Antidiarrheal characteristics of tempe produced traditionally and industrially in children aged 6-24 months with acute diarrhea

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ABSTRACT A randomized controlled double-blind clinical trial was conducted to evaluate tempe-based formulated foods for treatment of young Indonesian children suffering from acute diarrhea. A total of 214 cases aged between 6 and 24 months visiting two teaching hospitals, i.e., Sardjito hospital (n=102) in Yogyakarta and Karyadi hospital (n=112) in Semarang. Two cases from one hospital were dropped because they moved to other towns. In addition to their hospital food given during hospitalization and daily food at home, 72 cases were given tempe-based formulated foods with tempe produced traditionally (group TT), 72 were given tempe produced industrially (group IT), whereas a control group of 68 received soybean powder formulated foods (group IS). Formula feedings were started immediately following WHO (world health organization) standard oral rehydration therapy (ORT) and continued at the patients homes for up to 90 consecutive days, including feedings during hospitalization. Follow-up observations at patients homes were conducted twice weekly. The initial clinical characteristics of the cases in each group were similar. Using analysis of variance there was a non significant trend towards a shorter duration of diarrhea in the groups using tempe based formula (p=0.079). Using the t-test, the duration of diarrhea appeared to be significantly shorter only for the group using formula with traditional tempe compared with the group using soy formula (p=0.035). The total amount of feeding formula and the total amount of calories consumed at the hospital and at home was similar for all three groups, although the group receiving the control formula consumed a somewhat higher amount of breast milk (p=0.045) and a lower amount of solid food at home. Weight for age was below normal at the start of the study or after rehydration (Z-score between -1.0 and -1.4) and approached the normal value at the end of the study for all three groups (Z-score between -0.51 and -0.27). The increase in Z-score was highest in the groups receiving tempe based formula (+1.0 in the TT group and +0.9 in the IT group) and lowest in the IS group (+0.7). This implies that a tempe based formula can diminish the duration of acute diarrhea and improve weight gain following an episode of acute diarrhea. [Paediatr Indones 2001; 41:88-95]

Keywords: acute diarrhea, feeding therapy, fermented soya.

Despite a remarkable decrease of mortality in children with acute diarrhea due to the success of oral rehydration therapy (ORT), the importance of current WHO recommendations on continuous feeding during and after acute diarrhea is neglected even by medical personnel. This is perhaps due to the more chronic and often non dramatic impact of weight loss, compared to the obvious, acute and frightening impact of fluid loss happening in children with acute diarrhea. Diarrheal episodes have their impact on nutritional status through increased stool output, vomiting, anorexia, damage of the intestinal mucosa, the catabolic effect of the infection and the withholding
of food due to ignorance. The proper feeding of children with diarrhea has to fulfill the following requirements: the food must be easily digestible and absorbable, have a low content of lactose and contain vitamins like vitamin A and minerals like zinc and mangan (Mn).

Tempe is a popular Indonesian food of soaked, cooked soy beans, fermented by Rhizopus species moulds.\textsuperscript{1} It is easily digestible due to the high amount of free fatty acids, peptides and amino acids.\textsuperscript{2} Apart from an improvement: of flavor, texture and appearance, fermentation of soy beans by Rhizopus leads to the synthesis of B-vitamins.\textsuperscript{3,4} Another benefical effect is that during the tempe production process the amount of the sugars raffinose and stachiose, which are responsible for increased flatulence is strongly reduced.\textsuperscript{5} During the fermentation process the phytate content is also reduced, leading to increased bioavailability of Zn, Fe, Mn, Ca and P.\textsuperscript{6,7} Astuti et al.\textsuperscript{8,9} showed that tempe powder added to the diets of anemic rats enhanced serum hemoglobin, iron and zinc levels. Former studies in Indonesia by Mien\textsuperscript{10} and Karyadi\textsuperscript{11} showed that a tempe based formula may shorten the duration of acute diarrhea in comparison with a rice based formula and that in individual cases of chronic diarrhea tempe based formula may improve post diarrheal weight gain. Tempe might therefore serve very well as the basic ingredient for a formula for children recovering from an episode of acute diarrhea. As tempe lacks a high caloric value and does not contain digestible carbohydrate however, digestible carbohydrates like rice, maltodextrin, and/or saccharose should be added to optimize the caloric value of the product.

The aim of the present study was to compare the antidiarrheal properties of a formula containing tempe, either traditionally or industrially prepared, with a formula based on non fermented soy beans. The study was performed in children aged 6-24 months with acute diarrhea, in a randomized double blind trial.

**Methods**

**Subjects**

This study was carried out at the Child Health Departments in the Medical Faculty of Gadjah Mada University in Yogyakarta (Sardjito hospital) and Medical Faculty of Diponegoro University in Semarang (Karyadi Hospital). Children aged 6-24 months suffering from acute diarrhea were enrolled if they fulfilled the following criteria: 1) purging soft or watery stool more than 3 times a day; 2) stool contains blood or mucus; 3) diarrhea before admission that had lasted less than 7 days; 4) living less than 17 km from the hospital; 5) willing to stay in the hospital until the diarrhea resolved; and 6) their parents or child-caregivers agreed to sign an informed consent. Patients who had severe diarrheal disease and had experienced other episodes of diarrhea within the previous 2 weeks were excluded. The research protocol was approved by the ethics committee of Gadjah Mada University (GMU) Faculty of Medicine.

**Study formulas**

The powdered formulas were made by PT Nutricia Indonesia Sejahtera and the products were packed in a double-blind method. The Industrial tempe formula was produced by Nutricia Research, Zoetermeer, The Netherlands. This tempe was dried in a drum dryer. The traditional tempe was produced according to traditional Indonesian methods in a forced-air cabinet dryer at the Nutrition Research and Development Center, Bogor, Indonesia. The control formula was produced by Nutricia Research in the Netherlands from soybeans that were soaked and cooked in the same way as the soybeans for tempe production. The only difference in production was that the soybeans for the soybeans powder were not fermented with Rhizopus oligosporus. The same lot of soybeans was used for the production of all three study formulae. It was of Chinese origin and purchased in Indonesia. Ingredients other than tempe or soya were exactly the same in all three formulas. The soybeans were dehulled in the Nutricia Research and Development Center at Bogor, Indonesia and then sent to the Nutricia Research, The Netherlands for processing into soybeans powder or industrially prepared tempe powder. Oven-dried Indonesian tempe was also sent to Nutricia Research for further processing. The study formulas were packed in 25 gram sachets and 4 sachets were placed in one box. Sachets and boxes were labeled with a random number that became the identification (ID) number of the recipient. The ID numbers were obtained by a cluster-randomization, so that in a group of 9 recipients there were three of each formula.
Patients who met the inclusion criteria were randomly assigned to three treatment groups. Children in one group were treated with a soya based formulated food, whereas patients in the other two groups were treated with tempe-based formulated food. One of the groups treated with tempe-based formula was treated with formula using tempe produced industrially, another one was treated with formula using tempe produced traditionally. The randomization list was established at Nutricia Research, The Netherlands. Individual patient’s assignments, corresponding to the master randomization list were then placed in sealed serially numbered envelopes and sent to Nutrition Research and Development Centre, Indonesia, for being sent to the investigators. The composition of the formulas is shown in Table 1.

### TABLE 1. COMPOSITION STUDY-FORMULAS PER 100 GRAMS

| Nutrient | Tempe powder (g%) | Soya powder g% |
|----------|-------------------|---------------|
|          | Industrial | Traditional |               |
| moisture | 5.1       | 3.8          | 5.6           |
| protein  | 19.0      | 20.5         | 19.1          |
| fat      | 9.9       | 10.2         | 6.9           |
| carbohydrate | 63.7 | 64.1        | 67.0          |
| ashes    | 1.4       | 1.4          | 1.4           |
| kcal/100g| 424       | 430          | 407           |

### TABLE 1. COMPOSITION STUDY-FORMULAS PER 100G

| Component | Tempe-Formula | Soya-Formula |
|-----------|--------------|--------------|
| tempe powder | 36.5     | 36.5         |
| soybean powder | 36.5 | 36.5         |
| rice flour | 36.5       | 36.5         |
| saccharose | 24.5       | 24.5         |
| salt       | 1.0         | 1.0          |
| banana flavor | 1.5    | 1.5          |

Examinations and observations

Each patient admitted into this study was given an ID number and anthropometric data, grade of dehydration, accompanying disease, blood, urine and stool analysis were assessed and recorded. Clinical assessment of vital signs and hydration status was repeated 1 hour after rehydration treatment was started, then repeated every 2 hours until all signs of dehydration resolved. After the patients rehydrated, observations were made every six hours, blood analysis included hemoglobin and hematocrit at the time of admission and 6 hours later. Each day stool volume, stool frequency and stool aspect were assessed.

The stool specimens were examined for the presence of rotavirus by ELISA (enzyme linked immunosorbent assay) using rotavirus wellcozyme of Welcome Foundation Limited, Diagnostic Division, Dartford, England DA 15 AH as described by Haksohusodo et al. Fresh stool specimens were obtained by rectal swab shortly after admission and cultured for Salmonella, Shigella V cholera as described by Dibley. Serotyping for E coli was not conducted because of its expense and lower prevalence in this age group.

### Treatments

Oral rehydration therapy (ORT) using ORS (oral rehydration salts) solution was given to all patients according to World Health Organization guidelines. All children received ORS solution in volumes of 400-800 ml (based on their body-weight) during the first 4 hours until full correction of all signs of dehydration. The ORS was a standard glucose-electrolyte solution consisting of 3.5 g sodium chloride, 1.5 g potassium chloride, 2.9 g trisodium citrate, and 20 g glucose per liter. On going losses of liquid stool and vomit were replaced volume for volume using the same ORS solution. Treatment with one of the study formulas was started when there was no sign of dehydration. Each patient was given 2 sachets of formula daily, in addition to their normal daily menu provided by the hospital. The formula was served as a porridge by dissolving it in 100-120 ml of preboiled water.

Patients were hospitalized until diarrhea ceased. During hospitalization each patient’s mother was instructed in the preparation and feeding of her child with the study formula. The feeding with the study formula was continued at home for up to 90 consecutive days including feedings during hospitalization, in addition to their daily menu prior to their illness.

### Treatment failures

Treatment failures were defined as: 1) diarrhea lasting longer than 7 days at the hospital, or 2) recurrent dehydration, if the children fell into dehydration again after being successfully rehydrated.
Follow-up observations

Follow-up observations at patients' homes were conducted twice weekly by residents in pediatrics and a psychologist who was also project manager, and her assistant. At each home visit, 6 or 8 sachets of the study formula were given to the patient's mother or child care-giver. During home treatment the patients were given their normal daily food. Their mothers were told to persuade their children to consume all the contents of two sachets. The anthropometric, food consumption, and diarrhea data were recorded.

Sample size estimates

The sample size estimates indicated that approximately 63 patients were needed per group to detect a difference of 250 g increase in weight gain and approximately 30% decrease of duration of the diarrhea with a probably of 0.05 and statistical power of 0.80.

Analysis of data

At the end of the study, the code was opened in the Netherlands. Data were analyzed using SPSS by Hadi & Pamardyanto and SPSS. Descriptive statistics of the crude and derived variables were reviewed, the etiologic agents associated with diarrhea were compared by chi-square analyses for discrete variables and the three dietary groups by analysis of variance and Duncan test for continuous variables. Probabilities (P) less than 0.05 were considered statistically significant. Based on the WHO definition, duration of diarrhea at the hospital was calculated from the day of admission until diarrhea had stopped, which was defined as no passage of watery stool more than 3 times a day, stool does not contain mucus or blood in addition stool volume is less than 100 ml/day as in the study of Myo-Khon. In addition, for the frequency and stool volume analysis, the definition of diarrheal was using Myo-Khon’s Study.

Results

Subjects characteristics

During the study, there were 214 patients involved, of which 112 were at Karyadi hospital and 102 at Sardjito hospital. Two cases in Karyadi hospital were dropped because they moved to other towns. At the end of the study there were 212 cases eligible for analysis.

Table 2 shows no significant differences between the three feeding groups with regard to age, weight, nutritional status, severity of diarrhea in terms of duration of diarrhea prior to the study, dehydration, type of stool, and also hemoglobin and hematocrit values. The most frequently identified microorganism was rotavirus (41.1% of 202 specimens). There was no significant difference in frequency among the three groups. Salmonella, Shigella and V cholera were found in a few cases. Although E. coli was found in 68.8% of 202 specimens, its serotyping was not conducted. However, this microorganism was also not different significantly in frequency among those three groups.

Outcome of therapy

Table 3 presents the outcome variables by the three feeding formula groups and summarizes the outcome variables for treatment with three different kinds of feeding formula. Using analysis of variance there was a non significant trend towards a shorter duration of diarrhea in both groups using tempe based formula (TT: 3.4+1.2 and IT: 3.5+1.3 days) compared with the control group IS: 3.9+1.2 days (p=0.079). Using the t-test, the duration of diarrhea appeared to be significantly shorter only for the group using formula with traditional tempe compared with the control group using soy formula (p=0.035). This table indicates that formulated food consumed in the hospital is almost 2 sachets per patient per day, whereas at home, the study subjects consumed approximately 1.5 sachets per day. Consumption of formulated foods during hospital and home treatments did not differ significantly. Hospital and home daily food were still given additionally. Unlike the home daily food, hospital daily food did not consist of tempe. There was a non significant difference in total energy intake (not including study formula) with or without breastmilk, between the tempe food groups TT and IT and the soya group IS.

Of the total patients at the hospital, 62.7% (133/212) were breast-fed. Only the amount of breastmilk given by mothers to the children at home in the soya feeding group was higher (p=0.045). Tempe consumption in terms of kcal, from additional foods during treatment is shown in Table 5. Consumption of 32.75 kcal from tempe equals to 25 g of fresh tempe. Data on tempe
consumption shown in Table 5 indicate that consumption of tempe from additional food has no correlation with the duration of diarrhea shown in Table 4a. Data on this Table show that tempe consumption from additional foods by most of the patients was less than 25g/patient/day. Consumption of tempe from tempe formulated food consumed equals to about 37.5 g fresh tempe.

During the 11-week home treatment there were six cases with diarrhea (2.8%), 2 cases in each group. Details of these cases will be presented separately.

| TABLE 2. CLINICAL FEATURES OF PATIENTS ON ADMISSION |
|---------------------------------|--------|--------|--------|--------|
|                                 | TT (n: 72) | IT (n: 72) | IS (n: 68) | p      |
| 1. Age (months)                | 11.3±4.7 | 11.9±4.5 | 11.6±4.4 | NS     |
| 2. Weight (Kg)                 | 8.0±1.2 | 8.0±1.2 | 8.2±1.5 | NS     |
| 3. Weight-for-age* (z-score)   | -1.2±1.7 | -1.4±1.7 | -1.0±1.7 | NS     |
| 4. Duration of diarrhea**      | 1.7±1.1 | 1.7±0.9 | 1.6±0.7 | NS     |
| (prior to admission)           | (n: 65) | (n: 63) | (n: 60) |         |
| 5. Degree of dehydration**     | No      | 23 (11.3) | 24 (11.6) | 23 (11.1) | NS     |
| (n: 71)                        | (n: 71) | (n: 65) |         |         |
| Some                           | 44 (21.3) | 43 (20.8) | 38 (18.4) | NS     |
| Severe                         | 4 (1.9) | 4 (1.9) | 4 (1.9) | NS     |
| 6. Stool (day 1)**             | No      | 427±22.2 | 380.2±21.3 | 433.2±25.8 | NS     |
| (n: 64)                        | (n: 62) | (n: 59) |         |         |
| frequency (x/day)              | 5.8±2.5 | 6.1±2.9 | 6.1±2.7 | NS     |
| (n: 70)                        | (n: 67) |         |         |         |
| appearance: bloody             | 3       | 3       | 1       | NS     |
| 7. Laboratory value**          | No      | 11.8±1.43 | 11.6±1.53 | 11.4±1.43 | NS     |
| Hemoglobine (g%)               | 49±3.9 | 49±4.0 | 48±4.1 | NS     |
| Hematocrit (%)                 | (n: 64) | (n: 63) | (n: 63) |         |
| 8. Rotavirus/N(%)              | Positive | 25 (37.3) | 32 (46.4) | 26 (39.4) | NS     |
| (n: 67)                        | (n: 69) | (n: 66) |         |         |
| Not examined                   | 7 (10.4) | 9 (13.0) | 4 (6.1) |         |
| 9. Enteropathogen bacteria/N (%)| Salmonella | 1 (1.5) | 0       | 0       |
| Shigella                       | 1 (1.5) | 1 (1.5) | 1 (1.5) |         |
| Vibrio cholera                 | 0       | 1 (1.5) | 1 (1.5) |         |
| E coli                         | 42 (62.7) | 49 (73.5) | 48 (72.0) | NS     |
| Not examined                   | 23 (34.3) | 18 (23.5) | 16 (24.0) |         |
|                                 | (n: 67) | (n: 69) | (n: 66) |         |

*After rehydration
**Total number of less than N: 212 (4) Some mothers/child-caregivers were not sure; (5) Not recorded in some cases; (6) The 1st-day admission was not a complete day; (7) Some refused.
TT: traditional tempe; IT: industrial tempe; NS: not significant
Rotavirus positive: 41.1% of all the 202 diarrhea cases
Serotyping for E coli was not conducted
No amoeba trofozoit was found

Body Weight
Weight for age Z-score (Table 4) was below normal at the start of the study, after rehydration (Z-score between -1.0 and -1.4) and approached the normal value at the end of the study for all three groups (Z-score between -0.27 and -0.51). The increase in Z-score was highest in the groups receiving tempe based formula (+1.0 in the TT group and +0.9 in the IT group) and lowest in the IS group (+0.7).
Several limitations were considered in reviewing the data. The test-weighing measurement for breast milk has some limitations, particularly when babies are fed frequently. However, several studies demonstrate close agreement with the deuterium dilution technique,18-20 and note that test weighing is likely to remain a valuable method.21

The weighing was only used at the hospital, whereas at home, the duration of active lactation was measured, to compare the groups. As this study was conducted in two different hospitals, several confounders were taken into consideration. Several meetings and training sessions prior to and during the study were held for the researchers and staff from the two study units, to minimize the disagreement. Another limitation is that stool cultures for some bacterial pathogens were not done, e.g. serotyping for *E. coli* Campylobacter, and Cryptosporidium due to the budget limitation.

However, as it was demonstrated by other studies including our own, rotavirus is the most important cause of diarrhea in this age group of children. In this study, rotavirus was found in almost half of the specimens; this finding is similar to findings conducted with similar sample populations elsewhere.14,22 In addition, the three groups were initially similar for all clinical as well as laboratory findings, thus the observed differences in treatment outcome might reasonably be attributed to the type of feeding treatment provided. There were no treatment failures; all groups responded to dietary interventions.

This study demonstrates the efficacy of tempe produced traditionally and industrially in comparison to soy formula in reducing symptoms of diarrhea in 6-24 months old children with acute diarrhea. In contrast to former studies conducted by our investigators the difference between the control and the treatment groups was not significant in this study. The reason may be that in previous studies the control group did

### TABLE 3. CLINICAL OUTCOMES OF THERAPY

| Outcomes                        | TT* (n=72) | IT** (n=72) | IS*** (n=68) | F       | p       |
|--------------------------------|------------|-------------|--------------|---------|---------|
| Duration of diarrhea (day)     | 3.4±1.2    | 3.5±1.3     | 3.9±1.4      | 2560    | 0.079   |
| Mean stool freq. day 1-4 (x/day) | 4.4±2.2   | 4.6±2.3     | 4.7±2.0      | 0.326   | 0.722   |
|                                | n: 70      | n: 69       | n: 67        |         |         |
| Difference in average stool volume, day 1-4 (ml) | -456.6±217.2 | -417.9±243.4 | -315.5±267.5 | 1919    | 0.156   |
|                                | n: 16      | n: 19       | n: 28        |         |         |
| Mean stool vol. day 1-4 (ml)   | 218.2±219.7| 239.5±175.3 | 222.9±162.2  | 0.214   | 0.807   |
|                                | n: 62      | n: 60       | n: 59        |         |         |
| Formulas acceptability (sachet/day) | 1.8±0.35  | 1.8±0.38    | 1.8±0.33     | 0.420   | 0.658   |
|                                | 1.5±0.56   | 1.5±0.53    | 1.5±0.53     | 0.029   | 0.972   |
| t-test (Duration of diarrhea):  |            |             |              |         |         |
| TT-IT = 0.47                   | p          |             |              |         |         |
| TT-IS = 2.13                   | 0.035      |             |              |         |         |
| IT-IS = 1.65                   | 0.102      |             |              |         |         |

*TT = traditional tempe (study group)
**IT = industrial tempe (study group)
***IS = industrial soya (control group)

### TABLE 4. WEIGHT FOR AGE Z-SCORE (WAZ) BY TREATMENT FORMULA

| TT | IT | IS | Time       | Times (day) |
|----|----|----|------------|-------------|
| -1.23 | -1.35 | -1.02 | After rehydration | 1          |
| -1.18 | -1.45 | -1.15 | on day of discharge | 8          |
| -1.26 | -1.45 | -1.13 | Home visit 1 | 11         |
| -0.94 | -1.13 | -0.83 | Home visit 8 | 31         |
| -0.59 | -0.81 | -0.52 | Home visit 16 | 61         |
| -0.27 | -0.51 | -0.33 | Home visit 24 | 91         |

Discussion

Several limitations were considered in reviewing the data. The test-weighing measurement for breast milk has some limitations, particularly when babies are fed frequently. However, several studies demonstrate close agreement with the deuterium dilution technique,18-20 and note that test weighing is likely to remain a valuable method.21 The weighing was only used at the hospital, whereas at home, the duration of active lactation was measured, to compare the groups. As this study was conducted in two different hospitals, several confounders were taken into consideration. Several meetings and training sessions prior to and during the study were held for the researchers and staff from the two study units, to minimize the disagreement. Another limitation is that stool cultures for some bacterial pathogens were not done, e.g. serotyping for *E. coli* Campylobacter, and Cryptosporidium due to the budget limitation.

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This study demonstrates the efficacy of tempe produced traditionally and industrially in comparison to soy formula in reducing symptoms of diarrhea in 6-24 months old children with acute diarrhea. In contrast to former studies conducted by our investigators the difference between the control and the treatment groups was not significant in this study. The reason may be that in previous studies the control group did
not receive any supplement in addition to their daily foods.\textsuperscript{10,23} Besides, the control group in this study consumed more breast milk significantly.

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**TABLE 5. FOOD INTAKE**

|                | TT       | IT       | IS       | F       | p       |
|----------------|----------|----------|----------|---------|---------|
| 1. Study formula (kcal/kg/day) |          |          |          |         |         |
| at hospital    | 20.2±8.6 | 19.2±8.9 | 19.4±7.5 | 0.232   | 0.784   |
| at home        | 18.9±8.8 | 18.3±8.2 | 17.6±2.0 | 0.421   | 0.657   |
|                | (n: 72)  | (n: 72)  | (n: 68)  |         |         |
| 2. Breast milk (kcal/day)    |          |          |          |         |         |
| at hospital    | 193.6±153.6 | 180.0±147.8 | 193.2±162.3 | 0.118   | 0.888   |
| at home        | 187.2±80.9 | 212.9±92.9 | 236.4±113.5 | 3050    | 0.045   |
|                | (n: 47)  | (n: 42)  | (n: 48)  |         |         |
| 3. Bottle milk (kcal/kg/day) |          |          |          |         |         |
| at hospital    | 26.8±12.5 | 32.3±16.7 | 33.3±18.2 | 1108    | 0.336   |
| at home        | 19.1±15.3 | 19.1±14.8 | 22.4±16.8 | 0.507   | 0.604   |
|                | (n: 23)  | (n: 26)  | (n: 31)  |         |         |
| 4. Solid food (kcal/kg/day)  |          |          |          |         |         |
| at hospital    | 37.4±28.2 | 31.7±24.9 | 41.3±27.9 | 1167    | 0.315   |
| at home        | 57.8±28.3 | 53.6±21.6 | 48.7±20.9 | 2540    | 0.081   |
|                | (n: 31)  | (n: 40)  | (n: 33)  |         |         |
| 5. Tempe in solid food (kcal/kg/day) | |          |          |         |         |
| at home        | 1.6±1.1 | 2.1±2.6 | 1.8±1.7 | 0.993 | 0.373 |
|                | (n: 60) | (n: 63) | (n: 55) |         |         |
| 6. Total caloric intake (kcal/kg/day) | |          |          |         |         |
| at hospital    | 391.3±245.2 | 418.09±245.30 | 440.68±275.00 | 0.555 | 0.575 |
|                | (n: 66)* | (n: 66)* | (n: 58)* |         |         |
| at home        | 342.0±265.4 | 347.4±265.1 | 410.2±261.8 | 0.921 | 0.400 |
|                | (n: 46)** | (n: 53)** | (n: 43)** |         |         |
|                | 784.0±375.7 | 721.1±319.4 | 727.8±303.6 | 0.760 | 0.468 |
|                | (n: 72)* | (n: 72)* | (n: 68)* |         |         |
|                | 667.5±398.8 | 602.8±320.7 | 568.8±283.0 | 0.545 | 0.216 |
|                | (n: 72)** | (n: 72)** | (n: 68)** |         |         |

*not including study formula = total of 2, 3, 4, 5, and 6
**not including: study formula & breast milk = total of 3, 4, 5 and 6
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