinal contents DNA isolation

The cecum were sterilely removed and treated to collect microbial samples according to Hartono, Reed, Ankrah, Glahn, and Tako (2015) and Tako et al. (2008). The contents of the cecum were placed into a sterile 15 mL tube containing 9 mL of sterile PBS and homogenized by vertexing with glass beads (3 mm diameter) for 3 min. Debris was removed by centrifugation at 1000*g* for 5 min, and the supernatant was collected and centrifuged at 4000*g* for 10 min. The pellet was washed with PBS and stored at −20 °C until DNA extraction. For DNA purification, the pellet was re-suspended in 50 mM EDTA and treated with lysozyme (Sigma Aldrich CO., St. Louis, MO, USA; final concentration of 10 mg/mL) for 60 min at 37 °C. The bacterial genomic DNA was isolated using a Wizard Genomic DNA purification kit (Promega Corp., Madison, WI, USA).

2.10. Primers design and PCR amplification of bacterial 16S rDNA

Lactobacillus, Bifidobacterium, Clostridium and E. coli primers for were used according to Tako et al. (2008). PCR products were separated by electrophoresis on 2% agarose gel, stained with ethidium bromide, and quantified using the Quantity One 1-D analysis software (Bio-Rad, Hercules, CA, USA). All products were expressed as relative proportion

Table 1
DNA sequences of the primers.

Analyte	Forward Primer (5′–3′)	Reverse Primer (5′-3′)	Base Pair	GI Identifier
DMT1	TTGATTCAGAGCCTCCCATTAG	GCGAGGAGTAGGCTTGTATTT	101	206597489
Ferroportin	CTCAGCAATCACTGGCATCA	ACTGGGCAACTCCAGAAATAAG	98	61098365
DcytB	CATGTGCATTCTCTTCCAAAGTC	CTCCTTGGTGACCGCATTAT	103	20380692
Hepcidin*	GAGCAAGCCATGTCAAGATTTC	GTCTGGGCCAAGTCTGTTATAG	132	8056490
Δ-6-desaturase	GGCGAAAGTCAGCCTATTGA	AGGTGGGAAGATGAGGAAGA	93	261865208
ZnT1	GGTAACAGAGCTGCCTTAACT	GGTAACAGAGCTGCCTTAACT	105	54109718
ZnT7	GGAAGATGTCAGGATGGTTCA	CGAAGGACAAATTGAGGCAAAG	87	56555152
ZIP9	CTAAGCAAGAGCAGCAAAGAAG	CATGAACTGTGGCAACGTAAAG	100	237874618
CalbindD28K	CGGAGGAGAATTTCCTGTTGT	CCACTGTGGTCACTGTCATATT	97	396519
PMCA1b	TGCAGATGCTGTGGGTAAAT	CCATAAGGCTTCCGCAATAGA	100	NM_001168002.3
NCX1	CCTGACGGAGAAATAAGGAAGA	CCCAGGAGAAGACACAGATAAA	114	NM_001079473.1
MRS2	GCTGGTAACCGGGATTATGT	GCAGGAACATGAGGAGGTAAT	105	420820
TRPM6	ACAGATGCTGCTGACTGATATG	AAGATAGTGGGTGGTAGGAGAA	99	100859603
TRPM7	GCGTGGGATAGAGTTGACATT	TCACAAGGGCATCCAACATAG	100	427502
IL1β	CTCACAGTCCTTCGACATCTTC	TGTTGAGCCTCACTTTCTGG	119	88702685
IL6	ACCTCATCCTCCGAGACTTTA	GCACTGAAACTCCTGGTCTT	105	302315692
TNF-α	GACAGCCTATGCCAACAAGTA	TTACAGGAAGGGCAACTCATC	109	53854909
SI	CCAGCAATGCCAGCATATTG	CGGTTTCTCCTTACCACTTCTT	95	2246388
AP	CGTCAGCCAGTTTGACTATGTA	CTCTCAAAGAAGCTGAGGATGG	138	45382360
SGLT1	GCATCCTTACTCTGTGGTACTG	TATCCGCACATCACACATCC	106	8346783
G6PC*	GTCTGTCTGTCCCGCATTT	CATGGTAGATGGAGTGGATGTG	115	100857298
PCK1*	GCGATGGCTCAGAAGAAGAA	CTTGCTACGTCTCTTGGGTTAG	124	396458
PFKM*	AAGATGAAGACGACGGTGAAG	CCGTGTAGAGGTTGTAGATGAAG	94	374064
18S rRNA*	GCAAGACGAACTAAAGCGAAAG	TCGGAACTACGACGGTATCT	100	7262899

DMT1, Divalent metal transporter 1; Dcytb, Duodenal cytochrome *b*; Znt1, ZnT7, ZIP9, Zinc transporter 1; Calcium transporters: CalbindD28K, calcium binding protein 1; PMCA1b, plasma membrane calcium-transporting ATPase 1b; NCX1, Na(+)/Ca(2+) exchanger 1; Magnesium transporters: MRS2, mitochondrial RNA splicing 2, TRPM6 and TRPM7, transient receptor potential melastatin; IL1β, Interleukin 1 beta; IL6, Interleukin 6; TNF-α, Tumor Necrosis Factor Alpha; AP, Amino peptidase; SI, Sucrose isomaltase; SGLT1, Sodium-Glucose transport protein 1; G6PC (glucose-6 phosphatase); PCK1 phosphoenolpyruvate carboxykinase 1; PFKM, phosphofructokinase 1; 18S rRNA, 18S Ribosomal subunit.
* Liver analyses.

to the content of the universal 16s rRNA primer product and proportions of each examined bacterial group.

2.11. Morphological examination

Intestinal morphology was conduction according to Pereira da Silva et al. (2019). Duodenum samples were fixed in fresh 4% (v/v) buffered formaldehyde, dehydrated, cleared, and embedded in paraffin. Serial sections were cut at 5 μm and placed on glass slides. Sections were deparaffinized in xylene, rehydrated in a graded alcohol series, stained with hematoxylin/ eosin or Alcian Blue/Periodic acid-Schiff. Morphometric measurements of villi surface area (μM), villus height (μM), villus diameter (μM), depth of crypts (μM), goblet cell number and goblet cell diameter (μM) in the crypt and villi, in 4 segments for each biological sample with five biological samples per treatment group were performed with a light microscope using EPIX XCAP software (Standard version, Olympus, Waltham, MA, USA). The morphometric measurements from each experimental group are indicated by a representative duodenal histological cross section image (Fig. 4).

2.12. Statistical analysis

Quantitative data were expressed as means and standard deviation from eight biological samples per treatment group, except intestinal morphology parameters analyses which used five biological samples. Experimental treatments for the *intra amniotic administration* procedure were arranged in a completely randomized design. Effect of treatments were analyzed using one-way analysis of variance (ANOVA). For significant "*p*-value", post hoc Duncan test was used to compare test groups, establishing the significance level at $p < 0.05$. The SPSS version 26.0 software was used to perform the analysis.

3. Results

3.1. Concentration of dietary fiber, protein, iron, zinc, phytic acid, and phytate: Iron ratio in yacon flour and yacon flour soluble extracts.

Yacon flour soluble extract (YFSE) showed a higher concentration ($p < 0.05$) of protein, total dietary fiber in the soluble fraction, and higher iron, phytic acid and phytate: iron in comparison to the yacon flour ($p < 0.05$, Table 2).

3.2. Polyphenol profile in the yacon flour

The concentration of the most prevalent polyphenolic compounds found in the yacon flour is presented in Table 3. The yacon flour showed significant concentrations of epicatechin, myricetin, myricetin 3-glucoside and quercetin.

Table 2
Chemical composition of yacon flour and yacon flour soluble extract.

	Yacon Flour (g/100 g)	yacon flour soluble extracts (g/100 g)
Total dietary fiber	10.35 ± 0.08^{b}	23.26 ± 2.37^{a}
Soluble dietary fiber	1.71 ± 0.00^{b}	20.28 ± 1.45^{a}
Insoluble dietary fiber	8.65 ± 0.08^{a}	2.98 ± 0.91^{b}
Protein	2.94 ± 0.01^{b}	12.98 ± 0.51^{a}
Iron	14.86 ± 0.66^{b}	65.70 ± 3.46^{a}
Zinc	7.42 ± 0.21^{a}	18.19 ± 0.99^{a}
Phytic acid	0.08 ± 0.0^{b}	0.25 ± 0.01^{a}
Phytic acid: iron ratio	4.52 ± 0.35^{a}	3.22 ± 0.22^{b}

Values are means ± SEM, n = 3.
[a,b]Treatment groups not indicated by the same letter are different ($p < 0.05$), *t*-test.

Table 3
Polyphenol profile present in yacon flour.

Polyphenolic compounds	Peak area (mAU)	Concentration (μM)
Epicatechin	51.85	390.00 ± 0.20
Kaempferol 3-sambubioside	4.22	2.40 ± 0.10
Myricetin	6.92	229.50 ± 1.20
Myricetin 3-glucoside	9.82	24.70 ± 0.10
Protocatechuic Acid	1.07	2.10 ± 0.80
Quercetin	0.03	15.10 ± 1.60
Quercetin 3-glucoside	2.89	3.60 ± 0.50
Quercetin 3-rutinoside	4.06	3.40 ± 0.30

Values are means ± SEM, n = 5.

3.3. The intra amniotic administration assay (Gallus gallus model)

3.3.1. Effect of the yacon flour soluble extract on biometric parameters

The yacon treatment group showed similar ($p > 0.05$) body and cecum weight, liver weight and cecum weight/body weight ratio when compared to non-injected, 18MΩ H_2O, and inulin control groups. However, the liver weight and liver: body weight ratio in yacon group was lower relative to the non-injected group (Table 4).

3.3.2. Effect of the yacon flour soluble extract on iron and zinc concentration in serum and liver

The yacon treatment group did not affect ($p > 0.05$) serum iron and zinc concentrations and liver zinc concentration compared to all control groups. However, the yacon treatment group decreased ($p < 0.05$) iron concentration in the liver compared to the 18MΩ H_2O group (Table 5).

3.3.3. Effect of the yacon flour soluble extract on glycogen concentration in pectoral muscle

The yacon treatment group demonstrated increased ($p < 0.05$) glycogen concentrations in pectoral muscle compared to the non-injected and 18 Ω H_2O control groups. No difference ($p > 0.05$) was observed between yacon and inulin groups (Table 6).

3.3.4. Effect of yacon flour soluble extract on Genera- and species-level bacterial populations

The yacon treatment group increased ($p < 0.05$) the relative abundance of *Lactobacillus*, *Bifidobacterium, Clostridium* and *E. coli* compared to the non-injected, 18MΩ H_2O and inulin groups. *Bifidobacterium* abundance did not differ between the yacon group and inulin treatment groups ($p > 0.05$) (Fig. 1).

3.3.5. Effect of yacon flour soluble extract on gene expression of Fe, Zn, Ca and Mg-Related proteins

For Fe-related proteins gene expression, the yacon treatment group increased ($p < 0.05$) the expression of ferroportin and Duodenal cytochrome *B* (DcytB), decreased ($p < 0.05$) hepcidin in comparison to the inulin group and, was did not differ ($p > 0.05$) relative to the non-injected and 18MΩ H_2O groups (Fig. 2A, 2B). For Zn transporters gene expression, the yacon treatment group demonstrated an increased ($p < 0.05$) expression of all zinc transporters, ZnT1, ZnT7 and ZIP9, when compared to the inulin group and was did not differ ($p > 0.05$) relative to the non-injected and 18MΩ H_2O groups (Fig. 2A). Expression of Δ-6-desaturase in the yacon treatment group was similar ($p > 0.05$) to inulin group and was down regulated ($p < 0.05$) relative to the non-injected and 18MΩ H_2O groups.

As for Ca transporters gene expression, the yacon extract administration did not alter ($p > 0.05$) the expression of calbinD28K relative to the inulin and 18MΩ H_2O groups. In addition, the yacon group demonstrated a similar ($p > 0.05$) gene expression of plasma membrane calcium-transporting ATPase 1b (PMCA1b) and Na^{+}/Ca^{+2} exchanger 1 (NCX1) relative to the non-injected and 18MΩ H_2O groups, and down regulated expression ($p < 0.05$) in comparison to the inulin group (Fig. 2A). For Mg transporters gene expression, the yacon extract administration did not alter ($p > 0.05$) the expression of mitochondrial RNA splicing 2 (MRS2) relative to the inulin and 18MΩ H_2O group but up regulated ($p < 0.05$) the expression of transient receptor potential melastatin (TRPM7) compared to the inulin and non-injected groups. However, the yacon group demonstrated a similar ($p < 0.05$) gene expression of transient receptor potential melastatin (TRPM6) in comparison to the non-injected and 18MΩ H_2O groups and down regulation ($p < 0.05$) in the expression of TRPM6 in comparison to the inulin group (Fig. 2A).

3.3.6. Effect of soluble yacon flour extract on gene expression of brush border membrane functional proteins

The yacon extract administration did not alter ($p > 0.05$) the gene expression of brush border membrane digestive and absorptive proteins, aminopeptidase (AP), sodium-glucose transport protein 1 (SGLT1) and sucrase isomaltase (SI) , and relative to the non-injected and 18MΩ H_2O groups. The genes expression of SI was up regulated, SGLT1 was down regulated ($p < 0.05$), and AP was similar ($p > 0.05$) in the yacon group

Table 4
Effect of the intra-amniotic administration of yacon flour soluble extract on body weight, liver weight, cecum weight, and Cecum: Body weight ratio.

Treatment group	Body weight (g)	Liver weight (g)	Cecum weight (g)	Cecum: body weight ratio	Liver: Body weight (g)
Non-injected	46.9 ± 0.9[a]	1.4 ± 0.3[a]	0.5 ± 0.0[a]	0.01 ± 0.001[a]	0.03 ± 0.01[a]
18 Ω H_2O	47.8 ± 1.1[a]	1.0 ± 0.0[b]	0.4 ± 0.0[a]	0.01 ± 0.001[a]	0.02 ± 0.00[b]
Inulin	48.7 ± 0.8[a]	1.1 ± 0.1[ab]	0.5 ± 0.0[a]	0.01 ± 0.001[a]	0.02 ± 0.00[ab]
1% Yacon	48.7 ± 1.6[a]	0.9 ± 0.1[b]	0.5 ± 0.1[a]	0.01 ± 0.002[a]	0.02 ± 0.00[b]

Values are means ± SEM, n = 8.
[a,b]Treatment groups not indicated by the same letter are different ($p < 0.05$) by *post hoc* Duncan test.

Table 5
Effect of the intra-amniotic administration of yacon flour soluble extract on iron and zinc concentrations in liver and serum.

Treatment Groups	Liver (μg/g)		Serum (μg/g)	
	Iron	Zinc	Iron	Zinc
Non-injected	44.46 ± 3.90[ab]	10.36 ± 1.05[a]	3.89 ± 0.51[a]	1.57 ± 0.29[a]
18 Ω H_2O	48.76 ± 5.86[a]	10.11 ± 1.25[a]	4.11 ± 0.51[a]	1.55 ± 0.40[a]
Inulin	44.37 ± 6.63[ab]	11.79 ± 1.38[a]	4.50 ± 0.36[a]	1.12 ± 0.25[a]
1% Yacon	31.88 ± 2.30[b]	12.73 ± 1.04[a]	3.30 ± 0.55[a]	1.61 ± 0.60[a]

Values are means ± SEM, n = 8.
[a,b,c]Treatment groups not indicated by the same letter are different ($p < 0.05$) by *post hoc* Duncan test.

Table 6
Effect of the intra-amniotic administration of yacon flour soluble extracts on glycogen concentrations in pectoral muscle.

Treatment group	Glycogen (mg/g)
Non-injected	0.03 ± 0.02[b]
18 Ω H_2O	0.04 ± 0.00[b]
Inulin	0.12 ± 0.10[ab]
1% Yacon	0.21 ± 0.02[a]

Values are means ± SEM, n = 8.
[a,b]Treatment groups not indicated by the same letter are different ($p < 0.05$) by *post hoc* Duncan test.

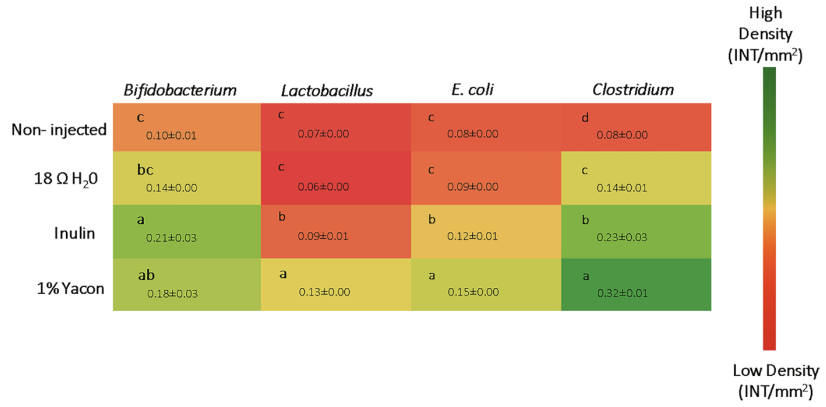

Fig. 1. Effect of the intra-amniotic administration of yacon flour soluble extract on genera- and species-level bacterial populations (AU) from cecal contents measured on the day of hatch. Values are the means ± SEM, n = 8. a–d Per bacterial category, treatment groups not indicated by the same letter are different ($p < 0.05$) by *post hoc* Duncan test.

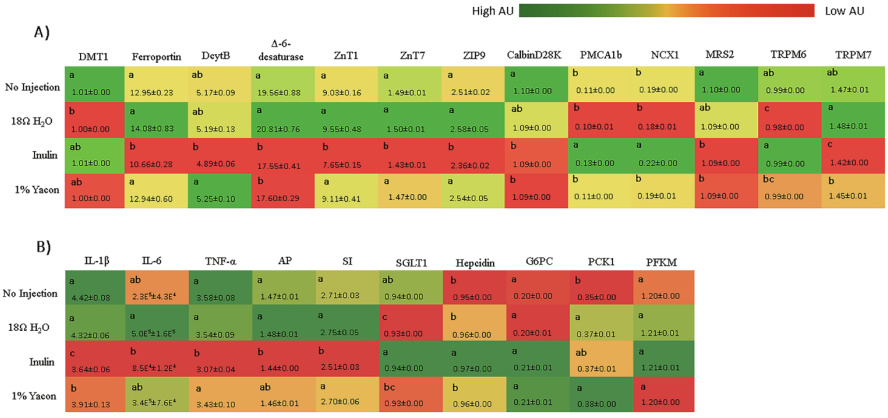

Fig. 2. Effect of the intra-amniotic administration of yacon flour soluble extract on intestinal and liver (hepcidin, G6PC, PCK1, PFKM) gene expression. Values are the means ± SEM, n = 8. a–c Per gene, treatments groups not indicated by the same letter are different ($p < 0.05$) by *post hoc* Duncan test. DMT1, Divalent metal transporter 1; DcytB, Duodenal cytochrome *b*; Zinc transporters: ZnT1, ZnT7, ZIP9; Calcium transporters: PMCA1b, plasma membrane calcium-transporting ATPase 1b; NCX1, Na(+)/Ca(2+) exchanger 1; Magnesium transporters: MRS2, mitochondrial RNA splicing 2, TRPM6 and TRPM7, transient receptor potential melastatin; IL1β, Interleukin 1 beta; IL6, Interleukin 6; TNF-α, Tumor Necrosis Factor Alpha; AP, Amino peptidase; SI, Sucrose isomaltase; SGLT1, Sodium-Glucose transport protein 1; G6PC (glucose-6 phosphatase); PCK1 phosphoenolpyruvate carboxykinase 1; PFKM, phosphofructokinase 1.

relative to the inulin group, respectively (Fig. 2B).

3.3.7. *Effect of soluble yacon flour extract on gene expression of inflammatory biomarkers, glycolysis and gluconeogenesis enzymes.*

The yacon extract administration down regulated the gene expression of Interleukin 1 beta (IL1β), did not affect the expressions of Interleukin 6 (IL6) and Tumor Necrosis Factor Alpha (TNF-α) relative to the non-injected and 18MΩ H_2O control groups. In addition, the yacon group demonstrated a similar ($p > 0.05$) expression of IL6 and up regulated ($p < 0.05$) expression of TNF-α and IL1β relative to the inulin group (Fig. 2B).

For the gene expression of gluconeogenesis enzymes -related proteins, the yacon extract administration did not alter ($p > 0.05$) the expression of glucose-6 phosphatase (G6PC) and phosphoenolpyruvate carboxykinase 1 (PCK1) compared to the 18MΩ H_2O and inulin groups, except for PCK1 expression which was up regulated in the yacon group ($p < 0.05$) compared to the non-injected group.

3.3.8. Effect of yacon flour soluble extract on duodenal morphometric parameters

The yacon treatment group demonstrated a similar ($p > 0.05$) villus surface area and villus diameter, shorter ($p < 0.05$) villus length and increased ($p < 0.05$) depth of crypts relative to the inulin group. Further, the yacon treatment group demonstrated a lower villus surface area, villus diameter and villus length relative to the non-injected group and, the yacon treatment group did not differ ($p > 0.05$) in villus diameter and villus length relative to the18MΩ H_2O group. However, depth of crypts of the yacon group was increased relative to all control groups ($p < 0.05$; Table 7, Fig. 3, Fig. 4).

The yacon treatment group demonstrated increased ($p < 0.05$) crypt goblet cell diameter and number, and villi goblet cell diameter compared to all other groups, indicating that yacon flour soluble extract had a positive effect on the intestinal development, through the proliferation of enterocytes. Further, the villi goblet cell number did not differ ($p > 0.05$) amongst all experimental groups (Table 8).

In relation to the types of goblet cells observed (acidic, neutral, mixed), the yacon group demonstrated an increased ($p < 0.05$) crypt acid and villi acid goblet cell type relative to all other groups. In addition, the administration of yacon flour soluble extracts decreased ($p < 0.05$) the number of crypt and villi neutrals and mixed goblet cell types compared to the inulin group (Table 9, Fig. 3, Fig. 4).

4. Discussion

Yacon flour is an abundant source of fructooligosacharides (FOS), a dietary soluble fiber, which was suggested to have a beneficial effect on host intestinal health. However, the potential effects of soluble extracts from yacon flour on the intestinal bacterial populations, brush border membrane (BBM) functionality, mineral uptake (Ca, Mg, Zn and Fe), glycogen status and intestinal morphology *in vivo* were yet to be investigated.

The current study indicates that the intra-amniotic administration (*Gallus gallus*) of yacon flour soluble extract (YFSE) positively affected gut health, increased the relative abundance of *Bifidobacterium*, upregulated the expression of proteins related to Fe transport (ferroportin and DcytB), Zn transport (ZnT1, ZnT7 and ZIP9), maintained Ca and Mg transporters expression and, upregulated or maintained the expressions of functional proteins involved in digestion and absorption of nutrients, improved energetic status (glycogen stores), down regulated the gene expression of inflammation protein biomarker and hepcidin, improved the intestinal morphology as demonstrated via increased depth of crypts, crypt goblet cell diameter, number and type (acid) and, villi goblet cell diameter and type (acid) (Fig. 4).

Further, the yacon flour contain significant concentrations of phenolic compounds, as epicatechin, myricetin, myricetin 3-glucoside and quercetin. Previous investigations reported that chlorogenic acid and caffeic acid derivatives, mainly esters of caffeic acid with the hydroxy groups of aldaric acid as the main phenolic compounds identified in yacon roots and leaves (Campos et al., 2012; Ueda et al., 2019). Compare to carioca bean flour (Dias et al., 2018), the polyphenolic profile of yacon flour showed higher concentrations of (epi) catechin and quercetin 3-glucoside.

Table 7
Effect of the intra-amniotic administration yacon flour soluble extract on the duodenal small intestinal villus and crypts.

Treatment group	Villi surface Area (mm^2)	Villus length (μM)	Villus diameter (μM)	Depth of crypts (μM)
Non-injected	71.41 ± 2.26^{a}	521.25 ± 8.89^{a}	83.64 ± 1.21^{a}	52.10 ± 0.99^{b}
18 Ω H_2O	54.76 ± 1.45^{c}	453.18 ± 8.19^{c}	75.25 ± 1.01^{c}	50.97 ± 0.87^{c}
Inulin	63.81 ± 1.57^{b}	496.76 ± 8.13^{b}	79.98 ± 1.07^{ab}	52.36 ± 0.92^{b}
1% Yacon	60.10 ± 1.73^{b}	470.03 ± 8.06^{c}	78.68 ± 1.09^{bc}	68.65 ± 0.91^{a}

Values are the means ± SEM, n = 5.
[a–d]Treatment groups not indicated by the same letter are different ($p < 0.05$) by *post hoc* Duncan test.

The intra-amniotic administration of YFSE increased the relative abundance of bacterial populations (at the genera and species levels) of *Lactobacillus*, *Bifidobacterium*, *Clostridium* and *E. coli* compared to the non-injected, 18MΩ H_2O and inulin groups. The YFSE presented high concentration of soluble dietary fiber (20.3%), and the yacon flour used in our study presented 52.2% of FOS and 6.6% of inulin (Grancieri et al., 2017). This suggests that the fermentation of the soluble dietary fiber, especially FOS in the 1% YFSE intra-amniotic administration treatment group resulted in a higher bifidogenic effect relative to 5% inulin (positive control) treatment group, inducing such effect that have probably led to increased *Bifidobacteria* abundance (in addition to the observed increased in *clostridium* and *E. coli*. *Bifidobacterium* species), which belongs to the *Actinobacteria* phylum, that produce acetate and lactate during carbohydrate fermentation (Rivière, Selak, Lantin, Leroy, & De Vuyst, 2016). This may also potentially lead to increased abundance of butyrate producing-bacteria that belong to the *Firmicutes* phylum, such as *Faecalibacterium prausnitzii,* and *Clostridium leptum* , part of the *Ruminococcaceae* family (Louis & Flint, 2009, 2017). In addition, yacon storage of monosaccharides (glucose, 3.76%) and disaccharides (fructose, 8.16%) (Grancieri et al., 2017), provides an adequate energy source for beneficial *E. coli* proliferation (Wang, Kolba, Liang, & Tako, 2019). These synergic effects may contribute to the increased bacterial production of short chain fatty acids (SCFA), mainly butyrate (Venegas et al., 2019). The main butyrate-producing bacteria are anaerobes, including the *Bacteroidetes* and *Clostridia*, and the low O_2 concentrations in the colon create a favorable niche for them, which can explain the increased abundance in general population of *Clostridium* by YFSE administration (Venegas et al., 2019).

Yacon flour demonstrated high concentrations of (Epi) catechins and myricetin. (Epi) catechins may influence the composition and function of human intestinal microbiota. Kutschera, Engst, Blaut, and Braune (2011) demonstrated that *Clostridium orbiscindens* converted the final product of (Epi) catechin cleavage by *Eggerthella lenta*. The main end metabolites of catechin metabolism of human intestinal microbiota are phenylpropionic acids, which presents anti-inflammatory effects. Dias et al. (2018) observed that the SCFA-producing *Firmicutes* had increased abundance in the intestinal contents of animals (*Gallus gallus*) fed Fe biofortified carioca bean-based diet, with high concentration of (epi) catechin, and specifically, *Eggerthella lenta* and *Clostridium piliforme*. The increased abundance of SCFA-producing bacteria may lead to an increased SCFA concentration in the intestinal lumen, which in return can promote intestinal cell proliferation. Previously, *E. coli,* bacterial genus that is part of the *Enterobacteriaceae* family, was suggested to be a candidate bacterial specie that is directly involved in the biotransformation of daidzein to its bioactive metabolites, equol and O-desmethylangolensin (Hartono et al., 2015), also, certain species of *Clostridium* have also been associated with daidzein metabolism (Schoefer, Mohan, Schwiertz, Braune, & Blaut, 2003), similarly to the observations in the current study, the above indicates that YFSE demonstrated a prebiotic effect, resulting in increasing *bifidobacterium* and *Lactobacillus* populations and potentially beneficial species within the *Clostridium* and *E. coli* populations (Fig. 4).

The intra-amniotic administration of YFSE increased the gene expression of Fe-metabolism proteins, ferroportin and duodenal cytochrome *B* (DcytB), and decreased ($p < 0.05$) hepcidin expression compared to the inulin group. These effects can be associated with the increased abundance of *Bifidobacteria* and *E. coli* populations which promote the production of short-chain fatty acids (SCFA) (Venegas et al., 2019). Such production leads to a reduction of intestinal luminal pH, which may increase the solubility of Fe^{3+} (Hartono et al., 2015) and increase DcytB expression, enzyme responsible to reduce Fe^{3+} to Fe^{2+} in

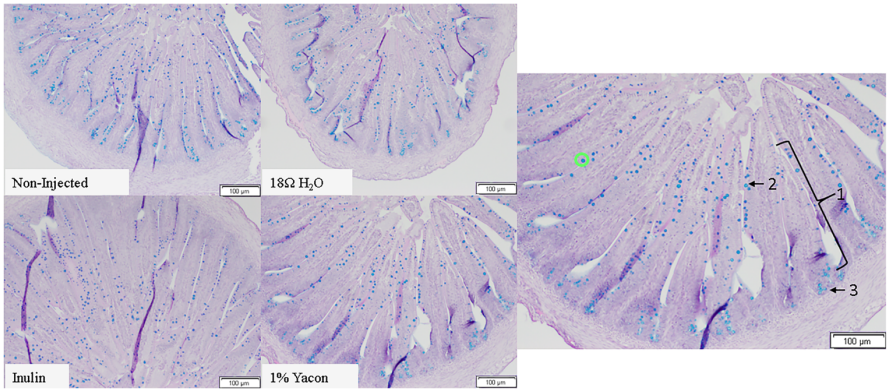

Fig. 3. Depicts a representation of intestinal morphology for each experimental treatment group, with Each parameter investigated is indicated as follows: (1) villi; (2) goblet cell; (3) Crypt. Acidity of goblet cells and crypts cells are depicted as follows; green circle is showing a bright blue crypt is acidic mucin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

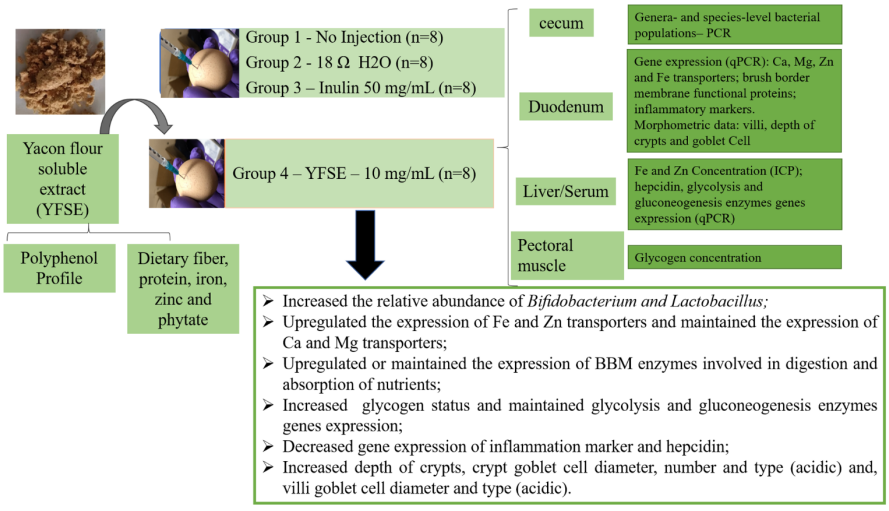

Fig. 4. Schematic diagram of proposed mechanisms of the suggested yacon flour soluble extract (YFSE) effects *in vivo*. The YFSE increased beneficial bacterial populations (*Bifidobacterium* and *Lactobacillus*, in addition *clostridium* and *E. coli*) which may promote the production of SCFA. This may lead for a luminal pH reduction; moreover; YFSE improved intestinal morphology (villus surface area, villus diameter, depth of crypt, crypt goblet cell diameter and number, villi goblet cell diameter, crypt acid and villi acid goblet cells), upregulated and DcytB), Zn transporters (ZnT1 and ZIP9), and maintained the expression of Fe transporters (ferroportin Ca and Mg transporters and, upregulated or maintained enzymes involved in digestion and absorption of nutrients, increased glycogen status, down regulated the gene expression of inflammatory marker and hepcidin. Blue arrow: YFSE maintained or increased parameter relative to 18 Ω H2O; red arrow: maintained or increased parameter relative to inulin. The injection site is amniotic fluid at day 17 of embryonic development and hatch on day 21. DMT1, Divalent metal transporter 1; DcytB, Duodenal cytochrome *b*; Zinc transporters ZnT and ZIP9; Calcium transporters: PMCA1b, plasma membrane calcium-transporting ATPase 1b; NCX1, Na(+)/Ca(2+) exchanger 1; Magnesium transporters: MRS2, mitochondrial RNA splicing 2, TRPM6 and TRPM7, transient receptor potential melastatin; IL1β, Interleukin 1 beta; IL6, Interleukin 6; TNF-α, Tumor Necrosis Factor Alpha; AP, Amino peptidase; SI, Sucrose isomaltase; SGLT1, Sodium-Glucose transport protein 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 8
Effect of the intra-amniotic administration yacon flour soluble extract on the goblet cells.

Treatment group	Villi goblet cell diameter (μM)	Villi goblet cell number	Crypt goblet cell diameter (μM)	Crypt goblet cell number
Non-injected	6.10 ± 0.05^{d}	38.86 ± 11.03^{a}	5.16 ± 0.03^{c}	8.49 ± 0.26^{b}
18 Ω H_2O	6.27 ± 0.05^{c}	24.37 ± 0.53^{a}	4.98 ± 0.03^{d}	7.15 ± 0.20^{c}
Inulin	6.53 ± 0.04^{b}	30.96 ± 0.24^{a}	5.43 ± 0.03^{b}	8.72 ± 0.24^{b}
1% Yacon	7.99 ± 0.04^{a}	33.00 ± 0.23^{a}	6.97 ± 0.03^{a}	9.53 ± 0.23^{a}

Values are the means ± SEM, n = 5.
$^{a–d}$Treatment groups not indicated by the same letter are different ($p < 0.05$) by *post hoc* Duncan test.

Table 9
Effect of the intra-amniotic administration of experimental Yacon solutions on the goblet cells types.

Treatment Group	Crypt Acid	Crypt Neutral	Crypt Mixed	Villi Acid	Villi Neutral	Crypt Mixed
Non-injected	7.32 ± 0.18^{c}	0.08 ± 0.02^{b}	0.13 ± 0.02^{c}	18.61 ± 0.43^{b}	0.00 ± 0.00^{b}	0.97 ± 0.07^{a}
Water	6.83 ± 0.15^{d}	0.04 ± 0.01^{bc}	0.28 ± 0.05^{b}	16.67 ± 0.35^{c}	0.01 ± 0.01^{b}	1.08 ± 0.07^{a}
Inulin	7.07 ± 0.14^{b}	0.18 ± 0.03^{a}	0.66 ± 0.07^{a}	19.85 ± 0.41^{b}	0.12 ± 0.03^{a}	0.96 ± 0.07^{a}
1% Yacon	10.48 ± 0.23^{a}	0.01 ± 0.01^{c}	0.29 ± 0.04^{c}	21.17 ± 0.35^{a}	0.06 ± 0.02^{b}	0.78 ± 0.07^{b}

Values are the means ± SEM, n = 5.
$^{a–d}$Treatment groups not indicated by the same letter are different ($p < 0.05$) by *post hoc* Duncan test.

the BBM of the enterocyte, as observed in our study. Furthermore, the increase in the abundance of *Lactobacillus*, *Bifidobacterium*, *Clostridium* and *E. coli* by YFSE may have led to an anti-inflammatory effect, decreasing the gene expression of Interleukin 1 beta (IL1β) and maintain Interleukin 6 (IL6) and Tumor Necrosis Factor Alpha (TNF-α) similar to the non-injected and 18MΩ H_2O control group, which decreased hepcidin (key iron-regulatory hormone that controls systemic Fe homeostasis), and up regulation of ferroportin gene expression, that transports Fe^{2+} from the enterocyte into the bloodstream. In addition, the YFSE increased the expression of zinc transporters, ZnT1, ZnT7 and ZIP9 compared to the inulin group and, Δ-6-desaturase (sensitive novel biomarker for evaluating Zn status) (Knez, Stangoulis, Glibetic, & Tako, 2017) expression in the YFSE group was similar to the inulin group which indicates on improvement in Zn physiological status. However, the YFSE did not change serum zinc nor iron, or liver zinc concentrations, yet, decreased iron concentration in hepatic tissue compared to the18MΩ H_2O group. The increased pectoral muscle glycogen levels induced by YFSE indicate on an improvement in energetic status, which may lead to enhancement of glycogen tissue reserves and thus availability. It is however necessary to assess this hypothesis in a future long-term feeding trial, as these results were evaluated immediately post hatch.

The biometric parameters collected in the yacon treatment group were similar to the control groups, except for liver weight and liver: body weight ratio which was lower compare to the non-injected group ($p < 0.05$). A possible explanation might be the phenolics compounds and soluble fiber profiles, specifically FOS in the YFSE, that may alter the lipid metabolism leading to a decrease in triacylglycerol and VLDL levels (Habib, Honoré, Genta, & Sánchez, 2011), which can promote less fat in the liver, thus, decreasing liver weight as observed in our study.

The YFSE group demonstrated increased ($p < 0.05$) glycogen levels in the pectoral muscle relative to the non-injected and 18 Ω H_2O groups, pectoral glycogen levels were similar ($p > 0.05$) to the inulin group. However, hepatic expressions of gluconeogenesis (glucose-6 phosphatase-G6PC), phosphoenolpyruvate carboxykinase 1 (PCK1), and glycolysis enzymes (phosphofructokinase 1-PFKM) were not altered. Thus, YFSE may have an indirect hormone-mediated effect on total muscle glycogen stores, as skeletal muscle requires insulin action for glucose uptake (Foye, Ferket, & Uni, 2007). A potential pathway is that YFSE may increase carbohydrates availability, as the source of monosaccharides and disaccharides, which may increase the uptake and storage of pectoral muscle glucose and in the form glycogen. Although the study reports that during the hatching process glycogen stores become depleted due to the high amount of energy expenditure needed, the YFSE keep a sufficient glycogen status in the birds post hatching (Foye et al., 2007).

The current study also demonstrated that yacon flour is a good source of calcium and magnesium, as intra-amniotic administration of YFSE improved the expression of calcium transporter, calbinD28K and similar to the inulin and 18MΩ H_2O groups. Further, the gene expression of calcium-transporter ATPase 1b (PMCA1b) and Na^{+}/Ca^{2+} exchanger 1 (NCX1) were similar to the non-injected and 18MΩ H_2O groups ($p > 0.05$), these findings agree with previous observations (Delgado et al., 2013). In related to magnesium metabolism transporters, the YFSE administration maintained the mitochondrial RNA splicing 2 (MRS2) expression similar to the inulin and 18MΩ H_2O groups ($p > 0.05$). The transient receptor potential melastatin (TRPM6) gene expression was similar ($p > 0.05$) to the non-injected and 18MΩ H_2O groups, and transient receptor potential melastatin (TRPM7) expression was up regulated ($p < 0.05$) relative to the inulin and non-injected groups. Thus, these results suggest that YFSE can potentially increase Ca and Mg absorption efficiency in a long-term feeding trial, which may be mediated by the gut microbiota, specifically *Bifidobacteria* populations that were also elevated ($p < 0.05$) in the current study, and relative to controls.

The intra-amniotic administration of YFSE increased ($p < 0.05$) sucrase-isomaltase (SI) gene expression compared to the inulin group, SI is the major disaccharidase located on the duodenal BBM, and hydrolyzes disaccharides or oligosaccharides to monosaccharides for absorption, which in the current study is associated with the elevation in glycogen levels, and as was previously demonstrated (Diaz-Sotomayor et al., 2013). In addition, YFSE administration did not affect ($p > 0.05$) the gene expression of aminopeptidase (AP), an exopeptidase that cleaves amino acids from the N-terminus of peptides (Hou, Kolba, Glahn, & Tako, 2017); sodium-glucose transport protein 1 (SGLT1) responsible for dietary glucose absorption (Oguma et al., 2015); and SI gene expression relative to the non-injected and 18MΩ H_2O groups. Our results indicate that yacon prebiotics, YFSE, improved BBM functionality. In previous studies where the intra-amniotic administration approach was utilized, the authors reported that the raffinose and stachyose (50 mg/mL and 100 mg/mL) increased the gene expression of AP, SI, SGLT-1 compared to non-injected and 18MΩ H_2O groups (Pacifici et al., 2017); in another study, wheat bran extract (100 mg/mL) increased SGLT-1 and AP gene expression compared to the arabinose group (50 mg/mL) and increased SGLT-1 compared to the cellulase wheat bran group and, SI expression did not differ amongst treatment groups (Wang et al., 2019). Further, different concentrations of soluble chia extract (5, 10, 25 e 50 mg/mL) (Pereira da Silva et al., 2019), and the administration of black, carioca and white beans extracts (50 mg/mL) (Dias et al., 2019), did not affect the BBM functional genes expression. Thus, considering that *Gallus gallus* embryos have a limited ability to digest and absorb nutrients prior to hatch (Uni, Noy, & Sklan, 1999), YFSE administration demonstrated a beneficial effect via the up-regulation of the BBM functional genes, reflecting an improvement in the intestinal BBM functionality, and overall nutrients digestive and absorptive capabilities (Fig. 4).

The intra-amniotic administration of YFSE had a positive prebiotic effect on intestinal development, as demonstrated by the increased

enterocyte proliferation. The YFSE administered group demonstrated a similar ($p > 0.05$) villus surface area and villus diameter, shorter villus length and deeper ($p < 0.05$) of crypts relative to the inulin group. Furthermore, the YFSE group did not differ in villus diameter and villus length ($p > 0.05$) relative to the 18MΩ H_2O group. Yet, these parameters were higher in the non-injected group, suggesting that the YFSE treatment have affected cellular (including enterocyte) proliferation and improved tissue's digestive and absorptive capabilities, and as was previously described (Hou & Tako, 2018). However, YFSE increased depth of crypts ($p < 0.05$) compared to all other groups. The shorter villus observed and relative to the inulin group may have occurred due to the increased BBM functionality induced by YFSE administration, as suggested by Mišta et al. (2017) that observed that intra-amniotic administration of inulin increased crypt depth, however the villus lengths and villus length: crypt depth ratio was not altered. Pacifici et al. (2017) observed that intra-amniotic administration of raffinose and stachyose compounds significantly increased villus surface area.

The YFSE that is rich in soluble fiber, mainly FOS, increased proliferation of the crypt and villi goblet cell number and diameter, and crypt acidic and villi acidic goblet cells type in comparison to the non-injected and 18MΩ H_2O groups. This indicates on an increased synthesis and secretion of mucus, a polysaccharide/protein rich layer, by duodenal goblet cells (Hou & Tako, 2018). The mucus layer provides a physical shield for intestinal epithelial cells. This layer also functions as the mucosal habitat that supports probiotic populations as *Bifidobacterium* and therefore, supports the host's immune system (Hou & Tako, 2018). Similarly, and in the current study, the YFSE treatment group demonstrated an increased abundance of *Bifidobacteria* and BBM down-regulation of proinflammatory cytokine IL1β- gene expression. Further, acidic mucus has been shown to protect against bacterial translocations, as sulphated mucins appear to be less degradable by the bacterial glycosidases and host proteases (Uni et al., 1999). Previous studies aimed to assess potential prebiotic effects and by utilizing the intra-amniotic administration approach, reported on a positive effect on intestinal morphology induced by Fe/Zn biofortified and nicotianamine enhanced wheat extracts, which resulted in increased intestinal villi length and goblet cells number (Beasley et al., 2020). Further, different concentrations of soluble chia extracts increased the villus surface area, villus length, villus width and villi goblet cells number (Pereira da Silva et al., 2019); chickpea and lentil extracts administration increased the villus surface area and goblet cell diameters (Hou et al., 2017); and carbohydrate (maltose, sucrose and dextrin) administration improved the mucosal function and increased the proportion of goblet cells containing acidic mucin (Smirnov, Tako, Ferket, & Uni, 2006).

Thus, the present study, and for the first time, demonstrates the positive prebiotic effects of YFSE administration on the intestinal microbiome, morphology and functionality, with potential enhancement of the digestive and absorptive capabilities of the of the BBM *in vivo*.

5. Conclusion

Intra-amniotic administration of yacon flour soluble extract positively affected gut health and function, by increasing the relative abundance of *Bifidobacterium*, and *Lactobacillus*, upregulating or maintaining Fe-Zn-Ca-Mg transporters and metabolism related proteins expressions, downregulating inflammation related and hepcidin genes expression, while improving the intestinal morphology. Thus, soluble extract from yacon flour that are rich in fiber and phenolic compounds demonstrated potential prebiotic properties that improved intestinal functionality, and therefore, improved mineral uptake. These findings suggest that yacon is a promising target staple food crop that can be used as a source of dietary fiber and phenolic compounds, with additional nutritional benefits associated with increased abundance of health promoting bacterial populations, and improvement in dietary mineral bioavailability.

CRediT authorship contribution statement

Hércia Stampini Duarte Martino: Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **Nikolai Kolba:** Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **Elad Tako:** Data curation, Formal analysis, Investigation, Methodology, Writing - original draft, Supervision, Resources.

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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