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Florescent in situ hybridization (FISH) method for quantification colonic microbiota *Sprague Dawley* rats with diet containing inulin from lesser yam tubers (*Dioscorea esculenta* L.)

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Abstract

Lesser yam (*Dioscorea esculenta* L.) is one of the many types of *Dioscorea* spp. growing in Indonesia and its tuber contain the highest amount of inulin (14.77% db) among others (Winarti *et al.*, 2011). The tuber is very important as an alternative source of carbohydrate in the countryside. Isolation and characterization of inulin from lesser yam tubers have been performed. The aim of study was investigated the effect of lesser yam inulin to change of the colonic micro biota (*Bifidobacteria, Lactobacillus* and *E. coli*) and produced SCFA (Short Chain Fatty Acid) in *Sprague Dawley* Rats. Quantification of total microbes in the cecum performed with the molecular approach, namely FISH (fluorescence In situ hybridization) method. The rats were divided into 4 groups, each group 8 rats, group I were fed a standard (AIN '93), group II were fed a standard which dietary fiber was replaced by dried inulin (Inulin KR), group III were fed a standard which dietary fiber was replaced by inulin powder (inulin FM), and the fourth group were fed a standard which dietary fiber was replaced by commercial inulin (inulin SD). The results showed that lesser yam inulin was increased the *Bifidobacterium* from 7.46±0.5 log cfu/gr to 8.64±0.69 log cfu/g, and *Lactobacillus* from 7.71±0.72 log cfu/g to 9.05±0.94 log cfu/g. Lesser yam inulin was reduced *E. coli* from 7.70±0.79 log cfu/g to 6.99±0.33 log cfu/g. Lesser yam inulin was increased the production of SCFA (acetate, propionate and butyrate). This result indicated that the lesser yam inulin can be controlled colonic micro biota *Sprague Dawley* (SD) rats, so it can be used as a prebiotics.

Introduction

The role of gastrointestinal micro biota or bacteria is essential for health of humans and animals. Some of degenerative diseases are strongly influenced by intestinal micro biota ecosystem. The majority of gastrointestinal desease were caused by bacterial pathogens that infected in the human gut. Factors that influence the composition of gut bacteria is food consumed (diet), beside factors in the digestive tract itself (Gibson, 2004). Bacterial populations in the ecosystem digestive tract of healthy people who eat a balanced diet are generally stable. Changes in lifestyle, diet and health conditions are to change the stability of ecosystem digestive tract. Accordingly, to achieve optimal health and performance (well-being) we mush to manage of intestinal micro biota, that are increased the proportion of beneficial bacteria, and reduced amount of harmful bacteria, by consuming probiotics and provide the appropriate nutrients for bacteria probiotic (good bacteria in digestive tract) (Pompei *et al*., 2008). Nutrients can stimulate the growth of probiotic bacteria are called prebiotics.

Prebiotics are food components that cannot be digested and can selectively stimulate the growth and activity of beneficial bacteria in digestive tract, especially bifidobacteria and lactobacilli (Gibson, 2004; Pompei *et al*., 2008; Gaggia *et al*., 2010). Prebiotics are carbohydrates that generally cannot be digested, but it has good effects on ecosystems of probiotic micro biota in the gut so it can provide health effects on humans and animals. In the large intestine, prebiotic ingredients are fermented by probiotic bacteria, especially *Bifidobacterium* and *Lactobacillus*, and produce short chain fatty acids (SCFA), that are acetic, propionic, butyric, and lactic acid. Short chain fatty acids can be used as an energy source by the body. One components widely used in food formulations is inulin (Roberfroid, 2005).

Inulin is a dietary fiber chemically composed of a

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mixture of oligo- and/or polysaccharides constituted of fructose unit chains linked by β-(2,1)-D-fructocyl-fructose bonds of various length, terminated by a single glucose unit linked by an α-D-glucopyranocyl bond (Roberfroid and Delzenne, 1998). Inulin is a group fructan β-(2-1) consisting of a mixture of oligo- and polysaccharides, in which almost every linear chains of fructose have GFn structure (with G=unit glucocyl, F=fructocyl unit and n=number of units of the chain fructocyl each other), for example inulin extracted from chicory has the chemical structure of α-D-Glu-(1-2)-[(β-D-Fru-(1-2)-]n, as a polymer molecules with a length between DP (Degrees of Polymerization) 3 to DP 60 (Crittenden, 1999).

Inulin cannot be digested by enzymes present in the digestive tract of mammals but can be selectively fermented by colonic bacteria, so it is positively effect on the health of its host. Several types of Bifidobacteria can utilize inulin as an energy source by producing extracellular inulinase that hydrolyzes the β-(2-1)-D-fructose-fructocyl bond to fructose (Robertfroid, 2005).

Inulin is widely used in food industry in Europe, USA, Canada and Indonesia as a component (ingredient) in various of food products. It is naturally found as plant storage carbohydrates in Jerusalem artichoke *(Helianthus tuberosum)* (Lingyun *et al*., 2007), roots of *Morinda officinalis* (Yang *et al.*, 2011), Agave tequilana (Arrizon *et al*., 2010), dahlia tubers, bananas and wheat (Roberdfroid, 2005), commercial inulin produced from *Cychorium intybus* tubers (Toneli *et al*., 2008).

Dioscorea esculenta is one of many types of *Dioscorea* spp. growing in Indonesia and its tuber contains the highest amount of inulin (14,77% db) among others (Winarti *et al.*, 2011). The tuber is very important as an alternative source of carbohydrate in the countryside. Isolation and characterization of inulin from *D. esculenta* tuber (lesser yam) have been performed, we have found that the yield of inulin is 21.33%, purity 73.585%, average solubility 76.77%, water content 13.68% and DP is 6 (Winarti et al., 2013). However, information about the effect of inulin from lesser yam tuber on the colonic micro biota is limited. The objective of this research was to evaluate the effect of inulin from lesser yam tuber on the colonic micro biota. Determination of the colonic micro biota was done by fluorescence in situ hybridization (FISH) method.

Fluorescence in Situ Hybridization (FISH) method one of the molecular diagnostic technique using DNA probes labeled with fluorescent substance to detect or confirm gene or chromosome DNA samples. Basic mechanisms of diagnostic test that use nucleic acid probes are hybridizations. This technique is based on the combination of two nucleotide bases and the complementary nucleic acid chains (DNA or RNA to DNA and RNA with RNA). This method is used to overcome weaknesses plate count method, among others, the overlap cells in colonies, microbes are unculturable and often the selective medium are not completely selective for certain bacteria (Bezirtzoglou *et al*., 2006). This technique is a technology that uses genetic material as the basis for testing and faster than convensional method (Zwirglmaier *et al.*, 2003; Lahtinen *et al*., 2006; Collado *et al.*, 2006; Collado and Sanz, 2007).

Materials and Methods

Materials

Raw materials used in this study was lesser yam inulin (*Dioscorea esculenta* L.), were dried with cabinet dryer (Inulin KR) and foam mat dryer (Inulin FM) and commercial inulin (Februline Instant, native chicory inulin DP 10) produced by Cosucra Groupe Warcoing SA, Belgium (Inulin SD). Animal for the research were Sprague Dawley (SD) males rats age 2 months, obtained from BP POM Jakarta. Feed rats were used in research standard fed was AIN ‘93 and for treatment was formulated with inulin.

DNA probe S-G-Bif-0164-a-A-18 (5’Cy/CATCCGGCATTACCCCC) for *Bifidobacterium* strain, S-G-Lab-0158-a-A-20 (5’Cy/GGTATTAGCACCTGTTTCA) for *Lactobacillus* strain and Eco16S07C (5’Cy/CTCCTTCCCTCATTTCA) for *E.coli* strain obtained from PT. Genetica Science Indonesia.

The equipments were used in the research that are autoclave, sentrifuse, shaker waterbath, GC 8A Shimadzu with coloum GP 10% SP 1200/1% H3PO4 on 80/100 Cromosob WAW diameter in 3 mm, coloum length 2 m and detector FID with condition for operation coloum temperature 140°C, detector temperature 240°C, gas flow N_2, pressure 1,5 kg/cm2 and sample injection 1 µl, Epifluorescence Microscope BX-53 CCD Camera Olympus DP-72, Software cellsens Standard 1.6 (Olympus Corporation), Microscopic field = PxL = 139.03 µm x 104.63 µm.

Treatment of rats

Amount 32 male white rats of *Sprague Dawley* strain aged 2 months were used in this study. The rats were divided into 4 groups each group was 8 rats, group I were fed a standard (AIN’93), group II were fed a standard with dietary fiber was replaced by dried inulin (Inulin KR), group III were fed a standard with

dietary fiber was replaced by inulin powder (inulin FM), and group IV were fed a standard with dietary fiber was replaced by commercial inulin (inulin SD). Before treated, the rats conditioned for 7 days with standard fed, over that the rats were treated according to treatment for 15 days. Each rat was placed in the individual cages. Every day rat cage, food containers and drink bottles that have been replaced with washed and disinfected. During maintenance the body weight of rats were weighed every two days and feed intake were calculated every day. After the rats dissected and taken care the cecum for analized.

Cecum from each rat all of the groups taken aseptically and collected in sterile botles. Analysis was performed on cecum weight; water content, total bacteria, total *Bifidobacteria, Lactobacillus, E.coli* and levels of SCFA (Short Chain Fatty Acid).

Complementary DNA probe assay

Complementary of DNA probe assay was conducted to determine that the DNA probe were used for microbial targets were matched (complementary). Assay of DNA probe by FISH method were carried out in the same manner with pure cultures of *Bifidobacterium, Lactobacillus* and *E. coli*.

Preparations and fixations of digesta

Preparations and fixations of digesta was examined according to Lahtinen *et al.*, (2006). Amount of 0.1-0.3 g of digesta diluted with phosphate-buffer saline solution (PBS) (130 mM NaCl, 10 mM sodium phosphate buffer; pH 7.2). To remove solid material instead of the cell, the sample suspensions were centrifuged at low speed 200xg 4°C for five minutes and the supernatant was collected in a sterile tube. Pellet samples diluted in PBS solution and performed centrifugation and supernatant collection the same way three times. Combined supernatant was centrifuged at 9,000 g high speeds 4°C for five minutes to obtain the cell pellets of microbial digesta. Microbial cells were fixed using 4% (w/v) paraformaldehyde in PBS for 24 hours at 4°C. After washing the cells using PBS solution, cell pellets were stored in a solution of 50% (v/v) 99.5% ethanol-PBS at -20°C as a fixed cell until used for FISH method analysis.

Quantification of total micro biota by FISH method

Quantification of total micro biota by FISH method was examined according to Lahtinen *et al.*, (2006). Amount of 3 µL suspension of fixed cells exposed on a glass slide Teflon (ADCELL, 12 wells, diameter 5 mm, Erie Scientific Company, ports mouth, NH) and dried at room temperature. Cells then dehydrated with 50% ethanol solution, 80% and 99.5% (v/v) for 3 minutes respectively. Fixed cells put on each well at glass slides hybridized by addition of 8 mL of hybridization buffer solution (0.9 M NaCl, 0.01% sodium dodecyl sulfate, 20 mM Tris-HCl, 20% formamide pH 7.2) and 1 µL oligonucleotide probe (25 ng/µL) were labeled with Cy3 (probe SG-Bif-0164-aA-18, specific for bifido bacteria, probe SG-Lab-0158-aA-20, specific for lactobacilli and Eco16S07C probe specific for *E.coli*). Hybridization was carried out at 46°C for 16 hours and then cells were washed with washing buffer (225 mM NaCl, 0.01% sodium dodecyl sulfate, 20 mM Tris-HCl; pH 7.2) at temperature of 48°C for 20 min. After the cells immersed in DAPI (4',6-diamidino-2-phenylindole di hydrochloride n-hydrate) solution for 5 min at room temperature for staining chromosomes all of the cells cecum micro biota, then cell soaked in distilled water for 5 min at room temperature and dried in dark conditions. Cells at the glass slides that have been dried were coated with a solution of Vectashield (Vector Laboratories Inc., Burlingame, Calif.), then keep with a cover glass and observed with a microscope epifluorecent Olympus BX-53 (Olympus Corporation, Japan) which equipped with filters for Cy3 and DAPI with DP72 CCD camera (Olympus Corporation, Japan). Cell image capture was done using cell Sens Standard 1.6 software (Olympus Corporation, Japan).

Calculation of cells bacteria

Calculation of cells bacteria was examined according to Lahtinen *et al.*, (2006). The proportion of target microbial cells (%) in the medium assay was calculated as the average ratio of the number cells that appear in the Cy3 filter (lactobacilli) with the number of cells that appear in the DAPI filter (total microbe) in the same locations the microscopic observation.

Calculation of the proportion of target cells following formula:

$$\text{Proportion (\%)} = \frac{\text{Total cells on cy3}}{\text{Total cells on DAPI}} \times 100\%$$

Calculation of total cells following to the formula:

$$\text{Total cell (cell/g)} = \frac{\text{Average total cells x microscopic factor x dilution x resuspention volume}}{\text{Volume sample in well x sample weight}}$$

Description:

The average number of cells = the mean of replications number of cells detected in wells at the same DAPI (cell)

Microscopic factor = value of extensive pitting

division with extensive microscopic field (= 9753)
Dilution = dilution factor to obtain the distribution of fixed sample cell can be calculated (= 4)
Resuspension volume = volume of fluid used for resuspension of solid samples (= 0.5 mL)
The volume of sample in the wells = fixed sample volume placed in the wells (= 0.003 ml)
Weight sample = weight of sample analyzed (samples A = 0.29 g, B = 0.13 g C = 0.30 g; D = 0.14 g)

Analysis of levels SCFA (short chain fatty acid)

Levels and profiles of acetic acid, propionic and butyric in digesta rats analyzed using a Shimadzu GC 8A with columns GP 10% SP 1200/1% H_3PO_4 on 80/100 WAW Cromosob 3 mm in diameter, 2 m long column and FID detector types with test conditions column temperature 140°C, detector temperature 240°C, carrier gas N_2, 1.5 kg/cm^2 pressure and the amount of sample injected 1 µL.

Statistical analysis

All the analysis was perform in triplicate, result were expressed as means ± standard deviation. The differences between the mean values of multiple groups were analyzed by one-way analysis of variance (ANOVA) with Tukey Methods range tests. ANOVA data with a $P < 0.05$ was classified as statistically significant. MINITABS 16.0 software, Origin 75 and Microsoft Excel 2007 program were used to analyze the data.

Results and Discussion

The cecum weight

The results showed that the cecum weight of the rats with fed containing lesser yam inulin (KR Inulin and Inulin FM) were decreased from 4.676 g (on diet standard AIN '93) to 1.406 g and 1.301 g on diet containing inulin KR and inulin FM respectively. Cecum weight in rats with fed containing commercial inulin (inulin SD) was slightly higher than inulin KR and inulin FM, was 2.448 gr.

Declined of the cecum weight of rats fed with inulin due that the inulin was fermented by bacteria in the lower gastrointestinal tract (colon), especially *Bifidobacterium* and *Lactobacillus* into simple compounds and released the amount of water. Futher more, the water was release absorbed by surface of the intestine, so that the water content of digesta declined, so its reduced of the cecum weight. Whereas in rats fed a standard feed containing high cellulose, the cecum weight higher than the inulin fed, because cellulose is difficult to fermentation by bacteria in the colon that is still a lot of water trapped in the cellulose.

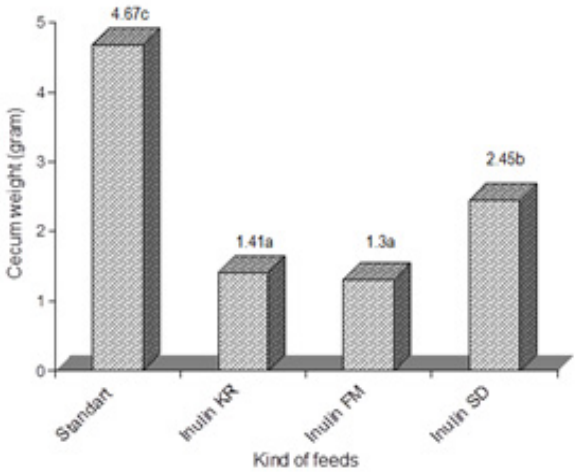

Figure 1. The average of cecum rats weight with different fed. (The hystogram followed by different letter indicates significant different p≤0.05)

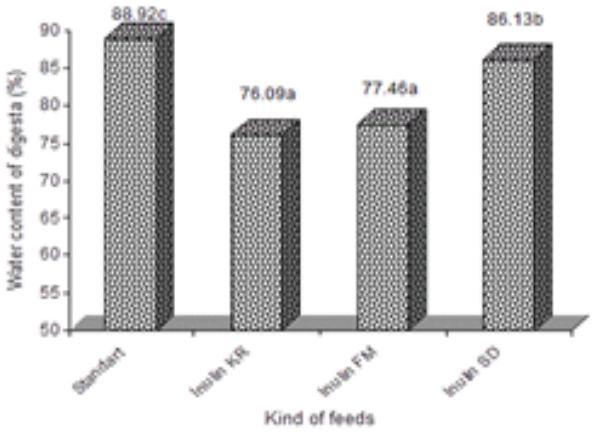

Figure 2. The average of water content of cecum rats with different fed. (The hystogram followed by different letter indicates significant different p≤0.05)

In the large intestine inulin or oligo-saccharides and polysaccharides that are not fully digested in the upper intestine, will be fermented by the micro biota in the large intestine, producing SCFA (short chain fatty acid) and gas (Roberfroid, 2005). SCFA produced from the fermentation of carbohydrates is acetate, propionate and butyrate and produced format, valerate, caproat, and branched-chain fatty acids isobutyrate (Macfarlane and Macfarlane, 2003).

The water content of digesta

The result of this research showed that significant decrease in the average of water content in digesta rats from 88.92% at (on standard fed AIN '93) to 86.13%, 77.46% and 76.09% at formulation fed with inulin SD, inulin FM and inulin KR respectively. It was because the standard fed (AIN'93) containing CMC which is a derivative of cellulose. Cellulose

Table 1. The average of total bacteria, total *Bifidobacteria, Lactobacillus* and *E. coli* on cecum rats with different fed

Groups of bacteria	Painting/ DNA probe	Amount and prosentase of bacteria on rat digesta with different fed (n=10)			
		AIN '93	Inulin KR	Inulin FM	Inulin SD
Total bacteria	DAPI	4,61x10^{9}	2,03x10^{9}	6,73x10^{9}	1,71x10^{9}
Prosentase Bif	Cy3	0,7	1,9	0,9	0,5
Bifidobacteria	Bif-0164m	2,89x10^{8}	4,32x10^{8}	3,74x10^{8}	8,72x10^{8}
Prosentase Lac	Cy3	1,1	5,3	0,9	0,8
Lactobacillus	Lab-0158	5,09x10^{7}	1,13x10^{8}	6,37x10^{7}	1,28x10^{8}
Prosentase coli	Cy3	1,5	0,07	0,1	0,1
E.coli	Eco-16S07C	4,95x10^{7}	9,8x10^{6}	6,72x10^{6}	9,78x10^{6}

is one of the insoluble dietary fiber that has a long polymer with a degree of polymerization between 300-1500 glucose molecules with β-(1,4)-glicosidic bond, so as to trap/absorb the water, and the trapped water can no longer move freely (Fenema and Playne, 1999). CMC has the form of extended or stretched conformation ribbon (ribbon type), the type formed from 1,4-D-glukopiranosil that of the cellulose chain. The shape of the ribbon conformation linked by zig-zag geometry bonding monomers with hydrogen bridges 1,4-D-glucopiranocyl with other, causing difficulty for the trapped water is released back (Belitz and Grosch, 1986). The amount of water that is trapped in the digesta mice resulted in increased digesta weight in rats.

Inulin is a soluble dietary fiber with a lower molecular weight than cellulose, so the ability to absorb water molecules smaller than cellulose. Inulin is a polymer of fructose units linked by bonds β-(2.1)-glicosidic with terminal groups of glucose. The degree of polymerization (DP) of inulin ranges from 3-60. Smaller molecules called fructo-oligo-saccharide of inulin (FOS). The smallest FOS was containing 2 molecules of fructose and one glucose molecule (Roberfroid, 2005). At least the water is trapped in the rats digesta leads to lower weight.

The pH of digesta rats

The pH of digesta rats was a decreased, which the rats given a formulation fed with inulin. The analysis indicates that the type of feed significantly ($p \leq 0.05$) on the pH of the digesta rats. The declined in the digesta rats from 6.73 ± 0.027 with the standard fed AIN '93 to 6.19 ± 0.03 with the formulation fed with inulin KR and 6.18 ± 0.032 with the formulation fed with inulin FM and 6.21 ± 0.039 which with the formulation fed with inulin SD (commercial inulin). The decrease of pH due inulin which is polyfructose metabolized by the glycolytic pathway to produce pyruvate, then pyruvate is converted into Acetyl-co-A, lactate and succinate. Acetil-co-A then can be converted to acetate and butyrate, whereas succinate is converted propionate and format to converted produced H_2, CH_4 and/or H_2S (Macfarlane and Macfarlane, 2003). Acetic and lactic acid contributes to a decrease in the pH of digesta.

Fermentation of inulin in the large intestine can be done by microbes that colonize in the colon is particularly Bifidobacterium spp. to produce fructose. Then the fructose subsequently hydrolyzed by Bifidobacterium spp. to produce energy and acetic acid and/or lactic acid. The acids can lower the pH of the medium in vitro as well as in the lower gastrointestinal tract of its host (Roberfroid, 2005).

Complementerization of DNA probe

Complementary test were done to prove that a specific piece of DNA that has been labeled with Cy3 fluorescent indo cyanine compounds suitable for detecting microbial targets. The test results showed that the DNA probe SG-Bif-0164-aA-18 (5'Cy3/ CATCCGGCATTACCCCC) complementary to Bifidobacteria, SG-Lab-0158-aA-20(5'Cy3/ GGTATTAGCACCTGTTTCA) complementary to the Lactobacillus and Eco16S07C (5 'Cy3/ CTCCTTCCCTCATTTCA) complementary to the *E. coli.*

Collado and Sanz (2007) stated that Bif 164, Lac 168 and Eco11513 a complementary DNA probes to detect *Bifidobacteria, Lactobacillus* and *E.coli* respectively in mucosa calves. Lahtinen et al. (2006) and Bezirtzoglou *et al.* (2011) stated that the quantification of Bifidobacteria by FISH method, the DNA probes Bif164 can used.

Colonic microbiota rats

Total bacteria in the rats digesta, which detected on DAPI was 4.61 x109 cells/g (9.66 ± 0.29 log cfu/g) at a standard fed AIN '93, it was not significant different with at formulations fed by lesser yam inulin or commercial inulin (Table 1). Average number of log total bacteria, *Bifidobacteria, Lactobacillus* and *E.coli* can be seen in Figure 3.

The results showed that the number of *Bifidobacteria* in digesta rats with diet containing inulin were increase, it is because inulin can be used/fermented by colonic micro biota particularly Bifidobacteria for the growth. Roberfroid (2005) showed that several types of Bifidobacteria produce inulinase extra celuller enzyme that can hydrolyze inulin to fructose, then the fructose used as an energy source for growth.

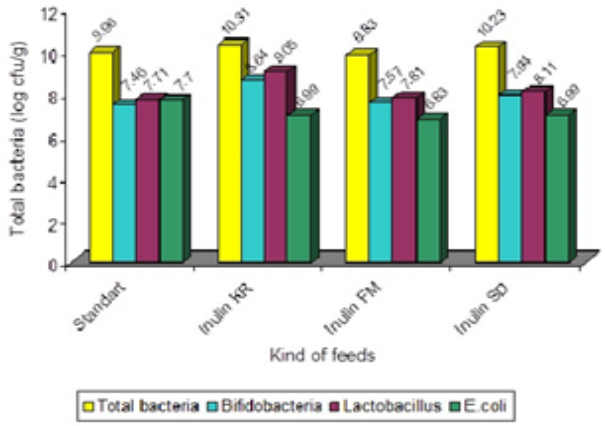

Figure 3. The average of total bacteria, *Bifidobacteria, Lactobacillus* and *E.coli* in cecum rats with different fed

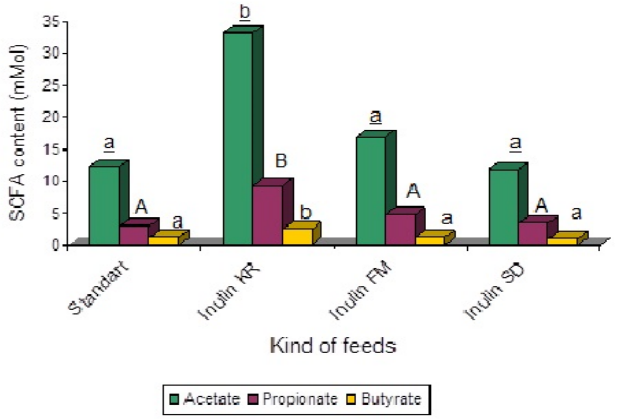

Figure 4. The average of SCFA in cecum rats with different fed. (The hystogram followed by different letter indicates significant different p≤0.05)

Fermentation of inulin in the large intestine can be done by the colonizing micro biota in the large intestine is particularly Bifidobacteria. This is because some of Bifidobacteria produce extracellular inulinase enzymes that can hydrolyze of β-(2→1)-fructocyl-fructose bond to produce fructosa that can be used as an energy source for the growth of Bifidobacteria (Roberfroid, 2005).

An increase in the total number of *Lactobacillus* in the digesta rats fed inulin formulations because the inulin can be used/fermented by colonic micro biota especially *Lactobacillus* for growth. Inulin was fermented by anaerobic bacteria in the colon produce short-chain fatty acids (SCFA), thus stimulating the growth of various bacteria, including lactobacilli and bifidobacterium, and can produce gas (Pompei *et al.*, 2008). Lesser yam inulin can increase the Bifidobacteria and Lactobacillus higher than commercial inulin due the degrees of polymerization lesser yam inulin lower than commercial inulin, which it easier on degradation by colonic bacteria. Degrees of polymerization of lesser yam inulin was 6, while inulin from the market (chicory inulin) was 10.

Lesser yam inulin and commercial inulin can reduce the growth of *Escherichia coli* in the rats cecum compared with the rats fed a standard AIN'93 contain cellulose. This is due to *Escherichia coli* was not able to degrade a low complex carbohydrates such as inulin (Pelczar, 1988). More ever, the decrease the number of *E. coli* in rat digesta because the increasing number of *Bifidobacteria* and *Lactobacillus* in rats digest, so resulting in several possibilities, among others, some lactic acid bacteria such as *Lactobacillus* and *Bifidobacteria* produce acid that lower the pH which can inhibit the growth of pathogenic bacteria such as *E. coli* (Matteuzzi *et al*., 2004). Number of lactic acid bacteria also produced antimicrobial compounds that can inhibit pathogenic bacteria such as *E.coli* and *Salmonella*. Some lactic acid bacteria can increase the ability of immune cells that can fight bacterial pathogens, and are also capable of blocking the attachment of pathogens on the surface of the gut (Gibson and Cartney, 2005). *Bifidobacteria* and *Lactobacillus* more dominant than *E. coli,* thus better able to compete in getting the nutrients in the large intestine compere to *E.coli* (Louis *et al*., 2007).

Pompei *et al.* (2008), showed that inulin can increase the growth of *Bifidobacterium adolesentis, Bifidobacterium infantis, Bifidobacterium breve, Bifidobacterium longum, Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus reuteri* and *Lactobacillus delbruechii* can inhibit the growth of *E. coli* and *Clostridia*.

The SCFA Content in rats cecum

SCFA levels (acetate, propionate and butyrate) in the cecum rats with feed formulation by inulin increased compared with standard feed containing cellulose. This is because fructans inulin compounds that were metabolized by colonic microorganisms, including Bifidobacteria through the glycolytic pathway to produce pyruvate, then pyruvate converted to Acetyl-CoA, lactate and succinate. Acetyl-co-A can be further converted to acetate and butyrate, whereas succinate converted to propionate, format converted to gas H_2, CH_4 and/or H_2S (Macfarlane and Macfarlane, 2003). Hexoses used by Bifidobacteria through an unusual metabolic pathway called "bifid shunt" (Robertfroid, 2005; Tannock, 2010). Genus of Bifidobacteria have a unique metabolic pathway that produces the enzyme fructose-6-phosphate phosphoketolase to ferment oligosaccharides. The enzyme is a key enzyme to recognize the genus of Bifidobacteria (Sela *et al.*, 2010).

Some species of bacteria can produce SCFA

by metabolic cross-feeding (cross- consumption products from metabolism), such as *Eubacterium halii* and *Anaerostipes caccae* can convert lactate and acetate to butyrate (Gibson *et al*., 1995). Conversion of lactate into butyrate cannot be done by *Bifidobacterium* adolescentis, but by butyrate-producing bacteria that can not directly use FOS as an energy source (Louis *et al*., 2007).

Formation of acetic, propionic and butyric acid in the human colon is highly dependent on the diet consumed (Pryde *et al*., 2002), and indirectly influenced by colon pH, because pH affects on micro biota composition (Louis *et al*., 2007). Lesser yam inulin (Inulin KR and Inulin FM) and commercial inulin (Inulin SD) can increase the molar ratio of propionic acid, but not in acetic and butyric acid. It is because at pH 5.5-6.5 bacteri butyrogenic less than propiogenic bacteria so that more formation of propionate than butyrate (Pryde *et al.*, 2002).

Conclusion

Fluorescent in situ hybridization (FISH) method was very suitable and accurate for the quatification the colonic micro biota rats. Lesser yam inulin can be controlled colonic micro biota *Sprague Dawley* rats, it can be increased the number of *Bifidobacterium* and *Lactobacillus* but reduced of *E.coli.* Lesser yam inulin increased of SCFA (acetate, propionate and butyrate), however only propionate molar ratio was increased.

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