The Effect of Yacon Tubers as Prebiotics and Sinbiotics against *Salmonella typhimurium* IFO 12529 on *Rattus norvegicus*

L Yuanita¹, P R Wikandari¹, Isnawati², W B Sabtiawan³ and D A P Sari³

¹Department of Chemistry, Universitas Negeri Surabaya, Indonesia
²Department of Biology, Universitas Negeri Surabaya, Indonesia
³Department of Science, Universitas Negeri Surabaya, Indonesia

Corresponding Author: lennyyuanita@unesa.ac.id

**Abstract.** This study is to obtain the role of yacon tubers through storage and boiling variations as prebiotics and sinbiotics against *Salmonella typhimurium* pathogenic bacteria (*in-vivo*). There are 80 of *Rattus norvegicus* that were divided into 10 groups: five of them by yacon tubers prebiotic supplements and five other feed by yacon tubers sinbiotics with *Bifidobacterium longum* Reuter ATCC 15707, infected on days 22 and 23. *Salmonella typhimurium* IFO 12529 infections is on days 29 and 30. Total length of yacon tubers supplements is 40 days. There are four kinds of treatment for yacon tubers, namely, T1 (without storage and boiling), T2 (without storage, 60 minute boiling), T3 (14 day storage, no boiling), T4 (14 day storage, 60 minute boiling). The results of the study: 1) The use of prebiotic yacon tubers inhibits growth or decreases the amount of *Salmonella typhimurium*, and it is more effective to use yacon tubers sinbiotics along with *Bifidobacterium longum*, 2) Among the yacon tubers treatment supplements, the T1 is the most effective treatment for decreasing the amount of *Salmonella typhimurium*, with or without *Bifidobacterium longum*.

1. **Introduction**

The yacon tubers [*Smallanthus sonchifolia* (Poepp.et Endl.) H.Robinson] are known as a source of FOS prebiotic bioactive compounds (fructooligosaccharide); containing 70-80% dry weight saccharide, consisting of 350.1 fructose, glucose 158.3, sucrose 74.5, FOS (GF2-GF9) 206.4, 13.5 mg inulin, also chlorogenic acid antioxidant 48.5 mg / kg dry weight [1]. FOS is the oligosaccharides of the fructosyl unit binding β-2,1 with glucose, 2-9 degree of polymerization (glucose- (fructose) n). The main components of FOS are 1-kestose (GF2), nystose (GF3), and 1-b-D-fructofuranosylnystose (GF4). Chlorogenic acid is a polyfenol compound that can be used as a specific growth substrate, intestinal bacteria include Lactobacillus and *Bifidobacterium* strains, because they can metabolize chlorogenic acid into caffeine and quinic acid [2].

One of the factors that affect the levels of bioactive compounds FOS and chlorogenic acid, is the activity of enzymes. The fructan 1-exohydrolase enzyme catalyzes the breakdown of FOS to fructose and glucose [3]. Miyaguchi et al [4] revealed that the FOS’s yacon will drop when it is heated in 100°C after it is peeled and cut. After 6 days storage in the shade, the concentration of yacon tubers’ FOS decreased from 50-62 to 36-48% dry weight, while the concentration of fructose, glucose, sucrose increased from 29-34 to 48-52% [5]. Six days sun heating decreased FOS into 29-44% and increasing the saccharide level into 45-51% dry weight. Chlorogenic acid is easily oxidized and polymerizes to form quinone compounds that cause brown color. In the case of boil tubers, there is a significant decrease of chlorogenic acid content, it isomerized with 4-O-caffeoylquinicacid (4-O-CA) and 5-O-caffeoylquinicacid (5-O-CA) [6][7].
Gastric acid and gastrointestinal enzymes that can reach the large intestine cannot digest FOS and inulin, but they are fully fermented to the cecum and colon by some bacterial strains such as *Bifidobacterium* and *Lactobacillus*, selectively to stimulate its growth; this is due to the production of β-fructofuranosidase / fructanase [8]. Sghir et al. [9] study showed that *Lactobacillus* and *Bifidobacterium* use FOS for its growth, but *Bifidobacterium* grows faster in FOS than *Lactobacillus*.

Fermentation of intestinal microflora to FOS-inulin will simultaneously produce various short chain fatty acids (SCFA), CO₂, CH₄, H₂, lactic acid, thus decreasing the pH of the large intestine, which will inhibit the growth of pathogenic bacterial groups such as *Salmonella typhii*, reducing the formation or accumulation of carcinogenic compounds and harmful metabolites. Tanner et al [10] found that the addition of FOS greatly inhibited the growth of *Salmonella enterica* Typhimurium N-15 in the PolyFermS porcine model, and correlated with increased production of SCFA, especially acetate and propionate.

One way to stabilize and enhance the effect of probiotics is by combining probiotics and prebiotics, called symbiotics. According to Suskovic et al. [11], symbiotics increase the survival of probiotic bacteria, decrease the amount of *Clostridia*, *Enterobacteriaceae* and *E. coli*. Gmeiner et al. [12] obtain symbiotic *L. acidophilus* 74-2 with FOS, increase SCFA and β-galactosidase activity that has a role in the hydrolysis of galactooligosaccharide.

The result showed that through in vitro method yacon tubers as a source of FOS prebiotics and symbiotics along with *Lactobacillus acidophilus* IFO 13951, *Bifidobacterium longum* Reuter ATCC 1570, can inhibit the growth of *Salmonella typhimurium* IFO 12529 [13]. The purpose of this research is to find yacon tubers’ role through variations of storage and boiling time as prebiotics and symbiotics with *Bifidobacterium* against *Salmonella typhimurium* on *Rattus norvegicus*.

2. Method

There are four groups of yacon tubers involving T₁ (no storage and no boiling), T₂ (no storage, with boiling 60 minutes), T₃ (14 days of storage, without boiling), and T₄ (14 days of storage, with boiling 60 minutes). There are 5 feed treatments involving standard (S), the T₁, the T₂, the T₃, and the T₄ (containing 10% FOS = 250 g yacon / 1000 g). 80 tested animals, wistar *Rattus norvegicus* strain, are divided into 10 groups, the first five groups without the addition of *Bifidobacterium longum* Reuter ATCC 15707 and the second five groups with the addition of *Bifidobacterium longum* Reuter ATCC 15707. Each animal is placed in individual cages and drinks in ad libitum. 4-day environmental adaptation with standard feed, and then with the feed containing the treatment yacon for 17 days. On the 22nd and 23rd days, an infection is performed by oral isolates *Bifidobacterium longum* Reuter ATCC 15707 @ 1/2 x z 1010 cfu (suspension in 1 ml of saline 1:3 w/v and containing 3% NaHCO₃ newly made) to 5 groups [14][15]. At days 29 and 30, all of an animal model to infected *Salmonella typhimurium* IFO 12529 isolates orally @ 1 / 2x2x108 cfu (suspension in 1 ml of saline 1:3 w/v and containing 3% of newly-created NaHCO₃) [14][15]. After an incubation period of ten days, the tested animal is killed. Feces collected on the first day and the tenth incubation period. Then analyzed the animals model: 1) *Bifidobacterium* and *Lactobacillus* in colon (cfu), and 2) *Salmonella* feces at the beginning and end of incubation (cfu). Total plate count is used to count the bacteria in this research. MRS De Man, Rogosa and Sharpe broth / media (MRS) are used for *Lactobacillus acidophilus*, MRS-cysteine HCl 0.05% for *Bifidobacterium longum*, while bismuth sulfite media for *Salmonella typhimurium*. The result of measurement has been analyzed quantitatively.

3. Results and Discussion

Yuanita et al. [16] results showed that increasing storage (0,7,14 days) and boiling time (0, 30, 60 minutes) would decrease of FOS and increase of saccharides concentrations. Yacon tubers contain FOS as prebiotic, which trigger a probiotic population growth of lactic acid bacteria, especially *Bifidobacterium* and *Lactobacillus* that naturally found in *Rattus norvegicus* colonic. This is due to FOS can be ingested through hydrolysis as a result of β-fructofuranosidase of probiotic microorganisms, produced glucose and fructose. The result of fermentation reaction of glucose fructose lowers pH
bowel/colon because the resulting acetic, propionic, butyric and lactic acids. According to Gibson and Roberfroid [17], the fermentation reaction stoichiometry described as follows:

\[
1 \text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 1.5 \text{CH}_3\text{COOH} + 0.33 \text{CH}_3\text{CH}_2\text{COOH} + 0.15 \text{CH}_3(\text{CH}_2)_2\text{COOH} + 0.33 \text{CH}_3\text{CHOHCOOH} + 0.33 \text{CO}_2 + 0.33 \text{H}_2\text{O}
\]

At the end of the treatment, it is obtained the number of *Bifidobacterium* and *Lactobacillus* colon bacteria (Table 1 and 2), and *Salmonella* in the feces of *Rattus norvegicus* (Table 3).

### Table 1 The average number of *Bifidobacterium* and *Lactobacillus* of *Rattus norvegicus* colon (10^3) cfu.

| Group | Feed Treatment | Bifidobacterium | Lactobacillus | Total |
|-------|----------------|-----------------|---------------|-------|
| 1     | S              | 0.60            | 1.80          | 2.40  |
| 2     | T1             | 33.40           | 38.60         | 72.00 |
| 3     | T2             | 19.80           | 20.80         | 40.60 |
| 4     | T3             | 7.20            | 11.80         | 19.00 |
| 5     | T4             | 7.80            | 12.60         | 20.40 |

S: standard feed, T1= fresh and without boiling; T2= fresh and 60 minutes boiling time; T3= 14 days storage time and without boiling; T4= 14 days storage time and 60 minutes boiling time.

### Table 2 The average number of *Bifidobacterium* and *Lactobacillus* of *Rattus norvegicus* colon with infection of *Bifidobacterium longum* Reuter ATCC 15707 (10^3) cfu.

| Group | Feed Treatment | Bifidobacterium | Lactobacillus | Total |
|-------|----------------|-----------------|---------------|-------|
| 6     | S + *B. longum* | 12.70           | 11.90         | 24.60 |
| 7     | T1 + *B. longum* | 39.50           | 50.45         | 89.95 |
| 8     | T2 + *B. longum* | 24.80           | 50.50         | 75.30 |
| 9     | T3 + *B. longum* | 11.70           | 17.05         | 28.75 |
| 10    | T4 + *B. longum* | 14.80           | 21.70         | 36.50 |

S: standard feed, T1= fresh and without boiling; T2= fresh and 60 minutes boiling time; T3= 14 days storage time and without boiling; T4= 14 days storage time and 60 minutes boiling time.

The average number of *Bifidobacterium* on the data Tables 1 and 2 can be described as follows in Figure 1, while the average number of *Lactobacillus* in Figure 2.

![Figure 1](image1.jpg)

**Figure 1.** The influence of yacon tubers supplement against average number of *Bifidobacterium* colonic of *Rattus norvegicus*.
Figure 2. The influence of yacon tubers supplements against average number of Lactobacillus colonic of Rattus norvegicus.

From the results in Table 1 and 2, it can be argued that:

1. The use of yacon tubers synbiotic supplement with Bifidobacterium longum is more effective against a number of colon Bifidobacterium and Lactobacillus compared to treatments with the yacon tubers prebiotic supplement alone. The combination of probiotics and non-digestible carbohydrates (FOS) may be a way of stabilization and/or improvement of the probiotic effect. The use of probiotic that orally administered can improve immune status by increasing circulating and local antibody levels, endurance live probiotic colon bacteria, increasing the activity of useful enzymes that degrade of prebiotic and supression of pathogenic microorganism in intestinal tract [11][12].

2. The use of yacon tubers as prebiotic or sinbiotic supplement, increasing the number of beneficial colon bacteria Bifidobacterium and Lactobacillus compared to treatments with the feed standards. This is because yacon tubers is a source of FOS serve as nutrients for the colon bacteria which have fructan 1-exohydrolase. Likewise, saccharides and chlorogenic acid of yacon tubers are useful for intestinal bacteria growth.

3. On the usage of yacon tubers as prebiotic or synbiotic, fresh and no boiling (T1) treatment most effective. May be due to the highest of FOS and chlorogenic acid on the T1 treatment yacon tubers. Chlorogenic acid can be used as a specific growth substrate, not degraded or absorbed in the upper digestive tract, but can be hydrolyzed by the intestinal bacteria produce quinic acids and hydroxycinnamate acids i.e. caffeic acids [2].

4. The yacon tubers supplement of T4 treatment (14 days storage and boiling 60 minutes) produced colon bacteria of Rattus norvegicus more higher than T3 yacon tubers supplement (14 days of storage, without boiling). May be due to glucose and fructose content in yacon tubers resulted in the T4 treatment more higher than T3, cause hydrolyzed during storage and boiling. Glucose and fructose also a source of carbon for growth of Bifidobacterium and Lactobacillus.

5. The amount of Lactobacillus colonie more higher than the Bifidobacterium, also against the standard feed groups. Lactobacillus have optimum growth at pH 6.5, while Bifidobacterium at pH 5-5.5. pH range of colon is 5.5 to 7, its mean that pH colonic is more suitable for the growth of Lactobacillus than Bifidobacterium. This sensitivity includes temperature, pH, the presence of nutrients according to their needs, and the presence of other compounds that interfere with its growth. Research of Sghir et al[9] showed that Lactobacillus and Bifidobacterium using FOS for its growth, but the Bifidobacterium grown faster on FOS than Lactobacillus. Then on infection of Bifidobacterium longum, competition for FOS limiting nutrients occurs by Bifidobacterium; while Lactobacillus using saccharides as a source of carbon for growth. Bifidobacterium is more affected than Lactobacillus,
population growth is limited, even rapidly entering the stationary phase and subsequent phase of death.

The average number of *Salmonella of Rattus norvegicus* feces through infections of *Salmonella typhimurium IFO 12529* on the usage of yacon tubers as prebiotic and symbiotic are provided in Table 3 and Figure 3.

**Table 3.** The average number of *Salmonella of Rattus norvegicus* feces through infections of *Salmonella typhimurium IFO 12529* (10$^3$ cfu).

| Group | Feed Treatment | Feces Early Treatment * | End Of Treatment * |
|-------|----------------|-------------------------|--------------------|
| 1     | S              | infinite                | 16.40              |
| 2     | T1             | infinite                | 1.20               |
| 3     | T2             | infinite                | 1.80               |
| 4     | T3             | infinite                | 5.40               |
| 5     | T4             | infinite                | 7.60               |
| 6     | S + b. longum  | infinite                | 19.20              |
| 7     | T1 + b. longum | infinite                | 0.60               |
| 8     | T2 + b. longum | infinite                | 0.80               |
| 9     | T3 + b. longum | infinite                | 3.60               |
| 10    | T4 + b. longum | infinite                | 4.20               |

S: standard feed. T$_1$= no storage and boiling; T$_2$= no storage, boiling 60 minutes; T$_3$= 14 days of storage, without boiling; T$_4$= 14 days of storage, boiling 60 minutes. * Early treatment: after 1 day, end of treatment: after 10-day treatment.

![Figure 3](image-url)

**Figure 3.** The influence of yacon tubers supplements against average number of *Salmonella of Rattus norvegicus* feces.

Table 3 showed that:
1. The use of yacon tubers symbiotic with *Bifidobacterium longum* is more effective inhibiting the growth of *Salmonella* than as prebiotics.
2. Yacon tubers as beneficial supplements inhibit the growth of *Salmonella*. This indicates the role of the yacon tubers FOS as prebiotic. The high levels of FOS is followed by growth of bacteria to intestinal generated high acidity; low pH will inhibit the growth of pathogenic bacteria.
3. T1 yacon tubers supplement has ability to suppress the growth of *Salmonella* in the feces of Rattus norvegicus. Moreover, similarly *Salmonella* bacteria not found in the cecum and colon [18]. This is due to the high concentration of FOS in fresh yacon tubers as a source of nutrients for colon bacteria. Fermentation of intestinal microflora against FOS simultaneously will produce short chain fatty acids, CO$_2$, CH$_4$, H$_2$, lactic acid, that is lowering the pH of the large intestine; by consequence,
it will inhibit the growth of pathogenic bacteria group. Undegraded or unhydrolyzed chlorogenic acid also suppress the growth of Salmonella. According to Karunanidhi et al. [19], several Gram-positive (Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus mutans and Bacillus subtilis) and Gram-negative (Salmonella typhimurium, Shigella dysenteriae, and Escherichia coli) bacterial pathogens have been reported to be highly susceptible to chlorogenic acid.

4. Increasing of average number of colonic bacteria, decreasing of Salmonella, except the T4 treatment.

It is due to the highest amount of glucose on the T4 yacon tubers. The glucose can be used for the growth of Salmonella through fermentation process.

4. Conclusion

To conclude, there are two main findings of the study, that usage of yacon tubers prebiotic inhibits the growth or decrease of the number of Salmonella typhimurium, but more effective in the use of yacon tubers symbiotic with Bifidobacterium longum and Among the supplements of treatment yacon tubers, the T1, fresh and no boiling yacon tubers supplement, is the most effective for lowering the number of Salmonella typhimurium, with or without a Bifidobacterium longum.

Acknowledgement

International Development Bank supported this research for the Development and Upgrading of Seven Universities in Improving the Quality and Relevance of Higher Education in Indonesia.

5. References

[1] Lachman J, Fernandez E C and OrsakM 2003 Plant Soil Environ. 49(6) 283.
[2] Couteau D, Mc Cartney AL, Gibson G R, Williamson G and Faulds CB 2001 J. Appl. Microbiol. 90(6) 873.
[3] Shiomi N, Benkeblia, N S, Onodera O T, Takahashi N, Fujishima M, Yoshihira T and Kosaka S 2007 J. Appl. Glycosci. 54 187.
[4] Miyaguchi Y and Inoue E 2012 Nippon Shokuhin Kagaku Chinese Story Kaishi. 59(4) January.
[5] Graefe S, Hermann M, Manrique I, Golombek S and Buerkert A 2006 Field Crops Research 86 157.
[6] Kan S, Cheung MW M, Zhou Y and Ho W S 2014 J. Food Sci. 79(2) C147.
[7] Xu B and Chang S K C 2008 J. Agric. Food. Chem. 56(16) 7165.
[8] Swennen K, Cauntin C M and Delcour A 2006 Food Sci. Nutr. 46 459.
[9] Sghir A, Chow J M and Mackie R I 1998 J Appl Microbiol. 85 769.
[10] Tanner SA., Chassard C, Berner A Z and Lacroix C 2014 Gut Pathogens 644.
[11] Suskovic J, KosB and Matosic S J G 2001 Food Biotechnol. Technol. 39(3) 227.
[12] Gmeiner M, Kneifel W, Kulbe K D, Wouters R, De Boever P, Nollet L and Verstraete W 2000 Appl. Microbiol. 53 219.
[13] Yuanita L, Wikandari P R and Isnawati 2015 The 5th Int. Conf. Indones. Chem. Soc. Proc. Samarinda, East Kalimantan. August, 30-31, 2015.
[14] Ten Bruggencate SJ M, Bovee-Oudenhoven IM J, Lettink MLG W and Van d M R 2005 J. Nutr. 135(4) 837.
[15] Boove OIM J, Termont DSML., Heidt P J and Van d M 1997 Gut. 40 497.
[16] Yuanita L, Wikandari P R and Sabtiawan W B 2017 Adv. Sci. Lett. 23(12) 11982.
[17] Gibson G R and Roberfroid M B 1995 J. Nutr. 125 1401.
[18] Yuanita L, Wikandari P R and Isnawati 2016 Yacon Tuber FOS [Smallanthus sonchifolia (Poepp.et Endl.) H. Robinson] as Prebiotic and Sinbiotik to inhibit the growth of pathogenic bacteria. Research Report PUPT-IDB. LPPM UNESA.
[19] Karunanidhi, Thomas R, Van B A and Neela V 2013 BioMed. Res. Inter. 2013 1.
