Effectiveness prebiotic and synbıotıc from composıte flour (Canna indica and Spirulina platensis)

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Effectiveness prebiotic and synbıotıc from composıte flour (*Canna indica* and *Spirulina platensis*)

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Abstract

Prebiotic can stimulate growth of non pathogenic microflora in intestinal such as Bifidiobacteria and Lactobacillus with the result that prevent benefit of health. Sources of prebiotic that contain oligosaccharide were *Canna indica* and *Spirulina platensis*. Information about the effectiveness of *C. indica* and *S. platensis* as prebiotic and component of synbiotic based on in vivo treatment were minimal. Aims of the research were to know the effect of flour composite from *C. indica* and *S. platensis* (Cannalina) as prebiotic and synbiotic on ability to suppress total of microbe and support the growth and hold *Lactobacillus* (LAB) in intestine male *Sprague dawley* . The methodology was experimental design which divided for 4 groups for treatment. There were control, prebiotic, probiotic and sinbiotic groups. The division of control group which was mice fed up standard ration and 1 ml NaCl physiology. The prebiotic groups fed up pellet from *Cannalina* flour and 1 NaCl physiology, probiotic groups fed up pellet standard and LAB cultured 6.5 x 10^8 log cfu / ml in 1 ml NaCl and synbiotic group fed up *Cannalina* formed as the pellets and LAB cultured 6.5 x 10^8 log cfu/ml in 1 ml NaCl. The number of *S. dawley* in each groups were six mice. Bacteria from mice feces were isolated and analyzed to find the total of microbes and number of LAB. The results from in vivo treatment showed the highest total of microbe was found in control. probiotic, prebiotic and syntiotic have ability stimulated growth and hold of LAB in intestine compared with control in 10 days treatment and 5 days after treatment.. On 5 days after treatment synbiotic group was highest hold log 10,59 cfu/ml of LAB, and probiotic group was the lowest total of mecrobe log 9,21 cfu/ml.

Keywords: *Cannalina, in vivo, pebiotics, probiotics, synbiotic,*

INTRODUCTION

Diabetes is one of the most metabolic disorder metabolic and significant decreasing health quality. Prevalence in low and middle income country in 2013 indicated from 130 country totally 382 million with over the next 22 years old and in 2035 was predicted 592 million. Indonesia people with 20-79 years old had diabetes total 14,1 million and 2035 included in top 10 countries for high prevalence (Guariguata *et al*, 2014). Probiotic and prebiotic have anti diabetic effect that improve insulin sensitivity. The other effect make the microbial community in the gut ecosystem and reducing intestinal endotoxin concentration (Kim *et al*, 2014). Lactobacillus in yogu[5] from goat has potency as anti diabetic food throught the mechanisme effect on bioactive peptide found to able to inhibit the activity α glucisidase (Lacroix & Li Chan, 2013). Combination probiotic supplemented with rosella extraction had the highest ant diabetic effect with 36.7% inhibition of α glycosidase before 15 days of cold storage (Wihansyah, *et al*). Flavonoid containing in rosella has link to α glucosidase inhibitor activities (Zeng *et al* 2016). *C. indica* is one of the plants that produce tubers and flavonoid containing, and invitro treatment combination with *S. platensis* stimulate growth of *Lactobacillus rhamnosus* .

M[4]TERIAL AND METHODE

Research design study used an experimental method with a completely randomized design (CRD) using 24 mice which divided into 4 groups. The division of control group which fed up standard ration and 1 ml NaCl physiology, prebiotic groups fed up pellet *Cannalina* and 1 NaCl physiology, probiotic groups fed up pellet standard and LAB cultured 6.5 x 10^8 log cfu / ml in 1 ml NaCl and synbiotic group fed up *Cannalina* pellets and LAB cultured 6.5 x 10^8 log cfu/ml in 1 ml NaCl. The research was conducted in Laboratory of microbiology and animal Laboratory Universitas Al Azhar Indonesia from December 2017 to June 2018. White *C. indica* obtained from UAI garden. The LAB

cultured obtained from the Microbiology Laboratory, Bogor Agricultural University. The mice for the in vivo experiment using a 2-month-old male *S. dawley* strain of obtained from the Animal Laboratory of the Food and Drug Supervisory Agency. Media were used aquadest, 70% ethanol, Lactose Broth (LB) (HIMEDIA), MRS (de Mann Rogosa) broth (HIMEDIA), MRS agar (HIMEDIA), 0.85% NaCl, and Mc Farland solution.

Procedure of the research

Ethical approval of test animals, material preparation, concert of Cannalina flour to Cannalina pellet, potential testing of prognostic in vivo, microbiological analysis (AOAC 2005). The Animal Ethics Approval got from the Health Research Ethics Committee of the Universitas Pembangunan Nasional Veterans Jakarta which was reviewed by Prof. Dr. M. Guritno Suryokusumo, Dr., SMHS, DEA with no B / 1413 / V / 2018 / KEPK (Appendix 1).

Preparation the cannalina flour was started by cleaned and washed the white *C. indica* tuber using running water and then peeling and slicing ± 1 mm thickness and were dried in an oven at 55^0 C for 20 hours. The sliced of dry *C. indica* tuber was blended. Making Cannalina pellet was carried out by *C. indica* flour mixed with *S. platensis* powder with a ratio of 10: 1, mixed with corn oil, sacatonic, tropicana slim salt and sweetener with a ratio of 97%: 1.2%: 0, 1%: 0.9%: 0.8%. All ingredients were stirred and add distilled water until the dough and could be formed according to the standard ration form (pellet) and put in the oven at 70 ° C for 2 hours.The standard ration used was a standard ration with the brand "HATORY", HATORY has the following composition:

Table 1. Composition of Hatory brand feeds

No	Composition	Percentage
1	Protein	20.5 %
2	Fat	9.6 %
3	Minerals	15.9 %
4	Rough fiber	20 %
5	Vitamin	20 %
6	Water	11 %
7	Salt	0.7 %

As supporting information chemical analysis used proximate test included analysis of water and ash content by the gravimetric method, protein content used the Kjeldahl method, lipid content used Soxhlet method, and analysis of carbohydrate levels by the method of difference. Refresher Culture of LAB used preserved LAB culture 1 ml was added to 9 ml of MRSB and was incubated at 37 ° C for 2 days for the culture refreshment period. After 2 days the refreshed LAB can be used immediately. BAL culture is refreshed then dilution is done and compared to McFarland's solution using a Spectrophotometer to obtain a cell count of 6.5 x 108 log cfu / ml. The McFarland solution used in this study was a McFarland solution with the composition 0.2 mL 1.0% Barium Chloride ($BaCl_2.2H_2O$) in 9.8 mL 1.0% Sulfuric Acid (H_2SO_4). Furthermore the culture was centrifuged to obtain sediment. The sediment was dissolved with 1 ml of physiological solution of NaCl. LAB cultures will be given to groups of prebiotic and synbiotic .. The research were carried out in 3 stage namely the adaptation period for 10 days, the treatment period 10 days and the post-treatment period for 5 days. Rations and drinking water are given for 25 days in ad libitum. The adaptation period only providing 20 gram and 50 ml drinking water adlibitum once a day.. Cannalina pellets were given to every rat in the prebiotic and sinbiotic groups every day as much as 20 grams for 10 days of treatment. The post-treatment period was carried out for 5 days by stopping administration rats were only given standard rations.

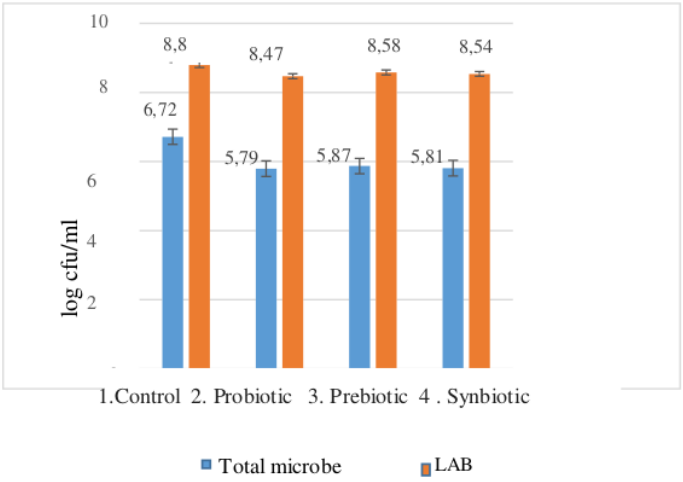
Sample preparation was found from 0.5 gram fecal samples diluted with Lactose Broth (LB) media as much as 4.5 ml and crushed with Mortar. Dilution 10^{-1} 10^{-2} , 10^{-3}, 10^{-4} dilution was made until the appropriate dilution rate with expected results 30-300 colonies. It was obtained and in 1 ml sample into 9 ml of physiological solution of NaCl . Then the sample suspension was used to analyze the total microbes and the amount of LAB. The feces from all groups were taken aseptically on 0, 1, 5, 10 days in treatments period and the fifth after treatment. Some feces will come out after the tail section and stored in sterile alumunium foil. Microbiology analysis based on AOAC. Total Microbes

was found by 6ml aseptically and poured into 3 sterile cups (triplo) added PCA medium and shaken to be homogeneou, and then incubated at 37 ° C for 48 hours. Calculation of the amount of LAB was carried out by the pouring method from the appropriate dilution level was piped 1 ml and poured into 3 petri dishes (Triplo)). After that, MRSA media was poured and shaken to be homogeneous and incubated at 37 ° C for 48 hours. The growing bacterial colonies were counted as LAB with characteristics of having milky white colonies but not slimy. Body weight of mice used in the study will be taken on days 0,10 and 15. Colony counts Total microbes, amount of LAB, and body weight obtained will be analyzed to know distribution and homogenity and continue to parametric analysis using one way ANOVA or non parametric.

RESULTS

Total of microbe and LAB before treatment

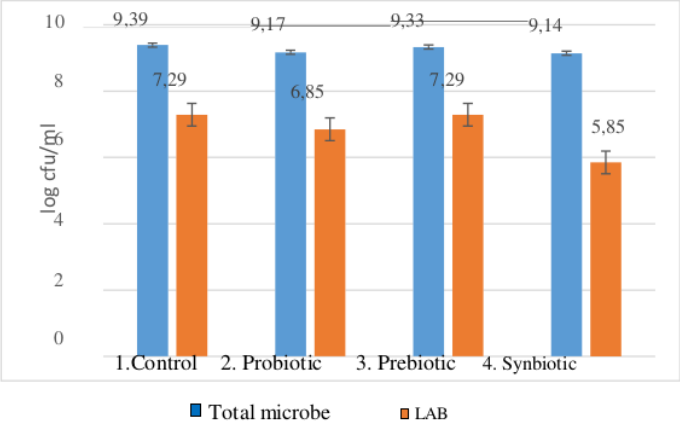
Total of microbe on fecal at 10th days adaptation (0 day) phase day for all groups based on Kruskal-Wallis (KS) analysis were not significantly different of 0.82 at P<0.05 in average of 6.05 log cfu / mL. The amount of LAB based on ANOVA analysis was not significantly different 0.37 at P<0.05. The average number of colonies issued was 8.59 log cfu/ml (Graph. 1)



Graph 1. Total Microbes and LAB in 0 day in fecal *S.dawley*

Total of microbe and LAB in 1th day treatment

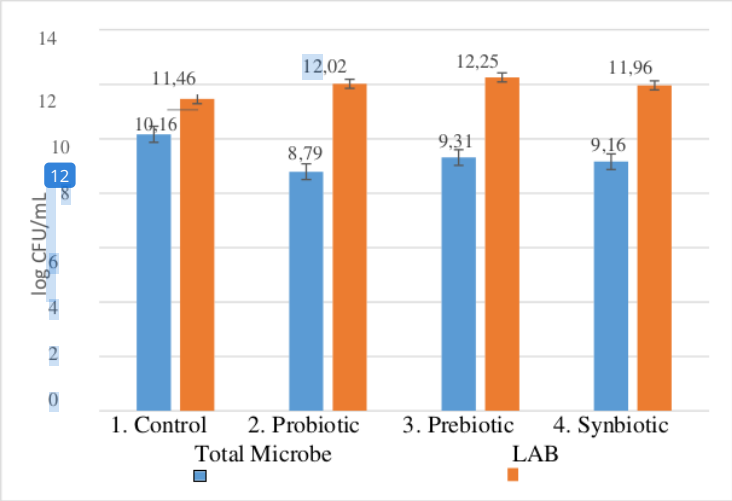
The results of the KS analysis of total microbe all groups after treatment showed significant difference 0.033 at P<0.05. The highest microbial total in fecal was shown by control group and prebiotic group 7.29 Log cfu/mL. The second group was given probiotic 6.85 Log cfu / mL and the lowest in the group synbiotic which was 5.85 Log cfu/mL. The results of KS analysis of the amount of LAB fecal showed no significant difference 0.68 at P<0.05 in all groups with an average LAB colony released at 9.027 Log cfu / mL (Graph 2).



Graph 2. Total Microbes and LAB in 1 st day treatment in fecal *S. dawley*

Total of microbe and LAB in 5th day treatment

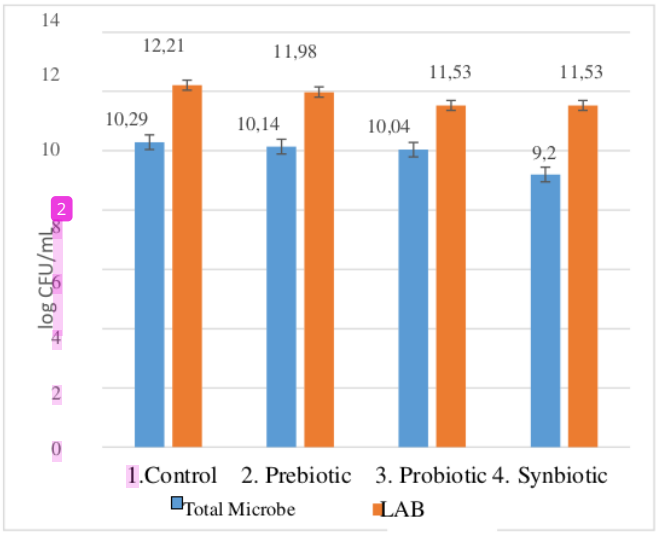
The results of ANOVA analysis on 5 th day showed that there were significant differences in total of microbe between treatment groups 1, 2, 3 and 4, however in groups 3 and 4 did not show significant differences with a significance value of 0,168 with P< 0.05. Number of LAB in the KS test showed no a significant difference 0.33 with P< 0.05 (Graph. 3).



Graph 3. Total Microbes and LAB in 5 st day treatment in fecal *S. dawley*

Total of microbe and LAB in 10th day treatment

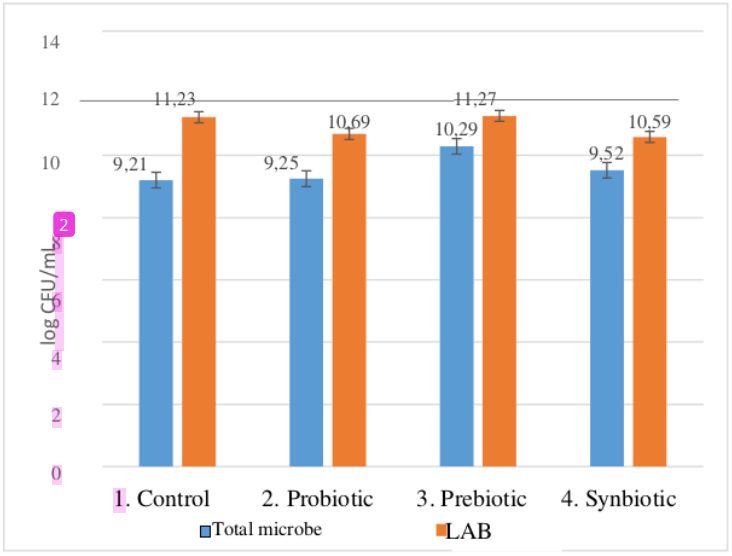
On 10th day of the total of microbe based on ANOVA test showed significant differences 0.00 at p < 0.05. On the 11th day the results of the KS test for the number of LAB showed significant differences in each treatment group with a significance value of 0.029 with p <0.05. The highest number of LAB was found from the 1st group with colonies of 12.21 Log cfu / mL. Then the second group with the number of colonies of 11.98 Log cfu / mL. Meanwhile groups 3 and 4 had the lowest number of LAB with colonies amounting to 11.53 Logs cfu / mL (Graph 4).



Graph 4. Total Microbes and LAB in 10 st day treatment of mice in fecal *S. dawley*

Total of Microbe and LAB in 5th day post treatment

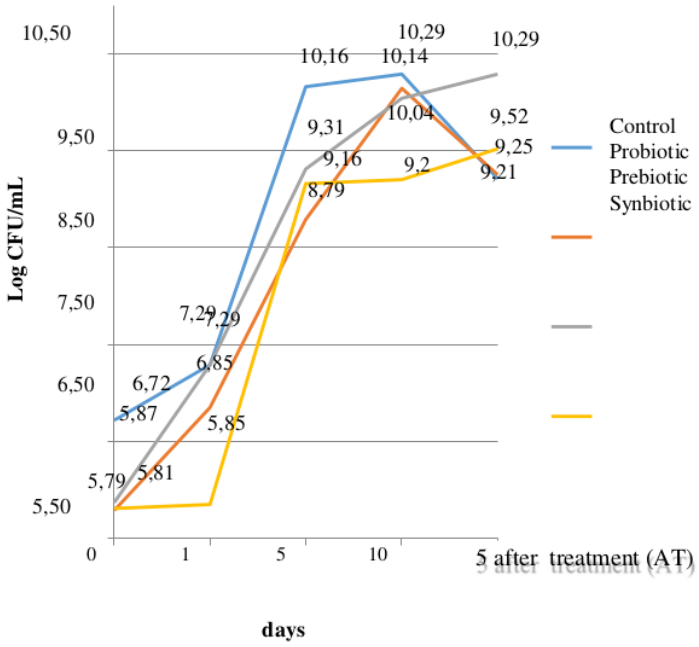
The KS test results showed no significance number of 0.83 at p<0.05. This shows that from day 0 to day 15 the total number of microbes similar. The results of the LAB showed significant differences 0.050 at p <0.05, it mean the synbiotic treatment has ability to hold LAB (Graph 5).



Graph 5. Total Microbes and LAB in 5 st day posttreatment in fecal *S. dawley*

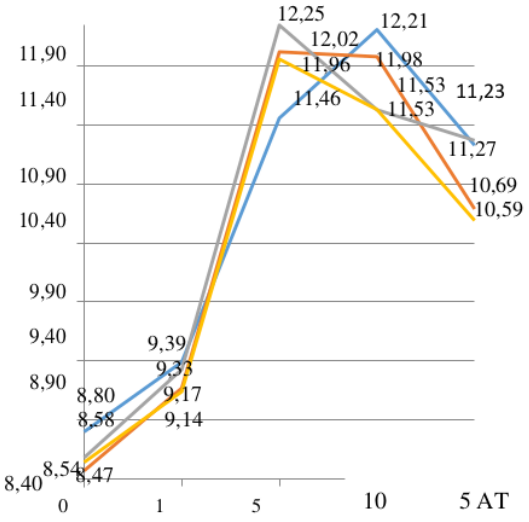
Analysis of total of microbe dan LAB

Trend of total microbe in 1 until 10 days treatment showed increasing, but in control group was the highest. In 1 and 5 days after treatment probiotic group is the lowest total of microbe (Graph 6)..



Graph 6. Total microbe control and treatment groups in 0 until 5 days after treatment

Total LAB in 10 days treatment and 5 days control groups was the highest but synbiotic group was lowest and followed in probiotic (Graph 7).



Graph 7. BAL control and treatment groups in 0 until 5 days after treatment

DISCUSSION

Total of microbe and LAB isolation from *S.dawley* fecal on day 0 for all groups were not significantly different. This indicates the health conditions the digestive animal was similar with the mean total microbe released 6.05 log cfu / mL and LAB 8.59 log cfu/ml. The highest microbe total in 1 day treatment was occurred in control group followed by the prebiotic, probiotic and synbiotic groups. The second group was given probiotic 6.85 Log cfu / mL and the lowest in the group synbiotic which was 5.85 Log cfu/mL. LAB was the highest total in control group. It means that the intestinum in control did not effective to retain LAB and more exited from disgestive system. The data indicated probiotic, prebiotic and synbiotic effective to suppress pathogen microbe by stimulated growth of LAB. The function of LAB in digestives attack of pathogenic microbe by secreted ant microbe substances such as lactic acid, peroxide, and bacteriocin (Sankarankutty and Palav 2016).According to Martin *et al.* (2013) prebiotics provide competitive advantages to specific native micro flora in the digestive tract such as LAB and Bifidobacteria which are the group of probiotics that can reduce pathogenic bacteria. When the probiotic bacteria fermentation process will produce active compounds such as lactic acid and bacteriocin (Syukur, et al, 2014). Bactericin protein substance has antimicrobial properties such as protease K and trypsin (Mukherjee, *et al* 2019). The other antimicrobial components are as diacetyl and hydrogen peroxide (Omemu, 2011). The highest LAB in prebiotic group was influenced by Cannalina rations which has a higher carbohydrate containing rather than the standard ration. C flour carbohydrate containing in cannalina 75,66% and standart ration 58.86%. LAB requires easily soluble carbohydrates to meet energy by broken down by the amylase enzyme (Paul, 2016).

Analysis on 5 th day showed total microbe in control group is the highest and significant differences with 2, 3 and 4 group. It means that probiotic, prebiotic and synbiotic effective supress pathogen microbe by stimulate growth of LAB. The results of the analysis of the number of LAB on the 5th day showed a significant difference. The highest number of LAB was in the prebiotic group with the number of colonies of 12.25 Log cfu / mL. Followed by probiotic group 12.02 Log cfu / mL and synbiotic . Meanwhile the lowest number of LAB was in control group of 11.46 Log cfu / mL colony and stimulate growth of LAB. It was caused the mice decreasing of eating activity on the fifth day of treatment day due to saturation in consuming rations containing probiotic, prebiotic and synbiotic. Decreasing of eat activity influenced decreasing the amount of LAB in the digestive system in prebiotic and probiotic and synbiotic group. After 5 days the rats in treatment groups adapted with their ration.

On the 10 th day the lowest total number of microbes was found in the synbiotic group group with colonies of 9.70 Log CFU / mL. This shows that the standard ration ability to suppress the number of microbes lower compared with the other groups . The highest number of LAB was found also in the control with colonies of 12.21 Log cfu / mL. Then the probiotic group with the number of colonies of 11.98 Log cfu / mL. Meanwhile groups prebiotic and synbiotic LAB with colonies 11.53 Logs cfu / mL. Both of groups received Cannalina pellet commonly given effect to attach LAB on intestinal epithelial cells and forming colonization of the the number of LAB (Brito *et al,* 2012).

On the 15th day (post treatment) the four groups showed total microbe were no significant difference. After 10 days treatment the consumtion of mice only standard rations. The results of the KS test for the number of LAB showed a significance value of 0.038 with $P < 0.05$. the lowest number of LAB is in the synbiotic group with a colony number of 10.59 log cfu / mL

CONCLUSIONS

LAB as probiotic, prebiotic from Cannalina flour and syntiotic from Cannalina flour and Lactobacillus have ability to suppress microbe specially pathogenic microbe. The others effect stimulate and hold LAB in intestine in 10 days treatment and 5 days after treatment.

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