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Original Research Article

Probiotic Effects of Feeding with Bifidobacterium Pseudocatenulatum G4 on Female Sprague-Dawley Rats

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ABSTRACT

Introduction: Studies on *In Vivo* effect of probiotics are limited.
Objective: To evaluate probiotics effects on selected microbial groups and related metabolic activity in rats received different *Bifidobacterium pseudocatenulatum* G4 preparations.
Methodology: 30 rats were acclimatized to the experimental condition for 10 days and randomly assigned into five different groups. Each group received for a period of 20 days either sterile water (control) or one of the following milk supplements: sterile non-fermented liquid milk (SM), sterile *B. pseudocatenulatum* G4 fermented milk (SG4FM), *B. pseudocatenulatum* G4 fermented milk (FG4M), and *B. longum* BB536 fermented milk (FBB536M).
Results and discussion: Compared with the control group bifidobacteria population in ceacum and colon significantly ($P < 0.05$) augmented in all supplements recipients groups. Consequently increases in short chain fatty acids (SCFA) including acetic, propionic, and butyric acids encountered without significant ($P < 0.05$) effect on nutrients availability. Lactobacillus and total anaerobes were increased; while total aerobes and potential pathogens (staphylococcus, enterococcus and enterobacteriaceae) were reduced; and salmonella and coliform maintained relatively unchanged.
Conclusion: Strain G4 supplements have promoted healthier microbiota communities in ceacum and colon of the treated rats. Therefore, further studies are recommended to approve other health benefits.

Keywords: *Bifidobacterium*, rat, microbiota, SCFA, nutrients availability, probiotics concept.

INTRODUCTION

Human gastrointestinal tract harbours up to 14 log CFU total bacteria that mediates the digestive functions of the colon. This complex microbiota includes beneficial microorganisms for host besides opportunistic pathogens. [1] The dominant groups of normal microbiota are obligate anaerobes of which 25% are bifidobacteria [2] presence together with *Lactobacillus*, bacteroides, eubacterium, *Fusobacterium*, *Clostridium* and anaerobic cocci. [3] Several factors concerning host, microbe and microbial interactions negatively disturb the intestinal proportion of bifidobacteria and lactobacillus, thus lead to illness. [4]

Prebiotics and probiotics have been successfully exploited to maintain optimum metabolic, trophic and protective activities that prevent disorders associated with lower concentrations of beneficial bacteria in the gastrointestinal tract. [5,6] Prebiotics are carbohydrate ingredients selectively stimulate the growth of bifidobacteria and lactobacillus in the colon. [1] Probiotics are microbial supplements capable to modulate microbiota components and related activity to beneficial state. [7, 8]

Lactobacillus and *Bifidobacterium* species constitute a significant proportion of probiotic cultures used in food applications. [9] *Bifidobacterium* has been reported to be one of the predominant genera in the intestinal microbiota of infants protecting them against many invaders. [10] Therefore, strains of this species are extensively screened and successfully used as probiotics. [11-13]

In this respect, research in our laboratory screened *Bifidobacterium pseudocatenulatum* isolates by RAPD and ERIC sequence-based PCR. [14] Recent findings initiated strain G4 isolate as a potential probiotic candidate. [15] More recently, investigation on BALB/c mice assessed the safety criterion of this strain. [16] However, to consider this strain as a probiotic, it should have capability to modulate populations and metabolic activities of the indigenous microbiota to state that brings about advantageous to the host. [17] Therefore, this study was conducted to explore probiotic effect of strain G4 in animal model using rats.

MATERIALS AND METHODS

Animals: Six weeks old female Sprague-Dawley rats with an average initial weight of 176 g ± 3.306 were purchased from institute of medical research (IMR, Kuala Lumpur, Malaysia). They were housed two per cage due to space limitation. A 12 h light dark cycle and a controlled atmosphere (22±2 °C; humidity 55±2%) were maintained throughout the study. Ten days acclimatization period under experimental condition was scheduled to observe any sign of illness, following which rats were randomly assigned into five different groups (n = 6) at random and treated for twenty days. Throughout the thirty days trial, the rats were offered sterile water and standard rodent feed (Lab-feed, Sydney, Australia) *ad libitum*. The bedding in cages was changed twice a week. Individual body weight of each rat was measured at ten days intervals.

Experimental design: The first group set as control was maintained only on sterile distilled water. Other treatment groups received reconstituted skim milk bases. Suspension of 10% (w/v) were sterilized by autoclaving at 121 C for 15 min and used directly for second groups (SM). The third group treated the sterile *Bifidobacterium pseudocatenulatum* G4 fermented cultured milks (SG4FM) to test effects of metabolic product. It was prepared by inoculating 10% *Bifidobacterium pseudocatenulatum* G4 culture to the skim milk suspension followed by incubation for 48 hour. Group four and five received the *Bifidobacterium pseudocatenulatum* G4 (FG4M) and *Bifidobacterium longum* BB536 cultured fermented milks (FBB536M), respectively. Commercial strain BB536 was considered to be a reference probiotic. All supplementary milks were freshly prepared and given daily. Bottles containing the drinking water were removed for a period of three hours followed by milk-based supplementations provided for 7 hours daily after 1: 1 dilution with sterile water.

The experimental protocol was approved by the Animal Ethics Committee of Universiti Putra Malaysia (Reference number: 2006/Dec/Nazrul/Biomedic/0089) and adhered to Guiding Principle in the Care and Use of Animals.

Bacteriological quantification in ceacum and colon contents: Four rats from each group were used for bacteriological analysis. Ceacum and colon contents were collected under strict aseptic conditions in sterile Eppendorf tubes to avoid any cross contamination. A suspension of 10% (w/v) was made with anaerobic buffered peptone water containing 0.5% g/l cysteine. The content was gently homogenized inside a cabinet and serially diluted prior to plating on different agar plates. Media used for total aerobe, total anaerobe, enterobacteriaceae and enterococcus reported previously by Santos et al. [18] Staphylococcus, coliform, and lactobacillus enumerated following Liong and Shah [19] method. While for bifidobacteria and salmonella, Tryptone peptone yeast agar and Brilliant green agar were used, respectively. Incubation conditions of media used for enumerations are shown in Table 1.

Analysis of total soluble solid (TSS), short chain fatty acids (SCFA) and glucose: All collected content of ceacum and colon of two rats from each group were stored at –40 ºC until analysis. Each sample was mixed with de-ionized water to prepare slurry used to test total soluble solid (TSS). The slurry was further centrifuged at 10000 rpm for 15 min, the supernatant were collected, filtered through a 0.2 µm nylon membrane filter and injected into HPLC to determine short chain fatty acids (SCFA) and glucose.

Profiles of SCFA were analyzed by high- performance liquid chromatography (HPLC) (Shimadzu LC-10AS Liquid Chromatography, Japan) with a Shimadzu SPD-10AV UV-VIS detector. An organic column packed with 9 µm of polystyrene divinylbenzene ion exchange resin (Aminex HPX-87H; 300 mm x 7.8 mm, Bio-Rad Laboratories, USA) maintained at 65 ºC was used. The UV detector was set at 220 nm and the mobile phase was 0.009 N sulphuric acid with a flow rate of 0.7 ml/ min.

Glucose content was analyzed by high- performance liquid chromatography (HPLC) system (Jasco Co., Tokyo, Japan), equipped with 250 mm x 4.6 mm Alltech Amino 5µ column (Alltech Associates, Inc., Deerfield, USA). The mobile phase used was 80% (v/v) acetonitrile (Merck, HPLC grade). The flow rate was set at 1.8 ml/min and analysis was carried at ambient temperature with RI detector (Jasco RI-1530, Jasco Co., Tokyo, Japan). Quantification of glucose was carried out by external standard method.

Statistical analysis: Analysis of normal data was carried out with MINITAB statistical software. [20] Two-way ANOVA was used to test for interaction, while one-way ANOVA was performed to examine significant difference between means of different groups. Probability levels of less than 0.05 were considered significant ($p < 0.05$). Tukey's-test was used to perform multiple comparisons between means.

Data on white blood cell (WBC), monocytes, eosinophil, red blood cell (RBC), mean corpuscular haemoglobin concentration (MCHC), Ca, Na, Cholesterol were not normally distributed. Randomization test was performed and the data were analyzed by Kruskal-Wallis test.

RESULTS AND DISCUSSION

Different microbial groups in ceacum and colon of rats: Selected microbial groups o in ceacum and colon of rats fed with different *Bifidobacterium* preparations are reported in Tables 1 and 2. Based on feeding trials, alterations in ceacum and colonic microbial groups of the rats are ensured.

Feeding with FBB536M induced the highest lactobacillus increase in colon by two fold (1.694 log CFU/g) than in ceacum (0.8235 log CFU/g) of the same group. The increases were also high in FG4M group recording population of 1.554 log CFU/g in

colon and 1.0905 log CFU/g in ceacum (Table 2).

Bifidobacteria exhibited the highest increased among all microbiota communities in ceacum and colon as well. However, the increases were significant (P <0.05) in groups received FG4M and FBB536M supplements (Table 2). Colonic increases were 2.238 and 2.235 CFU/ g; and ceacum of the same groups recorded rises of 1.868 and 1.713 log CFU/g, respectively. This met the ultimate intent of this investigation to fulfill probiotics concept via providing the gastrointestinal tract with elevated viable populations of beneficial bifidobacteria and lactobacillus. [21]

Total anaerobes of ceacum and colon increased in all groups of rats received the different *Bifidobacterium* supplements, but the increases were not significant as compared to the control. SFG4M group showed the highest increase in total aerobes in colon (0.5647 log CFU/ g) and ceacum (0.547 log CFU/ g). However, FBB536M group recorded reduction of 0.1643 log CFU/ g that might be due to activity of the viable supplements. SM recorded the highest increase of 1.4707 log CFU/ g in colon and FG4M was the highest stimulant in ceacum part (0.622 log CFU/ g).

The harmful pathogens of gut microbiota include (transients) species of staphylococcus, enterococcus, enterobacteriaceae, salmonella and coliform. Table 7 shows that coliform in colon and salmonella in ceacum remained unchanged. In ceacum coliform slightly increased, while salmonella in colon slightly decreased. Enterococcus and staphylococcus were reduced in all *Bifidobacterium* recipients groups compared with control (Table 3). In study to evaluate the impact of probiotic preparations on the composition of human intestinal microbiota, faecal concentrations of Streptococcus salivarius ssp. thermophilus, lactobacilli and bifidobacteria increased significantly in all treatment recipient individuals compared to those on basal level from the 20th day and remained stable throughout the study. It was also reported none significant increases of Bacteroides, clostridia, coliforms, total aerobic and anaerobic bacteria. [22] Overall observation on microbiota distribution showed higher population of total anaerobe, lactobacillus, bifidobacteria, Enterococcus and staphylococcus in colon. While total aerobes, coliform, enterobacteriaceae and salmonella were higher in ceacum (Tables 2 and 3). However, there were no significant differences in populations of lactobacilli, total anaerobes, total aerobes, bifidobacteria and streptococcus of ileal and ceacum in chickens fed with or without Lactobacillus cultures were reported, except after 30 days feeding trail. [23]

Short chain fatty acids (SCFA) in ceacum and colon of rats: The alterations in levels of each microbial group in ceacum and colon of rats (Table 2 and 3), revealed different activity (Table 4), thus encountered changes in ceacum and colonic short chain fatty acids. Potentials supplements for high SCFA productions were FG4M and FBB536M. The accumulation of high acetic was noted in ceacum while propionic and butyric were mostly found in colon part (Table 4). These acids are readily absorbed and metabolized in the liver and muscle tissues providing energy to the human body. [24, 25]

However, the amounts and types of short-chain fatty acid (SCFA) formation by intestinal bacteria is largely determined by host, environmental, dietary and microbiological factors, substrate availability, bacterial species composition of the microbiota, and intestinal transit time. [26]

Feeding with *Bifidobacterium* preparations significantly increased acetic acid in ceacum. In the colon, only FBB536

group recorded significant acetic increase (Table 4). In general, accumulation of high acetic acid in intestinal tract is favored. It is important source of fuel for skeletal muscles. [27]

Propionic acid increased in all groups received SM, SFG4M, FG4M, and FBB536M. In FG4M, and FBB536M groups, the acid increased by 315 and 282 mmol/g in ceacum and 205 and 245 mmol/g in colon, respectively (Table 4). Propionate is partially metabolized by the gut epithelium and liver takes up most of the remainder. It is the only SCFA that can be a major source of glucose; acetate, butyrate and longer chain SCFA. [28]

The results obtained on butyric showed that FG4M group recorded increase of 60 and 31.5 mmol /g in ceacum and colon, respectively. Similarly, feeding with FBB536 increased butyric by 36.6 and 22 ml mol in ceacum and colon, respectively (Table 4). Butyric acid has ability to block the cell cycle at mitosis, promote cell differentiation, protect against colonic cancer, [29] and represent important source of energy for human colonocytes. [30]

Table 1: Enumeration media and incubation conditions of different microbiota communities in ceacum and colon of rats fed different milk supplements[a]

Type of media	Bacterial group	Incubation****
Nutrient agar	Total aerobe**	Aerobic
Brain heart infusion agar	Total anaerobe**	Anaerobic
Eosin methylene blue	Enterobacteriacea*	Anaerobic
Macconky agar	Coliform**	Anaerobic
Esculin bile agar	Enterococcus***	Anaerobic
Brilliant green agar	Salmonella**	Aerobic
Mannitol salt agar	Staphylococcus**	Aerobic
De Man Rogosa Sharpe agar	Lactobacillus**	Anaerobic
Tryptone Phytone Yeast agar	Bifidobacteria**	Anaerobic

[a]All samples were incubated at 37 ºC.Incubation for one day.
** Incubation for two days** Incubation for three days****Anaerobic condition was created in anaerobic jars using gas-generating kits.

Table 2: Total aerobe and potential helpful population from ceacum and colon of rats received different Bifidobacterium supplements[a]

Bacterial groups	Location	Treatments[b]				
		Control[c]	SM[c]	SG4FM[c]	FG4M[c]	FBB536M[c]
Total anaerobe	Colon	7.35± 0.17	8.83±0.83	8.15±0.38	8.37±0.70	8.36±0.68
	Ceacum	7.61± 0.61	8.02±0.36	8.19±0.36	8.23±0.33	8.19±0.22
Total aerobe	Colon	7.23 ±0.16	7.52±0.51	7.80±0.49	7.32±1.15	7.07±0.58
	Ceacum	7.53 ±0.55	7.54±0.21	8.08±0.30	7.54±0.56	7.86±0.26
Lactobacillus	Colon	7.24±0.59	8.66±0.99	8.51±0.54	8.79±1.06	8.93±0.62
	Ceacum	7.17±0.17	8.26±0.29*	8.24±0.12*	8.26±0.31*	8.00±0.25*
Bifidobacteria	Colon	6.72±0.62	7.89±0.15	8.73±0.20	8.96±0.85	8.96±0.65
	Ceacum	6.33±0.53	7.59±0.52	8.17±0.26	8.40±0.70*	8.25±0.41*

[a]Values are means ± STD of four rats [b]Treatment groups received the followings: Control on sterile water, SM on sterile liquid milk, SG4FM on sterile G4 fermented milk, FG4M on G4 fermented milk, FBB536M on BB536 fermented milk.[c]Means of same bacterial group in column for each treatment are P≥ 0.05.[*]Means of same bacterial group in row at specific intestinal region are significantly different (P≤ 0.05).

Table 3: Potential pathogens (Log CFU/g) from ceacum and colon of rats received different *Bifidobacterium* supplements[a]

Bacterial groups	Location	Treatments[b]				
		Control[c]	SM[c]	SG4FM[c]	FG4M[c]	FBB536M[c]
Enterococcus	Colon	6.25±0.55	5.38±0.75	4.67± 0.14	5.12±0.36	4.89±0.45
	Ceacum	5.64±0.25	5.51±0.40	4.57 ±0.32	4.96±0.99	4.67±1.44
Coliform	Colon	3.63±0.11	3.44±0.13	3.61± 0.28	4.16±0.63	3.65±0.29
	Ceacum	3.59±0.13	3.67±0.38	4.17± 0.62	4.29±0.95	4.14±0.91
Enterobacteriacea	Colon	5.44±0.91	4.18±0.15	3.81±1.66	2.99±0.67	2.77±0.53
	Ceacum	5.99±0.13	4.83±0.94	4.97±1.26	3.91±0.81	4.67±1.44
Staphylococcus	Colon	5.57±0.33	5.57±0.95	5.19±0.90	4.75±0.98	4.71±0.92
	Ceacum	5.14±0.70	5.53±0.40	4.89±0.58	4.69±0.66	4.48±0.47
Salmonella	Colon	4.33±1.03	4.67±1.00	4.07±0.32	4.08±0.25	3.96±0.41
	Ceacum	4.32±0.19	3.67±0.24	4.14±0.70	4.28±0.75	4.37±0.61

[a]Values are means ± STD of four rats [b] Treatments groups received the followings: Control on sterile water, SM on sterile liquid milk, SG4FM on sterile G4 fermented milk, FG4M on G4 fermented milk, FBB536M on BB536 fermented milk.[c]Means of same bacterial group in column for each treatment are P≥ 0.05.[d]Means of same bacterial group in row at specific intestinal region P≥ 0.05.

Table 4: Short chain fatty acids in ceacum and colon of rats received different Bifidobacterium supplements for a period of 20 days[a]

Acids	Location	Treatments[b]				
		Control	SM	SG4FM	FG4M	FBB536M
Acetic acid	Ceacum	11.37±2.52	7.803 ± 0.658	28.00±15.90*	31.38±2.94*	31.79±0.817*
	Colon	11.18± 0.80	16.24±1.34	15.37±5.15	15.96±9.12	22.00±3.08*
Probionic acid	Ceacum	440±113	636± 160	669±152	755±82**	722±197**
	Colon	589±205	528± 188	744±168	794±227**	834±186**
Butyric acid	Ceacum	76.0±20.30	113.10±32.1	105.10±27.20	136.00±54.00	112.60±28.00
	Colon	110.90±23.60	119.80±33.50	120.00±30.20	142.40±22.90	132.00±33.30

[a]Values are means ± STD of two rats [b]Treatments groups received the followings: Control on sterile water, SM on sterile liquid milk, SG4FM on sterile G4 fermented milk, FG4M on G4 fermented milk, FBB536M on BB536 fermented milk. *Means of same bacterial group in row at specific intestinal region are significantly different ($P \leq 0.05$). **Means of same bacterial group in row at specific intestinal region are significantly different ($P \leq 0.01$).

Levels of nutrient availability: Table 5 and 6 describes changes in nutrient availability and glucose in ceacum and colon of rats treated different milk supplements. No significant differences were found based on the TSS and glucose determined. Nevertheless, the TSS and glucose of ceacum and colon remained higher in all recipient groups than in the control. Regarding nutrient availability, the highest records in ceacum were in groups received SG4FM, FBB536M, and FG4M groups in descending order; while the level of availability in colon were SM, FBB536 and then SG4F in descending order (Table 5).

Table 5: Total soluble solid (TSS) in ceacum and colon of rats received different Bifidobacterium supplements for a period of 20 days[a]

Treatments[b]	Intestinal region	
	Ceacum	Colon
Control	5.54 ± 1.89	5.51 ± 1.37
SM	5.78 ± 0.09	7.65 ± 2.08
SG4FM	6.99 ± 1.48	6.25 ± 0.16
FG4M	5.86 ± 1.58	5.77 ±1.39
FBB536M	6.46 ± 0.72	6.70 ± 0.21

[a]Mean ± STD of two rats. P > 0.05 (control vs. supplement recipient groups) [b] Treatments groups received the followings: Control on sterile water, SM on sterile liquid milk, SG4FM on sterile G4 fermented milk, FG4M on G4 fermented milk, FBB536M on BB536 fermented milk.

Substrate availability is a major factor used to monitors the bacterial growth and a subsequent metabolic product during colonic fermentation. Supplement recipient groups contained relatively similar glucose except SM group where the concentration was the highest. In addition, contributions of pre-and post fermentation were evident by lower concentrations of glucose in FG4M and FBB536M groups (Table 6). This might indicate higher metabolic activity in colon region due to viable *Bifidobacterium* supplements.

Table 6: Glucose content (mg/g sample) in ceacum and colon of rats received different Bifidobacterium supplements for a period of 20 days[a]

Treatments[b]	Intestinal region	
	Ceacum	Colon
Control	278.40 ± 49.30	201.30 ± 57.10
SM	403.20 ± 24.00	417.98 ± 8.91
SG4FM	385.00 ± 187	418.00 ± 172
FG4M	326.50 ± 37.40	313.60 ± 81.30
FBB536M	338.11 ± 2.01	245.40 ± 49.20

[a]Mean ± STD of two rats. P > 0.05 (control vs. supplement recipient groups) [b]Treatments groups received the followings: Control on sterile water, SM on sterile liquid milk, SG4FM on sterile G4 fermented milk, FG4M on G4 fermented milk, FBB536M on BB536 fermented milk.

CONCLUSION

Feeding with *Bifidobacterium* preparation increased intestinal *Bifidobacterium* and *Lactobacillus*, which promote healthier gut environment for well-being. They produce SCFA and other active metabolites, which have inhibitory effects against most potential pathogens.

Due to feeding the *Bifidobacterium* preparations, intestinal pathogens declined. Via extending treatment period to more than 20 days, further decline in pathogens could be attained. Overall modifications on intestinal microbial groups and related positive activity of rats fed with *Bifidobacterium* G4 preparations demonstrated that the concept of probiotics

is met. Strains G4 supplements have promoted healthier intestinal environment.

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REFERENCES

1. Gibson GR, Roberfroid MB. Colonic Microbiota, Nutrition and Health. Dordrecht: Kluwer Academic Publishers; 1999.
2. Gibson GR, Probert H M, Loo JV, Rastall RA, Roberfroid MB. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutrition Research Reviews. 2004; 17: 259 – 275.
3. Macfarlane GT, Cummings JH. The colonic microflora, fermentation and large bowel digestive function. In: The large intestine: Physiology, Pathophysiology and Disease. New York: Raven Press; 1991. Pp. 51 - 92.
4. Mitsuoka T. Microbes in the Intestine. Tokyo (Japan): Yakult Honsha CO. Ltd; 1989.
5. Huis in't Veld JHJ, Have-naar R. Probiotics and health in man and animal. Journal of Chemistry Technology and Biotechnology.1991; 51: 562 – 577.
6. FAO/WHO. Reports of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Cardoba (Argentina): American Cordoba Park Hotel; 2001, 1 - 4 October.
7. Fuller R. Probiotics in man and animals. Journal of Applied Bacteriology. 1989; 66: 365 - 378.
8. Charteris WP, Kelly PM, Morelli L, Collins JK. Selective detection, enumeration and identification of potentially probiotic *Lactobacillus* and *Bifidobacterium* species in mixed bacterial populations. International Journal of Food Microbiology.1997; 35: 1 - 17.
9. Gomes AMP, Malcata FX. *Bifidobacterium* spp. and *Lactobacillus acidophilus*: biological, biochemical, technological and therapeutical properties relevant for use as probiotics. Trends in Food Science and Technology. 1999; 10: 139 - 157.
10. Favier CF, Vaughan EE, de Vos WM, Akkermans ADL. Molecular monitoring of succession of bacterial communities in human neonates. Appl. Environ. Microbiol. 2002; 68: 219 - 226.
11. Zhou JS, Shu Q, Rutherfurd K J, Prasad J, Birtles MJ, Gopal PK, Gill HS. Safety assessment of potential probiotic lactic acid bacteria strains *Llactobacillus rhamnosus* HN001, *LB. Acidophilus* HN017, and *Bifidobacterium lactis* HN019 in BALB/C mice. International Journal of Food Microbiology. 2000; 56: 87 - 96.
12. Biavat B, Vescovo M, Torriani S, Bottazzi V. Bifidobacteria: history, ecology, physiology and applications. Annual Microbiology. 2000; 50: 117 – 131.
13. Holzapfel WH, Haberer P, Geisen R, Björkroth J, Schillinger U. Taxonomy and important features of probiotic microorganisms in food and nutrition. American Journal of Clinical Nutrition. 2001; 73: 365S - 373S.

14. Shuhaimi M, Ali AM, Norihan MS, Yazid AM. Classification of *Bifidobacterium* isolates from infant faeces using PCR-based and 16s rDNA partial sequences analysis methods. Bioscience Microflora. 2002; 21: 155 - 161.
15. Wong S , Wendy Y K , Kabeir BM, Shuhaimi M, Rosfarizan M, Yazid AM. Survival of Bifidobacterium pseudocatenulatum strains isolated from beast-fed infants to simulated gastric pH environment. Malaysian Applied Biology. 2006; 35: 57 - 62.
16. Kabeir B M , Yazid AM, Alhakim M, Wong S, Anas M, Shuhaimi M. Safety assessment of Bifidobacterium pseudocatenulatum G4 in BALB/c mice. Letters in Applied Microbiology. 2008; 46: 32 - 37.
17. Fooks LJ, Gibson GR. Probiotics as modulators of the human gut flora. British Journal of Nutrition. 2002; 88: s39 - s49.
18. Santos A, San Mauro M, Diaz DM. Prebiotics and their long-term influences on the microbial populations of the mouse bowel. Food Microbiology. 2006; 23: 498 - 503.
19. Liong MT, Shah NP. Effects of a Lactobacillus casei synbiotics on serum lipoprotein, intestinal microflora, and organic acids in rats. Journal of Dairy Science. 2006; 89: 1390 - 1399.
20. MINITAB. Statistical software, Release 14 for Windows. State College (US): Minitab Inc; 2006.
21. [Coussement P. Pre- and synbiotics with inulin and oligofructose: promising developments in functional foods. Food Technology in Europe. 1996; 2: 102 -104.
22. Venturi A, Gionchetti P, Rizzello P, Johansson R, Zucconi E, Brigidi P, Matteuzzi D, Campieri M. Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. Aliment Pharmacol Ther. 1999; 13: 1103 – 1110.
23. Jin Z, Ho Y W, Abdullah N, Ali MA, Jalaludin S. Effects of adherent Lactobacillus cultures on growth, weight of organs and intestinal microflora and volatile fatty acids in broilers. Animal feed science and technology. 1998; 70: 197 - 209.
24. Cummings JH, Pomare EW, Branch WJ, Naylor CPE, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut. 1987; 28: 1221 - 1227.
25. Hiroshi D. Physiological effects of short-chain fatty acid produced from Prebiotics in the colon. Bioscience and microflora. 2002; 21: 35 - 42.
26. Macfarlane S, Macfarlane GT. Regulation of short-chain fatty acid production. Proceedings of the Nutrition Society. 2003; 62: 67 - 72.
27. Snoswell AM, Trimble RP, Fishlock RC, Storer GB, Topping DL. Metabolic effects of acetate in perfused rat liver. Studies on ketogenesis, glucose output, lactate uptake and lipogenesis. Biochimica et Biophysica Acta.1982; 716: 290 - 297.
28. Goodlad JS, Mathers JC. Large bowel fermentation in rats given diets containing raw peas (*Pisum sativum*). British Journal of Nutrition. 1990; 64: 569 - 587.
29. Mandal M, Olson DJ, Sharma T, Vadlamudi RK. Butyric acid induces apoptosis by up-regulating

bax expression via stimulation of the c-Jun N-terminal kinase activation protein-1 pathway in human colon cancer cells. Gastroenterology. 2001; 120: 71 - 78.

30. Roediger WEW. Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. Gut. 1980; 21: 793 - 798.

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