# COMPARISON OF EFFICACIES BETWEEN LIVE AND KILLED PROBIOTICS IN CHILDREN WITH LACTOSE MALABSORPTION

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**Abstract.** The objective of this study was to determine the efficacy of live and killed probiotics to decrease the presence of hydrogen using the breath hydrogen test (BHT). This pretest-posttest control group design single blinded randomized study was performed in 5 government elementary schools in Tuminting subdistrict, Manado, Indonesia from March to May 2008. The study for inclusion as subjects consisted of healthy 10-12 year old children with heights and weights within normal limits using the Centers for Disease Control (CDC) criteria whose BHT was ≥20 parts per million (ppm), indicating lactose malabsorption. One hundred thirty children were screened, 86 met criteria, 43 children were randomized into two groups. Thirty-nine children who were given live probiotic and 40 children who were given killed probiotic completed the study. There was a significant difference when comparing the BHT results before and 120 minutes after giving probiotic for the children taking both the live and the killed probiotic ($p < 0.001$). When the children taking the live and killed probiotics were compared, there was no difference in the BHT at 120 minutes of probiotic ($p = 0.453$) by *t*-test. The administration of live or killed probiotic for 2 weeks can decrease the results of a BHT in children with lactose malabsorption. No adverse reactions attributable to treatment were noted.

**Key words:** live probiotic, killed probiotic, lactose malabsorption

## INTRODUCTION

Disaccharide sucrose and lactose are the most important carbohydrates in daily food (Hay, 2007). Lactose present in mammalian milk is a source of calories (Rauf *et al*, 1998; Heyman, 2006). Lactose malabsorption is a condition usually caused by deficiency of lactase enzyme as a result of damage of the mucosal epithelium of the ileum (Warouw, 2001; Wilson, 2005; Montalto *et al*, 2006). Lactose malabsorption can be diagnosed by symptoms of lactose intolerance (history of borborygmi, frequent flatus, bloating, flatulence, nausea, vomiting, abdominal pain, abdominal distention and diarrhea after ingestion of lactose) and by a breath hydrogen test (BHT) results ≥20 parts per million (ppm) after lactose ingestion. Lactase enzyme can be detected from the third month of gestation and reaches a maximum in infants

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two to four weeks of age (Rauf *et al*, 1998; Dharmasetiawani, 2005). Activity of the lactase enzyme in premature babies is the same as term babies by two weeks if the child is breastfed. This enzyme activity is sustained as long the child receives breast milk (Dharmasetiawani, 2005).

Lactose malabsorption, or lactose intolerance, is treated using lactose free milk, yogurt or fermented milk, probiotics and lactase enzyme (Heyman, 2006). Low lactose diets or lactose free diets are often used to overcome primary or secondary lactase deficiencies (Warouw, 2001).

Probiotics, especially *Lactobacillus* and *Bifidobacterium* species, which are commonly used in fermented milk, may decrease lactose malabsorption or lactose intolerance. These species may have natural antibacterial properties against some gram-negative pathogenic bacteria. They also increase the activity of lactase enzyme in the intestine, which helps to ferment lactose (Rolfe, 2000; Sudarmo 2003; Young and Huffman, 2003; Subijanto and Ranuh, 2005). Milk containing a probiotic may, at correct concentrations, be an alternative for patients with lactose malabsorption or lactose intolerance (Talwakar and Kailasapathy, 2004).

To date, there have been published studies comparing the efficacy of live probiotics with killed probiotics in the treatment of children with lactose malabsorption or lactose intolerance. Therefore, we conducted a study of the efficacy of live versus killed probiotics in children with lactose malabsorption using the BHT.

## MATERIALS AND METHODS

This research was carried out using a pretest-posttest control group design in children with a history of lactose intolerance (symptoms of borborygmi, frequent flatus, bloating, nausea, vomiting, abdominal pain, abdomen distention and diarrhea) or lactose malabsorption detected by BHT. The sample size was calculated to be a minimum 35 children in each study group ($\alpha = 0.05$; power 80%; S = 2.5 ppm; d = 1.05 ppm) (Madiyono *et al*, 1995). Using multistage sampling we selected 5 out of 33 government elementary schools in Tuminting subdistrict, numbers 114, 46, 19, 22 and 111, from March 1 to May 3, 2008.

Inclusion criteria were otherwise healthy children age 10-12 years old (class V and VI in school) with good nutritional status based on Centers for Disease Control (CDC) criteria lactose malabsorption as seen with a BHT ≥ 20 ppm in whom written informed consent was given by the authors. Exclusion criteria were fasting less than six hours; current or recent (within 1 week prior to the study period) probiotic supplement use, having received antibiotics (kanamycin, neomycin, colistin), methothrexate, acetyl salicylic acid, metochlorpramide, a laxative, radiation therapy a week before enrollment, children with respiratory or bowel disease (*eg*, persistent diarrhea), and those who did not follow protocol or who dropped out. All patients were evaluated by one of the authors. The study was approved by the Ethics Committee of the University of Sam Ratulangi, Medical School.

We determined the nutritional status of the children by measuring the body weight (BW) and body height (BH) and plotting it on a CDC growth chart. Nutritional status was determined based on BW per BH (Pusponegoro *et al*, 2004).

Post-lactose injection BHT was used to determine lactose malabsorption. After an overnight fast (minimum 6 hours), breath samples were collected using a portable LCD 4 digit Lactometer (version 1.0 CvO-HLMT Hoek Loos, Netherlands) at

Table 1
Distribution of children based on nutrition status and type of probiotic.

| Nutrition status | Live probiotic (%) | Killed probiotic (%) | Total (%) |
|---|---|---|---|
| Adequate | 36 (52.9) | 32 (47.1) | 68 (100) |
| Overweight | 1 (20.0) | 4 (80.0) | 5 (100) |
| Obese | 2 (33.3) | 4 (66.7) | 6 (100) |
| Total | 39 (49.4) | 40 (50.6) | 79 (100) |

Table 2
Children distribution based on symptoms before probiotic.

| Symptom | Live probiotic (%) | Killed probiotic (%) | Total (%) |
|---|---|---|---|
| Abdominal pain | 26 (66.7) | 29 (72.5) | 55 (69.6) |
| Nausea | 1 (2.6) | 0 (0) | 1 (1.3) |
| Bloating | 1 (2.6) | 0 (0) | 1 (1.3) |
| Diarrhea | 1 (2.6) | 0 (0) | 1 (1.3) |
| More than 1 symptom | 3 (7.6) | 7 (17.5) | 10 (12.5) |
| Asymptomatic | 7 (17.9) | 4 (10.0) | 11 (13.9) |
| Total | 39 (100) | 40 (100) | 79 (100) |

Table 3
Distribution of symptoms after taking probiotic.

| Symptom | Live probiotic (%) | Killed probiotic (%) | Total (%) |
|---|---|---|---|
| Abdominal pain | 11 (28.2) | 12 (30.0) | 23 (29.1) |
| Nausea | 0 (0) | 1 (2.5) | 1 (1.3) |
| Bloating | 1 (2.6) | 2 (5.0) | 3 (3.8) |
| Flatus | 0 (0) | 1 (2.5) | 1 (1.3) |
| More than 1 symptom | 2 (5.1) | 3 (7.5) | 5 (6.3) |
| Asymptomatic | 25 (64.1) | 21 (52.5) | 46 (58.2) |
| Total | 39 (100) | 40 (100) | 79 (100) |

0, 30, 60, 90 and 120 minutes after ingestion of lactose (2 g / kg body weight; maximum 50 g) given as a 20% aqueous solution. Lactose malabsorption was defined as an increase in hydrogen concentration to greater than 20 ppm above the fasting average baseline value at 60, 90, and 120 minutes post-ingestion (Di Stefano *et al*, 2004).

The children with lactose malabsorption were randomized into 2 groups to receive either live or killed probiotic. The live probiotic group took 1 capsule (Lacidofil) daily for 2 weeks and the killed probiotic group took 2 sachets (Dialac) daily for 2 weeks. They were followed up to determine if they developed any symptoms or adverse reactions. Two weeks later the

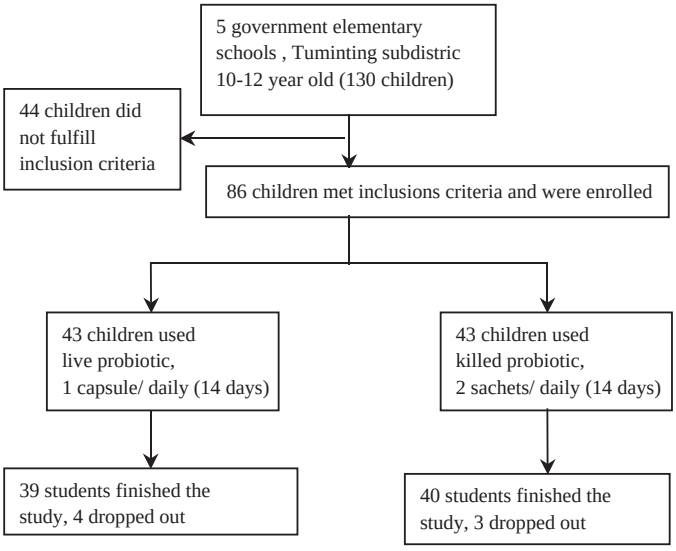


Fig 1–Flow chart of the children in this study.

BHT was repeated using the same procedure as described above.

We compared the BHT results before and after administration of live and killed probiotic at 120 minutes in children with lactose malabsorption. Data were analyzed using Statistical Package Software version 15 (SPSS 15); a *t*-paired test and *t*-independent test were used to analyze the data.

## RESULTS

Samples were taken from March 1 to May 3, 2008 (nine weeks). One hundred thirty children were studied. Of these, 86 (66.5%) were diagnosed with having lactose malabsorption, but only 79 children completed the study: 39 children in the live probiotic group and 40 children in the killed probiotic group. The male to female ratio was approximately equal (41 males : 38 females). Seven participants dropped out due to respiratory or bowel symptoms (*eg*, persistent diarrhea) during the research period: 4 from the live probiotic group and 3 from the killed probiotic group (Fig 1).

Of the 79 children with lactose malabsorption, 36 (92.3%) from the live probiotic group and 32 (80%) from the killed probiotic group had adequate nutritional status (Table 1).

The symptoms experienced before administration of probiotic are shown in Table 2. Fifty-five children (69.6%) had abdominal pain, 11 (13.9%) were asymptomatic and 10 (12.5%) had more than 1 symptom. The symptoms experienced after administration of probiotic are shown in Table 3. Forty-six children (58.2%) were asymptomatic, 23 (29.1%) had abdominal pain and 5 (6.3%) had more than 1 symptom.

The mean BHT before administration of live probiotic was 34.5 (SD 10.4) which decreased to 22.1 (SD 12.4) 120 minutes after administration of live probiotic; the difference was significant ($p < 0.001$) (Table 4). The mean BHT before administration of the killed probiotic was 36.0 (SD 10.2) decreased to 20.3 (SD 8.9) at 120 minutes after administration of killed probiotic. The difference was significant ($p < 0.001$) (Table 5). The mean BHT after administration of live probiotic was 22.1 (SD 12.4) and the mean BHT after administration of killed probiotic was 20.3 (S.D 8.9). The difference in BHT between the live and killed probiotic groups 120 minutes after ingestion of lactose was not significantly different ($p = 0.453$) (Table 6).

## DISCUSSION

In this study, lactose malabsorption was found in 86 out of 130 children (66.5%)

Table 4
Results of BHT before and after administration of live probiotic.

| Minutes | Group | *N* | Mean (SD) | T | *p* |
|---|---|---|---|---|---|
| 120 | Before | 39 | 34.51 (10.35) | 9.556 | < 0.001 |
| | After | 39 | 22.13 (12.41) | | |

Table 5
Results of BHT before and after administration of killed probiotic.

| Minutes | Group | *N* | Mean (SD) | T | *p* |
|---|---|---|---|---|---|
| 120 | Before | 40 | 36.00 (10.18) | 8.545 | < 0.001 |
| | After | 40 | 20.30 (8.86) | | |

Tabel 6
Results of BHT test after administration of live and killed probiotic.

| Minutes | Group | *N* | Probiotic | Mean (SD) | T | *p* |
|---|---|---|---|---|---|---|
| 120 | After | 39 | Live | 22.13 (12.41) | 0.755 | 0.453 |
| | | 40 | Killed | 20.30 (8.86) | | |

enrolled in 5 government elementary schools in Tuminting subdistrict. The prevalence in Jakarta, Indonesia was 57.1% (Hegar *et al*, 1999). The differences in prevalence between the studies maybe caused by better adaptation to milk of children from Jakarta than children from Manado.

In this study, we only included children with adequate nutritional status and excluded those with lower nutrition and malnutrition because both groups have atrophy of the intestinal mucosa and decreased lactase production (Heyman, 2006). We used probiotic for 2 weeks because previous studies found using probiotic for 2 weeks increased tolerance to lactose, decreased 95% of symptoms and changed 85.7% of subjects from having a positive BHT to a negative BHT (Kocian, 1994; Arifin *et al*, 2005).

The most common symptom prior to ingestion of probiotic (Table 2) were abdominal pain in 55 children (69.6%), having more than one symptom in 10 children (12.5%). A previous study showed nausea and bloating usually arise within 30 minutes of lactose ingestion, while abdominal pain, flatulence, borborygmi and diarrhea, arise within 1-2 hours after lactose ingestion (Buller, 1990). Ten children with elevated BHT had no symptoms of lactose intolerance; these are dependent on many factors, including the rate of gastric emptying, small intestine motility, sensitivity of colonic flora, and the amount and manner of lactose ingestion (Montes and Perman, 1991). False positives were mini-

mized with exclusion criteria.

Symptoms improved with probiotic (Table 3). Forty-six (58.2%) children became asymptomatic and only 5 (6.3%) had more than 1 symptom. This may be due to the natural antibacterial properties of the probiotics and due to the increased activity of lactase enzyme in the intestine.

The results of the BHT before and after receiving live probiotic are shown in Table 4. The difference was statistically significant ($p < 0.001$), similarly the difference in BHT before and after receiving killed probiotic was also significant ($p < 0.001$) (Table 5). However, the difference in the BHT between the groups after receiving live and killed probiotic was not significant ($p = 0.453$) (Table 6).

These are similar to the results seen in a study from Paris, France where 16 subjects (20-33 years old) had a positive BHT for lactose malabsorption, but after 2 weeks of probiotic using yogurt there was a significant decrease in the BHT result (Saviano *et al*, 1987). In a study from Baltimore, USA, 23 subjects (4-16 years old) with lactose malabsorption had a significant decrease in BHT after receiving probiotic (Shermak *et al*, 1995). In another study from the USA, 10 subjects (24-40 years old) with lactose malabsorption, had significant improvement, compared to the control group in symptoms after receiving yogurt (Lin *et al*, 1991). Another study from the USA concluded probiotic can decrease lactose intolerance symptoms (Lin *et al*, 1998). Arifin *et al* (2005) studied 42 junior high school students with symptoms of lactose intolerance. They were giving full cream milk with a probiotic to drink for 2 weeks. In 95% of the children the symptoms subsided and 86% had a negative BHT.

Dehkordi *et al* (1995) compared the efficacy of milk containing *Lactobacillus acidophilus* and *Bifidobacterium* and found a negative BHT without symptoms of lactose intolerance in any of the studied groups. In Turkey, subjects who consumed food without fermentation had positive BHT and symptoms of lactose intolerance (Mustapha *et al*, 1997). Warouw *et al* (2007) studied the efficacy of live probiotic (lacbon) and killed probiotic (dialac) in patients (4-60 months old) with acute diarrhea and concluded that there were no significant differences in the frequency of diarrhea and duration of illness between the two groups. Tlaskal *et al* (2006) studied the use of live and killed probiotics in patients with acute diarrhea and concluded the live probiotic (lacidofil) can shorten the duration and reduce the clinical symptoms of acute diarrhea. Xiao *et al* (2003) studied the efficacy of live and killed probiotics in adult patients with chronic diarrhea and found killed probiotics were more effective than live probiotics in decreasing abdominal pain, abdominal distention and improving fecal consistency.

The interpretation of that study was probiotic in the form of yogurt, sachets, capsules or fermented milk, can make a change in lactose metabolism by interference with the activity of β-galactosidase. Yogurt has a higher viscosity and a lower pH, which results in a slower rate of gastric emptying, reaching the colon more slowly and hydrolyzing lactose in the small intestine, decreasing the BHT (De Vrese *et al*, 2001). During the study period, there were no side effects reported in children receiving live or killed probiotic.

In conclusion, the administration of live and killed probiotic for 2 weeks is effective in treating children with lactose malabsorption, which is evident by a decrease in BHT. No adverse reactions were attributable to the treatment.

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