



A story with two versions: yacon root pulp on experimental asthma in different animal facilities

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ABSTRACT: The intestinal microbiota has an important role in animal health. Therefore, prebiotics have gained interest in the scientific community for their role in manipulating populations of intestinal microorganisms. Among prebiotics, there is *Smallanthus sonchifolius* Poepp. Endl (yacon) root, which can be ingested *in natura* or in different forms, such as flours and extracts. This prebiotic has been associated with beneficial effects in different diseases, including metabolic (like type 2 diabetes) and immunological disorders. Thus, mouse models of human diseases caused by immunological factors have been used to better understand the prebiotic effects of yacon. Since prebiotics interfere with animal microbiota, it is important to take into consideration the quality level of mouse facilities. In this way, the beneficial effects of a yacon root pulp were tested in a mouse model of allergic asthma and, considering how animal facility conditions could affect those results, we performed experiments both in conventional facilities and specific pathogen-free (SPF) ones. Our results showed that better prebiotic effects were observed in a SPF facility than in a conventional one and, in some cases, evidence of yacon beneficial effects was observed exclusively in a SPF facility. For example, there were reductions of 63.6% and 58.0% in IgE and eosinophils, respectively, in immunized/yacon-treated animals compared to immunized mice only. Taken together, our results not only showed the beneficial effects of yacon root pulp in an asthma model but also demonstrated the relevance of performing experiments with prebiotics in SPF facilities.

Key words: *Smallanthus sonchifolius*, IgE, eosinophils, allergy, lactobacilli.

Uma história com duas versões: efeito da polpa da raiz de yacon na asma experimental em biotérios distintos

RESUMO: A microbiota intestinal desempenha um papel importante na saúde dos animais. Portanto, os prebióticos têm despertado interesse na comunidade científica pelo seu papel na manipulação das populações de microrganismos intestinais. Entre os prebióticos, encontra-se a raiz de *Smallanthus sonchifolius* Poepp. Endl (yacon), que pode ser ingerida *in natura* ou em diferentes formas, como farinhas e extratos. Esse prebiótico tem sido associado aos efeitos benéficos em diferentes doenças, incluindo distúrbios metabólicos (como diabetes tipo 2) e distúrbios imunológicos. Assim, modelos de camundongos de doenças humanas causadas por fatores imunológicos têm sido usados para melhor entender os efeitos prebióticos do yacon. Uma vez que os prebióticos interferem na microbiota animal, é importante levar em consideração o nível de qualidade das instalações dos camundongos. Dessa forma, os efeitos benéficos da polpa de raiz de yacon foram testados em um modelo de camundongo de asma alérgica e, considerando como as condições da instalação dos animais poderiam afetar esses resultados, realizamos experimentos tanto em instalações convencionais quanto em instalações livres de patógenos específicos (LPE). Nossos resultados mostraram que melhores efeitos prebióticos foram observados em uma instalação LPE do que em uma convencional e, em alguns casos, evidências dos efeitos benéficos do yacon foram observadas exclusivamente em uma instalação LPE. Por exemplo, houve reduções de 63,6% e 58,0% nos níveis de IgE e eosinófilos, respectivamente, em animais imunizados/tratados com yacon em comparação com camundongos imunizados apenas. Em resumo, nossos resultados não apenas mostraram os efeitos benéficos da polpa de raiz de yacon em um modelo de asma, mas também demonstraram a relevância de realizar experimentos com prebióticos em instalações LPE.

Palavras-chave: *Smallanthus sonchifolius*, batata yacon, IgE, eosinófilos, lactobacilos.

INTRODUCTION

The mammalian digestive tract comprises the mouth, oral cavity, pharynx, esophagus, stomach, and small and large intestines (CHIVERS & LANGER, 1994). Despite the differences found in

each mammal species, it is accepted that microbiota has an important role in digestive system function and animal health. For example, it has been shown that early-life microbial interactions can modulate immune system development and the subsequent development of atopic diseases. These authors

highlighted that the composition of the microbiota during early life could have lasting effects on immune system maturation and function (DONALD & FINLAY, 2023). Disruptions in this delicate balance between the microbiota and the immune system have been associated with increased susceptibility to allergic conditions. Furthermore, other authors explored the role of gut microbiota in infectious and inflammatory diseases. They highlighted the intricate relationship between the microbiota and the immune system in maintaining gut homeostasis and protecting against pathogenic infections. Dysbiosis, an imbalance in the composition of the gut microbiota, has been implicated in various inflammatory diseases, including inflammatory bowel disease (IBD) and autoimmune disorders (MACIEL-FIUZA et al., 2023).

Regarding this last part, prebiotics have gained an increased interest in the scientific community because it is based on the manipulation of microbiota by modulation of animal diets (GIBSON et al., 1995; GIBSON & ROBERFROID, 1995; GUARNER, 2007). In this way, a prebiotic is defined as a beneficial non-digestible food ingredient that affects the growth and/or activity of a limited number of bacteria in the intestine, improving the host's health (GIBSON et al., 1995; GIBSON & ROBERFROID, 1995; GUARNER, 2007). But what types of food can be used to enrich diets for this purpose?

There are a lot of different foods that can be used as prebiotics. Among them, yacon root (*Smallanthus sonchifolius* Poepp. Endl) is one of the many types of tubers and roots (NABESHIMA et al., 2020) that has been shown to have beneficial effects on body health (ADRIANO et al., 2019; CAETANO et al., 2016; DELGADO, et al., 2013; DELGADO, et al., 2012; SILVA et al., 2017; HABIB, et al., 2011; KHAJEHEI et al., 2018; MOURA et al., 2012). This root is rich in fructooligosaccharides (FOS) that can increase the amounts of lactobacilli and Bifidobacterium sp. bacteria *in vitro* (PEDRESCHI et al., 2003) and *in vivo* (RODRIGUES, et al., 2012). Despite all the beneficial effects, one single case of yacon allergy after consumption was described (YUN et al., 2010), demonstrating that certain caution on its intake should be considered.

The beneficial health effects of yacon seem to be related to a myriad of factors, including alterations in the functions of many body systems. For example, it was shown that postprandial glucose and insulin levels were decreased in yacon-treated groups, indicating that FOS present in yacon root pulp

was responsible for altering the endocrine system of patients (ADRIANO et al., 2019). Other studies have shown that yacon constituents could suppress *in vitro* nitric oxide production by RAW 264.7 macrophages (HONG et al., 2008), improve THP-1 cell (human monocyte cell line) phagocytosis of yeasts (PAREDES et al., 2018), trigger activation of peritoneal macrophages for IL-1 production and increased IgA production by B cells in mice (DELGADO, G. T. et al., 2012), and increase gut immune responses against intestinal infections (VELEZ et al., 2013). However, more studies are needed to understand yacon effects on different body systems or even inside a particular one. This better understanding should take into consideration the environmental conditions where the experimental groups are set. In other words, animal facility conditions must have good sanitary control; otherwise, mouse guts infection with different types of microorganisms can lead to a different interpretation of prebiotic results.

This study investigated the effects of yacon as a prebiotic on the immune response, specifically focusing on its potential to decrease allergy-related parameters such as IgE production and eosinophil infiltrates in immunized mice. Additionally, the study explored the influence of sanitary conditions of animal facilities on these results, with a particular emphasis on observing whether the beneficial effects of yacon root pulp are more pronounced in mice kept under specific pathogen-free (SPF) conditions.

MATERIALS AND METHODS

Animals

Female BALB/c mice (*Mus musculus*), 12 to 16 weeks old and with an average weight of 25 g, were used in our experiments. They were located in two different animal facilities: a conventional animal facility (conventional), with filtered water, non-sterile mouse food (Nutri, Nutriave Ltda, Brazil), and sterile bedding (121 °C, for 20 min); other mouse groups were kept in a specific pathogen-free animal facility (SPF), with sterilized water, cages (both at 121 °C, for 20 min), bedding (gamma-irradiated pinnus flakes, Granja R.G. Ltda, Brazil), and mouse food (gamma-irradiated, Nuvilab CR-1, Quimtia Ltda, Brazil). In both animal facilities, all mice were kept under a light/darkness cycle of 12 h, 22 ± 2 °C, 55 % humidity, and cage exchanges were performed inside a sterile flow hood (Pachane Ltda, Brazil). All procedures were approved by the “Universidade Vila Velha” ethics committee for animal research (CEUA-UVV) under protocol number 583/2020.

Sample size calculation was performed according to the formula (MIOT, 2011):

$$n = (Sa^2 + Sb^2) \cdot \left(\frac{Z_{\alpha/2} + Z_{\beta}}{d} \right)^2$$

In our experiments, the tolerance to detect differences when they do not exist (type I error) or failure to do so between the groups when they exist (type II error) was standardized as errors α and β , and values of 5% (bilateral) and 20% were adopted, respectively. Therefore, each equation symbol is defined below:

n: sample size; Sa and Sb: standard deviation of the variable in each group; $Z_{\alpha/2}$: value of error α , chosen as 1.96 (5%); Z_{β} : value of error β , chosen as 0.84 (20%); d: minimum difference between the mean values. Taking into consideration published data in an asthma model, for IgE levels, Lactobacilli amounts, and Eosinophil infiltrates, we considered Eosinophil infiltrates from previous published data (ÖZKAN et al, 2021), since this was the variable with the largest number of mice needed. Then, according to the data presented in the paper above cited, we assumed Sa = 1, Sb = 2,5, d = 5 [values from eosinophil percentages inside lung samples from mice immunized with Complete Freund's Adjuvant (CFA) + Ovalbumin (OVA)] and the result was 3.76. This corresponds to a sample size (n) for each group of 4 mice as described in the item "*Diet and experimental groups.*" Randomization was performed by selecting 1 animal from 4 different litter cages until the total amount per cage was reached. There was no control for confounders. Only the first authors were aware of the group treatments during the whole procedure.

Yacon root pulp (YRP)

Yacon tuberous roots were bought in a local market (Vila Velha, Espírito Santo, Brazil) and processed. First, all inappropriate parts for consumption and bark were removed, and the roots were washed in running water. After that, the weight of root slices was measured for further citric acid (Sigma Aldrich Inc., USA) addition (0.05 % in distilled water). These root slices were heated at 100 °C, for 4 min, according to a described protocol to avoid oxidation (VASCONCELOS et al., 2015). After heating, root slices were transferred to ice at a root-water proportion of 1:1. After ice melting, water was removed and the roots were ground in a kitchen processor (Walita, Philips, Brazil) until a homogeneous aggregate was produced. For further sterilization, this aggregate was autoclaved for 20 min, 121 °C, and sampled in 2 ml Eppendorf tubes (Eppendorf, Germany), kept frozen at -20 °C until use.

YRP characterization

Centesimal composition analyses were performed according to "INSTITUTO ADOLFO LUTZ" and the ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS methods (HORWITZ, 1975; ZENEBO; PASCUET; TIGLEA, 2008) (HORWITZ, 1975; ZENEBO; PASCUET; TIGLEA, 2008). Humidity was determined by drying at 105 °C until the samples attained constant weight. The amounts of fructooligosaccharides (FOS), fructose, glucose, saccharose, and inulin were determined by HPLC at AGRILAB (Laboratório de Análises Agrícolas e Ambientais, Botucatu, SP). Briefly, sterile yacon pulp samples were centrifuged (model 206, FANEM Inc., Brazil) at 4,000 rpm, for 5 minutes, to separate the solid material. The supernatant was transferred to 2.0 ml tubes (Eppendorf Inc, USA) and centrifuged again (model NT805, Novatécnica Inc., Brazil) at 12, 000 rpm and at a temperature of 10 °C. The supernatants were filtered (Millipore polyvinylidene difluoride (PVDF) membrane, 0.22 μ m) and injected in a Shimadzu chromatograph, model 10A, with a refractive index detector (RID-10A. The isocratic pump, model LC-10AD, was used with a mobile phase flow of 0.6 ml/min and purified water. The working temperature was set at 80°C, controlled by an oven, model CTO-10A. The stationary phase used was an Agilent HI-PLEX PB 7.7 X 300 mm 8 UM column (lead stationary phase), and the sample injection volume was 20 microliters. The collected data formed a sequence of peaks that were compared to the curves of standard patterns such as Dália inulin standard, Chicory inulin standard, FOS (commercial fructooligosaccharide PA), sucrose, glucose, and fructose at pre-defined concentrations and injected into the equipment. The retention time of each standard was observed for sugar identification, and the area of each peak was determined using the N2000 Chromatography Data System software for sugar concentration calculation.

The fractionated and identified sugars were calculated by comparing their areas with those of their respective standards and multiplying by the sample dilution.

Diet and experimental groups

After FOS determination, the amount of YRP in ml was determined for a dose of 0.14 g.kg⁻¹ of FOS per day (GENTA et al., 2009). Four groups of mice were established in our "Conventional" or "SPF" facilities, each containing 4 mice/group. Thus, we had these groups: control (CTR), water gavage for 42 consecutive days and non-immunized

mice; treated with YRP only (YRP), yacon root pulp gavage for 42 consecutive days and no immunization; immunized with ovalbumin (OVA), water gavage for 42 consecutive days and ovalbumin immunization; treated with YRP and immunized with ovalbumin (YRP+OVA), yacon root pulp gavage for 42 consecutive days and immunization with ovalbumin. Table 1 shows a schematic view of these groups.

Immunization and challenge with OVA

For mice immunization, on YRP/water gavage days 19 and 33, 200 μ l of a mix of OVA (1 $\text{mg}\cdot\text{ml}^{-1}$) and aluminum hydroxide (10 $\text{mg}\cdot\text{ml}^{-1}$) was intraperitoneally injected in each mouse (TADOKORO et al., 1996).

For pulmonary challenging, each animal was anesthetized by an intraperitoneal injection of 100 $\text{mg}\cdot\text{kg}^{-1}$ ketamine (Cetamin[®], Syntec Ltda, São Paulo, Brazil) and 10 $\text{mg}\cdot\text{kg}^{-1}$ xylazine (Xilazin[®], Syntec Ltda, São Paulo, Brazil); later, each nostril received 10 μ l of OVA suspension (1 $\text{mg}\cdot\text{ml}^{-1}$) in PBS. This challenge was performed on days 40, 41, and 42 after the beginning of gavage (LAFAILLE et al., 2008).

Blood samples

All animals were euthanized on day 43 after YRP/water gavage and tissue samples were harvested. Among them, blood samples were harvested by heart puncture, and serum was separated by centrifugation (400g, 10 min). IgE was measured according to manufacturer conditions (Kit BD OptEIA[™], catalog # 555248, BD Biosciences Inc., USA). For evaluation of eosinophil percentages, blood or lung cells were stained with a panel of monoclonal antibodies (MAbs).

First, blood samples had their erythrocytes removed by lysis (BD FACS[™] lysing solution, cat. # 349202, BD Biosciences Inc., USA), according to manufacturer procedures. After that, blood and lung samples were incubated with a “FcBlock” MAb (anti-CD16/CD32, catalog # 553141, BD Biosciences Inc., USA), for 20 min., R.T. Later, an antibody cocktail was added to each sample, containing anti-Siglec-F PE-Cy7 (catalog # 25-1702-82, Thermo Fisher Scientific Inc., USA), anti-CD11b APC-eFluo780 (catalog # 47-0118-42, Thermo Fisher Scientific Inc. USA), and anti-Ly6G FITC (catalog # 561105, BD Biosciences Inc., USA). Eosinophils were identified as Siglec-F⁺ cells inside a population of CD11b^{low} and LY6G⁻ population from the whole blood/lung sample (GESLEWITZ et al., 2018). These samples were incubated for 30 min at R.T. After this period, each sample was centrifuged (400 g, 10 min), resuspended in FACS buffer (PBS containing 2% FCS and 0.1% Sodium Azide), and percentages of eosinophils determined by flow cytometry. We used a NovoCyte 3000 from Agilent Inc., USA.

Lung samples

The right side of the lungs was harvested and washed in PBS. Later, each lung sample was transferred to a 24-well plate (Kasvi Inc., USA), received a collagenase-type IV solution (Thermo Fisher Inc., USA) at 2 mg/ml in RPMI-1640 (Thermo Fisher Inc., USA), and was minced in small pieces with scissors. These samples were incubated for 2 h in a cell culture humidified incubator, at 36.5 °C and 5% CO₂. Then, each sample was filtered in 70 μ m diameter cell strainers (Corning Inc., USA), which were washed with 12 ml of RPMI-1640 medium.

Table 1 - Mouse facilities, mouse groups, and treatments.

Facility	Mouse groups ^a	Ingestion ^b	Immunizations ^c	Challenge ^d
Conventional	CTR	Water	No	Yes
	YRP	Yacon root pulp	No	Yes
	OVA	Water	Yes	Yes
	YRP+OVA	Yacon root pulp	Yes	Yes
SPF	CTR	Water	No	Yes
	YRP	Yacon root pulp	No	Yes
	OVA	Water	Yes	Yes
	YRP+OVA	Yacon root pulp	Yes	Yes

^a Each group had 4 mice from a conventional or Specific Pathogen Free (SPF) mouse facility.

^b Each mouse received water or yacon root pulp by gavage, for consecutive 42 days.

^c Each immunized mice received intraperitoneal injections of an ovalbumin solution mixed with aluminum hydroxide, on days 19 and 33, after the beginning of gavages.

^d Each mouse received an intranasal ovalbumin solution on days 40, 41, and 42 after the beginning of gavages.

After new centrifugation (400 g, 5 min), each sample was resuspended in complete medium (RPML-1640 containing 10 % FCS, Glutamin 2 mM, penicillin/streptomycin 100 $\mu\text{g}\cdot\text{ml}^{-1}$, 2-mercaptoethanol 0.05 M). From each of these samples, 20 μl was used to stain for flow cytometry as described for blood samples.

Fecal sampling and microbiological analyses

Fecal samples were harvested from each group of mice on day 41 after the beginning of YRP/water gavage. Later, 1 g of fecal samples were diluted in 9 ml of buffered peptone water (Merck Inc., Germany) at 0.1%. From this suspension, 10^{-1} to 10^{-5} dilutions were prepared in this same buffer, and 100 μl of each 10^{-3} , 10^{-4} , and 10^{-5} suspensions were plated in Man Rogosa & Sharpe solid agar (Merck Inc., Germany) for lactobacilli colony forming units (CFU) countings, 72 h after culture at 36.5 °C in anaerobiosis.

For counting yeast CFU, other 100 μl samples from 10^{-1} , 10^{-2} , 10^{-3} foecal dilutions were plated on Sabouraud Dextrose with Chloramphenicol solid agar (Kasvi Inc., USA), 48 h after culture at 36.5 °C in aerobiosis.

Finally, for thermotolerant coliform determination, we used the most probable number method where replications of the fecal dilutions were cultured to determine the presence of these bacteria. Initially, we prepared multiple dilutions of 10^{-1} to 10^{-5} samples, and the presumptive phase was performed using lactose broth (Merck Inc., Germany) for 48 h at 36.5 °C. Later, the confirmative phase was performed in positive tubes from the presumptive phase, where these microorganisms were then cultured in Brilliant Green Bile broth (Himedia Inc., India) for another 48 h at 36.5 °C, and EC Broth (Acumedia Ltda, Brazil), for 48 h at 45 °C. The results were obtained based on a reference table previously published elsewhere (SILVA & JUNQUEIRA, 2017).

Statistical analyses

All data was submitted to one-way Analysis of Variance (ANOVA) with Tukey correction to determine whether there is a difference between the mean of all possible pairs. All F and R^2 data are provided in each Figure legend, and $P < 0.05$ was considered statistically significant. All data were processed in a Prism 5 software (GraphPad Inc., USA).

RESULTS AND DISCUSSION

Composition of YRP and FOS concentration

Our results indicated that YRP treatment can decrease experimental allergic asthma, altering

IgE (Figure 1), eosinophils (Figure 2), and microbiota levels (Figure 3). However, before describing these results, we initially evaluated the composition of our YRP preparation, which allowed the determination of YRP doses to be delivered by gavage to our mice. Most of YRP is composed of water, around 93% of its weight (Table 2), and it is not affected by sterilization, as the carbohydrate contents were. However, when we checked the FOS amounts in our samples, sterilization decreased about 43% of its content (Table 2). This decrease in FOS amounts was also observed by other authors who submitted their preparations to 60 °C (SILVA et al., 2020) or 120 °C (L'HOMME et al., 2003). It is interesting to note that glucose and fructose concentrations were unchanged (Table 2). Since we established a dose of 0.14 $\text{g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ for each mouse, the amount of YRP injected by gavage into our mice was two doses of 270 μl per day. This volume was in accordance with the physiological mouse stomach capacity for normal ingestion of food.

In vivo YRP effects on IgE production

Classical asthma models in mice show that compromised animals had increased production of IgE against the allergen (LAMBRECHT et al., 2019). Therefore, we used one of these protocols to trigger asthma in our mice, which were treated or not with YRP. We could observe that allergic and YRP-treated mice (YRP + OVA group) had a decrease in IgE levels in their serum, when compared with allergic mice (OVA group); these differences were better observed in animals kept in SPF than conventional mouse facilities

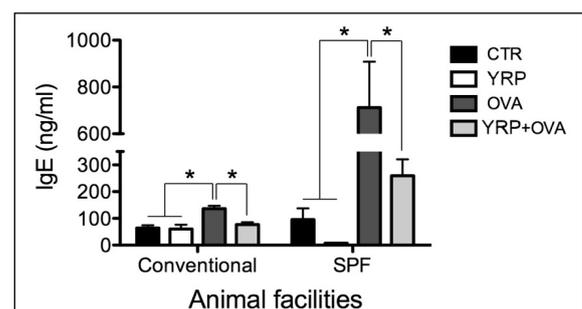


Figure 1 - IgE production is curbed by yacon root pulp (YRP) ingestion. Each animal received YRP or sterile water by gavage, and they were immunized or not with ovalbumin (OVA). On day 43, mice were euthanized, and blood samples harvested. The total IgE amount was quantified by ELISA and the results are representative of 2 independent experiments. Each column represents mean and standard deviations of each group. * $P < 0.05$. Conventional: $F = 10.40$; $R^2 = 0.7394$. Specific Pathogen Free (SPF): $F = 12.61$; $R^2 = 0.8631$.

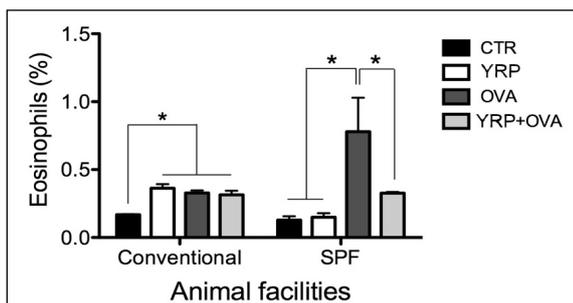


Figure 2 - Eosinophil infiltration is curbed by yacon root pulp (YRP) ingestion in Specific Pathogen Free (SPF) mice. Each animal received YRP or sterile water by gavage, and they were immunized or not with ovalbumin (OVA). On day 43, mice were euthanized, and lung samples harvested. The eosinophil percentages were determined by flow cytometry (FACS) and the results are representative of 2 independent experiments. Each column represents mean and standard deviations of each group. * $P < 0.05$. Conventional: $F = 11.35$; $R^2 = 0.7731$. Specific Pathogen Free (SPF): $F = 4.542$; $R^2 = 0.6023$.

(Figure 1). Thus, a diet supplemented with YRP can suppress the production of this classical asthma-related immunoglobulin (MATUCCI et al., 2021). Differences between IgE levels in OVA immunized groups from different animal facilities were also observed ($P < 0.0001$), in which an 80.8% reduction in IgE production in animals kept in the conventional facility

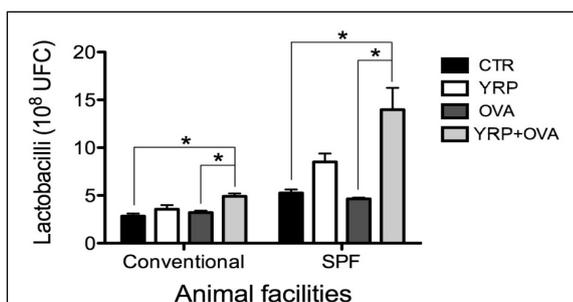


Figure 3 - Lactobacilli Colony Forming Units (CFU) are high in the intestines of yacon root pulp (YRP)-treated mice. Each animal received YRP or sterile water by gavage, and they were immunized or not with ovalbumin (OVA). On day 43, fecal samples from these mice were harvested and the number of lactobacilli CFU determined. These CFU were counted in plates with Man Rogosa & Sharpe solid agar, and the results are representative of 2 independent experiments. Each column represents mean and standard deviations of each group. * $P < 0.05$. Conventional: $F = 8.489$; $R^2 = 0.7610$. Specific Pathogen Free (SPF): $F = 11.83$; $R^2 = 0.8160$.

was observed when compared with SPF facility data (Figure 1). In terms of IgE production, the OVA+YRP treated groups exhibited reductions of 43.5% and 63.6% in IgE levels under Conventional and SPF conditions, respectively, compared to those immunized with OVA alone. This underscores the importance of maintaining specific animal facility conditions for ensuring the quality of final data.

In vivo YRP effects on eosinophils

Eosinophils are frequently present in tissues where the allergic response is active (LU et al., 2010), so we decided to evaluate the amount of these cells in lung and blood samples. Despite no differences reported in blood samples (data not shown), the amounts of eosinophils in the allergic group (OVA) of animals kept in SPF conditions (Figure 2) was higher than in groups of animals not allergic (CTR or YRP); even more interesting (Figure 2), eosinophil levels were lower in OVA-immunized animals that ingested YRP (YRP+OVA group) than OVA-immunized mice (OVA group). These data corroborate with lower IgE levels in YRP+OVA mice (Figure 1), confirming that YRP has an anti-allergic effect.

If the conditions of the animal facility have already been shown to affect IgE levels (as illustrated in Figure 1), the significance of these conditions becomes even more apparent when considering the presence of eosinophil infiltrates within the lungs. Specifically, it is noteworthy that a marked YRP effect on eosinophil levels was observed exclusively in animals housed under specific pathogen-free (SPF) conditions (as depicted in Figure 2). Similar to IgE levels in OVA immunized groups, Eosinophil accumulation in lungs from different animal facilities was observed ($P = 0.0006$), in which a 58.0% reduction in eosinophil accumulation in animals kept in the Conventional facility was noticed when compared with SPF facility data (Figure 2).

YRP effect on intestinal microbiota

It is already established by other authors that prebiotics (KOLIDA et al., 2002) and oligosaccharides (BOUHNİK et al., 2007; GIBSON et al., 1995; SHINOHARA et al., 2020) can alter intestinal microbiota, allowing fewer inflammation responses and, apparently, fewer Th2 type of responses (LIM et al., 1997; LIU et al., 2015; MATSUZAKI et al., 1998; SEGAWA et al., 2008). Therefore, we checked for the presence of some intestinal bacteria and fungi to check if any correlation between low IgE/eosinophil amounts and beneficial microorganisms could be observed.

Table 2 - Composition of yacon root pulp (YRP), before and after sterilization.

Component	Non-sterile	Sterile
Humidity	93.42 %	93.45 %
Dry matter	6.58 %	6.55 %
Carbohydrate	6.25 %	5.12 %
Glucose	1.37 mg/ml	1.29 mg/ml
Fructose	1.66 mg/ml	1.72 mg/ml
Sacarose	0.98 mg/ml	0.77 mg/ml
Inulin D	0.55 mg/ml	0.37 mg/ml
Inulin ch	0.55 mg/ml	0.32 mg/ml
FOS	1.14 mg/ml	0.65 mg/ml

^a Sterile YRP was prepared as described and sterilized at 120 °C for 20 min.

Initially, we could not find any differences in thermotolerant coliforms and yeasts in fecal samples from the mice (data not shown). These results indicated that the YRP mechanism of action does not involve a decrease in the amounts of these microorganisms. However, lactobacilli amount in fecal samples from YRP-treated groups were higher than other groups (Figure 3) regardless of whether mice were kept in conventional or SPF facilities; although, this difference was better observed in SPF conditions (Figure 3). Thus, these results indicate that lactobacilli inside YRP-treated mice would be involved in the control of these allergic reactions. It is possible that lactobacilli interfere with a typical Th2 type of response, as shown by other authors (MATSUZAKI et al., 1998; SEGAWA et al., 2008), but the molecular mechanisms involved in the suppression here reported is still to be further determined. Lactobacilli amounts were also compromised in the Conventional animal facility; for example, the number of lactobacilli in animals from the SPF facility, treated with OVA+YRP, had 2.84 higher counts than OVA+YRP treated mice from the Conventional facility (Figure 3).

CONCLUSION

Our findings shed light on the role of yacon in allergy and emphasized the significance of conducting prebiotic studies under controlled environmental conditions. It is crucial to recognize that poor sanitary conditions can result in underestimated interpretations of prebiotic effects or even the complete loss of observable effects. Therefore, investigations involving the modulation of intestinal microbiota and systemic effects should be carried out

in specific pathogen-free (SPF) conditions. Notably, yacon root pulp exhibits significant prebiotic effects in experimental asthma by reducing IgE production and eosinophil infiltrates. However, eosinophil reduction was observed in SPF conditions only, highlighting the importance of using such facilities when considering prebiotic effects.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest for this article. The founding sponsors had no role in the study design, collection, analysis, or interpretation of data, in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors conceived and designed the experiments. RMAM, GRC, CMA, JFD, and CET performed all experiments. FC, DCOG, and CET supervised and coordinated the experiments. CET performed statistical analyses of experimental data. FC, DCOG, and CET prepared the draft manuscript. All authors critically reviewed the manuscript and approved the final version.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

This study was approved by the Ethics Committee on Animal Use (CEUA), Protocol n° 583/2020 of Universidade Vila Velha (UVV).

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