



Development of symbiotic yoghurt and biological evaluation (New Zealand White Rabbits) of its functional properties

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Abstract

Functional foods are those that bring benefits to human health beyond nutrition such as the prevention of cardiovascular diseases, allergies and intestinal problems. Among the functional foods highly consumed worldwide, yogurt is highlighted, especially in the probiotic and symbiotic versions. The aim of this study was to use the Jerusalem artichoke tuber flour (*Helianthus tuberosus* L.) as a new prebiotic source for the development of functional yogurt. The identification and quantification of the fructans of the Jerusalem artichoke flour was performed and later yoghurt formulations were elaborated. These yogurts were submitted to analyses of centesimal composition, shelf life and sensorial evaluation. For the biological evaluation, a symbiotic yogurt formulation was chosen (the best accepted in sensorial evaluation) and included in the feed of New Zealand white rabbits, in the growth and final phase. The in vivo experiment had three distinct groups (control group, probiotic group and symbiotic group). At the end of 50 days of experiment, the animals were euthanized and submitted to the serological and cecal microbiota evaluation. Through this study, the prebiotic/bifidogenic capacity of Jerusalem artichoke flour was evidenced.

Keywords: functional foods; biological evaluation; *Helianthus tuberosus* L.; symbiotic yogurt.

Practical Application: New prebiotic/ functional ingredient alternative for application in fermented dairy products.

1 Introduction

Since 1991, there has been food regulation called “Foods for Specified Health Use” (FOSHU). In this context, it is possible to observe a growing consumer demand for food that offers health benefits, such as disease prevention and, consequently, an improvement in their quality of life (Santos et al., 2006).

In order to meet this food profile, the food industry, science and technology have advanced in the use of functional ingredients (Pinto & Paiva, 2010). Functional foods are found virtually in all food categories, however products are not homogeneously scattered over all segments of the market. The development and commerce of these products is rather complex, expensive and risky, as special requirements should be answered. Besides potential technological obstacles, legislative aspects, as well as consumer demands need to be taken into consideration when developing functional food. In particular, consumer acceptance has been recognized as a key factor to successfully negotiate market opportunities (Siró et al., 2008).

Yogurts, cereals, fermented milk and other functional products are trend in this niche market. This is due to the benefits these foods bring to human health. For instance, aid in curing or prevention of diseases such as those affecting the cardiovascular system, many types of allergies and intestinal problems, as well as the growing concern with health and well-being, and the

growing scientific evidence of the relationships existing between diet and health (Raud, 2008).

Moreover, as an ingredient used in functional food formulations, the so-called prebiotics stand out. They are defined as nondigestible food ingredients that beneficially affect the organism by selectively stimulating the growth and/or activity of bacteria in the colon. This is a substance that modifies the composition of the colonic microbiota in such a way that the bacteria with health promotion potential become the predominant majority (Capriles et al., 2005).

Prebiotics promote the survival or persistence of probiotic strains, enhance defense mechanisms of the host, increase resistance to various health disorders, and modify human gastrointestinal tract troubles (Singh & Singh, 2010; Bañuelos et al., 2008; Mountzouris et al., 2006).

On the other hand, probiotics are live microorganisms which when administered in adequate amounts and frequency confer a health benefit on the host (Food and Agriculture Organization of the United Nations, 2001; Roberfroid et al., 2010). The beneficial influence of probiotics on the human intestinal microbiota includes factors such as antagonistic effects, competition and immunological effects, resulting in increased resistance against pathogens (Uyeda et al., 2016).

Received 14 Jun., 2018

Accepted 19 Nov., 2018

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A product in which a probiotic and a prebiotic are combined is called symbiotic. Interaction between the probiotic and the prebiotic in vivo might be favored by an adaptation of the probiotic to the prebiotic substrate prior to consumption, but the bacteria should be able to survive in the food (Moroti et al., 2014).

Among probiotic and symbiotic functional foods, dairy products, especially fermented milks, are market leaders and are considered research priorities in many countries. The growing appreciation of functional foods around the world is promoting innovations in food products and stimulating the consumption of foods with nutritional and therapeutic value (Kumar et al., 2015).

Helianthus tuberosus L. (*Asteraceae*), popularly known in Brazil as *tupinambor* and worldwide as Jerusalem artichoke, is a perennial herb originating from eastern North America. It was introduced and extensively cultivated in temperate areas for edible tubers. *Helianthus Tuberosus* L. has high stem, large leaves, bright yellow flowers similar to sunflowers and potato tubers. As a source of inulin, tubers have been used as a popular medicine for the treatment of diabetes and rheumatism as well as for a variety of pharmacological activities such as laxative, diuretic, spermatogenic, stomach and tonic (Pan et al., 2009).

The Jerusalem artichoke accumulates similar levels of fructan to the roots of chicory (16-20%) and is one of the most interesting plants for the industrial production of inulin, since it is possible to cultivate it at low cost, with low application of fertilizers in any type of soil and conditions cold weather (Franck, 2000). The fructans, considered prebiotics, are able to resist the hydrolysis of digestive enzymes in the part of the human gastrointestinal tract and, therefore, they have a low caloric value for humans (1.5 kcal g⁻¹) and perform similar functions to dietary fiber (Genta et al., 2009).

The fructans are the main storage carbohydrate of *H. tuberosus* L., representing between 70 and 80% of the dry matter of the tubers, which ranges from 18 to 25% (Losavio et al., 1997). This species is considered as one of the most important candidates for use as a raw material for the industrial production of biological fructose and inulin (Baldini et al., 2004; Kays & Kultur, 2005).

Fructans of the inulin type (fructooligosaccharides, oligofructose and inulin) are considered prebiotics, composed of β -linked fructosyl units (2-1) with or without terminal D-glucose at the reducing end. They have different degrees of polymerization (DP) and can originate naturally as native components in many plants or derive by biochemical/enzymatic techniques (Bañuelos et al., 2008; Genta et al., 2009).

In view of the above, the aim of this work was to elaborate symbiotic yogurt, using as a prebiotic ingredient the tuber of "Tupinambor" (*Helianthus tuberosus* L.), and as probiotic the culture of *Bifidobacterium lactis* HN019. Furthermore, this study also identifies and quantifies the fructans in the prebiotic flour, characterises physicochemically, perform the shelf-life of the yogurt and analyse the sensory acceptance and then evaluate its symbiotic efficiency by means of biological assay in vivo.

2 Materials and methods

2.1 Obtaining Jerusalem artichoke flour

The Jerusalem artichoke (*Helianthus tuberosus* L.) was commercially purchased in the form of flour.

2.2 Identification and quantification of fructans in Jerusalem artichoke flour

The chromatographic profile of sugars was determined by high performance liquid chromatography (HPLC) in a VARIAN chromatograph model Pro Satr 410. The following chromatographic conditions were used: chromatographic column Bio Rad no. 125-0128; column Bio Rad AMINEX HPX-87P (300 × 7.8 mm); column temperature at 80 °C; refractive index detector (RID); eluent deionized water purified on ion-exchange column, filtered on a 0.22 μ m pore polyethylene filter and then degassed in an ultrasonic bath; volume of the injected sample 20 μ L. The samples were filtered through a membrane filter (Durapore) in PVDF, with 0.22 μ m pore and 13 mm diameter. The results were compared to Sigma standards, analysed under the same conditions described above. The determination of the sugar concentration was calculated in relation to standard solutions at 1%.

2.3 Production of yogurts

The raw material used for the preparation of the yogurts was ultra high temperature (U.H.T.) whole milk, the lyophilized commercial starter cultures were: *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* (traditional culture) and the probiotic culture was *Bifidobacterium lactis* HN019, both from Danisco®.

A completely randomized experimental design with factorial 2² was used to evaluate the effect of the different components used to prepare the yogurts on the characteristics of the same, with two independent variables and a control treatment, totaling five treatments and three replicates. The independent variables were the amount of sugar (80 and 100 g) and Jerusalem artichoke flour (50 and 70 g).

The formulations were as follows: standard (probiotic yogurt – 1% probiotic culture + 8% sugar), F1 (symbiotic yogurt 5% tupinambor flour/ 10% sugar), F2 (symbiotic yogurt 5% tupinambor flour / 8% sugar), F3 (symbiotic yogurt 7% tupinambor flour / 8% sugar) e F4 (symbiotic yogurt 7% tupinambor flour / 10% sugar).

2.4 Centesimal composition and shelf life

The determination of the centesimal composition (moisture, ashes, lipids, protein and carbohydrates) of yogurts was performed according to the Association of Official Analytical Chemists (2007). The total dietary fiber and its soluble and insoluble fractions were determined by the enzymatic-gravimetric method 991.43 (Association of Official Analytical Chemists, 2007). For the evaluation of shelf life of the yogurts, the microbial count of bifidobacteria in the modified MRS culture medium was performed with the addition of 0.2% (m/v) lithium and 0.3% (m/v) dicloxacillin, besides monitoring the titratable

acidity and pH (Digimed, São Paulo, Brasil). Serial dilutions of the samples were performed, plating in triplicate in depth, using 0.1 mL aliquots. The plates were incubated in anaerobiosis at 37 °C for 72 hours (Vinderola & Reinheimer, 2000).

2.5 Sensory analysis

A total number of 76 untrained judges, consisting of yogurt consumers, of both genders and aged between 18 and 50, participated in the study. The samples temperature was 5 °C. The analyses were performed in a sensory analysis laboratory, in individual booths and with white lighting. Acceptance testing was applied through a structured hedonic scale of nine points, (1- I greatly disliked to 9- I greatly liked), as described by Lawless & Heymann (2010). A preference testing by ordering from the most preferred to the least preferred, as suggested by Dutcosky (2011), was also performed.

The untrained judges received a portion of each sample (approximately 20 mL) in white plastic cups encoded with three-digit numbers, in a balanced and randomized manner, accompanied by a glass of water to make the blank between samples.

The project was approved by the Research Ethics Committee of the Federal University of Santa Maria (UFSM), under the Certificate of Presentation for Ethical Appreciation (CAAE) - 56769116.9.0000.5346.

2.6 In vivo experiment

A number of 40 New Zealand white rabbits with initial age of 35 days and weighing on average 917g were used. The rabbits were housed in individual cages with semi-automatic feeder and automatic nipple drinking fountain. From 35 to 40 days of age, the animals went through the adaptation period. The rabbits were obtained through the Laboratory of Teaching, Research, Extension and Production (LEPEP) – Cuniculture of the Federal Institute Farroupilha - Júlio de Castilhos. The experiment was conducted with the approval of the Committee on Ethics in the Use of Animals under protocol number 9412161017.

Subsequently, the animals were distributed in a completely randomized experimental design with three treatments of 10 replicates each, for a total of 30 experimental units. The treatments were composed by different experimental groups: control group (CG) - commercial feed only, probiotic group (PG) - commercial feed + probiotic yogurt and symbiotic group (SG) - commercial feed + symbiotic yogurt. The yogurts were given to the animals through gavage, and 10 mL were administered per animal per day.

At the end of the 50-day experiment, the animals were euthanized through cranial concussion followed by exsanguination, where blood collection was performed for centrifugation and separation of serum and blood plasma for serological analysis and the collection of cecal material for microbiological evaluation.

Furthermore, serological analysis of total cholesterol, HDL cholesterol (High Density Lipoproteins), LDL cholesterol (Low Density Lipoproteins), triacylglycerols, and glucose were determined using colorimetric enzyme kits and the reading was performed in a spectrophotometer. On the other hand, for the

microbiological determination of the cecal content, 1.0g of the intestinal content was weighed and transferred aseptically with a sterile spatula into a test tube containing 9.0 mL of diluent. Then, the necessary, decimal dilutions were performed for the plating in depth using MRS agar medium plus lithium chloride and dicloxacillin, according to the methodology proposed by Castele et al. (2006).

2.7 Statistical analysis

The data were submitted to the univariate analysis of variance and its averages compared by the Tukey test at 5% of significance, using Statistica software version 9.1 (Statsoft Inc., Tulsa - OK, USA).

3. Results and discussion

3.1 Identification and quantification of fructans in the *Helianthus tuberosus* L. flour

According to the fructan chromatographic profile of the *Helianthus tuberosus* L. flour (Table 1), it can be observed that it has a considerable content of inulin (44.44%), when compared to the yacon potato, for instance, which has in general terms from 60 to 70% of inulin and fructooligosaccharides (Vilhena et al., 2000). Inulin is a fructooligosaccharide which, unlike most others, is not digested in the stomach. Its caloric contribution is rather small: about 1.5 kilocalories per gram, against 4 kcal/g of sugar and 9 kcal/g of fat (Rensis & Souza, 2008).

According to Hua et al. (2007), the values of inulin found in the tubers of *Helianthus tuberosus* can often exceed 50% of their composition, reaching values of 50,20% and 78,16 as already presented by other authors (Tientgam et al., 2015; Afoakwah et al., 2015). Inulin values may vary according to plant variety. Petkova et al. (2014) found values between 40.5 and 68.7% of inulin for different varieties of Jerusalem artichoke.

3.2 Centesimal composition and shelf life

Table 2 shows the values of the centesimal composition and dietary fiber of the standard yoghurt and the different symbiotic yogurt formulations.

The moisture, ash, lipids, protein, carbohydrate and dietary fiber contents presented little difference between the formulations, since the variation of sugar and flour ingredients of Jerusalem artichoke had little influence on the bromatological composition of the formulations. The similar contents of ashes, proteins and lipids of the formulations can be explained because sugar and

Table 1. Identification and quantification of fructans in the *Helianthus tuberosus* L. flour.

Components	Results (g 100 g ⁻¹)
Fructose	0.67 ± 0.01*
Glucose	1.66 ± 0.01
Sucrose	9.24 ± 0.01
Fructooligosaccharide	0.38 ± 0.01
Inulin	44.44 ± 0.01

*Values are the mean of the triplicate, followed ± standard deviation.

inulin (*Jerusalem artichoke* flour) are exempt of these nutrients in their physicochemical composition (Universidade Estadual de Campinas, 2011), without altering the nutritional status of the products. Santos et al. (2014) found similar data by adding inulin in yogurt formulations. All yogurt formulations meet Brazilian legislation in force (Brasil, 2000) and according to resolution GMC 47/97 yogurts are classified as whole given its fat content (Brasil, 1997).

Moreover, in relation to functional properties, it is possible to emphasise the dietary fiber fraction, in which dietary recommendations for daily fiber consumption are 20-40 g per day (World Health Organization, 1998). The Brazil National Health Surveillance Agency (ANVISA) recommends that the product registered in the category of functional foods and/or health must present the claim according to the list of approved claims. Food fiber source should contain in the portion of the product ready for consumption a minimum of 3g of fiber if the food is solid and 1.5g if it is liquid (Brasil, 2002). Therefore, the Jerusalem artichoke flour may have the functional claim (fiber source), when added to the food products, in this case the yoghurt, provided it is in accordance with its respective portion established by the RDC no. 359 of ANVISA (Brasil, 2003).

The stability of the yogurts during their refrigeration storage was evaluated by the parameters of pH, acidity and microbial counts for 30 days (Tables 3 and 4), where measurements were performed every 15 days.

The average initial pH of the yogurts was 4.93 but by the end of the 30 days of storage, it dropped to 4.77. The pH reduction during storage can be explained by the lactose consumption by the lactic bacteria present in yogurts. Moreover, it is also possible to affirm that the Jerusalem artichoke flour had no influence

on this parameter. On the other hand, acidity had stable values throughout the storage period.

When evaluating the results of Table 4, it can be observed that the yoghurts presented counts of lactic bacteria and bifidobacteria compatible with the Technical Regulation of Identity and Quality of Fermented Milks during the storage period. The regulation recommends the total lactic acid bacteria count of 10^7 colony forming units per gram (CFU/g) of the product and 10^6 CFU/g for bifidobacteria (Brasil, 2007).

In the case of functional foods, the minimum viable quantity for probiotics should be in the range of 10^8 to 10^9 CFU/g in the daily recommendation of the product ready for consumption. This corresponds to the consumption of 100g of a product containing 10^6 to 10^7 CFU/g, i.e. from 6 to 7 log CFU/g. This result is also in accordance with the amount recommended by several authors so that the probiotic microorganisms produce the desired physiological effect (Jelen & Lutz, 1998).

According to the results, the prebiotic effect of the Jerusalem artichoke flour is evidenced, where at the end of the 30 days of refrigerated storage (5 ± 1 °C) the symbiotic yogurt presented one logarithmic cycle more than the probiotic yogurt. This result matches with data obtained from previous research in which bifidobacteria counts were evaluated in fermented milks with and without inulin addition and found average values of 5-6 log CFU/g without inulin addition and 6-7 log CFU/g with inulin addition (Gallina et al., 2011; Trento et al., 2009). In the yogurts added with Jerusalem artichoke flour, the bifidobacteria count remained constant throughout 30 days of refrigerated storage (5 ± 1 °C), whereas in the standard yogurt without the presence of Jerusalem artichoke flour there was a decrease of one logarithmic cycle after 30 days of storage.

Table 2. Centesimal composition of symbiotic yogurt formulations.

Fraction (g/100g)	Standard	F1	F2	F3	F4	VC (%)
Moisture	85.03 ^a ± 0.00	84.56 ^a ± 0.03	82.66 ^c ± 0.00	82.36 ^c ± 0.11	83.03 ^b ± 0.04	1.80
Ashes	0.74 ^{ab} ± 0.45	0.73 ^{ab} ± 0.02	0.72 ^b ± 0.05	0.76 ^{ab} ± 0.03	0.77 ^a ± 0.04	2.63
Lipids	3.09 ^a ± 0.25	3.19 ^a ± 0.11	3.16 ^a ± 0.10	3.48 ^a ± 0.21	3.44 ^a ± 0.08	1.32
Protein	2.96 ^c ± 0.26	3.45 ^b ± 0.04	3.59 ^{ab} ± 0.19	4.15 ^a ± 0.27	4.10 ^a ± 0.19	5.78
Carbohydrates	8.81 ^a ± 0.00	8.23 ^b ± 0.00	8.36 ^b ± 0.00	9.11 ^a ± 0.00	9.39 ^a ± 0.00	1.51
Dietary fiber	1.93 ^a ± 0.00	1.81 ^b ± 0.00	1.83 ^b ± 0.00	2.00 ^a ± 0.00	2.06 ^a ± 0.00	1.51

Different letters in the same line present significant difference at the level of 5% ($p < 0.05$). Reading: Standard (probiotic yogurt - 1% probiotic culture + 8% sugar), F1 (symbiotic yogurt 5% tupinambor flour / 10% sugar), F2 (symbiotic yogurt 5% tupinambor flour / 8% sugar), F3 (symbiotic yogurt 7% tupinambor flour / 8% sugar) and F4 (symbiotic yogurt 7% tupinambor flour / 10% sugar), VC (coefficient of variation).

Table 3. pH and titratable acidity values of the yogurt formulations during storage.

Product	pH			Titratable acidity		
	Day 01	Day 15	Day 30	Day 01	Day 15	Day 30
S	4.93 ^{aA} ± 0.01	4.85 ^{bB} ± 0.00	4.78 ^{aA} ± 0.00	0.6 ^{aA} ± 0.00	0.7 ^{aA} ± 0.00	0.7 ^{aA} ± 0.00
F1	4.92 ^{aB} ± 0.01	4.87 ^{abA} ± 0.00	4.78 ^{aA} ± 0.00	0.7 ^{aA} ± 0.00	0.7 ^{aA} ± 0.00	0.7 ^{aA} ± 0.00
F2	4.93 ^{aA} ± 0.01	4.87 ^{abA} ± 0.01	4.77 ^{aA} ± 0.01	0.7 ^{aA} ± 0.00	0.7 ^{aA} ± 0.00	0.7 ^{aA} ± 0.00
F3	4.94 ^{aA} ± 0.01	4.87 ^{abA} ± 0.01	4.77 ^{aA} ± 0.01	0.7 ^{aA} ± 0.00	0.7 ^{aA} ± 0.00	0.7 ^{aA} ± 0.00
F4	4.92 ^{aB} ± 0.01	4.87 ^{aA} ± 0.01	4.77 ^{aA} ± 0.01	0.7 ^{aA} ± 0.00	0.7 ^{aA} ± 0.00	0.7 ^{aA} ± 0.00

Different lowercase letters in the same row and uppercase in the same column present a significant difference at the 5% level ($p < 0.05$). Reading: S - standard (probiotic yogurt - 1% probiotic culture + 8% sugar), F1 (symbiotic yogurt 5% tupinambor flour / 10% sugar), F2 (symbiotic yogurt 5% tupinambor flour / 8% sugar), F3 (symbiotic yogurt 7% tupinambor flour / 8% sugar) e F4 (symbiotic yogurt 7% tupinambor flour / 10% sugar).

3.3 Sensorial evaluation

The sensorial evaluation of this work had the aim to reach the ideal formulation of symbiotic yogurt for subsequent experiment *in vivo*. All the yoghurts evaluated presented average acceptance allocated between the hedonic terms “greatly disliked” and “greatly liked”.

According to Table 5, for all attributes the standard formulation (probiotic, without Jerusalem artichoke flour) was the one that obtained the highest scores. However, for the other formulations added of Jerusalem artichoke flour, no significant

Table 4. Microbial counts values measured on days 1, 15 and 30 of storage at 5 ± 1 °C.

Product	Determinations	Counts (Log CFU/g)		
		Day 1	Day 15	Day 30
S	Lactic acid bacteria	9.15	8.20	6.51
	Bifidobacteria	8.0	7.93	6.06
F1	Lactic acid bacteria	9.53	8.32	7.53
	Bifidobacteria	9.20	9.00	8.06
F2	Lactic acid bacteria	9.52	9.10	7.08
	Bifidobacteria	8.83	8.00	7.00
F3	Lactic acid bacteria	9.20	8.83	7.52
	Bifidobacteria	9.0	8.16	8.03
F4	Lactic acid bacteria	9.22	9.05	7.07
	Bifidobacteria	9.50	8.86	8.23

CFU/g – Colony Forming Unit per gram of product. Reading: S - standard (probiotic yogurt – 1% probiotic culture + 8% sugar), F1 (symbiotic yogurt 5% tupinambor flour / 10% sugar), F2 (symbiotic yogurt 5% tupinambor flour / 8% sugar), F3 (symbiotic yogurt 7% tupinambor flour / 8% sugar) e F4 (symbiotic yogurt 7% tupinambor flour / 10% sugar).

Table 5. Results of the sensorial evaluation regarding the acceptance test of five yogurt formulations.

Samples	Color	Flavor	Taste	Texture	Overall Appearance
S	8.17 ^a	7.91 ^a	8.20 ^a	8.09 ^a	8.25 ^a
F1	6.74 ^b	7.02 ^b	6.81 ^b	6.77 ^b	6.93 ^b
F2	6.81 ^b	7.10 ^b	6.68 ^b	6.90 ^b	6.75 ^b
F3	6.75 ^b	6.66 ^b	6.40 ^b	6.74 ^b	6.74 ^b
F4	7.02 ^b	6.93 ^b	6.58 ^b	7.08 ^b	7.13 ^b
VC (%)	23.32	23.21	26.38	23.64	23.64

Averages in the same column with different envelopes differ significantly ($p < 0.05$); VC - coefficient of variation. 1 = I really disliked it, 2 = I really disliked it, 3 = I disliked it, 4 = indifferent, 5 = I liked it, 6 = I liked it a lot and 7 = I liked it a lot. Reading: S - standard (probiotic yogurt – 1% probiotic culture + 8% sugar), F1 (symbiotic yogurt 5% tupinambor flour / 10% sugar), F2 (symbiotic yogurt 5% tupinambor flour / 8% sugar), F3 (symbiotic yogurt 7% tupinambor flour / 8% sugar) e F4 (symbiotic yogurt 7% tupinambor flour / 10% sugar).

Table 6. Results of serological evaluation of rabbits in different groups.

Group	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Triglycerides (mg/dL)	Glucose (mg/dL)
Control	50.66 ^{a*} ± 4.16	28.0 ^a ± 1.52	26.0 ^a ± 1.73	55.66 ^a ± 2.30	136.66 ^a ± 0.57
Probiotic	45.33 ^{ab} ± 4.04	27.33 ^a ± 0.57	18.66 ^b ± 2.08	46.66 ^b ± 2.23	114.33 ^b ± 4.16
Symbiotic	40.33 ^b ± 2.08	29.66 ^a ± 4.05	27.33 ^a ± 2.88	42.0 ^b ± 3.46	88.0 ^c ± 1.00
VC (%)	7.83	11.10	8.89	5.59	3.09

*Averages in the same column with different envelopes differ significantly ($p < 0.05$); VC - coefficient of variation.

difference was observed, but in the preference test between the yogurts added of Jerusalem artichoke flour, the yogurt F1, with 5% of flour and 10% of sugar, was preferred by the untrained judges and, therefore, was the formulation defined to be used in the biological assay.

3.4 In vivo evaluation

Weight gain

At the beginning of the experiment, the animals weighed an average of 0.917 kg and at the end 2.348 kg, representing an average gain of 1.431 kg. The groups submitted to the consumption of probiotic and symbiotic yogurts presented an increase in weight gain, whereas the control group obtained a final average weight of 2.170 kg, compared to 2.470 and 2.403 kg of the probiotic and symbiotic groups, respectively. Nonetheless, this difference was a consequence of the increase in caloric intake by the groups that consumed yogurt and commercial feed in relation to those who consumed commercial feed only.

Serological evaluation

Table 6 shows the values of total cholesterol, HDL, LDL, triglycerides and glucose of the different animal groups. These results indicate a reduction in total cholesterol levels in relation to the control where the symbiotic yogurt group obtained a value of 40.33 mg/dL against 50.66 mg/dL from the control group. There was no significant difference for the HDL levels, however for the LDL levels the group fed with probiotic yogurt presented lower levels when compared to the control and symbiotic groups.

The simultaneous use of probiotics and prebiotics has been studied as a way to improve the viability of probiotic microorganisms and to potentiate the control of individual cholesterol levels attributed to probiotics and prebiotics (Liong & Shah, 2006).

The prebiotic action of inulin using yogurt-fed rabbits as a biological model is not usual in the literature, but studies such as Maertens et al. (2004) analysed this process by adding 2% of inulin or oligofructose to the feeding of nine week old rabbits. After ten days submitted to this, the rabbits were euthanized for further evaluation of their intestinal content. However, the presence of β -fructan (2-1) at the level of the small intestine (ileum) was not detected in rabbits that were fed with control feed. On the other hand, rabbits fed with feed added with inulin or phospholigosaccharides (POS) presented lower degradation rates - 49.2% and 35.3%, respectively. Yet, at the level of the large intestine (cecum) and fecal matter of all rabbits, including those fed with inulin and POS, no type of fructan was detected, which

confirms its complete fermentation by the microbial flora located therein and consequently its prebiotic effect.

In addition, other studies, such as the one conducted by Rossi et al. (2008), who used probiotic cultures in soybean water extract and obtained a lipid-lowering effect, that is, a control of cholesterol levels in rabbits (total cholesterol = - 18.4%; HDL-C = + 17.8%) and mice (non-HDL cholesterol = - 23.2%).

From the results presented in Table 6, we can observe that there was a significant difference ($p < 0.05$) in the triglycerides where the control group obtained a value of 55.66 mg/dL, in comparison to 42 mg/dL of the symbiotic yogurt group. The control value is quite similar to the one found by Torres et al. (2009), who evaluated this parameter in 42 day old rabbits and obtained the value of 59.7 mg/dL. This difference in triglyceride contents in both control and symbiotic groups may indicate an influence of the symbiotic product also in triglyceride levels.

Rodrigues et al. (2004), by verifying glucose in New Zealand white rabbits at 31 days of age fed exclusively with feed obtained levels of 130.64 mg/dL. In the present study, under the same dietary conditions, the glucose value of the control group was 136.66 mg/dL, whereas the symbiotic yogurt group had significantly lower levels (88.0 mg/dL).

Overall, the decrease in the levels of cholesterol, LDL, triglycerides and serological glucose in animals receiving symbiotic yogurt when compared to the control animals is due to the presence of inulin, which being a soluble fiber that cannot be digested by the intestine, causes a reduction in glycaemia and in the concentration of free fatty acids and plasma cholesterol levels. In addition, soluble fibers also “steal” bile salts and thus contribute to lowering cholesterol levels (Capriles et al., 2005; Stefe et al., 2008; Uyeda et al., 2016).

Evaluation of the cecal microbiota

As can be observed in Figure 1, animals receiving symbiotic yogurt had a higher bifidobacteria count ($9.93 \log 10 \text{ mL}^{-1}$) when compared to animals receiving only probiotic yogurt

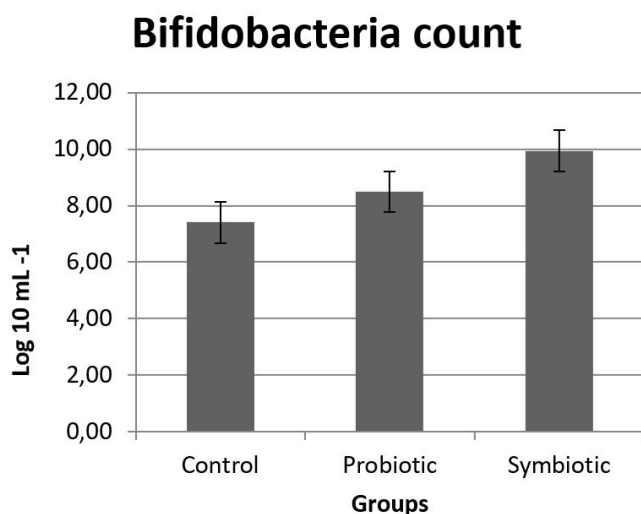


Figure 1. Cecal microbiota from rabbits in different treatments.

($8.49 \log 10 \text{ mL}^{-1}$) and with the control group ($7.41 \log 10 \text{ mL}^{-1}$), which is accordance with the bifidogenic potential of the Jerusalem artichoke tuber flour.

Lima et al. (2018), when studying supplementation with oligosaccharides and fructooligosaccharides in mice obtained values of bifidobacteria in the contents of the ascending colon of $9.0 \log 10 \text{ mL}^{-1}$. Similarly, Lemos et al. (2010) by adding prebiotics in the diet of the mice found values of $10.0 \log 10 \text{ mL}^{-1}$ of bifidobacteria in the intestinal contents. However, Rodrigues et al. (2012) used yacon flour as a prebiotic source in yogurts and obtained a higher bifidobacteria count when purchased with the probiotic product. As previously discussed, there are no studies with prebiotic yoghurt supplementation in rabbits in the literature, yet, the comparison with the biological model most used in research is valid.

4 Conclusion

Through this study, it can be concluded that the use of Jerusalem artichoke (*Helianthus tuberosus* L.) flour as a prebiotic ingredient in yoghurt performs a bifidogenic function, modulating the microbial flora and conferring health benefits, speciality with a significant reduction in the cholesterol and glucose levels in the animals evaluated, according to the serological and microbiological parameters evaluated.

Acknowledgements

The authors would like to thank the Coordination for Higher Education Staff Development (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Capes, Brazil).

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