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Prebiotic and Synbiotic Effects of *Lactobacillus rhamnosus* Isolated from Iraq on Intestinal Tract Microflora in Mice

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Abstract: In this study, Kunming mice were fed with galactooligosaccharide and *Lactobacillus rhamnosus* which isolated in former study from breast fed healthy infants feces in Basrah province, Iraq and chose as potential probiotic. The feed conversion efficiency and body weight of mice, contents of water and short chain fatty acid of mice feces and intestinal tract microflora in mice, were systematically investigated. The results revealed that the best treatment was when the mice (group f) were fed with infant formula plus galactooligosaccharide and *L. rhamnosus*, that gave best feed conversion efficiency (36.49), an increasing in *Lactobacilli* (9.45 cfu) and *Bifidobacterium* (7.35 cfu) counts and decreasing in *Staphylococci* (4.44 cfu) and *Coliform* (5.46 cfu) counts in mice feces, decreasing fecal pH (5.92), increasing fecal water content (69.71%) and best increasing in fecal SCFAs concentration of formic (1.066 $\mu\text{mol/g}$), acetic (24.766 $\mu\text{mol/g}$), propionic (16.644 $\mu\text{mol/g}$) and butyric (4.842 $\mu\text{mol/g}$) after 6 weeks of assay comparing with other groups.

Key words: Kunming mice, galactooligosaccharide, *Lactobacillus rhamnosus*

INTRODUCTION

Consumption of foods containing live bacteria is the oldest and still most widely used way to increase the numbers of beneficial bacteria in the intestinal tract (Kotikalapudi, 2009). Such bacteria now called 'probiotics' were defined as viable microbial food supplements which beneficially influence the health of the host (Schrezenmeir and De Vrese, 2001). Up to now, probiotics have been predominantly selected from the genera *Lactobacillus* and *Bifidobacterium*, both of which have been extensively studied and established as valuable native inhabitants of the (GIT) gastrointestinal tract (Fuller, 1989; Salminen *et al.*, 1998; Capela *et al.*, 2006).

The term "prebiotics" was coined in 1995 and defined as "non-digestible 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" (Gibson and Roberfroid, 1995). Since then, a range of prebiotic products have been developed and many are now available on the supermarket. Although a more recent development than probiotics, prebiotics overcome many of the traditional limitations of introducing food-borne bacteria into the gastrointestinal tract.

Firstly, prebiotics are non-viable food components, which mean that they do not encounter the problems associated with the use of probiotic strains in foods, in relation to microbial survival. Similarly, because the majority of prebiotics are carbohydrates they can be added to a much broader range of food products (e.g. confectionary and baked foods as well as more

traditional fermented milk food products and fruit drinks). Furthermore, prebiotics do not share the problem of probiotic survival upon ingestion by the consumer. Acidic conditions in the stomach coupled with digestive secretions of the small intestine and a high degree of microbial competition in the colon present a significant hurdle to the colonization or even survival of introduced probiotic strains in the gastrointestinal tract. Conversely, prebiotics (by definition) evade the degradative capabilities of the upper gut and reach the colon intact (Gibson *et al.*, 2000).

The concept of synbiotic, a combination of pre- and probiotics components has been proposed to characterize health enhancing foods and supplements used as functional food ingredients in humans (Gibson and Roberfroid, 1995).

Health benefits associated with the ingestion of probiotic bacteria includes: reduction in colon irritation, constipation, traveler's diarrhea, inhibition of the adhesion of pathogenic genera in intestinal lumen, synthesis of B vitamins, lowering of blood ammonia levels, cholesterol absorption and inhibition of tumor formation (Ziemer and Gibson, 1998). While prebiotic ingestion has many benefits such as stimulating carbohydrate metabolism in colonic bacteria, increasing bacterial mass, SCFA production, source of low-calorific sweeteners, bifidogenic effect, stool bulking/alleviation of constipation and Improving calcium bioavailability (Tanaka *et al.*, 1983; Ito *et al.*, 1990; Bounnik *et al.*, 1997).

The impact of diet upon gut populations and their activity has been illustrated effectively by an experiment where

a collection of bacterial species which suppressed the growth of *E. coli* in mice fed a refined diet, failed to exert the same effect when an alternative diet was fed (Freter, 1988).

Xiao *et al.* (2009) have examined the effects of (FOS), (GOS), (MOS) and (COS) on concentrations of cecal SCFAs and fecal pH of mice. After 14-day treatment, SCFA in mice cecum was significantly increased ($p < 0.05$) by intake of oligosaccharides, especially FOS and GOS while fecal pH values were lower ($p < 0.05$) in the FOS and the GOS groups. Thus, providing these oligosaccharides as ingredients in nutritional formulas may benefit the gastrointestinal tract. Acetate, propionate and butyrate concentrations ranged (30.09-47.59), (7.43-9.04) and (2.59-6.40) $\mu\text{mol/g}$ respectively.

Ramya *et al.* (2010) assessed the probiotic potential of two *Streptococcus thermophilus* strains (RD102 and RD104) isolated from Indian fermented milk products by both *in vitro* and *in vivo* tests. During the *in vivo* feeding trial in mice the strains showed a viable count of about 7 log cfu/g feces and 6 log cfu/g of large intestine, respectively.

Since the effect of prebiotic and synbiotic on microbial balance of GIT has not been fathomed nor studied in Iraq well, this study comes to determine the effect of prebiotic and synbiotic on gut health.

MATERIALS AND METHODS

Strain: *L. rhamnosus* was isolated in former study from collecting 68 samples of feces from healthy infants aged 2-6 weeks (Basrah/Iraq), fed on their mothers' breast milk, by using KB009-HiCarbohydrate™ Kit (Hi-media-India) as standardized colorimetric identification system and confirmative PCR identification.

Test animals: Thirty six Kunming male mice of 3 weeks old, were purchased from The Center for Disease Control of Hubei province, Wuhan, China.

Saccharide: The Galactooligosaccharide (GOS) "Tian men Hylae inulin R&D company Ltd. (Shanghai, China), was investigated *in vivo* for its effects on mice gastrointestinal tract balance.

Mice diet and infant formula: We bought the mice diet from The Center for Disease Control of Hubei province and infant formula from Chinese market and their composition were showed in Table 1 and 2, respectively.

Feeding plan: Thirty six mice were divided into 6 groups and fed for 6 weeks, each group has 6 mice as below:

- A. Mice diet as a control
- B. Mice diet + 5% GOS
- C. Mice diet + 5% GOS + *L. rhamnosus*
- D. Infant formula as a control
- E. Infant formula + 5% GOS
- F. Infant formula + 5% GOS + *L. rhamnosus*

Table 1: Mice diet composition

Ingredients	Content % (dry weight)
Soybean meal	50
Soy oil	6
Com. starch	36
Cellulose	1
Vitamin mixture	6
Mineral mixture	1

Table 2: Energy and nutritional ingredient content of infant formula

Composition	Per 100 g of prepared formula
Energy	497.00 kcal
Carbohydrate	56.30 g
Fat	24.00 g
Protein	13.70 g
Minerals (Ash)	3.00 g
Moisture	3.00 g
Vitamin A	1242.50 I.U
Vitamin E	6.00 I.U
Vitamin D3	300.00 I.U
Calcium	518.00 mg
Potassium	661.00 mg
Chloride	398.00 mg
Phosphorous	386.00 mg
Magnesium	53.00 mg
Sodium	186.00 mg
Iron	5.00 mg
Zink	2.49 mg
Vitamin B1	0.30 mg
Vitamin B2	0.79 mg
Vitamin B6	0.37 mg
Pantothenic acid	1.51 mg
Vitamin B12	1.10 μg
Biotin	17.00 μg
Folic acid	45.00 μg

Mice (3 weeks old) were used in this study, chosen with weights (15-20) g, housed under controlled circumstances (temperature 18-22°C, humidity 60-65%, lights on from 6:00 to 18:00) with wood shaving as a ground and gave for all mice 14 days as acclimatization to environment before test beginning. After acclimatization, all the mice were weighed and randomly assigned to 6 groups of 6 mice and fed with 6 different diets. The composition of mice diet and infant formula are shown in Table 1 and 2, respectively. Feed and water were available throughout this study.

Treatment of bacteria prior to oral injection: *L. rhamnosus* was propagated in MRS broth (Beijing land bridge Technology Co., Ltd. China) overnight in anaerobic jar at 37°C, then harvested by centrifugation (10,000 x g, for 10 min) at 4°C and washed twice with phosphate buffer saline (Oxiod, Australia).

The optical density of the bacterial suspensions at 600 nm was adjusted with PBS to 0.5±0.02 giving a count that varied between 10⁶ and 10⁸ cfu/ml. then administrated 1 ml/d for each mouse in group C and F (Rowaida *et al.*, 2007).

Body weight and food conversion efficiency: Body weight of each mouse was assessed every week by using a sensitive balance (Adventurer, OHAUS, USA) during the study period.

Food weight of each treatment was assessed daily (weekly average) using sensitive balance during study period. Food conversion efficiency calculated according to the equation below (Makni *et al.*, 2008).

$$\text{Food conversion efficiency} = \frac{\text{Feed intake (g)}}{\text{Weight gained (g)}}$$

for each treatment.

Bacterial enumeration of mice feces: Feces samples from mice were collected to determine *Lactobacilli*, *Bifidobacterium*, *Staphylococci* and *Coliform* counts after 0, 2, 4 and 6 weeks. Feces samples were directly transferred into sterile tubes and kept at 4°C. One gram of each sample was 10-fold serially diluted (10^{-2} to 10^{-12}) in 0.15% sterile peptone water diluents (Oxoid, Australia).

Enumeration was carried out using the pour plate technique. Anaerobic jar was used for creating anaerobic condition for *Lactobacilli* and *Bifidobacterium*. Bacterial count was performed on MRS-agar for *lactobacilli*, Beerens agar (Oxoid, Australia) for *bifidobacteria*, mannitol salt agar (Beijing land bridge technology co., ltd) for *staphylococci* and MacConkey agar (Beijing land bridge technology co., ltd) for *coliform*. Plates containing 30 to 300 colonies were enumerated and recorded as log₁₀ colony forming units (cfu) per gram of mice feces.

Determination of water contents, pH and fecal short chain fatty acid in mice feces

Fecal water content: The water content of feces samples was measured using a drying oven (105°C, 24 h). Fecal water content (%) was calculated according to the equation:

$$\text{Fecal water content (\%)} = \frac{W_{\text{wet}} - W_{\text{dry}}}{W_{\text{wet}}} \times 100$$

Where W_{wet} and W_{dry} are the weight of the fecal sample before and after drying in the oven (Do Kyung *et al.*, 2009).

pH: Feces samples were diluted in distilled water (1:4, w/v) and homogenized with pipette tips. Feces pH was determined using pH meter (AOAC, 1990).

Short Chain Fatty Acid (SCFA): Feces samples from mice were collected after 0 and 6 weeks and stored at freezer. They were thawed and then processed to determine fecal SCFA by gas chromatography. The SCFA analysis was carried out according to Campbell *et*

al. (1997a,b) with modification. One gram of homogenized feces sample was dissolved in 4 ml methanol (Sinopharm chemical reagent Co. Ltd., China). The mixture was centrifuged at 13,500 rpm, at 4°C for 15 min. The supernatant was transferred and subjected to SCFA (formic, acetic, propionic and butyric) analysis. FFAP 35 m x 0.25 mm I.D. x 0.33 µm column was used on a Gas Chromatography (9790, China).

Nitrogen was used as the carrier gas with the flow rate of 12 cm/sec. Oven temperature program was isothermal at 60°C Hold 1 min and romped to 90°C at 5°C/min and further to 200°C at 10°C/min and lastly hold on 200°C for 1 min to flush out all the impurities. Injection temperature was 240°C, FID temperature was 240°C, Injection volume was 0.1 µL. Formic, acetic, propionic and butyric acid were analytical grade (>97%), were obtained from Shanghai chemical pharmacy company (Shanghai, China). The fecal short-chain fatty acids concentrations were determined at weeks 0 and 6 respectively.

Statistical analysis: Data obtained for given parameters were statistically analyzed through General Linear Model (GLM) technique using SPSS, (1998) statistical software to compare the means.

RESULTS AND DISCUSSION

Mice weekly weight: All mice were generally healthy throughout the feeding trial period. Mice body weights were measured weekly starting from week 0 till week 6 as shown in Table 3. Although no significant differences ($p < 0.05$) among the 6 groups in final body weight, body weight showed a tendency to increase especially in groups D, E and F comparing with groups A, B and C. This means that the weights of all mice are increasing weekly, all of mice were healthy and there were no side effect of any group that may affect on the use of prebiotic or synbiotic. These results were in agreement with David *et al.* (1998); Ichiro *et al.* (2004); Liang and Shah (2006) and in contrary with Do Kyung *et al.* (2009).

The increasing of weight for groups D, E and F may be attributed to the rich formula of infants with many nutrients that may affected on increasing of the weight of these groups comparing with groups A, B and C that fed on mice diet supplemented with prebiotic or synbiotic.

Weekly feed intake: Table 4 shows the weekly feed intake of 6 groups for 6 weeks. The results illustrate that the control groups A and D required too much diet during 6 weeks to gain their final weights comparing with groups B, C, E and F which required lower diet to gain nearly the same weight.

It appears that GOS had a tendency to increase body weight gain and initiate a decrease in food intake as compared with the control groups (A and D). This decrease of feed intake and nutritional efficiency was observed by feeding indigestible polysaccharides to rats (Ikegamsi *et al.*, 1983).

Table 3: Mice weekly weight (Values presented as summation for 6 mice for each group)

Groups and weights (g)						
Weeks	A	B	C	D	E	F
0	170.75	170.3	175.24	167.85	176.3	177.30
1	182.95	176.3	179.00	176.30	186.2	182.69
2	189.20	180.7	185.70	187.94	198.3	188.96
3	194.65	189.1	190.84	197.36	207.8	196.88
4	201.61	193.6	197.48	209.31	213.0	208.57
5	208.02	205.3	203.73	217.22	221.6	215.93
6	216.90	209.4	209.79	222.95	226.3	221.00

Table 4: Consumed food (g) by mice

Treatments						
Weeks	A	B	C	D	E	F
1	398.57	240.00	155.19	280.96	304.06	210.21
2	252.58	169.14	249.57	373.10	380.34	213.80
3	260.50	296.39	166.84	333.27	328.68	254.86
4	299.22	163.74	225.52	430.31	185.34	388.62
5	278.92	388.78	216.12	337.04	289.16	229.70
6	365.29	152.55	204.04	233.15	164.94	158.79
Sum	1855.10	1410.60	1217.30	1987.80	1652.50	1456.00

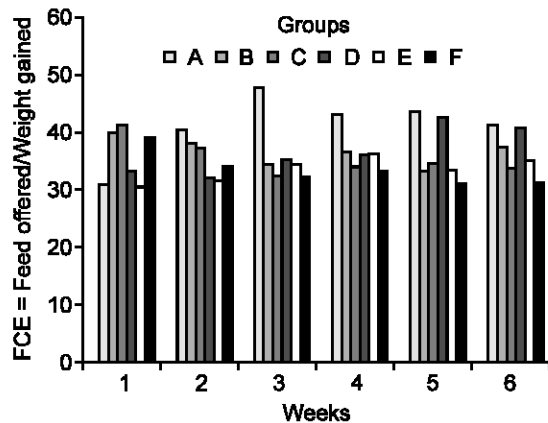


Fig. 1: Feed Conversion Efficiency (FCE) of mice groups

Feed Conversion Efficiency (FCE): In animal husbandry, Feed Conversion Ratio (FCR), feed conversion rate, or Feed Conversion Efficiency (FCE) is a measure of animal's efficiency in converting feed mass into increased body mass. There are no measurement units associated with FCE. Animals that have a low FCE are considered efficient users of feed (Brown *et al.*, 2001). The results shown in Fig. 1 illustrate that the feed conversion efficiency at week 1 was 32.66, 40, 41, 27, 33.24, 30.62 and 39 for groups A-F respectively. This ratio increased for control groups A and D during 2, 3, 4, 5 week and reached at week 6 to 41.13 and 40.68 respectively. While this ratio decreased for groups B, C, E and F during 2, 3, 4, 5 week and reached at week 6 to 37.2, 33.66, 35.01 and 31.31 respectively. This means, synbiotic group (F) was the best feed conversion ratio followed by synbiotic group (C), prebiotic group (E) and prebiotic group (B) respectively. In general, study results

in agreement with Bruzzese *et al.* (2006) who reported that synbiotic and prebiotic groups had a favorable influence on the small bowel by improving sugar digestion and absorption, glucose and lipid metabolism. It means increasing the ability of absorbing the nutrients inside the gut through increasing the digestibility and absorbability.

Lactobacilli, Bifidobacterium, Staphylococci and Coliform counts of mice feces: Fig. 2 shows bacterial counts of *Lactobacilli*, *Bifidobacterium*, *Staphylococci* and *Coliform*.

Lactobacilli at week 0 ranged 7.53, 7.74, 7.94, 7.88, 7.51 and 8.02 log cfu/g of mice feces in groups (A-F) respectively with significant differences ($p < 0.05$) for C, D and F groups comparing with A, B and E. The increasing of *Lactobacilli* was significant ($p < 0.05$) in B, C, E and F groups after 2, 4 and 6 weeks of assay comparing with control groups A and D. After 6 weeks *Lactobacilli* ranged 8.95, 9.26, 9.20 and 9.45 log cfu/g for B, C, E and F groups respectively while no significant increase ($p > 0.05$) was noticed in control groups A and D at the end of assay and the counts of *Lactobacilli* were 7.57 and 7.92 log cfu/g respectively.

The best group in *Lactobacilli* counts was F followed by C with significant differences ($p < 0.05$) followed by B and E respectively with no significant differences ($p > 0.05$).

Bifidobacterium counts at week 0 were 5.97, 5.93, 5.74, 5.69, 5.90 and 5.87 log cfu/g for groups (A-F) respectively with no significant differences ($p > 0.05$) among A, B, E and F groups comparing with C and D groups. *Bifidobacterium* increased significantly ($p < 0.05$) during 2, 4 and 6 weeks in B, C, E and F groups comparing with control groups A and D. *Bifidobacteria* counts after 6 weeks were 6.49, 6.92, 6.83 and 7.35 log cfu/g for B, C, E and F groups respectively while no significant ($p > 0.05$) and slow increase was noticed in control groups A and D at the end of assay and *Bifidobacterium* counts were 6.00 and 5.74 log cfu/g respectively. The best group in *Bifidobacterium* counts was F with significant differences ($p < 0.05$) followed by E, C and B respectively with no significant differences.

Staphylococci counts were 5.46, 5.59, 5.60, 5.51, 5.56 and 5.64 log cfu/g at week 0 for the groups (A-F) respectively with no significant differences ($p > 0.05$) among (B-F) groups comparing with group A.

Staphylococci counts were decreased significantly ($p < 0.05$) in B, C, E and F groups after 2, 4 and 6 weeks of assay comparing with control groups A and D. After 6 weeks *Staphylococci* ranged 4.90, 4.63, 4.61 and 4.44 log cfu/g for B, C, E and F groups respectively while a slightly increase in control groups A and D at the end of assay was noticed and counts of *Staphylococci* were 5.47 and 5.56 log cfu/g respectively.

The lowest group in *Staphylococci* counts was F and E with significant differences ($p < 0.05$) comparing with groups C and B respectively.

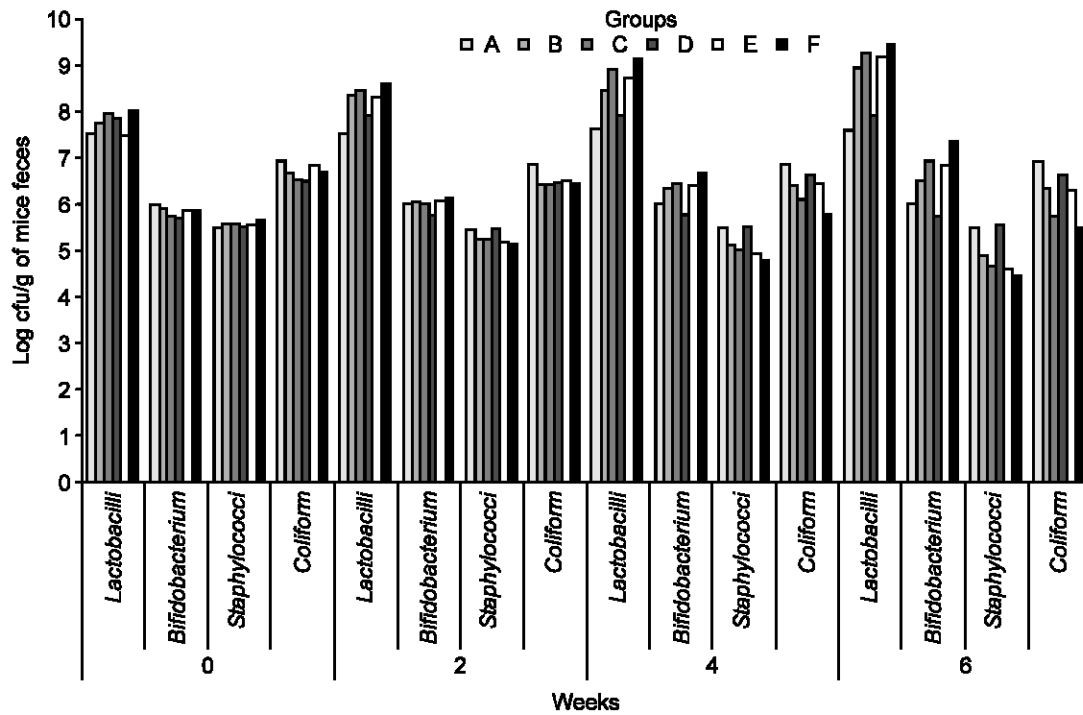


Fig. 2: *Lactobacilli*, *Bifidobacterium*, *Staphylococci* and *Coliform* counts of mice feces after 0, 2, 4 and 6 weeks

Coliform counts were 6.91, 6.65, 6.55, 6.50, 6.84 and 6.68 log cfu/g at week 0 for the groups (A-F) respectively with significant differences ($p < 0.05$) for A and E groups comparing with F, B, C and D respectively.

Coliform counts were decreased significantly ($p < 0.05$) in C, E and F groups after 2, 4 and 6 weeks of assay comparing with group B and control groups A and D despite the decreasing in group B. After 6 weeks, *Coliform* counts were 6.32, 5.75, 6.29 and 5.46 log cfu/g for B, C, E and F groups respectively while slightly increase was noticed in control groups A and D at the end of assay and *coliform* numbers were 6.92 and 6.62 log cfu/g respectively. The best group in decreasing *Coliform* counts was F followed by C with significant differences comparing with groups E and B respectively. In general, F was the best group followed by C, E and B respectively. An increase in the concentration of lactic acid bacteria in the feces of the mice was observed after the consumption of mice diet and infants formula supplemented with prebiotic (E and B groups), further increase happened by consumption of mice diet and infants formula supplemented with synbiotic (F and C groups) comparing with control groups (A and D). In return the decreasing in concentrations of pathogenic bacteria was wide in groups (F and C) followed by groups (E and B) comparing with control groups (A and C).

These results were agreed with Tanaka *et al.* (1983); Mutai and Tanaka (1987); Ito *et al.* (1990); Rowland and Tanaka (1993); Moro *et al.* (2002); Ichiro *et al.* (2004); Liu

et al. (2004); Silvia *et al.* (2005); Liong and Shah (2006); Monique and Jan (2006); Delphine *et al.* (2008) and Xiao *et al.* (2009) who reported same results.

In contrary with Alles *et al.* (1999) who found that ingestion of GOS at either 7.5 g/day or 15 g/day for a 3 week period had a little effect upon numbers of fecal bacteria or fecal microbial metabolites. Kullen *et al.* (1998) who showed that the cecal population of *Bifidobacteria* was not altered significantly by feeding 2% FOS to rats.

Prebiotic carbohydrates are, by definition, metabolized only by selected members of the gastrointestinal tract. Accordingly, these sugars have the ability to influence the population of the gastrointestinal tract due to their selective utilization. Organisms that rapidly ferment prebiotic sugars are enriched, presumably at the expense of those that do not. The effectiveness of a prebiotic depends, therefore on its ability to be selectively fermented by and to support growth of specific targeted organisms.

It is well known that some LAB are able to produce bacteriocins which have a broad spectrum of activity and the inhibitory effect shown by *L. rhamnosus* may be due to the presence of bacteriocins or metabolites similar to bacteriocins. Anti-pathogenic effects of SCFA, as the major end products of fermentation, decrease intestinal pH, which prevents colonization of pathogenic bacteria and stimulates health promoting bacteria (Swennen *et al.*, 2006). Because many intestinal pathogens and putrefactive bacteria prefer a neutral pH (Wang and Gibson, 1993; Marteau *et al.*, 1990).

Lactobacilli have been reported to inhibit the binding of enteropathogenic *E. coli* to intestinal cells (Bernet *et al.*, 1994) and the survival of *Lactobacilli* in the intestinal tract attributed to their adhesion ability (Jacobsen *et al.*, 1999). Although most inhibition of pathogenic bacteria has been contributed by a decrease in intestinal pH (Swanson *et al.*, 2002).

The increased amounts of acetic and lactic acids produced by the fermentation of prebiotics by lactic acid bacteria will decrease the pH in the large intestine and provide a better gut environment for *Bifidobacteria* and *Lactobacilli* bacteria. The decreased pH may suppress the growth of pathogenic bacteria like *E. coli* and thereby the incidence of diarrhea may be reduced (Gabert and Sauer, 1994).

In generally, GOS acts as a fuel for predominance of LAB that utilize it. Because GOS works as growing factor for LAB comparing with pathogenic bacteria that can't utilize GOS. Adding activated *L. rhamnosus* makes the balance goes towards increase of the beneficial bacteria upon pathogenic bacteria, due to the decreasing of gut pH by producing SCFA, lactic acid, bacteriocins and bacteriocins like products. In addition to the adhesion ability of LAB comparing to pathogenic bacteria, all of that make prebiotic and synbiotic effective to use in changing gut balance towards the beneficial bacteria.

Mice fecal pH: Figure 3 shows that fecal pH values at week 0 was 7.81, 7.63, 7.51, 7.76, 7.48 and 7.49 for groups A-F respectively. After 2 and 4 weeks fecal pH values increased slightly and reached 7.83 and 7.81 after 6 weeks in control groups A and D respectively.

In group B, C, E and F fecal pH values were decreased dramatically after 2, 4 and 6 weeks and reached 6.22, 6.05, 6.12 and 5.92 respectively.

More decreasing group in their fecal pH values was F with significant differences ($p < 0.05$) followed by C, E and B respectively. These results in agreement with Rowland and Tanaka (1993); Chonan and Watanuki, (1995, 1996); Moro *et al.* (2002); Astrid *et al.* (2005); Liong and Shah (2006); Dar'io *et al.* (2007) and Xiao *et al.* (2009) who reported decreasing in fecal pH through the feeding of prebiotic and synbiotic.

The actual production of SCFA in the colon may be reflected by fecal pH (Cummings and Englyst, 1987). Since short chain fatty acids are not only absorbed in the large intestine and subsequently utilized as an energy source, but also lower pH in the intestine lumen. The lower pH prevents enteric colonization of potentially pathogenic bacteria and growth of putrefactive bacteria (Gibson and Roberfroid, 1995).

The reduction of fecal pH in groups F, C, E and B was due to increase of SCFA produced from GOS utilization by LAB and the increase of LAB counts, might increase productions of lactic acid and other substances that decrease the pH.

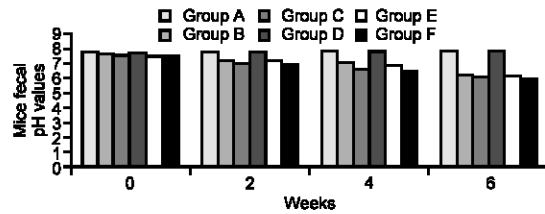


Fig. 3: Mice fecal pH after 0, 2, 4 and 6 weeks

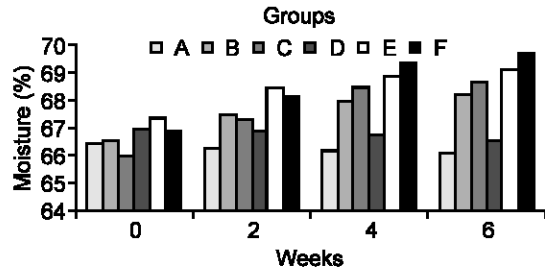


Fig. 4: Moisture content of mice feces after 0, 2, 4 and 6 weeks

The present results indicated that the fecal pH was reduced by feeding the GOS diets. The fecal pH tended to be reduced more by feeding synbiotic comparing with prebiotic and the control diet.

Mice fecal moisture: Figure 4 illustrates mice fecal moisture content after 0, 2, 4 and 6 weeks. At week 0 the mice fecal moisture content was 66.42, 66.54, 65.98, 67, 67.36 and 66.9% for groups A-F respectively. It decreased significantly ($p < 0.05$) in control groups A and D after 2, 4 and 6 weeks and reached to 66.1 and 66.52% respectively while in groups B, C, E and F it increased significantly ($p < 0.05$) after 2, 4 and 6 weeks and reached to 68.2, 68.66, 69.13 and 69.71% respectively. These results were in agreement with Gibson *et al.* (1995); David *et al.* (1998) and Liong and Shah (2006) who reported increasing in fecal moisture content through the feeding of prebiotic and synbiotic.

Mul and Perry (1994) showed that high levels of dietary FOS fed to weaned pigs will stimulate fermentation in the large intestine. This may increase the passage rate of digesta, which, in turn, might lead to soft feces.

Study results showed that the prebiotic and synbiotic diets contributed to higher fecal moisture compared with the control diet.

Tsuchihashi *et al.* (1988); Djouzi and Andrieux (1997) reported, that GOS diet has a bulking up effect on intestinal lumen due to its low absorbability, because GOS are not hydrolyzed by digestive enzymes.

The fact that, in this study, the parameter related to the water content of the feces volume, obtained significantly better values in the prebiotic and synbiotic groups may be a consequence of the higher SCFAs concentrations in the participants belonging to these groups.

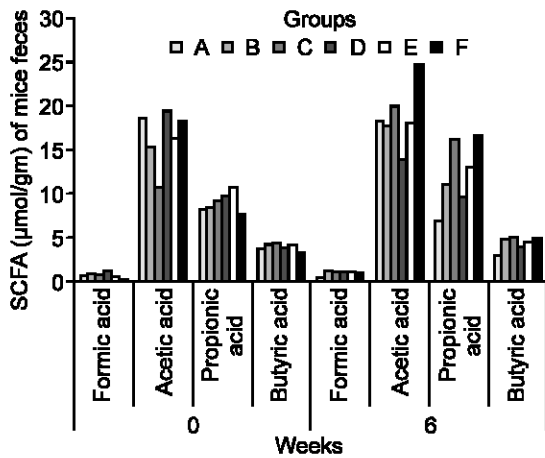


Fig. 5: SCFA ($\mu\text{mol/g}$) of mice feces after 0 and 6 weeks

Short chain fatty acid of mice feces: Figure 5 shows that formic acid concentrations at week 0 were 0.605, 0.901, 0.783, 1.185, 0.536 and 0.166 ($\mu\text{mol/g}$ of mice feces) in groups A-F respectively.

Acetic acid concentrations at week 0 were 18.656, 15.347, 10.765, 19.426, 16.273 and 18.275 ($\mu\text{mol/g}$ of mice feces) in groups A-F respectively.

Propionic acid concentrations at week 0 were 8.346, 8.444, 9.217, 9.642, 10.732 and 7.633 ($\mu\text{mol/g}$ of mice feces) in groups A-F respectively.

Butyric acid concentrations at week 0 were 3.678, 4.218, 4.266, 3.783, 4.038 and 3.32 ($\mu\text{mol/g}$ of mice feces) in groups A-F respectively.

At week 6 formic acid increased significantly ($p < 0.05$) in groups B, C, E and F to reach 1.181, 1.091, 1.141 and 1.066 ($\mu\text{mol/g}$ of mice feces) respectively while decreased significantly in control groups A to 0.452 ($\mu\text{mol/g}$ of mice feces) and decreased non significantly ($p > 0.05$) in control group D to 1.163 ($\mu\text{mol/g}$ of mice feces).

Acetic acid concentrations increased significantly ($p < 0.05$) at week 6 for groups B, C, E and F to 17.737, 19.899, 18.048, 24.766 ($\mu\text{mol/g}$ of mice feces) while it decreased significantly ($p < 0.05$) in control groups A and D to 18.338 and 13.930 ($\mu\text{mol/g}$ of mice feces) respectively.

Propionic acid concentrations increased significantly ($p < 0.05$) at week 6 for groups B, C, E and F to 11.025, 16.174, 13.079, 16.644 ($\mu\text{mol/g}$ of mice feces) while it decreased significantly ($p < 0.05$) in control groups A to 7.035 ($\mu\text{mol/g}$ of mice feces) and decreased non significantly ($p > 0.05$) in control group D to 9.685 ($\mu\text{mol/g}$ of mice feces).

Butyric acid concentrations increased significantly at week 6 for groups B, C, E and F to 4.847, 4.884, 4.408, 4.842 ($\mu\text{mol/g}$ of mice feces), while it decreased significantly ($p < 0.05$) in control groups A to 2.942 ($\mu\text{mol/g}$ of mice feces) and non significantly ($p > 0.05$) in control group D to 3.836 ($\mu\text{mol/g}$ of mice feces).

The best group in increasing formic acid concentration was F followed by E, C and B respectively. Best group in increasing acetic acid concentration was C followed by F, B and E respectively. The best group in increasing propionic acid concentration was F followed by C, B and E respectively. Best group in increasing butyric acid concentration was F followed by B, C and E respectively. In totally Group F was the best in increasing SCFA followed by C, E and B. This means synbiotic groups F and C were the best at all followed by prebiotic groups B and E.

The increase in SCFA is in agreement with many authors who found increasing in SCFA concentrations in cecal and fecal samples after the feeding on GOS or synbiotic such as Ichiro *et al.* (2004); Tzortzis *et al.* (2004); Michelle *et al.* (2007) and Xiao *et al.* (2009).

In contrary with the finding of Alles *et al.* (1999), Yap *et al.* (2005) and Liang and Shah (2006) who reported decreasing in SCFA concentrations after feeding on GOS or synbiotic.

SCFA such as acetic, propionic and butyric acids are the principal products of fermentation, through their absorption and metabolism, the host is able to salvage energy from food that is indigestible in the upper intestine (Topping and Clifton, 2001).

The fecal concentration of SCFA, a parameter related to the fermentation of some carbohydrates by lactic acid bacteria and other gut bacteria, was higher in the synbiotic groups (F and C) followed by prebiotic (E and B) groups when compared to the control groups (D and A). SCFA are the main energy source for colonocytes and contribute to several gut functions including carbohydrate and lipid metabolism, control of the colonic pH, maintenance of the integrity of the colonic mucosa, intestinal motility or absorption (Yajima, 1985; Robertfroid *et al.*, 1995; Mortensen and Clausen, 1996). This study demonstrated that the oral administration of local strain of probiotic *L. rhamnosus* and GOS, was well tolerated and exerts a beneficial effect on the bowel function. The addition of *L. rhamnosus* affected the production of SCFA comparing with GOS alone. This suggests that the bacterial addition was present in the fermentation.

The present study has shown that oligosaccharide demonstrated potentially prebiotic properties (i.e. increase SCFA concentrations), the prebiotic effect was further magnified by the addition of *L. rhamnosus*.

In vitro pure culture studies have shown that GOS are readily utilized by *Bifidobacteria* and *Lactobacilli* (Tanaka *et al.*, 1983). So when *L. rhamnosus* accompanied to GOS could create a good environment inside the gut that would encouraged fermentation to shift from proteolytic (putrefaction) to a more saccharolytic colon physiology which enhanced pH decreasing.

pH decreasing with increasing SCFA production that produced by utilization of GOS will provide a suitable environment to make beneficial bacteria predominant upon pathogenic bacteria.

SCFA, as the major end products of fermentation, may decrease intestinal pH, which may prevent colonization of pathogenic bacteria and may stimulate health promoting bacteria (Swennen *et al.*, 2006).

Conclusion: The consumption of infants formula + synbiotic (group F) by mice gave the best results dealing with increasing *Lactobacilli* and *Bifidobacterium* counts and decreasing *Staphylococci* and *Coliform* counts, decreasing fecal pH, increasing fecal moisture and increasing in SCFA concentration.

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