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Metabolic effects of goat milk yogurt supplemented with yacon flour in rats on high-fat diet

Emanuel Fabersani[a,1,2], María Virginia Grande[b,1,3], María Victoria Coll Aráoz[c,d,4], Martin L. Zannier[e], Sara S. Sánchez[b,4], Alfredo Grau[d,e], Ruben Oliszewski[a,4,*], Stella M. Honoré[b,4,*]

[a] Facultad de Agronomía y Zootecnia, Universidad Nacional de Tucumán, Florentino Ameghino s/n B° Mercantil, T4105 – El Manantial, Tucumán, Argentina
[b] Instituto Superior de Investigaciones Biológicas (INSIBIO, CONICET-UNT), Instituto de Biología "Dr. Francisco D. Barbieri", Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Chacabuco 461, T4000ILI – San Miguel de Tucumán, Tucumán, Argentina
[c] PROIMI-Biotecnología CONICET, Av. Belgrano y Pasaje Caseros -T4001MBV, San Miguel de Tucumán, Tucumán, Argentina
[d] Facultad Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán, Miguel Lillo 251, T4000ILI – San Miguel de Tucumán, Tucumán, Argentina
[e] Instituto de Ecología Regional (IER), Facultad de Ciencias Naturales, Universidad Nacional de Tucumán, CC34, 4107, Yerba Buena, Tucumán, Argentina

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ABSTRACT

This study aimed to evaluate the effects of addition of yacon flour on the quality parameters of goat milk yogurt and investigate the metabolic effects of its regular consumption on high fat diet-fed *Wistar* rats (30 days). The formulation containing 7% (w/v) yacon flour had higher nutritional values, acceptable sensory attributes and higher count (10^7 cfu/g) of viable probiotic microorganisms, with shelf life of at least 30 days. 7% yacon flour addition improved goat yogurt sugar profile, reducing lactose (0.94%) and increasing prebiotic fructooligosacharides (4.55%) content in the final product. Supplementation of goat yogurt + yacon to a high fat diet resulted in lower body weight, body mass index, fasting glucose levels, HOMA-IR and atherogenic indices of rats, improving the effects of goat yogurt or yacon flour alone ($P < 0.05$). Our results showed conclusive evidence indicating that goat yogurt + yacon is an excellent functional food that avoids the metabolic impact of high fat feeding.

1. Introduction

The increasing value of functional foods throughout the world has encouraged innovation in the production of new food products, stimulating their use (Kumar et al., 2015).

The dairy industry has been a pioneer in this regard, by encouraging the production of a large number of functional products, by adding prebiotic and probiotic agents to foods (Lollo et al., 2012, 2015a; Moura et al., 2016). Fermented milk and cheeses have been the subject of several studies focused on technological aspects and health benefits (Shiby and Mishra, 2013; Tripathi and Giri, 2014; Lollo et al., 2015a; Sperry et al., 2018).

Yogurt is the most popular of fermented milks and is considered a rich source of calcium and milk proteins with higher biological value (Sivieri et al., 2017). The regular incorporation of yogurt in the diet provide the balance of the intestinal microbiota improving the immune system (Lollo et al, 2013; Nabavi, Rafraf, Somi, Homayouni-Rad, & Asghari-Jafarabadi, 2015; Liu, Tang, Yu, Zhang, & Li, 2017). Although most yogurts are prepared from bovine milk, other mammalian species such as goat and sheep, are being recently used due to their intrinsic nutritional composition. In this sense, goat milk yogurt has been considered a functional food potentially useful in medicine and human nutrition (Clark and García, 2017; Ranadheera, Naumovski, & Ajlouni, 2018).

Yogurt has also been shown to be an excellent delivery vehicle for functional ingredients (Champagne, da Cruz, & Daga, 2018; Ranadheera et al., 2018; Zuidam and Velikov, 2018). Inulin, fructooligosaccharides (FOS), galactooligosaccharides, polydextrose and

* Corresponding authors.
E-mail addresses: rubenoliszewski311@yahoo.com (R. Oliszewski), smhonore@fbqf.unt.edu.ar (S.M. Honoré).
[1] Both authors contributed equally.
[2] Recipient of FONCYT Fellowship.
[3] Recipient of CONICET Fellowship.
[4] Career Investigators of CONICET.

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resistant starch are the main prebiotics incorporated in the food systems to improve sensory, rheological, physicochemical and physiological properties (Yoo and Kim, 2016; Batista et al, 2017). FOS are soluble fibers consisting of fructose subunits (2–16), linked to each other by β (2 → 1) bonds. They are metabolized by specific groups of bacteria in the colon providing, numerous local and systemic benefits to the host (Roberfroid, 2007, Cao et al., 2018).

Smallanthus sonchifolius (yacon) roots are considered the best natural source of FOS (Cao et al., 2018). Yacon pulp and root concentrate have been incorporated on yogurts to confer different physicochemical and sensory characteristics (Parra, 2014; Montarroyos Padilha et al., 2017). Additionally, yacon flour has been used as an ingredient to elaborate cow light yogurt with low fat content and a high concentration of soluble fiber, suggesting possible benefits for customers health (Mileib Vasconcelos et al., 2012).

Yacon roots consumption has been associated with several health benefits, such as improvement in gastrointestinal motility (Geyer, Manrique, Degen, & Beglinger, 2008), increased bone calcium retention (Lobo, Colli, Alvares, & Filisetti, 2007), triacylglycerols-lowering effects (Genta, Cabrera, Grau, & Sánchez, 2005), positive impact on glucose homeostasis (Genta et al., 2009; Honoré, Genta, & Sánchez, 2013), antioxidant activity (Habib, Serra-Barcellona, Honoré, Genta, &

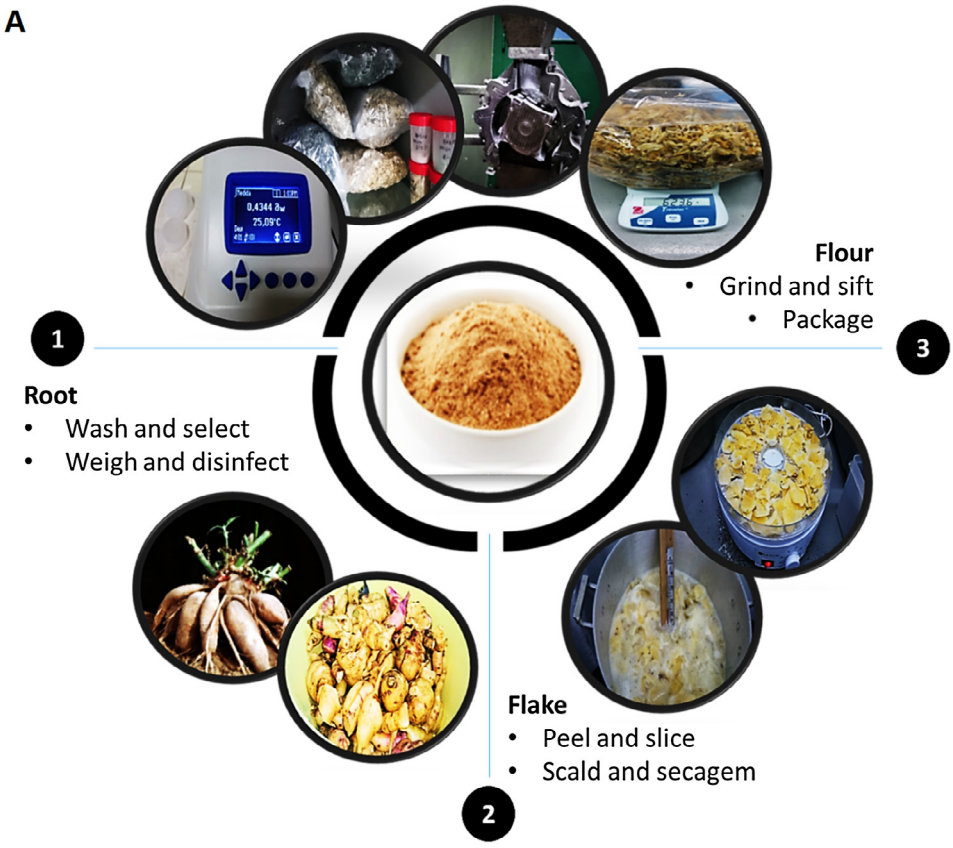

Fig. 1. Schematic summary of yacon roots processing for obtaining flour with high quality of FOS (A). Experimental design (B).

Sánchez, 2015), immune system stimulation (Delgado, Thomé, Gabriel, Tamashiro, & Pastore, 2012), and protective effects on colon carcinogenesis (de Moura et al., 2012). However, *in vivo* studies about the metabolic effects of the dairy products with yacon remain scarce.

In this context, this study purposed to manufacture a probiotic yogurt using goat's milk as a raw material with the addition of yacon flour and analyze its metabolic effects in a high-fat diet rodent model.

2. Material and methods

2.1. Yacon flour

2.1.1. Plant material and yacon flour preparation

The *Smallanthus sonchifolius* (yacon) roots (Clone LIEY97-1) were harvest between July and August 2017 of an experimental field at the Instituto de Ecología Regional, Horco Molle, province of Tucumán, 26° 47′ S, 65° 19′ W Argentina. A voucher specimen is deposited in the herbarium of "Instituto Miguel Lillo", San Miguel de Tucumán, Tucumán, Argentina (no. 607173LIL).

The roots were harvested, weighed, washed and disinfected by immersing in a solution of 10% sodium hypochlorite for 20 min. Tubers with signs of putrefaction, microbial contamination and very small size (< 50 g) were discarded. The peeled roots were cut into thin slices (PEABODY® CRUSHER SLICER (M520R) and subjected to scalding process, placing the slices in hot water between 80 and 90 °C for 20 min. The slices were dehydrated at 60 °C (BLANIK BDA020 food dehydrator) until obtaining croquettes with constant weight. The dry product (with less than 7% residual moisture) was crushed in a blender before being incorporated in a hammer mill (Wiley Mill) to obtain yacon flour with particles smaller than 1 μm (Fig. 1A). The yacon flour was pasteurized at 90 °C for 25 min, cooled and stored under vacuum until use.

2.2. Goat milk yogurt

2.2.1. Preparation of the starter

Strains were activated first via sterilization of the skim milk powder at 105 °C, with 10% (wt/wt) solids concentration. The lyophilized powder of *Streptococcus thermophilus* (*St*), *Lactobacillus delbrueckii* subsp. bulgaricus (*Lb*), was activated in the skim milk and then stored at – 20 °C until its use. Starter culture was prepared by dissolving 1 pouch of 50 μL in 250 mL of sterile skim milk (1000 ×).

2.2.2. Yogurt preparation

Yogurt was elaborated using commercial goat milk powder (La Primera, Córdoba, Argentina, RNE 004004768; RNPA04050997) resuspended in 13% sterile distilled water. The milk was pasteurized for 15 min at 90 °C, and cooled to 45 °C. Then, goat milk was inoculated with the starter culture containing the mixture of *St* and *Lb,* (FD-DVS YC-X16-YO-FLEX, Chr. Hansen, Denmark), stirred gently for 10–15 min to dissolve correctly the starter as indicated by the manufacturer. Finally, it was incubated at 43 °C for 4 h, with pH monitoring up to 5–4.8 and then cooled to 4 °C. The yogurt was stored at 4 °C until analysis was undertaken.

2.2.3. Microbiological analysis

The yogurt was manufactured and analyzed microbiologically weekly for a period of 30 days to ensure the viability of the strains used, at the time corresponding to the shelf life of commercial yogurts. The flour (1 g)/milk (1 mL) or yogurt (1 mL) supplemented with yacon flour were subjected to the microbiological analysis at 1, 10, 20 and 30 days since its manufacture. The samples were transferred to tubes with screw cap containing 9 mL of sterile peptone water solution 0.1% w/v and subsequent dilutions were made for microbial counts. The count of lactic acid bacteria was determined in a M.R.S. agar medium in atmosphere with 5% CO_2, at 35–37 °C for 24–72 h (B0220506 M.R.S. Agar; Britania, Buenos Aires, Argentina) according to De Man, Rogosa, and Sharpe (1960). Gram-negative bacteria were determined in the *Violeta rojo y Bilis Agar* medium (B0214306, Britania, Buenos Aires, Argentina), in aerobiosis, at 35–37 °C for 18–24 h, as indicated by the manufacturer. Fungi and yeasts were determined in HyM agar at 20–25 °C, for 5–7 days. Finally, the determination of pH was made through direct reading with a digital pH meter (AD1030 Professional pH-ORP-TEMP Bench Meter, Szeged - Hungary) using a 10 mL yogurt sample from each experimental unit.

2.3. Centesimal composition (nutritional values)

The crude protein content was determined using the Kjeldahl method (920.87AOAC, 2005); total lipid contents were determined by Soxhlet extraction method (925.38 AOAC, 2005); ash was determined by incinerating at 550 °C in a muffle furnace for 6 h (923.03 AOAC, 2005), the moisture was determined by AOAC 925.09 method, and total dietary fiber content (TDF) was determined according to the enzymatic gravimetric method of AOAC17, using the enzyme kit (Total Dietary Fiber Assay Kit, TDF100A SIGMA).

The carbohydrates were determined by high performance liquid chromatography (HPLC) (see Section 2.3.1). The caloric value of the foods studied was calculated considering 16.736 kJ/g (4 kcal/g) for proteins and carbohydrates, 37.656 kJ/g (9 kcal/g) for lipids and 62.76 kJ/g (1.5 kcal/g) for FOS.

2.3.1. FOS determination

FOS were determined by HPLC. Samples of yacon flour, caprine milk and goat yogurt with and without the addition of yacon flour were extracted with 70% ethanol at 80 °C (Pollock, 1982). The extract was dissolved in water and de-salted with a mixed exchange resin (Amberlite MB3, Sigma).

Samples were chromatographed on a Rezex RSO Oligosaccharide Phenomenex column 200 × 10 mm (4% crosslinked resin, silver ionic form) with a Rezex RSO Oligosaccharide guard column 60 × 10 mm (with pre-column), using deionized water as mobile phase at 70 °C. Flow rate: 0.3 mL/min. A Gilson 322 HPLC pump with a refractive index detector and Rheodyne injector with a 20 μL loop were employed. Carbohydrate peaks were identified using analytical standards of fructose, glucose, sucrose, nystose, kestose and fructofuranosylnystose (from SIGMA-ALDRICH). Simultaneously, total sugar was estimated by the phenol-sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.4. Animals and diets

Male Wistar rats weighing 400–410 g were maintained under standard conditions with controlled light (12:12 h light/dark schedule), temperature (23 ± 1 °C) and relative humidity (60 ± 5%). All experiments were conducted in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and were approved by the Institutional Animal Care and Use Committee of the Universidad Nacional de Tucumán (CICUAL-UNT, protocol No. 005/18).

The standard diet contained 12.08 kJ/g: 69.5% from carbohydrates, 5.6% from fat, and 24.9% from protein (Association de Cooperativas Argentinas-S.E.N.A.S.A. No. 04-288/A). The guaranteed analysis of the standard chow indicated: 24.90% crude protein, 5.60% fat (ether extract), 3.60% fat (acid hydrolysis), 4.2% crude fiber, 32.2% starch, 5.0% saccharose, 6.0% ash, mineral and vitamin mix. The mineral mix (expressed as mg/kg) contained calcium, 9200; phosphorus, 7000; Manganese, 30.0; iron, 100.0; cooper, 9.5; selenium, 0.4; iodine; 0.6; zinc; 50.0. The vitamin mix (in mg/kg, except as noted) contained thiamin, 21.0; riboflavin, 6.8; niacin, 80; pantothenic acid, 25.0; pyridoxin, 6.8; cyanocobalamin, 0.5; a-tocopherol, 49.0; menadione, 1.4; folic acid, 3.0; biotin, 0.2; choline, 2000; retinol, 26 IU/g; cholecalciferol, 5.2 IU/g. The dietary ingredients specified by the manufacturer

Table 1
Nutritional composition of yacon flour and dairy products.

	yacon	goat milk	yogurt	yogurt + yacon (7%)
Carbohydrates (g)	82.72 ± 2.67	4.867 ± 0.47	3.6 ± 0.19	6.74 ± 0.34
FOS (DP2 – DP ≥ 10) (g)	65 ± 1.53	0.47 ± 0.05	1.9 ± 0.07	4.55 ± 0.33
Lactose (g)	n/d	3.17 ± 0.42	0.95 ± 0.08	0.94 ± 0.06
Glucose (g)	3 ± 1.01	1.2 ± 0.12	0.4 ± 0.10	0.47 ± 0.02
Fructose (g)	9.12 ± 1.06	n/d	0.3 ± 0.05	0.63 ± 0.05
Sacarose (g)	5.6 ± 1.29	0.027 ± 0.01	0.05 ± 0.01	0.15 ± 0.012
Proteins (g)	2.7 ± 0.15	3.59 ± 0.09	3.7 ± 0.12	4 ± 0.15
Fat (g)	0.17 ± 0.01	3.51 ± 0.14	3.8 ± 0.18	4 ± 0.10
Saturated fats (g)	–	2.015 ± 0.14	2.28 ± 0.11	2.29 ± 0.12
Unsaturated fats (g)	–	0.65 ± 0.03	1.45 ± 0.08	1.6 ± 0.05
Trans Fat (g)	–	0.05 ± 0.01	0.07 ± 0.01	0.06 ± 0.01
Fiber (g)	9 ± 1.08	n/d	n/d	0.5 ± 0.02
Sodium (g)	–	0.035 ± 0.01	0.035 ± 0.01	0.04 ± 0.01
Moisture	6 ± 0.75	–	–	–
pH	-	6.65 ± 0.2	4.8 ± 0.25	4.7 ± 0.15
Energy value (kJ)	760 ± 31.06	255 ± 13.63	250 ± 12.63	285 ± 7.77

The values were expressed as grams each 100 g or 100 mL of food. (n = 3). n/d, not detected.

were as follows: ground grains (corn, sorghum, oats, barley, and wheat), alfalfa meal, extraction flour of soybean, peanut and sunflower, fish meal, porcine meat meal. The high fat diet was standard-based diet enriched with edible lard and carbohydrates (modified from Zhang et al., 2014). The calorie of fat diet was 17.40 kJ/g: 36.0% from carbohydrates, 41.0% from fat, and 23.0% from protein.

2.4.1. Experimental design

Rats were randomly divided into 5 experimental groups receiving the specified diets for 30 consecutive days: the control group (Control), standard diet; group 2 (HFD), high fat diet; group 3 (HFD-yogurt), high fat diet plus yogurt; group 4 (HFD-yacon), high fat diet plus yacon flour; and group 5 (HFD-yogurt + yacon) high fat diet plus yogurt containing yacon flour (Fig. 1B).

Goat yogurt (2 mL) was administered by oral gavage every day at approximately 05:00p.m., before the administration of food. Yacon flour contained the desired intake levels of 680 mg FOS/kg body weight/day (Honoré et al., 2013), was mixed into yogurt (HFD-yogurt + yacon group) or water (HFD-yacon group) before being supplied to the animals.

At the end of the experimental period rats were fasted overnight and deeply anaesthetized with 1:1 xilazine–ketamine. Blood samples were collected by cardiac puncture and serum used for biochemical determinations. Liver, spleen, pancreas and total fat pads (epididymal, perirenal and mesenteric) were removed and rinsed thoroughly with ice-cold saline, blotted, weighed and fixed in 4% formaldehyde saline for histological analysis.

2.4.2. Morphometric and nutritional determinations

Rats food intakes were recorded daily and their body weights were monitored once a week during 30 days of treatment. The body weight and body length were used to determine the following anthropometrical parameters: Body mass index (BMI) = body weight (g)/length^2 (cm^2). Nutritional parameters were calculated based on food and caloric intake: Energy intake (kJ/day) = mean food consumption x dietary metabolizable energy; FER (food efficiency ratio) = (body weight gain/food intake) × 100 (Novelli et al., 2007).

2.4.3. Biochemical determinations

Triacylglycerols, total cholesterol, high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein (LDL-c) concentrations were determined using a *Cobas® c-311* (Roche) autoanalyzer. The Friedewald's equation was applied to calculate the following other plasma lipid fraction: VLDL = TG/5 (Friedewald, Levy, & Fredrickson, 1972). Atherogenic index was calculated as (TG/HDL-c) = Trigliceride/HDL-c (Dobiášová and Frohlich, 2001). Blood glucose concentrations were measured using a glucose meter (Roche Diagnostics GmbH, Mannheim, Germany) Insulin was determined using available commercial kit (RAB0904 SIGMA Aldrich, St. Louis, MO, USA), according to the manufacturer's instructions. The HOMA-IR (Homeostasis model assessment of insulin resistance) index was calculated as [fasting glucose × fasting insulin/22.5] to assess insulin resistance.

2.4.4. Oral glucose tolerance test (OGTT)

Rats were administered orally with 50% D-glucose (2 g/kg body weight) after a 12-h fast. Blood glucose concentration was measured with a Glucometer (Accu-Check; Roche Diagnostics, GmbH, Mannheim, Germany) with blood from tail-tip bleedings at 0, 15, 30, 60 and 120 min. Area under the curve (AUC) was calculated as changes from 0 to 120 min and expressed in (mg/dl/min).

2.4.5. Insulin tolerance test (ITT)

Rats were injected intraperitoneally with 0.75 IU/kg body weight porcine Insulin (Betasint, BETA laboratory) after a 4 h fast. Blood glucose concentration was measured at 0, 15, 30 and 60 min. The areas under the curve (AUC 0–60 min) were calculated and expressed in (mg/dl/min).

2.5. Statistical analysis

All results are presented as the mean ± standard deviation. Graphs were obtained using Prism 6.05 (GraphPad Software Inc., San Diego, CA, USA). Comparisons between two groups were performed by the independent *t* test or analyzed by one-way ANOVA followed by Tukey's post-test when more than one group was compared with one control. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Nutritional value of yacon flour and its derived dairy products

Table 1 shows the obtained results for macronutrients composition, individual sugars and energetic value of the yacon flour, goat milk, yogurt and yogurt + yacon. Yacon flour had low protein (2.7 ± 0.15%), fat (0.17 ± 0.01%) and humid content (6 ± 0.75%). The predominated nutrients in the flour were carbohydrates (82.72 ± 2.67%) and fiber (9 ± 1.08%), inside the carbohydrates we mainly found FOS (65 ± 1.63%), fructose (9.12 ± 1.06%), sucrose (5.6 ± 1.29%) and glucose (3 ± 1.01%). In dairy products (milk and

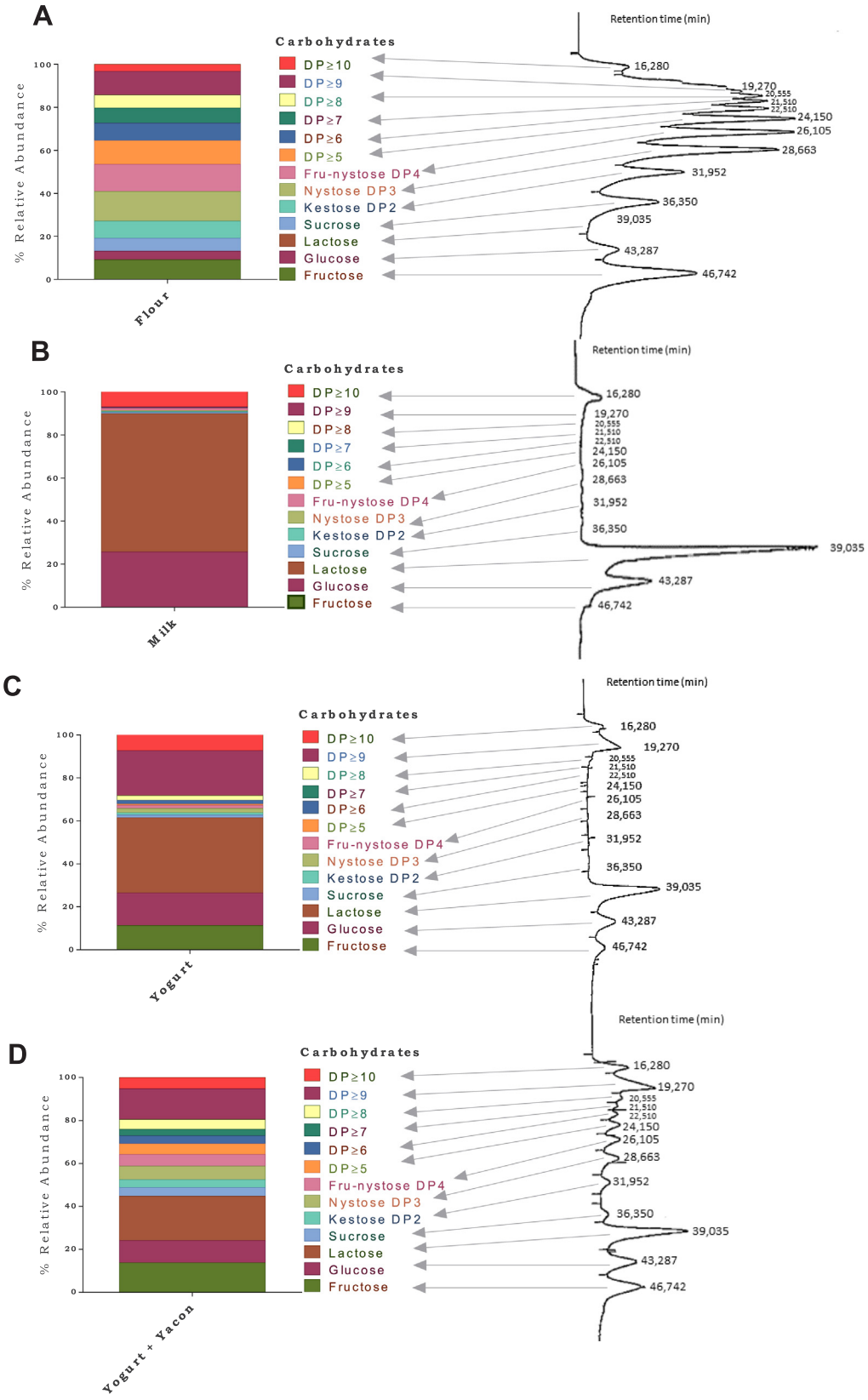

Fig. 2. Relative abundance of carbohydrates in yacon flour and dairy products. Representative histograms and chromatograms of different samples of yacon flour (A), milk (B), yogurt (C) and yogurt with yacon (D) assyed by HPLC.

yogurt) the three main macronutrients were balanced with similar contents of fats (3.5–3.8%), proteins (3.5–3.7%) and carbohydrates (3.6–4.8%), low content in FOS (0.4–1.9%) and absence of fiber. This trend was reversed with the addition of 7% yacon flour to yogurt, reaching a 2.4-fold increase in FOS content (4.55 ± 0.33%) and detectable levels of fiber (0.5 ± 0.02%).

The relative abundance of carbohydrates in yacon flour and dairy products detected by HPLC is shown in Fig. 2. Interestingly, in addition to a high concentration of FOS, we obtained a yacon flour with high abundance in fructans of different degree of polymerization, where the peaks with DP3 (13.8%), DP4 (12.6%) and P ≥ 5 (11%) predominated and to a lesser extent P ≥ 6 (8.2%), P2 (8%), P ≥ 7 (7%) and P ≥ 8 (6%) (Fig. 1S, Supplementary data).

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jff.2018.08.042.

The pH values for yogurt + yacon was 4.8 ± 0.25 and 4.7 ± 0.15 for yogurt (Table 1).

3.2. Microbiological analysis

The goat yogurt obtained was homogeneous, fine, white, uniform, creamy consistency, fragrant, typical of yogurt, pleasant sour taste. The average count of lactic acid bacteria colonies (*St* and *Lb*) on MRS agar was 3.6 ± 1.1 or 2.7 ± 1.7 × 10^7 (cfu/mL) at day 1 (yogurt or yogurt + yacon respectively) up to 3.9 ± 1.3 or 3.5 ± 1.2 × 10^8 (cfu/mL) at day 30 (yogurt or yogurt + yacon respectively) showing an acceptable concentration of lactic acid bacteria for this product and an increase during the shelf lifetime (30 days at 5 °C) (Table 1S, Supplementary data). This increase in lactic acid bacteria was accompanied by a decrease in pH from 4.91 ± 0.07 or 4.94 ± 0.05 at day 1 (for yogurt or yogurt + yacon) to 4.43 ± 0.08 or 4.56 ± 0.06 at day 30 (for yogurt or yogurt + yacon respectively). On the other hand, coliform microorganisms, fungi and yeasts were not detected in all the analysed samples during the shelf lifetime. Pasteurized (20 min at 95 °C) yacon flour did not show the presence of microorganisms in the evaluated media (MRS, HyM, and Mac Conkey).

3.3. Body weight, food intake

As shown in Table 2, the final body weight, body weight gain and BMI index of rats in the HFD group were greater than the values for the Control rats ($p < 0.05$). Supplementation of the HFD-animals with yogurt + yacon significantly reduced the analyzed parameters ($p < 0.05$). When yacon flour was administered to HFD-rats a decrease in final body weight, body weight gain and BMI index was also observed ($p < 0.05$). However, the consumption of yogurt alone, showed a tendency to decrease these parameters in HFD-rats.

In addition, HFD-rats showed increased food and energy intake compared to Control rats ($p < 0.05$) (Table 2). The administration of yogurt + yacon or yacon flour alone to HFD-rats significantly decreased the intake values ($p < 0.05$) and reduced the food efficiency ratio (FER) ($p < 0.05$). However, yogurt supplementation to HFD-rats only showed a tendency to decrease the food and energy intake and FER values (Table 2).

3.4. Body composition

Table 3 shows the effects of the different supplements on the relative organ weights of the animal groups. Supplementation with yogurt + yacon or yacon normalized the increased small intestine weight of HFD-rats ($p < 0.05$). These supplements also reduced the relative weights of spleen ($p < 0.05$). The administration of yogurt to HFD-rats decreased the cecum relative weight. No significant changes were observed in the weights of liver, kidney, heart, colon and pancreas in all the studied groups ($p > 0.05$).

As shown in Table 3 and Fig. 3(A–D), the total body fat (epididymal, perirenal and mesenteric fat) was increased in HFD-fed rats compared with Control group ($p < 0.05$). Supplementation with yogurt + yacon to HFD-animals significantly decreased the amount of total fat, mainly by the reduction of mesenteric fat ($p < 0.05$). Only a slightly decrease was observed in HFD-animals treated with yacon or yogurt. Additionally, the size of adipocytes from HFD-yogurt + yacon and HFD-yacon mesenteric fat-pads were smaller (60% and 50% respectively), compared with adipocytes from control HFD-animals ($p < 0.05$) (Fig. 3E).

3.5. Lipid profile

The high-fat diet induced a serum elevation of triacylglycerols and VLDL-c, and a reduction of HDL-c in HFD-group. The addition of yogurt + yacon in the diet significantly decreased triacylglycerols and VLDL-c levels compared with HFD-fed rats ($p < 0.05$) (Table 4). Furthermore, HDL-c showed a tendency to increase in these animals. Interestingly, a significantly decrease of triacylglycerols and VLDL-c concentrations in HFD-yacon and HFD-yogurt rats was also observed ($p < 0.05$). No significant differences in total cholesterol and LDL-c were shown in all experimental groups. In addition, the TG/HDL-c index was significantly reduced in all supplemented groups particularly in HFD-yogurt + yacon group after 30 days of treatment ($p < 0.05$).

3.6. Glucose metabolism

The fasting glucose and insulin levels, HOMA, OGTTs and ITT were analyzed to verify the effect of the different supplements on HFD-fed rats glucose metabolism (Fig. 4). The study showed that fasting glucose levels were higher in HFD-rats compared to Control rats ($p < 0.05$). A slight increase in basal plasma insulin was also observed. The incorporation of yogurt + yacon in the diet, reduced blood glucose and improved insulin levels of HFD-rats (Fig. 4A and B). Moreover, HOMA-IR index, taken as a measure of insulin resistance, was significantly reduced after 30 days of yogurt + yacon supplementation ($p < 0.05$).

As shown in Fig. 4D, after oral glucose loading, the HFD-group achieved significantly higher plasma glucose concentrations than the Control group at 15, 30, 60 and 120 min. Thus, the increment in AUC of HFD was significantly higher ($p < 0.05$) compared with the Control group. The glycemic response of the HDF-yogurt + yacon group

Table 2
Clinical and nutritional parameters of rats fed experimental diets.

	Control	HFD	HFD-yogurt	HFD-yacon	HFD-yogurt + yacon
Initial body weight (g)	408.5 ± 21.8	407.2 ± 26.8	404.3 ± 27.7	403.1 ± 38.5	402.4 ± 42.8
Final body weight (g)	456.0 ± 39.4	497.1 ± 41.1[a]	477.1 ± 29.5	465.1 ± 34.2[b]	452.3 ± 27.8[b]
Body weight gain (g/30 day)	52.3 ± 12.5	87.3 ± 2.6[a]	74.2 ± 18.2	60.3 ± 24.4[b]	53.9 ± 16.3[b]
BMI (%)	97.5 ± 1.9	109.1 ± 6.9[a]	100.2 ± 5.4	93.7 ± 3.6[a,b]	97.2 ± 4.5[b]
Food intake (g/animal/day)	18.3 ± 1.1	22.85 ± 0.21[a]	21.66 ± 0.91	20.38 ± 0.80[b]	19.04 ± 1.52[b]
Energy intake (KJ/animal/day)	234.2 ± 14.1	397.6 ± 7.6[a]	377.4 ± 14.5[b]	352.6 ± 14.7[b]	331.7 ± 21.4[b]
FER (%)	0.74 ± 0.06	0.74 ± 0.04	0.62 ± 0.17[a,b]	0.47 ± 0.28[a,b]	0.50 ± 0.16[a,b]

Values are means ± SD of n = 6 rats/group. [a]$p < 0.05$ compared to the Control group. [b]$p < 0.05$ compared to the HFD group.

Table 3
Body composition of rats fed experimental diets.

	Control	HFD	HFD-yogurt	HFD-yacon	HFD-yogurt + yacon
Total fat (% bw)	4.69 ± 1.78	6.47 ± 0.86[a]	6.03 ± 1.54	5.91 ± 0.98	5.47 ± 1.08[b]
Liver (% bw)	2.66 ± 0.17	2.89 ± 0.16	2.67 ± 0.18	2.72 ± 0.23	2.66 ± 0.28
Kidney (% bw)	0.34 ± 0.02	0.28 ± 0.04	0.30 ± 0.02	0.30 ± 0.02	0.29 ± 0.03
Heart (% bw)	0.29 ± 0.01	0.30 ± 0.04	0.29 ± 0.03	0.29 ± 0.02	0.28 ± 0.02
Small Intestine (% bw)	0.96 ± 0.09	1.30 ± 0.14[a]	1.03 ± 0.25	1.01 ± 0.20[b]	0.98 ± 0.16[b]
Cecum (% bw)	0.17 ± 0.02	0.17 ± 0.03	0.15 ± 0.01[a,b]	0.17 ± 0.01	0.17 ± 0.02
Colon (% bw)	0.26 ± 0.04	0.20 ± 0.04	0.18 ± 0.04	0.21 ± 0.01	0.20 ± 0.02
Spleen (% bw)	0.14 ± 0.01	0.15 ± 0.02	0.14 ± 0.02	0.13 ± 0.01[b]	0.13 ± 0.01[b]
Páncreas (% bw)	0.32 ± 0.06	0.34 ± 0.04	0.27 ± 0.11	0.27 ± 0.05	0.27 ± 0.09

Values are means ± SD of n = 6 rats/group. [a]$p < 0.05$ compared to the Control group. [b]$p < 0.05$ compared to the HFD group.

significantly improved, being similar to the Control-group ($p < 0.05$). Furthermore, insulin sensitiveness was improved in HFD-yogurt + yacon and also in HFD-yacon animals during the ITT test (Fig. 4E).

4. Discussion

Nowadays, changes in lifestyle and dietary habits have led to several health problems and chronic metabolic diseases (Yoo and Kim, 2016). In this regard, the development of new functional foods is gaining public acceptance around the world (Kumar et al., 2015; Yahfoufi, Mallet, Graham, & Matar, 2018). In this work we presented a yogurt elaborated from powdered goat's milk supplemented with yacon roots flour and evaluated its metabolic effects in HFD-fed rodents.

Fermented dairy products as yogurt have great importance at industrial level since they provide viable lactic bacteria, recognized organisms for their fermentative capacity, as well as for their nutritional and health benefits (Shiby and Mishra, 2013). In this study, goat milk was used as raw material in the formulation of the yogurt due to its high nutrients content, monounsaturated and polyunsaturated fatty acid profile, better digestibility, pH and good buffering capacity in comparison with bovine's milk (Balthazar, , 2017). On the other hand, from an immunological point of view, goat's milk has been reported to have better gastrointestinal tolerance and lower allergene properties than cow's milk (Clark, 2017). These unique characteristics provide technological advantages in yogurt manufacture process and also convert goat dairy products into excellent carrier matrices, by facilitating the survival of viable probiotic bacteria throughout the storage (Balthazar et al., 2017; Ranadheera et al., 2018).

Probiotics used in goat dairy products development have been mostly added as part of the fermentation process, either with starter culture microorganisms or alone (Ranadheera et al., 2018). In the present work, the interactions between *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* used as a starter culture in goat yogurt manufacture, resulted in a product with satisfactory characteristics of texture and acidity (Kristo, Biliaderis, & Tzanetakis, 2003). Total counts of the lactic bacterial strains present in yogurt, were in the range of 10^7–10^8 cfu/mL, maintaining, the viability of the probiotic microorganisms throughout the shelf life. This viable cell content was higher than the minimum required by international regulations (United Nations Organization for Food and Agriculture, 2003) and by Argentine legislation (10^7 cfu/mL) (Argentina, 1969) and would comply with the 'therapeutic minimum' that should be consumed regularly for the transfer of the “probiotic” effect to consumers, as suggested by Lourens-Hattingh and Viljoen (2001). In addition, the safety of the product during the storage is guaranteed by the absence of contaminating microorganisms such as coliforms, fungi and yeasts, probably due to the antimicrobial potential of lactic bacteria (Champagne et al., 2018; Ranadheera et al., 2018).

The efficacy of yogurt as probiotic matrix against other beverages has been demonstrated (Lollo et al., 2013). As we mentioned, different dairy products like cheese and ice cream have also been successfully used as vehicles for the incorporation of probiotic cultures (Lollo et al., 2012, 2015b; Sperry et al., 2018). However, despite the fact that these products can offer a number of advantages compared to fermented milk, their relatively higher content of fat limits their consumption for dietary purposes. Different technical adaptations have been used to increase the viability of probiotics in the final product without additional protein or fat incorporation in manufacturing process (Champagne et al., 2018). Direct supplementation of polysaccharides of short chain into foods, often results in stability of probiotic cultures (Parussolo et al., 2017). In this sense, the incorporation of yacon flour in ice cream has been shown to significantly improve the viability of *Lactobacillus acidophylus* NCFM, maintaining a minimum prebiotic count (10^7 cfu/g) over a 120-day storage period (Parussolo et al., 2017). Our results show that the addition of yacon flour (7%) to goat yogurt increased culture viability during the storage period but not affected the pH of the products, turning it into important alternatives in the development of healthy food. According with our results, Parra (2014) reported that the supplementation of yacon concentrate to bovine milk yogurt leads to a final product with low fat/low caloric value and higher concentration of lactic culture.

S. thermophilus and *L. bulgaricus* are widely and traditionally used as a starter in manufacturing dairy products particularly yogurt. Multiple studies have shown traditional yogurt alone, may be considered to have probiotic activity, since it can mediate improved lactose digestion and absorption in the gastrointestinal tract reducing symptoms of lactose intolerance (Guarner et al., 2005; Sivieri et al., 2017). In line of this, goat yogurt elaborated in this study showed reduced levels of lactose (0.94 ± 0.06%), probably due to *S. thermophilus* and *L. bulgaricus* lactose-hydrolyzing activity in the yogurt, suggesting potential health benefits (Guarner et al., 2005). Furthermore, the addition of 7% yacon flour improved the sugar profile of goat yogurt, increasing the FOS content (4.55 ± 0.33%) in the final product.

Yacon roots, are well-characterized according to its chemical composition and prebiotic properties (Genta et al., 2005, 2009; Lobo et al., 2007; de Moura et al., 2012; Honoré et al., 2013; Delgado et al., 2009). 70–80% of the total dry matter content of yacon tubers consists in FOS as main component and carbohydrates as fructose, glucose, and sucrose (Cao et al., 2018). As the majority of non-digestible carbohydrates, FOS represents an excellent substrate for lactic acid bacteria fermentation. They are selectively fermented by *Bifidobacteria* and *Lactobacillus* in the colon to produce short-chain fatty acids (acetic acid, propionic acid, and butyric acid), causing significant changes in the composition of the gut microflora and affecting gastrointestinal functions (Roberfroid, 2007). Based on these facts, yacon flour could be considered a source of prebiotic fiber, suitable to develop novel enriched foods (Mileib Vasconcelos et al., 2012; Parra, 2014; Parussolo et al., 2017). In addition, dried yacon roots has been recently proposed as an efficient probiotic carrier of some *Lactobacillus* strains (de Souza Leone et al., 2017).

FOS content in yacon roots have a great variability by intrinsic and extrinsic factors (cultivars used, physiological factors, harvest and post-

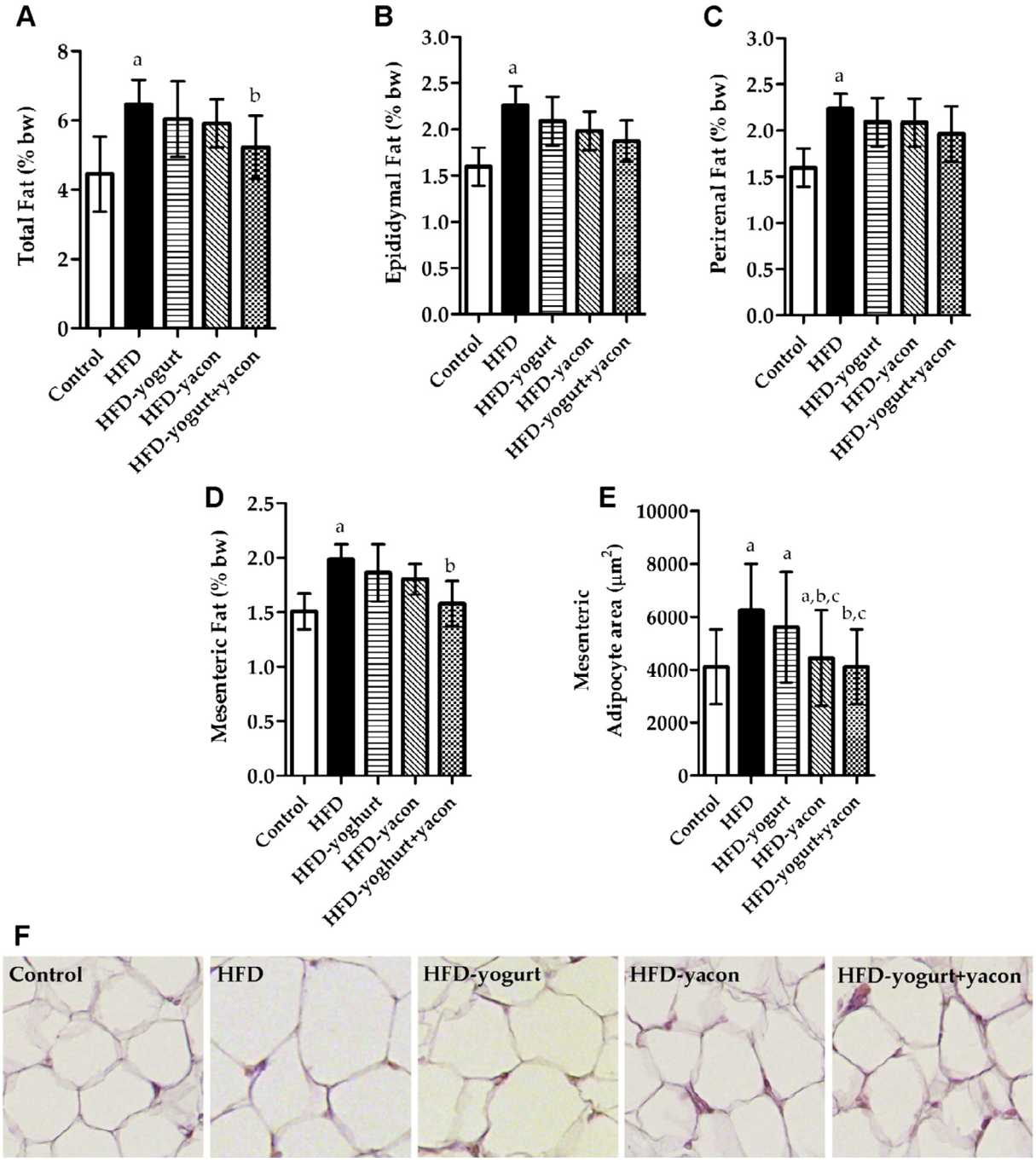

Fig. 3. Effects of goat yogurt + yacon flour on adipose tissue of HFD-fed rats. Epididymal (A) retroperitoneal (B), and mesenteric (C) adipose pad weights in rats fed standard diet (Control) or high-fat diet (HFD) supplemented or not with yogurt (HFD-yogurt), yacon flour (HFD-yacon) or yogurt plus yacon (HFD-yogurt + yacon) after 30 days of treatment. D: Histological pictures from visceral adipose tissue (Magnification 126×). E: Adipocytes size in mesenteric adipose tissue. Data are mean ± SD (n = 6/group). $^{a}p < 0.05$ vs. Control, $^{b}p < 0.05$ vs. HFD, $^{c}p < 0.05$ vs. HFD-yogurt, $^{d}p < 0.05$ vs. HFD-yacon.

Table 4
Lipid profile of rats fed experimental diets.

	Control	HFD	HFD-yogurt	HFD-yacon	HFD-yogurt + yacon
Triglyceride (mg/dl)	70.8 ± 14.9	165.0 ± 11.8[a]	145.47 ± 16.4[a,b]	126.1 ± 15.1[a,b]	113.6 ± 7.3[a,b]
Total cholesterol (mg/dl)	56.0 ± 8.9	68.6 ± 10.6	58.7 ± 4.1	63.5 ± 2.4	60.1 ± 3.7
HDL-c (mg/dl)	39.7 ± 6.3	28.6 ± 4.4[a]	30.6 ± 2.2	34.0 ± 1.8	35.6 ± 3.6
LDL-c (mg/dl)	11.4 ± 1.8	17.4 ± 3.8	14.9 ± 3.6	15.5 ± 4.6	11.2 ± 2.8
VLDL-c (mg/dl)	14.9 ± 2.9	33.0 ± 2.4[a]	20.1 ± 3.3[a,b]	25.4 ± 0.5[a,b]	22.8 ± 1.5[a,b]
TG/HDL-c	1.8 ± 0.6	5.9 ± 1.2[a]	4.3 ± 0.3[a,b]	3.7 ± 0.5[b]	3.2 ± 0.3[b]

Values are means ± SD of n = 6 rats/group. $^{a}p < 0.05$ compared to the Control group. $^{b}p < 0.05$ compared to the HFD group.

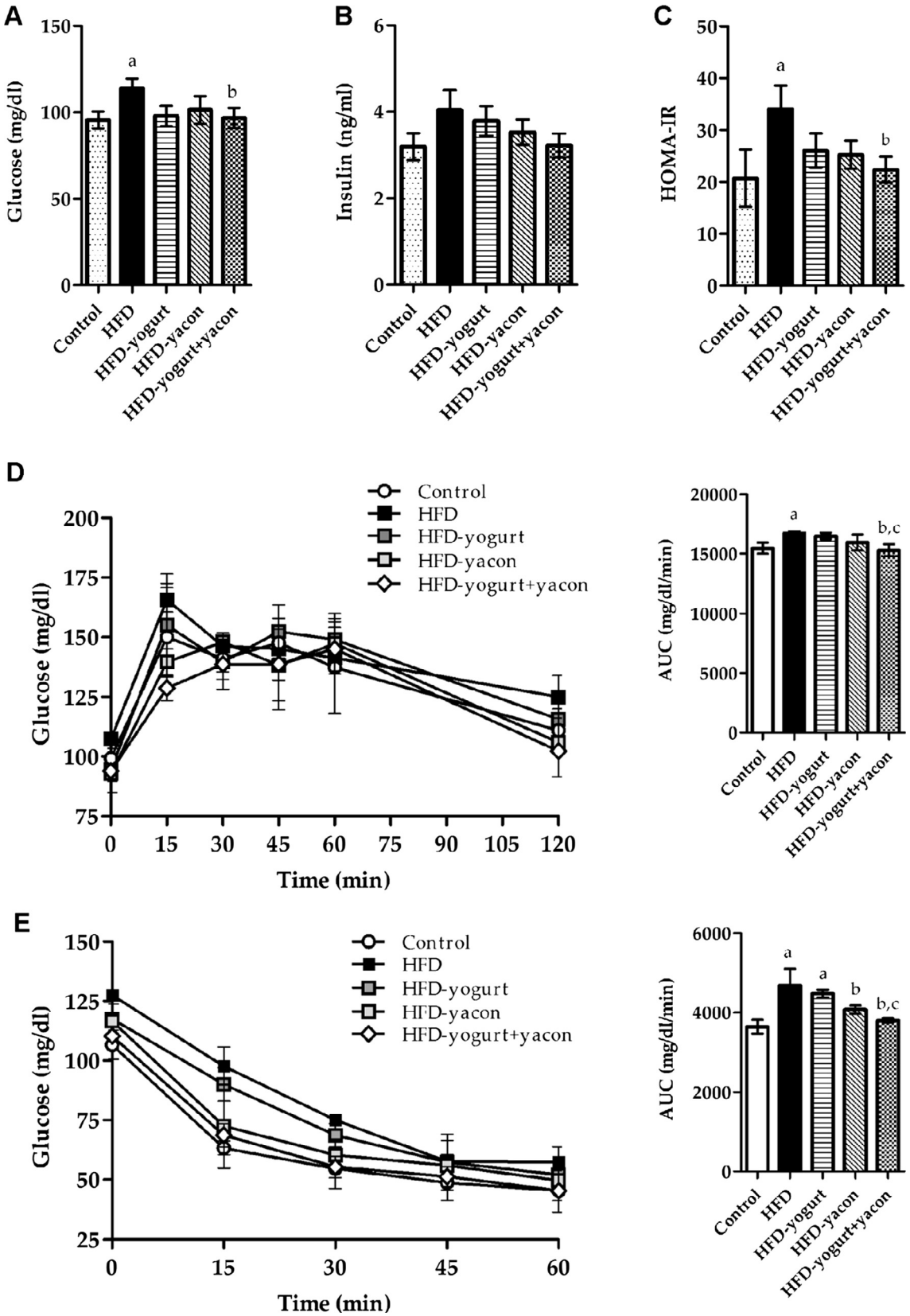

Fig. 4. Effects of yogurt + yacon on HFD-induced metabolic disease. A: Fasting glucose concentration of rats fed standard diet (Control) or high-fat diet (HFD) supplemented or not with yogurt (HFD-yogurt), yacon flour (HFD-yacon) or yogurt plus yacon (HFD-yogurt + yacon) at the end of the experimental period. B: Fasting plasma insulin concentrations at the end of the experimental period. C: Homeostasis model assessment of insulin resistance (HOMA-IR) index. D: Time course of glycemia in response to oral glucose overload (2 g/kg body weight) at 30 days (Insert: area under the curve of blood glucose following glucose overload). E: Time course of glycemia following a single intraperitoneal (i.p.) injection of insulin (0.75 U/kg bw) after 30 days of supplementation (Insert: area under the curve of blood glucose following insulin injection. Data are expressed as the mean ± SD (n = 6/ group). $^{a}p < 0.05$ vs. Control, $^{b}p < 0.05$ vs. HFD, $^{c}p < 0.05$ vs. HFD-yogurt.

harvest conditions, climatic conditions). Then, it is essential to maintain a high concentration of these oligosaccharides over time. In this sense, the methodology used in this work, allowed us to obtain a yacon flour with high concentration of FOS, with different degree of polymerization, being one of the highest concentrations reported in the literature (Kortsarz, Zannier, & Grau, 2015).

Yacon FOS and inulin have been used to improve the texture and organoleptic characteristics of dairy products, replacing fat and carbohydrates, enriching the product in dietary fibers (Mileib Vasconcelos et al., 2012; Parra, 2014; Montarroyos Padilha et al., 2017). However, as far as we know, there is no evidence regarding the *in vivo* metabolic effects of goat yogurt with the addition of yacon flour.

In the present study, the negative effects attributed to the high fat diet (body weight gain, increased body fat, altered glycemic response and lipid metabolism) were significantly improved by the administration of goat yogurt + yacon, even under hypercaloric diet. Nevertheless, the consumption of each products separately, only partially improved these metabolic parameters.

In our study we observed that the supplementation of HFD-rats with yogurt + yacon or yacon flour showed a significant decrease in food intake and in body weight gain. The main action could be related to the combination of probiotics and prebiotics effects. It is known that the presence of short-chain fatty acids not only brings a unique flavor to goat milk, but it can have beneficial effects at the gastrointestinal level improving the gut microbiota (Sivieri et al., 2017). In addition, soluble fibers, also play a regulatory role in intestinal biology, promoting L-cell differentiation and modulating the production of gastrointestinal peptides such as Glucagon-like protein 1 (GLP-1), peptide YY, Glucose-dependent insulintropic peptide (GIP) and ghrelin. These gut hormones called incretins, plays a key role in food intake regulation through central mechanisms (Cao et al., 2018). Habib, Honoré, Genta, and Sánchez (2011) have shown that consumption of yacon flour increases the intestinal production of incretins, particularly GLP-1, with physiological effect on satiety and glucose metabolism in normal and diabetic animals. These established mechanisms may explain the lower food intake and the reduced fat mass development in the HFD-fed animals supplemented with yogurt + yacon. These results are in agreement with the previous observation about the positive effect of yacon syrup on weight loss and visceral adipose tissue mobilization in obese women (Genta et al., 2009).

Visceral fat accumulation in HFD-fed animals has been associated with disturbances in glucose and lipid metabolism (Honoré et al., 2013). In the present work, yogurt + yacon supplementation to HFD-fed rats improved fasting glucose and insulin levels with a reduction in HOMA-IR index, ameliorating peripheral insulin sensitivity. These results could be related to the effects of goat yogurt on blood glucose, as was suggested by Sujono, Hikmawan, Saga, danYuananda (2016). In addition, it has been shown that yacon flour also reduces both postprandial glycemia and plasma insulin in diabetic animals, by improving pancreatic function (Habib et al., 2011).

A variety of *in vitro* experiments and *in vivo* trials have provided evidence to support the role of probiotic products in lowering serum lipids (Sperry et al., 2018; Moura et al., 2016). Our results showed that diet supplementation with 2 mL/animal/day of goat-yogurt improved the lipid metabolism in HFD-fed Wistar rats. Sujono and Dan Putra (2017) showed that yogurt developed from goat milk administered in a higher portion size of 10 mL/day, was effective in lowering the levels of blood triacylglycerols, cholesterol and LDL-c, improving blood HDL-c, in rats under standard chow. Interestingly, a greater effect on triacylglycerols and VLDL-c were seen when the animals were supplemented with goat yogurt + yacon. The effects of yacon flour on lipids metabolism has been previously reported in normal and diabetic animals and was related to the modulating effects of dietary oligofructose in the liver (Genta et al., 2005; Habib et al., 2011).

5. Conclusions

In this research we have successfully developed a functional product by combining a probiotic goat yogurt with yacon flour and evaluated its maintenance during storage. The appearance, consistency, texture, color, flavor and acidity were found to be adequate, and the starter culture was shown to survive in numbers above the recommended minimum limit (10^7 CFU/g) until 30 days. The addition of yacon flour enhanced the number of viable probiotic microorganisms, demonstrating that this ingredient has potential for use as a prebiotic in the food matrix. The addition of yacon flour improved the concentration of fiber in the goat yogurt and the formulation met the standards for the microbiological and physicochemical quality of the final products. Future clinical studies should focus on the study of the effect of this novel food product on the gut microbiota. This dairy product is an interesting technological option for small and medium-size dairy enterprises to enter to the market of functional dairy foods.

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Conflict of interest

The authors did not report any conflict of interest.

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