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# Effect of Daily Consumption of a Synbiotic Food on Pregnancy Outcomes: A Double-Blind Randomized Controlled Clinical Trial

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**Abstract**

**Background:** To our knowledge, no reports are available indicating the effects of synbiotics on pregnancy outcomes and maternal biochemical indices in pregnant women.
**Objectives:** This study was designed to evaluate the effects of daily consumption of a synbiotic food on pregnancy outcomes and maternal biochemical indices among Iranian pregnant women.
**Patients and Methods:** This double-blind randomized controlled clinical trial was conducted among 52 pregnant women, primigravida, aged from 18 to 35 years with singleton pregnancy at their third trimester. After a 2-week run-in period, pregnant women were divided into two groups to receive either a synbiotic (n = 26) or control food (n = 26) for 9 weeks randomly. The synbiotic food contained a probiotic viable and heat-resistant *Lactobacillus sporogenes* ($1 \times 10^7$ CFU) and 0.04 g inulin (HPX)/g as the prebiotic. Subjects were requested to consume the synbiotic food two times a day in 9 g portions, followed by checking for newborn's weight, height, head circumference, Apgar score, and hyperbilirubinemia. Fasting blood samples were taken at the beginning of the study and 9 weeks after the intervention to measure serum calcium, iron, and magnesium and liver enzymes.
**Results:** Supplementation with synbiotic food for 9 weeks among pregnant women did not affect any significant differences in the cesarean section rate, gestational age, newborn birth size and Apgar score. Synbiotic-supplemented women had a significant rise in serum calcium levels ($+0.2 \pm 1.0$ vs. $-0.5 \pm 0.8$ mg/dL, $P = 0.005$) compared to women in the control group, while no significant differences were found between the two groups in terms of their effects on serum iron, magnesium and liver enzymes levels.
**Conclusions:** Taken together, consumption of synbiotic food among pregnant women for 9 weeks has resulted in increased levels of serum calcium compared to the control food, but did not affect pregnancy outcomes, serum iron, magnesium and liver enzymes.

*Keywords:* Synbiotics, Pregnancy Outcome, Calcium, Iron, Liver Enzymes, Pregnant Women

## 1. Background

Complications during pregnancy are associated with several adverse outcomes for mother and newborns in the short and long term (1). During pregnancy, the alterations in the gut and vaginal microbiome (2), might affect the maternal metabolic profiles, biomarkers of inflammation and oxidative stress and also contribute to the metabolic and immunological health of the offspring (3). On the other hand, demand for micronutrients including calcium, iron and magnesium increases to cover the need of developing fetus (4-6). Calcium deficiency during pregnancy would result in pre-eclampsia (7), abnormal foetal programming (8) and hypocalcaemia in newborns (9). In addition, iron deficiency anemia (IDA) among pregnant women is associated with more than two-fold increase in the risk of preterm delivery (5).

Current strategies to prevent and decrease maternal morbidity and mortality and increase perinatal health are focused on specific aberrations during pregnancy. Various factors including diet, prebiotics, pharmaceutical agents, antibiotics, and probiotics may influence the composition of the microbiome (10). A synbiotic food contains a mixture of probiotic and prebiotic in a synergistic form (11). Previous randomised clinical trials have reported that probiotic supplementation in pregnancy led to decreased pregnancy complications such as gestational diabetes mellitus (GDM) (12) and preterm delivery (13). Other studies have evaluated the beneficial effects of probiotic supplementation in pregnancy on infant health and development (14, 15). In addition, our previous study showed that consumption of probiotic yogurt for 9 weeks by pregnant women maintained serum calcium levels compared with the conventional yogurt, but had no effect on serum iron and liver enzymes levels (16). However, a significant increase in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels was found from weeks 4 to 12 in infected fish fed with S. parauberis ($2.1 \times 10^7$ CFU/

mL) compared to fish fed without supplementation diet (17). About the effect of prebiotics on minerals status, some studies have shown that prebiotic intake stimulated the absorption of calcium (18, 19) or magnesium (20), whereas others found no significant effects (21). In addition, treatment with prebiotic has also led to a decreased ALT activity compared to controls 24 hours after lipopolysaccharide (LPS) administration in rats (22).

The modification of the gut microbiome following the probiotics supplementation may serve to prevent pregnancy complications and improve outcomes (1). We are aware of no randomized controlled trial that investigated the effects of synbiotic food consumption on pregnancy, serum calcium, iron, magnesium and liver enzymes among pregnant women.

## 2. Objectives

The aim of this study was to investigate the effects of daily consumption of a synbiotic food on pregnancy, serum calcium, iron, magnesium and liver enzymes among Iranian pregnant women.

## 3. Patients and Methods

### 3.1. Participants

This randomized, double-blind, controlled trial was conducted on pregnant women recruited from the medical outpatient maternity clinic affiliated to Kashan University of Medical Sciences (KUMS), Kashan, Iran between June-February 2012. A randomized clinical trial sample size formula was used to for estimating the sample size where type one (α) and type two error (β) were 0.05 and 0.20 (power = 80%), respectively. The change in mean (d) of iron as main variable was expressed by 74.6 as SD and 66.0 mg/dL based on a previous trial (16), where 21 pregnant women were needed in each group. Considering 5 dropouts in each group, the final sample size was determined to be 26 subjects per group. The study included primigravida, pregnant women, aged from 18 to 35 years with singleton pregnancy at their third trimester. The gestational age was assessed from the date of last menstrual period and concurrent clinical assessment. The exclusion criteria were pregnant women with multiparity, maternal hypertension, liver or renal disease and GDM, complete bed rest (CBR), intra uterine fetal death (IUFD), intrauterine growth retardation (IUGR), placenta abruption, preterm delivery, and hospitalization. The study was done according to the declaration of Helsinki guidelines and approved by the Ethics Committee of KUMS. All participants provided written informed consents. The trial was registered in the Iranian website (www.irct.ir) under clinical trials (IRCT code: IRCT201204065623N3).

### 3.2. Study Design

To obtain detailed data about the dietary intakes of the study participants, all pregnant women were entered into a 2-week run-in period where all subjects had to refrain from taking probiotic, prebiotic and synbiotic foods. At the end of the run-in period, signifying week 27 of gestation, subjects were randomly divided into two groups to receive 18 g/d of synbiotic (n = 26) or control food (n = 26) for 9 weeks. All pregnant women were requested not to change their routine physical activity or usual diets and not to consume any synbiotic products other than the one provided to them by the investigators. Synbiotic or control foods were provided for participants every week. Compliance with synbiotic or control foods was monitored once a week through telephone calls. All individuals were checked for three dietary regimens, once on the weekend and twice during the week and three physical activity records by telephone calls to verify that they maintained their usual diet and physical activity during the intervention. Both dietary recalls and physical activity records were taken at weeks 2, 4, 6, and 8 of the intervention. To obtain information on participant nutrient intake based on these three-day diets, we used Nutritionist IV software (First Databank, San Bruno, CA) modified for Iranian foods.

### 3.3. Synbiotic and Control Foods

The synbiotic food contained a probiotic viable and heat-resistant *Lactobacillus sporogenes* ($1 \times 10^7$ CFU), 0.04 g inulin (HPX) as prebiotic with 0.38 g isomalt, 0.36 g sorbitol, and 0.05 g stevia as sweetener per 1 g. Participants urged to receive the synbiotic food two times a day in a 9 g package. Therefore, all pregnant women consumed $18 \times 10^7$ CFU *Lactobacillus sporogenes* and 0.72 g inulin every day. Control food included the same ingredients without probiotic bacteria and prebiotic inulin presented in identical packages and coded by the producer to guarantee blinding. The *Lactobacillus sporogenes*, rather than other *Lactobacillus* species, was selected because of its viability against high temperature, acidity of the stomach, bile acids and growth at physiological conditions, as well as favorable effects on the intestinal environment, stool frequency and characteristics (23). The synbiotic and control foods were produced by Sekkeh Gaz Company, Isfahan, Iran.

### 3.4. Assessment of Anthropometric Indices

Anthropometric measurements of height and weight were made at the beginning and end of trial (9 weeks after the intervention) in both groups. Body weight was measured in an overnight fasting state, with barefoot and minimal clothing by a digital scale (Seca, Hamburg, Germany) to the nearest 0.1 kg (24). Body height was determined using a non-stretched tape measure (Seca, Hamburg, Germany) to the nearest 0.1 cm (24). BMI was calculated by dividing participant's weight in kg by his/or her height in $m^2$. All the measurements were determined by a trained nutritionist to decrease the error rate.

### 3.5. Outcomes

The primary study outcomes reflected serum calcium, iron, magnesium and liver enzymes including ALT, AST, and alkalin phosphatase (ALP). Fasting blood samples (5 mL) were taken from each pregnant women after overnight fasting at the KUMS reference laboratory, both at the beginning and the end of the study and were immediately centrifuged (3000 × g, 10 minutes, 4°C). The serum samples were then stored at -70°C until used. Commercial kits were used to determine serum calcium, iron, magnesium, AST, ALT and ALP levels (Pars Azmun, Tehran, Iran). All inter- and intra-assay coefficient of variations (CVs) for calcium, iron, magnesium, AST, ALT and ALP levels were less than 5%. Measurements of calcium, iron, magnesium and liver enzymes were done in a blinded fashion, in duplicate, before and after intervention simultaneously and in the same analytical run, and in random order to reduce systematic error and inter-assay variability.

The secondary outcomes were gestational age, cesarean section, newborn's size, Apgar score, and hyperbilirubinemia. Newborns diagnosed with hyperbilirubinemia if the total serum bilirubin levels was at or above 15 mg/dL (257 mol/L) in infants 25 to 48 hours old, 18 mg/dL (308 mol/L) in infants 49 to 72 hours old, and 20 mg/dL (342 mol/L) in infants older than 72 hours (25).

### 3.6. Statistical Analysis

In the present study, Kolmogrov-Smirnov test was used to examine the normal distribution of variables. Independent samples Student's t-test was used to detect differences in general characteristics, dietary intakes, the effects of synbiotic food consumption on serum calcium, iron, magnesium and liver enzymes between the two groups. Within-group differences were identified using paired-samples t-tests. The Pearson Chi-square test was used to compare categorical variables. To assess if the magnitude of the change in dependent variables depended on maternal age, baseline values and baseline BMI, all analyses were controlled, using analysis of covariance (ANCOVA), for maternal age, baseline values and baseline BMI to avoid possible potential bias. $P < 0.05$ was considered as statistically significant. All statistical analyses were done using the Statistical Package for Social Science version 17 (SPSS Inc., Chicago, Illinois, USA).

## 4. Results

There were no serious adverse reactions in the pregnant women following consumption of synbiotic food throughout the study. Mean age in the control food group (29.3 ± 5.4 years) was higher than that in the synbiotic food group (25.9 ± 5.7 years), (P = 0.02). Baseline (weight: 71.3 ± 11.5 vs. 71.2 ± 14.3 kg, P = 0.98; BMI: 27.8 ± 4.1 vs. 27.9 ± 5.0 $kg/m^2$, P = 0.95) and end-of-trial (weight: 74.8 ± 11.5 vs. 74.7 ± 13.6, P = 0.98; BMI: 29.2 ± 4.1 vs. 29.3 ± 4.8 $kg/m^2$, P = 0.93) were not significantly different comparing synbiotic and control foods groups.

Based on the three-day dietary records obtained throughout the intervention, no statistically significant difference was seen between the two groups in terms of dietary intakes of energy, total dietary fiber (TDF), calcium, iron, magnesium, manganese, zinc and vitamin C (Table 1).

Supplementing for synbiotic food among pregnant women for 9 weeks did not affect the cesarean section rate, gestational age, newborn birth size and Apgar score (Table 2).

Synbiotic supplementation has resulted in a significant rise in serum calcium levels (+0.2 ± 1.0 vs. -0.5 ± 0.8 mg/dL, P = 0.005) compared with the control group, while no significant differences were found between the two groups in terms of their effects on serum iron, magnesium, AST, ALT and ALP levels (Table 3).

The analysis of the results obtained showed no significant changes in serum calcium, iron, magnesium, AST, ALT and ALP levels between synbiotic and control groups, after adjustment for maternal age, baseline BMI and baseline values (Table 4).

**Table 1.** Dietary Intakes of Study Participants Throughout the Study[a,b]

| | Control Food | Synbiotic Food | P[c] |
|---|---|---|---|
| **Energy, kcal/d** | 2393 ± 259 | 2368 ± 218 | 0.70 |
| **TDF, g/d**[d] | 19.4 ± 4.0 | 20.0 ± 3.5 | 0.58 |
| **Calcium, mg/d** | 1161.1 ± 170.0 | 1092.5 ± 222.8 | 0.21 |
| **Iron, mg/d** | 15.1 ± 3.2 | 15.5 ± 3.7 | 0.68 |
| **Magnesium, mg/d** | 289.1 ± 53.8 | 282.1 ± 77.4 | 0.70 |
| **Manganese, mg/d** | 2.3 ± 0.8 | 2.2 ± 0.9 | 0.56 |
| **Zinc, mg/d** | 11.3 ± 2.3 | 10.2 ± 2.7 | 0.12 |
| **Vitamin C, mg/d** | 188.2 ± 76.3 | 181.7 ± 92.5 | 0.78 |

[a]Data are expressed as mean ± SD.
[b]N = 26.
[c]Obtained from independent t-test.
[d]TDF: total dietary fiber.

**Table 2.** The Association of Synbiotic Supplementation with Pregnancy Outcomes[a,b]

| | Control Food | Synbiotic Food | P[c] |
|---|---|---|---|
| **Cesarean Section, %** | 15 (57.7) | 9 (34.6) | 0.09[d] |
| **Gestational Age, weeks** | 39.3 ± 0.9 | 39.1 ± 1.3 | 0.38 |
| **Newborns' Weight, g** | 3286.9 ± 428.2 | 3280.8 ± 424.3 | 0.95 |
| **Newborns' Length, cm** | 50.9 ± 2.2 | 49.8 ± 2.6 | 0.10 |
| **Newborns' Head Circumference, cm** | 34.5 ± 1.3 | 34.8 ± 2.0 | 0.49 |
| **1-min Apgar Score** | 8.9 ± 0.2 | 8.9 ± 0.2 | 1.0 |
| **5-min Apgar Score** | 9.9 ± 0.2 | 9.9 ± 0.2 | 1.0 |
| **Newborns' Hyperbilirubinemia, %** | 11 (42.3) | 7 (26.9) | 0.24[d] |
| **Newborns'Hospitalization, %** | 9 (34.6) | 4 (15.4) | 0.10[d] |

[a]N = 26.
[b]Values are mean ± SD for continuous measures and number (%) for dichotomous variables.
[c]Obtained from independent t-test.
[d]Obtained from Pearson Chi-square test.

**Table 3.** Mean (± Standard Deviation) of Serum Calcium, Iron, Magnesium and Liver Enzymes at Baseline and After the Intervention[a,b]

| | Control Food | | | | Synbiotic Food | | | | P[c] |
|---|---|---|---|---|---|---|---|---|---|
| | Wk0 | Wk9 | Change | P[d] | Wk0 | Wk9 | Change | P[d] | |
| **Calcium, mg/dL** | 8.9 ± 0.6 | 8.4 ± 0.4 | -0.5 ± 0.8 | 0.004 | 8.6 ± 1.0 | 8.8 ± 0.6 | 0.2 ± 1.0 | 0.21 | 0.005 |
| **Iron, mg/dL** | 119.9 ± 100.9 | 110.1 ± 51.9 | -9.8 ± 77.6 | 0.52 | 128.9 ± 112.9 | 136.9 ± 118.2 | 8.0 ± 175.9 | 0.81 | 0.64 |
| **Magnesium, mg/dL** | 2.0 ± 0.4 | 1.9 ± 0.4 | -0.1 ± 0.4 | 0.95 | 2.2 ± 0.3 | 2.1 ± 0.4 | -0.1 ± 0.4 | 0.28 | 0.46 |
| **AST, IU/L** | 7.1 ± 4.1 | 5.9 ± 4.8 | -1.2 ± 4.2 | 0.19 | 8.2 ± 5.4 | 8.4 ± 5.8 | 0.05 ± 8.6 | 0.97 | 0.54 |
| **ALT, IU/L** | 16.7 ± 6.4 | 16.5 ± 7.0 | -0.2 ± 7.2 | 0.90 | 18.4 ± 8.2 | 20.2 ± 10.4 | 1.8 ± 12.8 | 0.47 | 0.49 |
| **ALP, U/L** | 200.7 ± 102.1 | 245.1 ± 166.8 | 44.4 ± 167.0 | 0.18 | 223.6 ± 124.4 | 219.2 ± 117.0 | -4.4 ± 172.2 | 0.89 | 0.30 |

[a]Abbreviation: ALP, alkalin phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.
[b]N = 26.
[c]The differences between the two groups (independent samples t-test).
[d]Within-group differences (paired samples t-test).

**Table 4.** Adjusted Changes in Calcium, Iron, Magnesium and Liver Enzymes in Pregnant Women That Receiving Either Synbiotic or Control Foods[a,b]

| | Control Food[c] | Synbiotic Food[c] | P[d] |
|---|---|---|---|
| **Calcium, mg/dL** | 0.3 ± 0.5 | 0.1 ± 0.5 | 0.007 |
| **Iron, mg/dL** | -15.0 ± 95.7 | 13.2 ± 95.7 | 0.30 |
| **Magnesium, mg/dL** | -0.1 ± 0.5 | -0.02 ± 0.5 | 0.61 |
| **AST, IU/L** | -1.5 ± 5.6 | 0.5 ± 5.6 | 0.21 |
| **ALT, IU/L** | -0.3 ± 8.6 | 1.9 ± 8.1 | 0.37 |
| **ALP, U/L** | 28.9 ± 149.1 | 11.0 ± 149.1 | 0.67 |

[a]Abbreviation: ALP, alkalin phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.
[b]All values are expressed in mean ± SD.
[c]N = 26.
[d]Adjusted for maternal age, baseline BMI, and baseline values with ANCOVA.

## 5. Discussion

This study demonstrated the effects of 9 weeks synbiotic supplementation on pregnancy outcomes, serum calcium, iron, magnesium and liver enzymes levels among pregnant women. The major finding was that taking synbiotic food increased serum calcium levels, without affecting pregnancy outcomes, serum iron, magnesium and liver enzymes concentrations. To the best of our knowledge, this is the first study to report on the effects of synbiotic supplementation on pregnancy outcomes, serum calcium, iron, and magnesium and liver enzymes in pregnant women.

Pregnant women are very susceptible to several pregnancy complications especially in the third trimester (1). The present study revealed that synbiotic food administration for 9 weeks did not influence pregnancy outcomes compared with control food group. In this context, no statistical difference was found in adverse pregnancy outcomes, including the number of spontaneous abortions, pre-term births as well as a low birth weight after ingestion of *Lactobacillus* in early pregnancy (26). Furthermore, a recent meta-analysis study showed that consumption of probiotic among pregnant women after week 36 of gestation did not affect the incidence of caesarean section, and gestational age at birth and birth weight (27). However, few studies have shown beneficial effects of probiotic supplementation on pregnancy complications such as GDM (12) and preterm delivery (13). Differences between our results and similar findings are probably due to diversity in cultures and used dosages of inulin and probiotic, as well as duration of the study.

The findings of the study showed that consumption of Synbiotic food was associated with a significant increase in serum calcium levels compared with that of control food group, while it could not affect serum iron and magnesium concentrations. Earlier studies have reported beneficial effects of synbiotics, pro-, and prebiotics on serum minerals predominately in animal models. In line with our study, Naughton et al., (28) demonstrated that intake of synbiotic containing *Lactobacillus* GG, *Bifidobacterium lactis* (BB12), and oligofructose-enriched inulin increased plasma calcium levels after 21 days in rats. Furthermore, our previous study on pregnant women revealed that taking 200 g/d probiotic yoghourt containing two strains of lactobacilli (*Lactobacillus acidophilus* LA5) and bifidobacteria (*Bifidobacterium lactis* BB12) of minimum $1 \times 10^7$ CFU in total for 9 weeks led to maintaining serum calcium levels compared with the conventional yogurt, but did not affect serum iron levels (16). A significant rise in serum calcium concentration following consumption of a probiotic preparation was also observed in broiler chickens, but had no effect on serum iron levels (29), and some other studies reported no such significant effect (21), whereas the intake of inulin stimulated the absorption of calcium or magnesium as reported by other investigations (18-20). Deficiency of calcium during pregnancy, may be associated with some complications such as abnormal fetal development, pregnancy induced hypertension, and preterm delivery (30). Consumption of synbiotic results in increased production of organic acids and short chain fatty acid (SCFA) (31). This in turn, causes calcium release from organic compositions and its higher absorption. Furthermore, fermentation of inulin in synbiotic food in gut increases SCFA production (32), which reinforces absorption of calcium.

We found no significant effect of synbiotic food consumption on serum ALT, AST and ALP levels compared to control food. Our findings were in agreement with those of our previous report that demonstrated probiotic yogurt consumption for 9 weeks did not affect serum liver enzymes levels (16). In addition, no significant effects were observed in patients with primary sclerosing cholangitis (PSC) on serum ALT, AST and ALP concentrations after consumption of probiotics containing four *Lactobacillus* and two *Bifidobacillus* strains after 3 months (33). However, Kirpich et al., (34) showed a significant reduction in serum ALT and AST levels following supplementation of *Bifidobacterium bifidum* and *Lactobacillus plantarum* 8PA3 alcohol-induced liver injury in human after 7 days. The inconsistency between our findings and some previous studies on the effect of synbiotic on liver enzymes levels might be explained by the different study designs, lack of considering baseline levels of dependent variables, discrepancy in participants and sample size, using different cultures and dosages of inulin and probiotic, as well as duration of the study.

Several limitations in the present study were due to budget limitations, where we did not assess other biomarkers related to calcium, iron and magnesium metabolism in the body. In addition, we used synbiotic food with a strain of probiotic. Future studies are recommended to evaluate the effects of synbiotic consumption on biomarkers of inflammation, oxidative stress, serum minerals and liver enzymes involving higher dosage and combination of bacterial strains in pregnant women. Furthermore, we did consider all dependent variables so called serum minerals as primary outcomes in the present study. Iron was used to estimate sample size as the largest sample size was taken on using this variable. The sample sizes obtained according to this variable covered the required sample size for all other variables. Therefore, more extensive trials are needed to confirm our findings.

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## Footnotes

**Authors' Contribution:**Mohsen Taghizadeh and Sabihe-Alsadat Alizadeh contributed in the conception, data collection and drafting of the manuscript. Zatollah Asemi contributed in conception, design, statistical analysis and drafting of the manuscript. All authors read and approved the final version of the paper. Zatollah Asemi is the guarantor of this work.

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