

## The correlations between gut microbiota of Muslim Thai lactating women and their dietary intake and gut microbiota of breastfed infants

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### ABSTRACT

**Introduction:** Foods and nutrients are essential not only for human health, but also for the balance of gut microbiota. This research aimed to correlate the gut microbiota of lactating women with their food/ nutrient intakes, as well as with their infants' gut microbiota. **Methods:** A cross-sectional study was conducted in 27 pairs of mothers and their exclusively breastfed infants. For lactating women, the dietary assessment was conducted by 24-hour recall, and food groups were assessed following the Food and Agriculture Organization's guidelines, while nutrient intake was analysed using INMUNCAL V3 programme. Gut microbiota of mothers and infants were measured in stool samples using fluorescent *in situ* hybridisation technique. **Results:** It was found that energy intake of mothers was only 66% of the recommended Thai Dietary Reference Intakes (DRIs). Most micronutrient and dietary fibre intakes were below the Thai DRIs. Vitamin A (VA)-rich fruits and vegetables food group correlated positively with *Lactobacillus* species (spp). The association between gut microbiota and nutrient intake of lactating women showed that total protein, phosphorus, and VA were positively correlated with *Bifidobacterium* spp.; while  $\beta$ -carotene and vitamin C were also positively correlated with *Lactobacillus* spp. In contrast, consumption of eggs and calcium correlated negatively with *Clostridium* spp./ *Enterobacter* spp. *Bifidobacterium* spp. and *Lactobacillus* spp. of lactating women and breastfed infants showed strong correlations. **Conclusion:** Food and nutrient intakes of lactating women were correlated with their *Clostridium* spp./ *Enterobacter* spp., *Bifidobacterium* spp. and *Lactobacillus* spp. Furthermore, *Bifidobacterium* spp. and *Lactobacillus* spp. of mothers and breastfed infants showed strong correlations.

**Keywords:** correlation, exclusively breastfed infants, food group, gut microbiota, lactating women, nutrient intakes

### INTRODUCTION

Formerly, newborn infants were considered to be in a sterile intrauterine environment (Karakochuk *et al.*, 2017), with their first exposure to microbes

occurring during breastfeeding. However, recent studies have suggested that the foetus does not reside in a sterile intrauterine environment, since commensal bacteria from the maternal

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gut/bloodstream can enter the amniotic fluid by crossing the placenta (Walker, 2017). In addition, newborns are also exposed to microbes at birth. The first gut microbiota that interact with an infant have significant roles in stimulating the immune system, facilitating maturation of the immune system, programming a healthy immune development, and metabolic programming (Karakochuk *et al.*, 2017).

The bacterial composition of gut microbiota in breastfed infants is closely related to their mothers'. González *et al.* (2013) found a positive correlation between maternal milk and infant's faecal microbiota for *S. epidermidis* group, analysed by polymerase chain reaction (PCR). There are several reasons why a correlation exists between a lactating woman and her infant's gut microbiota. The maternal gut microbiota may promote microbiota colonisation of the infant gut prior to birth *in utero*, after delivery, or later through breastfeeding (McDonald & McCoy, 2019). Oligosaccharides in breast milk promote the growth of *Lactobacillus* and *Bifidobacterium*, which dominate the infant gut (Mueller *et al.*, 2015). Maternal microbiota at different body sites and in the gut provide exposure to an infant at the initial stages of life from holding, cuddling and kissing (Farren & Turner, 2017), supplying viable microbiota.

An association between the gut microbiota and the infant's nutritional status was found for *Streptococcus*, *Clostridium leptum*, and *Clostridium coccoides*, which were significantly increased in infants with malnutrition (González *et al.*, 2013). A recent study has shown that changes in gut microbiota profile among Thai urban children were related to the types of food consumed (Nakayama *et al.*, 2015). The types of food and nutrients consumed in the dishes are primary factors influencing gut microbiota. For

example, fermentation of undigested dietary components generates short-chain fatty acids (SCFA) that are essential for the growth of beneficial bacteria in the intestine (Telle-Hansen, Holven & Ulven, 2018). Polyphenols, fibres, and carbohydrates are dietary factors that can modify the balance of gut microbiota, whereas illness, stress, ageing, bad dietary habits, and lifestyle can cause changes in the gut microbiota (Zhang *et al.*, 2015).

Recently, a study of the interrelation between nutrient/nutritional status and gut microbiota has recognised this as a determinant of human health (Hills *et al.*, 2019). However, there are needs of supporting evidence, especially of the relationship between gut microbiota in lactating women with their diet and nutrient intakes; as well as of the gut microbiota in their exclusively breastfed infants. Our focus was on southern peninsular Thailand, where the culture and eating habits differ from other parts of the country. Thus, this study aimed to correlate the gut microbiota in lactating women with their food/nutrient intakes, and with their exclusively breastfed infants' gut microbiota.

## **MATERIALS AND METHODS**

### **Study site and participants**

A descriptive cross-sectional study was conducted in a sample of 27 Thai Muslim lactating women and their exclusively breastfed infants. This study was part of a main study named "the efficacy of vitamin A (VA) fortified rice in lactating Thai women". The details of the study protocol and sample size calculation are described in a previous report (Pinkawee *et al.*, 2021). Briefly, healthy women with single birth, no gestational diseases, in the age range of 20-40 years old, and their exclusively breastfed infants with a birth weight of >2,500g, were recruited for the study from January 2017 to

February 2018. The study site was located in the Muang district of Pattani province, in southern Thailand.

Before recruitment, details of the study protocol were explained to the potential participants. Informed consent was obtained from the participants, as well as on behalf of their infants.

### Data collection

#### Dietary intake

Dietary intakes of the lactating women were assessed by 24-hour recall for three non-consecutive days (two weekdays and one weekend). The data consisted of food items, types of food, food components, cooking method, and the amount (weight/size) of the consumed portion. The data were analysed by INMUCAL V3 programme (Mahidol University, Thailand) for nutrient intakes and reported in % Thai Dietary Reference Intake (DRI).

In addition to that, data of food items from the 24-hour recall were also grouped. The nine food groups assessed were according to the Food and Agriculture Organization (FAO) guidelines (FAO, 2011). The amounts consumed for (1) starchy staples, (2) dark green leafy vegetables, (3) other VA-rich fruits and vegetables, (4) other fruits and vegetables, (5) organ meat, (6) meat and fish, (7) eggs, (8) legumes, nut and seeds, and (9) milk and milk products, were estimated by using the same programme (INMUCAL V3 programme).

### Gut microbiota determination

Stool samples of lactating women and their breastfed infants were collected during the first month of breastfeeding. At the time of stool sample collection, the participants were informed about instructions that must be followed on collecting the stool samples. Stool samples of both mother and infant had to be collected on the same day by using

a sterile plastic teaspoon and placed into a sterile small plastic box. Then they were brought to the laboratory and kept in a freezer at  $-80^{\circ}\text{C}$  for gut microbiota analysis.

All stool samples were analysed specifically for the bacteria *Bifidobacterium* species (spp.) and *Lactobacillus* spp. as representatives of beneficial bacteria, and *Clostridium* spp./*Enterobacter* spp. as representative of pathogenic bacteria, using DNA probes (Bif164, Lab158, Chris150) (Lucigen, Wisconsin, USA). The fluorescent *in situ* hybridisation (FISH) technique was used to measure the gut bacteria in stool samples following laboratory protocols of the Nutraceutical and Functional Laboratory (Faculty of Agroindustry, Prince of Songkla University, Hatyai, Thailand) (Plongbunjong *et al.*, 2017). Data were reported as  $\log_{10}$  cell/g faecal sample.

### Ethical clearance

The study protocol was approved by the Research Ethics Committee for Science, Technology and Health Science, Prince of Songkla University, Pattani campus (ERC No. psu.pn.1-005/59). The protocol was registered with ClinicalTrials.gov (ID: NCT03056625).

### Statistical analysis

The gut microbiota enumeration by FISH technique was presented as  $\log_{10}$  cell/g faecal sample. Data on nutrient intakes were analysed with INMUCAL V-3 programme (Mahidol University, Thailand) and were presented as % Thai DRI. Data were normally distributed as checked by Shapiro-Wilk test ( $p>0.05$ ) and were expressed as mean $\pm$ standard deviation (*SD*). The correlation between gut microbiota in lactating women versus nutrient intakes, amount of food consumption (nine groups), and gut microbiota of infants were assessed

using Pearson's correlation. All data were statistically analysed with R programme i386 version 3.5.2.

## RESULTS

The average age of mothers and infants were 29.2 years old and one month old, respectively. All infants were exclusively breastfed, and 70.4% were born via vaginal delivery. All of the participants were Muslim. The average *Bifidobacteria* spp., *Lactobacillus* spp., and *Clostridium* spp./*Enterobacter* spp. in lactating women were  $9.70 \pm 0.28$ ,  $9.75 \pm 0.36$ , and  $9.76 \pm 0.42$   $\log_{10}$  cell/g faecal sample, respectively. For infants, the average *Bifidobacteria* spp., *Lactobacillus* spp., and *Clostridium* spp./*Enterobacter* spp. were  $9.82 \pm 0.35$ ,  $9.71 \pm 0.36$ , and  $9.70 \pm 0.60$   $\log_{10}$  cell/g faecal sample, respectively.

### Dietary intake of lactating women

#### *Food group consumption of lactating women*

The amounts of daily consumption (per day) for the nine groups of food, as presented in Table 1, were assessed following the FAO guidelines (FAO, 2011). A total of 642 g of starchy staple food

was consumed daily, and this was the main food group consumed by lactating women. This was followed by other fruits and vegetables (117.8 g), milk and milk products (113.8 g), and meat and fish (112.0 g). Other VA-rich fruits and vegetables group had the lowest (11.5 g) intake.

#### *Nutrient intakes of lactating women*

On average, the energy intake of the participants was 1,476 kcal/day and this only accounted for 66% of the Thai DRI. The energy distribution of carbohydrate:protein:fat was in the normal range (64:15:21). Figure 1 showed that both the energy and protein intakes of participants were below the Thai DRIs and estimated average requirement (EAR~80% of Thai DRIs).

Most of the micronutrients (Figure 1) and dietary fibre consumed were below the Thai DRIs. Daily VA and iron consumption, which are essential for lactating women, were found to be deficient compared to the recommendation (14% and 17% of Thai DRIs, respectively). The consumption of other important micronutrients by

**Table 1.** The average consumption of each food group by lactating women (g/day)

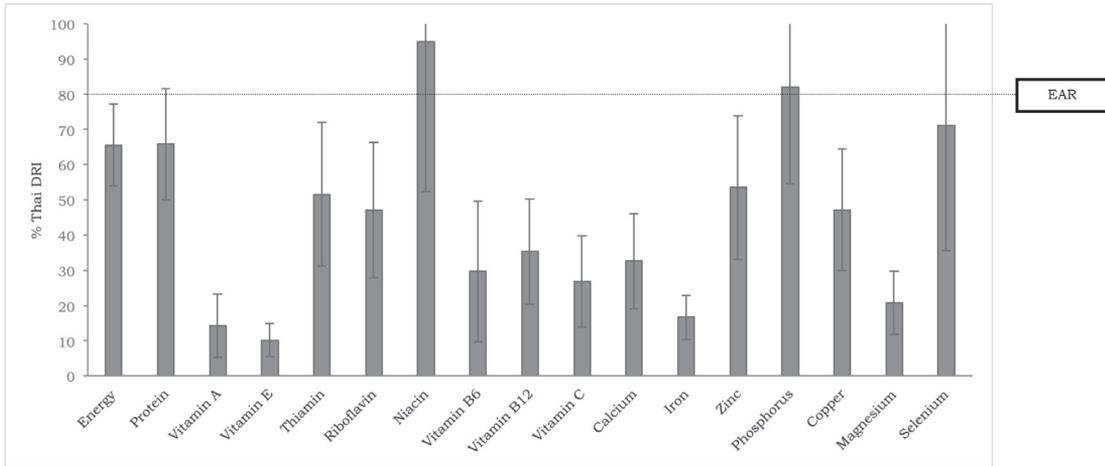
<i>Food group</i>	<i>Total (n=27)</i>
Starchy staples <sup>†</sup> (g)	642.0±217.3
Dark green leafy vegetables (g)	13.1±2.6
Other vitamin A-rich fruits and vegetables <sup>‡</sup> (g)	11.5±4.3
Other fruits and vegetables <sup>§</sup> (g)	117.8±50.8
Organ meat (g)	41.8±10.4
Meat and fish <sup>¶</sup> (g)	112.0±41.4
Eggs (g)	36.8±9.7
Legumes, nuts and seeds (g)	18.9±3.0
Milk and milk products (g)	113.8±28.8

<sup>†</sup>The starchy staples food group is a combination of cereals and white roots and tubers.

<sup>‡</sup>The other VA-rich fruits and vegetables group is a combination of VA-rich vegetables and tubers and VA-rich fruits (e.g. the plants with red, yellow, orange colour).

<sup>§</sup>The other fruits and vegetables group is a combination of other fruits and other vegetables (excluding dark green leafy vegetables and other vitamin A-rich fruits and vegetables<sup>‡</sup>).

<sup>¶</sup>The meat group is a combination of meat and fish.



**Figure 1.** The nutrient intakes of lactating women relative to Thai DRIs. The dotted line stands for EAR=estimated average requirement (IOM, Food and Nutrition Board, 1998)

lactating women, such as calcium, zinc, thiamine, and vitamin C, were also below the EARs (33%, 53%, 52%, and 27% of Thai DRIs). The intake of dietary fibre was only 6.8 g/day compared to the recommended daily intake of 25 g/day. Phosphorus and niacin were the only two nutrient intakes higher than the EARs (>80% of Thai DRIs.)

**Correlation of gut bacteria and dietary intake of lactating women**

*Correlation of gut bacteria and food group intakes of lactating women*

Table 2 shows the associations between the group of foods consumed by lactating women and *Bifidobacterium* spp., *Lactobacillus* spp., and *Clostridium* spp./*Enterobacter* spp. Among the nine

**Table 2.** Correlation coefficients (cc) of food group consumption and gut bacteria in lactating women

Food group	Correlation coefficients		
	<i>Bifidobacterium</i> spp.	<i>Lactobacillus</i> spp.	<i>Clostridium</i> spp./ <i>Enterobacter</i> spp.
Starchy staples	-0.15	0.31	0.09
Dark green leafy vegetables	0.23	-0.05	-0.19
Other vitamin A rich fruits and vegetables	-0.03	0.47*	-0.03
Other fruits and vegetables	-0.16	-0.26	-0.23
Organ meat	-0.18	0.03	-0.27
Meat and fish	0.05	-0.15	-0.14
Eggs	-0.03	0.07	-0.45*
Legumes, nut and seeds	0.13	0.004	0.32
Milk and milk products	0.13	-0.15	0.11

\*correlation (Pearson's correlation) is significant at the 0.05 level (2-tailed)

groups of food consumed, *Lactobacillus* showed a positive correlation with “other VA-rich fruits and vegetables”. However, lactating women with high egg consumption showed a negative correlation with *Clostridium* spp./*Enterobacter* spp.

*Correlation of gut bacteria and nutrient intakes of lactating women*

The correlations of *Bifidobacterium* spp., *Lactobacillus* spp., and *Clostridium* spp./*Enterobacter* spp. and nutrient intakes are shown in Table 3. Proteins, protein from animal, phosphorus, and VA were positively correlated with

*Bifidobacterium*, while β-carotene and vitamin C were positively correlated with *Lactobacillus* spp. However, *Clostridium* spp./*Enterobacter* spp. was negatively correlated with calcium.

**Correlation of gut bacteria between lactating women and their breastfed infants**

As shown in Table 4, *Bifidobacterium* spp. and *Lactobacillus* spp. of lactating women and infants were positively correlated. However, no correlation was detected for *Clostridium* spp./*Enterobacter* spp.

**Table 3.** Correlation coefficients (cc) of nutrient intakes with gut bacteria in lactating women

Nutrient	Correlation coefficients		
	<i>Bifidobacterium</i> spp.	<i>Lactobacillus</i> spp.	<i>Clostridium</i> spp./ <i>Enterobacter</i> spp.
Energy	0.21	0.06	0.28
Carbohydrate	0.31	0.18	0.39
Protein	0.55*	0.27	0.17
Protein-animal	0.55*	0.22	0.23
Protein-vegetable	0.39	0.31	0.32
Fat	-0.19	-0.21	-0.35
Calcium	-0.20	0.15	-0.50*
Phosphorus	0.50*	0.19	0.02
Iron	0.31	-0.08	0.07
Copper	-0.07	-0.15	0.28
Magnesium	0.27	0.32	-0.12
Selenium	0.33	0.15	0.31
Zinc	0.22	-0.10	-0.06
Vitamin A	0.50*	0.37	0.27
Retinol	-0.21	0.06	-0.07
Beta-carotene	-0.32	0.49*	-0.16
Thiamin	0.15	0.01	0.01
Riboflavin	-0.08	-0.37	-0.39
Vitamin B6	0.34	-0.14	0.01
Vitamin B12	0.25	-0.10	-0.09
Vitamin C	-0.29	0.47*	0.24
Niacin	0.28	0.20	0.08
Vitamin E	-0.16	-0.40	-0.29
Dietary fibre	0.13	-0.07	0.04

\*correlation (Pearson’s correlation) is significant at the 0.05 level (2-tailed)

**Table 4.** Correlation coefficients (cc) of gut bacteria in lactating women and in their infants

Bacteria	Lactating women bacteria		
	<i>Bifidobacterium</i> (cc)	<i>Lactobacillus</i> (cc)	<i>Clostridium/</i> <i>Enterobacter</i> (cc)
Infant bacteria			
Bifidobacterium (cc)	0.53**	0.47*	0.36
Lactobacillus (cc)	0.62**	0.51*	0.29
Clostridium/Enterobacter (cc)	0.17	0.40	0.01

\*correlation (Pearson's correlation) is significant at the 0.05 level (2-tailed)

\*\*correlation (Pearson's correlation) is significant at the 0.01 level (2-tailed)

cc, correlation coefficient

## DISCUSSION

Data on the average food consumption (nine food groups) and nutrient intakes of mothers revealed that lactating women in this study area may have a risk of undernutrition, especially a lack of vitamin A. This is because most of the nutrient and energy intakes of lactating women were below the Thai DRIs and EARs. The average energy intake was only 1,476 kcal/day, which accounted for 66% of the Thai DRI, while the average VA intake of participants was only 14% of the Thai DRI. The percentage of VA was lower than in other developing countries such as Indonesia and China. VA intake of lactating women in Indonesia was 64% of Angka Kecukupan Gizi (AKG), while China had 100.1% of its Chinese Recommended Nutrient Intake (RNI) (Chen *et al.*, 2012; Madanijah *et al.*, 2016). The main study had revealed that almost 50% of subjects had VA deficiency based on total VA liver reserve (Pinkaw *et al.*, 2021), while the average body mass index (BMI) was 22.5±3.9 kg/m<sup>2</sup> (Azis, Pinkaw & Wichienchot, 2021). However, according to Swalarz *et al.* (2018), BMI is not the best indicator of malnutrition, because malnutrition already occurs much earlier and there is no cut-off for lactating women. The subjects had low energy intake compared to the requirement for lactating women, mainly due to low socio-economic status

with an average family income of USD500 per month to support around five people in a family (Azis *et al.*, 2021). Moreover, food taboos of the Muslim people in the study area, which only allowed the consumption of rice and grilled or fried fish during the first period of lactation (30 days – three months) may have further contributed to the lack in energy intake.

Among the nine food groups consumed by lactating women (FAO, 2011), rice was the main starchy staple with the highest amount of consumption (642 g/day). This was the food group that contributed the main portion of calories and other nutrients to these lactating women.

The correlations between gut bacteria in lactating women and food groups showed that the “other VA-rich fruits and vegetables” was positively correlated with *Lactobacillus* spp. A study showed that *Lactobacilli* are able to use foods as their carbon source to support their growth and maintain the environmental pH (Walter, 2008). Previous *in vivo* and *in vitro* studies have found an interrelationship between *Lactobacillus* spp. and VA group of foods (Lee & Ko, 2016). However, the underlying mechanism has not been clearly elaborated. Cell differentiation and maintenance of healthy skin, mucous membranes, and soft tissues,

are the major functions of VA (Huang *et al.*, 2018). Therefore, VA could potentially help in promoting differentiation of healthy villi in the gastrointestinal tract. Moreover, fruits and vegetables are known as important sources of carbohydrates and prebiotics, such as fructooligosaccharides, oligofructose, inulin, galactose, and xylose, which contain oligosaccharides as energy sources used by the gut microbiota (Azad *et al.*, 2018).

The results revealed that *Bifidobacterium* spp. was positively associated with total protein, protein from animal sources, phosphorus, and VA intake. Garcia-Mantrana *et al.* (2018) reported that protein-rich foods have significantly high bifidobacteria counts and significant positive correlations with various groups of butyrate-producing bacteria within the faecal microbial composition, along with higher concentration of SCFA. In haemodialysis patients, *Bifidobacteriaceae* was positively correlated with phosphorus. *Bifidobacterium* spp. may regulate the absorption of phosphate in the intestine and use it as one of their energy sources (Miao *et al.*, 2018).

*Lactobacillus* spp. was positively correlated with  $\beta$ -carotene and vitamin C.  $\beta$ -carotene is a good source of VA. Therefore, this could support our finding of a positive correlation between "other VA-rich fruits and vegetables" and *Lactobacillus* spp. A prior study found that beta-carotene as pro-VA carotenoid promoted *Bifidobacteria* and *Lactobacillus* via fermentation of the substances bound with  $\beta$ -carotene in the food matrix (O'Callaghan & van Sinderen, 2016). The proposed mechanism of the correlation between vitamin C and *Lactobacillus* spp. is that vitamin C might stimulate the growth of *lactobacilli* by increasing the acidity of the medium, as probiotics prefer an acidic gastric

environment (Stadler & Viernstein, 2003). *Lactobacilli* of intestinal origin are considered intrinsically resistant to acidic environments and are often employed in fermented foods as probiotics (Corcoran *et al.*, 2005). On the other hand, calcium was negatively correlated with *Clostridium* spp./*Enterobacter* spp. Previously, calcium was also reported to be negatively correlated with *Clostridium coccoides* and *Clostridium leptum* in obese mice, which is in line with our finding. Calcium can modulate gut microbiota in a specific and prebiotic manner (Chaplin *et al.*, 2016).

The association between gut microbiota of lactating women and those of their breastfed infants has been confirmed in our Muslim population. *Bifidobacterium* spp. and *Lactobacillus* spp. in lactating women and infant pairs were positively correlated. According to Karakochuk *et al.* (2017), the gut microorganisms in mothers are closely similar with their infants'. This is because of their food pattern, environment, activity, and daily interaction, as well as the mode of delivery. *Bifidobacteria* species e.g. *B. breve*, *B. longum* spp., *B. bifidum*, *B. adolescentis*, *B. dentium*, and *B. pseudocatenulatum*, which were all identified in infant faeces, were also found in the corresponding milk samples of the mother (Duranti *et al.*, 2017). The transmission of *Bifidobacteria* and *Lactobacillus* of lactating women to her infant suggests that human milk is a potential vehicle to facilitate this acquisition.

This is the first study on Thai Muslim population that presented a correlation between gut bacteria and dietary/nutrient intakes of lactating women, and also of gut bacteria in their exclusively breastfed infants. Our data showed that *Lactobacillus* spp. of lactating women was positively correlated with VA-rich fruits/vegetables food group

(providing carotenoids),  $\beta$ -carotene (major source of carotenoids in the diet), and *Lactobacillus* spp. of their breastfed infants. Thus, VA/  $\beta$ -carotene in the diet of mothers could be a factor promoting the growth of *Lactobacillus* spp., not only in the mothers themselves, but also in their infants.

A limitation of this current study was the rather small sample size (27 pairs of lactating women and breastfed infants) due to the high rate of dropouts, mainly caused by inability to exclusively breastfeed. As a limitation of descriptive cross-sectional study, it was not possible to establish a true cause and effect. Therefore, a longitudinal or experimental study should be conducted to confirm this finding. In addition to that, aside from diet/nutrients, several other factors such as maternal size, mode of delivery, feeding practices, and body contact are also known to affect the gut microbiota of lactating women and infants; these could be addressed by a future study together with nutritional factors.

The findings from this study supported the need for encouraging exclusive breastfeeding for the first six months of a newborn's life, together with giving nutrition education to mothers. These strategies could shape the nutritional status and promote the growth of beneficial bacteria (*Lactobacillus* spp. and *Bifidobacterium* spp.) in both mothers and infants.

## CONCLUSION

Based on the dietary assessment, the lactating women in this study area were at risk of undernutrition and rice was consumed as the main staple food. The "VA-rich fruits and vegetables" food group correlated positively with *Lactobacillus* spp. Egg consumption correlated negatively with *Clostridium* spp./*Enterobacter* spp. Regarding associations between

gut microbiota and nutrient intakes in lactating women, total protein, protein from animal sources, phosphorus, and VA were positively correlated with *Bifidobacterium* spp. Beta-carotene and vitamin C were also positively correlated with *Lactobacillus* spp., but calcium was negatively correlated with *Clostridium* spp./*Enterobacter* spp. Finally, *Bifidobacterium* spp. and *Lactobacillus* spp. of lactating women and their breastfed infants showed strong correlations.

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## Authors' contributions

LA, conducted the dietary assessment, measured gut microbiota enumeration, statistically analysed the data, and drafted the manuscript; SW, designed the research and supervised gut microbiota determination; SP, designed the research, conducted the dietary assessment, and statistically analysed the data. All authors read and approved the final manuscript.

## Conflict of interest

LA, SW and SP have no conflicts of interest.

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