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Effects of consumption of galactooligosaccharides obtained through whey enzymatically modified on the faecal flora and nutritional parameters of hamsters

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Abstract

The aim of this research was to evaluate the influence of whey enzymatically modified rich in galactooligosaccharides in the nutritional characteristics and effects in the microflora of cecum contents by the study with Golden Syrian hamsters *(Mesocricetus auratus)* for 28 days (controlled conditions). Three isoproteic diets were prepared (20% w/w): C (casein), W (whey) and G (whey modified). The groups studied differed positively from the C regarding feed and protein efficiency ratio. The relationships (w/w) of organ/body were found proportional in all diets. The counts of probiotics from the cecum contents the groups showed no difference. The pHs of studied groups were lower than C, this acidity can at impairs the ability of pathogens to grow in the intestine. Results suggest that using whey enzymatically modified rich in galactooligosaccharides could replace the standard diet with nutritional efficiency and possible inhibit the microorganisms pathogenic without induce damage in health.

Introduction

Whey is the major byproduct waste of the dairy industry, it has a high pollution potential that has imposed strict controls on the disposal of effluents into the environment. Rich in organic matter, the whey is characterized by its elevated biochemical oxygen demand values, in the range of 30–60 kg/m^3, mainly resulting from its high lactose content, which usually accounts between 4 and 8% (w/v). Milk proteins are the second major constituent of whey, at a concentration of 1% (w/v). Therefore, as a source of nutrients, it has stimulated interest in developing commercially viable processes to convert whey into a value-added nutritive bioingredients (Belen and Lee, 1998; Hatzinikolaou *et al*., 2005).

The whey has already been used as a stabilizer of emulsions (Viljanen *et al.*, 2005), affect the rheological characteristics of reduced-fat food like low-fat ice cream (Akalin and Erisir, 2008) and development of different nutritious beverages (Djuric *et al.*, 2004). The saccharides and whey has been studied in the synthesis of prebiotics that can be used in functional foods (López-Leiva and Guzman, 1995; Adamczak *et al*., 2009; Li *et al*., 2010; Cardelle-Cobas *et al*., 2011).

Prebiotics have been defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, between these are the oligosaccharides are commonly used as prebiotics. (Gibson and Roberfroid, 1995).

According to Mussato and Mancilha (2007), galactooligosaccharides (GOS) are prebiotics cited in the literature as bifidogenic factors that can be used as sweetener in fermented milk products, breads, jams, beverages and confectionery. Currently milk drinks supplemented with GOS are commercially available in Japan and Europe. Child food and special foods for the elderly and hospitalized people are promising to increase the application of GOS, as these people are more susceptible to changes in intestinal development. Thus, GOS have unique characteristics to be used in foods, but substantial scientific research on their biological effects is still needed for this to become a reality (Sako *et al*., 1999). Santos *et al.* (2009) showed that prebiotic sugars are the most effective ones, especially in children, due to increasing the physiological immunity as well as preventing diarrhea and cramps.

Based on the lack of toxicologically relevant effects on other parameters in the study, the no-observable- adverse-effect level (NOAEL) for Vivinal® GOS syrup is 5000 mg/kg bw/day when

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administered by gavage for 90 consecutive days in male and female Sprague Dawley rats (Anthony *et al*., 2006). The use of rats has been replaced frequently by the use of hamsters in biological evaluation involving experiments that aims to obtain responses to drugs and diets on lipid metabolism, and atherosclerosis, mainly for the difference between this animal model and human (response to diet, HDL as the main carrier of serum cholesterol levels and resistance to the formation of atherosclerotic plaques) (Shefer *et al*., 1992). Hamsters have lipoprotein profile similar to the human being the lipoprotein LDL the main carrier of cholesterol ester and triglycerides, CETP (Cholesteryl Ester Transfer Protein), including low basal rate of hepatic cholesterol synthesis and a comparable composition of the bile acid pool, features that are not present in rats (Navarro *et al*., 2005).

Therefore, this study aimed to biologically evaluate the influence in the nutritional characteristics and possible effects in the microflora intestinal of GOS obtained whey enzymatically modified in added to diets for hamsters compared to whey in natura and diet control for can be utilized like safety prebiotic ingredient in new products without induce damage in human health.

Materials and Methods

GOS synthesis

The reactions of enzymatic synthesis of GOS in aqueous medium were performed in batch reactors under agitation at 180 rpm and 40°C. The reaction system was composed of powder whey (ELEGÊ, RS, Brazil), featured on the composition (AOAC, 2000), suspended in phosphate buffer pH 7.0 in order to result in a concentration of 40% lactose and 10 U/ml activity enzyme β-galactosidase from Kluyveromyces lactis Lactozym® 6500L (Novozymes Latin America LTDA) (evaluated through of the hydrolysis of substrate synthetic which one activity unit (U) corresponds to the quantities of enzyme to release of 1 µmol of o-nitrophenol per minute (Inchaurrondo *et al*., 1994).

These conditions lead to obtain a product containing (g/l) 35.9 of galactose, 119.12 of glucose, 122.1 of lactose and 119.8 of GOS determined through the system Dionex DX-500 (Sunnyvale, CA, USA), using an anion exchange column CarboPac PA1 (250 x 4 mm) and a guard column CarboPac PA1 (Lisboa *et al*., 2012).

Test animals

For *in vivo* experiments 18 Golden Syrian hamsters *(Mesocricetus auratus)*, males, recently weaned (21 days) from Federal University of Pelotas, Brazil, were randomly divided into 3 groups (n=6), being housed in cages of polypropylene.

Table 1. Composition of diets control (C), whey (W) and whey enzymatic modified rich in GOS (G)

Ingredients (g/kg)	C	W	G
Whey	-	-	388
Whey enzymatic modified rich in GOS	-	412	-
Casein (>85% protein)	200	169.5	168.3
Soybean oil	70	68.5	68.5
Mineral mix*	35	intrinsic	Intrinsic
Vitamin mix*	10	10	10
L-cystine	3	3	3
Choline Cholridrate	2.5	2.5	2.5
Wheat bran (43%)	50	intrinsic	Intrinsic
Sucrose	100	intrinsic	Intrinsic
Corn starch**	529.5	334.5	359.7

*Prepared according to AIN93G. ** Added to supplement the diet.

Composition of diets

Three diets were prepared following the determinations of the AIN-93G (Reeves *et al*., 1993). The protein content was adjusted to 20% and protein sources were: Control diet C: commercial casein, Diet W: 41.2% (w/w) whey, (3) Diet G: 38.8% (w/w) (Table 1). The formulation of the control diet (C), despite the recommendation of eating 20% protein for growing rodents, was calculated to provide 20% of this nutrient (Miller and Bender, 1955; Sgarbieri, 1996; Jood and Singh, 2001). The ingredients were recommended (Reeves *et al*., 1993) and corn starch added to diet supplement 1000 g. Formulation of diets for W and G the calculation was based on the proximal composition (Table 1), the amount of daily intake by rodents (13 g/rat/day) (Souza-Soares, 2009); and the limit set by the maximum daily consumption as fiber and minerals.

Analytical methods

The whey, whey enzymatically modified rich in galactooligosaccharides and diets were analyzed following the methods described by the Association of Official Analytical Chemists (AOAC, 2000).

Growth experiment

The experiment was performed over 28 days; the 7 first days were for adaptation of the animal to the environment. During the experiment, the laboratory remained under light conditions (12 h photoperiod) and temperature (25 ± 2°C) controlled, as also under

renovation air by exhaust system. The water were offered ad libitum and the diets daily (13 g/hamster) with the weighing the remainder of the same to determine the daily intake. The animals' body weight was recorded every 7 days for evaluation of weight gain from them. After euthanasia, animals were laparatomised, and livers, small intestines, cecum and kidneys were collected and washed in physiological solution, dried in filter paper, and weighed for calculations of their relationships to body weight. The implementation of the experiment followed the standards of the Ethics Committee of UFPEL-RS, Brazil (Case No. 23110.010120/2011-34) and the Brazilian College of Animal Experimentation-COBEA.

Nutritional evaluation

The following determinations were made to evaluate the protein quality of diets in the study: Food Efficiency Ratio (FER), given by the ratio between the weight gain (g) and total dietary intake during the experiment (g) Protein Efficiency Ratio (PER), they were calculated as the ratio of weight gain (g) and total (Sgarbieri, 1996; Souza-Soares, 2009).

Analysis of cecum content

Cecum contents of the animals were subjected to analysis pH and enumeration of probiotic microorganisms (Vinderola and Reinheimer, 1999) expressed as colony forming units (cfu) using the technique of pour plate method and incubated inverted. For enumeration of *L. acidophilus* was performed using MRS medium (Man, Rogosa and Sharp) and incubated at 37°C for a period of 72 h. For the enumeration of *Bifidobacterium* spp. using the MRS medium was added sodium propionate (0.3%) and lithium chloride (0.2%) and incubated in anaerobic using the GasPak (Gas PakSystem-Oxoid, Basingstoke, Hampshire, England) at 37°C for 72 h.

Statistical analysis

The results were expressed as mean ± standard deviation, subjected to analysis of variance (ANOVA) and compared through Tukey's test at a 5% significance level. In microbiological analysis, the Student t test was applied in the dependent variables ($p<0.05$).

Results and Discussion

The weight gain of animals and their daily feed intake were measured weekly during 28 days of monitoring. Figure 1 shows the weight variation curve for animals submitted to control (casein) and

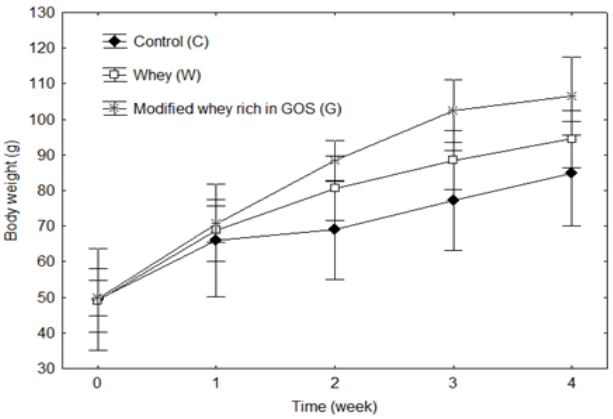

Figure 1. Body weight of hamsters fed with different diets, control (C), whey (W) and whey enzymatic modified rich in GOS (G)

studied diets (whey and the enzymatic modified whey rich in GOS).The weight gain profile for all groups showed a similar pattern, with an average weight of 49.4 g on the first day of experiment, incrementally progressing every week.

At the initial time and during the first week, the weights of hamsters were not statistically different ($p<0.05$) among themselves. There was a significant difference in their weights from the second to the fourth week. Groups receiving diet G differed from diet C, but diet W did not differ from diets C and G. From third to fourth week, diets G and W had a tendency toward stabilization of weight, and this reaction was expected because weight change occurs until the animal reaches adulthood (Melo *et al*., 2007). According to Chaud *et al.* (2008), reduced feed efficiency occurs due to the body's decreased metabolism, causing stagnation or reduction in body weight, as the diet consumed is less effective for the organism.

The Table 2 shows the weights of animals at the start of experiment (after 7 days of adaptation when all animals were fed with control diet) and by the end of 28 days of experiment, when hamsters were fed with diets C, W and G. Also, the total and daily consumption, total and daily weight gain, FER and PER after 28 days are shown. The three groups did not differ in total intake ($p<0.05$), which demonstrates that the diets were equally accepted by animals.

After 28 days of experiment (4 weeks), the animals had weights of (g) 84.73, 94.45 and 106.52 (Table 2) for groups C, W and G, respectively. Thus, the GOS diet (G) was found to give greater weight gain to animals, compared to the other diets, while the whey diet (W) did not differ from diets C and G.

The same result was obtained for daily food intake, with values of 5.87, 6.25 and 6.78 g for diets C, W and G, respectively, and no significant differences

Table 2. Weight gain, diet and protein biological values for hamsters fed with different diets, control (C), whey (W) and whey enzymatic modified rich in GOS (G)

Nutritional response	C	W	G
Initial weight*[1]	49.33±14.31[a]	49.13±8.92[a]	49.77±5.00[a]
Final weight on day 28[1]	84.73±14.79[b]	94.45±8.07[ab]	106.52±10.97[a]
Total food intake on Day 28[1]	164.51±21.95[a]	169.01±15.44[a]	189.94±16.86[a]
Daily food intake[1]	5.87±0.78[a]	6.25±0.57[a]	6.78±0.60[a]
Total weight gain on Day 28[1]	35.40±7.64[b]	45.32±5.86[ab]	56.75±10.04[a]
Daily weight gain[1]	1.26±0.27[b]	1.62±0.21[ab]	2.03±0.36[a]
FER	0.22±0.04[b]	0.27±0.02[a]	0.30±0.03[a]
PER	1.08±0.22[b]	1.59±0.12[a]	1.54±0.16[a]

Within the same line, means having different superscripts are significantly different by ($p<0.05$; n=6) Tukey test. *After 7 days of adaptation.[1]g/animal. FER: food efficiency ratio, PER: protein efficiency ratio.

were found among them ($p<0.05$). However, whey and the enzymatic modified whey rich in GOS sensory improvements compared to the control diet. However, with regard to total weight gain, group G was superior to group C in 56.75 g, while group W did not differ from others. In view of that, diet G was found to provide a high digestive utilization, showing that a higher weight gain was obtained for a total daily food intake with no differences among the groups. This behavior may be explained because of the proteins found in whey and the enzymatic modified whey rich in GOS, since whey proteins have almost all essential amino acids at levels above those recommended, except for aromatic amino acids (phenylalanine, tyrosine), which meet the recommendations for all ages as well as have high concentrations of tryptophan, cysteine, leucine, isoleucine and lysine. Moreover, whey proteins are highly digestible and quickly absorbed by the body, stimulating the synthesis of blood and tissue proteins, also termed as fast metabolism proteins, very suitable for situations of metabolic stress, where the replacement of proteins in the body becomes an emergency (Sgarbieri, 2004). Another possible reason is that the enzymatic modified whey rich in GOS contains monosaccharides, which are better metabolized in face of the starch found in greater quantity in control diet and no added sucrose in diets W and G.

Feddern *et al.* (2008) obtained a weight gain of 49.67 g for female Wistar rats fed with a multi-mixture made of wheat bran and cassava leaf during 28 days, this result is less than one obtained with diet G in this work. Statistically, the daily weight gain had the same pattern of the total weight gain. This demonstrates that the diets of whey and the enzymatic modified whey rich in GOS had higher values, and diet G differed from diet C ($p<0.05$). FER is related to total body weight gain and intake of diets throughout the experiment period. As shown in Table 2, the groups W and G differed positively from group C ($p<0.05$) in FER, reaching 0.27 and 0.30 respectively and having a better conversion of ingested food.

Moreover *et al.* (2008) reached the lowest conversion of food to body weight (FER of 0.12) for female Wistar rats fed with a diet based on the multi-mixture (wheat bran and cassava leaf) for 28 days, used to increase the availability of nutrients and improve the food digestibility. Anthony *et al.* (2006) evaluated diets supplemented with the GOS Vivinal® (45% GOS, 15% lactose, 14% glucose and 1% galactose) with 2.5 and 5.0 g/kg daily body weight, and achieved a FER of 0.19 for Sprague-Dawley rats fed during 28 days for both diets. Moreover, for the standard diet RODI (reverse osmosis deionized) the authors found 0.17 and 0.20 for the control diet containing FOS (fructooligosaccharides). These results confirm the potential of the diets under study, prepared with whey and the enzymatic modified whey rich in GOS.

A similar pattern was observed for PER. The

Table 3. Biometric measures, weight of organs and ratios between organ weight and body weight for hamsters fed with different diets, control (C), whey (W) and whey enzymatic modified rich in GOS (G)

Parameter	C	W	G
Forelimbs (cm)*	10.1±0.4^{b}	10.5±0.3^{ab}	10.6±0.3^{a}
Vertex-coccyx (cm)**	14.2±0.6^{b}	14.4±0.3^{ab}	14.9±0.3^{a}
Liver (g)	3.2±0.7^{a}	3.1±0.5^{a}	3.6±0.7^{a}
Small intestine (g)	2.0±0.2^{a}	2.3±0.4^{a}	2.2±0.4^{a}
Cecum with content (g)	1.8±0.6^{b}	3.6±0.5^{a}	2.6±0.9^{b}
Kidney (g)	0.8±0.2^{a}	0.9±0.3^{a}	0.9±0.2^{a}
Liver weight/body weight (%)	3.7±0.4^{a}	3.5±0.5^{a}	3.4±0.4^{a}
Small intestine weight/body weight (%)	2.4±0.3^{ab}	2.7±0.6^{a}	2.1±0.3^{b}
Cecum weight/body weight (%)	2.2±0.9^{b}	4.2±0.7^{a}	2.4±0.9^{b}
Kidney weight/body weight (%)	1.0±0.1^{a}	1.1±0.4^{a}	0.9±0.2^{a}

Within the same line, means having different superscripts are significantly different by ($p<0.05$; n=6) Tukey test. * Measure between front legs. ** Measure of the initial portion of the snout to the beginning of the tail.

groups G and W differed positively from group C ($p<0.05$), demonstrating that the incorporation of whey and the enzymatic modified whey rich in GOS could replace part of the protein source of control diet. This was evidenced by improved protein efficiency, compared to control diet. Feddern *et al.* (2008) found values ranging between 1.27 and 1.65 for female Wistar rats fed for 21 days with diets of different multi-mixtures, fermented or not fermented, composed of wheat bran, rice bran and cassava leaf. However, these values were lower and statistically different ($p<0.05$) from the PER of 2.30 of the standard diet based on casein used as control diet.

The nutritional results obtained suggest that the whey and the enzymatic modified whey rich in GOS have characteristics that allow their use in food in accordance with the properties shown in Table 2.

Table 3 shows the weight parameters concerning the growth of animals in groups C, W and G. An average size of 16 cm from snout to tail in adult hamsters is recommended by Barrie (1993), which is close to the value found in both groups. Regarding the body measures of animals, the group showing the greatest distance from arm to arm (10.6 cm) and from apex to coccyx (14.9 cm) was the one fed with a diet based on GOS, differing statistically only from group C ($p<0.05$). These weight parameters for animal growth are in accordance with the results related to weight gain shown in Table 2, where it is observed that the group with the highest development of body extremities also had the same results in relation to weight gain.

As shown in Figure 1, for 28 days the curves showed a weight gain tendency, assuming these hamsters reached values very similar to those applied by Barrie (1993).

This pattern of proportionality with FER and PER shows the diets efficiency in being metabolized and converted into animal growth. The weight gain adequate to biometric measurements indicates that the diets did not provide fat accumulation and obesity to hamsters fed with all diets (C, W and G) during the period of 28 days. The biometric evaluations for animals in groups C, W and G, expressed by weight of organs and their relation with the weight of animals were presented in the Table 3. The calculation of the ratios of organ weights with body weight is essential for a reliable interpretation of results, which demonstrate their proportionality.

There was no significant difference among the groups ($p<0.05$) concerning liver weight, which shows that there were no changes in this organ in response to the different diets given. However, it is noteworthy the relationship of liver weight with the body weight of animals, and this confirms the above, since all groups showed similar results among themselves. Moreover, the low ratio of liver weight/animal weight may be associated with liver weight loss in order to ensure the energy availability to major organs like heart and brain. During normal feeding, after 30 days of age, the liver of male rats shows an average weight of 2.80 g (Guzmán-Silva *et*

Table 4. Count of probiotic micro-organisms and pH of the cecum contents of hamsters fed with different diets, control (C), whey (W) and whey enzymatic modified rich in GOS (G).

Measure	C	W	G
*L. acidophilus**	8.30±0.02^{b}	8.30±0.01^{b}	8.70±0.01^{a}
Bifdobacterium spp*	7.00±0.01^{a}	7.00±0.03^{a}	7.00±0.01^{a}
pH	7.90±0.01^{a}	7.16±0.06^{c}	7.45±0.04^{b}

Within the same line, means having different superscripts are significantly different by ($p<0.05$; n=6) Student t test. * log cfu/g.

al., 2004), this value is lower than the one presented for the hamsters in this work.

With respect to the small intestine weight, there was no significant difference among the groups ($p<0.05$). However, the relationship of this organ with the body weight of animals showed that hamsters fed with GOS diets had lower values, differing from group W in 28.57%, but similar to group C, yet 14.29% inferior. These results provide evidence that the prebiotics have greater capacity to improve the intestinal transit by increasing the excretion of dry matter due to increased moisture in the stool through the osmotic pressure, thus decreasing the retention of the stool inside the intestine. According to Fooks and Gibson (2002) this fact contributes to minimize the invasion and colonization of microorganisms undesirable to this vital organ and is known as barrier effect provided by oligosaccharides along the mucosal surface of the human gut, which is cited as their primary beneficial effect on human health.

The results of cecum content weight (3.6 g) and its relationship with the weight of animals (4.2%) showed that group W differed from the other groups for being 75% and 91% higher than groups G and C, respectively. This may must have occurred by the absence of dietary fiber, thus decreasing the intestinal flow and increasing its content, given that such organ is responsible for handling it. The weight ratio between cecum and animal for diets C and G are 48% and 43% lower than those obtained by group W.

Mussatto and Mancilha (2007) argued that the fermentation of prebiotic fiber – such as wheat bran used in diet C and GOS in diet G – within the cecum produce health benefits, such as: increased excretion of dry mass, reduced intestinal constipation, diarrhea inhibition, modification of colonization by beneficial microflora, decrease in pH, production of nutrients such as B vitamins and folic acid, improved metabolism of carbohydrates and lipids, increased ability to absorb minerals, reduced cancer risks, protection of gastrointestinal, urogenital and respiratory tracts against infections, among others. However, among the functional benefits of prebiotics, Brazilian legislation recognizes only their contribution to intestinal flora balance, such as inulin and FOS, and their benefit in the intestine functioning (AOAC, 2000).

There were no differences among groups C, G and W ($p<0.05$) for kidney weights, as well as for their relationship with the body weight of hamsters. Besides, these kidneys of 30 day-old male rats under normal feeding have an average weight of 0.71 g and no changes were observed in this organ, like kidney stones or malfunctions (Guzmán-Silva *et al*., 2004). After euthanasia, evaluations of the cecum contents of animals were conducted. Table 4 gives the results of the counts of lactobacilli and bifidobacteria as well as determination of pH.

The composition of human intestinal flora is affected by many factors, such as age, susceptibility to infections, nutritional requirements, the immune status of host, as well as pH, transit time, availability of fermentable material in the gut, and interactions between flora and components within it. Bifidobacteria and lactobacilli together with the intestinal mucosa may act as a barrier to invasion by potential pathogens such as *E. coli, Campylobacter* and *Salmonella* spp. The lactic microflora of the human gastrointestinal tract is capable of inhibiting pathogens, interfering with the colonization through the following mechanisms: production of final metabolites, such as acids excreted by decreasing the pH to form a micro-niche with conditions in which the pathogens are unable to compete; natural competitive effect of space occupation; natural direct antagonism through excretion of antimicrobial agents; competition for nutrients; increase in the immune system (Gibson *et al*. 2005).

The presence of prebiotics reduces the risk of microorganisms associated with gastroenteritis, positively influencing the beneficial flora and increasing colonization with potential strength. As shown in Table 4, it is possible to verify the counts of probiotic microorganisms present in cecum contents.

The group fed with diet G had a higher content of lactobacilli when compared with groups C and W, showing significantly difference ($p<0.05$). This may be due to the metabolic process of fermentation, where the GOS is a source of energy with a stimulatory effect to microorganisms and probiotics, developing an effective anti-pathogene mechanism. Furthermore, GOS resist the digestive process in the small intestine and are hydrolyzed to small oligomers or monomers by anaerobic bacteria of the colon, such as lactobacilli (Gibson *et al.*, 2005; Roberfroid, 2007).

To investigate the prebiotic potential of alginate oligosaccharides (AOS), Wang *et al.* (2006) prepared through enzymatic hydrolysis of alginate polymer, the effects of AOS on bacterial growth were studied. Alginate oligosaccharides stimulated the growths of *Bifidobacterium bifidum* ATCC 29521 and *Bifidobacterium longum* SMU 27001 more significantly in comparison with FOS. In vivo studies showed that AOS selectively stimulated the cecal and fecal microflora of male Wistar rats. The number of fecal bifidobacteria of the rats fed a diet supplemented with 2.5% AOS for 2 weeks increased by 13-fold and 4.7-fold when compared with those fed the control diet and a diet supplemented with 5% FOS, respectively. The number of lactobacilli of the rats fed the diet containing 2.5% AOS increased by 5-fold compared with the controls. In contrast, AOS significantly decreased the abundance of enterobacteriaceae and enterococci (Wang *et al*., 2006).

The principal concept is that the prebiotic has a selective effect on the microbiota that results in an improvement in health of the host, these definitions arose from observations that particular dietary fibers bring about a specific modulation of the gut microbiota, particularly increased numbers of bifidobacteria and/or lactobacilli, and that ingestion of these compounds was associated with improved host health, however, as our ability to determine the microbial ecology of the gastrointestinal microbiota increases, along with our understanding of how this complex and diverse collection of bacteria functions, we now recognize that a beneficial modulation of the microbiota encompasses far more than bifidogenesis (FAO/WHO, 2007).

So, in determining the pH of the cecum contents of animals, the mean values of 7.90, 7.45 and 7.16 were obtained for the groups C, W and G, respectively, showing statistically difference ($p<0.05$). Groups W and G had lower pH values, probably because lactose is metabolized by microorganisms favoring lactic acid production and reducing this parameter. Some optimal conditions for probiotics can inhibit *Bacteroides, Clostridium* and coliform bacteria, among which are the low pH, with an antagonistic effect on the proliferation of pathogenic microorganisms, benefiting the host (Collins and Gibson, 1999; Gibson *et al.,* 2005; Wang *et al.*, 2006; FAO/WHO 2007; Roberfroid, 2007). However, substantiation of a claim should be based on studies with the final product type, tested in the target host, with safety assessment in humans (FAO/WHO, 2007).

Conclusion

The presence of whey and the enzymatic modified whey rich in galactooligosaccharides in diets showed acceptance through the consumption results. Animals that ingested diets containing the enzymatic modified whey rich in galactooligosaccharides tended to have higher weight gain. This was reflected in the food efficiency coefficients, which were found higher than the ones for control diet. Weight gains of animals ingesting the studied diets during 28 days also were found higher than those of control diet. Therefore, it is evident that the diets under study were well used by the organism, showing values above to those of control diet with the results of protein efficiency ratio. Through the results of monitoring and organs we realized that there was no negative effect on the use of galactooligosaccharides obtained enzymatic synthesis using whey that too demonstrated to promote beneficial intestinal flora. We show that this prebiotic can become a potential alternative ingredient in developing symbiotic functional foods.

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