

THE PREBIOTIC EFFECTS OF DIETARY JERUSALEM ARTICHOKE (KAENTAWAN) IN HEALTHY THAI ADULTS

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Abstract

Jerusalem artichoke or “kaentawan” (*Helianthus tuberosus* L.) is a wild relative of the cultivated sunflower that has recently experienced an upsurge of interest as a source of prebiotics. The polysaccharides in kaentawan (fructo-oligosaccharides (FOS) and inulin) are rich and could have the potential for use as a health-promoting dietary supplement (prebiotic) that modulates intestinal pathogens by increasing the amount of the probiotics. Reports on the prebiotic effect of dietary kaentawan in healthy Thai adults have hardly been found recently. Therefore, the present study aims to determine the prebiotic effect of kaentawan consumption in 90 healthy Thai subjects. The amounts of probiotics from their feces were used as an indicator. The subjects were randomly assigned into 3 groups as follows: the 1st group was normal consumption, the 2nd group was 85 grams of fresh kaentawan consumption per day, and the 3rd group was commercial 10 grams of inulin and 5 grams of FOS consumption per day. The amounts of probiotic bacteria, *Bifidobacterium* spp. and *Lactobacillus* spp., were enumerated from each subject’s feces 6 times using the

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real-time polymerase chain reaction technique. The results showed that the amount of probiotics in the feces of subjects who consumed fresh kaentawan had significantly increased ($P<0.05$) compared to the subjects who had a normal consumption. There was no significant difference of probiotic amounts between the subjects who consumed commercial prebiotics and those who consumed fresh kaentawan. These results reveal that kaentawan can be used as a prebiotic and promoted to replace imported commercial prebiotics.

Keywords: Jerusalem artichoke, kaentawan, prebiotics, *Bifidobacterium* spp., *Lactobacillus* spp.

Introduction

Inulin and fructo-oligosaccharides (FOS) are the polysaccharide linkages of D-fructose with the end of glucose residue (fructans). Because of these polysaccharide linkages, they resist digestion in the gut and colon. This beneficially affects health by selectively stimulating the growth and activity of a limited number of health-promoting bacteria (probiotics) in the colon such as bifidobacteria and lactobacilli (Gibson and Roberfroid, 1995; Van Loo *et al.*, 1999). Both inulin and FOS are current global market leaders for prebiotics due to their role as soluble dietary fibers, and these substances have been claimed to improve some physiological functions in humans such as relieving constipation (Roberfroid, 1993). A recent study on the prebiotic effects of inulin and FOS found that fruits and vegetables containing inulin and FOS at the level of 5 g/d significantly increased bifidobacteria (Ramnani *et al.*, 2010).

Fructans are found in a wide variety of plants such as chicory root, garlic, asparagus, onion, wheat, and particularly in Jerusalem artichoke (kaentawan) (van de Wiele *et al.*, 2007). Most fructans are synthesized or prepared commercially from inulin-rich plant sources (Gibson *et al.*, 1995; Kleessen *et al.*, 2007; Kleessen *et al.*, 1997; Li *et al.*, 2008; Roberfroid, 2005). Sixteen varieties of Jerusalem artichoke in Thailand were collected and analyzed by the Institute of Nutrition, Mahidol University. The results showed that the Jerusalem artichoke strain CN52867 or kaentawan has the highest levels of inulin and FOS (20.37 g

and 5.16 g/100 g fresh samples, respectively). This study demonstrated that kaentawan contains the highest amount of inulin and FOS when compared with other plants (Judprasong, *et al.*, 2011; Kleessen *et al.*, 2007).

The prebiotic effects of Jerusalem artichoke (fresh and heat dried) were tested among healthy volunteers in the United Kingdom. The results showed that the number of fecal bifidobacteria was significantly increased after 4 weeks' consumption, but there was no statistically significant difference in the amount of fecal bifidobacteria between fresh and heat dried kaentawan. However, the studies showed that kaentawan may cause bowel symptoms. Ingesting 40 g/d can cause diarrhea, flatulence, colic, and nausea (Ramnani *et al.*, 2010).

However, there have only been a few studies on the prebiotic effects of kaentawan in volunteers in Thailand. So, this human study was designed to assess the prebiotic capability of kaentawan. The effect of inulin and FOS present in kaentawan compared to commercial products upon the relative numbers of intestinal microbiota was determined using a real-time polymerase chain reaction (PCR) assay. Daily gastrointestinal symptoms were monitored over the course of the trial. It was expected that this study could beneficially demonstrate the consumer's decision for choosing kaentawan as a prebiotic source instead of imported commercial inulin and FOS powder which might decrease the cost about ten times.

Materials and Method

Subjects

Ninety healthy human subjects were recruited from Nong Khang Khok village, Muang district, Chonburi province, Thailand. Written consent was obtained from all the subjects, and they were assessed for good health before the start of the trial according to the inclusion and exclusion criteria. The study protocol was reviewed and approved by the Ethical Committee of the Faculty of Tropical Medicine, Mahidol University, Thailand according to document approval no. TMEC 15-071.

Inclusion and Exclusion Criteria

The inclusion criteria were a signed consent form, age 18-60 years inclusive, non-smoking, BMI 18.5-24.9 kg/m² inclusive, and good general health as determined by medical questionnaires.

The exclusion criteria were: evidence of physical or mental disease or major surgery which might limit participation in the study, or completion of the study, or interfere with the outcome of the study; a history of drug and alcohol abuse, severe allergy, abnormal drug reaction, pregnancy, lactation, or planned pregnancy. The intake of an experimental drug within 4 weeks before the study, former participation in probiotics, prebiotics, or a laxative trial within the previous 3 months, use of antibiotics within the previous 6 months, a history of chronic constipation, or diarrhea, or other chronic gastrointestinal complaint, and the intake of other specific prebiotics or probiotics, drugs active on gastrointestinal motility, or a laxative of any class within the 4 weeks before the start of the run-in period of the study were prohibited.

Requirements for Diet and Medication during Study

Subjects were instructed not to consume any additional prebiotics (such as oligosaccharides e.g. fructo-oligosaccharides and inulin). Several vegetables with fructans were recommended to be avoided during the study including Thai garlic (fructans 22.4 g/100 g), Chinese garlic

(24.3 g/100 g, about 30 cloves), big garlic (29.2 g/100 g, 20 heads), Indian onion (3.09 g/100 g, 10 heads), and shallots (4.98 g/100 g, 15 heads) (Judprasong *et al.*, 2011), probiotics (e.g. live yoghurts), drugs active on gastrointestinal motility, antibiotics, and laxatives. Subjects were not allowed to participate in any other nutritional or pharmaceutical trials for the duration of the trial. Any medication taken was recorded in the subjects' diaries. Participants were advised not to alter their usual diet or fluid intake during the trial period.

Jerusalem Artichoke (Kaentawan) Preparation

Organic Jerusalem artichoke (kaentawan) tubers were purchased from a gardening centre in Saiyok district, Kanjanaburi province. They were analysed for inulin and FOS contents with the standard method (gas chromatography) by the Institute of Nutrition, Mahidol University laboratories. The actual amount of kaentawan (ranging from 80-100 g) for the participants' daily intake had been calculated to gain 10 g inulin and 5 g FOS. They were washed, cleaned, peeled, and 85 g was placed in each ziplock bag for daily intake and then kept at 4-8°C until used. Fresh kaentawan packs were distributed to the participants once a week (7 bags per week).

Study Design

The feeding trial comprised a three-arm parallel; controlled randomization with 3 groups of 30 healthy human subjects. The subjects were assigned a specific code. The codes were then placed in 3 boxes randomly, at 30 codes per box. There was a completed total of 90 subjects.

The subjects were assigned to consume 85 g of kaentawan (approximately containing 10 g inulin and 5 g FOS), a mixture of 10 g inulin and 5 g FOS (Beneo- Orafit SA, Tienen, Belgium), or consume a usual meal, as shown in Figure 1.

In the run-in period, they were instructed to refrain from consuming foods that contain inulin and FOS in large quantities for 1 week. After that they were asked to consume the products once daily, at 1 time in the morning for 6 weeks. The treatment was followed by

a 1-week wash-out period during which no prebiotics were consumed.

Subjects were asked to keep diaries for the baseline, treatment, and wash-out periods to record fecal frequency and changes in intestinal comfort (abdominal pain, stomach or intestinal bloating, and flatulence). Any concomitant medication and adverse events or subject comments on the products were recorded. They were instructed to keep the products in a refrigerator at 4-8°C. There were 9 participants who dropped out before the end of the run-in period.

Fecal Sample Preparation and Processing

Fresh fecal samples were collected in sterile plastic pots at the beginning of the run-in (week 0), treatment (weeks 1, 2, 4, and 6), and wash-out periods (1 week after the study period, i.e. week 7), respectively. Samples were processed within 15 min of defecation. Samples were kept in 5 ml 70% alcohol and delivered to the laboratory within 24 h. The fecal consistency was also recorded as soft, formed, or hard.

DNA Preparation

Approximately 220 mg of feces was transferred to a 2.0 ml micro-centrifuge for further DNA preparation. DNA from each fecal sample was prepared using a previously described method (Arbolea *et al.*, 2012) as follows: fecal samples were mixed and diluted 1/10 in sterile phosphate buffered saline

solution, and homogenized using a homogenizer at full-speed for 4 min.

DNA was extracted from the homogenized feces, as well as from bacterial cloning cultures (*Bifidobacterium longum subsp.* ATCC 15707 and *Lactobacillus casei subsp. rhamnosus* ATCC 7469; Thailand Institute of Scientific and Technological Research) used for standard curves, by the QIAamp DNA stool kit (Qiagen GmbH, Hilden, Germany), following the manufacturer's specifications, as previously described (Gueimonde *et al.*, 2004). The DNA concentration was measured by using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The extracted DNA was kept at -80°C until analysis.

Bacterial Enumeration by SYBRgreen Real-time PCR Assay

Quantitative analysis of bacteria from the intestine was performed at the Center of Excellence for Antibody Research, Faculty of Tropical Medicine, Mahidol University, using a quantitative real-time polymerase chain reaction (qPCR) by following the method as previously studied (de Vrese *et al.*, 2005).

Quantification of bacteria in the feces was carried out by qPCR using the primers LacF: TGGAACAGGTGCTAATACCG/ LacR: CCATTGTGGAAGATTCCC (Heilig *et al.*, 2002) to enumerate lactobacilli bacteria and Bif-tF: GTCCGTGACCTCCTCGAC/ Bif-tR: GTGGAAGGTCTCGATGGAG to enumerate bifidobacteria (Sheu *et al.*, 2010). All reactions were performed on a Biorad CFX96 thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using the SYBRGreen PCR Master Mix (Applied Biosystems Corp., Foster City, CA, USA). A 0.5 microliter of template fecal DNA (~5 ng) and 0.5 µM of each primer was mixed in the 10 µL PCR tubes. Thermal cycling consisted of an initial cycle of 95°C for 10 min, followed by 95°C for 10 sec and annealing at 55°C for 10 sec for both the Bifidobacteria and Lactobacillus primers, and extension at 72°C for 10 sec for 45 cycles. The melting curve was determined step-by-step by slowly heating at 0.1°C/ sec increments from 55°C to 95°C with a simultaneous

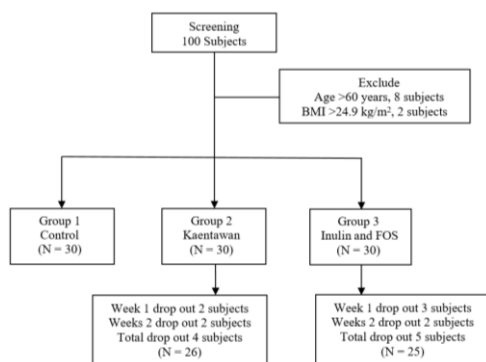


Figure 1. Schematic diagram for enrollment

measurement of the SYBR Green I signal intensity. Quantitation was done using standard curves made from the known concentrations of plasmid DNA containing the respective amplicon for each set of primers (*tuf* gene for *Bifidobacterium* spp. and 16SrRNA gene for *Lactobacillus* spp.). Standard curves were constructed using the threshold cycle (Ct) from each dilution of the standard sample (1 ng, 100, 10, 1 pg, and 100 fg). The DNA concentrations of *Bifidobacterium* spp. and *Lactobacillus* spp. in the samples were quantified by comparing the Ct of the feces with the Ct of the standard curve as shown in Figure 2.

Assessment of Gastrointestinal Symptoms

During the study period, the subjects kept a daily chart and recorded subjective intestinal characteristics, fecal frequency, and fecal consistency. Gastrointestinal complaints concerning flatulence, stomach pain, nausea, and vomiting could be selected as being absent (1), mild (2), moderate (3), or severe (4). Fecal consistency was also scored from hard (1), soft (2), and formed (3).

Statistical Analysis

Data were processed with the Statistical Package for Social Science, version 18 (SPSS

Inc., Chicago, IL, USA). Normal distribution of analytical variables was checked using the Shapiro-Wilk test. The Kruskal-Wallis H test was used to compare the median of the concentration of *Bifidobacterium* spp. and *Lactobacillus* spp. before and after the intervention of the 3 groups and the Mann-Whitney U test was used to compare the median of the concentration of *Bifidobacterium* spp. and *Lactobacillus* spp. before and after the intervention between the 2 groups. The *P* value of <0.05 was considered significantly different (Siegel and Castellan, 1988).

Results

Baseline Characteristics and Compliance of Subjects

In the intervention period, 4 subjects from the kaentawan group and 5 subjects from the mixture of inulin and FOS- consumed group withdrew from the experiment, giving a reason of not favoring the consumption of kaentawan and the mixture of inulin and FOS (Figure 1). Finally, 81 participants from the 3 treatment groups (28 men and 53 women) were enrolled in this study. In terms of age and body mass index (BMI), there was no significant

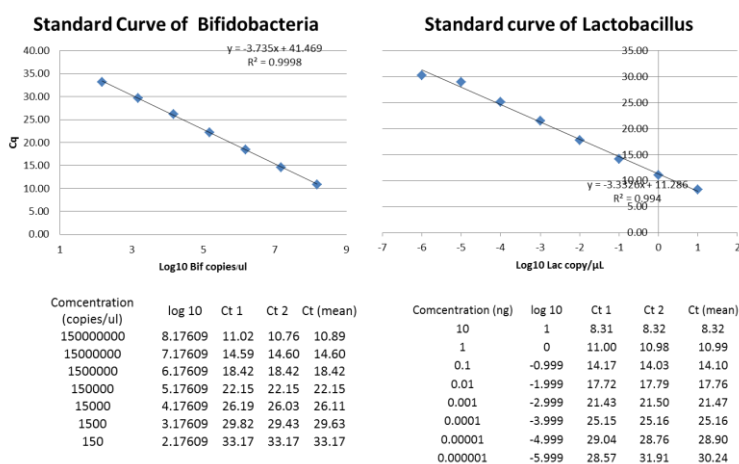


Figure 2. The standard curve which obtain from pure cultures of the reference strains of *Bifidobacterium longum* (A) and *Lactobacillus rhamnosus* (B) Y- axial are the Ct-value and X- axial are bacterial concentration

difference among the 3 groups; the usual meal (control), kaentawan, and the mixture of inulin and FOS. The data can be broken down as follows: age (42.73 ± 12.42 , 46.38 ± 11.10 , and 40.72 ± 12.25 years, respectively) and BMI (21.00 ± 1.31 , 22.26 ± 2.12 , and 22.69 ± 1.73 kg/m², respectively) (Table 1).

Fecal Consistency and Medication Adverse Events

Before the trial, all subjects were interviewed. Their fecal consistency was documented. Briefly, 5 subjects (16.67%) from the control group, 6 subjects (23.08%) from Kaentawan group, and 5 subjects (20.0%) from mixture powder of inulin and FOS group reported a hard fecal problem. After the study period, the fecal consistency was investigated in all groups. Hard stool problem was found in 3 (11.54%) subjects in Kaentawan group and 3 subjects (12.0%) in mixture powder of inulin and FOS group, a decreasing in the hard fecal consistency in the Kaentawan and mixture of inulin and FOS groups were found. On the other hand, there was no change in fecal consistency in control groups with normal diet.

No serious adverse events were recorded by the subjects. They were asked to report by an individual diary. A variety of mild symptoms, such as stomach pain, flatulence, vomiting, diarrhea or nausea, were scattered over the study periods. In the control group that had normal diet, mild symptoms of gastrointestinal tract effects were found; diarrhea was the most common symptom found among all subjects in the control group (11 cases), while abdominal pain, nausea and

vomiting were the least common issues for all subjects (1 cases). Moreover, flatulence symptoms were not found in this group. For the gastrointestinal tract effects of subjects who consumed fresh Kaentawan for 6 weeks, it was found that seven subjects had diarrhea but this symptom was no longer found in subjects after week 5 until the end of the study. For the gastrointestinal tract effects of subjects that consumed a mixture powder of inulin and FOS for 6 weeks, diarrhea was found to be the most common symptom. Seven cases (28%) were diagnosed as diarrhea at week 1 (3 cases, 12.0%), week 2 (2 cases, 8.0%), week 3 (1 cases, 4%) and week 4 (1 cases, 4%). Moreover, 6 (24%) cases with nausea and vomiting symptoms were found, and 3 cases (12.0%) of flatulence were investigated after having consumed a mixture powder of inulin and FOS for 3 weeks.

Intestinal Micro biota (Probiotics Bacteria)

All fecal samples were collected at the beginning of the intervention (week 0, baseline), during the intervention (week 1, 2, 4, the end of the intervention period (week 6)) and wash-out periods (week 7) to investigate fecal *Bifidobacterium* and *Lactobacillus* using real-time PCR. The results in Table 2, presented that fecal *Bifidobacterium* strongly increased in fresh Kaentawan as well as in mixture of inulin and FOS groups, at the end of the intervention period (median 0.4013 min 0.0151 max 29.2227 copies/μg of feces): when compared with baseline (median 0.0283 min 0.0001 max 1.1623 copies/μg of feces) in Kaentawan group and in mixture of inulin and FOS group at the end of the intervention period (median 0.9750

Table 1. Summary of baseline characteristics

Characteristics	Subjects				P-value
	Control (N=30)	Kaentawan (N=26)	Inulin&FOS (N=25)	Total (N=81)	
Gender (%)					
Male	12 (40.0)	7 (26.9)	9 (36.0)	28 (34.6)	0.9148
Female	18 (60.0)	19 (73.1)	16 (64.0)	53 (65.4)	
Age (years)					
range	18-58	21-59	21-56	18-59	0.2344
(mean ± SD)	(42.7±12.4)	(46.4±11.1)	(40.7±12.0)	(43.3±12.0)	
BMI (kg/m²)					
range	18.56-22.95	18.73-24.67	19.03-24.78	18.56-24.78	0.1934
(mean ± SD)	(21.0±1.3)	(22.3±2.1)	(22.7±1.7)	(21.9±1.9)	

min 0.0194 max 38.2318 copies/μg of feces), when compared with baseline (median 0.0931 min 0.0029 max 1.0286 copies/μg of feces). The fecal *Bifidobacterium* increased at week 1, week 2, week 4 and at the end of the intervention period. After that fecal *Bifidobacterium* decreased at wash-out periods (week 7). In addition to *Lactobacillus* level, the significant rise in the fecal *Lactobacillus* was also found in both Kaentawan and mixture powder of inulin and FOS groups at the end of the intervention period: when compared with baseline (median 3.4659 min 0.2472 max 38.0136 copies/ μg of feces and median 47.5705 min 0.0151 max 29.2227 copies/μg of feces in Kaentawan group and median 2.3704 min 0.0019 max 31.5182 copies/μg of feces and median 38.4455 min 0.3744 max 596.3636 copies/μg of feces in mixture of inulin and FOS group, respectively). The fecal *Lactobacillus* increased at week 1, week 2, week 4 and at the end of the intervention period. After that, fecal *Bifidobacterium* decreased at wash-out periods (week 7). It is noteworthy that, fecal *Lactobacillus* level was higher than that of *Bifidobacterium* level in all three group (Table 2).

Table 3 showed the difference of fecal *Bifidobacterium* and *Lactobacillus* at each week of the intervention period (week 1, 2, 4, 6) compared with those of the baseline and the difference of each fecal bacteria at wash out period (week 7) compared with those at the end of the intervention period (week 6)

(respectively). In Kaentawan group, for *Bifidobacterium*, the difference at week1-week 0 was reported as median at 0.0116 min -0.72 max 1.03 copies/μg of feces, week 2-week 0 as median at 0.0197 min-0.87 max 7.39 copies/μg of feces, week 4-week 0 at median 0.0905 min 0.0905 min-0.17 max 10.87 copies/μg of feces, week 6-week 0 at median 0.1637 min-0.09 max 28.06 copies/μg of feces, after that they were decrease in wash-out periods (week 7-week 6) at median-0.1413 min-4.89 max 0.13 copies/μg of feces. In mixture of inulin and FOS group, for *Bifidobacterium*, the difference at week1-week 0 was reported as median at 0.0684 min 0.01 max 1.38 copies/μg of feces, week 2-week 0 at median 0.4100 min 0.02 max 20.75 copies/μg of feces, week 4-week 0 at median 0.5973 min-0.02 max 29.13 copies/μg of feces, week 6-week 0 at median 0.8583 min 0.00 max 38.06 copies/μg of feces and after that they were decrease in wash-out periods (week 7-week 6) at median-0.1864 min-8.38 max 1.20 copies/μg of feces.

In addition to *Lactobacillus* level, the fecal *Lactobacillus* increased in both Kaentawan and mixture powder of inulin and FOS groups. In Kaentawan group, the difference at week1-week 0 was reported as median at 9.4993 min max 75.59 copies/μg of feces, week 2-week 0 as median at 14.4068 min-35.09 max 190.55 copies/μg of feces, week 4-week 0 at median 30.8998 min-35.09 max 264.25 copies/μg of feces, week 6-week 0 at median 41.6318 min-

Table 2. Fecal *Bifidobacterium* and *Lactobacillus* in subjects from 3 groups, using real-time PCR

Bacteria	Groups	The fecal <i>Bifidobacterium</i> and <i>Lactobacillus</i> (DNA copies / μg of feces)						
		n	Week 0	Week 1	Week 2	Week 4	Week 6	Wash-out period
<i>Bifidobacterium</i>	Control	30	Med = 0.5089 Min = 0.0027 Max = 7.1365	Med = 0.1761 Min = 0.0019 Max = 5.9273	Med = 0.3896 Min = 0.0263 Max = 3.8996	Med = 0.4565 Min = 0.0083 Max = 3.8927	Med = 0.2508 Min = 0.0081 Max = 3.1359	Med = 0.3883 Min = 0.0281 Max = 3.1359
			Med = 0.0283 Min = 0.0001 Max = 1.1623	Med = 0.0559 Min = 0.0004 Max = 2.1896	Med = 0.0812 Min = 0.0006 Max = 8.5546	Med = 0.1628 Min = 0.0191 Max = 12.0273	Med = 0.4013 Min = 0.0151 Max = 29.2227	Med = 0.1659 Min = 0.0001 Max = 24.3364
			Med = 0.0931 Min = 0.0029 Max = 1.0286	Med = 0.1704 Min = 0.0311 Max = 1.8850	Med = 0.4977 Min = 0.0349 Max = 20.8409	Med = 0.6850 Min = 0.0239 Max = 29.2227	Med = 0.9750 Min = 0.0194 Max = 38.2318	Med = 0.6191 Min = 0.0029 Max = 31.5182
	Kaentawan	26	Med = 7.3909 Min = 0.0027 Max = 83.0455	Med = 5.7750 Min = 0.0837 Max = 186.4091	Med = 4.4361 Min = 0.0317 Max = 127.5909	Med = 3.9261 Min = 0.0840 Max = 266.6818	Med = 3.0348 Min = 0.0016 Max = 230.2273	Med = 6.8932 Min = 0.0711 Max = 171.0455
			Med = 3.4659 Min = 0.2472 Max = 38.0136	Med = 13.3896 Min = 0.6691 Max = 97.3182	Med = 27.6090 Min = 0.0125 Max = 212.2727	Med = 38.0227 Min = 0.0019 Max = 297.6364	Med = 47.5705 Min = 0.4823 Max = 1598.1818	Med = 19.8205 Min = 0.0013 Max = 2200.0000
			Med = 2.3704 Min = 0.0019 Max = 31.5182	Med = 6.8227 Min = 0.0739 Max = 297.6364	Med = 8.9546 Min = 0.2561 Max = 216.4091	Med = 15.7318 Min = 0.0048 Max = 116.3636	Med = 38.4455 Min = 0.3744 Max = 596.3636	Med = 24.5455 Min = 0.1815 Max = 297.6364
	Inulin&FOS	25	Med = 7.3909 Min = 0.0027 Max = 83.0455	Med = 5.7750 Min = 0.0837 Max = 186.4091	Med = 4.4361 Min = 0.0317 Max = 127.5909	Med = 3.9261 Min = 0.0840 Max = 266.6818	Med = 3.0348 Min = 0.0016 Max = 230.2273	Med = 6.8932 Min = 0.0711 Max = 171.0455
			Med = 3.4659 Min = 0.2472 Max = 38.0136	Med = 13.3896 Min = 0.6691 Max = 97.3182	Med = 27.6090 Min = 0.0125 Max = 212.2727	Med = 38.0227 Min = 0.0019 Max = 297.6364	Med = 47.5705 Min = 0.4823 Max = 1598.1818	Med = 19.8205 Min = 0.0013 Max = 2200.0000
			Med = 2.3704 Min = 0.0019 Max = 31.5182	Med = 6.8227 Min = 0.0739 Max = 297.6364	Med = 8.9546 Min = 0.2561 Max = 216.4091	Med = 15.7318 Min = 0.0048 Max = 116.3636	Med = 38.4455 Min = 0.3744 Max = 596.3636	Med = 24.5455 Min = 0.1815 Max = 297.6364

Note: FOS = Fructo-oligosaccharide, n = number of subject, Med = Median value of fecal *Bifidobacterium* and *Lactobacillus* (DNA copies/μg of feces), Min = Minimum value of fecal *Bifidobacterium* and *Lactobacillus* (DNA copies/μg of feces), Max = Maximum value of fecal *Bifidobacterium* and *Lactobacillus* (DNA copies/μg of feces)

17.75 max 1595.99 copies/ μ g of feces, after that they decreased in wash-out periods (week 7-week 6) at median-14.2682 min-1598.18 max 2100.00 copies/ μ g of feces. In mixture of inulin and FOS group, the difference at week1-week 0 was reported as median at 3.3773 min -6.60 max 291.20 copies/ μ g of feces, week 2-week 0 at median 5.7478 min-7.32 max 184.89 copies/ μ g of feces, week 4-week 0 at median 13.1098 min-7.47 max 122.67 copies/ μ g of

feces, week 6-week 0 at median 38.0210 min-25.60 max 596.26 copies/ μ g of feces and after that they were decrease in wash-out periods (week 7-week 6) at median-19.1091 min-298.73 max 284.26 copies/ μ g of feces. (Table 3).

In Table 4, all data from Table 3 on the difference of fecal *Bifidobacterium* and *Lactobacillus* levels during intervention period to baseline (week 1-week 0, week 2-week 0, week 4-week 0 and week 6-week 0) and wash-

Table 3. The difference of fecal *Bifidobacterium* and *Lactobacillus* in subjects of 3 groups using real-time PCR

Bacteria	Groups	The different of fecal <i>Bifidobacterium</i> and <i>Lactobacillus</i> in feces (DNA copies / μ g of feces)					
		n	Week 1 – Week 0	Week 2 – Week 0	Week 4 – Week 0	Week 6 – Week 0	Week 7 – Week 6
<i>Bifidobacterium</i> spp.	Control	30	Med = - 0.0025	Med = 0.0095	Med = - 0.0064	Med = 0.0011	Med = 0.0485
			Min = - 7.10	Min = - 7.11	Min = - 6.73	Min = - 7.02	Min = - 1.98
			Max= 1.40	Max= 1.75	Max= 1.62	Max= 1.32	Max= 0.79
	Kaentawan	26	Med = 0.0116	Med = 0.0197	Med = 0.0905	Med = 0.1637	Med = - 0.1413
			Min = - 0.72	Min = - 0.87	Min = - 0.17	Min = - 0.09	Min = - 4.89
			Max= 1.03	Max= 7.39	Max= 10.87	Max= 28.06	Max= 0.13
	Inulin and FOS	25	Med = 0.0684	Med = 0.4100	Med = 0.5973	Med = 0.8583	Med = - 0.1864
			Min = 0.01	Min = 0.02	Min = - 0.02	Min = 0.00	Min = - 8.38
			Max= 1.38	Max= 20.75	Max= 29.13	Max= 38.06	Max= 1.20
<i>Lactobacillus</i> spp.	control	30	Med = 0.2581	Med = 0.0238	Med = 0.6330	Med = - 0.3782	Med = 0.1490
			Min = - 48.34	Min = - 48.84	Min = - 56.24	Min = - 51.83	Min = - 207.09
			Max = 152.28	Max = 127.08	Max = 253.04	Max = 216.58	Max = 112.10
	Kaentawan	26	Med = 9.4993	Med = 14.4068	Med = 30.8998	Med = 41.6318	Med = - 14.2682
			Min = - 26.95	Min = - 35.09	Min = - 35.09	Min = - 17.75	Min = - 1598.18
			Max = 75.59	Max = 190.55	Max = 264.25	Max = 1595.99	Max = 2100.00
	Inulin and FOS	25	Med = 3.3773	Med = 5.7478	Med = 13.1098	Med = 38.0210	Med = - 19.1091
			Min = - 6.60	Min = - 7.32	Min = - 7.47	Min = - 25.60	Min = - 298.73
			Max = 291.20	Max = 184.89	Max = 122.67	Max = 596.26	Max = 284.26

Note: FOS = Fructo-oligosaccharide, Note, n = number of subject, Med = Median value of fecal *Bifidobacterium* and *Lactobacillus* (DNA copies/ μ g of feces), Min = Minimum value of fecal *Bifidobacterium* and *Lactobacillus* (DNA copies/ μ g of feces), Max = Maximum value of fecal *Bifidobacterium* and *Lactobacillus* (DNA copies/ μ g of feces)

Table 4. The difference of fecal *Bifidobacterium* and *Lactobacillus* in subjects o 3 groups using real-time PCR

Bacteria	Groups	The different of fecal <i>Bifidobacterium</i> and <i>Lactobacillus</i> in feces (DNA copies / μ g of feces)					
		n	Week 1 – Week 0	Week 2 – Week 0	Week 4 – Week 0	Week 6 – Week 0	Week 7 – Week 6
<i>Bifidobacterium</i>	control	30	Med = - 0.0025	Med = 0.0095	Med = - 0.0064	Med = 0.0011	Med = 0.0485
			Min = - 7.10	Min = - 7.11	Min = - 6.73	Min = - 7.02	Min = - 1.98
			Max = 1.40	Max = 1.75	Max = 1.62	Max = 1.32	Max = 0.79
	Kaentawan	26	Med = 0.0116	Med = 0.0197	Med = 0.0905	Med = 0.1637	Med = - 0.1413
			Min = - 0.72	Min = - 0.87	Min = - 0.17	Min = - 0.09	Min = - 4.89
			Max = 1.03	Max = 7.39	Max = 10.87	Max = 28.06	Max = 0.13
	Inulin and FOS	25	Med = 0.0684	Med = 0.4100	Med = 0.5973	Med = 0.8583	Med = - 0.1864
			Min = 0.01	Min = 0.02	Min = - 0.02	Min = 0.00	Min = - 8.38
			Max = 1.38	Max = 20.75	Max = 29.13	Max = 38.06	Max = 1.20
	P-value		<0.05	<0.05	<0.05	<0.05	<0.05
<i>Lactobacillus</i>	control	30	Med = 0.2581	Med = 0.0238	Med = 0.6330	Med = - 0.3782	Med = 0.1490
			Min = - 48.34	Min = - 48.84	Min = - 56.24	Min = - 51.83	Min = - 207.09
			Max = 152.28	Max = 127.08	Max = 253.04	Max = 216.58	Max = 112.10
	Kaentawan	26	Med = 9.4993	Med = 14.4068	Med = 30.8998	Med = 41.6318	Med = - 14.2682
			Min = - 26.95	Min = - 35.09	Min = - 35.09	Min = - 17.75	Min = - 1598.18
			Max = 75.59	Max = 190.55	Max = 264.25	Max = 1595.99	Max = 2100.00
	Inulin and FOS	25	Med = 3.3773	Med = 5.7478	Med = 13.1098	Med = 38.0210	Med = - 19.1091
			Min = -6.60	Min = -7.32	Min = -7.47	Min = - 25.60	Min = - 298.73
			Max = 291.20	Max = 184.89	Max = 122.67	Max = 596.26	Max = 284.26
	P-value		0.08	<0.05	<0.05	<0.05	<0.05

P-value using Kruskal Wallis H <0.05, when compared between 3 group. Note: FOS = Fructo-oligosaccharide, n = number of subject, Med = Median value of fecal *Bifidobacterium* and *Lactobacillus* (DNA copies/ μ g of feces), Min = Minimum value of fecal *Bifidobacterium* and *Lactobacillus* (DNA copies/ μ g of feces), Max = Maximum value of fecal *Bifidobacterium* and *Lactobacillus* (DNA copies/ μ g of feces)

out period (week7-week 6), among three groups (control, Kaentawan and Inulin and FOS groups) were compared and analyzed using Kruskal Wallis H test to find the statistically significant difference between, at least, one pair of the groups (p -value<0.05). All the difference of fecal *Bifidobacterium* and *Lactobacillus* levels (respectively) at each period among three groups were found to be statistically significant different (p -value <0.05) except the difference of fecal *Lactobacillus* levels at week1-week 0 (p -value at 0.08). (Table 4). This analyzed result showed that there may be

at least one pair from these three groups that has statistically significant difference of fecal *Bifidobacterium* and *Lactobacillus* levels. However, the statistically significant difference between which pair of these three group (control, Kaentawan and Inulin and FOS groups) are still not exactly known. Thereby, this needs to be further analyzed using Man Whitney U test.

Table 5 uses Mann Whitney U test, to compare the difference of fecal *Bifidobacterium* and *Lactobacillus* levels between two groups. First, between control and Kaentawan groups,

Table 5. The difference of fecal Bifidobacterium and Lactobacillus between control and Kaentawan groups using real-time PCR

Bacteria	Groups	The difference of fecal <i>Bifidobacterium</i> and <i>Lactobacillus</i> in feces (DNA copies / μ g of feces)					
		n	Week 1 – Week 0	Week 2 –Week 0	Week 4–Week 0	Week 6 –Week 0	Week 7–Week 6
<i>Bifidobacterium</i>	control	30	Med = - 0.0025 Min = - 7.10 Max = 1.40	Med = 0.0095 Min = - 7.11 Max = 1.75	Med = - 0.0064 Min = - 6.73 Max = 1.62	Med = 0.0011 Min = - 7.02 Max = 1.32	Med = 0.0485 Min = - 1.98 Max = 0.79
	Kaentawan	26	Med = 0.0116 Min = - 0.72 Max = 1.03	Med = 0.0197 Min = - 0.87 Max = 7.39	Med = 0.0905 Min = -0.17 Max= 10.87	Med = 0.1637 Min = - 0.09 Max = 28.06	Med = - 0.1413 Min = - 4.89 Max = 0.13
	P-value		0.237	0.308	<0.05	<0.05	<0.05
	control	30	Med = 0.2581 Min = - 48.34 Max = 152.28	Med = 0.0238 Min = - 48.84 Max = 127.08	Med = 0.6330 Min = - 56.24 Max= 253.04	Med = - 0.3782 Min = -51.83 Max= 216.58	Med = 0.1490 Min = -207.09 Max= 112.10
	Kaentawan	26	Med = 9.4993 Min = -26.95 Max= 75.59	Med = 14.4068 Min = -35.09 Max= 190.55	Med = 30.8998 Min = -35.09 Max= 264.25	Med = 41.6318 Min = -17.75 Max= 1595.99	Med = -14.2682 Min = -1598.18 Max= 2100.00
	P-value		0.068	<0.05	<0.05	<0.05	<0.05

P-value using Mann Whitney U<0.05, when compared between control group with Kaentawan group, Note: FOS = Fructo-oligosaccharide, n = number of subject, Med = Median value of fecal *Bifidobacterium* and *Lactobacillus* (DNA copies/ μ g of feces), Min = Minimum value of fecal *Bifidobacterium* and *Lactobacillus* (DNA copies/ μ g of feces), Max = Maximum value of fecal *Bifidobacterium* and *Lactobacillus* (DNA copies/ μ g of feces)

Table 6. The different of fecal Bifidobacterium and Lactobacillus in subjects between control with mixture powder of inulin and FOS consumed group by the real-time PCR

Bacteria	Groups	The different of fecal <i>Bifidobacterium</i> and <i>Lactobacillus</i> in feces (DNA copies/ μ g of feces)					
		n	Week 1 – Week 0	Week 2 –Week 0	Week 4–Week 0	Week 6 –Week 0	Week 7–Week 6
<i>Bifidobacterium</i>	control	30	Med = - 0.0025 Min = - 7.10 Max = 1.40	Med = 0.0095 Min = - 7.11 Max = 1.75	Med = -0.0064 Min = - 6.73 Max = 1.62	Med = 0.0011 Min = - 7.02 Max = 1.32	Med = 0.0485 Min = - 1.98 Max = 0.79
	inulin&FOS	25	Med = 0.0684 Min = 0.01 Max= 1.38	Med = 0.4100 Min = 0.02 Max = 20.75	Med = 0.5973 Min = - 0.02 Max= 29.13	Med = 0.8583 Min = 0.0080 Max= 38.06	Med = -0.1864 Min = -8.38 Max= 1.20
	P-value		<0.05	<0.05	<0.05	<0.05	<0.05
	control	30	Med = 0.2581 Min = -48.34 Max= 152.28	Med = 0.0238 Min = -48.84 Max= 127.08	Med = 0.6330 Min = - 56.24 Max= 253.04	Med = -0.3782 Min = -51.83 Max= 216.58	Med = 0.1490 Min = -207.09 Max= 112.10
	inulin&FOS	25	Med = 3.3773 Min = -6.60 Max= 291.20	Med = 5.7478 Min = -7.32 Max= 184.89	Med = 13.1098 Min = -7.47 Max=122.67	Med = 38.0210 Min = -25.60 Max= 596.26	Med = -19.1091 Min = -298.73 Max= 284.26
	P-value		0.06	<0.05	<0.05	<0.05	<0.05

P-value using Mann Whitney U<0.05, when compared between controlled group with mixture powder of inulin and FOS consumed group, Note: FOS = Fructo-oligosaccharide, n = number of subject, Med = Median value of fecal *Bifidobacterium* and *Lactobacillus* (DNA copies/ μ g of feces), Min = Minimum value of fecal *Bifidobacterium* and *Lactobacillus* (DNA copies μ g of feces), Max = Maximum value of fecal *Bifidobacterium* and *Lactobacillus* (DNA copies/ μ g of feces)

the fecal *Bifidobacterium* levels of Kaentawan groups were statistically and significantly increased at week 4, at the end of intervention period (week 6) when compared with baseline (week 0) and wash out period (week 7) when compared with at the end of intervention period (week 6), whereas *Lactobacillus* levels of Kaentawan groups were significantly increased at week 2, week 4, week 6 when compared with baseline (week 0) and wash out period (week 7) when compared with at the end of intervention period (week 6) (p-value<0.05) (Table 5).

As shown in Table 6, statistically significant difference was found in fecal *Bifidobacterium* levels between control and the mixture of inulin and FOS groups (p-value <0.05) at week 1, week 2, week 4, week 6, when compared with baseline (week 0) and wash out period (week 7) when compared with at the end of intervention period (week 6) while fecal *Lactobacillus* levels was found significantly different at week 2, week 4, week 6 when compared with baseline (week 0) and wash out period (week 7) when compared with at the end of intervention period (week 6). The difference of fecal *Bifidobacterium* and *Lactobacillus* levels between Kaentawan and mixture of inulin and FOS consumed groups, was significantly different only in fecal *Bifidobacterium* at week 1, week 2, week 4

when compared with baseline (week 0). However, there was no significant change in the difference of fecal *Bifidobacterium* levels between two groups in wash-out periods (week 7) when compared with the end of intervention period (week 6). The fecal *Lactobacillus* was not significantly different between Kaentawan and mixture powder of inulin and FOS consumed groups, as shown in Table 7.

Discussion

Eighty-one subjects completed the study with a very strict compliance to background diet and restrictions in the consumption of prebiotics and probiotic foods. No extremities in medication or adverse events were observed. Only one subject reported diarrhea, but this subject was not excluded from the control treatment group.

The result showed that, consumption of Kaentawan by healthy adult people modified their fecal *Bifidobacterium* and *Lactobacillus* numbers the same as mixture powder of inulin and FOS. It was clear that the bacteria were significantly increased when compared with the control treatment group. This observation was consistent with the previous studies (Kleessen *et al.*, 2001; Kleessen *et al.*, 2007; Ramnani *et al.*, 2010) However, the bacteria

Table 7. The different of fecal *Bifidobacterium* and *Lactobacillus* in subjects between Kaentawan and mixture of inulin and FOS consumed group by the real-time

Bacteria	Groups	The different of fecal <i>Bifidobacterium</i> and <i>Lactobacillus</i> in feces (DNA copies/μg of feces)						
		n	Week 1–Week 0	Week 2–Week 0	Week 4–Week 0	Week6–Week 0	Week 7–Week 6	
<i>Bifidobacterium</i>	Kaentawan	26	Med = 0.0116 Min = -0.72 Max=1.03	Med = 0.0197 Min = -0.87 Max= 7.39	Med = 0.0905 Min = -0.17 Max= 10.87	Med = 0.1637 Min = -0.09 Max= 28.06	Med = -0.1413 Min = -4.89 Max= 0.13	
		inulin&FOS	25	Med = 0.0684 Min = 0.01 Max= 1.38	Med = 0.4100 Min = 0.02 Max= 20.75	Med = 0.5973 Min = -0.02 Max= 29.13	Med = 0.8583 Min = 0.00 Max= 38.06	Med =-0.1864 Min = -8.38 Max= 1.20
			P-value	<0.05	<0.05	<0.05	0.059	0.34
	<i>Lactobacillus</i>	Kaentawan	26	Med = 9.4993 Min = -26.95 Max= 75.59	Med = 14.4068 Min = -35.09 Max= 190.55	Med = 30.8998 Min = -35.09 Max= 264.25	Med = 41.6318 Min = -17.75 Max= 1595.99	Med = -14.2682 Min = -1598.18 Max= 2100.00
			inulin&FOS	25	Med = 3.3773 Min = -6.60 Max= 291.20	Med = 5.7478 Min = -7.32 Max= 184.89	Med = 13.1098 Min = -7.47 Max=122.67	Med = 38.0210 Min = -25.60 Max= 596.26
P-value				0.34	0.61	0.20	0.57	0.98

P-value using Mann Whitney U<0.05, when compared between Kaentawan consumed group with mixture of inulin and FOS consumed group, Note: FOS = Fructo – oligosaccharide, n = number of subject, Med = Median value of fecal *Bifidobacterium* and *Lactobacillus* (DNA copies/ μ g of feces), Min = Minimum value of fecal *Bifidobacterium* and *Lactobacillus* (DNA copies/ μ g of feces), Max = Maximum value of fecal *Bifidobacterium* and *Lactobacillus* (DNA copies/ μ g)

numbers decreased twice during the study period, after treatment stopped (wash-out period), indicating that the bacteria not only survived transit of the gastrointestinal tract, but they persisted in the colonic ecosystem. This was not consistent with previous reports, the bacteria numbers returned to original baseline levels at the end of wash-out period (Gibson *et al.*, 1995; Tuohy *et al.*, 2001; Kruse *et al.*, 2005). This evidence demonstrated that long-term persistence in the gut of *Bifidobacterium* and *Lactobacillus* could occur, but it may depend on the participant's metabolism as previous description (Fujiwara *et al.*, 2001). Although, the bacteria were found in fecal samples after treatment stopped, it was in decline. Further study is needed to unravel the exact mechanism underlying the decreasing of bacteria in the time following dietary intervention.

It is noteworthy that the increasing number of *Bifidobacterium* and *Lactobacillus* in the present study was paralleled by a decrease of hard fecal consistency and an increase of flatulence, nausea and vomiting. Therefore, the decreasing rate of bacteria after treatment has ceased should be further observation to apply it for inulin or Kaentawan usage to avoid side effect such as flatulence, nausea and vomiting as found in several studies including the present study.

Moreover, this study revealed that, the number of *Bifidobacterium* population in feces of healthy adult people was very few compared with the number of *Lactobacillus* population. The reduction in numbers of *Bifidobacterium* in the human intestine was observed with aging, whereas there were increased numbers of the other bacteria. It is widely known that fecal microbiota in bowel disease generally comprise a decreasing number or disappearance of *Bifidobacterium* (Arbolea *et al.*, 2016; Davis and Milner, 2009; Kleessen *et al.*, 2007). This indicated that *Lactobacillus* is common among this population and only *Bifidobacterium* could not be an indicator for follow up the bowel diseases as seen in this result, although the number of *Bifidobacterium* was very low and there were no reported cases of serious intestinal discomfort in this study.

In summary, the result indicates that a high-dose long-term application of 85 g of Kaentawan (approximately contained 10 g inulin and 5 g FOS) is practicable. Alteration of the colonic microbiota in favor of *Bifidobacterium* and *Lactobacillus* was a clear effect of Kaentawan administration. Since the majority of subjects in the study did not feel significantly affected by symptoms of discomfort, Kaentawan could be a useful imported inulin and FOS replacer. Future studies have to find out whether higher dose of Kaentawan administration may influence long-term persistence of *Bifidobacterium* and *Lactobacillus* in the human gut.

Conclusions

The study has found that both the consumption of normal diet plus 85 g/day of Kaentawan and the consumption of a mixture of inulin and FOS can increase the concentration of *Bifidobacterium* spp. and *Lactobacillus* spp. in the subjects' colon. Notably, the increased concentration is significantly different from that of subjects in the control group, who consumed only normal diet. Even though there is no significant difference of the concentration of *Bifidobacterium* spp. and *Lactobacillus* spp. among subjects in Kaentawan group and mixture of inulin and FOS group, the concentration of *Bifidobacterium* spp. and *Lactobacillus* spp. in Kaentawan group is slightly higher. This shows that Kaentawan can be recommended as an alternative prebiotic to inulin and FOS. Kaentawan is not only cheaper and easily grown, but also more favorable among people and easy to consume. Therefore, the study demonstrates that Kaentawan can be used as food supplement and an alternative crop for the health promotion in Thai population.

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