Original Article

Effects of *Bifidobacterium animalis lactis* HN019 (DR10^{TM}), inulin, and micronutrient fortified milk on faecal DR10^{TM}, immune markers, and maternal micronutrients among Indonesian pregnant women

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Background and Objectives: Maternal nutrition affects fetal growth and development. This study evaluates the effects of milk powder fortified with micronutrients, docosahexaenoic acid (DHA), a prebiotic, and probiotic *Bifidobacterium animalis* subsp. *lactis* HN019 DR10^{TM} on the micronutrient status, as well as the presence of faecal probiotic and immune markers in pregnant women. **Methods and Study Design:** This randomised, double-blind, placebo-controlled trial was conducted at Budi Kemuliaan and Cipto Mangunkusumo Hospital in Jakarta from 2013 to 2014. A total of 104 participants were randomly allocated to receive either completely enriched milk powder (intervention group) or iron- and vitamin folic-acid-enriched milk powder (control group). Data were collected using standardised measures and were statistically analysed using the independent t or Mann–Whitney test. **Results:** At the baseline, the micronutrient status of the participants was acceptable, except for 25-OH-vitamin D, in both the intervention and control groups. Vitamin B-1, zinc, total free fatty acid, linoleic acid, arachidonic acid, and DHA were significantly higher in the intervention group in the second trimester (p=0.014, 0.028, 0.023, 0.014, 0.001, and 0.032, respectively). Interleukin-6 and tumor necrosis factor-α levels did not significantly vary during pregnancy. *B. animalis* subsp. *lactis* DR10^{TM} was present in the faeces of the intervention group but not the control group (61.1% vs 0%). **Conclusion:** Milk fortified with a prebiotic, probiotic, DHA and micronutrients increases the faecal concentration of the organism used for fortification in Indonesian pregnant women. This may represent an improvement in intra-partum maternal gut health.

Key Words: probiotic, micronutrient, milk, pregnant, digestive health, vitamin folic-acid, homocysteine

INTRODUCTION

Maternal nutrition affects fetal growth and development, and inadequate dietary intake is one of the causes of poor maternal nutritional status. The Indonesian Recommended Dietary Allowances (RDAs) suggest that pregnant women should consume additional calories, protein, and certain micronutrients [(additional 180 kcal during trimester 1 and 300 kcal during trimesters 2 and 3), 20 g of additional protein, 300 ug of additional retinol equivalent of vitamin A during trimester 1 and 2, 350 ug during trimester 3, 200 μg of folic-acid, 200 mg of calcium, 2-10 of zinc, and 5 mg of selenium].[1,2] Although there is no recommended higher intake of iron (from 12 mg), iron intake is often low in women before pregnancy. Therefore, the Indonesian government recommends iron supplementation in addition to folic-acid supplementation for pregnant women. The Indonesian RDAs for pregnant women can be achieved by consuming a balanced diet. However, surveys conducted on pregnant Indonesian women have revealed that many women do not achieve their recommended calorie or protein intake, and the status of key nutrients such as vitamins (A, folic-acid, and D), iron, and zinc may be highly inadequate.[3-5] Nutrient supplementation, both food- and pharmaceutical-based, is an appropriate strategy for improving the nutritional status of pregnant women. Among the food-based nutrient supplements, milk is a particularly efficient vehicle for delivering additional nutrients to pregnant women.[6,7] Evidence suggests that moderate milk consumption during pregnancy is positively related to fetal growth and infant birth weight.[8]

Maternal immune function is an important determinant of pregnancy outcomes. During pregnancy, the maternal

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immune system changes to enable tolerance to paternal antigens expressed on fetal cells. Hormonal changes during pregnancy facilitate this event at the feto–maternal interface. Furthermore, the cytokine profile is modified, in which the Th2 cytokines are enhanced, whereas the Th1 response is inhibited.[9] Maternal nutrition can play a crucial role in immune function during pregnancy.[10,11] In association with immune function, the commensal gut microbiota plays major roles in human health by providing several mechanisms including a natural defence mechanism against invading pathogenic bacteria.[12] However, no studies have sufficiently explained this phenomenon during pregnancy.

The probiotic strain *Bifidobacterium animalis* subsp. *lactis* HN019 (DR10™) has proven gut function and immune modulation benefits.[13-16] These positive effects may influence numerous factors in pregnancy including nutritional status, gut symptoms, immune status, and other health outcomes. The present study evaluated the effects of milk powder fortified with micronutrients, docosahexaenoic acid (DHA), a prebiotic (inulin), and *B. animalis* subsp. *lactis* DR10™ on the micronutrient status of as well as detection of faecal probiotic and immune markers in pregnant women.

MATERIALS AND METHODS

Study design

Pregnant women were examined in this randomised, double-blind, placebo-controlled trial. The inclusion criteria were women who were aged 18–35 years, were residents of Jakarta and likely to remain in the city for 1 year, had singleton pregnancy at 8–12 weeks of gestation, were apparently healthy, and had an uncomplicated pregnancy. Exclusion criteria were milk avoidance, hyperemesis gravidarum, use of antibiotic and anti-inflammatory drugs, regular consumption of prebiotic- and probiotic-supplemented yogurt, allergy to any ingredients in the investigational products, and a previous neural tube defect (NTD)-affected pregnancy.

This study assessed the primary outcome of DR10™ faecal concentration and evaluated the effects of probiotics and micronutrient-fortified milk consumption during pregnancy. A sample size of 50 participants per group was calculated to be necessary for the detection of a clinically relevant difference of 0.6 standard deviations, with a power of 80%. The study recruited 60 participants per group to allow for an anticipated 20% dropout rate. The study was exploratory for only secondary outcomes, such as birth anthropometrics, because it may not be adequately powered. This dropout rate corresponds to the exclusion criteria such as not consuming milk for 1 week and fetal defects that require the termination of pregnancy.

After written informed consent was obtained, the participants were enrolled in the study (n=143) and stratified into two groups: control (n=74) and intervention (n=69) groups (Figure 1). The control milk powder was fortified with folic-acid and iron, and the intervention milk powder was fortified with folic acid, iron, DHA, *B. animalis* subsp. *lactis* DR10™, and inulin (Table 1). The study milk powders were identical in colour, taste, and smell, and they were manufactured by Fonterra Brands (Singapore) Pte Ltd. The participants were asked to consume 75 g of milk powder daily, which was reconstituted as 37.5 g of powder in 200 mL of water twice daily (morning and

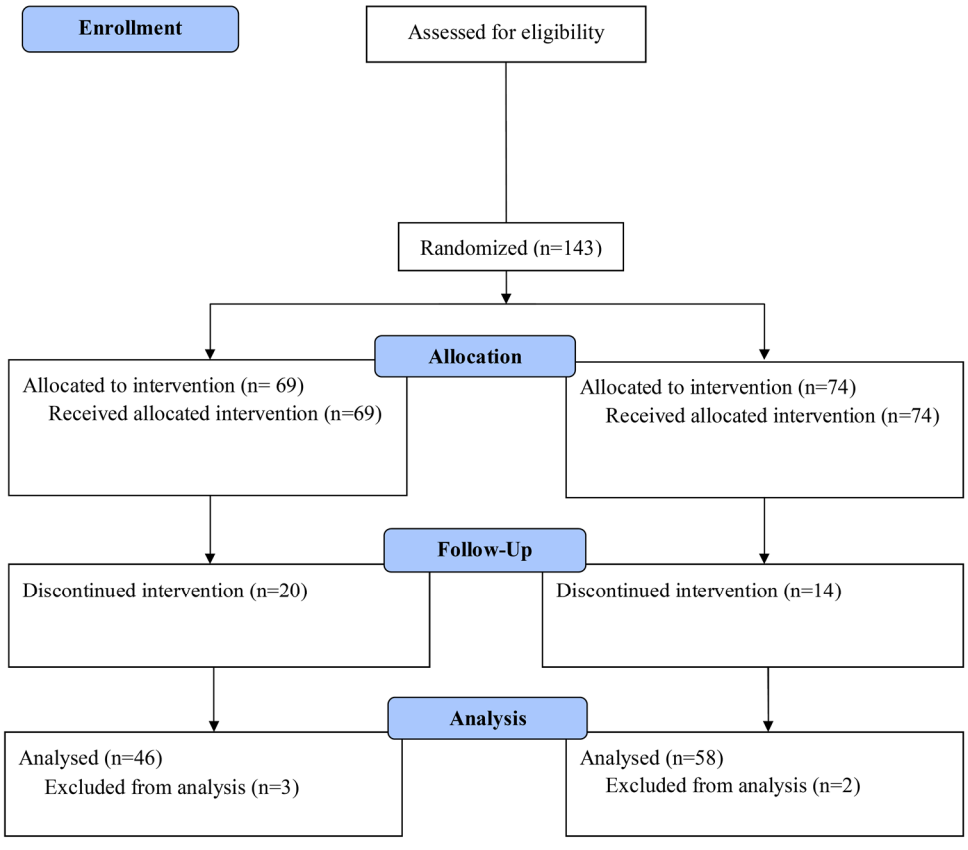

Figure 1. Flow diagram of the subjects.

Table 1. Product nutritional profile, per serve, 2 x 200ml serves per day required

Nutrient	Control milk powder (2 serves X per day)†	Intervention milk powder (2 serves X per day)
Energy, kcal	150	150
Protein, g	8.9	8.9
Carbohydrate, g	17	17
Fat, g	4.4	4.4
Iron, mg	8.25	8.25
Folic acid, ug	200	200 ug
Prebiotics (FOS/Inulin), g	-	2.5
Probiotic *Bifidobacteria animalis* subsp. *lactis* (DR10^{TM}), cfu	-	5 x 10^6
Calcium, mg	360	500
Zinc, mg	1.3	5.25
Vitamin A, ug	56	230
Vitamin D, ug	0.4	2.5
Thiamin, mg	0.1	0.6
Riboflavin, mg	0.7	0.7
Vitamin B-12, ug	1.3	1.3
Vitamin C, mg	4.2	42

†Fortified only with iron and folic acid, all other nutrients are natural levels.

evening) during the supplementation period. Baseline examinations included weight and height measurements, blood pressure assessment, general health examinations, faecal *Bifidobacteria animalis* subsp. *lactis* (DR10^{TM}) detection, immune and micronutrient status assessment. All participants attended monthly monitoring or check-ups at the maternity clinic for weight and blood pressure measurement, underwent fetal growth detection through ultrasound, and completed a gut symptom questionnaire. The nutrient intake was analysed from the dietary 24-hour recall and semiquantitative food frequency questionnaire data by converting it using the free Nutrisurvey 2007 food-processor software. For the actual nutrient intake, the 24-hour recall data were more relevant for analysis.

All participants were monitored for compliance and general health by conducting a survey for illnesses, infections, hospital visits, and antibiotic use. At the routine visits, the participants were asked a series of questions about their general health and well-being including gastrointestinal symptoms (experience of nausea, vomiting, constipation, bloating, and diarrhoea), cold and flu symptoms, and tiredness. Any non-pregnancy-related complaints or extra doctor visits were recorded, including the reason for the complaint and any medications prescribed.

Study overview

This study was undertaken at Cipto-Mangunkusumo and Budi Kemuliaan hospital in Jakarta during 2013 until 2014. The participants provided written informed consent before recruitment and enrolment to the study. The study protocol was approved by the Ethics Committee of the University of Indonesia (71/H2.F1/ETIK/2013) and registered at clinicaltrials.gov.

Data collection

Blood samples were collected and analysed by using standardized laboratory test for nutritional status at the baseline (8–10 weeks) and at 24–26 and 36–38 weeks of gestation by measuring changes in haemoglobin; transferrin; complete blood count; serum ferritin, retinol, 25-OH-vitamin D, B-12, zinc, and phospholipid; plasma folic acid; DHA; and vitamins B-1 and B-2. All the water- and fat-soluble vitamins was measured by reversed-phase high-performance liquid chromatographic tandem mass spectrometry (RP-HPLC-MS/MS) procedures.

Moreover, we analysed the maternal microbiota in faecal samples collected at the aforementioned time points through qPCR for *B. lactis* HNO19 (DR10^{TM}). The DNA level expression measure is by enzymatic amplification on the DNA segment target selectively based on the chosen primer in thousand per-million copies to be detected.

The immune system profile was analysed for tumour necrosis factor (TNF)-α, interleukin (IL)-6, and polymorphonuclear leukocyte phagocytosis by using peripheral blood samples collected at the baseline (8–10 weeks) and at 24–26 and 36–38 weeks of gestation. Tumor necrosis factor (TNF)-α and IL-6 was measured by ELISA.

At 36–38 weeks of gestation, birth outcomes (including delivery date and mode, birth weight, and APGAR score of the newborns) were recorded. Compliance was assessed through standardised telephone calls every 3 weeks and by using a monthly structured questionnaire.

Statistical analyses

All data was presented in accordance to its normality distribution, and analysed by using independent-T test or Mann-Whitney test.

RESULTS

Among the 143 eligible participants, we collected data from 104 (intervention group, n=46; control group, n=58; Table 2). Most participants dropped out of the study because they could not be contacted to complete the compliance measures. This is a typical challenge in conducting an intervention study for such a long period of pregnancy.

Maternal energy and nutrient intake in trimester 1 (baseline)

The baseline 24-hour dietary recall data (Table 3) revealed that the average total energy and nutrient intake (protein; iron; calcium; zinc; and vitamins A, B-12, folic-

Table 2. Baseline characteristics of the subjects

Variables	Intervention (n=46)	Control (n=58)
Age, years	29.4±4.5	29.8±4.1
Gestational age, week by USG	10.1±2.5	9.6±2.6
Systolic BP, mmHg	110 (90–144)	110 (90–140)
Diastolic BP, mmHg	70 (68–75)	70 (56–90)
Hemoglobin, g/dL	12.3±1.0	12.4±0.9
Serum ferritin, ng/mL	68.8 (8.0–483.7)	66.7 (7.2–180.7)
Hematocrit, %	36.3±2.9	36.5±2.3
GTT, mg/dL	78.9±8.0	78 (66–100)
GTT 2-h pp, mg/dL	111±31.9	108±32.5
Serum albumin, g/dL	4.2±(3.9–4.8)	4.3±0.3
Height, cm	156±4.9	156±5.2
Weight, kg	60.8±12.3	58.4±10.1
BMI, kg/m^2	25±4.5	23.9±3.9

BP: blood pressure; GTT: glucose tolerance test.
Data presented as mean±SD or median (minimum–maximum) based on the normality of data distribution.

Table 3. Dietary energy and nutrient intakes at baseline

Variables	Intervention (n=46)	Control (B) (n=58)
Total energy intake, kcal	1339±340	1352±352
Protein intake, g	47.0 (15.3–91.7)	49.8±14.5
Iron intake, mg	9.6 (3.2–53.7)	8.9 (2.9–67.8)
Calcium intake, mg	411 (108–1488)	442 (20.5–1619)
Zinc intake, mg	2.55 (0.4–33.0)	2.8 (0.5–56.9)
Vitamin A intake, mcg	245 (3.5–4423)	819 (0.5–9063)
Vitamin B-12 intake, mcg	1.0 (0–17.2)	1.2 (0–45.6)
Folate intake, mcg	251 (26.7 –1043)	225 (41.3–1089)
Vitamin C intake, mg	30.2 (0–224)	42.2 (0.3–408)
Vitamin D intake, mcg	0 (0–8)	0.5 (0–20.6)

Data presented as mean±SD or median (minimum–maximum) based on the normality of data distribution

acid, C, and D) was lower than the recommended intake. More than 90% of all participants had inadequate energy, carbohydrate, calcium, zinc, magnesium, and vitamin D intake relative to the Indonesian RDA criteria. Moreover, more than 80% of all participants had an inadequate intake of protein; iron; and vitamins C, B-12, and folic-acid.

Maternal micronutrient level during pregnancy

At the baseline (Table 4), the status of micronutrients, except for 25-OH-vitamin D, was acceptable in both the intervention group and control group. Throughout pregnancy, the status of all micronutrients, including 25-OH-vitamin D, was acceptable in both groups. However, the vitamin B-1 and zinc levels were significantly higher in the intervention group in the second trimester (p=0.014 and 0.028, respectively) than in the control group. Furthermore, total free fatty acid (FFA), linoleic acid, and arachidonic acid (ARA) were significantly higher in the intervention group in the second trimester (p=0.023, 0.014, and 0.001, respectively). The DHA status was significantly higher in the intervention group in the second trimester (p=0.032), but it was significantly lower in the third trimester (p=0.041).

Maternal inflammation biomarker level during pregnancy

No significant differences were observed in IL-6 levels throughout pregnancy (Table 4); however, the TNF-α level was transiently higher in the intervention group in the second trimester (p=0.003).

Maternal faecal microbiota during pregnancy

B. animalis subsp. *lactis* DR10^{TM} predominated the faecal microbiota of the intervention group compared with that of the control group (61.1% vs 0%; Table 5).

Maternal and fetal wellbeing during pregnancy

No significant differences were observed in maternal weight increment during pregnancy (Table 6) between the study groups (p>0.05). Estimates of the fetal weight (p=0.008), head circumference (HC) (p=0.007), abdominal circumference (AC) (p=0.018), and occipito–frontal diameter (OFD) (p=0.007) were significantly higher in the intervention group in the second trimester; however, these outcomes did not vary in the third trimester. The crown–rump length (CRL) was not significant (p>0.05) between the study groups in either the second or third trimester. No difference was observed in symptoms and signs of the digestive system and general health between the groups.

DISCUSSION

The present study determined that at the beginning of pregnancy, more than 80% of the participants had a low intake of energy and specific nutrients important for a healthy pregnancy. A large proportion of the participants had lower than recommended dietary intake levels for calcium, zinc, magnesium, and vitamin D in the early

Table 4. Maternal micronutrient status and inflammatory biomarkers during pregnancy in accordance with the combined prebiotic and probiotic intervention

Biomarkers	Intervention (n=46)			Control (n=58)		
	1st trimester	2nd trimester	3rd trimester	1st trimester	2nd trimester	3rd trimester
Vitamin A, pg/mL	523±153	476±142	459±153	507±122	479±119	432±121
Vitamin B-1, ng/mL	37.6 (16–113)	42.2±14.1	38.4±12.6[a]	38 (17–94)	37.8±13.7	32.6±8.3[b]
Vitamin B-12, pg/mL	484±161	314 (150–571)	311±96.1	445 (216–896)	308 (159–670)	261 (115–799)
Folic acid, ng/mL	19.9 (13.4–32.8)	24.5 (10.0–32.5)	18.9 (10.7–40)	19.1 (11.7–34.6)	26.8 (11.7–37.4)	26.0 (11.3–39.5)
Vitamin D 25OH, ng/mL	11.2±4.7	12.9±5.6	13.6±6.1	11.6±4.5	12.0 (5.7–39.9)	12.6 (4.6–28.7)
Calcium, mg/dL	8.9±0.4	8.5 (7.9–9.7)	8.7±0.4	8.9 (8.4–9.8)	8.6 (8.0–9.6)	8.7 (8.2–9.4)
Zinc, mcg/mL	63.2±13.9	52.0±11.6[a]	50 (34–70)	61 (39–102)	55.5 (34.0–87.0)[b]	48.8±8.6
Total FFA, mcm	2674±1446	4194 (864– 14602)[a]	4115±2533	2608 (238–10473)	3586±1796[b]	3847 (582–14004)
Linoleic, mcm	2115±1283	3504 (689–12376)[a]	3115±2286	2064 (120–8987)	2854±1530[b]	3055 (232–11817)
Arachidonic acid, mcm	279 (44.9–993)	283 (88.3–638)[a]	248±139	271±135	223±130[b]	257±163
DHA, mcm	16.0 (3.1–98.4)	15.5 (2.4–59.4)[a]	12.9±7.0[a]	12.9 (1.2–96.4)	12.6 (1.0–57.3)[b]	14.0 (6.6–51.8)[b]
IL–6, pg/mL	1.6 (0.4–3.8)	1.6 (0.7–15.9)	1.8 (0.8–13.8)	1.4 (0.6–4.1)	1.3 (0.7–7.1)	1.8 (0.7–10.6)
TNF–α	1.2±0.3	1.4 (0.6–4.8)[a]	1.5 (0.7–2.0)	1.1 (0.2–6.7)	1.1±0.4[b]	1.3 (0.7–4.8)

a and b, signify differences between intervention and control groups by trimester where significance at the $p<0.005$ level. Data are presented as mean±SD or median (minimum–maximum) based on the normality of data distribution.

Table 5. Changes in fecal microbiota of the subjects during the intervention

Variables	Intervention (n=46)	Control (n=58)	*p*–value
Fecal total bacteria, log^{10}DNA			
Baseline	8.38±0.50	8.40±0.53	0.815^{T}
Final	8.56 (7.28–13.88)	11.56 (7.59–13.50)	0.096^{MW}
Fecal DR 10, log^{10}DNA, n (%)			
Baseline	0	0	
Final	22 (61.1)	0	

Data presented as mean±SD or median (minimum–maximum) based on the normality of data distribution.

Table 6. Maternal and fetal health during pregnancy based on intervention

Variables	Intervention (n=46)	Control (n=58)	*p*–value
Weight increment, kg			
2nd trimester	6.5±3.9	5.0 (0–29)	0.393^{MW}
3rd trimester	10.9±4.6	11.4±5.1	0.661^{T}
Fetal weight estimation, g			
2nd trimester	905 (450–2011)	800 (104–1696)	0.008^{MW}
3rd trimester	2859±358	2782±312	0.273^{T}
Fetal CRL estimation, mm			
1st trimester	28.7±18.1	23.7 (1.4–180)	0.942^{MW}
Fetal HC estimation, mm			
2nd trimester	245±22.0	233±20.5	0.007^{T}
3rd trimester	319±12.4	317 (206–344)	0.502^{MW}
Fetal AC estimation, mm			
2nd trimester	220±22.6	208±24.9	0.018^{T}
3rd trimester	320±20.7	314±19.6	0.187^{T}
Fetal OFD estimation, mm			
2nd trimester	84.3±7.8	80.0±8.1	0.007^{T}
3rd trimester	106 (99.3–125)	107±6.3	0.674^{MW}

CRL: crown-rump length; HC: head circumference; AC: abdominal circumference; OFD: occipito-frontal diameter; T: independent-T test; MW: Mann-Whitney test.
Data presented as mean±SD or median (minimum–maximum) based on the normality of data distribution.

stages of pregnancy (i.e. first trimester), and this finding is a concern. If not corrected, this can result in an increased risk of preeclampsia and preterm birth. Evidence indicates that preeclampsia or gestational hypertension is one of the leading causes of maternal mortality and morbidity. However, other micronutrients, such as zinc, in addition to copper, magnesium, and selenium, can increase complications in pregnancy and fetal development. Moreover, evidence shows that zinc supplementation is associated with reduced preterm birth.[17]

Vitamin A, an essential micronutrient, is required in small amounts. In a study conducted in Nepali pregnant women, the daily intake of vitamin A reduced the maternal mortality rate by 40%; the reduction is apparently caused by less susceptibility to infection. Moreover, additional vitamin A has an advantage in increasing the haemoglobin level.[2]

Vitamin B-2, B-6, or B-12 deficiency leads to elevated plasma homocysteine concentrations, in which homocysteine exerts a higher risk of adverse pregnancy outcomes and low-birth-weight infants. Vitamin B-2 alone can reduce the plasma homocysteine concentration.[2] Vitamin B1 is a cofactor for several enzymes involved in carbohydrate metabolism and neural function. Severe vitamin B-1 deficiency causes beriberi.[17] However, suboptimal vitamin folic-acid status is strongly related to the increased risk of NTDs.

In this study, we provided milk fortified with micronutrients to pregnant women to meet the increased nutritional requirements during pregnancy. Among the policy and programme responses for improving the nutritional status (i.e. food-based strategies), dietary diversification and food fortification as well as nutrition education and supplementation can facilitate overcoming poor nutritional status among pregnant women.

In this study, throughout pregnancy, the study participants had an acceptable concentration of micronutrients, except for vitamin D. This is a serious concern because of the established role of vitamin D in bone formation, brain development, and immune system modulation. Severe vitamin D deficiency can cause rickets in infants and children as well as osteomalacia in adults.[17-19] Some transient changes in vitamin B-1 and zinc levels were observed between the groups across the pregnancy stages. Levels of vitamins A, B-2, B-12, folic-acid, D, and E exhibited no change throughout pregnancy. This finding could be explained by physicians who would have provided their patients with a correction whenever they identified micronutrient deficiency during pregnancy. Medical intervention providing additional nutrient supplements may also have skewed the data in a way which affected outcome measures. In this study, folic-acid and B-12 levels did not vary between the groups in the second and third trimesters because both intervention and control milks provided the same amount of folic-acid. Blood calcium levels are tightly regulated; therefore, the calcium level showed no change.

The fortified milk increased the maternal FFA level. In this study, significant differences were observed in the concentrations of total FFA, linoleic acid, ARA, and DHA between the control and intervention groups, notably in the second trimester. Eicosapentaenoic acid (EPA) converts alpha-linoleic acid to DHA, and linoleic acid is metabolised to ARA. These FAs are required to form functional and structural lipids in tissues, and DHA, EPA, and ARA are present in cellular membranes. EPA influences the function of endothelial cells, monocytes, and platelets, as well as smooth muscle cells. In addition, EPA influences immune reactions and inflammatory processes.[20]

These FAs are important for brain development because lipids are the predominant constituents of brain tissue. Fetal brain development is accelerated during the second half of pregnancy, lasting until late adolescence. Among the essential FAs, DHA is the most abundant in the human brain. Therefore, pregnant and lactating women are recommended a dietary intake of n-3 long-chain polyunsaturated FAs supplying at least 200 mg/day of DHA. During pregnancy, maternal dietary intake of fatty fish or oils providing n-3 long-chain polyunsaturated FAs is associated with fetal visual and cognitive development.[20] Atalah et al reported the consumption of the fortified dairy drink during pregnancy and lactation produced a significant increase of DHA intake, and an improved content of DHA on red blood cell membranes as well as in human milk.[21]

During pregnancy, the immune system plays a very important role in preventing infection and inflammatory processes. Micronutrients also play a role in supporting the function of the immune system. Furthermore, vitamin D plays a pivotal role in regulating immune responses by promoting Th2 responses and suppressing Th1 responses. Vitamin D supplementation was reported to significantly reduce TNF-α and interferon (IFN)-γ production.[22] Palmer et al reported that maternal vitamin A supplementation in undernourished populations enhanced the natural antibody concentration in preadolescents.[23]

In addition to micronutrients, the gastrointestinal microbiota plays a key role in supporting the function of the immune system. Prescott et al reported that the consumption of probiotics during pregnancy could increase IFN-γ, transforming growth factor-β, and immunoglobin A.[13] However, in the current study, TNF-α was significantly changed in the intervention group in the second trimester; this change was not apparent in the third trimester, and cytokine measurements varied. Therefore, the results must be verified before being interpreted.

B. animalis subsp. *lactis* DR10™ was significantly higher in the faecal microflora in the intervention group (61.1% vs 0 respectively), thus supporting the effectiveness of this strain in fortified milk. In particular, no differences were observed in symptoms and signs of the digestive system and general health between the study groups, supporting the safety of this probiotic strain in this Indonesian population. Furthermore, Bisanz et al reported that among pregnant women living in rural Tanzania, the daily consumption of micronutrient-supplemented probiotic yogurt provides a safe, affordable food option.[24] However, this supplement result did not affect micronutrient levels during pregnancy.

The maternal micronutrient status is considered to reflect the neonatal outcome. However, in this study, no significant differences were observed in the third trimester. A single micronutrient is unlikely to affect fetal growth, unless the micronutrient is crucial. In a study conducted in Nepal, multiple micronutrients increased the birth weight by 64 g and reduced the percentage of low birth weight babies by 14%.[25] Regarding maternal health, no significant differences were observed in the complaints of the digestive system. Fekete et al conducted a systematic review and indicated a 2% increase in birth weight for every twofold increase in folic-acid intake; however, no beneficial effects were observed in the placental weight or gestational length.[26]

Hoa et al reported significant increases in the transferrin concentration in the iron-fortified milk group compared with the placebo and nonfortified milk groups. They concluded that iron supplementation and fortification are effective in pregnant women.[7] A study conducted in Spain revealed that fortification with folic-acid can positively affect the nutritional status of women without increasing the risk of excessive folic-acid exposure.[27]

In contrast to food fortification, a study conducted in China reported the use of daily milk supplementation; this study revealed that the daily consumption of milk can increase the serum folic-acid concentration in pregnant women and may enhance the birth outcomes.[28] Infants born to women drinking milk had higher term birth weight.[28] A large study conducted in the United Kingdom reported a significant decrease in the number of newborns with a low birth weight [relative risk (RR): 0.88)] or low gestational age (RR: 0.9) and stillbirths (RR: 0.91) in a group administered multiple micronutrients with iron and folic-acid, compared with a group administered multiple micronutrients with or without folic-acid.[29]

Mardones et al reported that a dairy product fortified with multiple micronutrients and omega-3 FAs resulted in increased mean birth weight.[30] DHA and EPA consumption reflects fetal brain development. However, Makrides et al conducted a randomised controlled trial and reported that the use of DHA-rich fish oil capsules, rather than vegetable oil capsules, during pregnancy did not improve cognitive and language development during early childhood.[31]

Limitations include the high drop-out rate among subjects. Medical intervention required for clinical ethical reasons may also have skewed the data in a way which affected outcome measures.

In conclusion, evidence on the micronutrient deficiency status in the first trimester must be comprehensively addressed by several programmes, such as nutrition counselling and education. As the main finding, this study confirms that milk fortified with *B. animalis* subsp. *lactis* DR10™ is safe and well tolerated. Fortified milk enhances the concentration of *B. animalis* subsp. *lactis* DR10™ in faeces.

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AUTHOR DISCLOSURES

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