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Original Article

POTENTIAL EFFECTS OF DUKU (*LANSIUM DOMESTICUM CORR*) AND LANGSAT (*LANSIUM DOMESTICUM JACK)* EXTRACTS ON THE GROWTH OF *BIFIDOBACTERIA* SPP.

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ABSTRACT

Objective: *Lansium domesticum Corr*. is a fruit tree species belongs to the family *Meliaceae*. There are numerous forms of the species and grouped into two main types: Duku and Langsat. The objective of this study is to screen the ability of adding extracts of freeze-dried duku and langsat to stimulate the growth and stability of selected *Bifidobacteria* spp in skimmed milk.

Methods: Samples were prepared by adding either 5% or 12% of oligosaccharides from duku, langsat, inulin, galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS) to 5% and 12% (w/v) reconstituted nonfat dry milk (NDM), respectively. The specific growth rates (μ) for each sample were calculated. All experiments were replicated ten times.

Results: The mean doubling time (Td) for *Bifidobacterium longum*, was lowest in the presence of freeze-dried duku and langsat compared to GOS, FOS and inulin. Retention of the viability of five *Bifidobacterium* species was greatest in the presence of freeze-dried duku and langsat followed by GOS, FOS and inulin. The highest percentage of acetic and lactic acids were produced by *B. longum*, *B. infantis* and *B. adolescentis* with freeze-dried duku and langsat. The pattern of results was similar to the commercial product, oligosaccharides (inulin, GOS and FOS).

Conclusion: Therefore, this study provides promising results on promoting growth and probiotic activity of natural oligosaccharides compound from freeze-dried duku and langsat.

Keywords: Duku (*Lansium Domesticum Corr*), Langsat *(Lansium Domesticum Jack), Bifidobacteria* Spp

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INTRODUCTION

In current years, local species of plant and fruit tree have been scientifically studied for their potential medicinal applications [1]. *Lansium domesticum Corr.* is a fruit tree species derived from the family of Meliaceae. This popular tropical fruit found mainly in South-East Asia, especially in the Philippines, where it is known as Lanzones, and South Sumatra in Indonesia [2]. It also grows in Surinam, Puerto Rico and Australia [3]. Although many species have been ascribed to the genus Lansium, Mabberley [4] recognized only three species, *L. membranaceum Kosterm*, *L. domesticum Correa. agg.* and *L. breviracemosum Kosterm*. In Peninsular Malaysia, the genus is represented by only one species, *L. domesticum* [4, 5]. There are numerous forms of the species, and these belong to four main types: Dokong, Duku, Langsat and Duku-langsat. There is a fairly clear distinction between the two major types of L. *domesticum*: Langsat and Duku. Langsat fruits are oblong, thin-skinned and possess latex, while Duku fruits are round, thick-skinned and latex-free. The Duku-langsat (or Duku Terengganu) is the intermediate type, generally regarded as superior to both Duku and Langsat [5]. Duku, Langsat and Duku-langsat are natives of Peninsular Malaysia while Dokong originates from southern Thailand, and has been cultivated in Peninsular Malaysia for more than 10 y. Langsat can be found throughout the peninsular, but predominates in the north, while Duku occurs in the southern region [6]. Duku-langsat is a popular fruit tree on the east coast and is mainly cultivated in the states of Terengganu and Kelantan.

Duku and langsat raw fruit are green in color, and the flavor is very sour gummy. As the fruit matures, the skin will turn into yellow and the flesh of the fruit will taste sweet. Most of the fruit is eaten fresh as Duku and Langsat flesh only. The nutritional composition of duku and langsat has reported that in every 100 grams of duku and langsat contained 70-74 calories, 1.0-1.5 g protein, 0.2-0.5 g fat, 13-15 g carbohydrates, 0.7-1.0 g minerals, 18-20 mg calcium, 9-11 g phosphorus and 0.9-1.5 mg of iron. For the caloric content, it shows that the level of mineral and iron found in duku and langsat are higher than imported fruits like apples and oranges [4]. Duku and langsat fruits are relatively high in dietary fiber, which gives great benefits for the digestive system in preventing cancer of the colon and act to cleanse the body from cancer-causing free radicals. In addition to healthy fresh fruit, the fruit peel and seeds are also useful for anti-diarrhea medicine raw materials as well as to reduce fever. Traditionally, the barks of duku and langsat tree are often used to treat venomous insect bites, dysentery medicine and eradicate cancer cells [7].

Bifidobacteria is considered to be one of the most important genera of bacteria in terms of human health. They account nearly 85 to 99% of the intestinal flora in infants [7, 8]. All species derived from human are non-spore forming, non-motile, anaerobic Gram-positive bacteria. In a healthy adult, *Bifidobacteria* constitutes third to the fourth largest group of microflora in the lower gastrointestinal tract, while Coliforms, *clostridia* and *lactobacilli* normally account for less than 15% of the intestinal flora [8]. Recently, there has been an increasing interest in the incorporation of the intestinal species *Lactobacillus acidophilus* and *Bifidobacterium* species into fermented milk products [9]. *Lactobacilli* colonizing in the human body such as oral cavity and gastrointestinal tract; in which fruits and fermented foods are the main sources of probiotics [10]. These species are frequently associated with health promoting effects in human and animal intestinal tract. These probiotic effects are generally related

to inhibition of pathogenic species, reducing the risk of colon cancer, increasing the immune response and decreasing the concentration of cholesterol in blood plasma [11]. *Bifidobacteria* is not true lactic acid bacteria in the sense of a *Lactococcus* or *pediococcus* [12]. *Bifidobacteria* produce both acetic and lactic acids as primary metabolites in the molar ratio of 3:2 [13]. Glucose is degraded characteristically by the fructose 6-phosphate shunt metabolic pathway [14].

Furthermore, milk is one of the best sources of nutrients for child growth. The previous study showed that *Bifidobacterium spp.* can be isolated mostly from the feces of infant milk feed the baby. These gut floras have to digest the milk-based food and compromise the major line of defense against the pathogenic bacteria [15]. The addition of fruit extracts may enhance the growth of *Bifidobacteria* by providing essential nutrients and further improve the sensory quality of the products since the flavor of bifido culture in milk is not favorable to test and provide consumers with certain nutrients especially minerals and energy at the same time. Therefore, the objectives of the study were to screen the ability to add extracts of freeze-dried duku and langsat to stimulate the growth and stability of selected *Bifidobacteria* Spp in skimmed milk.

MATERIALS AND METHODS

Fruits

Commercially matured Duku (*Lansium Domesticum Corr*) and Langsat (*Lansium Domesticum Jack)* fruits were obtained from a local plantation in Terengganu, Malaysia within a week of harvest. They were cleaned and peeled manually prior to extraction. Exposure to light was consciously avoided to reduce possible losses of nutrient.

Sample extraction procedure

The methods introduced by Xiaoli *et al.* [16] were used during the extraction procedure. The fruits were carefully washed under running tap water, dried with a soft cloth and the skin was carefully peeled. Lipid was removed from the sample (500 g) using petroleum ether (boiling range temperature of 37-55 °C). The optimal conditions selected for the extraction of oligosaccharides in duku and langsat fruits were carried out as follows: Exactly 1.0 g of fruit sample were extracted 3 times with 10 ml/50% ethanol-water, at a ratio of 10:1 (solvent to fruit extracts) in a water bath at a temperature of 50 °C for 60 min. After each extraction, the samples were centrifuged at 2500 g for 20 min. Supernatants from the three cycles of extraction were combined and concentrated by using a rotary vacuum evaporator (Heidolph, Germany) then freeze-dried.

Bifidobacterial strain

Lyophilized *B. longum* (ATCC 15707) and *B. breve* (ATCC 15700) were purchased from American Type Culture Collection (Rockville, MD). Forty-eight hours prior to the start of each experiment, cultures were revived by a series of two inoculations into 10 ml of MRSL [MRS Broth added with 5% Lactose [12] and incubated at 37 °C for 48 h in an anaerobic jar (Merck, Germany) with Anaerocult® A (Merck, Germany) as an anaerobic reagent to generate an anaerobic medium. After 48 h, the turbidity of the MRSL broth was measured at 640 NM using a UV-Vis spectrophotometer (Secoman, France). The bifidostrain is considered healthy and ready to be used as inoculate prebiotic bacteria if the turbidity of MRSL broth absorbance reading value shows more than 0.50 nm.

Bifidobacterial growth study evaluation

The combination method proposed by Ustunol and Gandi [17] and Hughes and Hoover [12] was used for this purpose. Samples were prepared by adding either 5% or 12% of oligosaccharides from duku, langsat, inulin, GOS and FOS to 5% and 12% (w/v) reconstituted NDM, respectively. A control of NDM without oligosaccharides was also prepared. The samples were pasteurized at 70 °C for 30 min and cooled in the ice bath to 37 °C within 4 min. Each sample was divided into 2 portions and inoculated at the 5% level with *B. longum* (ATCC 15707) and *B. breve* (ATCC 15700) propagated in MRS medium with 5% lactose. The inoculated milk sample was incubated at 37 °C for 60 h. A total of 1 ml of sample was taken every 12 h and diluted (1:10, v/v) with 0.2% EDTA (pH 12) and turbidity was measured at 640 nm. Non-inoculated NDM was used as a control. The specific growth rates (μ) for each sample were calculated according to the following equation introduced by Hughes and Hoover [12].

$$\mu = \frac{(\text{Ln } X_2 - \text{Ln } X_1)}{(t_2 - t_1)}$$

Where X_2 and X_1 are the cell densities at t_2 and t_1, respectively.

Mean doubling time (Td) for *Bifidobacteria* was calculated as follows:

$$\text{Td} = \frac{\text{Ln}2}{\mu}$$

All experiments were replicated ten times.

Determination of *Bifidobacterial* activity

The activities of each culture in the presence of different prebiotics were determined by measuring the products of fermentation (lactic and acetic acids) using high-performance liquid chromatography (Shimadzu HPLC system: Shimadzu, Japan). Samples were prepared by adding either 5% or 12% of oligosaccharides from duku, langsat, inulin, GOS and FOS, respectively. An NDM control without oligosaccharides was also prepared. Samples were pasteurized at 70 °C for 30 min and cooled to 37 °C within 4 min. Each sample was divided into five portions and inoculated at a 5% level of *B. longum* and *B. breve* propagated in MRS medium with 5% lactose. Inoculated milk samples were incubated at 37 °C for 60 h. A total of 100 μl 15.8 M HNO_3 and 14.9 ml of 0.009 M H_2SO_4 were added to 1.5 ml of the sample and centrifuged at 4000 x g for 10 min using bench top centrifuge (Sigma-Aldrich, USA). The supernatant was filtered using 0.22 mm Millipore filters (Whatman, USA) and 2 ml aliquots was stored in HPLC vials at a temperature of-20 °C until analyzed.

The methods introduced by Ustunol and Gandi [17] were used for HPLC procedure with modification. The HPLC system consists of a pump and a 10A refraction index detector. A LiChroCART® 250-4 LiChrosper® NH2, 5 μm column and a guard column with disposable cartridges (Merck, Germany) maintained at 65 °C for organic acids to be quantified. The standard solutions or organic acids (lactic and acetic acids; Sigma, St Louis, MO., USA) were prepared with water (HPLC grade) to establish election times and calibration curves. The retention time for lactic acid and acetic acid were ≈ 25.7 min and ≈ 16.2 min respectively. All experiments were replicated ten times.

Statistical analysis

Data were express as mean±standard deviation (SD) in 10 replicates. Statistical analysis was performed with a single factor and One-way ANOVA to identify the significant difference based on the effects of oligosaccharides from duku and langsat on the growth, viability, and activity of *B. longum* and *B. breve.*

RESULTS AND DISCUSSION

Clinical studies have associated other beneficial effects such as immune enhancement and anti-carcinogenicity with the presence of *Bifidobacteria* in the gastrointestinal tract. One approach for ensuring or increasing the presence of healthful colonic bacteria is to utilize them as a prebiotic. A prebiotic is a live microbial food supplement, which beneficially affects the host organism by improving its intestinal microbial balance [14]. Another approach used for increasing the numbers of *Bifidobacteria* in the gastrointestinal tract is the incorporation of prebiotics in the diet. A prebiotic is a non-digestible dietary supplement that modifies the balance of the intestinal microflora stimulating the growth and activity of beneficial organisms and suppressing potentially deleterious bacteria [17]. However, not all dietary fibers are prebiotics, and certain criteria need to be recognized before sorting dietary carbohydrate as prebiotics [18]. Thus, it is important to select appropriate prebiotics to improve retention viability of *Bifidobacteria* in dairy food with an ultimate goal of delivering a large number of viable *Bifidobacteria* and stimulating *Bifidobacteria* growth in the colon.

Bifidobacterial growth study evaluation

In general, the mean doubling time was used to measure the efficacy of various carbon sources in modulating the growth rate of lactic acid bacteria known as probiotics. The growth of *B. longum* in skimmed milk was significantly stimulated by increasing the concentration of prebiotic extracts after being incubated anaerobically at the temperature of 37 °C for 72 h as shown in fig. 1. The growth promotion of *B. longum* by prebiotics of duku, langsat, inulin, GOS and FOS was obtained over the range either at 5% or 12% as evidenced by decreased mean doubling time with increased concentration of prebiotics indicating that *B. longum* strains grew faster in the presence of these carbohydrates compared to the controls.

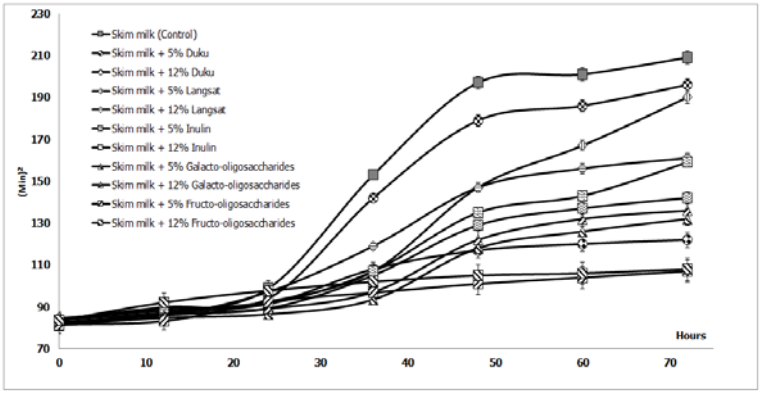

Fig. 1: Doubling time of *B. longum* in skimmed milk containing prebiotics

Among the carbohydrate sources tested, a 12% supplementation of FOS, duku and GOS showed to be most effective in enhancing the growth rate of *B. longum* in skimmed milk. The mean doubling time of *B. longum* after 72 h of incubation was significantly reduced ($p<0.05$) compared to the controls. The addition of 12% FOS significantly decreased ($p<0.05$) the doubling time value as compared to the other samples. The supplementation of 12% GOS and duku also decreased the doubling time value after 72 h of fermentation. The supplementation of 5% and 12% oligosaccharides extracted from duku also helped in stimulating the growth of *B. longum* when added to skimmed milk. It showed a similar pattern of positive results as observed in the supplementation of 12% FOS. As compared with 12% supplementing of GOS, inulin and other carbohydrate sources tested, their mean doubling time was shorter than control except for the 12% supplementation of FOS.

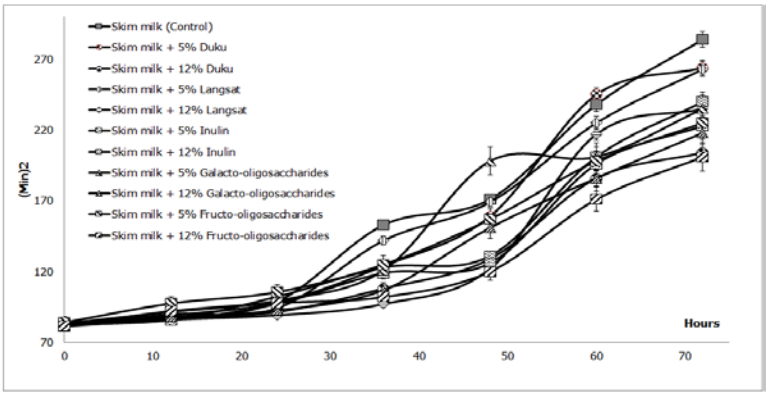

Fig. 2: Doubling time of *B. breve* in skim milk containing prebiotics

In general, doubling time of *B. breve* grown with prebiotics significantly decreased ($p<0.05$) compared to the control as shown in fig. 2. From the results, the most effective supplements that enhanced the growth rate of *B. breve* in skim milk were 12% supplementation of FOS, duku and inulin. This study also found that the supplementation of inulin, GOS and FOS were equally enhancing the growth rate of *B. breve* in skim milk. The supplementation of 12% FOS provided the shortest mean doubling time, followed by 12% inulin, 12% GOS, 5% GOS, and 5% FOS. Both supplemented with 5% FOS and 5% GOS were found numerically higher, but not significant in stimulating the growth rate of *B. breve* in skim milk. For the fruit prebiotics tested, duku and langsat were significantly lower in enhancing the growth rate of *B. breve* in skim milk. The supplementation of 12% oligosaccharides from duku also helped stimulate the growth of *B. breve* when added to skim milk. It showed a similar pattern of positive results as with the supplementation of 12% FOS. As compared to supplementations of 12% GOS, 12% inulin and other carbohydrate sources tested, their mean doubling time was shorter than others except with 12% FOS. The concentration of more than 5% oligosaccharides from duku and langsat was needed to stimulate ($p<0.05$) the growth of *B. breve* strains.

This study also showed that a supplementation of oligosaccharides from duku and langsat could effectively stimulate the growth of *Bifidobacterium* species when added to skimmed milk and showed a similar pattern of results as supplementation of FOS and GOS. These data could have an important nutritional significance since they indicate that the fruit prebiotics, specifically red pitaya supplementation, showed potential in improving the bifidobacterial *glucosidase* activity during skimmed milk fermentation. The supplementation of FOS and GOS showed a sharp decrease in mean doubling time as compared to other prebiotic samples. These results were consistent with previous reports by Shin *et al.* [19] on the ability of FOS to stimulate *Bifidobacterium* spp (Bf-1 and Bf-6) growth in skimmed milk containing oligosaccharides and inulin. The results also indicated the ability of FOS to stimulate the proliferation

of *Bifidobacteria* relative to other intestinal microflora *in vitro* culture models simulating the colon, as stated by Gibson and Wang [20]. The human colon was known to have over 400 distinct species of bacteria as resident flora (a population of up to 1010 bacteria per gram of colonic contents) [21]. The ingestion of GOS also been demonstrated to promote the increase number of fecal *Bifidobacteria* in human feeding trials [22, 23].

A previous study was done by Shin *et al.* [19] showed that inulin was found to be less effective in stimulating the growth of *Bifidobacterium* spp. However, in this study, inulin was shown to be effective in stimulating the growth of *Bifidobacteria*. The results of this study were similar and consistent with previous reports by Bruno *et al.* [22] on the ability of probiotics from Hi-maize, lactulose, raftilose and inulin in stimulating growth, viability, and activity of *Bifidobacterium spp* in skimmed milk. Gibson and Wang [20] reported on the ability of inulin to stimulate the proliferation of *bifidobacteria* relative to other intestinal microflora. Roberfroid [23] also reported positive outcomes *in vitro* fermentation of inulin by human fecal bacteria when molecules had DP>10. Overall, the results showed that there was a significant difference between the effect of oligosaccharides from duku and langsat on the growth activities of *B. longum* and *B. breve* in skimmed milk.

Determination of *Bifidobacterial* activity

According to Shin *et al.* [17], the *Bifidobacteria* fermentation pathway resulted in 3 moles of acetic acid and 2 moles of lactic acid per 2 moles of glucose in an ideal synthetic medium. The yields of the theoretical molar ratio (acetic: lactic) should be 1.5 as proposed by Scardovi and Trovatelli [24]. Although lactic acid production is desired in fermented dairy foods, a high concentration of acetic acid can result in distinct vinegar flavor in products, thus a high acetic to lactic acid ratio is typically undesirable in fermented dairy foods. In accordance with growth stimulation, both acetic and lactic acid production by *B. longum* and *B. breve* were enhanced by the presence of both fruit prebiotics and commercially available probiotics (table 1) in skimmed milk as compared to the controls. For the production of acetic acid by *B. longum*, the highest production level of acetic acid was produced with a supplementation of 12% oligosaccharides from duku followed by 12% oligosaccharides from langsat, 12% FOS, 12% GOS, 12% inulin, 5% oligosaccharides from duku, 5% oligosaccharides from langsat, 5% inulin, 5% FOS, while the lowest acetic acid level was produced by 5% GOS.

For lactic acid production by *B. longum*, the highest production level of lactic acid was produced with a supplementation of 12% oligosaccharides from langsat followed by 5% oligosaccharides from duku, 12% GOS, 5% oligosaccharides from langsat, 5% inulin, 5% FOS, 5% GOS, 12% FOS, 12% inulin and the lowest lactic acid production level was produced by supplementation of 12% oligosaccharides from duku. Acetic acid concentration produced by *B. longum* when combined with prebiotics of different concentrations were only significant ($p<0.05$) with 12% concentrations of supplementations of duku, langsat, inulin, GOS and FOS, compared to the controls. Lactic acid concentration produced by *B. longum* was significantly lower ($p<0.05$) compared to the controls when 12% oligosaccharides from duku, 12% inulin and 12% FOS were used. This study also observed a higher average molar ratio of acetic to lactic acid production by *B. longum* when 12% oligosaccharides from duku was added, followed by 12% FOS, 12% inulin, 12% GOS, 12% oligosaccharides from duku, 5% FOS, 12% oligosaccharides from langsat, 5% oligosaccharides from langsat, 5% inulin and 5% GOS. The control samples containing *Bifidobacteria* with no added prebiotics produced an average ratio of 1.27:1.

Table 1: Acetic and Lactic Acid Production by five strains of *Bifidobacterium* spp. in skimmed milk containing 5% and 12% fruit and commercial prebiotics

Prebiotics/Species		*B. longum* (ATCC15707)	*B. breve* (ATCC15700)
Control	Acetic Acid (mM)	54.90±1.50 [a]	39.80±1.20 [a]
	Lactic Acid (mM)	43.20±1.08 [3]	30.40±1.07 [2]
	Ratio[2]	1.27	1.31
5% Duku	Acetic Acid (mM)	59.40±0.70[a]	*91.90±0.60[d]
	Lactic Acid (mM)	32.30±0.90 [2]	45.68±0.20 [3]
	Ratio[2]	1.84	2.01
12% Duku	Acetic Acid (mM)	*99.40±1.70[c]	*91.90±1.15[d]
	Lactic Acid (mM)	*22.03±1.35 [1]	*22.98±1.32[1]
	Ratio[2]	4.51	3.99
5% Langsat	Acetic Acid (mM)	63.21±1.10[a]	*62.98±1.50[c]
	Lactic Acid (mM)	32.90±1.00 [2]	*21.90±1.04[1]
	Ratio[2]	1.73	1.72
12% Langsat	Acetic Acid (mM)	*96.78±1.01[c]	*97.56±1.50[d]
	Lactic Acid (mM)	38.45±0.80 [2]	43.90±1.09 [3]
	Ratio[2]	2.52	2.22
5% Inulin	Acetic Acid (mM)	59.21±0.90[a]	54.23±0.30[b]
	Lactic Acid (mM)	32.78±0.20 [2]	33.20±0.78[2]
	Ratio[2]	1.81	1.63
12% Inulin	Acetic Acid (mM)	*89.56±0.45[b]	*84.67±0.80[c]
	Lactic Acid (mM)	*22.90±0.20 [1]	*23.25±0.35[1]
	Ratio[2]	3.91	3.64
5% GOS	Acetic Acid (mM)	40.98±0.50[a]	42.98±0.90 [b]
	Lactic Acid (mM)	*23.09±1.10 [1]	*28.67±1.04[1]
	Ratio[2]	1.77	1.50
12% GOS	Acetic Acid (mM)	*90.21±0.10[c]	*92.67±0.35[d]
	Lactic Acid (mM)	33.89±0.30 [2]	*32.56±0.50[2]
	Ratio[2]	2.66	2.85
5% FOS	Acetic Acid (mM)	58.97±0.50[a]	54.56±1.02 [b]
	Lactic Acid (mM)	*23.20±0.70 [1]	*23.67±0.10[1]
	Ratio[2]	2.54	2.31
12% FOS	Acetic Acid (mM)	*95.23±0.10[c]	*94.89±0.50[d]
	Lactic Acid (mM)	*21.98±0.50[1]	*25.24±0.90[1]
	Ratio[2]	4.33	3.76
Average	Acetic Acid (mM)	*73.10±0.20	*71.57±1.07[c]
	Lactic Acid (mM)	30.61±0.78	*29.74±1.82[2]
	Ratio2	2.22	2.41

*Significantly different ($p<0.05$) from the control, [abc]Variation in the following letters between acetic acid indicates significant of difference by Duncan's test at 5% level ($p<0.05\%$), 12[3] Variation in the following numbers between lactic acid indicates significant of difference by Duncan's test at 5% level ($p<0.05\%$).

The supplementation of fruits or commercially available prebiotics were influenced by the production of acetic and lactic acids. It was similar in all prebiotics supplementation ($p<0.05$) and the results showed it did not depend on prebiotic concentration. In the case of *B. longum*, acetic and lactic acids were significantly enhanced ($p<0.05$) when *B. longum* was grown in the presence of 12% oligosaccharides from duku, 12% oligosaccharides from langsat, 12% FOS, 12% GOS and 12% inulin with the average ratio of 2.22:1, which is close to the theoretical molar ratio of 3 acetic acids to 2 lactic acids. For production of acetic acid level by *B. breve*, the highest production of acetic acid level was produced with supplementation of 12% oligosaccharides from langsat followed by 12% FOS, 12% GOS, 12% oligosaccharides from duku, 5% oligosaccharides from duku, 12% inulin, 5% oligosaccharides from langsat, 5% FOS, 5% inulin and 5% GOS. The highest production of lactic acid level by *B. breve* was produced with supplementations of 5% oligosaccharides from duku followed by 12% oligosaccharides from langsat, 5% inulin, 12% GOS, 5% GOS, 12% FOS, 5% FOS, 12% inulin and 12% oligosaccharides from langsat.

Acetic acid concentration produced by *B. breve* when combined with prebiotics of different concentrations was only significant ($p<0.05$) at 12% concentrations of oligosaccharides from duku, langsat, inulin, GOS and FOS when compared to the controls. While lactic acid concentration (by *B. breve*) was significantly lower ($p<0.05$) when compared to the controls in 12% oligosaccharides from duku and 12% inulin. This study also observed a higher average molar ratio of acetic acid to lactic acid production by *B. breve* when 12% oligosaccharides from duku was added (3.99:1) followed by 12% FOS (3.76:1); 12% inulin (3.64:1); 12% GOS (2.85:1); 12% oligosaccharides from langsat (2.58:1); 5% FOS (2.31:1); 5% oligosaccharides from duku (2.01:1); 5% oligosaccharides from langsat (1.72:1); 5% inulin (1.63:1) and 5% GOS (1.50:1). The control samples containing *Bifidobacteria* with no added prebiotics produced an average ratio of 1.31:1. The supplementation of oligosaccharides, either from fruits or those commercially available, was influenced by the production of acetic and lactic acids. It was similar in all prebiotic supplementations ($p<0.05$) and the results showed it did not depend on the prebiotic concentration. In the case of *B. breve*, acetic acid and lactic acid were significantly enhanced ($p<0.05$) when *B. breve* was grown in the presence of 12% oligosaccharides from duku, 12% oligosaccharides from langsat, 12% FOS, 12% GOS and 12% inulin with the average ratio 2.41:1, which is close to the theoretical molar ratio of 3 acetic acids to 2 lactic acids.

Overall results showed that lactic acid production was significantly enhanced ($p<0.05$) when *B. longum* and *B. breve* was grown in the presence of 12% oligosaccharides from duku with the production of lactic acid ranging from 3.29–4.51 and nearly 2.5 times greater as compared to inulin, GOS and FOS. *Bifidobacteria* produce lactic acid and acetic acid as the end product of sugar fermentation. This is an important characteristic of *Bifidobacteria*. The *Bifidobacterium* fermentation pathway resulted in 3 mol of acetic acid and 2 mol of lactic acid per 2 mol of glucose in an ideal synthetic medium, therefore generating a theoretical molar ratio (acetic: lactic) of 3:2 [24]. Although lactic acid production is desired in fermented dairy foods, a high concentration of acetic acid can result in distinct vinegar flavor in products. Thus, a high acetic to lactic acid ratio is typically undesired in fermented dairy products [17, 24].

CONCLUSION

The degree of enhancement of growth, activity and viability of *Bifidobacterium* spp. in skimmed milk are dependent on the carbon source and concentration as well as the strain of *Bifidobacterium* spp. The results from this study showed a certain stimulatory effect upon addition of fruit prebiotics from duku and langsat on *B. longum* and *B. breve* in skimmed milk. In general, doubling time of *Bifidobacterium* species grown with prebiotics decreased as compared to the control. Mean doubling times were significantly lower ($p<0.05$) following the addition of 5% and 12% fruit and commercial oligosaccharides with strains of *B. longum* and *B. breve* when compared to the control samples. The addition of 12% oligosaccharides from duku supplementation influenced further decrease of mean doubling time as compared to other fruit prebiotic samples.

Overall, the result showed there is a significant difference between the effect of oligosaccharides from duku and langsat on the growth activities of *B. longum* and *B. breve* in skimmed milk. The addition of 12% oligosaccharides from duku supplementation had greatly influenced the viability of *B. longum* as compared to other fruit prebiotic samples. This study also showed that a supplementation of oligosaccharides from duku significantly stimulated the growth of *B. longum* and *B. breve* when added to skimmed milk and it was more effective exhibiting similar result patterns as supplementations of FOS, GOS and inulin. The concentration of more than 5% oligosaccharides from duku and langsat was needed to stimulate ($p<0.05$) the viability of *B. bifidum* in skimmed milk.

Other than that, results of lactic acid showed that the production had significantly enhanced ($p<0.05$) when *B. longum, B. breve, B. infantis, B. bifidum* and *B. adolescentis* were grown in the presence of 12% oligosaccharides from red pitaya with the production of lactic acid ranging from 3.29–4.51 and nearly 2.5 times greater as compared to inulin, GOS and FOS. Thus, this study reveals that milk supplementation with oligosaccharides from duku and langsat had significantly improved the survival and viability of *Bifidobacteria* spp. cells and it showed potential in improving the bifido-bacterial *glucosidase* activity during skimmed milk fermentation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist

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