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Saccharomyces cerevisiae Yeast-Based Supplement and Breast Milk Supply: A Randomised Placebo-Controlled Trial

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ABSTRACT

Saccharomyces cerevisiae yeast-based supplements (SCYS) are frequently used as galactagogues with limited evidence of their efficacy. This study investigates the effect of SCYS on human milk oligosaccharide (HMO) concentration and indicators of milk supply. Sixty-eight breastfeeding women with a healthy singleton infant aged 1–7 months were randomly assigned to consume a SCYS product (5 g/day) or placebo for 4 weeks. The primary outcome was the change in the total HMO concentration. The secondary outcomes included participants' perceptions of milk supply, intervention effectiveness, postnatal distress, infant feeding patterns, infant anthropometry, and adverse effects. Intention-to-treat analysis was performed. Multivariable linear regression analysis showed no significant effect of SCYS on individual or total HMO concentrations. However, 65% of women in the SCYS group, compared to 35% in the placebo group, perceived an increase in milk production ($p < 0.05$). No significant differences were found for other secondary outcomes. However, mothers in the SCYS group had a small but significant improvement in perception of their milk quantity and quality ($p < 0.05$). SCYS use was also associated with significantly lower formula use at 6 months postpartum (4% vs. 27%, $p < 0.05$). While SCYS does not impact HMO concentration, it may improve women's perceptions of milk supply. A larger randomised controlled trial is needed to assess its potential effects on actual milk production and composition and address issues of perceived insufficient milk.

Trial Registration: This trial was registered at the Australian New Zealand Clinical Trials Registry (trial registration number: ACTRN12619000704190)

1 | Introduction

Breastfeeding promotes infant growth and development and benefits mothers' physical and mental health in the short and long term (Victora et al. 2016). The World Health Organization (WHO) and Aotearoa New Zealand (NZ) recommend exclusive breastfeeding to 6 months of age and prolonged breastfeeding to at least 2 years (Ministry of Health 2021a; World Health Organization 2022).

Perceived insufficient milk (PIM) is frequently reported by breastfeeding women as the primary reason to stop breastfeeding or to introduce infant formula (Gatti 2008; Morton et al. 2012). PIM is the mother's perception of her milk supply based. This is generally based on signs such as infant behaviour, infant growth, the appearance of the milk or breast fullness. In some cases, the milk supply may be insufficient for the infant while in other cases it is a misperception (Huang et al. 2022; Segura-Pérez et al. 2022). Breastfeeding women sometimes use galactagogues when they have

Summary

- Women consuming *Saccharomyces cerevisiae* yeast-based supplements grown on molasses at 5 g/day perceived the intervention as effective significantly more often than those receiving a placebo, despite no observed effects on human milk oligosaccharide or women's perception of their milk supply.
- Significantly fewer women in the SCYS group reported formula use at 6 months postpartum compared to the placebo group, suggesting that SCYS may support exclusive breastfeeding.
- Future studies with larger sample sizes are warranted to confirm these findings and explore potential mechanisms in greater detail.
- Healthcare professionals and breastfeeding women should be aware that the galactagogue efficacy and the effective dosage of brewer's yeast remain unclear.

concerns about their milk supply. Galactagogues are substances suggested to increase breast milk production and/or support breastfeeding outcomes (Foong et al. 2020). *Saccharomyces cerevisiae* yeast-based supplements (SCYS) such as brewer's yeast are popular galactagogues among breastfeeding women (Bazzano et al. 2017; McBride et al. 2021; Steyn et al. 2017). However, they have only been demonstrated to increase milk production in lactating ruminants and sows (Jia et al. 2021).

SCYS could be effective as a galactagogue by increasing breast milk volume or they could affect mothers' perception of their milk supply, for example by influencing human milk oligosaccharides (HMOs) and postnatal distress, therefore, improve breastfeeding outcomes (Jia et al. 2021).

Some indirect evidence suggests maternal SCYS supplementation may affect HMO concentrations. Having a high prebiotic fibre maternal diet was reported to affect oligosaccharides in rat milk, consequently influencing the gut microbiota in offspring (Hallam et al. 2014). Results in human studies also suggest that maternal diet influences HMO abundance and profile (Quin et al. 2020; Seferovic et al. 2020). HMOs benefit infant gut health, modulate immune responses, and promote brain development (Bode 2012; Oliveira et al. 2015). Thus, HMO concentration changes may impact infants, resulting in less unsettled and crying behaviour, influencing the mother's perception of her infant's satisfaction and, consequently, the perceived adequacy of her milk supply, i.e. PIM (Peacock-Chambers et al. 2017; Sacco et al. 2006; Safon et al. 2017).

Additionally, we propose that SCYS supplementation may contribute to reducing postnatal distress and, consequently, address PIM for some women. Symptoms of postnatal distress such as stress, anxiety and depression are reported to adversely influence milk ejection reflex (Massey et al. 2016) and decrease breastfeeding self-efficacy (Mercan and Tari Selcuk 2021), which can result in PIM. In an Australian study, participants who consumed spreads produced from *Saccharomyces cerevisiae* yeast extract exhibited significantly lower stress and anxiety scores compared to those who did not, suggesting that SCYS may relieve stress and anxiety in the general population (Mikkelsen et al. 2018). Supplementing with *Saccharomyces cerevisiae* yeast-derived beta-glucan has also significantly reduced Profile of Mood States scores in women with moderate stress, when a lower score denotes less stress (Talbott and Talbott 2012). No studies have been conducted on how SCYS supplementation influences postnatal distress in lactating women. However, if it is effective in reducing postnatal distress, it may result in improved milk ejection reflex and consequently decrease PIM.

Adverse effects of taking brewer's yeast supplements, including constipation, nausea, decreased appetite, and skin rash, were reported in two human studies investigating the health benefits of chromium-enriched yeast supplementation on type 2 diabetes (Kleefstra et al. 2006; Król et al. 2011). Studies on selenium-enriched yeast supplementation during lactation did not report any adverse effects (Kumpulainen et al. 1985; McGuire et al. 1993; Trafikowska et al. 1998; Trafikowska et al. 1996). Thus, it remains unknown if taking yeast-based supplements during lactation causes adverse effects on women or infants.

This study aims to evaluate the effect of maternal supplementation with SCYS on HMO concentration and indicators of milk supply and to assess the adverse effects of taking SCYS during lactation.

2 | Method

2.1 | Study Design

This was a randomised, double-blind, placebo-controlled trial with a two-arm, parallel design. The participants were randomly assigned to a placebo group or a SCYS group with an allocation ratio of 1:1.

2.2 | Setting

This trial was conducted in Palmerston North, NZ, between May 2019 and July 2021. Participants were recruited from Manawatū-Wanganui and Wellington regions in the North Island of NZ.

2.3 | Participants

Breastfeeding women first contacted the researcher after seeing the online or local advertisements. The study information sheet was then sent to the potential participants, and an online screening questionnaire was completed to check eligibility. Inclusion criteria included women over 16 years with a healthy, singleton infant 1–7 months of age; currently breastfeeding, including feeding directly from the breast, feeding with expressed milk or mixed feeding with formula (less than 100 mL/day). Mothers with the following problems were excluded: allergy to yeasts; taking medicines such as Phenelzine (Nardil), Tranylcypromine (Parnate), Selegiline (Ensam, Eldepryl), Isocarboxazid (Marplan) and Meperidine (Demerol) or any other medications containing Monoamine Oxidase Inhibitors; having health conditions or taking medications that could influence milk secretion-related hormones or milk supply; Crohn's disease; compromised immunity; or treatment for fungal infections. SCYS may contain

large amounts of tyramine that can interact with monoamine oxidase inhibitors. This interaction may cause a significant rise in blood pressure and increase the risk of heart attack or stroke (McCabe-Sellers et al. 2006; U.S. Food and Drug Administration 2010). As such, SCYS should be avoided when taking medications containing monoamine oxidase inhibitors.

The primary outcome of this study is the change in total HMO concentration from baseline to endpoint, calculated as the sum of the concentrations of individual HMOs, consistent with established methods reported in the literature (Austin et al. 2019; Azad et al. 2018; Ferreira et al. 2020; Lagström et al. 2020; Larsson et al. 2019; Miliku et al. 2018; Paganini et al. 2019; Plows et al. 2020; Samuel et al. 2019; Sprenger et al. 2017). HMO profiles and concentrations vary substantially between individuals, primarily due to genetic differences in glycosyltransferase enzymes encoded by the Secretor (Se) and Lewis (Le) genes (Plaza-Díaz et al. 2018; Urashima et al. 2018) Moreover, most HMO concentrations naturally decline over the course of lactation, particularly between one and 9 months postpartum, with exceptions such as 3-FL and LNFP-III (Austin et al. 2019; Ferreira et al. 2020; Larsson et al. 2019; Paganini et al. 2019; Plows et al. 2020; Samuel et al. 2019; Sprenger et al. 2017). Given this interindividual variability and the natural decline in HMO concentrations over time, using the change in total HMO concentration offers a more stable and feasible outcome measure in studies with smaller sample sizes.

The estimated sample size was 32 in each group. This calculation was based on an assumption to capture a 20% difference in total HMO concentration changes from the baseline to the endpoint between the SCYS group and the placebo group. It is further assumed that the total HMO concentration changes is skewed distributed and the variance equal to 0.3-fold the median of the total HMO concentration changes in both groups. These assumptions were based on findings from longitudinal studies on HMO concentration at 1–9 months postpartum (Austin et al. 2019; Ferreira et al. 2020; Larsson et al. 2019; Paganini et al. 2019; Plows et al. 2020; Samuel et al. 2019; Sprenger et al. 2017).

The following equation was used to calculate the sample size (O'Keeffe et al. 2017), where: $m1 = 1$ (median in the placebo group at the endpoint); $m2 = 0.8$ (median in the yeast supplement group at the endpoint); $\varphi 1 = \varphi 2 = 0.3$ (variances); $z\alpha/2 = 1.96$ (5% significance level); and $z\beta = 0.84$ (80% power).

$$n = \frac{\left(\sigma_1^2 + \sigma_2^2\right)\left(z_{\frac{\alpha}{2}} + \frac{z_\beta}{2}\right)^2}{(\log(m_1) - \log(m_2))^2}$$

$$= \frac{\left[\log\left(\frac{1}{2} + \sqrt{\frac{1}{4} + \frac{\phi_1^2}{m_1^2}}\right) + \log\left(\frac{1}{2} + \sqrt{\frac{1}{4} + \frac{\phi_2^2}{m_2^2}}\right)\right]\left(\frac{z_\alpha}{2} + \frac{z_\beta}{2}\right)^2}{(\log(m_1) - \log(m_2))^2}$$

2.4 | Intervention

An independent research technician used the toss-of-coin method for a block of four to randomly assign the eligible participants to the placebo or the SCYS group. Two separate independent technicians generated the randomisation sequences, assigned unique study codes, and securely stored the allocation to maintain concealment. During recruitment and data analysis, the researcher and the participants were blind to the intervention.

Participants received nine capsules daily for 4 weeks, containing either yeast (5 g/day, powdered brewer's yeast grown on molasses, purchased at a local market) or a placebo (corn starch, Novation 4600, Ingredion, NZ). The dose of SCYS was moderate compared to the common dose of brewer's yeast supplementation recommended by manufacturers, online recipes for lactation, and human studies investigating effects on diabetes. Capsule preparation was conducted manually at the Food Product Development Laboratory at Massey University, using orange-flavoured and coloured capsules (Capsuline, US) to mask the yeast's colour and flavour. Participants were instructed to swallow the capsules whole to avoid detecting any differences in taste. All participants received contacts for local breastfeeding support services.

2.5 | Compliance

Capsules were in containers labelled with the week and day of the study. Participants took capsules following the labels and left the missed dose in the container. A photo of the containers was taken weekly to count the number of capsules consumed. Consumption of more than 80% of capsules in total was considered good compliance.

2.6 | Data Collection

Data were collected at baseline, every week during the study period, and at follow-up at 6 months postpartum for the subgroup of infants younger than 150 days at baseline. At baseline and week four of the study (the endpoint), participants visited the Human Nutrition Research Unit at Massey University or met the researcher at their home as per their preference for milk sample collection and infant anthropometric measurements. Other data were collected using online questionnaires or paper forms.

2.6.1 | Milk Sampling and HMO Analysis

Approximately 50 mL of breast milk was collected at the baseline and the endpoint. Milk samples were collected using an electric breast pump between 9:30 am and 11:00 am to avoid milk composition variations caused by circadian rhythm (Italianer et al. 2020). A detailed milk collection procedure is in Appendix. The milk samples were kept at 4°C during transfer to the lab, then separated into 5 mL tubes and stored at −80°C until analysis.

HMOs were analysed by UHPLC with fluorescence detection after labelling with 2-aminobenzamide as described by Austin and colleagues (Austin et al. 2016), with online solid phase extraction clean-up (Bénet and Austin 2011). The total HMO

TABLE 1 | Human milk oligosaccharides included in the analysis.

Name	Abbreviation
Fucosylated HMOs	
2-fucosyllactose	2′-FL
3-fucosyllactose	3-FL
A-tetrasaccharide	A-Tet
Lacto-N-fucosylpentaose-I	LNFP-I
Lacto-N-fucosylpentaose-II	LNFP-II
Lacto-N-fucosylpentaose-III	LNFP-III
Lacto-N-fucosylpentaose-V	LNFP-V
Lacto-N-neofucosylpentaose	LNnFP
Sialylated HMOs	
3-sialyllactose	3′-SL
6-sialyllactose	6′SL
Non-fucosylated, non-sialylated HMOs	
Lacto-N-tetraose	LNT
Lacto-N-neotetraose	LNnT

concentration is calculated as the sum of the concentrations of individual HMOs. Twelve HMOs were quantified, including nine HMOs quantified directly with available standards (2′-FL, 3-FL, A-Tet, LNFP-I, LNFP-V, 3′-SL, 6′-SL, LNT, and LNnT) and three HMOs (LNFP-II, LNFP-III and LNnFP) quantified indirectly against LNFP-I assuming a similar detector response (Table 1). These HMOs are among the most frequently evaluated in the literature and account for approximately 80% of total HMOs (Austin et al. 2019; Azad et al. 2018; Ferreira et al. 2020; Lagström et al. 2020; Larsson et al. 2019; Miliku et al. 2018; Paganini et al. 2019; Plows et al. 2020; Samuel et al. 2019; Sprenger et al. 2017). The presence of 2′-FL was used to define the Secretor status of the women, which influences the concentration of 2′-FL and LNFP-I (Thurl et al. 2017).

2.6.2 | Mothers' Perceptions

Women completed questionnaires at baseline, every week during the study, and at follow-up for the subgroup regarding their perceptions of milk supply and infant behaviours. These questions were developed from previous research (Dennis 2003; Hill and Humenick 1996; Mccarter-Spaulding and Kearney 2001; Safon et al. 2017), interviews with breastfeeding women (Jia 2023) and consultation with midwives and IBCLCs. Questionnaires were refined using cognitive interviews with breastfeeding women and pretested for clarity.

PIM was evaluated by a single question asking if the participant believed she was producing the right amount of milk to satisfy her baby with five options: make too much, just right, sometimes not enough, often not enough, and not sure. Participants who selected "not sure" were asked to explain the reasons, and their responses were then coded to the above options according to their answers.

Perceived milk quantity, quality and infant behaviour were measured by five-point Likert scale items from five (strongly agree) to one (strongly disagree), with reverse scoring for one negative item of perceived infant behaviour. Scores of three items on perceived milk quantity and quality were summed to create the variable of perceived milk quantity and quality (PMQ); similarly, three items were combined for the variable of perceived infant behaviour (PIB).

An open-ended question at the endpoint visit evaluated the perceived effectiveness of the intervention. Participants were asked if they thought the intervention influenced their milk supply and the reasons. The answers were coded to "no influence," "no idea," and "effective." A binary variable of perceived effectiveness of the intervention (PEI) was created with "effective" coded as positive and "no influence"/"no idea" coded as neutral.

2.6.3 | Postnatal Distress

Postnatal distress was assessed by the DASS-21 scale (Lovibond and Lovibond 1995). This scale has 21 Likert scale (0–3) items, including three subscales of depression, anxiety, and stress. Each subscale contains seven items. Scores for each subscale were calculated by summing up the points for relevant items and multiplying by two; the total score was calculated by summing up the scores of the three subscales, and higher scores indicated more severe symptoms (Lovibond and Lovibond 1995). The DASS-21 scale has been used to evaluate psychological distress, depression, anxiety, and stress in pregnant women and mothers of young children in NZ (Lovell et al. 2015).

2.6.4 | Infant Breastfeeding Patterns

Infant feeding practices were recorded in the questionnaire at baseline, every week during the study and at follow-up after 6 months. A binary variable of breastfeeding status (exclusive breastfeeding vs other) was created to compare the proportion of women exclusively breastfeeding at baseline, endpoint, and follow-up. A variable of predominant breastfeeding was also created.

The WHO definition of exclusive breastfeeding was used in this study (Word Health Organization 2022). Predominant breastfeeding in this study means that from birth to each time point of the survey, the infant mostly received breast milk (including feeding on breasts, expressed milk and donor milk). In addition, the infant might also have received liquids (water and water-based drinks, fruit juice), ritual fluids and ORS, drops or syrups (vitamins, minerals and medicines), as well as a small amount of formula (< 100 mL/day) occasionally.

The participants recorded breastfeeding timing and duration for each feed over 24 h 1 day before the baseline and endpoint visits. Participants were asked to record one feed beyond 24 h to ensure the record covered 24 h. Both feeding on the breasts and feeding with expressed milk were recorded to calculate breastfeeding frequency in 24 h. Breastfeeding duration over 24 h was only calculated from feeds on the breast. The definition of one feed (on breast) was the completion of latching on, swallowing and latching off, regardless of the time between latching on and off. For example, if the infant latched on one side and fed for

1 min, then switched to the other side and fed for another 2 min, this was counted as two feeds. This definition of one feed differs from previous research, which limited the feeding time to at least 1 min (Oras et al. 2015; Oras et al. 2020; Vitzthum 1989) or 2 min (Gross and Burger 2002), without involving the time between the latches. This study focused on the effect of the intervention on infant feeding behaviour; therefore, even short feeds were recorded.

2.6.5 | Infant Anthropometry

The researcher measured the infant's weight, length and head circumference at baseline and endpoint visits. Infants were weighed using a baby weighing scale (Nagata Scale Co Ltd), and the weight was recorded to the nearest 5 g. Infant length was measured from heel to crown using an infant length board and recorded to the nearest millimetre. Infant head circumference was measured using a flexible, non-stretch tape (Gibson 1990) and recorded to the nearest millimetre.

2.6.6 | Adverse Effects

Self-reported frequency of symptoms related to potential adverse effects from SCYS, including constipation, decreased appetite, nausea, and skin rash, were collected at the baseline and weekly during the study by using a five-point Likert scale for each symptom ranking from five (always) to one (never). Participants were also asked to record any other adverse events they perceived related to taking the study capsules.

2.6.7 | Socio-Demographic and Maternal Factors

Socio-demographic and birth and infant information were collected at baseline. This information was used to describe the study sample.

2.7 | Data Analysis

Data analyses were performed using SPSS version 25.0 (IBM, Armonk, NY, US). Intention-to-treat analysis was used. All continuous variables were tested for normality using a combination of methods, including stem-and-leaf plot of residuals, normality plot, and Shapiro–Wilk test. Descriptive analysis was conducted for all the data. Data were summarised using mean ± standard deviation (SD) for normally distributed variables or median (Q1, Q3) for non-normally distributed variables. Categorical variables were summarised using frequencies (%).

The total concentration of fucosylated HMOs was calculated by summing the concentrations of 2′-FL, 3-FL, LNFP-I, LNFP-II, LNFP-III, LNFP-V and LNnFP. The concentration of sialylated HMOs was calculated by summing the concentrations of 3′-SL and 6′-SL. The total concentration of non-fucosylated, non-sialylated HMOs was calculated by summing the concentrations of LNT and LNnT. The total HMO concentration was calculated by summing all 11 of these HMOs. A-Tet was present in only 26% of milk samples ($n = 18$), and the concentration of A-Tet was only 1%–3% of the total HMO concentration in most milk samples. Thus, A-Tet concentration was excluded from the data analysis. Variables of total HMO concentration change and each individual HMO concentration change were established by calculating the concentration changes from baseline to endpoint. Multivariable linear regression with the bootstrap method (Astivia and Zumbo 2019; Williams et al. 2013) was used to address potential confounders and to examine the relationship between the effect of intervention and HMO concentration changes. The potential confounders included secretor status, maternal age, and breastfeeding status (Azad et al. 2018; Ferreira et al. 2020; McGuire et al. 2017). Thus, four variables we included in the model to evaluate individual and total HMO concentration change. The estimated sample was 53 participants to detect significant individual and total HMO concentration changes. The required sample size was calculated using G*Power for a multiple regression analysis with four predictors. Assuming a medium effect size ($f^2 = 0.25$), 80% power, and a 0.05 significance level (Faul et al. 2009).

A comparison of mothers' perceptions (PIM, PMQ and PIB) between the two groups was made using the Mann-Whitney U test. Friedman's ANOVA was used to compare the changes in mothers' perceptions over time within each group. The difference in PEI between the two groups at the endpoint was assessed using the Chi-square test. Postnatal distress (scores of DASS-21 scale and subscales), breastfeeding frequency and duration over 24 h, and infant growth were analysed by mixed ANOVA or nonparametric factorial ANOVA. Variables of breastfeeding duration and frequency were created by summing up breastfeeding time and number of feeds for the day (6 am–6 pm), night (6 pm–6 am the next day), and over 24 h. Variables related to infant growth were infant weight, length and head circumference, as well as weight-for-age Z-score (WAZ), length-for-age Z-score (LAZ), head circumference-for-age Z-score (HCAZ) and weight-for-length Z-score (WLZ), which were calculated using WHO Anthro software version 3.2.2 (World Health Organization 2011). The Aligned Rank Transform (ART) method was used for non-normally distributed variables during nonparametric factorial ANOVA (Elkin et al. 2021; Wobbrock et al. 2011). When significant differences were identified in ANOVA analysis, post hoc comparisons were conducted using the Wilcoxon signed-rank test with a Bonferroni correction to determine the specific time points at which the changes occurred. Perceived adverse effects between the two groups were compared using the Mann-Whitney U test. Other adverse effects reported were analysed using descriptive analysis. The difference between the number of participants who reported other adverse effects was compared by the Chi-square test.

In the subgroup of women with infants less than 150 days at baseline, the proportions of predominant breastfeeding, exclusive breastfeeding, and formula use between the two groups were compared by Fisher's Exact test. The change in mothers' perceptions (PIM, PMQ and PIB) from baseline, endpoint to follow-up after 6 months was evaluated using Friedman's ANOVA. PIM, PMQ, and PIB between the two groups were tested using the Mann-Whitney U test.

2.8 | Ethics Statement

Massey University Human Ethics Committee approved this study (SOA 18/80).

3 | Results

In total, 72 women signed up for this study, 68 participants completed the trial at the endpoint, and 46 completed the follow-up survey (Figure 1). Most participants had good compliance during the intervention: 57 participants consumed ≥ 90% of capsules, 10 participants consumed 80%– 89% of capsules (five in each group), and only one participant in the placebo group consumed < 80% of capsules.

Demographics and baseline characteristics were similar in the two groups (Table 2). The mean age of participants was 32.1 years, similar to the median age of women having an infant in NZ (31.2 years) (Statistics New Zealand 2023). Compared to the census data in 2018 (Statistics New Zealand 2020), there was a much higher proportion of participants who were Caucasian and had received tertiary education. About 60% of participants had an annual family income above the median income of NZ in 2020 (Statistics New Zealand 2021). Fewer women had a caesarean delivery than nationally (21% vs 29%) (Ministry of Health 2021b).

3.1 | The Effect on Human Milk Oligosaccharide Concentration

The change of HMO concentration from baseline to endpoint was evaluated in 67 participants (Table 3). There were significantly more secretors in the placebo group ($n = 26$, 72%) than in the SCYS group ($n = 19$, 53%, $p = 0.046$). Multivariable linear regression analysis was used to adjust the potential influences of secretor status and the other confounders (Azad et al. 2018; McGuire et al. 2017) (the regression models are in Appendix 2). The SCYS had no significant influence on the change in concentration of total HMOs or any individual HMO during the 4-week intervention (Figure 2). However, exclusive breastfeeding was significantly associated with the concentration change of 6′-SL and the concentration change of the total sialylated HMOs.

3.2 | Other Indicators of Breast Milk Production

3.2.1 | Participants' Perceptions

Most of the participants had positive perceptions of their milk supply (PIM), milk quantity and quality (PMQ) and infant behaviour (PIB) at baseline. There was no difference between mothers' perceptions in the placebo group and the SCYS group at baseline, each week, or at the study's endpoint (Table 4); however, PMQ in the SCYS group significantly changed over the 4-week study (χ^2 (4) = 11.88, $p = 0.018$). Post hoc analysis of the effect at a 0.05/4 = 0.0125 level of significance suggested that total scores of PMQ in the SCYS group significantly increased from baseline to endpoint ($T = 7$, $r = -0.31$). However, no significant changes for individual items of PMQ in the SCYS group were found.

There were significantly more participants in the SCYS group than in the placebo group who perceived that the intervention effectively increased their milk supply ($n = 22$, 65% and $n = 12$,

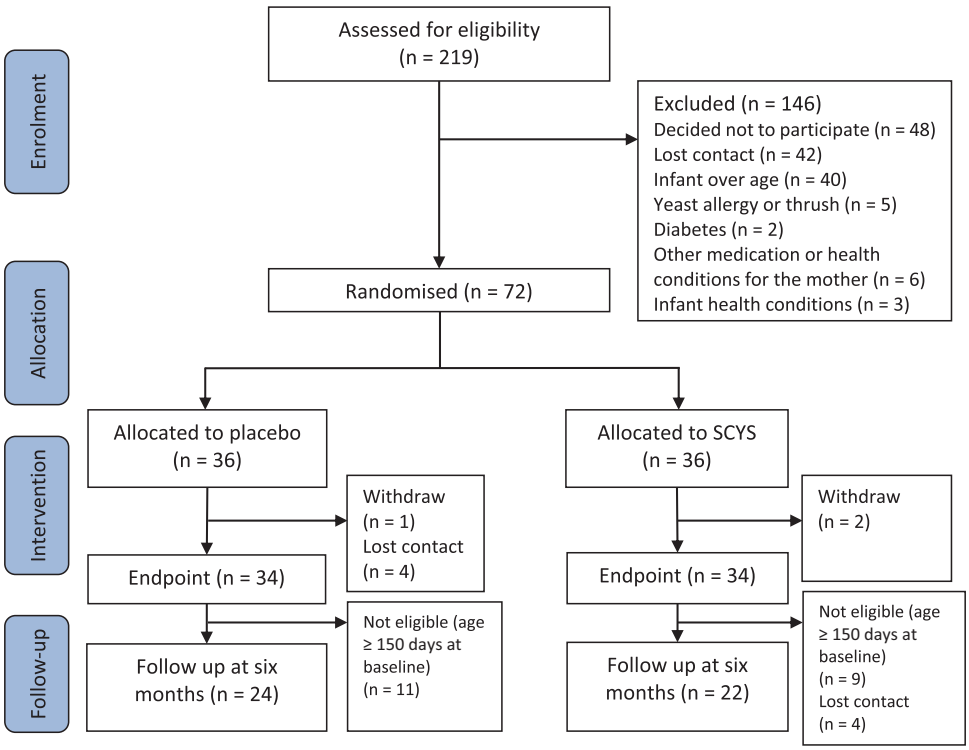

FIGURE 1 | Flowchart of participant recruitment.

TABLE 2 | Demographic and baseline characteristics.

Characteristics	Total ($n = 72$)	Placebo ($n = 36$)	Yeast ($n = 36$)
Maternal age, years (mean ± SD)	32.1 ± 4.9	31.9 ± 5.4	32.4 ± 4.3
Tertiary education (n, %)	56 (78)	28 (78)	28 (78)
Ethnicity – Caucasian (n, %)[a]	59 (82)	31 (86)	28 (78)
Ethnicity – Māori (n, %)	6 (8)	5 (14)	1 (3)
Ethnicity – Asian (n, %)	9 (13)	4 (11)	5 (14)
Ethnicity – Other (n, %)	3 (4)	1 (3)	2 (6)
Annual household income, $n = 68$ (Above median, n, %)[b]	43 (63)	23 (64)	20 (56)
Live with partner and child(ren) (n, %)	68 (94)	35 (97)	33 (92)
Number of children – 1 (n, %)	33 (46)	17 (47)	16 (45)
Number of children – 2 (n, %)	22 (31)	9 (25)	13 (36)
Number of children – ≥ 3 (n, %)	17 (23)	10 (28)	7 (19)
Smoking (n, %)	2 (3)	1 (3)	1 (3)
Drinking alcohol (n, %)	43 (60)	20 (56)	23 (64)
Drinking < 1 standard drink each time (n, % in drinking alcohol)	32 (74)	13 (65)	19 (83)
Caesarean delivery (n, %)	21 (29)	11 (30)	10 (28)
Gestation age, week (median [Q1, Q3])	40 (39, 40.8)	40 (38.9, 40.9)	39.8 (39.4, 40.7)
Infant age, days (median [Q1, Q3]) at the first visit	108 (70, 166)	108 (66, 170)	105 (76, 157)
Infants birth weight, grams (median [Q1, Q3])	3678 (3335, 3975)	3740 (3465, 3975)	3575 (3255, 4005)
Male infant (n, %)	40 (56)	23 (64)	17 (47)
Breastfeeding status – predominant breastfeeding (n, %)[c]	64 (89)	33 (92)	31 (86)
Breastfeeding status – exclusive breastfeeding (n, %)	34 (47)	18 (50)	16 (44)
Breastfeeding status – currently formula (n, %)	5 (7)	2 (5)	3 (8)
Breastfeeding status – started solids (n, %)	22 (30)	10 (28)	12 (33)

[a]The frequencies of ethnicity do not add up to 100% as some participants selected more than one ethnicity group.
[b]Median annual household income based on Statistics New Zealand is 87,607 New Zealand Dollars for the year ended June 2020 (Statistics New Zealand 2021).
[c]The frequencies of breastfeeding status do not add up to 100% as three infants who were mixed-fed with breast milk and formula had started consuming solids.

35%, respectively, $p = 0.043$). Participants self-reported the signs and reasons for reporting the effectiveness of the intervention. These included, in order of frequency of mention, breast fullness and/or leaking, faster and stronger milk ejection, more settled infant or improved infant sleep, increased amount of expressed milk, and more efficient or shorter time at each feeding (the latter reported only in the SCYS group). While individual participants mentioned more than one sign/reason for the effectiveness of the intervention, the limited number of women perceiving the intervention to be effective made it impossible to conduct further quantitative analyses.

3.2.2 | Postnatal Distress

DASS-21 score significantly decreased from baseline to the study endpoint, suggesting that postnatal distress improved over time. However, there was no interaction between time and the intervention. Compared to the placebo, taking the yeast supplement did not significantly relate to the DASS-21 score or score of depression, anxiety, or stress (data shown in Appendix 3).

3.2.3 | Infant Anthropometry Measurements

There was no interaction between time and the intervention. There were no differences in WAZ, LAZ, HAZ or WLZ between the SCYS and placebo groups. Taking SCYS was not associated with infant growth (data shown in Appendix 4).

3.2.4 | Breastfeeding Pattern

After the 4-week intervention, the proportion of predominant breastfeeding ($n = 27$, 79% in the placebo group and $n = 26$, 77% in the SCYS group) and exclusive breastfeeding ($n = 20$, 59% in the placebo group and $n = 19$, 56% in the SCYS group) were similar in the two groups.

The total breastfeeding time and the number of breastfeeds over 24 h (6 am – 6 am the next day) significantly decreased from baseline to endpoint in both groups (data shown in Appendix 5). There were no differences in total breastfeeding time and number of feeds during the day (6 am – 6 pm), night

TABLE 3 | Human milk oligosaccharide concentrations at the baseline and endpoint.

HMO concentration, mg/L (median [Q1, Q3])	Baseline		Endpoint	
	Placebo ($n = 33$)	Yeast ($n = 34$)	Placebo ($n = 33$)	Yeast ($n = 34$)
2′-FL	1639 (1021, 2215)	1475 (0, 2744)	1490 (1000, 2163)	1461 (0, 2550)
3-FL	1747 (1022, 2655)	2034 (1099, 3313)	1936 (1218, 2491)	2405 (1094, 3769)
LNFPI	330 (107, 550)	204 (2, 700)	269 (140, 482)	128 (2, 595)
LNFPII	559 (291, 994)	611 (326, 1175)	511 (304, 942)	571 (296, 1085)
LNFPIII	502 (365, 676)	523 (411, 639)	527 (408, 643)	492 (408, 667)
LNFP-V	67 (41, 111)	74 (45, 139)	58 (35, 111)	57 (40, 134)
LNnFP	21 (11, 41)	16 (10, 28)	17 (11, 34)	12 (6, 27)
Total Fucosylated	5286 (4814, 6082)	5502 (4951, 6444)	5250 (4818, 6141)	5524 (4897, 6809)
3′-SL	179 (149, 206)	171 (145, 209)	181 (163, 236)	169 (145, 206)
6′-SL	116 (70, 241)	181 (75, 257)	98 (66, 160)	113 (56, 177)
Total Sialylated	311 (241, 466)	365 (237, 419)	288 (241, 466)	294 (217, 357)
LNT	1088 (714, 1423)	984 (689, 1534)	917 (672, 1272)	841 (606, 1159)
LNnT	145 (67, 216)	113 (40, 212)	123 (60, 194)	101 (29, 189)
Total Non-fucosylated, non-sialylated	1175 (965, 1552)	1106 (833, 1707)	984 (805, 1462)	980 (714, 1303)
Total	6682 (6135, 8156)	7128 (6340, 8266)	6870 (5979, 7494)	6849 (5947, 8366)

Abbreviations: HMO, human milk oligosaccharides; 2′-FL, 2-fucosyllactose; 3-FL, 3-fucosyllactose; LNFP-I; Lacto-N-fucosylpentaose-I; LNFP-II, Lacto-N-fucosylpentaose-II; LNFP-III, Lacto-N-fucosylpentaose-III; LNFP-V, Lacto-N-fucosylpentaose-V; LNnFP, Lacto-N-neofucosylpentaose; 3′-SL, 3-sialyllactose; 6′-SL, 6-sialyllactose; LNT, Lacto-N-tetraose; LNnT, Lacto-N-neotetraose.

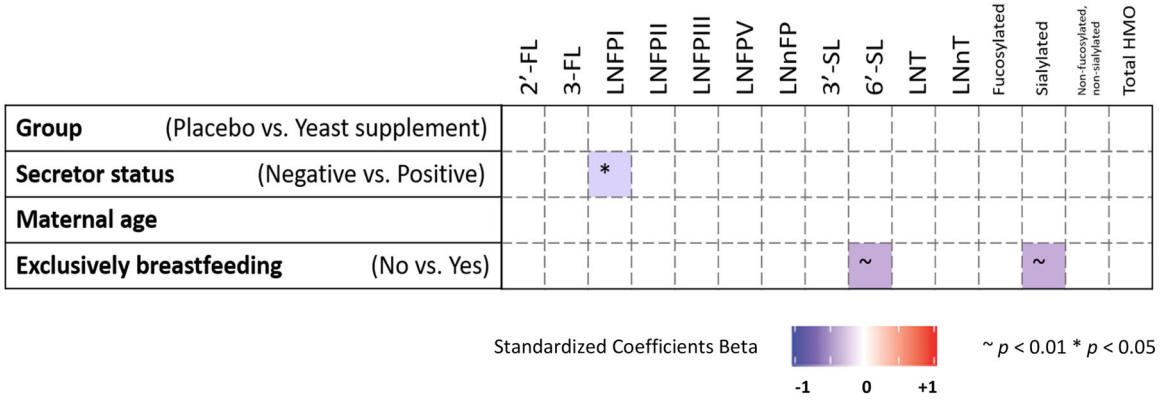

FIGURE 2 | Summary of multivariable linear regression of the intervention (group) and confounders (secretor status, maternal age, and breastfeeding status) and HMO concentration change among 67 participants. Beta estimates are from multivariable linear regression models with the bootstrap method for the concentration of each HMO at the endpoint. Colouring reflects the direction and magnitude of the coefficient beta. ~$p < 0.01$, *$p < 0.05$. The first category of each factor is the reference category. Abbreviations: HMO, human milk oligosaccharides; 2′-FL, 2-fucosyllactose; 3-FL, 3-fucosyllactose; LNFP-I; Lacto-N-fucosylpentaose-I; LNFP-II, Lacto-N-fucosylpentaose-II; LNFP-III, Lacto-N-fucosylpentaose-III; LNFP-V, Lacto-N-fucosylpentaose-V; LNnFP, Lacto-N-neofucosylpentaose; 3′-SL, 3-sialyllactose; 6′-SL, 6-sialyllactose; LNT, Lacto-N-tetraose; LNnT, Lacto-N-neotetraose.

(6 pm – 6 am the next day) or over 24 h between the SCYS group and the placebo group. Taking SCYS had no significant influence on infant breastfeeding patterns (data shown in Appendix 5).

3.3 | The Adverse Effects

There were no significant differences between the placebo and the SCYS group in the self-reported frequency of constipation, nausea,

TABLE 4 | Participants' perceptions of milk supply and effectiveness of the intervention at baseline, each week, and the endpoint ($n = 68$).

Perceptions	Baseline		Week 1		Week 2		Week 3		Endpoint	
	Placebo ($n = 34$)	Yeast ($n = 34$)	Placebo ($n = 34$)	Yeast ($n = 34$)	Placebo ($n = 34$)	Yeast ($n = 34$)	Placebo ($n = 34$)	Yeast ($n = 34$)	Placebo ($n = 34$)	Yeast ($n = 34$)
PIM (Median [Q1, Q3])	4 (3.75, 4)	4 (4, 4)	4 (4, 4)	4 (4, 4)	4 (4, 4)	4 (4, 4)	4 (4, 4)	4 (4, 4)	4 (4, 4)	4 (4, 4)
PMQ (Median [Q1, Q3])	14.5 (13, 15)	15 (13, 15)*	15 (13, 15)	15 (13, 15)	15 (13, 15)	15 (13, 15)	15 (13, 15)	15 (14.75, 15)	15 (13, 15)	15 (14, 15)*
PIB (Median [Q1, Q3])	13 (12, 14)	14 (12, 15)	13 (12, 15)	14 (12, 15)	14 (13, 15)	13.5 (12.75, 14)	14 (12, 15)	14.5 (13, 15)	14 (13, 14)	15 (13, 15)
PEI (n [%])	—	—	—	—	—	—	—	—	12 (35)	22 (65)**

Abbreviations: PIM, perceived insufficient milk supply; PMQ, perceived milk quantity and quality; PIB, perceived infant behaviour; PEI, Perceived effectiveness of the intervention. Mann–Whitney U test for PIM, PMQ and PIB scores found no significant differences between two groups at each time point. Friedman's ANOVA for PIM, PMQ and PIB scores within each group found no significant differences for PIM and PIB, but significant changes in PMQ within the yeast over the four-week study ($\chi^2(4) = 11.88$, $p = 0.018$).

** Post hoc* test using the Wilcoxon signed ranks test with a Bonferroni correction, compared with the yeast group at the baseline, $p = 0.011$.

****** Chi-square test for PEI at the endpoint ($\chi^2 = 4.099$, $p = 0.043$).

decreased appetite or skin rash at baseline, each week during the intervention, or at the endpoint (data shown in Appendix 6).

Twenty-two women (32%) reported adverse events, other than the four listed in the survey, either for themselves or for their infants, including eight (24%) in the placebo group and 14 (41%) in the SCYS group, with no significant difference between the two groups. Four women in the placebo group and seven in the SCYS group reported adverse effects in only one out of 4 weekly surveys, whereas the others reported the same problem in two or more weekly surveys. However, all these participants continued taking the capsules and completed the study. These adverse events reported by participants are summarised in Table 5.

3.4 | Breastfeeding Status and Participants' Perceptions at 6 Months Postpartum

Forty-six women completed the follow-up survey at 6 months postpartum. Two participants in the placebo group had ceased

TABLE 5 | Other adverse events reported by study participants.

Adverse events	Placebo group ($n = 34$)	SCYS group ($n = 34$)
Adverse events from the mother		
Upper back pain	1	
Headache		1
Abdominal pain	1	
Thrush		2
Itchy/dry skin		3
Orange aftertaste	1	1
Dry mouth	1	
Increased appetite		1
Thirsty		1
Heart burn	1	1
Reflux	1	2
Gassiness/bloating and flatulence	2	3
Decreased frequency of bowel movements	1	
Change the smell of urine		1
Adverse events from the infant		
Choke/cough during milk ejection		2
Increased spill after feedings		1
Nappy rash		1
Change the smell of faeces	1	
Decreased frequency of bowel movements	1	1

breastfeeding, and the other 44 women were breastfeeding their infants. There was no difference in maternal age, education level or family income in this subgroup between the placebo and SCYS groups. There was also no difference in mean infant age at baseline in this subgroup (88 ± 33.3 days in the placebo group vs. 96 ± 30.8 days in the SCYS group).

The proportion of predominant breastfeeding ($n = 11$, 50% in the placebo group and $n = 13$, 59% in the SCYS group) and the proportion of exclusive breastfeeding ($n = 8$, 36% in the placebo group and $n = 10$, 45% in the SCYS group) were similar in the two groups. No difference was observed in the proportion of infants who started complementary feeding ($n = 5$, 23% in the placebo group and $n = 7$, 32% in the SCYS group). However, the proportion of participants reporting any formula use in the SCYS group was significantly lower than in the placebo group ($n = 1$, 4% and $n = 6$, 27% respectively, $p = 0.047$).

Participants in both groups had positive perceptions of their milk supply and milk quality at 6 months postpartum, which were the same as those at the endpoint. There were no significant differences in PIM or PMQ between the two groups at 6 months postpartum. However, women in the SCYS group had significantly higher PIB scores than women in the placebo group at the baseline and 6 months (Table 6), Thus although the randomisation process was properly conducted, it did not result in balanced baseline characteristics within this smaller follow-up subgroup.

4 | Discussion

This is the first RCT to test the effect of maternal supplementation of SCYS on the concentration of HMOs, indicators of PIM, and potential adverse effects on mothers and their infants. Compared to the placebo, SCYS did not affect the concentration of individual HMOs or total HMOs. There was no significant difference in PIM, PMQ, PIB, postnatal distress, infant feeding pattern, infant anthropometry measurements or adverse effects between the SCYS and placebo groups at the endpoint. However, the endpoint PMQ score in the SCYS group was significantly increased compared to the baseline. Furthermore, compared to women who received the placebo, women who consumed SCYS were significantly more likely to report that the intervention was effective after the intervention (35% in the placebo group vs. 65% in the SCYS group, $p < 0.05$). Compared to the placebo group, significantly fewer women in the SCYS group reported using formula at 6 months postpartum.

In this study, SCYS supplementation did not affect the total HMO concentration or the concentration of any single HMO. Notably, only 11 HMOs were tested in this study, accounting for approximately 80% of the concentration of total HMOs (Thurl et al. 2017). This study did not measure a few neutral HMOs, reported to be at higher concentrations than 3-FL, such as TF-LNH, DF-LNH II and LNDFH-I (Thurl et al. 2017). These HMOs may impact the total HMO concentration of non-secretor's milk more than that of secretor's milk. Future research with a larger sample size should include these HMOs and compare the individual and total HMO concentrations of secretor's and nonsecretor's milk separately.

TABLE 6 | Participants' perceptions of milk supply at endpoint and follow-up at six months postpartum ($n = 44$).

	Baseline		Endpoint		Follow up	
Perceptions	**Placebo ($n = 22$)**	**Yeast ($n = 22$)**	**Placebo ($n = 22$)**	**Yeast ($n = 22$)**	**Placebo ($n = 22$)**	**Yeast ($n = 22$)**
PIM (Median [Q1, Q3])	4 (4, 4)	4 (4, 4)	4 (4, 4)	4 (4, 4)	4 (3.75, 4)	4 (4, 4)
PMQ (Median [Q1, Q3])	14.5 (13, 15)	15 (14, 15)	15 (14, 15)	15 (15, 15)	15 (13.5, 15)	15 (14.75, 15)
PIB (Median [Q1, Q3])	13 (11.75, 15)*	15 (13.75, 15)*	14 (13, 15)	15 (13.75, 15)	13 (11.5, 15)*	14.5 (13, 15)*

Abbreviations: PIM, perceived insufficient milk supply; PMQ, perceived milk quantity and quality; PIB, perceived infant behaviour.
Friedman's ANOVA for PIM, PMQ and PIB within each group. Mann-Whitney U test for PIM, PMQ, and PIB was conducted between the two groups at each time point.
*Mann–Whitney U test, $p < 0.05$.

Further, the significant interindividual variation in HMO concentration suggests a need for a larger sample size. In this study, the concentration of some HMOs decreased for some women but increased for others. This has also been observed in previous research (Sprenger et al. 2017). This interindividual variation may result from day-to-day variation of HMO concentration, which is poorly documented in the literature. Furthermore, HMO concentration is affected by not only fixed factors such as genetic secretor status and maternal age but also modifiable and environmental factors, including diet, season and geography that were not controlled in this study (Azad et al. 2018; McGuire et al. 2017; Quin et al. 2020). Future studies need to collect these data to control the confounders and check for the effectiveness of randomisation.

There was a significant inverse association between the exclusivity of breastfeeding and changes in the concentration of 6′-SL. This is consistent with previous findings reported by Azad et al. (2018) that 6′-SL was enriched in the milk of women feeding formula to their infants. However, our data do not permit us to explain the biological mechanism underlying this relationship. Future research should further explore the reasons behind this observed association, and the implications for infant growth, development, and maternal breastfeeding practices.

Participants in the SCYS group were more likely to report the intervention as positively impacting their milk supply than participants in the placebo group. However, there was no difference in PIM between the two groups after the intervention. This may be because most of the participants in both groups already had strong beliefs in their breast milk supply at baseline, so most participants were not likely to experience PIM regardless of intervention. In the SCYS group, the PMQ scores were significantly higher at the study's endpoint than at baseline. However, no differences between the two groups were found. The contradictory results could be due to an insignificant increase in the placebo group, leading to nonsignificant differences in PMQ at the endpoint. It is also possible that the sample size was too small to identify a significant difference in PMQ between the two groups. Replication in a larger sample would show if there were a difference in mothers' perceptions between the two groups.

At 6 months postpartum, significantly fewer women reported formula use in the SCYS group than in the placebo group, but no differences in exclusive breastfeeding or complementary feeding were observed. Women in the SCYS group also had significantly higher PIB scores than women in the placebo group. It is possible that women in the SCYS group perceived their infants to be more satisfied and settled, and, therefore, they decided not to feed formula at 6 months. However, in this subgroup, the baseline PIB score in the SCYS group was significantly higher than that in the placebo group, but no difference was found at the endpoint. All women reported improved infant behaviour at the endpoint, regardless of group. These results indicate that the women's perceptions were unrelated to the intervention, and differences were seen due to the ineffective randomisation for this small subgroup followed up at 6 months postpartum. The small sample size in this subgroup provided insufficient power to state if SCYS has any effect.

There were no differences between the two groups for postnatal distress, infant anthropometry measurements, or breastfeeding patterns in this study. The sample size was estimated based on the total HMO concentration changes, however, and, as such, the sample was too small to identify any differences in these indicators between the two groups.

This study found no evidence of major adverse effects of SCYS supplementation at this dose on women and/or their infants. The frequency of occurrence of four previously reported effects of SCYS (constipation, decreased appetite, nausea, and skin rash) was similar between the groups. Both groups reported several "adverse events," which were all common health problems and might be unrelated to supplementation. Moreover, all these adverse effects were rarely reported or reported similar numbers between groups, and all the participants completed the study. Thus, SCYS supplementation had limited adverse effects on mothers and their infants.

This is the first study that aimed to evaluate the effect of SCYS on breastfeeding. Randomisation with a double-blind design controlled for selection, performance and detection bias (Akobeng 2008). The low number of participants that withdrew from the study and the intention-to-treat analysis controlled for attrition bias (Akobeng 2008). However, there are a few limitations to this study. First, the failure of randomisation regarding secretor status made it impossible to compare individual and total HMO concentrations between the two groups. Currently, the practical method for identifying secretor status is based on the presence of 2′-FL in breast milk samples (Urashima et al. 2018). This was done retrospectively after data collection. Future research could include 2′-FL concentration tests during recruitment and use the block randomisation method to ensure that participants are evenly allocated across intervention and placebo groups. Second, the limited number of women with PIM was a limitation, as it reduced the ability to detect changes in the proportion of women reporting PIM. In this study, the PIM definition covered both actual insufficient milk supply and misperceptions. Recruiting a large number of women who are experiencing PIM could address this limitation. Increasing the sample size beyond the original calculation may be necessary because including women with actual insufficient milk introduces additional variability due to diverse underlying causes, and different intervention mechanisms may only be effective for certain subgroups of women. Additionally, recruiting women with PIM might result in higher formula use during the study, which could subsequently decrease breast milk production (Riordan and Wambach 2010; Weaver and Hernandez 2016) and result in introducing formula use as a confounder and potentially increase participant withdraw rates. Additionally, this study used a small dose of a SCYS (5 g/day) grown on molasses. It remains unknown if the high dose (i.e., > 20 g/day) recommended by manufacturers would impact women's milk production and composition. The results cannot be generalised to SCYS produced from the brewing process (Jia et al. 2021). Finally, this study lacked direct measures of breast milk volume, focusing instead on indicators of perceptions of milk supply.

5 | Conclusion

This is the first RCT that examines the effect of a SCYS grown on molasses (at a dosage of 5 g/day) on HMO concentration and indicators of milk supply. Participants had good compliance

and no strong adverse effects. No difference was observed in changes in the concentration of HMOs measured or in the measured indicators of milk supply between the two groups. However, participants who received the SCYS were more likely to perceive the intervention to be effective than those receiving the placebo. The low prevalence of PIM in both groups at baseline and endpoint may have contributed to the lack of difference in PIM between the SCYS and placebo groups. An RCT with a larger sample size, broader HMO profiles and milk composition measures, and direct measures of milk volume are needed to further evaluate the effects of SCYS on breast milk supply and composition.

Author Contributions

This research is part of Lili Jia's PhD thesis. Janet Weber and Louise Brough supervised Lili Jia's PhD. All authors contributed to the study conception, design and ethics application; Lili Jia prepared the study material, collected and analysed data, and prepared the original manuscript draft; Janet Weber and Louise Brough regularly discussed data analysis with Lili Jia, and reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

Akobeng, A. K. 2008. "Assessing the Validity of Clinical Trials." *Journal of Pediatric Gastroenterology and Nutrition* 47, no. 3: 277–282. https://doi.org/10.1097/MPG.0b013e31816c749f.

Astivia, O. L. O., and B. D. Zumbo. 2019. "Heteroskedasticity in Multiple Regression Analysis: What It Is, How to Detect It and How to Solve It With Applications in R and SPSS." *Practical Assessment, Research, and Evaluation* 24, no. 1: 1.

Austin, S., C. De Castro, T. Bénet, et al. 2016. "Temporal Change of the Content of 10 Oligosaccharides in the Milk of Chinese Urban Mothers." *Nutrients* 8, no. 6: 346. https://doi.org/10.3390/nu8060346.

Austin, S., C. A. De Castro, N. Sprenger, et al. 2019. "Human Milk Oligosaccharides in the Milk of Mothers Delivering Term Versus Preterm Infants." *Nutrients* 11, no. 6: 1282. https://doi.org/10.3390/nu11061282.

Azad, M. B., B. Robertson, F. Atakora, et al. 2018. "Human Milk Oligosaccharide Concentrations Are Associated With Multiple Fixed and Modifiable Maternal Characteristics, Environmental Factors, and Feeding Practices." *Journal of Nutrition* 148, no. 11: 1733–1742.

Bazzano, A. N., L. Cenac, A. Brandt, J. Barnett, S. Thibeau, and K. P. Theall. 2017. "Maternal Experiences With and Sources of Information on Galactagogues to Support Lactation: A Cross-Sectional Study." *International Journal of Women's Health* 9: 105–113. https://doi.org/10.2147/ijwh.s128517.

Bénet, T., and S. Austin. 2011. "On-Line Cleanup for 2-Aminobenzamide-Labeled Oligosaccharides." *Analytical Biochemistry* 414, no. 1: 166–168.

Bode, L. 2012. "Human Milk Oligosaccharides: Every Baby Needs a Sugar Mama." *Glycobiology* 22, no. 9: 1147–1162.

Dennis, C. L. 2003. "The Breastfeeding Self-Efficacy Scale: Psychometric Assessment of the Short Form." *Journal of Obstetric, Gynecologic & Neonatal Nursing* 32, no. 6: 734–744. https://doi.org/10.1177/0884217503258459.

Elkin, L. A., M. Kay, J. J. Higgins, and J. O. Wobbrock. 2021. "An Aligned Rank Transform Procedure for Multifactor Contrast Tests." *34th Annual ACM Symposium on User Interface Software and Technology*: 754–768.

Faul, F., E. Erdfelder, A. Buchner, and A.-G. Lang. 2009. "Statistical Power Analyses Using G*Power 3.1: Tests for Correlation and Regression Analyses." *Behavior Research Methods* 41, no. 4: 1149–1160. https://doi.org/10.3758/BRM.41.4.1149.

Ferreira, A. L., R. Alves, A. Figueiredo, et al. 2020. "Human Milk Oligosaccharide Profile Variation Throughout Postpartum in Healthy Women in a Brazilian Cohort." *Nutrients* 12, no. 3: 790. https://doi.org/10.3390/nu12030790.

Foong, S. C., M. L. Tan, W. C. Foong, L. A. Marasco, J. J. Ho, and J. H. Ong. 2020. "Oral Galactagogues (Natural Therapies or Drugs) for Increasing Breast Milk Production in Mothers of Non-Hospitalised Term Infants." *Cochrane Database of Systematic Reviews* 5: CD011505. https://doi.org/10.1002/14651858.CD011505.pub2.

Gatti, L. 2008. "Maternal Perceptions of Insufficient Milk Supply in Breastfeeding." *Journal of Nursing Scholarship* 40, no. 4: 355–363. https://doi.org/10.1111/j.1547-5069.2008.00234.x.

Gibson, R. 1990. *Principles of Nutritional Assessment*. Oxford University Press.

Gross, B. A., and H. Burger. 2002. "Breastfeeding Patterns and Return to Fertility in Australian Women." *Australian and New Zealand Journal of Obstetrics and Gynaecology* 42, no. 2: 148–154.

Hallam, M. C., D. Barile, M. Meyrand, J. B. German, and R. A. Reimer. 2014. "Maternal High-Protein or High-Prebiotic-Fiber Diets Affect Maternal Milk Composition and Gut Microbiota in Rat Dams and Their Offspring." *Obesity* 22, no. 11: 2344–2351.

Hill, P. D., and S. S. Humenick. 1996. "Development of the H &; H Lactation Scale." *Nursing Research* 45, no. 3: 136–140.

Huang, Y., Y. Liu, X. Y. Yu, and T. Y. Zeng. 2022. "The Rates and Factors of Perceived Insufficient Milk Supply: A Systematic Review." *Maternal & Child Nutrition* 18, no. 1: e13255.

Italianer, M. F., E. F. G. Naninck, J. A. Roelants, et al. 2020. "Circadian Variation in Human Milk Composition, a Systematic Review." *Nutrients* 12, no. 8: 2328. https://doi.org/10.3390/nu12082328.

Jia, L. 2023. *Use of Herbal and Food Galactagogues to Support Breastfeeding in Aotearoa New Zealand: A Thesis Presented in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Nutritional Science, College of Sciences, Massey University, Palmerston North, New Zealand* Massey University. Palmerston North, New Zealand. https://mro.massey.ac.nz/handle/10179/69271.

Jia, L. L., L. Brough, and J. L. Weber. 2021. "*Saccharomyces cerevisiae* Yeast-Based Supplementation as a Galactagogue in Breastfeeding Women? A Review of Evidence From Animal and Human Studies." *Nutrients* 13, no. 3: 727. https://doi.org/10.3390/nu13030727.

Kleefstra, N., S. T. Houweling, F. G. A. Jansman, et al. 2006. "Chromium Treatment Has No Effect in Patients With Poorly

Controlled, Insulin-Treated Type 2 Diabetes in an Obese Western Population." *Diabetes Care* 29, no. 3: 521–525.

Król, E., Z. Krejpcio, H. Byks, P. Bogdański, and D. Pupek-Musialik. 2011. "Effects of Chromium Brewer's Yeast Supplementation on Body Mass, Blood Carbohydrates, and Lipids and Minerals in Type 2 Diabetic Patients." *Biological Trace Element Research* 143, no. 2: 726–737. https://doi.org/10.1007/s12011-010-8917-5.

Kumpulainen, J., L. Salmenperä, M. Siimes, P. Koivistoinen, and J. Perheentupa. 1985. "Selenium Status of Exclusively Breast-Fed Infants as Influenced by Maternal Organic or Inorganic Selenium Supplementation." *American Journal of Clinical Nutrition* 42, no. 5: 829–835.

Lagström, H., S. Rautava, H. Ollila, et al. 2020. "Associations Between Human Milk Oligosaccharides and Growth in Infancy and Early Childhood." *American Journal of Clinical Nutrition* 111, no. 4: 769–778.

Larsson, M. W., M. V. Lind, R. P. Laursen, et al. 2019. "Human Milk Oligosaccharide Composition Is Associated With Excessive Weight Gain During Exclusive Breastfeeding–An Explorative Study." *Frontiers in Pediatrics* 7: 297.

Lovell, G. P., A. Huntsman, and J. Hedley-Ward. 2015. "Psychological Distress, Depression, Anxiety, Stress, and Exercise in Australian and New Zealand Mothers: A Cross-Sectional Survey." *Nursing & Health Sciences* 17, no. 1: 42–48.

Lovibond, P. F., and S. H. Lovibond. 1995. "The Structure of Negative Emotional States: Comparison of the Depression Anxiety Stress Scales (DASS) With the Beck Depression and Anxiety Inventories." *Behaviour Research and Therapy* 33, no. 3: 335–343.

Lovibond, S. H., and P. F. Lovibond (1995). Manual for Depression Anxiety Stress Scale, *2nd edition*.

Massey, S. H., K. A. Backes, and S. A. Schuette. 2016. "Plasma Oxytocin Concentration and Depressive Symptoms: A Review of Current Evidence and Directions for Future Research." *Depression and Anxiety* 33, no. 4: 316–322.

McBride, G. M., R. Stevenson, G. Zizzo, et al. 2021. "Use and Experiences of Galactagogues While Breastfeeding Among Australian Women." *PLoS One* 16, no. 7: e0254049.

McCabe-Sellers, B. J., C. G. Staggs, and M. L. Bogle. 2006. "Tyramine in Foods and Monoamine Oxidase Inhibitor Drugs: A Crossroad Where Medicine, Nutrition, Pharmacy, and Food Industry Converge." Supplement, *Journal of Food Composition and Analysis* 19, no. Suppl: S58–S65.

Mccarter-Spaulding, D. E., and M. H. Kearney. 2001. "Parenting Self-Efficacy and Perception of Insufficient Breast Milk." *Journal of Obstetric, Gynecologic & Neonatal Nursing* 30, no. 5: 515–522.

McGuire, M., S. Burgert, J. Milner, et al. 1993. "Selenium Status of Lactating Women Is Affected by the Form of Selenium Consumed." *American Journal of Clinical Nutrition* 58, no. 5: 649–652.

McGuire, M. K., C. L. Meehan, M. A. McGuire, et al. 2017. "What's Normal? Oligosaccharide Concentrations and Profiles in Milk Produced by Healthy Women Vary Geographically." *American Journal of Clinical Nutrition* 105, no. 5: 1086–1100.

Mercan, Y., and K. Tari Selcuk. 2021. "Association Between Postpartum Depression Level, Social Support Level and Breastfeeding Attitude and Breastfeeding Self-Efficacy in Early Postpartum Women." *PLoS One* 16, no. 4: e0249538.

Mikkelsen, K., K. Hallam, L. Stojanovska, and V. Apostolopoulos. 2018. "Yeast Based Spreads Improve Anxiety and Stress." *Journal of Functional Foods* 40: 471–476. https://doi.org/10.1016/j.jff.2017.11.034.

Miliku, K., B. Robertson, A. K. Sharma, et al. 2018. "Human Milk Oligosaccharide Profiles and Food Sensitization Among Infants in the Child Study." *Allergy* 73, no. 10: 2070–2073.

Ministry of Health. 2021a. *Healthy Eating Guidelines for New Zealand Babies and Toddlers 0–2 years old*. Ministry of Health. 20 April. https://www.health.govt.nz/system/files/2021-09/healthy-eating-guidelines-for-new-zealand-babies-and-toddlers-nov21-v3.pdf.

Ministry of Health. 2021b. *Report on Maternity Web Tool: Key Findings About Births in 2019*. Ministry of Health. 20 April. https://www.health.govt.nz/publication/report-maternity-web-tool.

Morton, S. M. B., P. E. Atatoa Carr, C. C. Grant, et al. 2012. Growing Up in New Zealand: A Longitudinal Study of New Zealand Children and Their Families. Report 2: Now We Are Born.

O'Keeffe, A. G., G. Ambler, and J. A. Barber. 2017. "Sample Size Calculations Based on a Difference in Medians for Positively Skewed Outcomes in Health Care Studies." *BMC Medical Research Methodology* 17, no. 1: 157.

Oliveira, D. L., R. A. Wilbey, A. S. Grandison, and L. B. Roseiro. 2015. "Milk Oligosaccharides: A Review." *International Journal of Dairy Technology* 68, no. 3: 305–321.

Oras, P., Y. T. Blomqvist, K. H. Nyqvist, et al. 2015. "Breastfeeding Patterns in Preterm Infants Born at 28-33 Gestational Weeks." *Journal of Human Lactation* 31, no. 3: 377–385. https://doi.org/10.1177/0890334415586406.

Oras, P., T. Ljungberg, L. Hellström-Westas, and E.-L. Funkquist. 2020. "A Breastfeeding Support Program Changed Breastfeeding Patterns but Did Not Affect the Mothers' Self-Efficacy in Breastfeeding at Two Months." *Early Human Development* 151: 105242. https://doi.org/10.1016/j.earlhumdev.2020.105242.

Paganini, D., M. A. Uyoga, G. A. M. Kortman, et al. 2019. "Maternal Human Milk Oligosaccharide Profile Modulates the Impact of an Intervention With Iron and Galacto-Oligosaccharides in Kenyan Infants." *Nutrients* 11, no. 11: 2596.

Peacock-Chambers, E., K. Dicks, L. Sarathy, A. A. Brown, and R. Boynton-Jarrett. 2017. "Perceived Maternal Behavioral Control, Infant Behavior, and Milk Supply: A Qualitative Study." *Journal of Developmental & Behavioral Pediatrics* 38, no. 6: 401–408. <Go to ISI>://WOS:000405266800007.

Plaza-Díaz, J., L. Fontana, and A. Gil. 2018. "Human Milk Oligosaccharides and Immune System Development." *Nutrients* 10, no. 8: 1038.

Plows, J. F., P. K. Berger, R. B. Jones, et al. 2020. "Associations Between Human Milk Oligosaccharides (HMOS) and Eating Behaviour in Hispanic Infants at 1 and 6 Months of Age." *Pediatric Obesity* 15: e12686.

Quin, C., S. D. Vicaretti, N. A. Mohtarudin, et al. 2020. "Influence of Sulfonated and Diet-Derived Human Milk Oligosaccharides on the Infant Microbiome and Immune Markers." *Journal of Biological Chemistry* 295, no. 12: 4035–4048.

Riordan, J., and K. Wambach. 2010. Breastfeeding and Human Lactation/Edited by Jan Riordan, Karen Wambach *[Standards/Specifications Bibliographies Non-fiction]* (4th ed.). Mass.: Jones and Bartlett Publishers, c2010. http://ezproxy.massey.ac.nz/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=cat00245a&AN=massey.b2202029&site=eds-live&scope=site.

Sacco, L. M., L. E. Caulfield, J. Gittelsohn, and H. Martinez. 2006. "The Conceptualization of Perceived Insufficient Milk Among Mexican Mothers." *Journal of Human Lactation* 22, no. 3: 277–286. https://doi.org/10.1177/0890334406287817.

Safon, C., D. Keene, W. J. U. Guevara, et al. 2017. "Determinants of Perceived Insufficient Milk Among New Mothers in León, Nicaragua." *Maternal & Child Nutrition* 13, no. 3, Article : e12369. https://doi.org/10.1111/mcn.12369.

Samuel, T. M., A. Binia, C. A. de Castro, et al. 2019. "Impact of Maternal Characteristics on Human Milk Oligosaccharide Composition Over the

First 4 Months of Lactation in a Cohort of Healthy European Mothers." *Scientific Reports* 9, no. 1: 11767.

Seferovic, M. D., M. Mohammad, R. M. Pace, et al. 2020. "Maternal Diet Alters Human Milk Oligosaccharide Composition With Implications for the Milk Metagenome." *Scientific Reports* 10, no. 1: 22092.

Segura-Pérez, S., L. Richter, E. C. Rhodes, et al. 2022. "Risk Factors for Self-Reported Insufficient Milk During the First 6 Months of Life: A Systematic Review." *Maternal & Child Nutrition* 18: e13353.

Sprenger, N., L. Y. Lee, C. A. De Castro, P. Steenhout, and S. K. Thakkar. 2017. "Longitudinal Change of Selected Human Milk Oligosaccharides and Association to Infants' Growth, an Observatory, Single Center, Longitudinal Cohort Study." *PLoS One* 12, no. 2: e0171814.

Statistics New Zealand. 2020. *2018 Census Ethnic Groups Dataset*. https://www3.stats.govt.nz/2018census/Ethnicgroupsummaries/2018-census-ethnic-group-summaries-csv-updated-14-8-20.zip?_ga=2.266037512.606967542.1688640439-2014068134.1688640439.

Statistics New Zealand. 2021. *Corrections Made to Household Economic Survey Data for Year Ended June 2020*. https://www.stats.govt.nz/methods/corrections-made-to-household-economic-survey-data-for-year-ended-june-2020.

Statistics New Zealand. 2023. *Population - Summary Figures*. https://www.stats.govt.nz/topics/population.

Steyn, N., M. Zunza, and E. H. Decloedt. 2017. "A Cross-Sectional Descriptive Study of Breastfeeding Behaviour and Galactogogue Use Among Private-Sector Patients in Cape Town, South Africa." *South African Journal of Obstetrics and Gynaecology* 23, no. 1: 20–23. https://doi.org/10.7196/SAJOG.2017.v23i1.1116.

Talbott, S. M., and J. A. Talbott. 2012. "Baker's Yeast Beta-Glucan Supplement Reduces Upper Respiratory Symptoms and Improves Mood State in Stressed Women." *Journal of the American College of Nutrition* 31, no. 4: 295–300.

Thurl, S., M. Munzert, G. Boehm, C. Matthews, and B. Stahl. 2017. "Systematic Review of the Concentrations of Oligosaccharides in Human Milk." *Nutrition Reviews* 75, no. 11: 920–933.

Trafikowska, U., E. Sobkowiak, J. A. Butler, P. D. Whanger, and B. A. Zachara. 1998. "Organic and Inorganic Selenium Supplementation to Lactating Mothers Increase the Blood and Milk Se Concentrations and Se Intake by Breast-Fed Infants." *Journal of Trace Elements in Medicine and Biology* 12, no. 2: 77–85. https://doi.org/10.1016/s0946-672x(98)80029-1.

Trafikowska, U., B. Zachara, M. Wiacek, E. Sobkowiak, and M. Czerwionka-Szaflarska. 1996. "Selenium Supply and Glutathione Peroxidase Activity in Breastfed Polish Infants." *Acta Paediatrica* 85, no. 10: 1143–1145. <Go to ISI>://WOS:A1996VN21300003.

Urashima, T., J. Hirabayashi, S. Sato, and A. Kobata. 2018. "Human Milk Oligosaccharides as Essential Tools for Basic and Application Studies on Galectins." *Trends in Glycoscience and Glycotechnology* 30, no. 172: SE51–SE65.

U.S. Food and Drug Administration. 2010. *Avoid Food and Drug Interactions* (Editor. U.S. Food and Drug Administration).

Vitzthum, V. J. 1989. "Nursing Behaviour and Its Relation to Duration of Post-Partum Amenorrhoea in an Andean Community." *Journal of Biosocial Science* 21, no. 2: 145–160. https://doi.org/10.1017/S0021932000017843.

Victora, C. G., R. Bahl, A. J. Barros, et al. 2016. "Breastfeeding in the 21st Century: Epidemiology, Mechanisms, and Lifelong Effect." *Lancet* 387, no. 10017: 475–490.

Weaver, S. R., and L. L. Hernandez. 2016. "Autocrine-Paracrine Regulation of the Mammary Gland." *Journal of Dairy Science* 99, no. 1: 842–853.

Williams, M. N., C. A. G. Grajales, and D. Kurkiewicz. 2013. "Assumptions of Multiple Regression: Correcting Two Misconceptions." *Practical Assessment, Research, and Evaluation* 18, no. 1: 11.

Wobbrock, J. O., L. Findlater, D. Gergle, and J. J. Higgins. 2011. "The Aligned Rank Transform for Nonparametric Factorial Analyses Using Only Anova Procedures." *Proceedings of the SIGCHI Conference on Human Factors in Computing Systems*: 143–146. https://dl-acm-org.ezproxy.massey.ac.nz/doi/abs/10.1145/1978942.1978963.

Word Health Organization. 2022. Defining Exclusive Breastfeeding. December 19, 2022. https://www.emro.who.int/nutrition/breastfeeding/index.html.

World Health Organization. 2011. *WHO Anthro for Personal Computers, Version 3.2.2: Software for Assessing Growth and Development of the World's Children*. In (Version 3.2.2) World Health Organization.

World Health Organization. 2022. *Infant and Young Child Feeding*. March 15. https://www.who.int/news-room/fact-sheets/detail/infant-and-young-child-feeding.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.
Appendices 1 to 6.