JOURNAL OF BIOMEDICINE AND TRANSLATIONAL RESEARCH

The Effects of Rusip on the Amount of Lactic Acid Bacteria in Gastrointestinal Tract. Experimental Study in Rats *Rattusnorvegicus Sprague Dawley* Strains

Wiwin Efrizal[1], HendroWahyono[2], Andrew Johan[3]

1. Health Office of Kepulauan Bangka Belitung
2. Department of Clinical Microbiology Faculty of Medicine UNDIP / Dr. Kariadi Hospital Semarang
3. Department of Biochemistry Faculty of Medicine UNDIP / Dr. Kariadi Hospital Semarang

Article info
History :
Received 24 November 2016
Accepted 25 December 2016
Available 29 December 2016

ABSTRACT

Background : Rusip consumption can change the microbial composition of the digestive system. However, the changes on lactic acid bacterial count due to rusip consumption have not been known yet.
Objective: The purpose of the study was to analyze the effect of rusip consumption to the number of lactic acid bacteria in the gastrointestinal tract.
Methods: Experimental study with post test only control group design using twelve Rattusnovergicus Sprague dawley strains aged 2-2.5 months which were divided into 2 groups. Rats adapted for a week, then they were given rusip 5.1 mg/g body weight for 14 days. Cecal content were taken on the 22th day to be analyzed for the lactic acid bacteria count.
Results : The number of lactic acid bacteria (LAB) in the group given rusip was lower compared to the control group, but not significantly different (p=0.180). Weight gain was greater in the treatment group compared to the control group. The given group tends to have more weight in cecal content and lower pH compared to the control group.
Conclusion : Rusip consumption does not increase the number of lactic acid bacteria in gastrointestinal tract.
Keywords : Rusip, cecum, fermentation, lactic acid bacteria, gastrointestinal tract.

INTRODUCTION

Gastrointestinal tract is a home to the largest collection of microbiota.[1]Most of microbiota is found in the colon with the amount up to 10^{11} - 10^{12} cells per gram and it becomes the largest number of cells which are noted in the microbial habitat.[2] A symbiotic relationship between the host and gut microbiota has been essential for normal microbiota in many functional aspects and intestinal development.[3]

Commensal bacteria has an important role for the digestion and absorption of nutrients, primarily carbohydrates that cannot be digested, and also the synthesis of vitamins which is essential, and regulation of metabolism as well as fat storage.[4] Intestinal microbiota also has a crucial role in the homeostasis of intestinal epithelium, angiogenesis, and the development of intestinal immune system.[5] A group of intestinal microbiota are dominated by *Firmicutes* and *Bacteroidetes* bacteria that form a component of largest colonies in the distal intestine between 100 phyla which is known in this domain of life.[1]

*Firmicutes*consists of bacteria from *Clostridia* class and a subset of *Mollicutes* and Basil, including Enterokokki, Lactobacilli, and Laktokokki, which are capable of oxidizing organic sugar through fermentation to produce a large amount of lactic acid and carbon dioxide.

The low amounts of other bacteria in a tight anaerobic environment such as intestine is caused by their inability to compete with the members of *Bacteroidetes* and *Firmicutes*.[6]

At this time, it is known that the diversity of subdominant species groups, for example *Lactobacillus*, is much less stable from time to time than the other dominant and the stability of the larger community is in the colon than in the ileum.[7] Diet plays an important role in terms of microbiota composition and the changes in diet are known turning the content of microbiota in gastrointestinal tract.[8] Human and microbiota symbiotic group of their intestine are very adaptive in response to the diet changes.[9]The limitations and the high cost of this study as well as the ethical problems lead the study about gastrointestinal microbiota to be conducted mostly in experimental animals.[10]

One of the traditional foods processed by fermentation that contain lactic acid bacteria (LAB) is rusip. Rusip is a typical food from Bangka Belitung[11] made within the household scale during the fishing season with a small selling scaleat the market or home. Rusip is usually consumed as a mixture for the sauce (*sambal*), either by being cooked first or directly consumed as a side dish without cooking (raw).[12]Rusip is often found in the typical food shops in Bangka Belitung, and some are being sold directly from home to home.

Rusip processing is conducted by traditional fermentation which generally takes place spontaneously. Components of the mixture which are used in the rusip production other than fish are salt and carbohydrates. The composition are still extremely diverse for the amounts and types.[11]The process of fermented fish preservation will include chemical and microbial enzymatic process during fermentation process that ultimately determine the microbiological and chemical characteristics of fermented fish.[13]Lactic acid fermentation takes place in anaerobic condition by anaerobic microbiota or obligate.[14]

The purpose of this study was to analyze the effect of rusip consumption to the amount of lactic acid bacteria in gastrointestinal tract of *Rattus novergicus Sprague Dawley* strain.

MATERIALS AND METHODS

This study was conducted after obtaining an approval from Health Research Ethics Committee of Faculty of Medicine Diponegoro University and Dr. Kariadi Hospital Semarang. This study design was posttest only control group. The experimental was carried out a measurement of lactic acid bacterial count after treatment.

This study used *Rattus novergicus Sprague Dawley* strain which were obtained from Integrated Research and Testing Laboratory of Gajah Mada University, as much as 12 rats that undergone the adaptation period for one week. They were divided into two groups randomly with each group consisted of six rats in accordance with the calculation based on WHO. The control group was given only the standard feed which its composition was based on *Official Methods of Analytical of the Association of Official Analytical Chemists (AOAC)* in 1990.[15] In the treatment group, it was given the standard feed added with rusip as much as 5.1 mg / g of weight based on the calculation using *Human Equivalent Dose (HED)* formula.[16]

Rusip production used raw material of Galer anchovy which was obtained from supermarkets and salt (15% w / w) as well as palm sugar (10% w / w) which were obtained from traditional market in Semarang city. The mixture was then left for ripening. While waiting for ripening of rusip, the adaptation period for experimental animal was started at the 10^{th} day of ripening. Then, at the 17^{th} day of ripening, the treatment period was started. This treatment was continued for 14 days.

The dependent variable was the amount of lactic acid bacteria (LAB) in gastrointestinal tract. The ratiosca lewas cultured from each cecal content of the samples in each group on the 22^{nd} day or a day after 14 days of rusip consumption. The color and consistency evaluation of cecal content were conducted subjectively by visual observation. The criteria for the color and consistency evaluation of cecal content are shown in Table 1

Table 1. Scoring for the Color and Consistency Evaluation of Cecal Content

Score	Color Criteria	Consistency Criteria
1	Cecal Content is black	Cecal Content is hard
2	Cecal Content is greenish black	Cecal Content is soft

The evaluation of lactic acid bacteria (LAB) counts used *de Mann ROGOSA Sharpe (MRS) Agar* media produced by Merck. The culture was obtained from ½ gram of cecal content which was dissolved in 4.5 ml physiological saline of diluent solution (NaCl 0.85%) in stages. The growth of colonies was calculated according to International Commission on Microbiological Specifications for Foods (ICMSF) standard, then morphology and catalase tests were conducted.

The results of the analysis were presented in tables and graphs. Statistical analysis uses t - test if 1there was normal distribution and uses Mann-Whitney-U test if the distribution was not normal. The data was considered significantly different if p <0.05 with 95% confidence level. The data processing used statistical software (SPPS version 21).

RESULTS

During the research, there was not found a dead or drop-outs rat. The examination of Total Bacteria Counts and Lactic Acid Bacteria Counts in rusip for 10 days of ripening (initial adaptation), 17 days of ripening (initial rusip consumption), and 30 days of ripening (final rusip consumption) were presented in Figure 1.

Figure 1. Total Plate Counts and Lactic Acid Bacteria Counts in Rusip (log cfu/g).

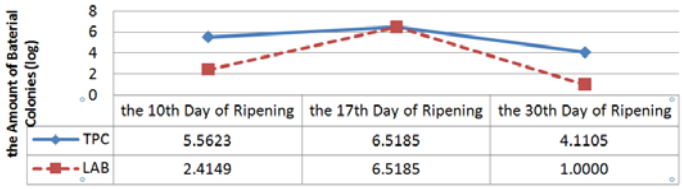

Figure 1. TPC (Total Plate Counts) was conducted by looking at the total amount of bacterial colonies that existed in rusip in the 10th, 17th, and 30th day (log) compared with the number of LAB in rusip on the same day using *de Mann ROGOSA Sharpe (MRS) Agar*.

The measurement of rats' weight was conducted at the beginning of adaptation period, the beginning of treatment period, the middle, and the end of treatment period in order to find out the amount of rusip consumption and the weight development of rats during the research. The mean results were presented in Table 2.

Table 2. The Mean Weight of Rats in the Beginning and the End of Research.

Parameters	n	Mean± sb
Initial Weight		
Control (C)	**6**	191,17±9,09
Treatment (T)	**6**	195,50±2,74
Final Weight		
Control (C)	**6**	206,33±8,98
Treatment (T)	**6**	222,67±3,83

The median of initial weight in the control group and the treatment group was not much different. It was equal to ± 1.5 grams, whereas in the end of research there was ± 14 grams of difference between the control group and the treatment group. The highest weight development during the research occurred in the treatment group than the control group. The weight development in the treatment group was± 1.8 times when it was compared to the weight development in the control group as presented in Table 3.

Table 3. The Mean of Rats' Weight Development During Research

Weight Development	n	Mean±sb	*p*
Control (C)	6	15,17±1,33	0,000
Treatment (T)	6	27,17±1,60	

The observation that was conducted on the color and consistency of cecal content is presented in Table 4. The mean weight ofcecal content and pH of cecal content were shown in Table 5. The control group has lower mean weightof the cecum by ± 0.2 than the treatment group. It also has higher mean pH of the cecum by ± 0.2 compared to the treatment group.

Table 4. The Observation Results of Cecal Content

Parameters	Control	Treatment
Color		
Black	3 (50%)	2 (33,3%)
Greenish Black	3 (50%)	4 (66,7%)
Consistency		
Hard	4 (66,7%)	3 (50%)
Soft	2 (33,3%)	3 (50%)

Table 5. The Mean Weight and pH of Rats' Cecal Content

Parameters	n	Mean± sb	*p*
Weight of cecal content			0,368
Control (C)	6	1,22±0,33	
Treatment (T)	6	1,43±0,45	
pH of cecal content			0,317
Control (C)	6	6,83±0,30	
Treatment (T)	6	6,61±0,41	

The mean of Lactic Acid Bacteria (LAB) counts obtained from each group was shown in Table 6. Testing for normality by using *Shapiro-Wilk* indicates that the data distribution of Lactic Acid Bacteria (LAB) counts was not normal with $p<0.05$. Homogeneity test by using Levene test showed homogeneous result ($p> 0.05$).

Table 6. Median of Lactic Acid Bacteria (LAB) Counts in Rats'Cecal Content(log cfu / g)

Groups	n	Median (minimum-maximum)	*p*
Control (C)	6	8,44 (0,00 - 9,07)	0,180
Treatment (T)	6	8,15 (0,00 - 8,27)	

Nonparametric test result of *Mann-Whitney-U* showed that there was no significant difference between Lactic Acid Bacteria (LAB) counts in rats which were given rusip with rats which were not given rusip.

DISCUSSION

Microbiota plays a crucial role to exert an important function for human health and welfare maintenance, such as stimulation of the immune system, antagonistic effect against pathogens, carcinogenic compounds detoxify, fermentation of foods that cannot be digested, and the release of various metabolites that are involved in the relation between microbiota and host.[17] Studying the flora of the colon is more appropriate than studying stool to determine the functions that occur in cecum, such as fermentation of dietary fiber and endogenous substrates, or for a disease that involves the right part of the colon, such as *ileocecal Crohn's* disease.[18]

If fermentation level of fibers in cecum is higher, the water absorption will be lower. This is due to fibers' role in binding the water decreases as the number of fibers components fermented. Consequently, the cecal content contained in the colon will be harder.[15] In this study, the weight and consistency of cecal content in the control group were not different with the treatment group. However, the mean value of the weight and consistency of cecal content in the control group which was lower than the treatment group showed that rusip assisted in binding water and other organic compounds, such as fat, cholesterol, bile acids, vitamins, and minerals. These components were accumulated in cecum.[19]Organic compounds and bacteria contained in rusip also supported in decreasing the mean pH of cecal content to become lower in the treatment group than the control group.

Giving fermented foods that contained LAB, which one of them was rusip, provided some main impacts with the optimization of colonic function and metabolism, such as an increase in the expression or an increase in the stool weight, a decrease in the colonic luminal pH, a decrease in final products of nitrogen and reductive to enzymes, an increase of the expression in protein binding or an active carrier connected by mineral absorption, and immune system modulation[20]. Bacterial interactions had a crucial role in the substrate metabolism and the final products. The balance between different functional groups might be essential to maintain the efficiency of degradation and fermentation of organic materials in intestine and maintain community stability. Bacterial host interactions were also relevant in controlling the composition of intestinal microbiota. Homeostasis of intestinal wall was the result of the bacterial interaction and the bacterial host interaction[21].

Carbohydrates from palm sugar and protein from anchovy in rusip which have been fermented became potential substrates for bacteria in the colon[11,22]. The changes of microbiota could be observed as a consequence to the diet variations, pathological conditions (such as enteral infection), antibiotic therapy, anti-acid medication, or immunosuppressant[21].

In this study, the total number of bacteria found in rusip reached the highest colonies on the 17th day of ripening (initial treatment) by 6.5185 log cfu / g. After reaching the highest amount, the value would decrease with more acid products and had a reduction in the provided nutrients.[11] Therefore, the amount of LAB that reached the highest amount by 6.5185 log cfu / g did not qualify the criteria of LAB's minimum amount as probiotic based on

WHO criteria which had the amount of LAB between 7.0792 to 8.1761 log cfu / g[20].

In this study, the low value of LAB in rusip was probably due to the usage of raw materials of different fish. Galer anchovy and the freshness level of fish obtained from supermarket with long distribution chain might not be checked either with chemical analysis (*Total Volatile Base / TVB*), or with microbiological (The Total Bacteria Count)[12]. The amount in added salt, brown sugar, the place/ equipments which were used, the conditions and duration of ripening depended on the habits of each process, caused the quality of rusip and the amount of LAB in the products being unstable and dissimilar[12]. Furthermore, the researchers were still lack of ability in making rusip.To overcome this problem, the researchers conducted several times in making rusip with the available materials.

The average amount of LAB in cecal content of the treatment group was slightly lower compared to the average amount of LAB in the cecal content of the control group. This indicated that the LAB in rusip can not contribute to the increased growth of LAB in gastrointestinal tract. This study did not conduct a resilience test of LAB in rusip towards stomach acids and bile acids[20,23], so the ability of rusip's LAB in reaching intestine was unknown. High salt level in rusip probably inhibited the growth of LAB in gastrointestinal tract, so that the ability of inhibitory effect to non LAB bacteria cannot be optimal.

The low average of LAB in cecal content of the treatment group was likely due to the amount of LAB in rusip which have not reached expected minimum limit, and also the growth which was declining, the temperature, time, and substances or dissolved organic materials such as lactic acid[24], as well as the stress which is caused by the treatment when giving rusip in rats using feeding tube, and the interaction of the rusip transit duration in the stomach and small intestine[3]. Diet changes will lead normal microbiota components to induce "physiological" inflammatory response in intestine followed by balanced and controlled response (*self-limiting response*)[23].

In this study, rusip usage as sauce that increased appetite[12,13,25]proved to cause an increase in the body weight of rats. Even so, the increase in body weight in the treatment group was not clearly known whether it occurred as a result of the increase in standard feed consumption or the additional nutrients derived from rusip. It was because there was no weighing of standard feed amount that has been consumed by rats.

CONCLUSION

Rusip consumption does not cause a difference in Lactic Acid Bacteria (LAB) counts of cecal content in gastrointestinal tract of *Rattus norvergicus Sprague Dawley* strain. Further study is needed to the LAB in rusip as functional foods and to determine composition patterns of LABi n gastrointestinal tract during rusip consumption in a longer period of time.

REFERENCES

1. Garrett WS, Gordon JI, Glimcher LH. Homeostasis and Inflammation in the Intestine. Cell. 2010;140(6):859-70.
2. Ross RP, Mills S, Hill C, Fitzgerald GF, Stanton C. Specific metabolite production by gut microbiota as a basis for probiotic function. Int Dairy J. 2010;20(4):269-76.
3. Vizoso Pinto MG, Franz CMAP, Schillinger U, Holzapfel WH. Lactobacillus spp. with in vitro probiotic properties from human faeces and traditional fermented products. Int J food microbiol. 2006;109(3):205-14.
4. Tannock GW. Microecology of the gastrointestinal tract in relation to lactic acid bacteria. Int Dairy J. 1995;5(8):1059-70.
5. Chen GY, ez GNn. Gut Immunity: A NOD to the Commensals. Cell. 2008;19(4):R171 - R4.
6. Duerkop BA, Vaishnava S, Hooper LV. Immune Responses to the Microbiota at the Intestinal Mucosal Surface. Immunity. 2009;31(3):368-76.
7. Dore J, Corthier G. The human interstinal microbiota. Gastroen Clin Biol. 2010;34:S7 - S15.
8. Dinan TG, Cryan JF. Regulation of the stress response by the gut microbiota : Implications for psychoneuroendocrinology. Psychoneuen - journal. 2012;37:1369 - 78.
9. Candela M, Maccaferri S, Turroni S, Carnevali P, Brigidi P. Functional intestinal microbiome, new frontiers in prebiotic design. Int J Food Microbiol. 2010;140(2–3):93-101.
10. Sousa T, Paterson R, Moore V, Carlsson A. The gastrointestinal microbiota as a site for the biotransformation of drugs. Int J Pharm. 2008;363:1 - 25. Epub July 2008.
11. Kusmarwati A, Heruwati ES, Utami T, Rahayu ES. Pengaruh Penambahan *Pediococcus acidilactici F-11* Sebagai Kultur Starter Terhadap Kualitas Rusip Teri (*Stolephorus sp.)*. JPB Perikanan 2011;6(1):13 - 26.
12. Sastra W. Fermentasi Rusip [Penelitian]. Bogor: IPB; 2008.
13. Yuliana N. Profil Fermentasi "Rusip" Yang Dibuat dari Ikan Teri (*Stolephorus sp*). Agritech. 2007;27(1):12 - 7.

14. Rosida, Rosida DF. Evaluasi Nilai Gizi Tepung Pra-Masak Pisang Tanduk dan Pisang Raja Nangka. JTIP. 2011;22(2):125 - 9.
15. Nurdin SU. Evaluasi Efek Laksatif dan Fermentabilitas Komponen Pembentuk Gel Daun Cincau Hijau (*Premna oblongifolia Merr.)*. JTIP. 2007;18(1):10 - 6.
16. FDA. Guidance for Industry : Estimating the Maximum Safte Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. In: (CDER) CfDEaR, editor. Rockville, USA: U.S. HHS; 2005. p. 1 - 27.
17. Corthier G, Doré J. A new era in gut research concerning interactions between microbiota and human health. Gastroen Clin Biol. 2010;34, Supplement 1(0):S1-S6.
18. Marteau P, Pochart P, Doré J, Béra-Maillet C, Bernalier A, Corthier G. Comparative study of bacterial groups within the human cecal and fecal microbiota. Appl Environm microbiol. 2001;67(10):4939-42.
19. Astawan M, Wresdiyati T, Hartanta AB. The Utilization of Seaweed as a Source of Dietary Fiber to Decrease the Serum Cholesterol in Rats. HAYATI J Biosci. 2009;12(1):23.
20. Douglas LC, Sanders ME. Probiotics and Prebiotics in Dietetics Practice. J Am Diet Assoc. 2008;108(3):510-21.
21. Montalto M, D'Onofrio F, Gallo A, Cazzato A, Gasbarrini G. Intestinal microbiota and its functions. Digest Liver Dis. 2009;3:30 - 4.
22. Mauliana D. Pengaruh Penambahan Berbagai Sumber Karbohidrat Terhadap Kadar Asam Laktat pada Fermentasi Rusip Ikan Bilis. Media Infotama. 2006;1(2):40 - 8.
23. Tiihonen K, Ouwehand AC, Rautonen N. Human intestinal microbiota and healthy ageing. Ageing Research Reviews. 2010;9(2):107-16
24. Parameswari A, Kuntari S, Herawati. Daya hambat probiotik terhadap pertumbuhan streptococcus mutans. Surabaya: Unair; 2009.
25. Koesoemawardani D. Analisis Sensori Rusip dari Sungailiat - Bangka. JTHP 2007;12(2):36 - 9.