THE EFFECT OF *MAGEU* CONSUMPTION ON SYSTEMIC INFLAMMATION AND NUTRITION IN BREASTFEEDING MOTHERS

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PLAGIARISM DECLARATION

I, Janine Amy Fredericks, hereby declare that the work, on which this thesis is based on, is my original work (except where acknowledgments indicate otherwise) and that neither the whole work nor any part of it has been, is being or is to be submitted for another degree in this or any other university.

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ABBREVIATIONS

AEs – Adverse Events

AGP - α(1)-acid glycoprotein

ANOVA – Analysis of Variance

BHI – Brain Heart Infusion

BLAST – Basic Local Alignment Tool

BMI – Body Mass Index

cfu– Colony Forming Units

CO_2 – Carbon Dioxide

CPM – Commercially Pasteurised *Mageu*

CRF – Case Report Form

CRP – C-reactive protein

CV – Coefficient of Variance

DAIDS – Division of AIDS

DPBS – Dulbecco's Phosphate Saline Buffer

E. coli – Escherichia coli

ELISA – Enzyme-Linked Immunoassay

FC – Fecal Calprotectin

FFQs – Food Frequency Questionnaires

GCP – Good Clinical Practice

GDM – Gestational Diabetes Mellitus

gDNA – genomic DNA

HIV – Human Immunodeficiency Virus

HREC – Human Research Ethics Committee

hs-CRP – High-sensitivity CRP

IFN-γ - Interferon-γ

IL-1β - Interleukin-1β

IL-6 – Interleukin-6

IBD – Inflammatory Bowel Disease

IQR – Interquartile Range

LAB – Lactic Acid Bacteria

LCFA – long-chain Fatty Acid

LCM – Live Culture *Mageu*

LCN2 – Lipocalin-2

LDL – Low-density Lipoprotein

LMIC – Low-and-Middle-Income Countries

LTFU – Lost to follow-up

MaMISA – *Mageu* for Health of Mothers and Infants in South Africa

MPO – Myeloperoxidase

NCCDs – Non-communicable Chronic Diseases

NCBI – National Center for Biotechnology

NGAL – Neutrophil Gelatinase-associated Lipocalin

NHLS – National Health Laboratory Service

NM – No *Mageu*

PCoA – Principal Coordinate Analysis

PCR – Polymerase Chain Reaction

PERMANOVA – Permutational Multivariate ANOVA

qPCR – Quantitative Polymerase Chain Reaction

RBP4 – Retinol Binding Protein 4

RCT – Randomised Controlled Trial

rRNA – Ribosomal Ribonucleic Acid

SAHPRA – South African Health Product Regulatory Authority

SANS – South African National Standards

SCFA – Short-chain Fatty Acid

SD – Standard Deviation

sTfR – Soluble Transferrin Receptor

T2DM – Type II Diabetes Mellitus

Tg – Thyroglobulin

TGF-β - Transforming Growth Factor-β

TNF-α - Tumour Necrosis Factor-α

UC – Ulcerative Colitis

UTI – Urinary Tract Infection

WHO – World Health Organization

ABSTRACT

Introduction:

Optimal maternal body mass index (BMI) and nutrition are critical for postpartum health. Malnutrition and abnormal BMI are associated with a range of postpartum complications, such as lactation difficulties and cardiovascular risk. Key micronutrients, such as iron, folate, calcium and vitamin D, are essential during this period. Inflammation, measured by acute phase reactants such as C-reactive protein (CRP), ferritin and α1-acid glycoprotein (AGP), alongside inflammatory cytokines, such as interleukin (IL)-6, IL-1β, tumour necrosis factor (TNF)-α and interferon (IFN)-γ, can further impact maternal health. Fermented foods containing live organisms may help regulate inflammation and support metabolic and immune function. *Mageu*, a traditional non-alcoholic fermented grain-based beverage, is commonly consumed in Southern Africa as a meal replacement or weaning food for infants. While traditionally fermented, live-culture *Mageu* has been valued for its anti-inflammatory and immune-modulatory properties, pasteurisation of commercially available *Mageu* eliminates most live microbes, thus reducing its probiotic potential. This study aimed to compare the effect of daily consumption of live culture *Mageu* (LCM), commercially pasteurised *Mageu* (CPM) versus no *Mageu* (NM) for six weeks on systemic and intestinal inflammation, nutritional markers and BMI in breastfeeding postpartum mothers, and explored relationships between these outcomes.

Methods:

The nutritional composition of CPM and LCM were evaluated prior to the study by external laboratories. CPM and LCM were cultured on selective media to ensure the absence of spore-forming, pathogenic *Clostridium* spp. and *Escherichia coli* (*E. coli*) in compliance with the South African National Standards (SANS) 1199:2011 guidelines. Total bacterial load of *Mageu* batches was determine by qPCR of the 16S rRNA gene. Forty-five eligible mothers were randomised 1:1:1 to NM, CPM or LCM within 10 days after delivery, and followed longitudinally over 15 weeks. After enrollment, women had a three-week washout period followed by six weeks of their randomised intervention or no *Mageu*. At each visit, maternal anthropometrics (height, weight) and health outcomes (including adverse events) were collected. To monitor compliance with the intervention, mothers completed daily monitoring sheets and were asked to return bottles. Maternal blood and stool were collected before starting the intervention (week 4), at completion (week 10) and 5 weeks after completion of the intervention (week 15). Maternal plasma was used to quantify systemic inflammatory markers (IL-6, IL-1β, TNF-α and IFN-γ) via Luminex® assay, intestinal inflammatory markers (lipocalin-2 and myeloperoxidase) via Enzyme-Linked Immunoassay (ELISA) and systemic micronutrients (ferritin, AGP, thyroglobulin, soluble transferrin receptor, CRP, retinol binding protein 4 (RBP4)) using a Q-Plex Human Micronutrient assay. Maternal serum was used to quantify nutritional markers (iron, serum ferritin, vitamin B12 and vitamin D) at the local National Health Laboratory Service Pathology

Laboratory. Maternal stool lysates were used to quantify fecal calprotectin using ELISA. The primary analysis was to compare the change in concentration of the markers between weeks 4 and 10 and weeks 4 and 15 between randomisation arms. Secondary analyses included cross-sectional comparisons of biomarker concentrations at weeks 4, 10 and 15. The relationships between these markers with BMI, and effect of the intervention, was described using Pearson correlations, Principal Coordinate Analysis (PCoA) and heatmaps.

Results:

LCM had a higher nutritional content than CPM. Both CPM and LCM batches were free from *E. coli* and pathogenic *Clostridium* spp. LCM batches had a higher proportion of obligate (38.36%) and facultative anaerobe spore-formers (87.67%) and non-*E. coli* (79.45%) species compared to CPM (4.55%, 40.91% and 3.03%, respectively). Common microorganisms found in LCM were *Clostridium beijerinckii*, *Clostridium manihotivorum*, *Bacillus* spp., and *Leuconostoc lactis*, among others. LCM batches had a higher 16S rRNA gene copy number per µL gDNA (median 31,550 [IQR 1802-104,858]) compared to the CPM product (median 126 [IQR 8-360], $p<0.0001$), suggesting higher bacterial load. Self-reported and objective *Mageu* adherence was high, with an average of 6.62 (SD 0.14) servings per week reported for CPM and 6.64 (SD 0.22) servings per week reported for LCM users. At week 4 (prior intervention), the mean BMI was 31.75 kg/m^2 (SD 6.77) and did not differ amongst randomisation arms, nor did it differ at weeks 10 (post-intervention) or 15. Generally, *Mageu* consumption did not impact the change of nutritional, systemic and intestinal inflammatory marker concentrations from week 4 to 10, nor from week 4 to 15. Cross-sectional analysis revealed that in NM users compared to *Mageu* users, ferritin was higher at week 10, and IL-6 and IFN-γ concentrations tended to be higher at week 15. Further, iron concentrations increased in NM users but decreased in the CPM users from week 4 to 10. When assessing correlations between concentrations of markers between time points, concentrations of all systemic inflammatory markers, vitamin B12 and vitamin D at week 4 correlated strongly with their concentrations at weeks 10 and 15, while intestinal inflammatory marker concentrations correlated moderately between weeks 4 and 10. This suggests that individual inflammation is consistent through time postpartum and the intervention had little influence. At week 4, BMI correlated positively with RBP4 (Pearson r=0.61, p=0.0090), but not with any other markers. Iron negatively correlated with AGP (Pearson r=-0.92, p=1.47x10-7) and CRP (Pearson r=-0.78, p=0.0002). Ferritin positively correlated with IFN-γ (Pearson r=0.57, p=0.0158) and TNF-α (Pearson r=0.52, p=0.0341), suggesting that ferritin levels may be reflective of inflammation rather than iron deficiency in this instance. Fecal calprotectin correlated negatively with myeloperoxidase (Pearson r=-0.50, p=0.0399) and IFN-γ (Pearson r=-0.56, p=0.0184). Systemic inflammatory markers (IL-6, IL-1β, TNF-α and IFN-γ) all positively and strongly correlated with each other. When nutritional and inflammatory data were integrated, no distinct clustering was observed between intervention arms, whether assessing changes from week 4 to 10 or from week 4 and 15.

Discussion and conclusion:

The LCM product had higher nutritional levels than commercial *Mageu*, suggesting that traditionally prepared *Mageu* products may offer superior nutritional value. LCM complied with SANS regulations as it was free from pathogens. Pasteurisation affected bacterial load. Overall, *Mageu* did not significantly decrease inflammation, improve nutritional status, or decrease BMI in this small cohort of postpartum women. However, we found that NM users had the highest change in concentration for some intestinal and systemic inflammatory markers over time compared to *Mageu* users, which might suggest that inflammation is dynamic postpartum in the absence of a nutritional intervention. More research is needed to understand the effects of locally produced live culture traditional fermented foods on inflammation and nutrition in South African mothers. Given the local context and relevance of these findings for maternal health, this should be explored in a larger cohort.

CHAPTER 1
LITERATURE REVIEW

1.1 Maternal health during pregnancy and postpartum periods

1.1.1 Nutrition, body weight, and health outcomes

South Africa is undergoing a rapid nutritional shift, marked by a growing double-burden of undernutrition and overnutrition, driven by changes in dietary patterns and urbanisation (Sebeta et al., 2022). The World Health Organisation (WHO) reports that up to 25% of women are obese during pregnancy, and this prevalence is rising (Ozdilek et al., 2019). Amongst reproductive aged women in South Africa, 31% are anaemic, 68% are overweight or obese and 46% are hypertensive. Maternal weight gain during pregnancy and inadequate weight loss postpartum can increase the risk of metabolic disorders and long-term obesity in women (Langley-Evans et al., 2022). Poor maternal health is associated with infection, like vaginal and urinary tract infections (UTIs) (González-Fernández et al., 2022), gestational diabetes mellitus (GDM), hypertension, preeclampsia, cardiovascular diseases, lactation complications and maternal mortality (Nguyen et al., 2017; Ozdilek et al., 2019; Simko et al., 2019).

Postpartum nutritional health is important for the health and well-being of both the mother and infant. Iron, folate, calcium and vitamin D are amongst the most essential nutrients needed during pregnancy, postpartum and lactation (Jaisamrarn et al., 2023). Food intake patterns may be significantly influenced by physiological needs and behavioural patterns postpartum (Schwedhelm et al., 2022). Lactating women require increased amounts of vitamins A, E, B6, B12, folate, iodine, omega-3 fatty acids and zinc, among other nutrients (Beluska-Turkan et al., 2019; Langley-Evans et al., 2022). The prevalence of postpartum anaemia is high (80%) in low-and-middle-income countries (LMICs), as well as in high-income countries (50%) (Butwick & McDonnell, 2021). A cross-sectional study on 405 postpartum women in Ghana found that dietary diversity may reduce the risk of postpartum anaemia (Wemakor et al., 2022). However, maternal nutritional health during the postpartum period has not been well-researched in the African context. The increase in nutritional demand during the postpartum and lactation period, along with poor diet, may predispose women to becoming overweight and/or undernourished (Schwedhelm et al., 2022; Sebeta et al., 2022). Certain dairy products, such as yoghurt or cheeses, in combination with exercise, may exert different effects on weight loss postpartum (Yuan et al., 2023).

Maternal nutrition and body weight can impact breastfeeding outcomes, while breastfeeding itself also influences maternal weight regulation. Breastfeeding frequency is associated with weight loss in overweight and obese mothers postpartum (Sebeta et al., 2022). Postpartum weight loss as a result of lactation is likely due to altered metabolic rate and mobilisation of energy stores. The amount of energy expended in mothers who exclusively breastfeed is approximately 500kcal/day, and is influenced by

volume and composition of breastmilk and the stage of lactation (Kieliszek, 2019). Conversely, the metabolic and hormonal effects of obesity may reduce the energy demands of lactation (Kieliszek, 2019). However, underweight, overweight and obese women frequently experience difficulties in lactation (Schwedhelm et al., 2022). Breastfeeding is important in delivering essential nutrients to the infant (de Seymour et al., 2022). Breastmilk composition is greatly influenced by maternal dietary intake during lactation and by adipose nutrient stores, which in turn, also influence breastmilk production (Schwedhelm et al., 2022). Nutrient inadequacies of magnesium, essential vitamins, folate, calcium and zinc in maternal diet may also affect breastmilk composition (Hart et al., 2022; Yuan et al., 2023). Exclusive breastfeeding for 6 months in women with GDM reduced the risk of type II diabetes mellitus (T2DM) and cardiovascular disease development for women (Marshall et al., 2022), suggesting health benefits to the breastfeeding mother.

1.1.2 Systemic inflammation

Inflammation is the body's response to infection or injury and can sometimes be pathological and cause damage, or serve as a means to combat pathogens (Chen et al., 2018; Kekkonen et al., 2008). Cytokines are protein mediators that regulate immune and inflammatory responses, both locally and systemically. They are often broadly categorized into pro-inflammatory cytokines and anti-inflammatory cytokines, based on their primary functions (Remick, 2014). Pro-Inflammatory cytokines promote inflammatory responses and include interleukin (IL)-1β, IL-6, IL-8 and tumour necrosis factor-alpha (TNF-α) (Remick, 2014; J. M. Zhang & An, 2007). Anti-inflammatory cytokines are immunoregulatory molecules that modulate or supress pro-inflammatory cytokine responses to maintain immune balance (Remick, 2014; J. M. Zhang & An, 2007) and examples include IL-10 and transforming growth factor-beta (TGF-β) (J. M. Zhang & An, 2007). Systemic inflammatory markers interact in a coordinated manner with one another during immune activation, often amplifying each other in a cascade that promotes inflammation to enhance the immune response against infection (Jarlborg & Gabay, 2022). For example (e.g.), interferon (IFN)-γ stimulates the production of TNF-α, IL-1β and IL-6, while TNF-α also stimulates the production of IL-1β and IL-6. IL-1β stimulates IL-6 and TNF-α and in turn, IL-6 regulates the expression of IL-1β and TNF-α (Al-Roub et al., 2021; Jarlborg & Gabay, 2022; Ledesma et al., 2004).

Diet rich in red and processed meat, whole milk, refined grains, simple sugars, trans and saturated fats are associated with an increase in the circulation of pro-inflammatory cytokines, whereas a diet rich in fruits, vegetables, seeds, nuts and whole grains is associated with an increase in the circulation of anti-inflammatory cytokines (Jaisamrarn et al., 2023; Zou et al., 2022). Increased levels of plasma IL-6 is associated with obesity and pregnancy (Madlala et al., 2024). A prospective study involving pregnant women with Human Immunodeficiency Virus (HIV) found that late pregnancy IL-6 secreted by adipose tissue was positively associated with adverse postpartum weight (Madlala et al., 2024). Serum levels of IL-6 is associated with GDM, tissue repair and immune regulation during and after pregnancy (Bränn

et al., 2019; Rojas-Quintana et al., 2025). In a cross-sectional, prospective study involving 16 women with confirmed diagnosis of GDM and 16 women with normal pregnancies, found that serum levels of IL-6 was significantly reduced in women with GDM compared to the control group (Rojas-Quintana et al., 2025). C-reactive protein (CRP), α(1)-acid glycoprotein (AGP) and serum ferritin are acute phase reactants and markers of systemic inflammation (Farrag et al., 2024). Chronic mildly elevated levels of CRP can be associated with the risk of cardiovascular diseases, obesity and other chronic inflammatory diseases (Wan et al., 2022). CRP is known to be elevated in insulin resistance and is a predictor of cardiovascular disease at levels >2 mg/l (Quansah et al., 2023). A secondary analysis of a randomised controlled trial (RCT) found that CRP during pregnancy and at 6-8 weeks postpartum predicted an increase in weight and insulin resistance at 1 year postpartum (Quansah et al., 2023). The association of CRP and increased insulin resistance is linked to the role of inflammation in impaired metabolism or adverse cardiometabolic diseases (Quansah et al., 2023). Increased levels of AGP have been associated with a higher body mass index (BMI) and body fat mass in humans (Alfadda et al., 2012; Maraj et al., 2021). Mediators of systemic inflammation play a central role in the pathogenesis of chronic diseases, including rheumatoid arthritis, Alzheimer's disease, obesity, T2DM and inflammatory bowel disease (IBD) (Holz et al., 2010; Hotamisligil, 2006; Remick, 2014). A cross-sectional and retrospective study investigated the effectiveness of ferritin as a marker for diagnosing iron-deficiency anaemia in the presence of inflammation. The study involved 118 participants previously diagnosed with IBD, whereby 38 had Crohn's disease, 47 had Ulcerative Colitis (UC) and 33 controls. They found a 29.76% increase in ferritin using CRP as an inflammatory marker, this then increased to 82.14% when AGP or both AGP and CRP ($p<0.05$) was used for detection of inflammation via ferritin levels (Farrag et al., 2024). This results suggest that ferritin alone was not sufficient in diagnosing iron-deficiency anaemia as it can fluctuate in the presence of inflammation.

Apart from systemic inflammatory markers described above, lipocalin-2 (LCN2), also known as neutrophil gelatinase-associated lipocalin (NGAL), fecal calprotectin (FC) and myeloperoxidase (MPO) can be used as markers of intestinal inflammation (de Moura Gondim Prata et al., 2016). LCN2 measured in urine, blood and faeces, serves as a biomarker in human inflammatory diseases and as an indicator of gut dysbiosis (de Moura Gondim Prata et al., 2016; Yadav et al., 2022). Increased levels of LCN2 expressed in intestinal innate and adaptive immune cells limits bacterial growth *in vivo,* through iron sequestration, which may suppress intestinal dysbiosis in the inflamed gut and within damaged tissue (de Moura Gondim Prata et al., 2016; Moschen et al., 2017). FC is a calcium and zinc binding protein that has antimicrobial and anti-proliferative functions that play a regulatory role in inflammation and induces apoptosis in cell culture (D'Amico et al., 2021). It is also an indicator of infectious and inflammatory conditions, like IBD, and elevated levels of FC have been associated with gut dysbiosis (de Moura Gondim Prata et al., 2016; Heinzel et al., 2024). A prospective cohort study evaluated the association of FC with gut microbial dysbiosis in 735 adults. The individuals were stratified into low

(0-50 μg/g), moderate (50-100 μg/g) and high (>100 μg/g) FC levels, and the authors found that pro-inflammatory gut microbial genera (*Haemophilus, Lactobacillus* and *Veillonella*) were significantly increased in the high FC group and had decreased genera of short-chain fatty acid (SCFA)-producing microbes (*Blautia, Clostridium XIVa* and *Turicibacter*) compared to the low FC group. They also noted that BMI was increased in the high FC group (Heinzel et al., 2024). These finding suggest that elevated levels of FC are associated with gut microbial dysbiosis and obesity. MPO is released after oxidative stress and in various inflammatory responses. Production of reactive oxygen and reactive nitrogen species underlies MPO's antibacterial activity (Khan et al., 2018). MPO observed in the intestinal mucosa can be used to monitor disease activity and treatment interventions for cardiovascular disease and other chronic diseases (Khan et al., 2018). MPO is not well studied in maternal health postpartum, recent literature mostly evaluates the association between MPO and preeclampsia (Modzelewski et al., 2023; Rocha-Penha et al., 2017; Van Rijn et al., 2016).

Achieving immune balance in response to pathogens and inflammation, together with optimal maternal nutrition, is crucial for supporting maternal and infant health (Zou et al., 2022). Dysregulated maternal immune responses during pregnancy can increase the risk of adverse pregnancy outcomes and increase long-term health risks for both mother and infant (Kwok et al., 2022; Sohn & Underwood, 2017). In particular, maternal cardiometabolic disorders and excessive inflammation (Ravi et al., 2022), which has been associated with increased maternal body weight or obesity (Kwok et al., 2022), GDM and preeclampsia, can increase the risks of miscarriage, preterm labour and lactation complications (Challis et al., 2009; Schwedhelm et al., 2022; Simko et al., 2019). Obesity-associated chronic inflammation has also been described as a risk factor for poor lactation, further being associated with lower levels of long-chain fatty acids (LCFAs) present in the breastmilk (Walker et al., 2022).

1.1.3 Gut Microbiota

The gut microbiota comprises a diverse community of microorganisms that regulate nutrient metabolism, the immune system and natural defence against gastrointestinal infection, all of which influences the host health (Sen et al., 2016). Both intrinsic (genetics, innate and adaptive immunity) and extrinsic factors (diet, lifestyle, geography, allergens, pathogens, antibiotics) shape the composition and function of the gut microbiota (Bander et al., 2020). Mode of delivery, breastfeeding status, and maternal microbiota are the biggest determinants of the gut microbiota in early life of infants (Bander et al., 2020; Shen et al., 2025). Early-life exposure to optimal maternal microbiota is crucial for infant immune development and aids in decreasing the risk of developing infections or allergies later in life (Bander et al., 2020; Shen et al., 2025). Breastmilk contains a diverse number of microbial species that influence the establishment of the gut microbiota of the newborn during the first 6 months of life (Shen et al., 2025).

Inflammatory cytokines modulate immune responses to the intestinal microbiota, contributing to intestinal homeostasis through microbiota-derived metabolites, such as SCFAs, which have regulatory

effects (Bander et al., 2020; Maciel-Fiuza et al., 2023) and can help supress inflammatory diseases such as IBD and obesity (Clemente et al., 2018). For example, in adipose tissue of overweight participants, propionate prevents obesity-related inflammation by increasing IL-4 in macrophages (Al-Lahham et al., 2012; Maciel-Fiuza et al., 2023). Patients with IBD exhibit low gut microbial diversity and stability, including a lower abundance of *Lactobacillus* spp. and *Bifidobacterium* spp., therefore resulting in reduced production of SCFAs, and an increase in *Proteobacteria* spp. and *Bacteroidetes* (Bander et al., 2020). Butyrate supplementation has also been shown to increase the growth of SCFA-producing bacteria, thus reducing inflammation in patients with IBD (Clemente et al., 2018). A high dietary fibre intake has been associated with a decreased risk of chronic inflammatory diseases due to shifts in the gut microbiome (Wan et al., 2022). Dysbiosis of the gut microbiota (Maciel-Fiuza et al., 2023) and elevated CRP levels (Wan et al., 2022) can result in metabolic dysfunction and the pathogenesis of cardiovascular and inflammatory diseases like T2DM, obesity and IBD (Maciel-Fiuza et al., 2023).

Diet is one of the key factors that drive gut microbial diversity and composition in adults (Shen et al., 2025). Gut microbial dysbiosis refers to an imbalance in the composition, diversity or function of the gut microbiota (Shen et al., 2025); and is associated with many non-communicable chronic diseases (NCCDs), obesity, T2DM and IBD (Cuffaro et al., 2021). Meat-abundant diets are associated with a dominance of *Bacteroides* spp. in the gut, compared to plant-abundant diets where a dominance of *Prevotella* spp. has been described (Bander et al., 2020). High-calorie, high-fat and low-fibre diets increase the abundance of *Proteus* spp. in the gut (Shen et al., 2025). Western diets rich in saturated fats are also associated with high inflammation and altered microbiota (Bander et al., 2020). Further, unhealthy eating patterns lead to an increased body weight or obesity, which in turn leads to increased inflammation (Alfadda et al., 2012), both diet and gut inflammation is associated with gut microbiota diversity of individuals (Allin et al., 2018; Bander et al., 2020; Kwok et al., 2022; Shen et al., 2025; Zinöcker & Lindseth, 2018). However, studies have questioned whether gut microbial dysbiosis leads to inflammation and its associated inflammatory diseases (Allin et al., 2018; Kwok et al., 2022; Zinöcker & Lindseth, 2018). A study investigating the intestinal microbiota of individuals with prediabetes found five bacterial genera that were differentially abundant in prediabetic individuals compared to the controls. This suggests that certain gut microbial compositions are associated with the development of diabetes (Allin et al., 2018). An RCT involving 53 participants with Ulcerative Colitis (UC) randomised to either anti-inflammatory diet (AID) or Canada's Food Guide (CFG)–recommended serving portions for each food group, found that dietary modification consisting of an increased intake of anti-inflammatory foods, combined with a decreased intake of pro-inflammatory foods, was associated with microbial changes and was effective in preventing or reducing inflammation (Keshteli et al., 2022). Another study (parallel-group, cross-over) involving participants diagnosed with UC in remission found that individuals on the low-fat, high-fibre diet (LFD) showed signs of a reduced gut dysbiosis and had an increased abundance of *Bacteriodes* spp. and decreased abundance of

Actinobacteria in their faeces compared to the improved standard American diet (iSAD). The increase in *Faecalibacterium prausnitzii*, along with other microbes, resulted in an anti-inflammatory shift within the gut microbiome, thus reducing inflammatory markers in UC participants on a LFD (Fritsch et al., 2021; Shen et al., 2025). Overall, these findings suggest that diet can significantly influence the gut microbiota and in turn, affect inflammation.

1.1.4 Association between gut microbiota, inflammation and maternal health

Dysbiosis in the maternal gut microbiota may result in weight gain, inflammation, hyperglycaemia and insulin resistance (Chen et al., 2019; X. Li et al., 2021). Alteration and composition of the gut microbiota may be associated with the regulation and secretion of immune and inflammatory cytokines and may decrease the risk of metabolic disorders, such as GDM and other inflammatory conditions (Chen et al., 2019; X. Li et al., 2021). GDM is associated with gut microbiota composition during pregnancy and postpartum, but whether there is causation is unknown. Decreased richness in gut microbiota has previously been associated with elevated levels of pro-inflammatory markers and insulin resistance (Crusell et al., 2018).

A study evaluated the gut microbiota composition in 50 women diagnosed with GDM and 157 healthy pregnant women in the third trimester who were followed up until 8 months postpartum. They found that GDM diagnosis in the third trimester was associated with gut microbiota disruption during pregnancy. This disruption was still present at 8 months postpartum (Crusell et al., 2018). They also found that women with previous GDM had a relatively lower abundance of *Firmicutes* at 3-16 months postpartum, typically associated with individuals with T2DM (Crusell et al., 2018). Furthermore, *Collinsella* was abundant in the gut microbiota of postpartum women who had previous GDM, *Collinsella* may contribute to later development of T2DM (Crusell et al., 2018). Gut microbial dysbiosis has also been associated with preeclampsia and with hypertension during and after pregnancy (Lv et al., 2019). A study evaluating newly diagnosed preeclampsia during the third trimester in 78 women found that preeclampsia was associated with disrupted gut microbiota composition, which extended up to 6 weeks postpartum, compared to women with uncomplicated pregnancies (Lv et al., 2019). Women with preeclampsia had gut microbiota significantly enriched in *Blautia, Ruminoccocus2, Bilophila* and *Fusobacterium* during the third trimester until 6 weeks postpartum (Lv et al., 2019). *Blautia and Ruminoccocus2* have been associated with obesity, gestational weight gain (GWG) and GDM, and *Bilophila* and *Fusobacterium* have been associated with an increase in inflammatory marker IL-6 in blood (Lv et al., 2019).

1.2 Microbiome based interventions to improve maternal health

1.2.1 Probiotics

Probiotics are live microorganisms that, when administered in adequate amounts, exert a proven health benefit on the human host (Abid et al., 2022). Lactic acid bacteria (LAB) (including *Lactobacillus*,

Bifidobacterium and *Streptococcus*) are the most commonly used organisms for probiotic product development (Das et al., 2022; Ranjha et al., 2021). Others include *Bacillus* spp., yeast (e.g. *Saccharomyces cerevisiae* and *Saccharomyces boulardii*) and filamentous fungi (e.g. *Aspergillus oryzae)* (Abid et al., 2022; Das et al., 2022; Franz et al., 2014; Ibrahim et al., 2023; Parvez et al., 2006). While the health benefits of probiotics are strain specific, broadly speaking, probiotics are suggested to have a range of beneficial effects, including improvement of intestinal microbial balance (Barkhidarian et al., 2021; Das et al., 2022; Parvez et al., 2006), pathogen interference, immune modulation, reduction of oxidative stress through cytokine modulation (Das et al., 2022; Kwok et al., 2022; Mohammadi et al., 2015), and maintenance of mucosal integrity (Das et al., 2022; Franz et al., 2014; Kwok et al., 2022). Probiotics are deemed relatively safe as they are usually commensal organisms of the human gut (Abid et al., 2022; Adekoya et al., 2019).

Certain probiotics have the ability to prevent and decrease the incidence of antibiotic-induced diarrhoea (Franz et al., 2014), prevent and treat lactose intolerance and IBD by enhancing the intestinal mucosal barrier function, immune system and by stimulating the release of anti-inflammatory factors that inhibit the growth of harmful bacteria in the gut (Damián et al., 2022; Wang et al., 2020). The immunomodulatory effects of probiotics have primarily been studied in inflammatory diseases and allergies (Alard et al., 2021; Asemi et al., 2011; de Brito Alves et al., 2019; Hegazy & El-Bedewy, 2010; Khan et al., 2021; Liao et al., 2021; Roškar et al., 2017; Song et al., 2020; Tonucci et al., 2017; L. Zhang et al., 2022). The use of probiotics during postpartum may confer health benefits, like reduced GDM and decreased weight (Sheyholislami & Connor, 2021). A RCT study of 256 healthy women showed that dietary interventions along with probiotics (*Lactobacillus rhamnosus* GG and *Bifidobacterium lactis*) decreased postpartum waist circumference and significantly reduced the incidence of GDM from 34-36% to 13% (Ilmonen et al., 2011; Sohn & Underwood, 2017; Luoto, 2010). A RCT with 256 pregnant women in their first trimester were randomised to receive dietary counselling with probiotics (*Lactobacillus rhamnosus* GG and *Bifidobacterium lactis*), or placebo (Microcrystalline, cellulose and dextrose) and a control group receiving no counselling evaluated changes in total and low-density lipoprotein (LDL) cholesterol until 12 months postpartum. Total and LDL cholesterol were lower in both dietary counselling groups (probiotic $p=0.027$ and placebo $p=0.012$ groups) compared to the control group. This suggests that dietary counselling during pregnancy was effective in decreasing the risk of developing cardiovascular diseases later on (Abbasi et al., 2021; Hoppu et al., 2014). However, for the Southern African context, fermented foods might provide an available, culturally-acceptable, and cheaper avenue to improve maternal postpartum health as compared to commercial probiotics.

1.2.2 Fermented Foods

1.2.2.1 Types of fermented foods and their benefits

Fermented foods are foods or beverages produced through controlled microbial growth and the conversion of food components through enzymatic action (Damián et al., 2022). This process is traditionally used to improve taste, texture, preserve and process foods and increase nutritional value (Knez et al., 2023; Moiseenko et al., 2021; Negrete-Romero et al., 2021). Fermented foods are traditionally produced on a household scale in rural communities; however, a number of fermentation processes have been industrialised for commercial use (Blandino et al., 2003; Gadaga et al., 1999; Moiseenko et al., 2021; Setta et al., 2020). The variation in fermented foods is derived from the factors involved in the fermentation process. For example, the type of microorganism used and its specific by-products, the ingredients present and the environmental conditions, all influence the final fermented food product (Dimidi et al., 2019). Fermentation can occur spontaneously, whereby the microorganisms are present naturally in the food, or it can occur via the addition of a starter culture, known as culture-dependent fermentation, whereby a fraction of the previously fermented batch is added to the food (Blandino et al., 2003; K. J. Li et al., 2021). There are four main fermentation processes known to date: (i) alcoholic fermentation, driven by yeast to produce ethanol (e.g. wines, breads and beers); (ii) lactic acid fermentation, carried out by lactic acid bacteria to produce fermented milks and cereals; (iii) acetic acid fermentation, which converts alcohol to acetic acid in the presence of oxygen and is responsible for the fermentation of vinegar and kombucha and finally (iv) alkaline fermentation, driven mostly by fungi, commonly used for the fermentation of meats and soy products, as well as the maturation of cheeses (Blandino et al., 2003; K. J. Li et al., 2021).

The microbiota of fermented foods mainly consist of LAB and yeast, the same microorganisms that can be found in probiotics (Pswarayi & Gänzle, 2019). Just like the microbes in probiotics, the microbes associated with the manufacture of fermented foods may influence the gut microbiota and other physiological functions (Savaiano & Hutkins, 2021). Fermented foods exert health benefits derived from the potential probiotic effects of the microorganisms used during the fermentation process. These include bioactive peptides and biogenic amines produced, the conversion of phenolic compounds to biologically active compounds and the reduction of toxins and anti-nutrients (Şanlier et al., 2019). Furthermore, antimicrobial metabolites produced during fermentation reduce the risk of contamination with pathogenic microorganisms, thus increasing the shelf life of the product (Şanlier et al., 2019). In addition, a number of B vitamins including folate, riboflavin, vitamin B12 and vitamin K2 (menaquinones) can be enriched through fermentation, thus elevating the nutritional quality of the product (Hayes & García-Vaquero, 2016). A review of recent clinical trials showed an improvement in nutritional levels of vitamin B complexes (folate and B12) (Challis et al., 2009; Soccol et al., 2010) and minerals (iron, zinc and calcium) following administration of cultured yoghurts with varied dosages (Challis et al., 2009). Previous observational cohort and interventional studies have linked fermented

food consumption to weight maintenance and a decreased risk of T2DM, cancer and cardiovascular diseases (Zeng et al., 2023). Fermented foods may also aid in the control of intestinal disorders, whereby the microorganisms present during fermentation outcompete pathogenic bacteria and produce immunoregulatory and neurogenic fermentation by-products (Dimidi et al., 2019; Hotamisligil, 2006).

A 10-week interventional study assessed the influence of high-fibre and high-fermented food diets on gut microbiota diversity and inflammation (Wastyk et al., 2021). They found that a high-fermented food diet gradually increased microbiota diversity and decreased inflammatory markers in relatively healthy North American adults (Wastyk et al., 2021). The increase in microbiota diversity of the participants consuming the high-fermented food diet during the intervention suggested gut ecosystem remodelling and not a reflection of the fermented food microbiota consumed. Indeed, another study showed that the gut microbiota of individuals who consumed fermented foods consisted of both fermented food-associated and non-fermented-associated microorganisms (Taylor et al., 2020). They also noted that IL-6, IL-10, and IL-12β, among other inflammatory factors, decreased in the high-fermented food diet arm during the course of the intervention (Taylor et al., 2020). A double-blind RCT involving 50 volunteers with T2DM consumed 120g/d daily of fermented milk for 6 weeks. The probiotic group consumed fermented milk containing *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 (10^9 colony forming units (cfu)/d each) and the control group consumed conventional fermented milk. They found that TNF-α was significantly reduced in both groups (SaeidiFard et al., 2020; Tonucci et al., 2017). However, a RCT found that consumption of a single meal daily containing fermented versus non-fermented dairy foods over a course of 4 weeks did not alter the concentrations of circulating inflammatory cytokines (Nestel et al., 2012; SaeidiFard et al., 2020). The differences in these finding may be due to the duration of the study, sample size and frequency and type of fermented foods consumed (SaeidiFard et al., 2020).

Although few studies of fermented foods have been conducted in postpartum mothers, these findings suggest that fermented foods may have the potential to decreased gut microbiota diversity and may also influence the immune system by decreasing inflammation (Zeng et al., 2023) – effects that could be beneficial to lactating mothers. More studies are needed to assess the health benefits of fermented foods in the postpartum period (Caffrey et al., 2025).

1.2.2.2 Potential evidence of fermented foods improving maternal health

Fermentation might be a low-cost intervention as compared to probiotics to improve maternal nutrition, particularly in LMICs (Setta et al., 2020; Simango, 2002). Fermented foods have been part of the human diet in Southern Africa throughout time and have been a point of interest due to their health and nutritional benefits (Ibrahim et al., 2023; Leeuwendaal et al., 2022). Traditional fermented foods are occasionally used for infant weaning in Southern Africa because of the improved nutritional quality, high digestibility and the potential to reduce the transmission of bacterial enteric pathogens in food

(Adekoya et al., 2019; Anyogu et al., 2021; Fadahunsi & Soremekun, 2017; Simango, 1997). Postpartum mothers' dietary choices may by be shaped by traditional cultural beliefs, or they may also opt for convenient, lower-quality foods that are not aligned with the recommended items listed in the South African Food Database (Bathula et al., 2024; Sebeta et al., 2022; Symington et al., 2018). A study evaluating the consumption of *Shameta,* a traditional cereal-based fermented product prepared exclusively for lactating mother in Wollega Zones, Ethiopia, enhanced postpartum recovery, strength and milk production of the lactating mothers (Kitessa et al., 2022, 2023). A RCT investigated the effects of consuming 200g probiotic yoghurt containing *Lactobacillus acidophilus La5* and *Bifidobacterium animalis BB12* daily for 9 weeks on inflammatory markers in pregnant women described a decrease of high-sensitivity-CRP (hs-CRP) in the probiotic compared to the conventional yoghurt group, but no difference in TNF-α (Asemi et al., 2011). These probiotic strains are similar to bacteria used as starter cultures in the preparation of fermented foods.

1.2.2.3 *Mageu* – a traditional fermented food in Southern Africa

Mageu is a traditional non-alcoholic fermented grain-based sour beverage consumed in Southern Africa. It is commonly used as a meal replacement, energy drink or weaning food in infants and children (Daji et al., 2023; Sibiya et al., 2022; Simango, 1997). This non-alcoholic sour beverage goes by many names depending on the region in Southern Africa, for example, in Zimbabwe it is called *Mahewu*, in the South African language isiZulu it is called *Amahewu*, in isiXhosa (the primary language spoken in the community where this study was conducted in) it is *AmaRhewu,* and in Sesotho it is called *Mahleu. Mageu* is often referred to as a general term (Eswatini, 2019; Fadahunsi & Soremekun, 2017; Mafukata et al., 2024).

While *Mageu* was traditionally produced on a household level, in the late 1900s, *Mageu* started being produced on an industrial scale and gradually increased in the market overtime (Holzapfel & Taljaard, 2004; Ray & Montet, 2017). Industrialisation of *Mageu* is still an experimental process, whereby decisions on the use of sweeteners or flavourants and the types of starter cultures to be used for food safety regulations are ever changing (Holzapfel & Taljaard, 2004; Ray & Montet, 2017). Various brands and flavours, such as banana, cream and strawberry are now readily available in supermarkets (Holzapfel & Taljaard, 2004; Ray & Montet, 2017), with "Number-1 Mageu" being the most popular choice for consumers in South Africa (Ray & Montet, 2017).

Recent literature has investigated the nutritional and microbial composition of *Mageu* and ways to improve its nutritional content through fortification (Daji et al., 2022; Fadahunsi & Soremekun, 2017; Mafukata et al., 2024; Mashau et al., 2020; Moiseenko et al., 2021). For example, a study investigated various percentages (1-5%) of whole and defatted *Moringa oleifera* leaf powder (MOLP) on the nutritional composition, physiochemical properties and protein digestibility of sorghum *Mahewu (Mafukata et al., 2024)*. They found that sorghum *Mahewu* enriched with whole and defatted MOLP had significantly higher amounts of protein and fibre compared to the non-enriched *Mahewu*. MOLP-

enriched sorghum *Mahewu* had more minerals, higher amino acid contents and better protein digestibility non-enriched control sample, and consumers preferred the sorghum *Mahewu* enriched with 1% defatted or whole MOLP (Mafukata et al., 2024). Another study investigated the nutritional and antioxidant properties of *Mahewu* when supplemented with flaxseed and soybean. The variations of *Mahewu* were stratified into C1 (maize), C2 (sorghum), C3 (1:1 maize & sorghum), C4 (8:8:3:1 maize, sorghum, soybean, flaxseed) and C5 (6:6:6:2 maize, sorghum, soybean, flaxseed) (Sibiya et al., 2022). They found that *Mahewu* made from maize and sorghum had higher contents of carbohydrates, while *Mahewu* fortified with soybean and flaxseed had higher contents of protein and fatty acids. The fortified *Mahewu* had improved micronutrient levels and antioxidant activity (Sibiya et al., 2022). These finding suggest that *Mahewu* fortification can improve nutritional quality of the product (Mafukata et al., 2024; Sibiya et al., 2022).

Mageu is also suggested to have anti-inflammatory and immune-modulatory properties, e.g. through organic acids that are produced during fermentation and inhibit many pathogenic bacteria (Fadahunsi & Soremekun, 2017; Shahbazi et al., 2021; Simango, 2002).

1.2.2.4 Preparation of *Mageu*

The traditional way of preparing *Mageu* is a natural process, whereby malt flour is added to the gelatinized, thin porridge (boiling water with maize meal flour) as an inoculum for the spontaneous fermentation process that takes place at ambient temperatures for about 24 hours, and is driven by LAB and yeast (Figure 1) (Taylor, 2004; Pswarayi & Gänzle, 2019; Simango, 2002). Lactic acid and amylolytic enzymes are produced as an end product after fermentation, thus reducing the pH of the beverage to about 3.5, which ultimately prevents the proliferation of undesirable microbes that could cause food spoilage, and results in the typical sour taste (Daji et al., 2023; Mashau et al., 2020; Simango, 2002). Following fermentation, *Mageu* contains ~11% protein, ~5% fat, ~1% fibre, and ~65% carbohydrate, with mineral content including sodium, potassium, calcium, iron, zinc and manganese (Fadahunsi & Soremekun, 2017; Oyeyinka et al., 2021).

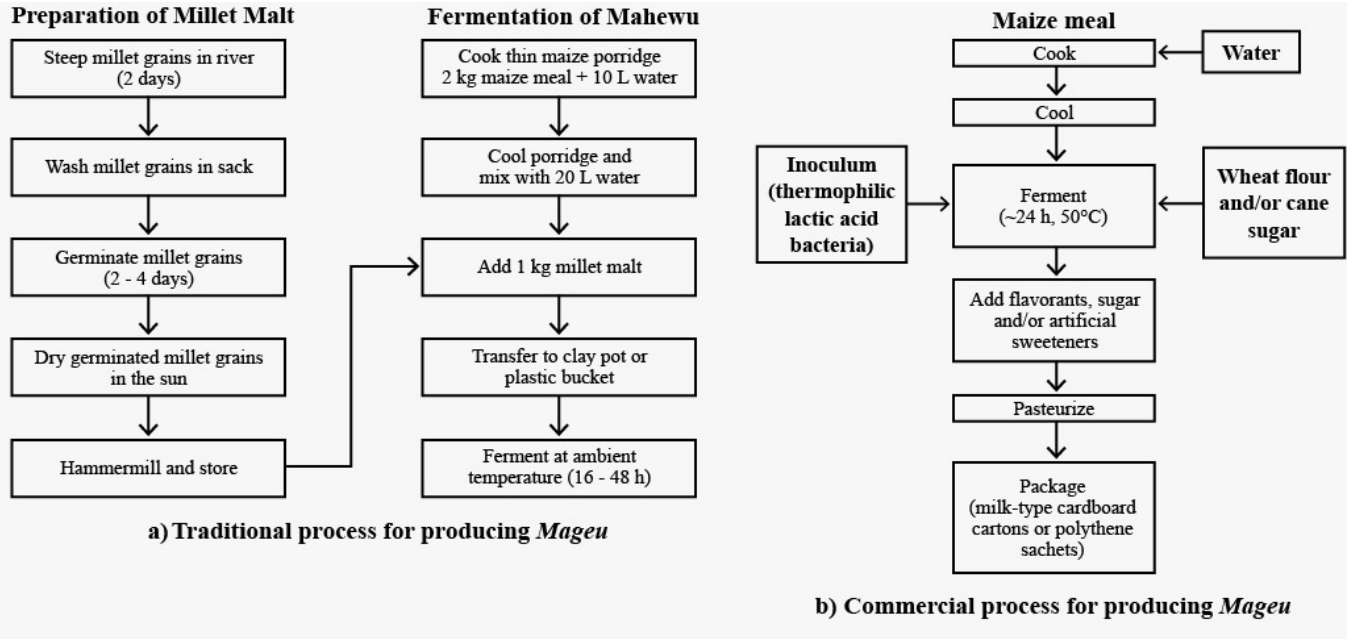

Figure 1: Traditional and commercial *Mageu*. a) Process for producing traditional *Mageu* (Pswarayi & Gänzle, 2019) or at the household level is much longer than the industrial process and does not involve pasteurisation steps or the addition of flavourants as shown in the b) commercial/industrial process for producing *Mageu* (J. Taylor, 2004).

The current industrially-produced versions of fermented foods and beverages, like *Mageu*, only serve as simulations to the traditionally produced fermented products, as they are often produced with different substrates and starter cultures that follow safe and hygienic food practices outlined by Food Regulatory bodies (Gadaga et al., 1999). In accordance with the SANS 1199:2011 regulation, "The production of mageu", the product should be free from spoilage organisms such as pathogenic spore-forming *Clostridium perfringens*, *Clostridium botulinum, Clostridium difficile* and *Escherichia coli* (*E. coli*). Importantly, heat sterilisation and pasteurisation (involving heating to 72°C for >15 seconds, followed by rapid cooling), (Figure 1) of the commercially available product increases the shelf life but also inactivates live LAB, and thus might minimise the impact of the fermented porridge on overall health (Dimidi et al., 2019; Taylor, 2004). To support this hypothesis, a previous study found that pasteurised yoghurt reduced lactase activity, resulting in an increase in lactose malabsorption compared to unpasteurised yoghurt, therefore minimising the impact LAB had on reducing lactose intolerance (Savaiano & Hutkins, 2021; B. C. Taylor et al., 2020). In Zimbabwe, a powdered concentrated form of *Mageu* is sold in local retailers, and these powders are fortified with minerals, vitamins and soy protein to potentially increase or replace the nutritional quality of the product that was originally lost during the pasteurisation process (Gadaga et al., 1999). Whether the health benefits of traditional, live culture *Mageu* and commercial, pasteurised *Mageu* differ has not been evaluated in South African postpartum mothers.

1.2.2.5 Microbial composition of *Mageu*

The microbes predominantly responsible for fermentation in *Mageu* include *Lactobacillus delbreuckii, Lactobacillus plantarum, Streptococcus thermophilus* and *Lactococcus lactis* subsp. *Lactis* (Blandino et al., 2003; Daji et al., 2022; Mashau et al., 2020; Tsafrakidou et al., 2020). As previously outlined, *Lactobacillus* spp., *Streptococcus* spp., *Bacillus* spp. and *Saccharomyces* spp. are commonly used in probiotic product development and as starter cultures in fermented foods because they are able to survive the acidic conditions in the gastrointestinal tract, compete with pathogens and exert health benefits in the human gut (Abid et al., 2022; Das et al., 2022; Franz et al., 2014; Ibrahim et al., 2023; Parvez et al., 2006). The symbiotic relationship between the LAB and yeast present in *Mageu*, such as *Saccharomyces cerevisiae* and the occasional *Candida* spp., can contribute to the nutritional quality of the *Mageu* product.

A study investigating the difference in bacterial community profile of optimal *Mahewu* prepared using white and yellow maize with different inocula found little difference in bacterial composition between the two types of maize. Bacterial taxa belonging to *Lactococcus, Enterococcus, Lactobacillus, Bacillus, Clostridium sensu stricto1, Streptococcus* and *Leuconostoc* were identified via 16S amplicon analysis in both white and yellow maize *Mahewu* (Daji et al., 2022), and were similar to taxa found in literature (Blandino et al., 2003; Pswarayi & Gänzle, 2019; Tsafrakidou et al., 2020). Another study evaluating the microbiological composition of *Mahewu* isolated *Lactobacillus brevis, Lactobacillus casei, Lactococcus lactis, lactobacillus plantarum, Saccharomyces cerevisiae* and *Saccharomyces pombe* (Fadahunsi & Soremekun, 2017). They noted that *Saccharomyces cerevisiae* had the highest occurrence (present in 70% of batches) in *Mahewu*, followed by *Lactobacillus brevis* found in 54% of *Mahewu* batches (Fadahunsi & Soremekun, 2017). *Leuconostoc* spp. produce ethanol, carbon dioxide (CO_2) and organic acids, while *Lactobacillus* spp. and *Streptococcus* spp. produce lactic acid. *Clostridium sensu stricto1* enhances the production of butyrate, an anti-inflammatory SCFA, and stimulates the proliferation of fibrolytic bacteria that degrade fibres to produce organic acids and monosaccharides (Daji et al., 2022; Ray & Montet, 2017).

The acidic environment that LAB provide to yeast for growth allows the yeast to produce bioactive compounds, such as proteins and antioxidants (Daji et al., 2022; Pswarayi & Gänzle, 2019; Simango, 2002). A benefit of consuming *Mageu* is not only the LAB present during the fermentation process but also the probiotic factors that these microbes may exert in the gut microbiota and on physiological functions (Daji et al., 2022; Savaiano & Hutkins, 2021). A well-balanced gut microbiota is essential for immune regulation and the prevention of inflammation that lead to diseases like obesity and T2DM, among others. Upon consumption of fermented foods, components like macronutrients, micronutrients, vitamins, phenolic compounds, minerals and bacterial components (fermentation by-products, metabolites and live microorganisms) enter the intestinal tract and interact with the gut microbiota to deliver their beneficial properties (Pswarayi, 2022; Tsafrakidou et al., 2020). Consuming fermented

foods like *Mageu* enriches the intestinal tract with these bacteria and yeasts however Western-based and Mediterranean-based diets, among others, may alter the microbial balance (Daji et al., 2022; Pswarayi, 2022; Tsafrakidou et al., 2020). Recent evidence on the impact of *Mageu* or fermented foods on maternal nutritional health and inflammation during the postpartum period remains limited (Hayes & García-Vaquero, 2016).

1.3 Study rationale

South Africa faces significant socio-economic challenges, including high levels of unemployment and widespread poverty. As a result, low- and middle-income households experience reduced access to sufficient, safe, and nutritious food (Department of Statistics South Africa, 2023). In 2021, it was estimated that 15% of households had inadequate access to food and 6% reported severely inadequate access to food (Department of Statistics South Africa, 2023). Furthermore, in 2018, it was estimated that only 75% of the South African population in the Western Cape reported access to medium or highly diversified diets (Swart, 2022). The survey also indicated that household dietary diversity levels increased with income, whereby 70% of high-income groups reported medium or high dietary diversity compared to 41% in the low-income groups (Swart, 2022). It is widely recognised that maternal health and nutrition are central to developmental outcomes of their children (Miele et al., 2023; Miko et al., 2022). With approximately 250 million children in LMIC at risk of not reaching their developmental potential (Nnam, 2015), there is an urgent need to prioritise research improving maternal nutrition (Miele et al., 2023; Nnam, 2015).

NCCDs are largely driven by chronic inflammation, and rates are increasing rapidly with industrialisation (Gille et al., 2018). Furthermore, women diagnosed with GDM, hypertension or preeclampsia during pregnancy are at a significantly greater risk of developing long-term NCCDs, such as cardiovascular diseases and T2DM, in the postpartum period (Sheiner et al., 2019). Chronic inflammation is closely linked to alterations in the gut microbiome (Bander et al., 2020; Maciel-Fiuza et al., 2023; Sen et al., 2016), and diet has emerged as a driving factor in microbiota composition and function (Gille et al., 2018). Throughout time, fermentation has served as a way to preserve and process foods, and there is a growing acknowledgment that fermented foods have nutritional and other health benefits (Castellone et al., 2021; Franz et al., 2014; Shahbazi et al., 2021). A meta-analysis of RCTs evaluated the effect of fermented foods consumption on inflammation and found that fermented foods may decrease important systemic biomarkers of inflammation, like TNF-α, although other inflammatory biomarkers like IL-6 and CRP remained unchanged (SaeidiFard et al., 2020). Fermented foods are also capable of inducing shifts in gut microbiota populations (Tonucci et al., 2017). Prospective clinical studies have shown reductions in the risk of cardiovascular diseases, T2DM, GDM and overall mortality with frequent yoghurt or probiotic intake, thought to be due to modulation of the gut microbiota (Gille et al., 2018). Therefore, fermented foods may serve as suitable vehicles for delivering beneficial bacteria, possibly influencing the gut microbiota, inflammation and overall health

(Tonucci et al., 2017). Yet, it is still unclear whether fermented foods with viable versus non-viable bacteria and their products would have differing effects.

In order to optimise maternal and infant outcomes, adequate nutrition is essential during pregnancy and postpartum periods (Nnam, 2015). Most South Africans consume commercially produced pasteurised *Mageu*, because it is readily available and has a longer shelf-life than traditionally produced *Mageu.* The effect of commercial versus live-culture *Mageu* on inflammation and nutritional markers in postpartum women has never been described, to my knowledge.

1.4 Aims and objectives

The overall aim of this study was to evaluate the microbial quality, safety and health effects, with an emphasis on nutritional and inflammatory markers, of *Mageu* in a pilot randomised trial enrolling 45 South African breastfeeding mothers.

The specific aims are:

1. **To assess the nutritional and microbiological quality and safety of live culture and pasteurised *Mageu* products prior to administration.**

   The nutritional composition of a 500ml serving of the live culture *Mageu* product will be obtained from an external laboratory and the pasteurised *Mageu* product will be obtained from the packaging provided by the manufacturer. Selective bacterial culture will be performed to detect the presence of *E. coli* and pathogenic *Clostridium* species. Quantitative polymerase chain reaction (qPCR) of the 16S ribosomal ribonucleic acid (rRNA) gene will be performed to assess total bacterial load.

   Hypotheses:

   - The live culture *Mageu* will have a higher nutritional content than the pasteurised *Mageu*.
   - Both *Mageu* products will comply with the SANS 1199:2011 food safety regulations, and contain no *E. coli* and pathogenic *Clostridium* ssp.
   - The pasteurised *Mageu* product will have a lower total bacterial load compared to the live culture *Mageu* product.

2. **To evaluate adherence to *Mageu* consumption and monitor adverse events, and to compare the effects of live culture, pasteurised, and no *Mageu* consumption on markers inflammation, nutritional status and BMI in postpartum women**.

   Adherence (assessed using self-report and objective measures) to the intervention and the incidence of adverse events will be assessed across study arms. Inflammatory markers (systemic and intestinal, assessed by Luminex® and Enzyme-Linked Immunoassay (ELISA), respectively), nutritional biomarkers (assessed by Q-Plex Human Micronutrient Array assay), and BMI will be compared to determine the physiological effects of *Mageu* consumption.

Hypotheses:

- Participants in both *Mageu* randomisation arms will adhere to the assigned product, and avoid consumption of additional fermented foods, as per protocol.
- *Mageu* will be well tolerated, with a low incidence of adverse events.
- The live culture *Mageu* group will have decreased systemic and intestinal inflammation and improved nutritional markers compared to the pasteurised and no *Mageu* groups.
- *Mageu* consumption, particularly the live culture product, will be associated with reductions in postpartum weight.

3. **To examine associations between inflammation, nutritional markers and BMI in postpartum women**.

   This aim will explore how maternal nutritional status and body weight relate to inflammatory responses during the postpartum period using data integration methods.

   Hypotheses:

   - Higher BMI will be associated with increased levels of inflammatory markers.
   - Higher BMI will be associated with a lower nutritional status.
   - Poor nutritional status will correlate with increased levels of inflammatory markers.

CHAPTER 2
MATERIALS AND METHODS

2.1 Study approval

The parent study was approved by the University of Cape Town Human Research Ethics Committee (HREC 004/2022), the South African Health Product Regulatory Authority (SAHPRA ref 20220305) and registered on the South African Clinical Trials Registry (DOH-27-072022-6097, registration approved on 27 July 2022). The trial was implemented in accordance with (International Conference on Harmonisation) ICH E6 and South African Good Clinical Practice (GCP). All women provided written informed consent. This MSc protocol was approved under HREC 901/2024.

2.2 *Mageu* manufacturing and assessment

2.2.1 *Mageu* preparation

The live-culture *Mageu* (LCM) was produced at the Centre for Bioprocess Engineering Research (CeBER) at the University of Cape Town (UCT) in line with the regulations stipulated in the South African National Standards (SANS) guideline SANS1199:2011 "The production of mageu". For each batch, a maize-meal in water suspension was prepared by mixing 500ml of boiling water with 100g of maize-meal to form a paste. A sterile pot containing 500ml of boiling water was heated on a stovetop, after which the paste was added and whisked to form a smooth porridge. The porridge was cooked on high temperatures (>90°C) for 15 minutes, stirring frequently. The porridge was then cooled to 25-30°C by placing the pot in an ice bath for approximately 1 hour. Once cooled, 10g flour and 20g sugar was added to the porridge and mixed well. The porridge was transferred to a sterile fermentation vessel, where the initial temperature and pH of the inoculated porridge was recorded. The fermentation vessel was labelled with the date of preparation and batch number, then allowed to incubate at 37°C. The temperature and pH were recorded again at 24 and 48 hours before dispensing into the sterile drinking containers for each participant (Figure 2). The final product had a maize-meal concentration of 10 wt% solids and final pH of 3.5. The nutritional quality of the final product was externally verified through an independent, accredited nutritional, microbial and pathogen testing laboratory (Microchem Specialised Lab Services, SA) prior to use in the randomised controlled trial (RCT). For comparison, commercial pasteurised *Mageu* (CPM) was purchased from local suppliers for use in the RCT and dispensed into identical containers. The nutritional quality of the CPM product was obtained from the manufacturer. This work was conducted as part of the parent RCT (HREC/REF 004/2022).

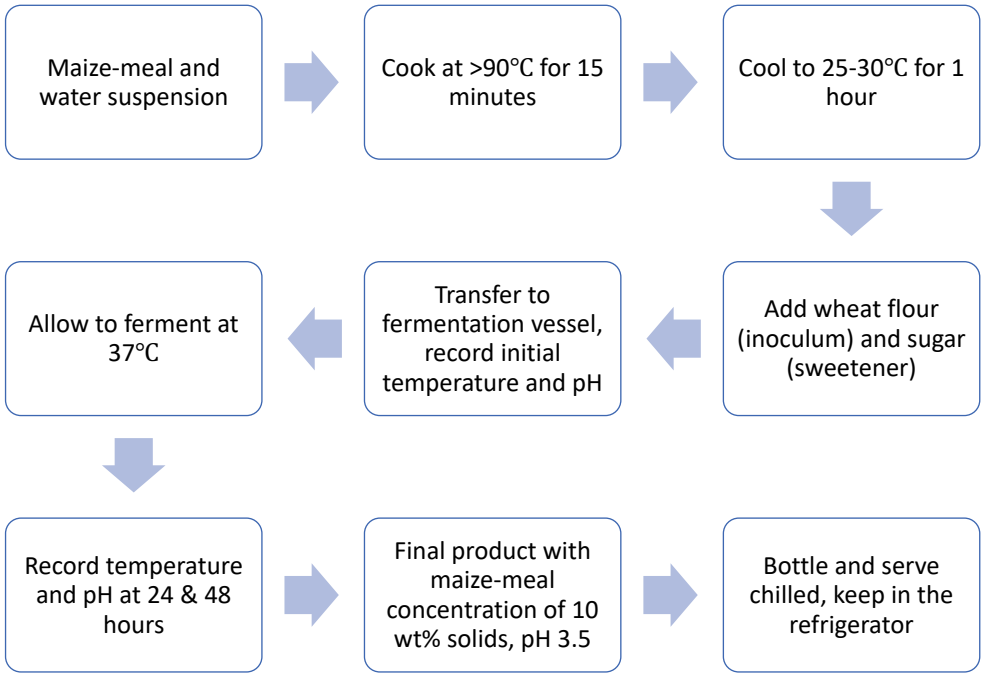

Figure 2: Preparation of the live culture *Mageu* study product

2.2.2 *Mageu* quality control

2.2.2.1 Screening of *Mageu* batches for *Clostridium* spp. and *Escherichia coli*

The bacterial culture quality control was performed according to SANS (SANS 1199:2011) for the presence of spoilage organisms *Escherichia coli* (*E. coli*) and mesophilic *Clostridium* spp. (that is (i.e.) *C. perfringens*, *C. botulinum* and *C. difficile*) using selective media. The quality control evaluations were performed for each batch of LCM and CPM. Five-hundred µl of *Mageu* was added to 500µl sterile Dulbecco's Phosphate Buffered Saline (DPBS) (Sigma-Aldrich, Cat. no.: D8537-500ml) in two labelled 1.5ml Eppendorf tubes for *E. coli* and *Clostridium* spp. identification, respectively, and vortexed. The samples used for the identification of *Clostridium* spp. were heat shocked to kill vegetative cells whilst preserving bacterial spores and cultured using a 10-fold dilution series on Brain Heart Infusion (BHI) agar plates [3.7g/L BHI [Bacto] (Merck, #53286) and 0.5g/L yeast extract (Sigma-Aldrich, #8D13-01.2), supplemented with 0.05g/L Cysteine-hydrochloride (HCL) (Merck, #C7880) and 10ml/L Haemin (Merck, #51280) + Menadione (Glentham Life Sciences, #GV3614) mixture, made up to 1L according to manufacturer's instructions]. The heat-shocked samples (100µl), used for the identification of spore-forming species, were plated on BHI agar and incubated anaerobically for 24h hours to allow for growth of oxygen sensitive colonies. These colonies were then incubated aerobically on BHI agar for 24 hours to distinguish between obligately anaerobic spores (*Clostridium* spp.) and facultative spore formers (non-*Clostridium* spp.). The samples used for the identification of *E. coli* were inoculated with 100µl of the non-heat shocked dilutions (10^1 to 10^5) on labelled MacConkey agar plates [50g/L MacConkey agar with crystal violet and salt (Millipore, #HG00002.500), made according to

manufacturer's instructions] and incubated aerobically at 37°C for 24 hours. All *E. coli* (dark pink) and *non-E. coli* (pale/colourless) colonies on MacConkey and BHI plates were counted to determine the number of colony forming units (cfu/ml) and confirmed by colony polymerase chain reaction (PCR) of the 16S rRNA gene and Sanger sequencing of the product (see section 2.2.2.2). This work was done as part of the parent RCT (HREC/REF 004/2022) and this protocol (HREC/REF 901/2024).

2.2.2.2 Colony PCR and sequencing

Template deoxyribonucleic acid (DNA) for PCR was prepared by picking single colonies from BHI and MacConkey plates that were suspended in 50 µl of lysis buffer containing 0.2mg/ml proteinase K (ThermoFisher Scientific, #AM2546) in Tris-ethylenediaminetetraacetic acid (EDTA) (TE) buffer [10mM Tris pH 7.7 (Melford, #T60040-1000.0), 1mM ethylenediaminetetraacetic acid (EDTA) (Melford, #E57020-250.0)]. The samples were incubated at 37°C for 20 minutes in a heating block and then at 80°C for 30 minutes to inactivate the proteinase K, allowing for downstream reactions. The samples were centrifuged for 3 minutes at 2348 xg and the supernatant was used as the template for a PCR reaction targeting the full-length 16S rRNA gene. The PCR mix contained 2X Kapa Taq Ready Mix [containing magnesium chloride ($MgCl_2$), deoxynucleotide triphosphates (dNTP's), buffer, Taq polymerase (Merck, #KK1006)], 10µm 27F & 1492R primers (Supplementary Table 1), and 4 µl DNA template, made up to a total volume of 50µl with nuclease-free water. The following PCR cycling parameters were used in a SimpliAmp™ Thermal Cycler system (Applied Biosystems, #A24812): an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of 30 seconds denaturation (95°C) , 30 seconds annealing (55°C) and a 1 minute 40 seconds elongation (72°C) with a final elongation step at 72°C for 7 minutes. The PCR products were electrophoresed on a 1% agarose gel (Bio-Rad, #162-0138) at 110V for 50 minutes, using 1X Tris-acetate-EDTA (TAE) buffer (Invitrogen, #24710-0R0) and visualised using 5µl CondaSafe Stain (Condalab, #4687). Once amplicons had been visualised, the remaining PCR products were sent to Inqaba Biotec for sequencing using the 907R primer (Supplementary Table 1). The sequencing data were compared to the National Center for Biotechnology Information (NCBI) 16S rRNA gene database using Basic Local Alignment Search Tool (BLAST) (Z. Zhang et al., 2004) to identify the cultured species. This work was done as part of the parent RCT (HREC/REF 004/2022) and this protocol (HREC/REF 901/2024).

2.2.2.3 Testing of total bacterial load in *Mageu* batches

A portion of the LCM and CPM batches was stored for genomic DNA extraction by adding 500µl of *Mageu* to 1ml Primestore® MTM (On Point Diagnostics, #PS-MTM 2ml) in sterile cryotubes, mixing well and subsequent storage at -80°C.

From the above, 400 µl of sample was used to extract genomic DNA (gDNA) using the DNeasy PowerSoil® Pro kit (Qiagen, #47014), according to manufacturer's instructions. Total bacterial load was assessed via the Bactquant assay (Liu et al., 2012): 1X Sso Advanced Universal Probes Supermix

[containing $MgCl_2$, dNTP's, Sso7d Fusion polymerase, stabilizers, ROX normalisation dyes (BioRad, #1725281)], 1.8µM Forward & Reverse Primer and 0.2µM probe (Supplementary Table 2), and 2µl of DNA template, made up to 10µl with nuclease-free water. A 1:10 serial dilution (10^1 to 10^8) of the 16S TOPO standard, containing a full length *E. coli* 16S rRNA gene sequence (Liu et al., 2012), was included, along with positive (gDNA extracted from stool) and negative (nuclease-free water) controls. All samples were run in duplicate. The following PCR cycling parameters were used a QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems, #4485701): 1 cycle of Uracil DNA glycosylase (UNG) treatment at 50°C for 3 minutes, followed by Taq activation for 10 minutes (95°C), 15 seconds denaturation (95°C) and 40 cycles of 1 minute annealing and extension (60°C). A standard curve with a coefficient of determination (R^2) of ≥ 0.9 and efficacy percentage of ≥ 80% was considered sufficient to determine the concentrations from the standard curves using the Design and Analysis Software (version 2.3.0). This work was done as part of this protocol (HREC/REF 901/2024).

2.3 Clinical trial conduct and assessments

2.3.1 Recruitment and study schema

In the parent study, entitled "*Mageu* for Health of Mothers and Infants in South Africa (MaMISA) " (HREC/REF 004/2022), 45 eligible mothers (Supplementary Table 3) and their infants were enrolled from the Midwife Obstetrics Unit (MOU) at Site B Khayelitsha between 21 June 2022 and 18 August 2023 prior to day 10 postpartum and followed up until week 15 postpartum. Written informed consent was obtained from all women prior to enrolment. At enrolment, mothers were randomised 1:1:1 to either LCM, CPM or no *Mageu* (NM) (Figure 3). The randomisation was performed using Excel and the confidential list was provided to the dispensing pharmacist at Site B MOU. All women received dietary counselling at enrolment, and were asked to refrain from consuming any fermented foods for the following 3 weeks (referred to as "washout period", from enrolment to approximately week 4 postpartum). After the washout period, mothers returned to the clinic and if assigned to a *Mageu* group (LCM or CPM) received their intervention product. The intervention period took place for 6 weeks, from week 4 to week 10 postpartum. The participants in the *Mageu* arms were blinded as to which type of *Mageu* they would be receiving, and women were instructed to consume one bottle of 500 ml daily for 6 weeks. During this time, participants in all arms were asked to continue their regular diet, and to not consume more than 500 ml of other fermented foods per week besides the assigned intervention. Food frequency questionnaires (FFQs) were completed daily by each participant during the intervention period and captured on REDCap (Version 14.0.39), (Harris et al., 2009, 2019) at every follow-up visit (Supplementary Figure 5). Follow-up visits took place at weeks 10 (end of intervention) and 15 (5 weeks post-intervention). Blood and stool samples were collected at every visit from the mother. For this study, samples and data collected at week 4, 10 and 15 were used for analysis (Figure 3).

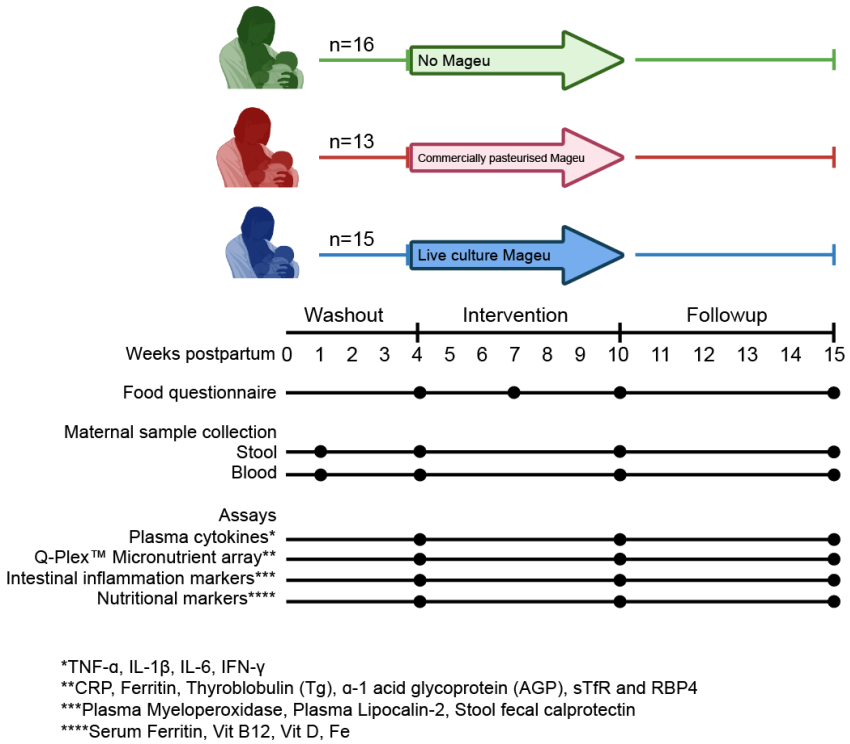

Figure 3: Study overview. Study scheme showing the groups and number of participants (n), timeline, sample type collection, and corresponding experimental platforms that are presented in this thesis.

2.3.2 Specimen collection and processing

A maximum of 6ml maternal whole blood was collected at each time point in both a Sodium Heparin Tube (NaHep) and Serum Separator Tube (SST), and then transported to the processing laboratory at room temperature (RT). Whole blood was centrifuged at RT for 10 minutes at 344 xg, after which 1 ml of plasma and serum was aliquoted into 2 appropriately labelled O-ring vials, respectively. Maternal stool samples were self-collected either at the Khayelitsha MOU or the participant's home in a sterile specimen collection cup. The stool samples were transported to the lab at 2-8°C and aliquoted into 2 appropriately labelled O-ring vials containing equal volumes of the sample. All samples were stored at -80°C for downstream analysis (Figure 3).

2.3.3 Demographic and clinical assessments

Maternal demographic factors, such as age, level of education, occupation, marital status, type of housing, access to running water, number of pregnancies and the use of antibiotics, concomitant medications and vitamins were collected from each participant at every study visit and recorded in standardised case report forms (CRFs) using REDCap by trained clinical research staff. Maternal height and weight were collected at each time point to calculate BMI (kg/m^2). BMI was categorised according to WHO cut-offs for underweight ($<18.5kg/m^2$), normal weight ($18.5\text{-}24.9kg/m^2$), overweight ($25.0\text{-}29.9kg/m^2$), obese class I, II and III ($>30kg/m^2$) categories. Breastfeeding frequency and symptoms of mastitis were recorded throughout the duration of the study on REDCap. Any adverse events (AEs) experienced by the mother was recorded throughout the duration of the study on REDCap. Severity and

relatedness to the study product was assessed by the study clinician using the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Paediatric Adverse Events, Corrected Version 2.1 (U.S. Department of Health and Human Services, 2017).

2.3.4 Assessment of adherence to the assigned intervention

To monitor compliance with the intervention (*Mageu* intake yes/no and if yes, amount), as well as daily intake from 10 food groups (FAO, 2021), participants completed daily monitoring sheets for the duration of the 6-week intervention. Participants were trained to mark food groups from which items had been consumed using a food photograph guide developed for these purposes. Monitoring sheets were reviewed at weeks 7 and 10 by the dietitian to assess adherence (Supplementary Figure 5). The daily food FFQs were used to assesses self-reported adherence. Participants were requested to circle the bottle (full, half empty or empty bottle) best representing the amount of product they consumed each day on the study monitoring sheet (Supplementary Figure 5). For subsequent data analysis, the average reported consumed servings per week for LCM, CPM, and other fermented foods from weeks 1 to 4 (washout period) and from weeks 5 to 10 (intervention period) was calculated. In addition, participants were asked to return bottles. The volume of *Mageu* consumed was measured by weighing each bottle returned and calculating the difference between distributed product (500ml) and any leftover product. The weight difference (in grams) was used to calculate servings per week consumed, averaged over the intervention period, which was defined as "objective adherence".

2.3.5 Measurement of systemic inflammatory markers

MILLIPLEX® Human High Sensitivity T-Cell Panel Premixed 13-plex assay (Merck, #HSTCMAG28SPMX13) was used to quantify interferon (IFN)-γ, tumour necrosis factor (TNF)-α, interleukin (IL)-6 and IL-1β in maternal plasma collected at weeks 4, 10 and 15, according to manufacturer's instructions. Plasma samples were thawed and 300µl of plasma was aliquoted for the assay. The plasma aliquots were centrifuged at 1000 xg for 15 minutes at 4°C to remove particulates from the sample. A 1:2 dilution of plasma with assay buffer (provided in the kit) was used. Samples were run in singlets, with standards and assay controls run in duplicates. The data was acquired using the Bio-Plex™ Suspension Array Reader (Bio-Rad Laboratories Inc®, USA), and a 5PL regression line was used to determine the cytokine concentrations from the standard curves using the Bio-Plex™ manager software. Three plasma samples were used for intra- and inter-plate controls to evaluate precision and repeatability of the assay. A Co-efficient of variance (CV) of ≤20% was considered an acceptable measure of precision and repeatability (Supplementary Table 4).

2.3.6 Measurement of intestinal inflammatory markers

Maternal plasma isolated at weeks 4, 10 and 15 was used to quantify Lipocalin-2 (R&D Biotechne, #DLCN20) and Myeloperoxidase (R&D Biotechne, #DMYE00B) by Enzyme-Linked Immunosorbent Assay (ELISA), according to manufacturer's instructions. Plasma was centrifuged at 10000 xg for 10 minutes at 4°C to remove particulates from the sample. A 1:20 dilution and a 1:150 dilution of plasma sample with calibrator diluent (provided in the kit) was used to assay lipocalin-2 and myeloperoxidase, respectively. Maternal stool lysates from weeks 4, 10 and 15 were used to quantify faecal calprotectin using the Human S100A8/S100A9 Heterodimer Quantikine ELISA kit (R&D Biotechne, #DS8900), according to manufacturer's instructions. As per manufacturer instructions, dry stool samples were thawed and 30mg of sample was mixed with 1.5 ml extraction buffer [0.1M Tris, 0.015M sodium chloride (NaCl) (Merck, #10.06404.0500), 1M Urea (Sigma-Aldrich, #U5378), 1mM calcium chloride ($CaCl_2$) (Sigma-Aldrich, #C5080-500G), 0.1M Citric Acid Monohydrate (Sigma-Aldrich, #C1909), 5mg/ml bovine serum albumin (BSA) (Sigma-Aldrich, #1003376231) and 0.25% Gentamycin Sulphate (Aspen, #30/20.1.1/0106) at pH 8.0] to generate stool lysates. The samples were vortexed for 10 minutes and centrifuged at 344 xg for 10 minutes at RT. After centrifugation, the samples were filtered through a 0.2µm filter (GVS ABLU, #FJ25ASCCA002DL01) to obtain the fecal extract supernatant. The total protein concentration for each sample was determined using a Nanodrop Spectrophotometer (ThermoFisher, #ND-1000) at A280nm. The input sample for ELISA was normalised to 30mg of total protein faecal extract. The faecal extract samples were centrifuged at 10000 xg for 10 minutes at 4°C to remove particulates from the sample. A 1:10 dilution of faecal extract sample with calibrator diluent (provided in the kit) was used to assay faecal calprotectin at each visit.

For all assays, samples were run in singlets, with standards run in duplicates, and data was acquired using the Agilent Biotek Absorbance Reader (800 TS Microplate Reader). Three samples were used for intra- and interpolate controls to evaluate precision and repeatability of the assay. A CV of ≤20% was considered an acceptable measure of precision and repeatability (Supplementary Table 4).

2.3.7 Measurement of systemic micronutrients

The Q-Plex™ Human Micronutrient v2 (7-Plex) Assay (Quansys Biosciences, Cat. no.: 355149HU) was performed at the University of Washington to measure micronutrient biomarkers in maternal plasma isolated at weeks 4 and 10, according to manufacturer's instructions. The assay quantified α(1)-acid glycoprotein (AGP), C-reactive Protein (CRP), Ferritin, Retinol Binding Protein 4 (RBP4), Serum Transferrin Receptor (sTfR) and Thyroglobulin (Tg).

2.3.8 Measurement of nutritional markers

Maternal serum isolated at weeks 4 and 10 was sent to the local National Health Laboratory Service Pathology Lab (NHLS) in Cape Town, South Africa, to quantify nutritional markers, namely serum ferritin, serum vitamin B12, 25-hydroxy vitamin D [25(OH)D] and iron. Nutritional status for each

participant was assessed using reference ranges from the 2nd Edition of the Ampath Desk Reference: Guide to Laboratory Tests (du Plessis et al., 2016).

2.4 Outcomes of interest

In line with the aims, the primary outcomes of interest were as follows:

Aim 1: (i) The nutritional composition of CPM and LCM, (ii) presence of spoilage organisms *E. coli* and mesophilic *Clostridium* spp. (i.e. *C. perfringens*, *C. botulinum* and *C. difficile*) in commercially pasteurised *Mageu* (CPM) and live cultured *Mageu* (LCM) batches, and (iii) the total bacterial load in CPM and LCM.

Aim 2: (i) Participant's adherence to the *Mageu* intervention and use of any other fermented foods throughout the study, and (ii) BMI at weeks 4, 10 and 15 by randomisation arm, and (iii) change of nutritional and inflammatory markers from weeks 4 to 10 and weeks 4 to 15. Secondary outcome included the cross-sectional differences at weeks 4, 10 and 15 between randomisation arms.

Aim 3: (i) Correlations between week 4 and week 10, and week 4 and week 15 for inflammatory and nutritional markers, (ii) correlations between BMI, inflammatory and nutritional markers at week 4, and (iii) overall impact on inflammatory and nutritional profiles by randomisation arm.

2.5 Data analysis

Statistical analysis was performed in Prism (version 10.4.1) and RStudio (version 2024.12.1). Differences in the frequency of bacterial culture screening between CPM and LCM were assessed using Chi-square or Fisher's exact test, as appropriate. Differences in median 16S rRNA gene copy number in CPM and LCM product batches were assessed using Mann-Whitney test. The demographic data were described using the mean (SD) and frequency for included participants in each randomisation arm. The frequency of breastfeeding between groups was compared using Fisher's exact tests. *Mageu* product adherence and the consumption of *Mageu* along with any other fermented foods during the washout and intervention period was compared between groups using two-way Analysis of Variance (ANOVA). Cross-sectional difference in mean BMI for each randomisation arm calculated at weeks 4, 10 and 15 was compared using ANOVA, while frequency of each BMI classification at weeks 4, 10 and 15 was compared using Fisher's exact tests.

The frequency of participants with optimal nutritional status – determined according to the South African reference ranges for women mentioned above – at weeks 4 and 10 was compared using Fisher's exact test. The change in concentrations of nutritional and inflammatory markers between weeks 4 to 10 and weeks 4 to 15 was calculated, and compared between groups using ANOVA for parametric data and Kruskal Wallis for non-parametric data. The cross-sectional comparison of concentrations of nutritional and inflammatory markers measured at weeks 4, 10 and 15 between groups was compared using ANOVA for parametric data and Kruskal Wallis for non-parametric data.

Correlations between the different inflammatory and nutritional markers measured at weeks 4 and 10 and weeks 4 and 15 was assessed using a Pearson correlation. Pearson correlations between BMI, inflammatory and nutritional markers measured at week 4 were analysed using a correlation matrix using the RStudio packages Hmisc (version 5.2-2) and corrplot (version 0.95).

The change in inflammatory and nutritional marker concentration from weeks 4 to 10 was visualized in Principal Coordinate Analysis (PCoA) plots using the RStudio packages vegan (version 2.7-1) and ggplot2 (version 3.5.1) and annotated by randomisation arm. The permutational multivariate analysis of variance (PERMANOVA) test was used to assess the heterogeneity of dispersion between randomisation arms. The log change in concentration was calculated for the inflammatory and nutritional markers at weeks 4 to 10 and for the inflammatory markers at week 4 to 15 to assess for clustering by randomisation arm using the R package Pheatmap (version 1.0.12). A P-value < 0.05 was considered statistically significant for all analyses. However, as this was a pilot trial, p-values were adjusted for multiple comparisons using Holm-Sidak's test, Dunn's test and Tukey's test.

CHAPTER 3
RESULTS

3.1 Quality control of *Mageu*

3.1.1 Nutritional composition of *Mageu*

The nutritional composition of a 500ml serving of the LCM product (obtained from an external laboratory) was compared to the nutritional composition for the CPM product (obtained from the packaging provided by the manufacturer). The LCM product contained more energy (855kJ), protein (5.2g) and carbohydrates (43.8g) per 500 ml serving compared to the CPM product (energy=569kj, protein=2.0g and carbohydrates=30g per 500 ml). The sugar content was higher in LCM (21.9g/500ml) than that of CPM (14.5g/500ml), while the fat content was lower in LCM (<0.05g/500ml) than in CPM (1.0g/500ml). The fibre content was also higher in the LCM product (2.6g/500ml) than in the CPM product (1.0g/500ml) (Table 1). These results suggest that traditional, live culture *Mageu* has a higher nutritional content than the mass produced, commercially available *Mageu*.

Table 1: Nutritional content of *Mageu* per 500ml serving

	CPM	LCM
Energy [kJ]	569	855
Protein [g]	2.0	5.2
Carbohydrates [g]	30.0	43.8
Sugar [g]	14.5	21.9
Fat [g]	1.0	<0.05
Saturated fat [g]	0	0
Trans fat [g]	0	0
Polyunsaturated fat [g]	0	0
Monounsaturated fat [g]	0	0
Cholesterol [mg]	0	<5
Fibre [g]	1.0	2.6
Sodium [mg]	10.0	28.2
Potassium [mg]	0	0

Values were obtained by Microchem Specialized Lab Services for LCM and the manufacturer for CPM.
CPM=Commercially Pasteurised *Mageu,* LCM=Live Culture *Mageu*

3.1.2 Bacterial culture screening of *Mageu*

Among *Mageu* batches manufactured during the trial, LCM had a significantly higher prevalence of obligate anaerobic spore-forming species (27/73, 36.99%) compared to CPM (3/66, 4.55%, Fisher's Exact p<0.0001), as well as a higher prevalence of facultative spore-forming species (64/73 [87.67%] vs. 27/66 [40.91%], respectively, Chi-square p<0.0001). Similarly, LCM had a higher proportion of batches containing non-*E. coli* species (58/73, 79.45%) compared to CPM (3/66, 4.55%, Fisher's Exact p<0.0001). None of the CPM and LCM batches contained *E. coli* (Table 2 and Figure 4).

Table 2: Bacterial culture screening of *Mageu* batches to identify food spoilage pathogens

Species [n (%)]	CPM (n=66)	LCM (n=73)	P-value
Obligate anaerobic spore-formers	3 (4.55)	28 (38.36)	<0.0001[a]
Facultative spore-formers	27 (40.91)	64 (87.67)	<0.0001[b]
E. coli	-	-	-
non-*E. coli* species	2 (3.03)	58 (79.45)	<0.0001[a]

CPM=Commercially Pasteurised *Mageu*, LCM=Live Culture *Mageu, E. coli = Escherichia coli*
P-value represents a) Fisher's exact analysis and b) Chi-square analysis

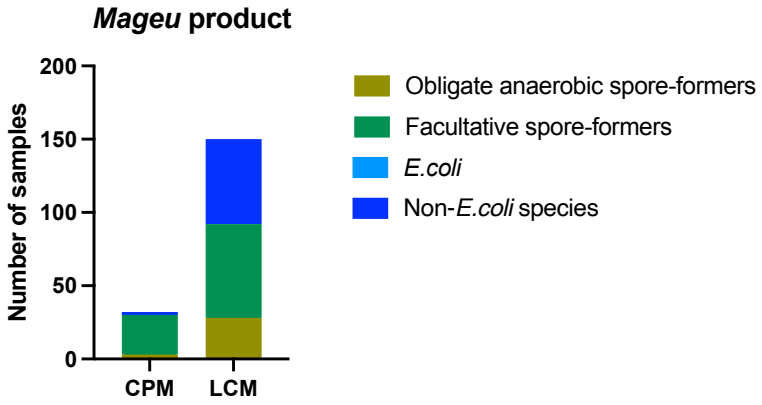

Figure 4. Bacterial culture screening for quality control. The presence of *E. coli* and *Clostridium* spp. in LCM and CPM batches manufactured during the trial was assessed by culture on MacConkey agar (to enrich for *E. coli*) and BHI agar (to enrich for *Clostridium* spp.). The graphs show the number of samples that had obligate anaerobic spore-forming species (brown), facultative spore-forming species (green), *E. coli* (light blue) or non-*E coli* (dark blue) present as determined by bacterial culture. CPM=Commercially Pasteurised *Mageu*, LCM=Live Culture *Mageu,* BHI= Brain Heart Infusion.

3.1.3 Assessment of total bacterial load in *Mageu*

Colony PCR and 16S rRNA sequencing data of oxygen-sensitive colonies (obligate anaerobic spore formers) cultured on BHI agar identified *Bacillus* spp., *Clostridium beijerinckii*, *Clostridium manihotivorum* and *Leuconostoc lactis* to be present in *Mageu*. The non-*E. coli* species cultured on MacConkey agar were *Klebsiella pneumoniae*, *Kosokonia cowanii*, *Citrobactor amalonaticus*, *Citrobactor freundii*, *Cronobacter* spp. and *Enterobactor* spp. These results confirm that the *Mageu* products contained no pathogenic *E. coli*, and no mesophilic *Clostridium* spp. associated with human diseases. Both CPM and LCM products contained live obligate anaerobic spore formers, including *C. beijerinckii* and *C. manihotivorum.* None of the identified *Clostridium* spp. are considered human pathogens but are known for their roles in biodegradation and fermentation (Cheawchanlertfa et al., 2020; Jones, 2024; Mete et al., 2024). To ensure fermented foods like *Mageu* is free from pathogenic species, sterile utensils and fermentation vessels should be used. Additionally, screening the flour and maize before inoculation for the presence of pathogenic species may increase product safety (Daji et al., 2022; Mishra et al., 2024).

Randomly selected *Mageu* batches were subjected to broad-range quantitative PCR (qPCR) to measure absolute 16S rRNA gene copy number, in order to compare the total bacterial load between CPM and

LCM batches (Figure 4). The 16S rRNA gene copy number per µl gDNA extracted from CPM batches (n = 11, median 125.6 [IQR 8.06-360.7]) was significantly lower as compared to LCM (n = 20, median 31550 [IQR 1802-104858] copies /µL gDNA, Mann-Whitney p<0.0001) (Figure 5). This suggests that the total bacterial load of LCM was higher than that of CPM, likely reflecting pasteurisation of CPM.

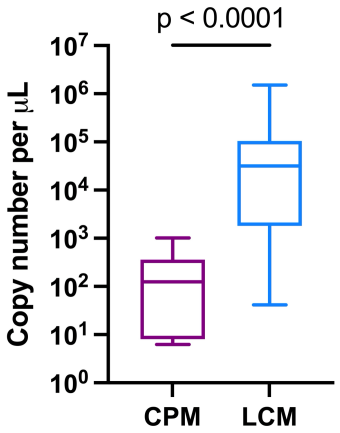

Figure 5. Assessing total bacterial load of *Mageu*. The 16S rRNA gene copy number per µl gDNA extracted from CPM (n = 11) and LCM (n = 20) batches was quantified using real-time qPCR. The median 16S rRNA gene copy number per µl gDNA was compared using Mann-Whitney Test. Graphs show a box-and-whisker plot with median, minimum and maximum. CPM=Commercially Pasteurised *Mageu*, LCM=Live Culture *Mageu*, qPCR=Quantitative Polymerase Chain Reaction.

3.2 The effect of *Mageu* consumption on maternal health, inflammation and nutritional status in South African postpartum women

3.2.1 Participant recruitment and follow-up

Of the 321 women assessed for eligibility, 45 women met the eligibility criteria and were randomised at Day 4-10 postpartum. This included 16 participants in the NM arm, 14 in CPM arm and 15 in the LCM arm. One participant from the CPM arm acquired HIV during the study and was excluded from all subsequent analyses. Of the remaining women without HIV, eleven (11) women in NM arm, 12 in the CPM arm and 11 in the LCM arm attended the week 4 visit (after 'washout' phase, pre-intervention). At the post-intervention visit (week 10), 12 women each in the NM and CPM arms and 11 women in the LCM arm were retained and therefore blood samples were available for analysis. At week 15 (5 weeks post-intervention), blood samples for 10 women each in the NM and CPM arms and 9 in the LCM arm were retained for analysis. Stool samples collected for analysis at follow-up visits were based on availability. Reasons for non-inclusion were lost to follow-up (LTFU) or discontinuation of the study (with reasons including relocation and return to work, among others) (Figure 6).

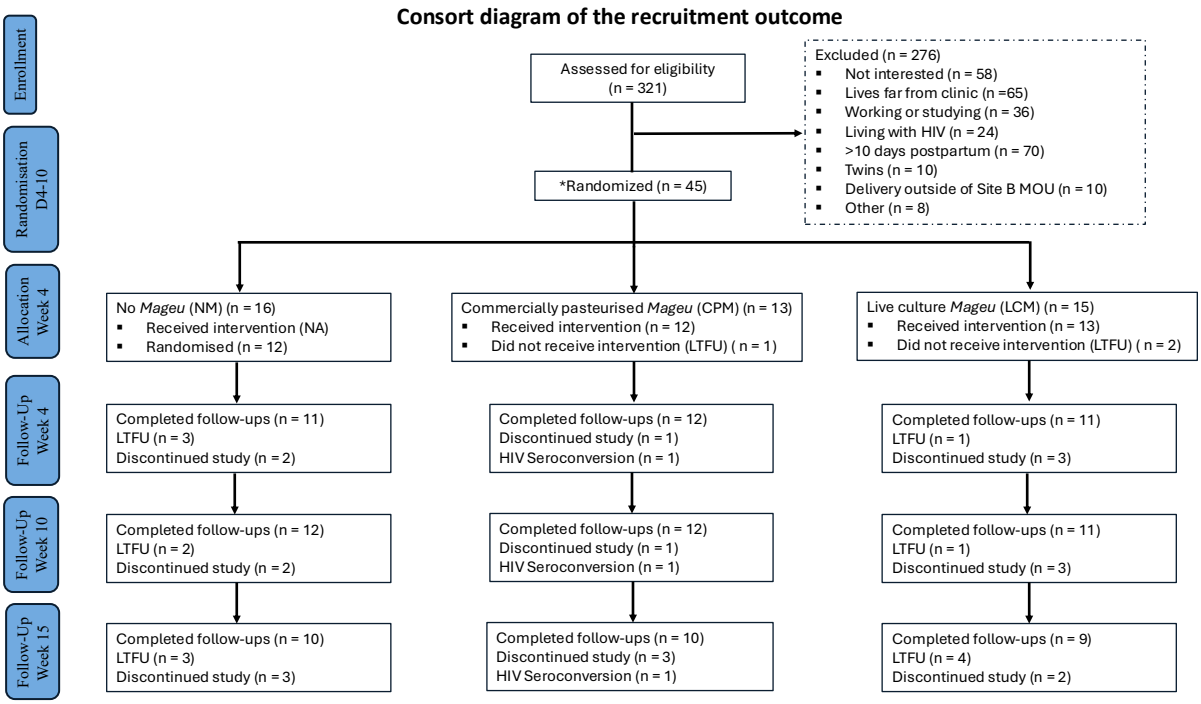

Figure 6. Consort diagram of the clinical trial. Consort flow diagram depicting participant enrolment, allocation, follow-up and analysis for each of the randomisation arms reflecting the time points that are presented in the study schema. *One participant from the CPM arm was excluded from the analysis as she acquired HIV after enrolment. LTFU=loss to follow-up, HIV=Human Immunodeficiency Virus, NA=Not Applicable.

3.2.2 Participant demographics

The mean age of the participants at enrollment was 28 years (SD 6.29), with a mean BMI of 31.75 kg/m^2 (SD 6.77). Most participants were single (27/44, 61.36%), with 79.55% (35/44) having secondary or high school as the highest level of education, and 90.91% (40/44) being unemployed (Table 3). Approximately half (23/44) of the participants lived in informal housing but most (38/44, 86.36%) had access to running water in the home. Many of the women had previously been pregnant (30/44, 68.18%) and none of the participants used antibiotics during pregnancy (Table 3).

Table 3: Demographics and adverse events of included participants

	All (n = 44)	NM (n = 16)	CPM (n = 13)	LCM (n = 15)
AGE (years) [mean (SD)]	28 (6.29)	27 (6.40)	26 (6.97)	31 (4.76)
***BMI (kg/m^2) [mean (SD)]**	31.75 (6.77)	32.76 (7.68)	31.55 (4.80)	30.84 (7.48)
MARRITAL STATUS [n (%)]				
Single	27 (61.36)	9 (56.25)	9 (69.23)	9 (60)
Living together	6 (13.64)	4 (25)	1 (7.69)	1 (6.67)
Married	11 (25)	3 (18.75)	3 (23.08)	5 (33.33)
LEVEL OF EDUCATION [n (%)]				
Secondary/High School	35 (79.55)	13 (81.25)	10 (76.92)	12 (80)
Higher Education	9 (20.45)	3 (18.75)	3 (23.08)	3 (20)
OCCUPATION [n (%)]				
Unemployed	40 (90.91)	15 (93.75)	12 (92.31)	13 (86.67)
Employed	4 (9.09)	1 (6.2)	1 (7.69)	2 (13.33)
TYPE OF HOUSING [n (%)]				
House	21 (47.73)	9 (56.25)	7 (53.85)	5 (33.33)
Informal housing	23 (52.27)	7 (43.75)	6 (46.15)	10 (66.67)
RUNNING WATER [n (%)]				
Yes	38 (86.36)	14 (87.50)	12 (92.31)	12 (80)
No	6 (13.64)	2 (12.50)	1 (7.69)	3 (20)
FIRST PREGNANCY [n (%)]				
Yes	14 (31.82)	6 (37.50)	4 (30.77)	4 (26.67)
No	30 (68.18)	10 (62.50)	9 (69.23)	11 (73.33)
ANTIBIOTICS USED DURING PREGNANCY [n (%)]	0 (0)	0 (0)	0 (0)	0 (0)

*No Body Mass Index (BMI) data available for n=5
SD=Standard deviation, NM=No *Mageu*, CPM=Commercially Pasteurised *Mageu*, LCM=Live Culture *Mageu*.

All women initiated breastfeeding at birth. All participants still breastfed at week 4 (Fisher's Exact p>0.9999). At week 10, all NM (12/12, 100%) and LCM users (11/11, 100%) still breastfed, while one CPM user had stopped breastfeeding (91.67% (11/12), Fisher's Exact p>0.9999). Breastfeeding rates remained high at week 15 (23/29, 79.3%), with no differences between randomisation arms (Fisher's Exact p=0.847) (Table 3). As expected, a slight decrease in the percentage of participants breastfeeding in each group was observed over time (NM p=0.071, CPM p=0.088, LCM p=0.273, Fisher's Exact), (Table 4). No problems or infections of the breasts were reported by any participants throughout the study.

Table 4: Breastfeeding frequency of included participants

	NM [n/ N (%)]	CPM [n/ N (%)]	LCM [n/ N (%)]	P-value
Week 4 (n=39)	14/14 (100)	12/12 (100)	13/13 (100)	>0.999
Week 10 (n=35)	12/12 (100)	11/12 (91.67)	11/11 (100)	>0.999
Week 15 (29)	8/10 (80)	7/10 (70)	8/9 (88.89)	0.846
P-value	0.071	0.088	0.273	

NM=No *Mageu*, CPM=Commercially Pasteurised *Mageu*, LCM=Live Culture *Mageu*
P-value represents a Fisher's exact analysis between arms and through time.

3.2.3 Adherence to *Mageu*

Participant adherence to the *Mageu* product was assessed using two methods: self-reported (captured through daily FFQs) and objective adherence (measured by the return and weighing of bottles by the distribution pharmacy).

During the washout period (enrollment to week 4), participants were advised to consume less than 5 servings of *Mageu* per week. Participants in the LCM arm reported to consume an average of 4.50 (SD 2.59) *Mageu* servings per week, while participants in the NM (mean 2.75 [SD 1.39]) and CPM arms (mean 2.75 [SD 2.47]) reported fewer servings (Figure 7a), although this was not statistically significant. The average servings per week for the LCM arm was still below the <5 servings per week limit, to see any effect in subsequent outcomes. Perhaps future studies could require participants to not consume any *Mageu* during the washout period to remove potential bias. In line with the study requirements, the CPM and LCM users increased their *Mageu* consumption during the intervention period (week 5 to week 10), while reported *Mageu* consumption in the NM arm remained low (ANOVA p<0.0001) during the intervention period (Figure 7a). As such, participants in the CPM arm self-reported an average of 6.44 servings of 500 ml *Mageu* (SD 0.51) per week during the intervention period, while participants in the LCM self-reported an average of 6.71 (SD 0.39) servings of 500 ml *Mageu* per week (Figure 7b). In agreement, the objective adherence was an average of 6.62 servings per week (SD 0.14) for women in the CPM arm, and an average of 6.64 servings per week (SD 0.22) for the LCM arm during the intervention period (Figure 7b).

Participants were advised to consume less than 5 servings of any other fermented food (besides the CPM or LCM) while enrolled into the study. In line with these instructions, most participants in all arms reported consumption less than 5 servings per week of any other fermented food during the washout and intervention periods, however 2 participants in the LCM arm consumed more than 5 servings per week (Figure 7c). *Amasi* was the most common additional fermented food consumed, specifically during the washout period (overall mean of 2.84 servings per week [SD 0.96]), with a mean of 3.76 servings per week (SD 3.16) in the LCM arm. During the intervention period, the reported number of servings of any other fermented foods was lower across all arms (mean 1.96 servings/week [SD 0.22]) (Figure 7c).

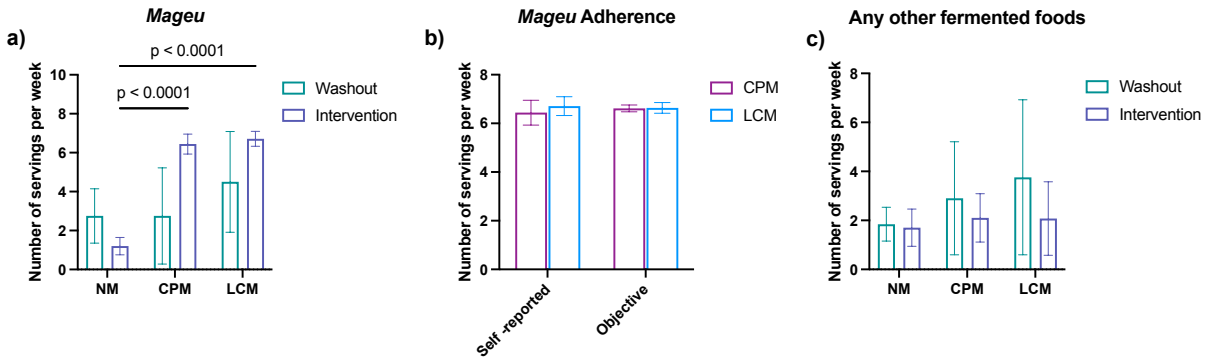

Figure 7. Consumption of *Mageu* and any other fermented foods. a) Participant FFQs were used to calculate the average frequency (number of servings consumed per week) of *Mageu* consumed during the washout (enrollment – week 4) and intervention (weeks 5-10) periods. Graphs show the mean and standard deviation. Statistics was calculated using Two-way ANOVA. b) The self-reported adherence to the *Mageu* study product was calculated by the frequency of consumption over the 6 weeks using the FFQs. The

objective adherence was calculated based on the amount of *Mageu* left upon return of the bottle by each participant. Statistics was calculated using Two-way ANOVA. c) Participant FFQs were used to calculate the average frequency (number of servings consumed per week) of any other fermented foods consumed during the washout and intervention periods. Statistics was calculated using Two-way ANOVA. FFQ = Food Frequency Questionnaires, NM=No *Mageu*, CPM=Commercially Pasteurised *Mageu*, LCM=Live Culture *Mageu*, ANOVA=Analysis of Variance.

3.2.4 Occurrence of adverse events during the trial

Few AEs occurred in the trial, and the frequency was comparable between arms. One participant in the NM arm experienced a runny nose and sneezing. In the CPM arm, 3 participants experienced AEs, namely headache, abdominal pain and diarrhoea, respectively. In the LCM arm, 2 participants experienced AEs, namely fever, bloating, diarrhoea and seizures, respectively. Abdominal pain, bloating and diarrhea were possibly related to *Mageu* consumption, while the remainder were considered unrelated. Thus, consumption of LCM and CPM was generally safe. Concomitant medication use was rare (paracetamol used by n=2 women) and did not differ by randomisation arm.

3.2.5 Effect of *Mageu* consumption on maternal BMI

The mean BMI at the beginning of the intervention (week 4) was 31.75 kg/m^2 (SD 6.77), with about half (18/39, 46.15%) of the women being obese (BMI ≥ 30 kg/m^2) and the remainder mostly being overweight (BMI 25-29.9 kg/m^2, 16/39, 41.03%) (Table 5). At week 4, BMI did not differ significantly between randomisation arms (ANOVA p=0.737): the mean BMI was 32.76 kg/m^2 (SD 7.68) in the NM arm, 31.55 kg/m^2 (SD 4.80) in the CPM arm, and 30.84 kg/m^2 (SD 7.48) in the LCM arm (Figure 8a). In agreement, the percentage of overweight (Fisher's exact p=0.839) or obese (Fisher's exact p=0.948) women did not differ by randomisation arm at enrollment (Table 5). Likewise, there were no significant differences in BMI cross-sectionally at week 10 (end of intervention, ANOVA p=0.758) or week 15 (5 weeks post-intervention, ANOVA p=0.703) by randomisation arm (Figure 8b, c). The percentage of overweight and obese women did not differ at any of the post-intervention time points either (p>0.9999, p=0.5418 at week 10, respectively, and p=0.762, p=0.290 at week 15, respectively, Fisher's exact) (Table 5). Perhaps changes in lifestyle habits, increased food consumption and lack of exercise that comes with caring for a newborn may have resulted in the stable BMI observed over time in this cohort.

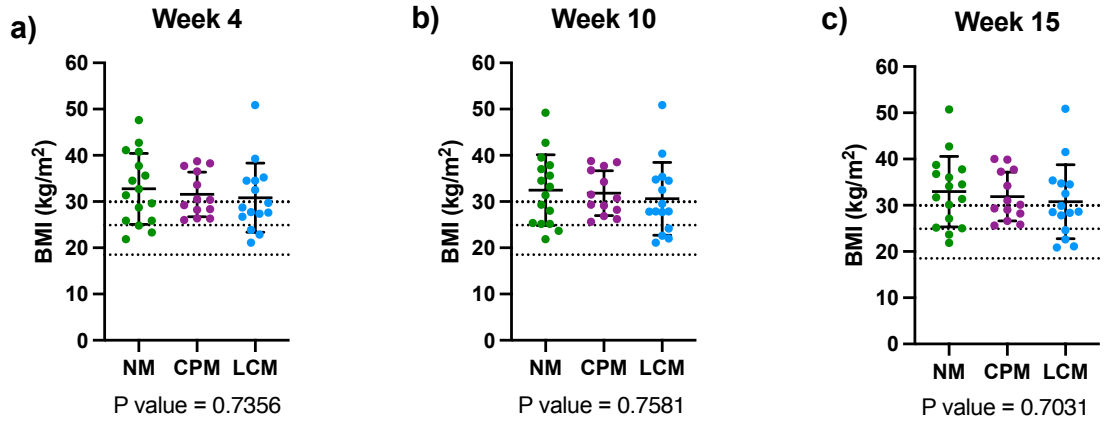

Figure 8. Cross-sectional comparison of participant's BMI at pre- and post-intervention time points. BMI was calculated according to the WHO formula at a) pre-intervention at week 4, and b) post-intervention at week 10 and c) week 15. The graphs show mean and standard deviation, and the BMI cut-offs for underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2), obese (>30 kg/m^2) are indicated by the dotted horizontal line. Statistics were calculated using ANOVA. NM=No *Mageu*, CPM=Commercially Pasteurised *Mageu*, LCM=Live Culture *Mageu,* WHO=World Health Organization, ANOVA=Analysis of Variance.

Table 5: Maternal BMI by randomisation arm

Time points	Weight classification	All [n/N (%)]	NM [n/N (%)]	CPM [n/N (%)]	LCM [n/N (%)]	P-value
Week 4	Normal	5/39 (12.82)	3/39 (7.69)	-	2/39 (5.13)	0.368
	Overweight	16/39 (41.03)	4/39 (10.26)	6/39 (15.38)	6/39 (15.38)	0.839
	Obese	18/39 (46.15)	7/39 (17.95)	6/39 (15.38)	5/39 (12.82)	0.948
Week 10	Normal	4/35 (11.43)	1/35 (2.86)	-	3/35 (8.57)	0.320
	Overweight	16/35 (45.71)	5/35 (14.29)	6/35 (17.14)	5/35 (14.29)	>0.999
	Obese	15/35 (42.86)	6/35 (17.14)	6/35 (17.14)	3/35 (8.57)	0.542
Week 15	Normal	2/29 (6.90)	-	-	2/29 (6.90)	0.326
	Overweight	10/29 (34.48)	2/29 (6.90)	4/29 (13.79)	4/29 (13.79)	0.762
	Obese	17/29 (58.62)	8/29 (27.59)	6/29 (20.69)	3/29 (10.34)	0.290

No Body Mass Index (BMI) data available for n=5 at Week 4, n=9 at Week 10, and n=13 at Week 15.
BMI used to categorize weight into normal (18.5 – 24.9 kg/m^2), overweight (25 – 29.9 kg/m^2) or obese (>30 kg/m^2).
NM=No *Mageu*, CPM=Commercially Pasteurised *Mageu*, LCM=Live Culture *Mageu*.
P-value represents a Fisher's exact analysis for BMI categories between randomisation arms.

3.2.6 Effect of *Mageu* on markers of maternal nutritional health

To assess the nutritional status of the women in our cohort, and the effect of the intervention, we assayed the nutritional markers iron, ferritin, vitamin B12 and vitamin D and systemic micronutrients α(1)-acid glycoprotein, thyroglobulin, sTfR and RBP4. As serum and plasma ferritin correlated strongly at both week 4 (Pearson p <0.0001, r=0.97) and week 10 (Pearson p <0.0001, r=0.95) (Supplementary Figure 1a-b), we only report serum ferritin in all further analyses.

We first compared the concentrations of nutritional markers and micronutrients to the South African nutritional reference ranges for women that were available for iron, serum ferritin, vitamin B12, vitamin

D and thyroglobulin (du Plessis et al., 2016). No local reference ranges were available for α(1)-acid glycoprotein, sTfR and RBP4.

According to the South African nutritional reference ranges, all participants in the NM arm (100% (11/11) had optimal levels of iron at week 4 (pre-intervention), while the proportions of women with optimal iron levels in the *Mageu* arms (CPM 66.67% (8/12) and LCM 63.64% (7/11)) were lower at this timepoint (Fisher's exact p=0.085). No significant differences were observed amongst randomisation arms for the proportion of women with optimal levels of iron at week 10 (post-intervention) (NM 81.82% (9/11), CPM 75% (9/12) and LCM 90.91% (10/11), Fisher's exact p=0.853) (Table 6). Further, no significant differences were observed between proportions of optimal levels of ferritin, vitamin B12, vitamin D and Thyroglobulin amongst randomisation arms for at both weeks 4 and 10 (Table 6). Of note, more than half of the women in the cohort were vitamin D deficient, in line with previous reports of high proportions of vitamin D deficiency among South Africans (Durazo-Arvizu et al., 2014; Mogire et al., 2020; Soepnel et al., 2023; Velaphi et al., 2019). These results suggest that, apart from vitamin D, most women in the cohort had nutritional marker levels within the optimal range, and consumption of *Mageu* had no measurable impact on these levels. As part of the study, intensive diet assessments were conducted. However, the intervention period was too short to see any differences amongst the arms.

Table 6: Nutritional status of postpartum women grouped by randomisation arm at week 4 and 10

Proportion of women with optimal levels* [n/N (%)]	Week 4				Week 10			
	NM	CPM	LCM	P-value[a]	NM	CPM	LCM	P-value[b]
Iron	11/11 (100)	8/12 (66.67)	7/11 (63.64)	0.085	9/11 (81.82)	9/12 (75)	10/11 (90.91)	0.853
Ferritin	11/11 (100)	12/12 (100)	11/11 (100)	>0.999	11/11 (100)	12/12 (100)	11/11 (100)	>0.999
Vitamin B12	11/11 (100)	12/12 (100)	11/11 (100)	>0.999	11/11 (100)	12/12 (100)	11/11 (100)	>0.999
Vitamin D•	5/11 (45.45)	5/12 (41.67)	4/11 (36.36)	>0.999	6/11 (54.55)	5/12 (41.67)	6/11 (54.55)	>0.999
Thyroglobulin	11/11 (100)	11/12 (91.67)	10/11 (90.91)	>0.999	11/11 (100)	12/12 (100)	10/11 (90.91)	0.647

NM=No *Mageu*, CPM=Commercially Pasteurised *Mageu*, LCM=Live Culture *Mageu*
*South African reference ranges for females according to the 2nd Edition Ampath Desk Reference: Guide to Laboratory Tests (du Plessis et al., 2016). Optimal levels are as follows: iron (*9.0–30.4 μmol/L), ferritin (10–120 ng/mL), vitamin B12 (107–443 pmol/L), vitamin D (20–100 ng/mL), Thyroglobulin (3.5–77 ng/mL).*
•Insufficient sample for n=2, for Vitamin D quantification at week 10 for NM and CPM.
P-value represents Fisher's exact analysis at week 4 (a) and week 10 (b) between randomisation arms.

To assess whether *Mageu* consumption led to subtle changes in nutritional marker and micronutrient concentrations—rather than shifts in the proportion of women with optimal levels—we next compared individual changes in each marker between week 4 and week 10, stratified by randomisation arm (Figure 9). No significant differences were observed in the change of concentrations of nutritional markers and micronutrients between arms from week 4 to week 10 (Figure 9).

In supplementary analyses, we conducted a cross-sectional comparison at week 4 and week 10 of the concentrations of nutritional markers between randomisation arms (Supplementary Figure 2). Although NM users (median 17.60, [IQR 11-20.50] had a significantly higher concentration of iron at week 4 (baseline) than CPM (median 9.50, [IQR 7.25-14.40], p=0.0321) and LCM users (median 10, [IQR 7.70-18.30], iron levels did not differ significantly between groups at week 10 (median [IQR]: NM 16.20 [10.00-17.30], CPM 12.05 [6.68-16.15] and LCM 13.60 [9.70-15.60], p=0.5627) (Supplementary Figure 2a, i), indicating that CPM and LCM users experienced a slight increase in iron levels at week 10, but not sufficient enough to increase the median change in concentration for *Mageu* users in Figure 10a.

The NM arm had significantly higher levels of ferritin at week 10 (median 84 [IQR 78-115]) compared to CPM (median 51 [IQR 24.75-99]) and LCM users (median 47 [IQR 40-64]), Kruskal-Wallis p=0.0388) but the week 4 concentrations also tended to be higher in the NM arm at week4/baseline (median [IQR]: NM 86 [IQR 64-112], CPM 54.50 [IQR 24.25-89.25] and LCM 48 [IQR 34-75], Kruskal-Wallis p=0.0939), (Supplementary Figure 2b, j). No significant difference was observed in the concentration of vitamin B12, vitamin D, α(1)-acid glycoprotein, thyroglobulin, sTfR and RBP4 at either time point between randomisation arms (Supplementary Figure 2). Overall, these results suggest that *Mageu* consumption did not increase iron and ferritin levels in *Mageu* users.

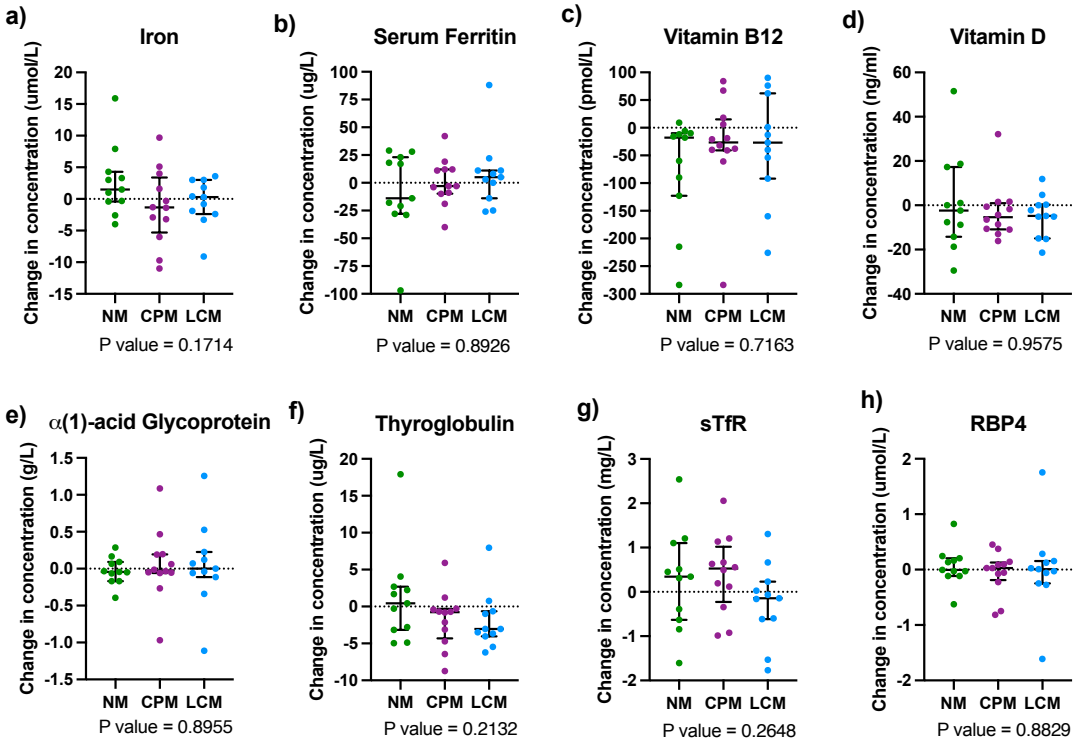

Figure 9. Change in concentration of nutritional markers from week 4 to week 10. The concentration for the nutritional markers a) iron, b) ferritin, c) Vitamin B12 and d) Vitamin D were quantified at NHLS. The concentration for the micronutrients e) α(1)-acid Glycoprotein, f) thyroglobulin, g) sTfR and h) RBP4 were quantified using a Q-plex micronutrient array assay. The change in concentration from week 4 to week 10 was calculated for each analyte. Graphs show the median and IQR. Comparisons were performed using

ANOVA for parametric data and Kruskal-Wallis for non-parametric data. NM=No *Mageu*, CPM=Commercially Pasteurised *Mageu*, LCM=Live Culture *Mageu*, sTfR=Soluble Transferrin Receptor, RBP4=Retinol Binding Protein 4, IQR=Interquartile Range, NHLS=National Health Laboratory Services, ANOVA=Analysis of Variance.

3.2.7 Effect of *Mageu* on systemic inflammatory markers

We assessed if *Mageu* consumption led to differential changes in inflammatory markers during and after the intervention period. We therefore compared the individual change in concentration of plasma IFN-γ, IL-1β, IL-6, TNF-α and C-reactive protein between paired samples from week 4 and week 10, and from week 4 and week 15, stratified by randomisation arm (Figure 10). While there were no significant differences in the change of inflammatory markers by randomisation arm from week 4 to week 10, there was a trend that IFN-γ (Figure 10f), IL-1β (Figure 10g) and IL-6 (Figure 10h) increased from weeks 4 and 15 in the NM arm, while there was very little change, or a decrease, in the CPM and LCM arms.

In supplementary analyses, we conducted a cross-sectional comparison of the concentrations of inflammatory markers between randomisation arms at weeks 4 (baseline), 10 and 15 but found no differences by randomisation arm at any time point (Supplementary Figure 3). CPM users had the lowest median concentration IFN-γ at week 10 (median 24.32 [IQR 17.68-31.92]) and week 15 (median 23.85 [IQR 19.50-29.57]) compared to NM at week 10 (median 29.17 [IQR 20.34-50.47]) and at week 15 (median 37.83 [IQR 19.30-48.07]), and LCM users at week 10 (median 32.34 [IQR 21-47.85]) and at week 15 (median 34.60 [IQR 18.63-43.17]), albeit this was not significant (Supplementary Figure 3f, k). NM users tended to have slightly higher concentrations of TNF-α at weeks 10 (median 17.19 [IQR 13.27-23.60]) and week 15 (median 20.36 [IQR 14.89-23.25]) compared to both CPM (week 10 median 15.14 [IQR 12.04-16.82], week 15 median 13.56 [IQR 11.80-17.76]) and LCM users (week 10 median 17.34 [IQR 13.06-20.80], week 15 median 15.05 [IQR 12.82-19.07]) (Supplementary Figure 3i, n). There were no differences between randomisation arms for IL-β at weeks 10 or 15 (Supplementary Figure 3g, l) or for C-reactive protein at week 10 (Supplementary Figure 3j). Overall, these results suggest that consumption of *Mageu* did not affect systemic inflammatory markers in South African postpartum women. Increased weight may have created an initially high inflammatory profile as seen with the elevated BMI in this cohort.

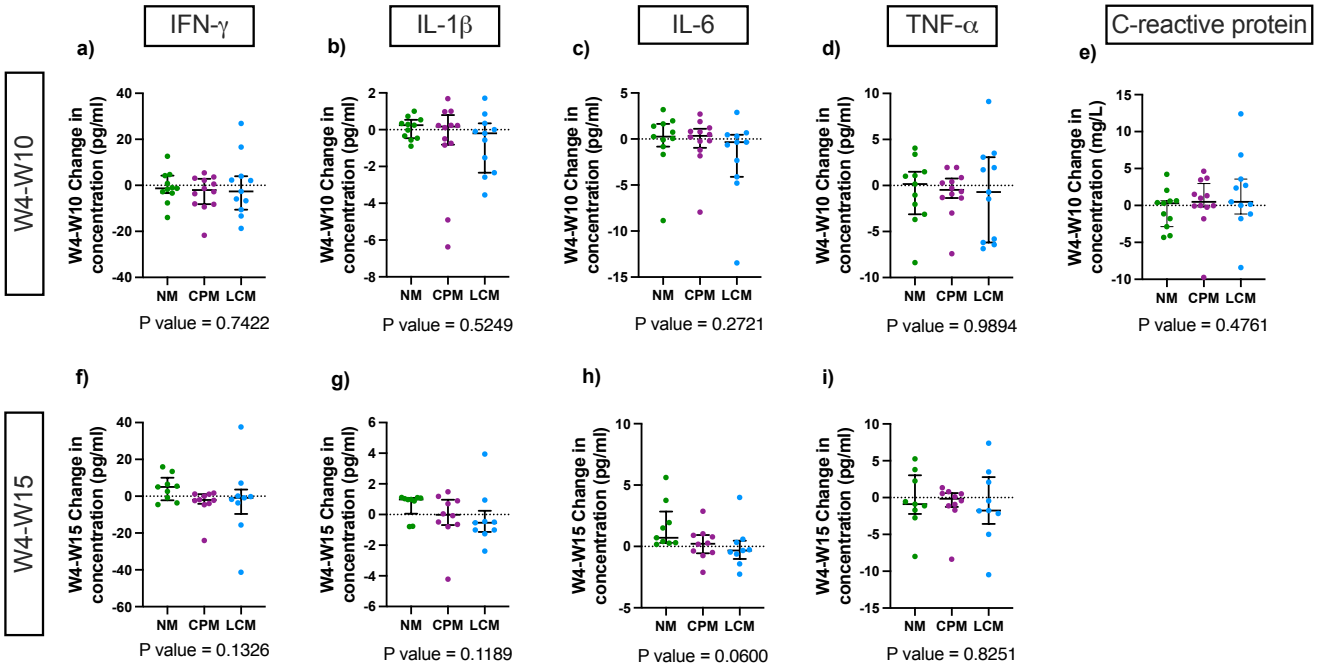

Figure 10. Change in concentration of systemic inflammatory markers during and after intervention. The concentration for the systemic inflammatory markers IFN-γ (a, f), IL-1β (b, g), IL-6 (c, h) and TNF-α (d, i) were quantified using Luminex® assay and C-reactive protein (e) was quantified using a Q-plex micronutrient array assay. a-e) Illustrated the change from W4-W10, and f-i) illustrated the change from W4-W15. Graphs show the median and IQR. Statistics was calculated using ANOVA for parametric data and Kruskal-Wallis for non-parametric data. NM=No *Mageu*, CPM=Commercially Pasteurised *Mageu*, LCM=Live Culture *Mageu,* IQR=Interquartile Range, ANOVA=Analysis of Variance.

3.2.8 Effect of *Mageu* consumption on intestinal inflammatory markers

We next assessed if *Mageu* consumption led to differential changes in the intestinal inflammatory marker lipocalin-2, myeloperoxidase and fecal calprotectin during or after the intervention period. We compared the individual change in concentration of each intestinal inflammatory marker between week 4 and week 10, and between week 4 and week 15 by randomisation arm (Figure 11). While there were no significant differences in the change of inflammatory markers by randomisation arm, fecal calprotectin tended to decrease in LCM user from weeks 4 to week 15, whereas they remained stable from weeks 4 to 15 in the other arms (Figure 11f).

In supplementary analyses, a cross-sectional comparison of the inflammatory markers between randomisation arms at weeks 4, 10 and 15 found no differences in the concentration of these markers (Supplementary Figure 4). LCM users had the highest median concentration of lipocalin-2 at week 10 (134.38 [IQR 93.77-168.36]) and week 15 (141.18 [IQR 80.05-154.94]) (Supplementary Figure 4d, g) versus CPM users (120.62 [IQR 100.47-132.64] and 135.50 [IQR 105.06-173.52], respectively) (Supplementary Figure 4d, g). However, NM users had slightly lower lipocalin-2 at baseline (median 99.40 [IQR 83.08-124.05]) compared to the other arms (median [IQR]: CPM 108.27 [90.86-153.53] and LCM 135.44 [97.91-153.69]) (Supplementary Figure 4a). Although the change between week 4

and 15 in fecal calprotectin tended to differ between arms, there were no differences in the concentrations of fecal calprotectin at week 15 between arms (NM median 152.39 [IQR 45.87-330.98], CPM median 69.68 [IQR 50.36-233.68] and LCM median 92.73 [IQR 26.78-357.75]) (Supplementary Figure 4i). However, sample size for stool collected at all visits were limited to provide sufficient data points for analysis.

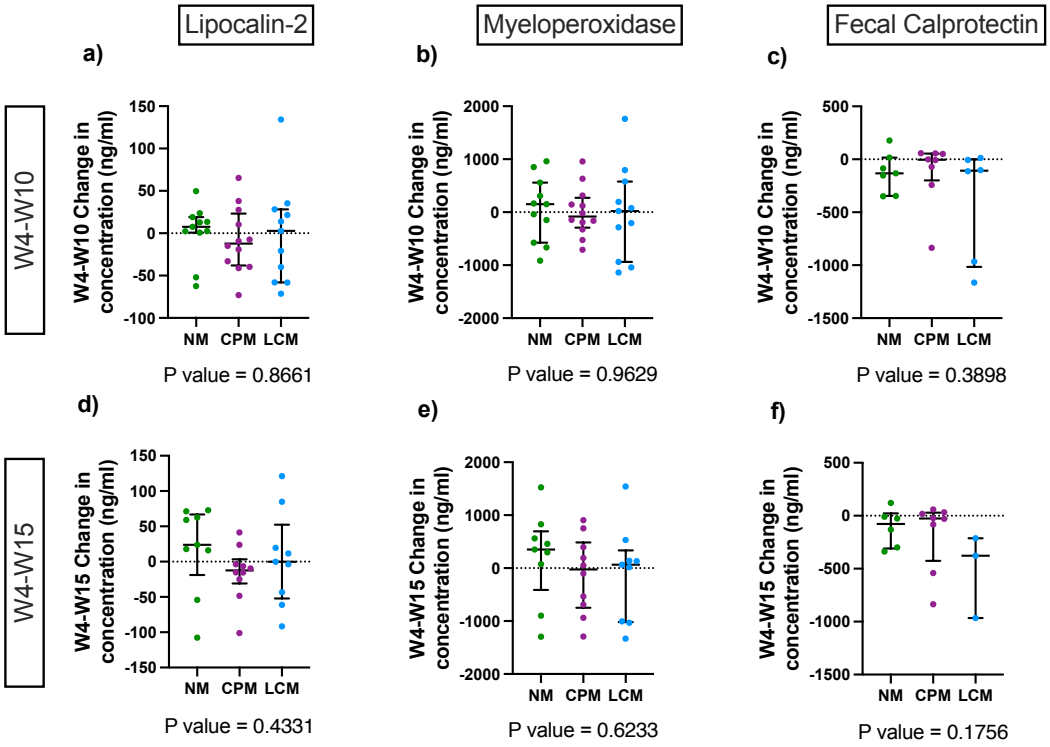

Figure 11. Change in concentration of intestinal inflammatory markers during and after intervention. The concentration for the intestinal inflammatory markers Lipocalin-2 (a, d), Myeloperoxidase (b, e) and Fecal Calprotectin (c, f) were quantified using ELISA. a-c) Illustrated the change from W4-W10, and d-f) illustrated the change from W4-W15. Graphs show the median and IQR. Statistics was calculated using ANOVA for parametric data and Kruskal-Wallis for non-parametric data. NM=No *Mageu*, CPM=Commercially Pasteurised *Mageu*, LCM=Live Culture *Mageu,* IQR=Interquartile Range, ELISA= Enzyme-Linked Immunosorbent Assay, ANOVA=Analysis of Variance.

3.3 The relationship between inflammation, nutritional markers and BMI in postpartum women

3.3.1 Correlations between concentrations of inflammatory and nutritional markers measured at week 4, 10 and 15

We evaluated correlations of concentrations of nutritional and inflammatory markers for all analytes at each time point in order to assess whether individual nutritional and inflammatory profiles are stable over time, or shift in response to the intervention. Strong correlations suggest that cytokine levels are intrinsically regulated, while weaker correlations may indicate intervention or other exogenous effects.

Significant positive correlations were observed between concentrations of all nutritional markers and micronutrients measured at weeks 4 and 10 (Figure 12). The strongest correlations were observed for

ferritin (Pearson r=0.72, p<0.0001), vitamin B12 (Pearson r=0.79, p<0.0001), vitamin D (Pearson r=0.80, p<0.0001), thyroglobulin (Pearson r=0.76, p<0.0001) and sTfR (Pearson r=0.77, p<0.0001), (Figure 12b, c, d, f, g,). More moderate correlation coefficients were observed for iron (Pearson r=0.52, p=0.0016) and RBP4 (Pearson r=0. 55, p=0.0007) (Figure 12a, h). A weak, positive correlation was observed for α(1)-acid glycoprotein (Pearson r=0. 37, p=0.030) (Figure 12e).

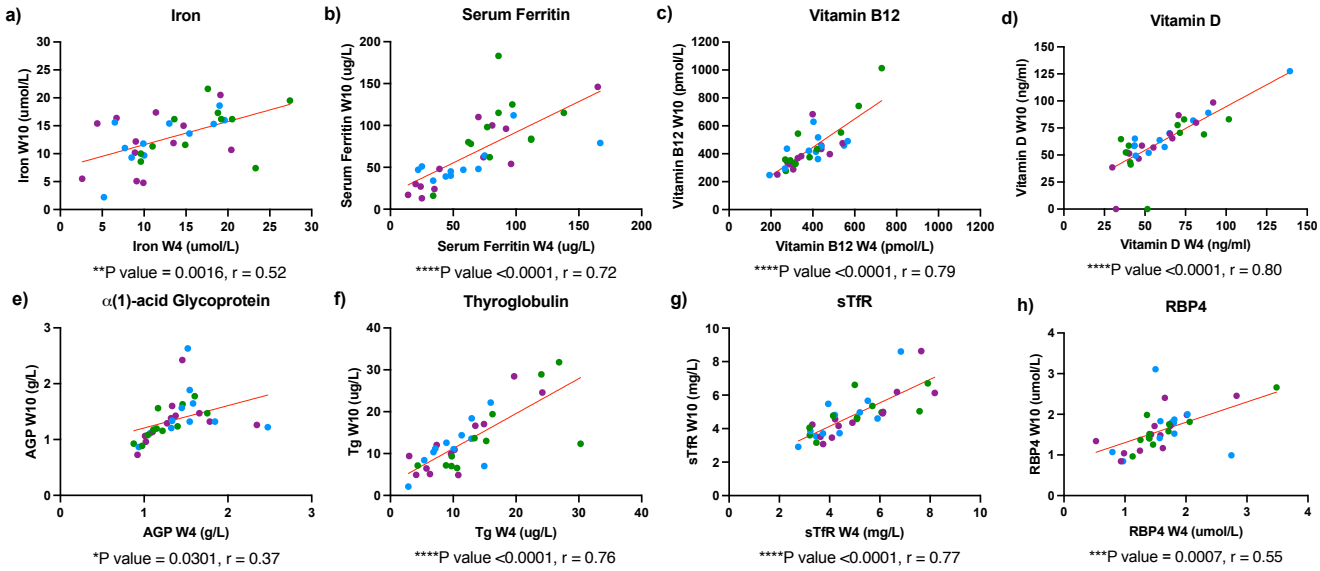

Figure 12: Relationship between week 4 to week 10 concentrations of nutritional markers. The concentration for the nutritional markers Iron (a), Serum Ferritin (b), Vitamin B12 (c) and Vitamin D (d) were quantified at NHLS. The concentration for the micronutrients α(1)-acid glycoprotein (e), Thyroglobulin (f), sTfR (g) and RBP4 (h) were quantified using a Q-plex micronutrient array assay. Statistics was calculated using Pearson correlation. sTfR=Soluble Transferrin Receptor, RBP4=Retinol Binding Protein 4. Colour-coded points represent randomisation arms (Green=NM, Purple=CPM and Blue=LCM). NM=No *Mageu*, CPM=Commercially Pasteurised *Mageu*, LCM=Live Culture *Mageu,* NHLS=National Health Laboratory Services.

Similarly, strong positive correlations were observed for all systemic inflammatory marker concentrations between week 4 and week 10, and between week 4 and week 15, (Figure 13). IFN-γ (Pearson r=0.85, p<0.0001), IL-1β (Pearson r=0.75, p<0.0001) and IL-6 (Pearson r=0.77, p<0.0001) showed the strongest correlation between weeks 4 and 10 (Figure 13a, b, c) and between weeks 4 and 15 (Pearson r=0.78, p<0.0001; Pearson r=0.82, p<0.0001; Pearson r=0.86, p<0.0001, respectively), (Figure 12f, g, h). Moderate correlations were observed for TNF-α (Pearson r=0.69, p<0.0001) and C-reactive protein (Pearson r=0.47, p=0.0050) concentrations measured at week 4 and 10 (Figure 13d, e), and a strong correlation was also observed for TNF-α between week 4 and 15 (Pearson r=0.77, p<0.0001) (Figure 13i).

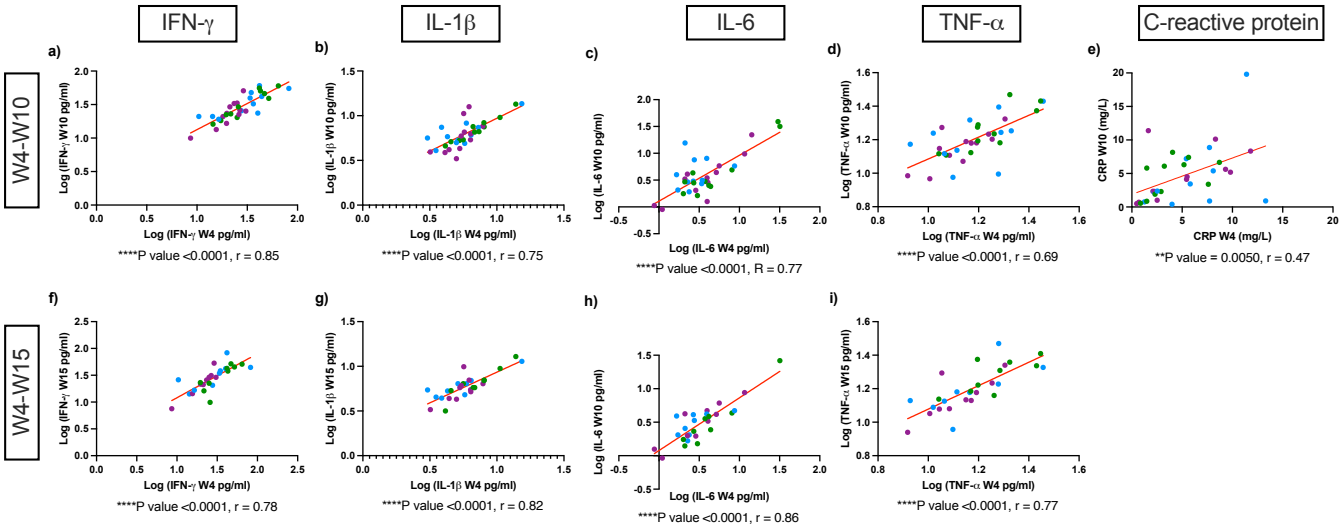

Figure 13: Relationship between week 4 to week 10 and week 4 to week 15 concentrations of systemic inflammatory markers. The concentration for the systemic inflammatory markers IFN-γ (a, f), IL-1β (b, g), IL-6 (c, h) and TNF-α (d, i) were quantified using Luminex® assay, while C-reactive protein (e) was quantified using Q-plex micronutrient array assay at weeks 4, 10 and 15. The $\log_{10}$ concentration of each marker was calculated and correlations between week 4 and week 10 (top) and week 4 and week 15 (bottom) were assessed. Statistics was calculated using Pearson correlation. Colour-coded points represent randomisation arms (Green=NM, Purple=CPM and Blue=LCM). NM=No *Mageu*, CPM=Commercially Pasteurised *Mageu*, LCM=Live Culture *Mageu.*

For the intestinal inflammatory markers, a weak positive correlation was observed between week 4 and week 10 concentrations for lipocalin-2 (Pearson r=0.42, p=0.0124, Figure 14a), myeloperoxidase (Pearson r=0.42, p=0.0129, Figure 14b) and fecal calprotectin (Pearson r=0.47, p=0.0377 Figure 14c). No relationship was observed between the week 4 and week 15 concentrations for any of these markers (Figure 14d, e, f).

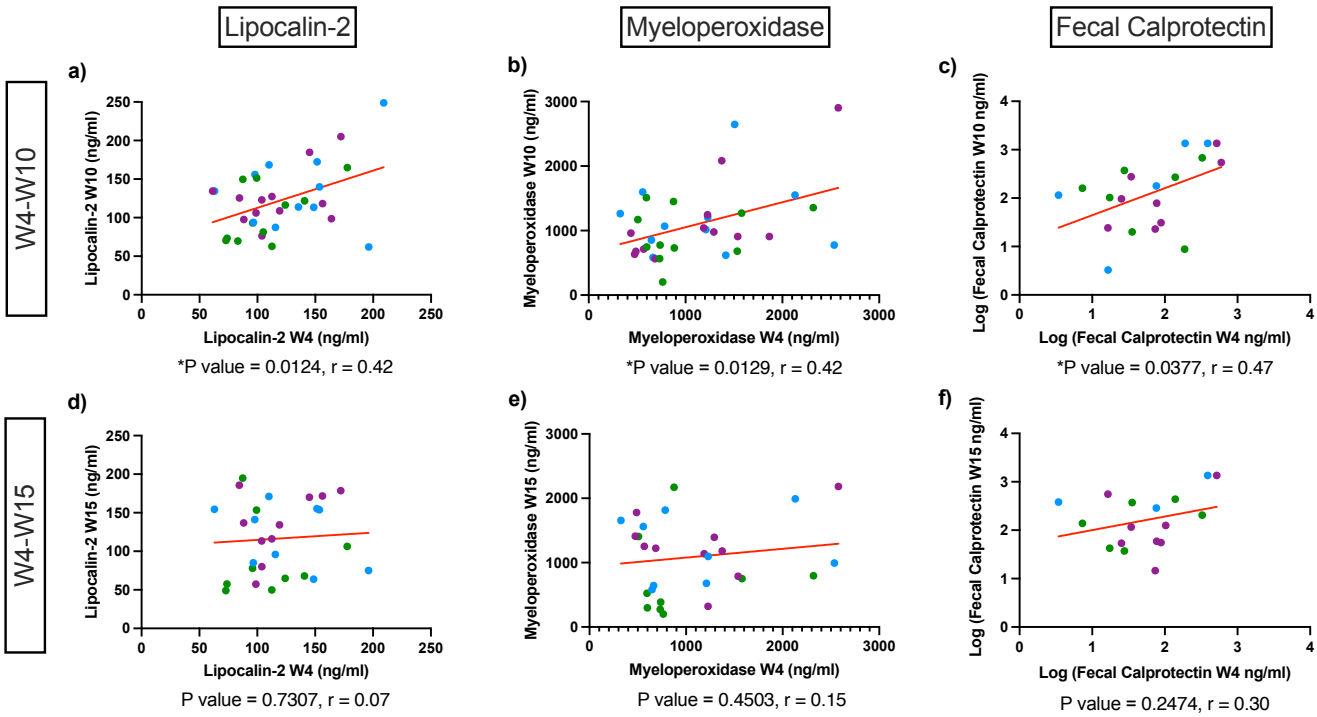

Figure 14: Relationship between week 4 to week 10 and week 4 to week 15 concentrations of intestinal inflammatory markers. The concentration for the intestinal inflammatory markers Lipocalin-2 (a, d), Myeloperoxidase (b, e) and Fecal Calprotectin (c, f) were quantified using ELISA at weeks 4, 10 and 15, and correlations between week 4 and week 10 (top) and week 4 and week 15 (bottom) were assessed. Statistics was calculated using Pearson correlation. Colour-coded points represent randomisation arms (Green=NM, Purple=CPM and Blue=LCM), ELISA= Enzyme-Linked Immunosorbent Assay, NM=No *Mageu*, CPM=Commercially Pasteurised *Mageu*, LCM=Live Culture *Mageu.*

In summary, these results suggest that participant was the strongest predictors of nutritional markers and micronutrient levels measured post-intervention, and that the intervention had little influence on ferritin, vitamin B12, vitamin D, thyroglobulin and sTfR levels. A similar pattern was observed for the systemic inflammatory markers (IFN-γ, IL-1β, Il-6, TNF-α). The measured concentrations of intestinal inflammatory markers (lipocalin-2, myeloperoxidase and fecal calprotectin) showed little correlation between week 4 and week 10, and no correlation between week 4 and week 15, this might suggest an exogenous modulation of these markers. However, the sample size was insufficient to compare correlations between randomisation arms, leaving the question of whether the intervention had an effect unresolved.

3.3.2 Correlations between quantities of inflammatory and nutritional markers and BMI

A correlation matrix was used to evaluate the relationship between BMI, inflammatory and nutritional markers at week 4 to assess body composition, immune and nutrient interactions (Figure 15). This analysis was limited to baseline measurements, as the previous section demonstrated correlations across time points, and the analysis was conducted independently of intervention assignment.

At week 4, BMI correlated significantly with RBP4 (Pearson r=0.61, p=0.0090) but not with any other inflammatory or nutritional markers (Figure 15), suggesting that higher adiposity may increase RBP4 production, potentially reflecting early metabolic disturbance or altered vitamin A handling.

RBP4 negatively correlated with α(1)-acid glycoprotein (Pearson r=-0.52, p=0.0341), lipocalin-2 (Pearson r=-0.61, p=0.0097) and vitamin D (Pearson r=-0.61, p=0.0099). A strong negative correlation was observed between iron and α(1)-acid glycoprotein (Pearson r=-0.92, $p=1.47x10^{-7}$) and between iron and C-reactive protein (Pearson r=-0.78, p=0.0002). Iron also significantly correlated with lipocalin-2 (Pearson r=-0.50, p=0.0411), an intestinal inflammatory marker, and vitamin D (Pearson r=-0.49, p=0.0461). These results suggest that systemic and intestinal inflammation (reflected by α(1)-acid glycoprotein, C-reactive protein, and lipocalin-2) is strongly associated with lower concentrations of iron, RBP4, and vitamin D.

As expected, sTfR correlated negatively with iron (Pearson r=-0.49, p=0.0466) and ferritin (Pearson r=-0.64, p=0.0059) concentrations. Since ferritin correlated positively with the systemic inflammatory markers IFN-γ (Pearson r=0.57, p=0.0158) and TNF-α (Pearson r=0.52, p=0.0341), this suggests that ferritin may be reflective of acute phase reaction rather than iron deficiency in this instance. The concentrations of systemic inflammatory markers strongly correlated with each other. As such, IFN-γ strongly correlated with TNF-α (Pearson r=0.93, $p=6.38x10^{-8}$) and IL-1β (Pearson r=0.87, $p=4.83x10^{-6}$). The same was observed for TNF-α with IL-1β (Pearson r=0.93, $p=5.09x10^{-8}$) and a moderate correlation was observed between TNF-α and IL-6 (Pearson r=0.58, p=0.0137). Finally, systemic inflammatory markers IL-6 and IL-1β strongly positively correlated with each other (Pearson r=0.71, p=0.0013) (Figure15). The persistent correlation of these markers could be indicative of chronic low-grade inflammation in the cohort. Fecal calprotectin negatively correlated with IFN-γ (Pearson r=-0.56, p=0.0184) and myeloperoxidase (Pearson r=-0.50, p=0.0399). Perhaps reflective of expression via different stimuli during inflammation (de Moura Gondim Prata et al., 2016).

α(1)-acid glycoprotein correlated positively with C-reactive protein (Pearson r=0.88, $p=2.94x10^{-6}$), a common non-specific marker of inflammation. α(1)-acid glycoprotein also moderately and positively correlated with lipocalin-2 (Pearson r=0.63, p=0.0068), a marker of intestinal inflammation. This suggests that the state of general systemic inflammation (α(1)-acid glycoprotein, C-reactive protein) is likely driven in part by intestinal inflammation (α(1)-acid glycoprotein, lipocalin-2). In agreement, C-reactive protein positively correlated with the intestinal inflammatory markers lipocalin-2 (Pearson r=0.58, p=0.0148) and myeloperoxidase (Pearson r=0.51, p=0.0376).

Vitamin D correlated strongly with C-reactive protein (Pearson r=0.69, p=0.0020), lipocalin-2 (Pearson r=0.74, p=0.0008), myeloperoxidase (Pearson r=0.69, p=0.0020), α(1)-acid glycoprotein (Pearson r=0.59, p= 0.0129) and ferritin (Pearson r=0.49, p= 0.0441). These strong positive correlations between vitamin D and several inflammatory and iron-related markers are biologically unexpected, as

inflammation is usually associated with lower vitamin D status (Azizieh et al., 2016; Etminan et al., 2020; Soepnel et al., 2023; Young et al., 2023).

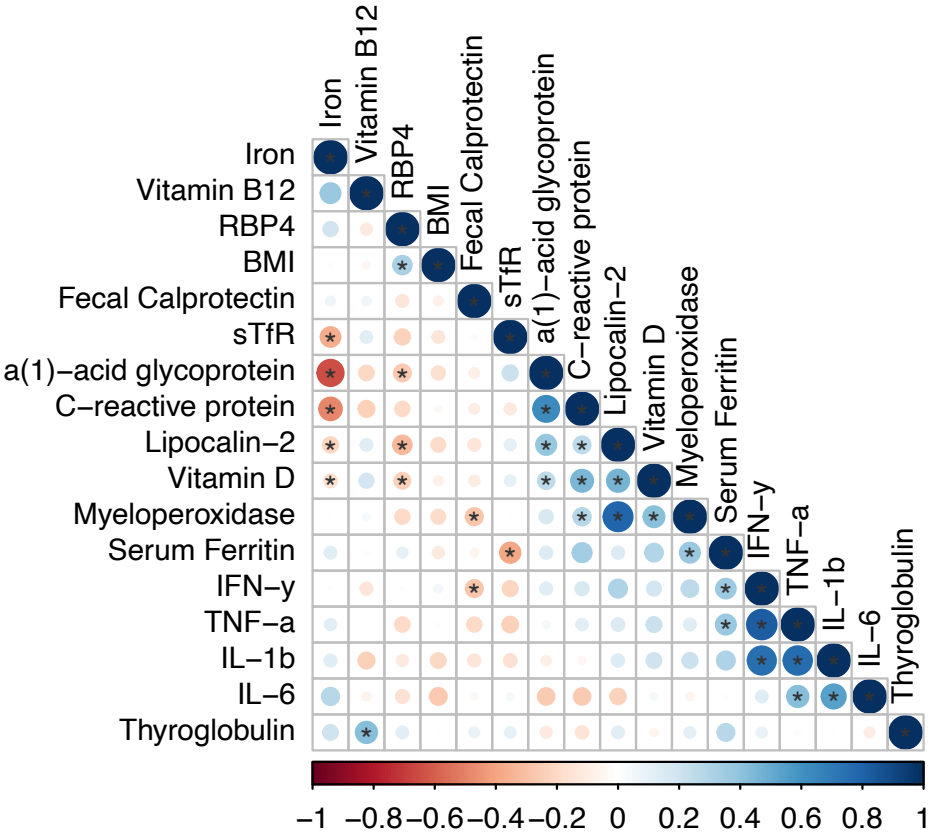

Figure 15: Correlations between BMI and inflammatory and nutritional markers. Intestinal inflammatory markers (Lipocalin-2, Myeloperoxidase and Fecal Calprotectin) were quantified using ELISA assay. Systemic inflammatory markers (TNF-α IFN-γ IL-6 and IL-1β) were quantified using Luminex® assay. Nutritional markers (Iron, Serum Ferritin, Vitamin B12 and Vitamin D) were quantified at NHLS. Micronutrients (α(1)-acid glycoprotein, C-reactive protein, RBP4, sTfR and Thyroglobulin) were quantified using a Q-plex Micronutrient array assay. Pearson correlations are shown. The size of the circle indicates the strength of the correlation, the star indicates the statistical significance and the colour indicates correlation coefficient. sTfR=Soluble Transferrin Receptor, RBP4=Retinol Binding Protein 4, BMI=Body Mass Index, ELISA= Enzyme-Linked Immunosorbent Assay, NHLS=National Health Laboratory Services.

3.3.3 Integrating nutritional and inflammatory responses to the *Mageu* intervention

We evaluated the impact of the intervention on overall change in all nutritional and inflammatory parameters between week 4 and 10 using PCoA plots (Euclidian distances) (Figure 16). There was no significant difference in the overall nutritional profile between the three arms (p=0.471, PERMANOVA), with a very small effect size (R^2=4.63%), indicating that randomisation arm does not strongly explain the variance (PC1=89.91% and PC2=9.08%) in the nutritional data (Figure 16a). There was no significant difference between randomisation arms for the change of systemic (p=0.790, R^2=3.53%, PERMANOVA) (Figure 16b) and intestinal (p=0.946, R^2=0.98%, PERMANOVA) inflammatory markers (Figure 16c) from week 4 to 10. For systemic inflammatory markers, PC1

accounted for 42.92% of the variation, while PC2 accounted for 29.9%. For intestinal inflammatory markers, PC1 accounted for 83.82% of the variation, PC2 accounted for 16.18%. Overall, this indicates that randomisation arm did not strongly explain the variance in inflammatory markers (Figure 16b, c). When including all the datasets into the analysis (Figure 16d), PC1 and PC2 accounted overall for 98% of the variation, with a very small effect size of R^2=1.05%. Again, no significant heterogeneity of dispersion was observed between randomisation arms (p=0.949, PERMANOVA). The lack of clustering by randomisation arm highlights biomarker responses were similar across the three arms and did not create large differences in the measured biomarkers (Figure 16).

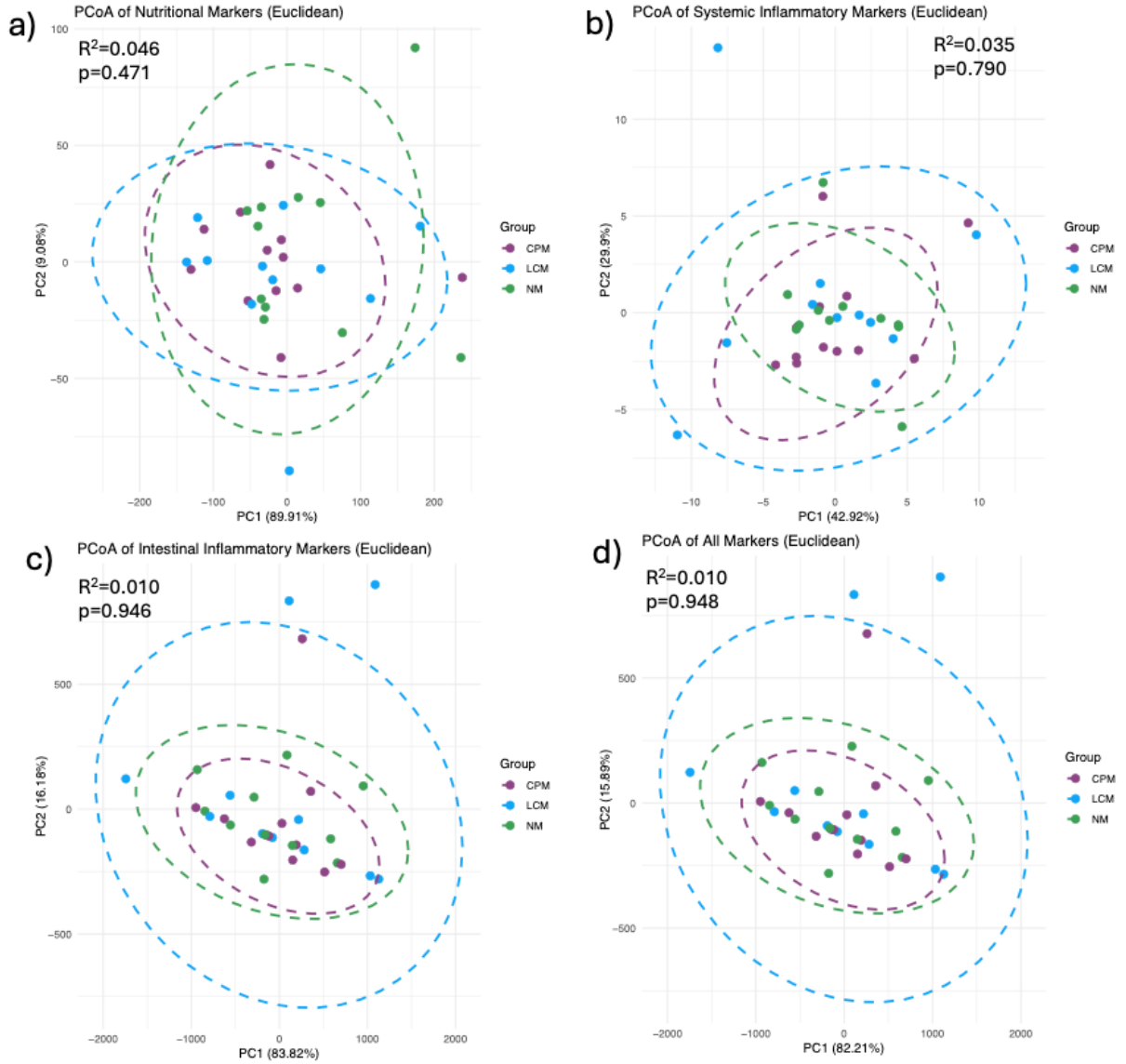

Figure 16: Change in overall nutritional and inflammatory markers from week 4 to 10. Principal coordinate analysis (PCoA) of Euclidian distances using a) nutritional markers (Iron, Serum Ferritin, Vitamin B12 and Vitamin D) were quantified at NHLS, micronutrients (AGP, sTfR, Tg and RBP4) were quantified using a Q-plex micronutrient array assay, b) systemic inflammatory markers (TNF-α, IFN-γ, IL-6 and IL-1β) were quantified using Luminex® assay, while systemic CRP was quantified using a Q-plex micronutrient array assay, c) intestinal inflammatory markers (Lipocalin-2, Myeloperoxidase and Fecal Calprotectin) were quantified using ELISA assay, d) all markers. The change in concentration was calculated

at week 4 to week 10. NM=No *Mageu*, CPM=Commercially Pasteurised *Mageu*, LCM=Live Culture *Mageu.* Tg=Thyroglobulin, AGP=α(1)-acid glycoprotein, CRP=C-reactive protein, sTfR=Soluble Transferrin Receptor, RBP4=Retinol Binding Protein 4, ELISA= Enzyme-Linked Immunosorbent Assay, NHLS=National Health Laboratory Services.

Finally, we constructed heatmaps based on changes in biomarker concentrations between weeks 4 and 10, and between weeks 4 and 15, to assess whether participants clustered by assigned intervention (Figure 17). No distinct clustering was observed by randomisation arm when assessing the log-change in concentrations of inflammatory and nutritional markers between week 4 and week 10, but there seemed to be a general decrease in concentrations of inflammatory markers (Figure 17a). When assessing the change from week 4 to week 15, there appeared to be a general increase in inflammatory marker concentrations (Figure 17b).

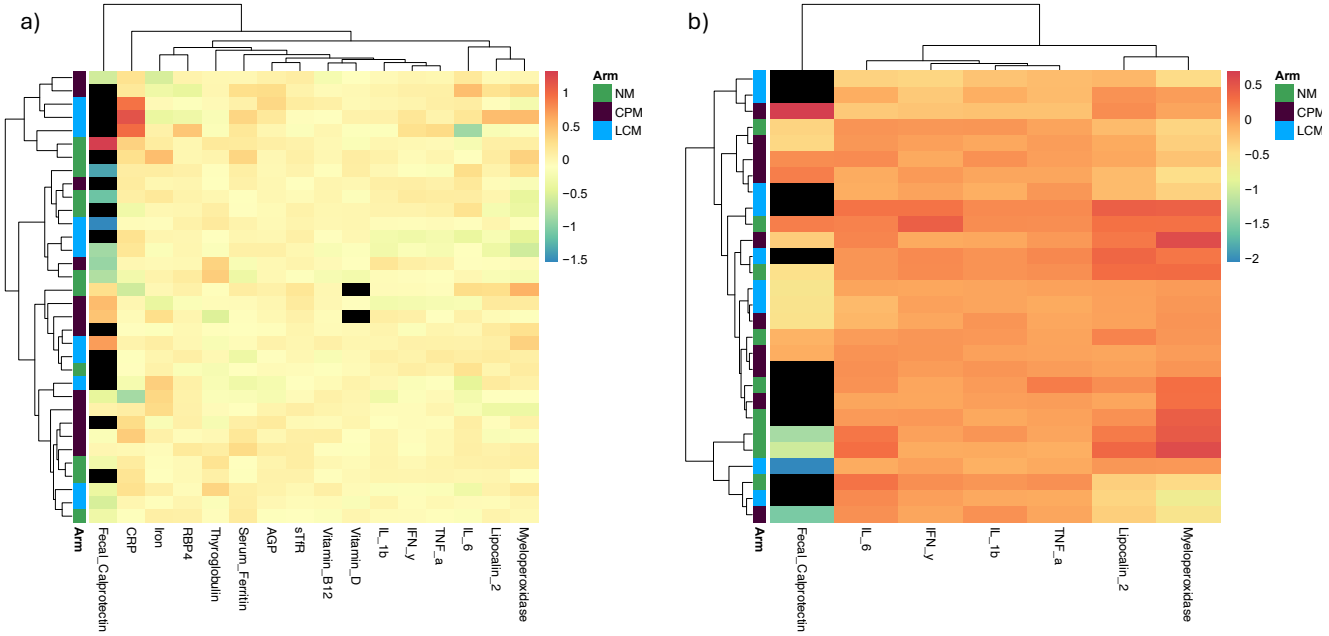

Figure 17: Change in concentration of inflammatory and nutritional markers. Intestinal inflammatory markers (Lipocalin-2, Myeloperoxidase and Fecal Calprotectin) were quantified using ELISA assay, systemic inflammatory markers (IFN-γ, IL-1β, IL-6 and TNF-α) were quantified using Luminex® assay at weeks 4, 10 and 15, nutritional markers (Iron, Serum Ferritin, Vitamin B12 and Vitamin D) were quantified at NHLS, inflammatory marker CRP and micronutrients (Thyroglobulin, AGP, sTfR and RBP4) were quantified using a Q-plex micronutrient array assay at weeks 4 and 10. The concentration of the inflammatory and nutritional markers was log transformed, and the change calculated from a) week 4 to week 10 and b) week 4 to week 15. Black indicates missing data. NM=No *Mageu*, CPM=Commercially Pasteurised *Mageu*, LCM=Live Culture *Mageu,* AGP=α(1)-acid Glycoprotein, CRP=C-reactive protein, sTfR=Soluble Transferrin Receptor, RBP4=Retinol Binding Protein 4, ELISA= Enzyme-Linked Immunosorbent Assay, NHLS=National Health Laboratory Services.

The general decrease in inflammatory markers in the early postpartum period, between week 4 and week 10, may reflect the natural resolution of inflammation related to delivery, irrespective of the nutritional intervention. The subsequent increase in inflammation between week 4 and week 15 could reflect delayed immune activation.

CHAPTER 4
DISCUSSION AND CONCLUSION

4.1 Discussion

As a first step, we analysed the nutritional composition of the LCM product at an external laboratory and compared it with the store-bought CPM product, based on the nutritional content specified by the manufacturer. Overall, the LCM had higher levels of energy, protein, carbohydrate, sugar, fibre and sodium, while the mass-produced commercially available *Mageu* (CPM) contained more fat. Published reports show considerable variability in the nutritional composition of *Mageu* products, likely reflecting differences in formulation and manufacturer, and fortification. For example, a study evaluating the nutritional composition of another commercially available *Mahewu* (MNANDI *Amahewu*, RCL Foods, South Africa) reported a fat content of <0.1g, 0.7g protein, 6.0g carbohydrates and 122kJ energy per liter (Moiseenko et al., 2021), contrasting with the higher macronutrient values in our CPM. In line with our findings, other studies reported that traditionally produced *Mageu* contains a higher nutritional content than the commercially available product (Awobusuyi & Siwela, 2019; Fadahunsi & Soremekun, 2017; Mafukata et al., 2024; Moiseenko et al., 2021; Oyeyinka et al., 2021; Qaku et al., 2020). Fortification of *Mageu* seems to further improve nutritional composition. As such, Mafukata et al., 2024 found that 100% sorghum *Mahewu* contained 0.85% fat, 1.67 % fibre, 11.11% protein and 32.86 % carbohydrate, all of which significantly increased by fortification with *Moringa oleifera* leaf powder. Similarly, a study evaluating the nutritional properties of *Amahewu* fortified with Provitamin A and Bambara (*Vigna Subterranea*) flour reported 82.8g carbohydrates per 100g, 14g protein/100g, and 0.05g fat/100g. The reported mean values were significantly lower than the enriched Provitamin A with Bambara flour *Mahewu* (Awobusuyi & Siwela, 2019; Oyeyinka et al., 2021). Overall, our findings and these results suggest that that while traditionally prepared *Mageu* products may offer higher nutritional content than some commercial versions, fortification strategies further enhance their nutritional profile.

Next, we analysed the nutritional composition of the LCM product at an external laboratory and compared it with the store-bought CPM product, based on the nutritional content specified by the manufacturer. Per a 500ml serving of *Mageu*, the LCM had a higher nutritional content than the mass-produced commercially available *Mageu* (CPM). Energy (kJ), protein (g), carbohydrates (g), sugar (g), fibre (g) and sodium (mg) in the LCM product were higher than the CPM product. However, the fat content of CPM was higher than the LCM product. Pasteurisation of the CPM product may have killed microorganisms responsible for lipid degradation and utilisation. It can also decrease lipase activity by microorganisms that produce lipase (Deeth, 2021; Rabbani et al., 2025). The high fat content can impact on weight, which may be the manufacturer's decision to address product enrichment. In the control sample (100% sorghum *Mahewu*) of a study evaluating the nutritional composition of *Mahewu* enriched with *Moringa oleifera* leaf powder (MOLP) reported a mean fat percentage (%) of 0.85 (SD=0.28),

fibre % of 1.67±0.58, protein % of 11.11±0.23 and carbohydrate % of 32.86±0.70. The reported mean values were significantly lower than the enriched *MOLP Mahewu* (Mafukata et al., 2024). A study evaluating the nutritional content of *Mahewu* reported the following composition percentages: 9.21% protein, 2.02% fat, 0.83% fibre and 63.01% carbohydrates, with a 0.051±0.00mg/kg of sodium (Fadahunsi & Soremekun, 2017; Oyeyinka et al., 2021). A study evaluating the nutritional properties of *Amahewu* fortified with Provitamin A and Bambara (*Vigna Subterranea*) flour, reported the mean nutritional composition (g/100g) in the control sample containing 82.8±1.45 carbohydrates, 14±0.14 protein, 0.05±0.04 fat and 74mg/kg sodium. The reported mean values were significantly lower than the enriched Provitamin A with Bambara flour *Mahewu* (Awobusuyi & Siwela, 2019; Oyeyinka et al., 2021). These results suggest that fortification is capable of improving the nutritional composition of *Mageu*. Another study evaluating the nutritional composition of commercially available *Mahewu* (MNANDI *Amahewu*, RCL Foods, South Africa), reported a fat content of <0.1g, 0.7g protein, 6.0g carbohydrates and 122kJ energy in a 1L sample (Moiseenko et al., 2021). The *Mahewu* product (Moiseenko et al., 2021) in this study was from a different manufacturer to our store-bought *Mageu*, perhaps explaining the difference in nutritional composition to our CPM product. Overall, the traditionally produced *Mageu*, whether fortified or not, contains a higher nutritional content than the commercially available product (Awobusuyi & Siwela, 2019; Fadahunsi & Soremekun, 2017; Mafukata et al., 2024; Moiseenko et al., 2021; Oyeyinka et al., 2021; Qaku et al., 2020).

Next, we conducted comprehensive quality control assessments to ensure the safety of the administered *Mageu* products. Food safety is a continuous concern to public health, especially in the preparation and storage of traditional fermented foods prepared at a household level (Anyogu et al., 2021; Tan et al., 2024). Because these foods rely on spontaneous fermentation or backslopping, there is an increased risk of introducing food spoilage or pathogenic microorganisms (Anyogu et al., 2021). Heat treatment or pasteurisation and low pH (~3.5 to 4.0) has been the standard practice in inhibiting the growth of food spoilage microorganisms, such as *E. coli* and *Clostridium* spp. in *Mageu* (Byaruhanga et al., 1999; Daji et al., 2022; Holzapfel & Taljaard, 2004; Moiseenko et al., 2021; Oyeyinka et al., 2021; Tan et al., 2024). Commercial pasteurised *Mageu* undergoes pasteurisation to extend the shelf-life of the product and reduce food spoilage microorganisms (Dimidi et al., 2019; Gadaga et al., 1999). We therefore conducted bacterial culture screening on both the CPM and LCM product to ensure its safety. Both *Mageu* product types were free from *E. coli.* LCM had a higher percentage of batches with *Clostridium* and non-*E. coli* species, such as *Bacillus* spp. and *Klebsiella* spp., present compared to the CPM. The CPM and LCM products were bottled and produced in a controlled laboratory setting; therefore, the risk of introducing contaminating food spoilage microorganisms was low. Thus, the difference observed may be a result of the lack of pasteurisation of the LCM, as seen in CPM (Byaruhanga et al., 1999; Holzapfel & Taljaard, 2004; Oyeyinka et al., 2021). Further, the *Clostridium spp.* identified were *C. beijerinckii*, a non-pathogenic spore-forming bacterium that ferments a wide variety of carbohydrates

(Mete et al., 2024) and *C. manihotivorum*, another non-pathogenic spore-former with capacity to digest raw starches (Cheawchanlertfa et al., 2020).

The other bacterial species that were identified in both CPM and LCM products were similar to those previously described in literature to be present in *Mageu*. A study on the identification of lactic acid bacteria in *Emasi* and *Emahewu* found that *Lactobacillus* spp. and *Leuconostoc lactis* were the most common microorganisms identified in *Emahewu* (Simatende et al., 2019). Another study evaluating microbial communities in *Mahewu* isolated the following enteric bacteria: *Klebsiella pneumoniae, E. coli, Enterobactor cloacae and Enterobactor sakazakii,* and the LAB *Leuconostoc lactis* from *Mahewu* (Simango, 2002). Our data is consistent with common microorganisms isolated from *Mahewu*, and the differences in organism isolated may be due to the environment, starter culture used, preparation methods and the use of heat sterilisation (Anyogu et al., 2021; Daji et al., 2022; Dimidi et al., 2019; Gadaga et al., 1999; Rezac et al., 2018). *Leuconostoc lactis* is a common organism involved in fermentation, thus explaining its presence in our *Mageu* products (Daji et al., 2022; Franz et al., 2014; Paul et al., 2023). A study evaluating the physicochemical properties and bacterial community of *Mahewu* prepared using white and yellow maize with different inocula isolated *Bacillus*, *Clostridium* and *Leuconostoc* spp. from both maize types (Daji et al., 2022). Additionally, in a study evaluating the composition and origin of fermentation microbiota of *Mahewu*, they found that a *Mahewu* produced with a 5-strain inoculum consisting of lactobacilli, *Klebsiella pneumoniae* and *Cronobacter sakazakii* outcompeted *Enterobacteriaceae* and *Bacillus* spp. found in the millet malt microbiota samples within the first 24 hours (Pswarayi & Gänzle, 2019). The pilot RCT of the parent study MaMISA (HREC 004/2022), compared the bacterial community between live culture *Mageu* (LCM), prepared in a controlled laboratory setting and store-bought *Mageu* (SBM) to determine whether pasteurisation reduced microbial community in the SBM (Happel et al., 2025). They found that LCM had a higher relative abundance of bacterial taxa belonging to *Kosakonia cowanii, Bacillus* spp., *Enterobactor kobei* and *Enterobactor hormaechi*, *Lactococcus lactis, Leuconostoc citreum and Leuconostoc pseudomesenteroides*, compared to SBM (Happel et al., 2025). These results suggest that the LCM has more similarity in microbial community to the traditionally produced *Mageu* than the industrialised version that underwent extreme heat sterilisation (Daji et al., 2022; Happel et al., 2025; Pswarayi & Gänzle, 2019). This may provide evidence that the commercially available *Mageu* requires fortification with probiotic-like bacteria to fully exert the *Mageu* health benefits (Abid et al., 2022; Das et al., 2022; Ibrahim et al., 2023; Savaiano & Hutkins, 2021). Overall, this suggests that the *Mageu* products manufactured during the trial were safe for consumption, and contained organisms commonly found in fermented grain products.

We next assessed the impact of pasteurization on bacterial load of *Mageu* products. LAB are the most commonly used organisms for fermented foods (Rezac et al., 2018). Traditionally produced and commercially produced fermented foods may contain probiotic-like bacteria, such as *Lactobacillus* spp.

(Daji et al., 2022; Rezac et al., 2018). However, commercial foods and beverages developed through fermentation processes may not necessarily contain live microorganism, due to heat-treatment, distillation or filtration that is used to enhance food safety and extend shelf-life (Rezac et al., 2018). These non-viable microorganisms, termed postbiotics and paraprobiotics, may still exert health benefits to the host by means of bioactive compounds (Castellone et al., 2021; Conte et al., 2021; Cuevas-González et al., 2020; Sugawara et al., 2016).

Hence, we aimed to determine the total bacterial load of both the CPM and LCM products through 16S rRNA gene copy number quantification. This method does not distinguish between DNA from viable versus non-viable organisms but rather confirms quantifies total bacterial DNA. CPM had a significantly lower bacterial load (copy number/µL) compared to LCM, as expected to be observed in a food product that underwent pasteurisation. Pasteurisation is known to decrease total bacterial load (Egbere et al., 2009; Mauer, 2003) and affect DNA integrity and degradation (Bitskinashvili et al., 2019; Liao & Liu, 2020). A study assessing yield, purity and integrity of DNA extracted from Ultra-High Temperature (UHT) treated milk showed that UHT treatment degraded the DNA into smaller fragments compared to the raw milk (positive control) (Liao & Liu, 2020). Additionally, the influence of heat processing on DNA degradation and PCR-based detection of maize, using temperatures (100°C and 121°C) that are frequently applied to food production and preservation, found that DNA integrity was more severely affected at 121°C than at 100°C. However, both temperatures proved to be capable of degrading DNA (Bitskinashvili et al., 2019). Overall, our results are in line with previous reports that suggest that pasteurization results in a lower bacterial load and decreased DNA integrity.

During the clinical trial, we monitored *Mageu* consumption to ensure participant's adherence to the study product. Both CPM and LCM users consumed more *Mageu* during the intervention period than NM users, which is in line with the study requirements as this includes the *Mageu* study product. The self-reported adherence was lower than the objective adherence for CPM users, possibly a result of imperfect completion of FFQs by participants or that the number of calories in *Mageu* made them feel full. The overall high adherence to the LCM intervention suggests that the *Mageu* product was acceptable. *Mageu* is typically consumed amongst rural communities in Southern Africa as a refreshing beverage for adults and a weaning food for infants because of its nutritional and anti-microbial properties (Fadahunsi & Soremekun, 2017; Oyeyinka et al., 2021; Pswarayi & Gänzle, 2019). Therefore, the strong adherence to the study product may be supported by the consistency of consuming these beverages postpartum as a result of social and religious beliefs amongst the community (Daji et al., 2022; Fadahunsi & Soremekun, 2017). Participants were advised to consume less than 5 servings of any other fermented food while enrolled into the study. In all arms, participants followed to this recommendation during the washout and intervention periods. *Amasi*, a traditional South African fermented milk product, was the most common additional fermented food consumed during the washout period, specifically in LCM users. However, during the intervention period, the number of servings

decreased across all arms, though not statistically significant. This may be due to reports of participants feeling full and satisfied after consuming the study product for 6 weeks.

All the participants in the LCM and CPM arms had a favorable response to the *Mageu* product, as there were only 5 adverse events reported that were possibly related to *Mageu* consumption. Fermented foods are known to exert a range of health benefits imparted by the presence of LAB, including relief of irritable bowel syndrome (IBS) and diarrhoea caused by foodborne pathogens (Daji et al., 2022; Mathur et al., 2020; Setta et al., 2020; Simatende et al., 2019). This may explain the low occurrence of adverse events experienced by participants consuming *Mageu* or other fermented foods. Very few concomitant medications were used, and use did not differ by randomisation arm. Therefore, adherence and concomitant medications likely did not affect our results.

BMI is commonly used amongst healthcare professionals to assess the health and risk factors associated with pregnancy. High pre-pregnancy BMI and gestational weight gain (GWG) are common contributors to risk factors associated with postpartum weight gain and retention (Jayasinghe et al., 2022; H. Zhang et al., 2024). High-postpartum BMI is associated with cardiovascular and metabolic diseases (H. Zhang et al., 2024), lactation complications (Nguyen et al., 2017; Ozdilek et al., 2019; Simko et al., 2019) and diabetes (Choudhary et al., 2018). We thus assessed whether *Mageu* consumption affected postpartum BMI. In this study, the average BMI at enrollment was 31.75 kg/m^2, with almost half of participants being obese. The percentage of overweight and obese women did not differ post-intervention, suggesting that *Mageu* consumption did not affect postpartum weight loss. However, it is important to note that these women were in their postpartum phase with no pre-pregnancy and pregnancy BMI recorded. Therefore, the estimation of their postpartum weight loss may have been subject to some inaccuracy. Fermented foods containing probiotic-like bacteria may be associated with a decrease in body weight (Kim et al., 2011; Rezac et al., 2018). A study evaluating the effect of yoghurt containing *Lactobacillus amylovorus* and *Lactobacillus fermentum* on body fat mass in healthy but overweight individuals found that body fat mass was reduced in all treatment arms, with *Lactobacillus amylovorus* supplementation showing the greatest improvement in weight loss (Omar et al., 2013). However, a pilot RCT investigated the consumption of fermented vegetables for six weeks on intestinal microbiota and inflammatory markers. Thirty females were randomised 1:1:1 into 3 groups: 100g/day of fermented vegetables (Group A), 100g/day of pickled vegetables (Group B) and no vegetables (Group C). They found no significant difference in BMI before and after the intervention of fermented vegetables, nor did they find any significant differences amongst the randomisation arms (Galena et al., 2022). In summary, while our finding contrasts some previous reports suggesting that probiotic-rich fermented foods may support weight loss, it aligns with other studies showing minimal or no effect of such interventions. The lack of impact observed here may reflect that the cohort had pre-existing overweight or obesity, where weight regulation is multifactorial and may require more intensive or prolonged interventions, or that a maize-based fermented food is higher in calories and sugar than other fermented

foods (Anyogu et al., 2021). Previous studies that have explored associations between BMI and concentration of RBP4 have had mixed results in normal-weight, overweight and obese participants (Fahim et al., 2022; Huang et al., 2012; Korek et al., 2018; Majerczyk et al., 2018; Wessel et al., 2019). Among Slum-dwelling lean adults in Bangladesh, including 135 underweight adults and 135 adults with normal BMI, there was a significant positive association between RBP4 and normal BMI (Fahim et al., 2022), while studies conducted by others noted that BMI or fat mass did not directly correlate with RBP4 (Huang et al., 2012; Korek et al., 2018; Majerczyk et al., 2018; Wessel et al., 2019). We observed a negative correlation between RBP4 and BMI. The majority of our participants were overweight and obese, providing evidence that elevated BMI is associated with RBP4 (Huang et al., 2012; Majerczyk et al., 2018; Wessel et al., 2019). Further research is needed to explore this observation.

Breastfeeding is an important determinant of infant health (de Seymour et al., 2022; Feldman-Winter et al., 2020; Von Salmuth et al., 2021). In out cohort, most women were still breastfeeding at week 4 and 10, but the proportion dropped by week 15. A study on the preparation and consumption of *Shameta*, an indigenous cereal-based fermented porridge, including 150 lactating women in Western Ethiopia, found that the frequency of *Shameta* consumption during lactation was 86%, with all women stating that the fermented porridge increased breastmilk production (Kitessa et al., 2023). The same authors found that nutritional composition and bioactive compounds of *Shameta* provided to exclusively lactating mothers contributed to the crude protein and energy intake required during lactation, and with fortification can contribute to the nutritional benefits required during lactation (Kitessa et al., 2022). An observational study on women with a median BMI of 39.6 kg/m^2 found that mothers with very low milk production had significantly higher obesity and inflammatory markers (Walker et al., 2022). Obesity is linked to a decreased duration of breastfeeding and lower breastmilk production (Walker et al., 2022). The effect of maternal weight on lactation is further supported in a review highlighting that increased weight and lack of nutrition can affect breastmilk quality and quantity (Hart et al., 2022). Thus, in our cohort of women with high BMI, it may have been difficulties in lactation explaining the decrease in breastfeeding rates by weeks 15. This trend we observed is consistent with previous studies in South African settings, which have documented a drop in continued breastfeeding rates within the first 3 - 4 months postpartum (Bhattacharjee et al., 2019; Maponya et al., 2021; Ngwenchi Nkeh-Chungag et al., 2023; Nieuwoudt et al., 2019; Quebu et al., 2023; Theodorah & Mc'Deline, 2021; Witten et al., 2020). Multiple factors perceived insufficient milk supply may contribute to early breastfeeding cessation, including return to work, limited maternity leave, lack of access to breast pumps in this poor-resourced setting, and lack of breastfeeding support in the workplace. These findings highlight the need for stronger structural and community-based support systems to enable sustained breastfeeding practices among postpartum women in South Africa.

We next assessed the nutritional status of the women throughout the intervention. Nutritional health postpartum is important for the health and well-being of the mother and infant. Malnutrition is

associated with both overweight and underweight postpartum, it may affect lactation and can contribute to postpartum anaemia (Butwick & McDonnell, 2021; Nguyen et al., 2017; Ozdilek et al., 2019; Simko et al., 2019). According to the South African nutritional reference ranges for females, most participants had adequate levels of ferritin, vitamin B12 and thyroglobulin. About three-quarters of the cohort had optimal iron levels at baseline. Although the NM arm had the highest proportion of women with optimal levels of iron before the intervention no differences in the prevalence of women with optimal iron levels were observed between randomisation arms after consuming *Mageu*. This suggest that *Mageu* consumption did not increase iron levels in *Mageu* users. The nutritional composition of *Mageu* varies depending on fortification and preparation methods (Oyeyinka et al., 2021). *Mageu* contains ~11% protein, ~5% fat, ~1% fibre, and ~65% carbohydrate, with mineral content including sodium, potassium, calcium, iron, zinc and manganese (Fadahunsi & Soremekun, 2017; Oyeyinka et al., 2021). Our LCM product contained 5.2g protein, <0.05g fat, 2.6g fibre and 43.8g carbohydrates, with a mineral content of 28.2g sodium. Of note, almost half of the cohort was vitamin D deficient. This is consistent with emerging evidence suggesting that vitamin D deficiency is common among black women of reproductive age in South Africa (Durazo-Arvizu et al., 2014; Soepnel et al., 2023; Velaphi et al., 2019), despite the country's relatively high year-round sun exposure. A prospective study investigating vitamin D status in 291 maternal-neonate pairs at birth in black South Africans, noted that the prevalence of vitamin D deficiency was 15.9% in mothers and 32.8% in cord blood. The results suggested that among black South African women, approximately one in six mothers have vitamin D levels reflective of vitamin D deficiency (Velaphi et al., 2019). In the postpartum period, vitamin D requirements may be elevated due to physiological recovery and lactation (Gellert et al., 2017; Hollis et al., 2015), further increasing the risk of deficiency. Given the role of vitamin D in immune function and bone health, these findings emphasize the need to include the importance of maternal micronutrient status in postpartum public health care.

Cross-sectionally at week 10, ferritin concentrations differed between randomisation arms, with ferritin being highest in the NM arms and lower in both *Mageu* arms. Ferritin and iron share a unique relationship. Ferritin can serve as a marker of iron stores as well as an acute phase reactant during inflammation (Dhondge et al., 2024; Knovich et al., 2009). Elevated levels of ferritin can suggest either iron overload or inflammation, while decreased levels of ferritin indicate iron deficiency (Dhondge et al., 2024; Knovich et al., 2009; World Health Organization, 2020). Therefore, our finding could either suggest lower iron stores in the *Mageu* arms, or lower systemic inflammation. Considering that there were no significant differences in iron after the intervention and there were slight differences in systemic inflammation as indicated by IL-6 concentrations, lower ferritin in the *Mageu* arms is likely indicating anti-inflammatory effects of the *Mageu*.

No significant differences were observed between randomisation arms for other nutritional markers, including vitamin B12, vitamin D and thyroglobulin. Fermented foods are usually fortified with a

desired vitamin or mineral content to appeal to the dietary needs of the consumer (Awobusuyi et al., 2021; Fauziyyah et al., 2018; Vieira & Souza, 2022; H. Zhang et al., 2007). Our LCM product was not fortified with any additional vitamins or minerals, which may account for the lack of observed changes in other nutritional biomarkers compared to the CPM product. Some plant-based cereal beverages may lack essential micronutrients (Hidalgo-Fuentes et al., 2024). However, fermentation with LAB is known to enhance and increase the bioavailability of vitamins and minerals (Hidalgo-Fuentes et al., 2024; Tsafrakidou et al., 2020). Further, it is important to note that fermentation with LAB is strain-specific when used as a starter culture or added culture to fortify naturally fermented products (Tsafrakidou et al., 2020). There is a lack of research highlighting the vitamin content of *Mageu* or *Mahewu*, most research speaks to the use of LAB in fermentation to enhance nutritional quality (Chandrasekar Rajendran et al., 2017; Chaves-López et al., 2020; De Angelis et al., 2014; Greppi et al., 2017; Madhu et al., 2010; Masuda et al., 2012; Padonou et al., 2023; Rekha & Vijayalakshmi, 2010; Tsafrakidou et al., 2020; Xie et al., 2019). Traditional *Mageu* is a LAB-fermented cereal beverage and primarily plant-derived, with essential minerals and vitamins (Chaves-López et al., 2020). The increased vitamin content in LAB-fermented foods has given rise to literature evaluating their nutritional content (Hidalgo-Fuentes et al., 2024; Tsafrakidou et al., 2020). Vitamin D and vitamin B12 is mostly found in meat products and eggs (de Seymour et al., 2022; Nguyen et al., 2017), however; fortification of fermented foods with these vitamins may increase its concentrations (Vieira & Souza, 2022). As women in all randomisation arms reported consumption of meat products, this may explain why no significant differences were observed between the NM and *Mageu* users.

Systemic inflammation is association with IBD, T2DM and cardiovascular diseases, amongst other adverse health outcomes (Clemente et al., 2018; Di Vincenzo et al., 2024; Ma et al., 2021). We thus next assessed the impact of *Mageu* on levels of key inflammatory markers. Previous studies have suggested that fermented foods can reduce inflammation and improve overall health, as these foods are rich in probiotic-like bacteria capable of increasing the gut microbiome diversity and subsequently decreasing inflammatory markers (Paul et al., 2023; SaeidiFard et al., 2020; Wastyk et al., 2021). We did not observe any significant differences in the change in IFN-γ, IL-1β, IL-6, TNF-α and C-reactive protein concentrations between baseline and week 10 or week 15 by randomisation arm. The concentration of IL-6 increased in the NM arm from weeks 4 and 15, while it decreased in the LCM arm. A 10-week interventional study assessed the influence of high-fibre and high-fermented food diets on gut microbiota diversity and inflammation. Similar to us, they found that a high-fermented food diet decreased IL-6 in relatively healthy North American adults (Wastyk et al., 2021). An *in vitro* study on oak kombucha showed a decrease in TNF-α and IL-6 in response to lipopolysaccharide stimulation in THP-1 human monocytic cells (Paul et al., 2023; Vázquez-Cabral et al., 2017). An RCT including 50 individuals consuming fermented milk daily for 6 weeks found that the consumption of fermented milk decreased TNF-*α* levels (SaeidiFard et al., 2020; Tonucci et al., 2017). Inflammation postpartum can

be both beneficial or detrimental to the health of the mother and infant (Zou et al., 2022). As mentioned previously, high levels of inflammation during and after pregnancy can lead to preeclampsia, postpartum weight retention and mastitis, among other adverse outcomes (Challis et al., 2009; Quansah et al., 2023; Walker et al., 2022). IL-6 is an innate, pro-inflammatory cytokine responsible for immune response to infections, tissue repair and lactation in postpartum mothers (Bränn et al., 2019; C. Li et al., 2018; Tuaillon et al., 2017; Van Rijn et al., 2016). None-the-less, our findings of decreased IL-6 in the LCM arm suggest that *Mageu* may have had a beneficial influence on inflammation, and although it may not have been statistically significant, it may be clinically relevant. C-reactive protein, an acute phase protein, is produced in response to the upregulation of IL-6 (Ali et al., 2023). We found no differences in the change in C-reactive protein between arms postpartum. Similarly, an RCT found no significant differences in mean levels of C-reactive protein amongst intervention arms, nor did they find any differences observed before and after the intervention, as well as for the change in concentration of C-reactive protein (Baron et al., 2024). Further analysis into the relationship between *Mageu* consumption an inflammation, as well as its potential clinical relevance, is warranted.

As the intestinal immune environment plays a central role in regulating systemic immune responses, we also assessed markers of intestinal inflammation. Lipocalin-2 is an acute and chronic inflammatory protein involved in immune response, metabolic regulation and iron homeostasis (Kubben et al., 2007). Increased levels of lipocalin-2 are associated with intestinal inflammation, high BMI and obesity (Auguet et al., 2011; Moschen et al., 2017). Lipocalin-2 concentrations increased from weeks 4 to 15 for the NM arm and decreased in the CPM arm, although this difference was not statistically significant. An evaluation of the upregulation of lipocalin-2 and its association with obesity in 90 women found that lipocalin-2 serum levels were significantly elevated in severely obese women (>40 kg/m^2) compared to normal weight women (<25 kg/m^2). They also noted that lipoclain-2 serum levels correlated with BMI values (Auguet et al., 2011). The high BMI in our cohort could be a contributing factor to observing no difference in the regulation of lipocalin-2. Fermented foods can improve gut dysbiosis and reduce intestinal inflammation, ultimately reducing the expression of lipocalin-2 (Hafeez et al., 2025; Jalili et al., 2023). Research focuses on the effect of fermented foods on lipocalin-2 regulation in murine models (Agista et al., 2021; Singh et al., 2019), with few reported in human studies. A pilot study investigating the effects of fermented pickle consumption on gut microbiota and inflammation in 223 women observed a negative association between inflammatory lipocalin-2 and gut microbiota diversity, suggesting beneficial effects of fermented foods in modulating inflammation (Hafeez et al., 2025). Although beyond the scope of the current study, future work should assess associations between lipocalin-2 levels and gut microbiota composition in this cohort.

Myeloperoxidase is an enzyme involved in immune defense by generating reactive oxygen species, antimicrobial activity and interacting with inflammatory cytokines (Frangie & Daher, 2022; Khan et al., 2018; Ndrepepa, 2019). We observed no significant difference in the change of myeloperoxidase

concentration amongst the three randomisation arms from week 4 to 10 or 15. Studies conducted in murine models provide promising evidence that fermented foods can reduce myeloperoxidase activity and inflammation (de Jesus et al., 2024; Jang & Min, 2020; Park et al., 2017; Varela-Mendoza et al., 2025), however, evidence in human studies are limited. Therefore, further clinical research is necessary to establish the effects of fermented food consumption on myeloperoxidase concentrations in humans, and its clinical relevance.

Fecal calprotectin is an indicator of infectious and inflammatory conditions in the gut, specifically in diagnosing IBD and gastrointestinal disorders (de Moura Gondim Prata et al., 2016; Dinçer et al., 2024; Heinzel et al., 2024). Increased levels of fecal calprotectin in stool is indicative of intestinal permeability and compromised gut barrier integrity (Heston et al., 2023; Schwiertz et al., 2018). In LCM users, fecal calprotectin decreased between weeks 4 and 15, while levels remained consistent in NM and CPM users from weeks 4 to 10 and weeks 4 to 15. The decrease in fecal calprotectin from week 4 to later time points in *Mageu* users could suggest an anti-inflammatory activity exhibited by *Mageu* consumption, and resulting improved gut permeability (D'Amico et al., 2021; de Moura Gondim Prata et al., 2016). Fermented foods may influence fecal calprotectin levels through gut microbial regulation (Heinzel et al., 2024), however, most clinical trial research focus on the beneficial effects of fermented foods on Ulcerative Colitis and limited research is available on healthy individuals. An RCT investigated the effects an anti-inflammatory diet (AID) and Canada's Food Guide (CFG) diet on inflammatory markers in Ulcerative Colitis patients in remission. They found that fecal calprotectin concentrations were increased in the CFG diet (184 μg/g) compared to the AID group (129 μg/g). They also noted that participants in the AID group had an increase in fecal *Bifidobacteriaceae, Lachnospiraceae*, and *Ruminococcaceae,* common microorganisms involved in fiber-rich fermented food consumption and associated with reduced fecal calprotectin levels (Gubatan et al., 2023; Keshteli et al., 2022). These and our data suggest that live bacteria in fermented foods may have activity in regulating inflammation in the gut through improvement of gut epithelial integrity. We acknowledge that the clinically relevance of these findings needs to be confirmed, and that sample size of stool samples received from the mothers at their follow up visits was low, thus limiting the strength of our results. Further, factors such as pregnancy and postpartum periods, obesity and age, among others, have been known to influence levels of fecal calprotectin (D'Amico et al., 2021; Dinçer et al., 2024; Doherty et al., 2020; Heinzel et al., 2024; Joshi et al., 2010). Fecal calprotectin levels were increased in pregnant women with a BMI >25 kg/m^2 (Doherty et al., 2020). A prospective controlled study also reported significantly elevated levels of fecal calprotectin in obese adolescents (Dinçer et al., 2024). We did not identify a correlation between fecal calprotectin and BMI in our cohort. This may be because our cohort was generally overweight and obese, it may have been difficult to assess subtle changes in fecal calprotectin. We had hypothesized that the BMI would correlate tightly with most inflammatory markers or acute phase proteins (C-reactive protein and α(1)-acid glycoprotein), but this was not the case. A high BMI is known to be

associated with inflammatory markers, similar to those assessed in our study (Auguet et al., 2011; Dinçer et al., 2024; Moschen et al., 2017). We however found that BMI only correlated with RBP4, but not with any other inflammatory or nutritional markers.

Next, we evaluated whether concentrations of nutritional and inflammatory markers correlated between time points to provides insight into the temporal stability of these biomarkers. The concentrations of all nutritional markers and micronutrients measured at weeks 4 and 10 were highly correlated, suggesting stability of nutritional markers postpartum. In the previous section we noted that overall, the participants had optimal concentrations of iron, ferritin and thyroglobulin at week 4 and week 10. Dietary patterns can influence the concentrations of nutritional markers (Bander et al., 2020; Gille et al., 2018). Participants in our cohort received dietary counselling from weeks 4 to 10 and were encouraged to eat balanced meals, other dietary staples such as fruits, vegetables and meat sources were consumed along with the fermented *Mageu* (CPM and LCM users exclusively). A pilot RCT investigated the effects of live culture (LCM) and store-bought (SBM) *Mageu* consumption for 6 weeks on gut health in lactating postpartum women of South Africa, found that gut alpha-diversity increased in the LCM group compared to the SBM and control groups (Happel et al., 2025). They also noted that bacterial, inflammation and nutritional signatures were associated with *Mageu* intake driven by ferritin, sTfR and *Eubacterium halli*. This study suggests that *Mageu* is capable of improving maternal gut health (Happel et al., 2025). Therefore, the influence of a balanced diet along with improved gut microbial diversity may have significantly contributed to the enhanced bioavailability of these essential nutritional markers (Awobusuyi & Siwela, 2019; Happel et al., 2025; Mafukata et al., 2024; Oyeyinka et al., 2021). α(1)-acid glycoprotein had the weakest correlation between week 4 and 10 values amongst all the nutritional markers. A meta-analysis on postpartum changes in maternal physiology and milk composition observed a 30% decrease in α(1)-acid glycoprotein levels by the end of week 4 postpartum and continued to decline up until 12 months postpartum (Deferm et al., 2025). This may explain the weaker correlation observed between week 4 and 10, as α(1)-acid glycoprotein levels naturally decline.

We observed strong, positive correlations for all quantities of systemic inflammatory markers measured at week 4 and week 10, or week 4 and week 15. Inflammatory cytokines are all interconnected, meaning that the expression of one cytokine can result in the reduction or upregulation of another through stimulation of signaling pathways (Al-Roub et al., 2021; Jarlborg & Gabay, 2022; Ledesma et al., 2004). This, together with immune response and microbial diversity, could explain the strong correlations observed between the abovementioned systemic inflammatory markers during the intervention period. At week 4 and week 15, the strong correlations remained consistent despite the smaller sample size, which may be a result of a prolonged inflammatory response in the later postpartum period.

The concentrations of intestinal inflammatory markers lipocalin-2, myeloperoxidase and fecal calprotectin all correlated between week 4 to week 10 but not between week 4 to week 15. This suggest

short term (week 4-10) stability of intestinal inflammation markers during the early postpartum period, and the lack of correlations between weeks 4 and 15 may greater inter-individual variability later in the postpartum period, or influence of external factors (like diet/*Mageu* consumption) emerging over time. However, we need to take into consideration that the number of missed visits and unavailable stool samples was higher at week 15, which might have impacted our ability to observe a correlation.

We found some noteworthy correlations between the nutritional and immune markers we measured. Iron correlated negatively with α(1)-acid glycoprotein and C-reactive protein, with α(1)-acid glycoprotein and C-reactive protein correlating positively with each other. Both α(1)-acid glycoprotein and C-reactive protein are acute phase proteins produced in response to inflammation (Farrag et al., 2024; Ko et al., 2024); therefore, their positive correlation is expected. Together, both α(1)-acid glycoprotein and C-reactive protein can be used to improve the detection of iron deficiency when correcting for inflammation (Farrag et al., 2024; Ko et al., 2024) and indirectly result in iron sequestration by upregulating hepcidin in macrophages to inhibit iron acquisition by infectious organisms (Rohner et al., 2017), which can explain the inverse relationship between iron and C-reactive protein/α(1)-acid glycoprotein. As expected, negative correlations were observed for sTfR with iron and ferritin. The negative correlation observed between sTfR and ferritin reflects their opposing roles in iron metabolism and regulation. Adequate iron stores will increase ferritin levels and decrease sTfR levels (Al-Saqladi et al., 2012; Ambroszkiewicz et al., 2017; Dhondge et al., 2024). However, ferritin is also an acute phase reactant that can be influenced by inflammation as well as iron stores (Al-Saqladi et al., 2012; Dhondge et al., 2024; Knovich et al., 2009). Both sTfR and ferritin are sensitive markers of iron-deficiency; however, sTfR is considered a more reliable marker for iron-deficiency as it remains unaffected by inflammation (Al-Saqladi et al., 2012; Ambroszkiewicz et al., 2017; Günther et al., 2022; Oustamanolakis et al., 2011).

Myeloperoxidase correlated negatively with fecal calprotectin. Myeloperoxidase and fecal calprotectin are produced by neutrophils but released via different stimuli in the presence of inflammation (de Moura Gondim Prata et al., 2016). As such, myeloperoxidase and fecal calprotectin are both effective biomarkers of intestinal inflammation but are independently associated with each other (Swaminathan et al., 2024). A study assessing fecal myeloperoxidase levels in children with Crohn's Disease described a strong positive correlation (Spearman $r = 0.81$, $P < .0001$) between these two markers (Edwards et al., 2025). Further mechanistic studies are warranted to clarify these dynamics in postpartum women. The negative correlation we observed could suggest that these markers peak at different phases of the inflammatory response—i.e., individuals with chronic gut inflammation may show high calprotectin but relatively low myeloperoxidase, compared to those with more acute inflammation.

Ferritin correlated positively with the pro-inflammatory markers IFN-γ with TNF-α, and the systemic inflammatory markers IL-1β, IL-6, IFN-γ and TNF-α all correlated with each other. Both IFN-γ and

TNF-α can stimulate ferritin production in macrophages as an immune response (Abreu et al., 2020; Karki et al., 2021). IFN-γ and TNF-α are also capable of stimulating IL-6 production in macrophages, dendritic cells and endothelial cells, which in turn stimulates the production of ferritin in hepatocytes and macrophages (Chen et al., 2023; Jarlborg & Gabay, 2022; Ueda & Takasawa, 2018). The systemic inflammatory markers assessed in our study were all pro-inflammatory markers. The positive correlations observed between these markers suggests an interconnecting role of stimulation with one another during immune activation. During this cascade, these markers upregulate each other to induce inflammation and respond to infection (Jarlborg & Gabay, 2022).

Vitamin D correlated positively with both lipocalin-2 and myeloperoxidase. Vitamin D has immunomodulatory and regulatory effects during inflammatory responses (Codoñer-Franch et al., 2012; Jones et al., 2023; Liao et al., 2022; Zhou et al., 2022), whereby it can induce the expression of lipocalin-2 and modulate neutrophil activity to increase the concentration of myeloperoxidase (Jones et al., 2023; Liao et al., 2022; Zhou et al., 2022). Therefore, upregulation of myeloperoxidase, lipocalin-2 and vitamin D during an inflammatory state may result in the observed positive correlation. Vitamin D has been described to be associated with BMI, whereby overweight and obese individuals have been reported to have vitamin D deficiency (Codoñer-Franch et al., 2012; Zhou et al., 2022). Similarly, a cross-sectional study found that insufficient vitamin D levels were detected in severely obese children with increased markers of oxidative stress and inflammation and may translate to adults as well (Codoñer-Franch et al., 2012). Our participants showed a high prevalence of vitamin D deficiency, with approximately 58% of the participants having less than optimal concentrations of vitamin D at week 4.

Finally, we assessed overall inflammatory and nutritional biomarker changes from week 4 to week 10 to evaluate the impact of the intervention on the three arms and determine distinct drivers of variation between biomarkers. We then assessed the change in biomarker concentrations from weeks 4 to 10 and the effect of the intervention on inflammatory markers (lipocalin-2, myeloperoxidase, fecal calprotectin, IFN-γ, IL-1β, IL-6, TNF-α and C-reactive protein) and nutritional markers (iron, serum ferritin, vitamin B12, vitamin D, α(1)-acid glycoprotein, thyroglobulin, sTfR and RBP4). No distinct clustering by randomisation arm was observed in any of the inflammatory and nutritional markers, suggesting that biomarker responses were similar across the randomisation arms and did not change over time. Overall, the above suggests that *Mageu* did not have a profound impact across all markers measured. Our cohort was generally overweight and obese at the start of the intervention and no change in weight was observed after the intervention. The subsequent increase in inflammation between week 4 and week 15 could reflect delayed immune activation, with potential drivers including nutritional shift and assigned intervention. Neutrophil-associated gut inflammation may be a dominant feature shaping the overall inflammatory profile in this cohort. Further research on the effects of local fermented food like *Mageu* on inflammation and nutritional health is needed to confirm these findings.

4.2 Conclusion

The *Mageu* product was free from *E. coli* and contained no mesophilic *Clostridium* spp. associated with disease in humans. The LCM batched contained a higher bacterial load than the CPM batches, as expected when comparing a pasteurised fermented product to a live culture fermented product. Participant adherence was high, with limited intake of additional fermented foods, in line with the protocol. Overall, *Mageu* did not significantly decrease inflammation, improve nutritional status, or decrease BMI in this small cohort of postpartum women. Although fluctuations in inflammatory markers were observed, NM users had the highest change in concentration for intestinal and systemic inflammatory markers over time compared to *Mageu* users. The change in concentrations over time showed iron, serum ferritin and thyroglobulin were higher in NM users than *Mageu* users. Elevated levels of serum ferritin may be a marker of inflammation as both intestinal and systemic inflammatory markers showed increased concentrations over time amongst the randomisation arms. Further research into fortifying *Mageu* may yield better outcomes for inflammation and nutrition.

The limitations of our study included small sample size, dosing and duration of the interventional product. The FFQs were estimated sizes calculated by the participant and based on recall, therefore it cannot serve as a true reflection of nutritional health. We did not have access to our participant's pre-pregnancy BMI and therefore, could not calculate an accurate BMI postpartum. Our participants were generally healthy in terms of no comorbidities, pregnancy complications or Tuberculosis and HIV, however the majority had a high BMI at the start of the intervention. Further research into the beneficial properties of local *Mageu* is needed to evaluate the effects on inflammation and nutritional health in South Africa.

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APPENDICES

Supplementary Table 1: Amplification primers for colony PCR and sequencing

Primer Name	5' to 3' Sequence
27F	5'-AGA GTT TGA TCM TGG CTC AG-3'
1492R	5'-TAC GGY TAC CTT GTT ACG ACT T-3'
907R	5'-CCGTCAATTCMTTTRAGTTT-3'

Supplementary Table 2: Amplification primers for Bactquant assay

Primer Name	5' to 3' Sequence
Forward	5'- CCT ACG GGD GGC WGC A-3'
Reverse	5'- GGA CTA CHV GGG TMT CTA ATC-3'
Probe	5'- CAG CAG CCG CGG TA-3'

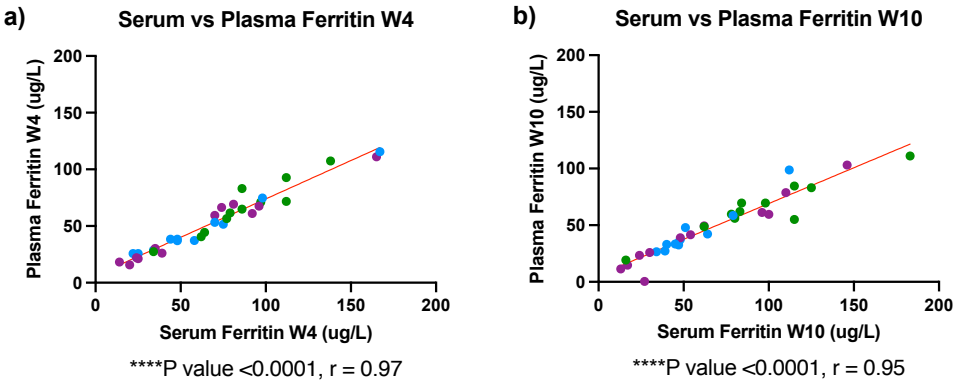
Supplementary Table 3: Inclusion and exclusion criteria

	Inclusion Criteria	Exclusion Criteria
Maternal Factors	1) Documented Human immunodeficiency virus (HIV) uninfected in the past 6 weeks 2) Age of mother ≥18 and <50 years 3) Mother has self-chosen to breast feed her infant 4) Mother is able and willing to provide informed consent and do the follow up assessments 5) Access to fridge and electricity 6) Willing to be randomised to daily *Mageu* or no *Mageu*	1) Complications during pregnancy and delivery such as gestational diabetes, obesity, (BMI >35 kg/m^2 prior to pregnancy), chorioamnionitis and eclampsia 2) Active Tuberculosis (TB) or other infectious diseases 3) Consumes fermented foods, including *Amasi*, *Mageu* alcohol > five times weekly 4) Administration of probiotics, prebiotics or immunoregulatory products
Infant Factors	1) Gestational age ≥ 37 weeks 2) Birth weight ≥ 2,4 Kg	1) Hypoxic injury/ seizures/ sepsis/ intrauterine growth retardation 2) Administration of probiotics, prebiotics or immunoregulatory products

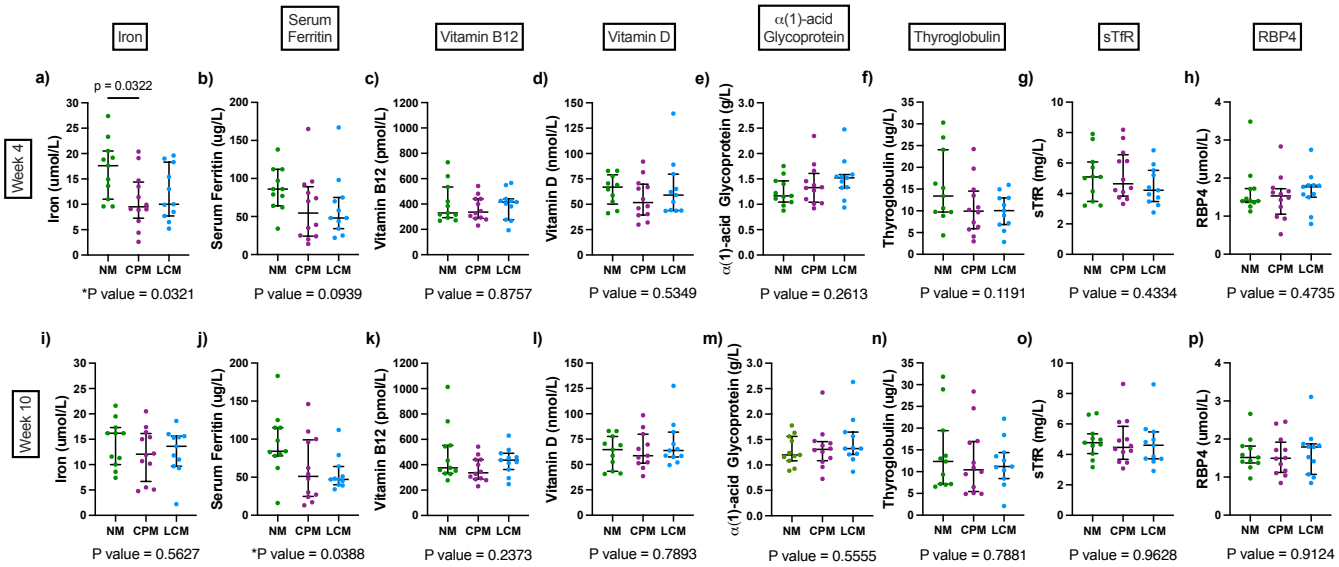
Supplementary Table 4: Inter- and intra-plate quality assessment of inflammatory markers and cytokine data across two plates

Marker	Samples with detectible concentrations (n/N) [%]	Intra-assay CV (%)	Inter-assay CV (%)
Interferon (IFN)-γ	(111/118) [94.1]	11.6	14.5
Interleukin (IL)-1β	(111/118) [94.1]	9.1	7.5
Interleukin (IL)-6	(111/118) [94.1]	12.4	4.9
Tumor necrosis factor (TNF)-α	(111/118) [94.1]	8.5	25.5
Lipocalin-2	(110/110) [100]	3.2	6.9
Myeloperoxidase	(111/111) [100]	16.7	15.5
Fecal Calprotectin	(84/90) [93.3]	9.0	21.1

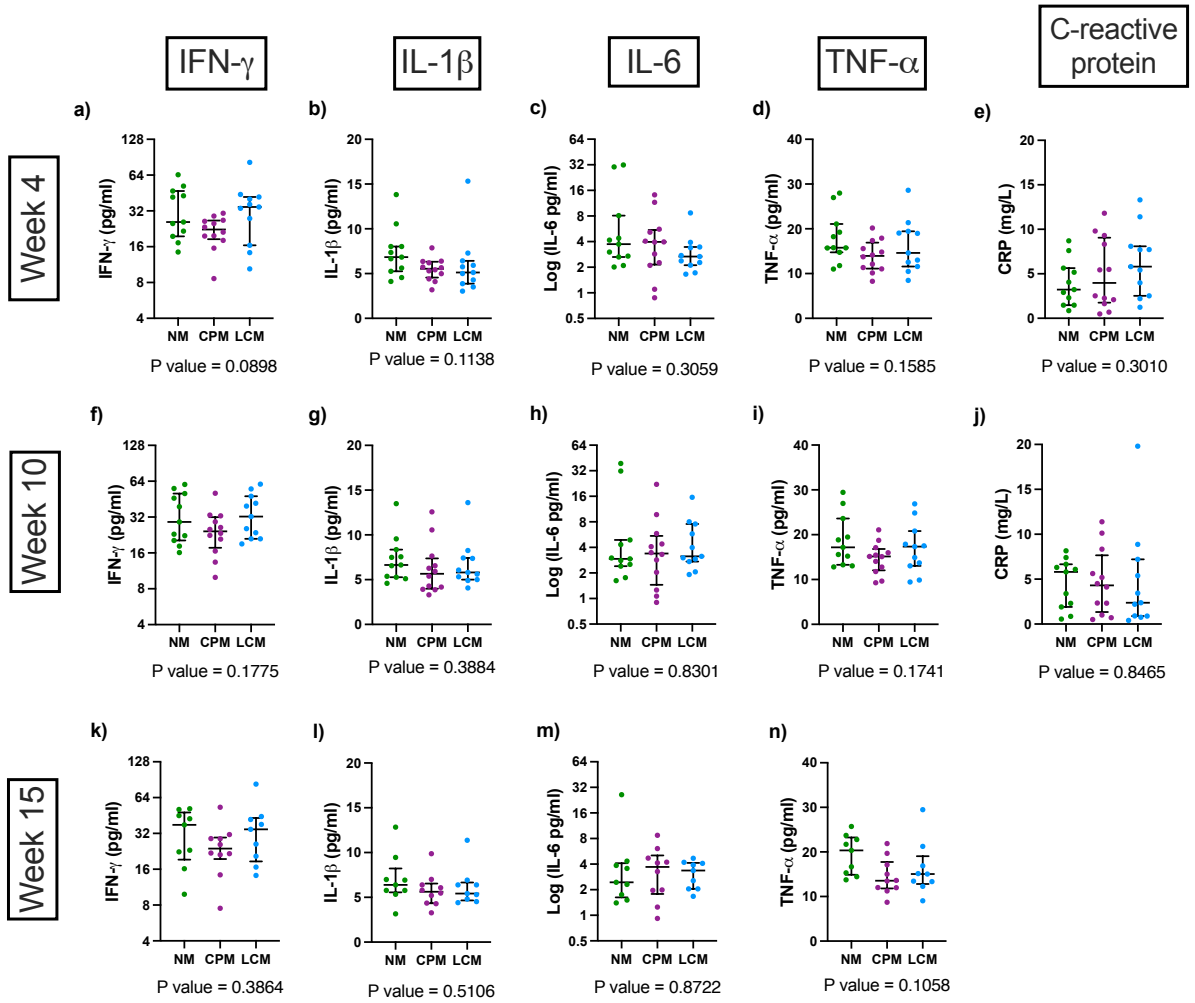
Intra-plate controls (n=3) were assayed in duplicates within a plate.
Inter-plate controls (n=3) were assayed in singlicate across all plates.
CV: Coefficient of variation for each participant across all plates is calculated for each cytokine by dividing the standard deviation by the average. The average CV is represented in the table.



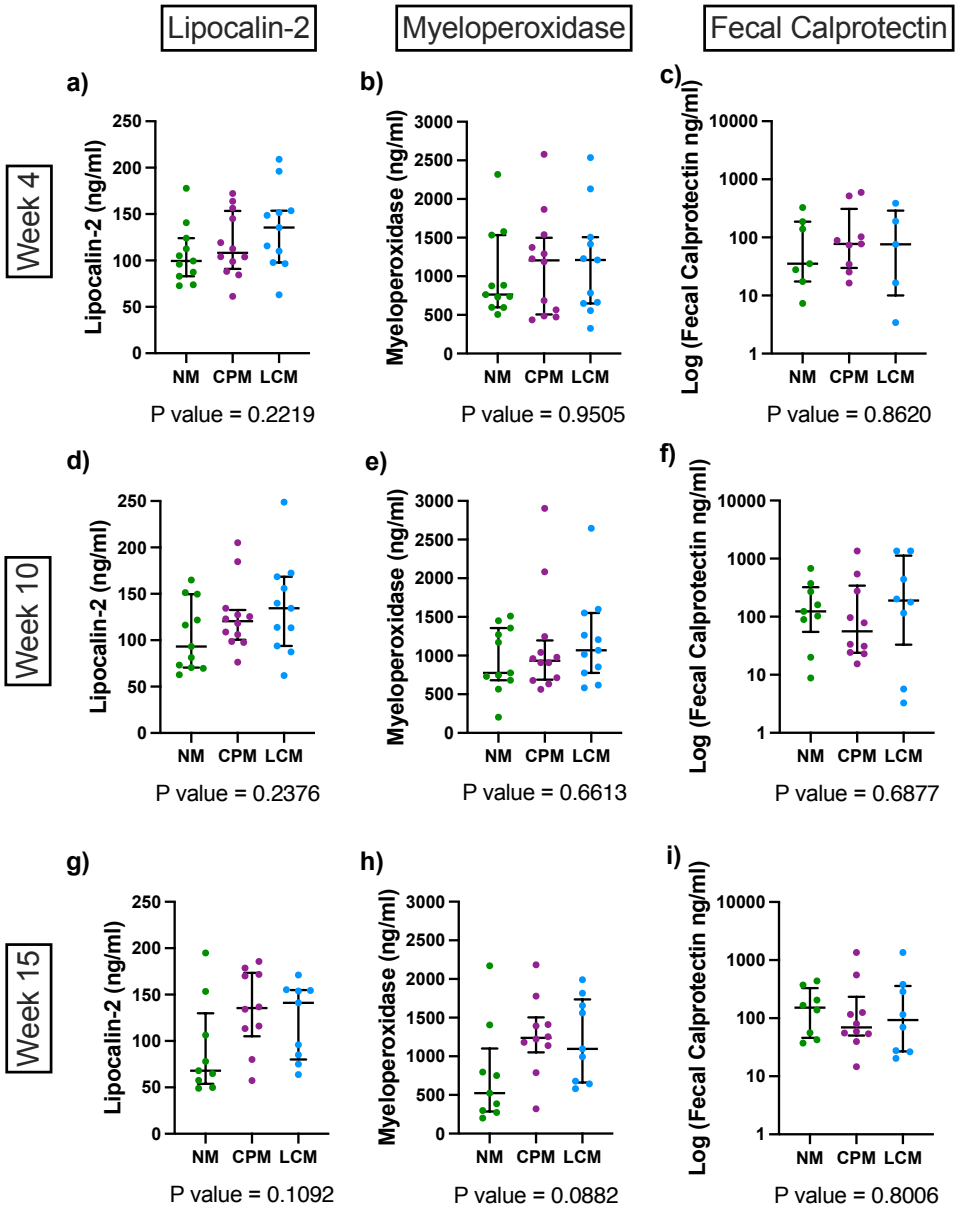
Supplementary Figure 1: Correlations of serum and plasma ferritin concentrations of week 4 and week 10. The concentration for serum ferritin was quantified at NHLS and plasma ferritin was quantified using a Q-plex micronutrient array assay at week 4 (a) and week 10 (b). Statistics was calculated using Pearson correlation. Colour-coded points represent randomisation arms (Green=No *Mageu,* Purple=Commercially Pasteurised *Mageu* and Blue=Live Culture *Mageu*). NHLS=National Health Laboratory Services.



Supplementary Figure 2: Cross-sectional comparison of the concentrations of nutritional markers and systemic micronutrients. The concentration for the nutritional markers iron (a, i), ferritin (b, j), Vitamin B12 (c, k) and Vitamin D (d, l) were quantified at NHLS. The concentration for the micronutrients α(1)-acid glycoprotein (e, m), thyroglobulin (f, n), sTfR (g, o) and RBP4 (h, p) were quantified using a Q-plex micronutrient array assay. Graphs show median and IQR at week 4 (top) and week 10 (bottom). Statistics was calculated using ANOVA for parametric data and Kruskal-Wallis for non-parametric data. NM=No *Mageu*, CPM=Commercially Pasteurised *Mageu*, LCM=Live Culture *Mageu*, sTfR=Soluble Transferrin Receptor, RBP4=Retinol Binding Protein 4, IQR=Interquartile Range, NHLS=National Health Laboratory Services, ANOVA=Analysis of variance.



Supplementary Figure 3: Cross-sectional comparison of the concentrations of systemic inflammatory markers. The concentration for the systemic inflammatory markers IFN-γ (a, f, k), IL-1β (b, g, l), IL-6 (c, h, m) and TNF-α (d, i, n) were quantified using Luminex® assay and C-reactive protein (e, j) was quantified using a Q-plex micronutrient array assay at weeks 4 (top), 10 (middle) and 15 (bottom). Graphs show median and IQR. Statistics was calculated using ANOVA for parametric data and Kruskal-Wallis for non-parametric data. IFN-γ=Interferon-γ, IL-1β=Interleukin-1β, IL-6=Interleukin-6, TNF-α=Tumour Necrosis Factor-α, NM=No *Mageu*, CPM=Commercially Pasteurised *Mageu*, LCM=Live Culture *Mageu*, IQR=Interquartile Range, ANOVA=Analysis of variance.



Supplementary Figure 4: Cross-sectional comparison of the concentrations of intestinal inflammatory markers. The concentration for the intestinal inflammatory markers Lipocalin-2 (a, d, g), Myeloperoxidase (b, e, h) and Fecal Calprotectin (c, f, i) were quantified using ELISA assay at week 4 (top), 10 (middle) and 15 (bottom). Graphs show median and IQR. Statistics was calculated using ANOVA for parametric data and Kruskal-Wallis for non-parametric data. NM=No *Mageu*, CPM=Commercially Pasteurised *Mageu*, LCM=Live Culture *Mageu*, IQR=Interquartile Range, ELISA= Enzyme-Linked Immunosorbent Assay, ANOVA=Analysis of variance.

Funding and Ethics:

This project is funded by The Bill & Melinda Gates Foundation (Gates Grand Challenges Fermented Foods, INV-033570).

This project is linked to HREC/REF 004/2022 (*MAGEU* for Health of Mothers and Infants in South Africa). This MSc project proposal was submitted to DRC and HREC (HREC REF: 901/2024).

All procedures of the parent study were conducted according to the International Conference on Harmonisation (ICH), Good Clinical Practices (GCP), South African Good Clinical Practices (SA GCP) guidelines and approved by the South African Health Products Regulatory Authority (SAHPRA) in Cape Town (Ref no.: 20220305).

Study Diary – Fermented Foods: Washout weeks 1,2,3 and 4

Participant Identification: _____

Protocol: Mageu

Study Doctor: Dr Heather Jaspan

Phone: 0786839806

Week 1: Start date		Day 1:	Day 2:	Day 3:	Day 4:	Day 5:	Day 6:	Day 7:
Foods eaten: Please make one tick for each time a food from a group was eaten on a specific **day**								
1	 Grains & starchy veg							
2	 Legumes							
3	 Nuts and seeds							
4	 Dark green leafy vegetables							
5	 Dark yellow and orange fruit & veg							
6	 Other vegetables							
7	 Other fruits							
8	 Eggs							
9	 Milk and milk products	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other
10	 Meat, poultry, fish							
11	 Mageu							
12	 Traditional beer							
 **Medications**								
Medication 1: Name								
Dosage?								
Why taken?								
Medication 2: Name								
Dosage:								
Why taken?								

If you are experiencing serious symptoms, please contact the study doctor/ follow the adverse event process

Please keep the containers of any medicines you used and bring them with you when you come to the clinic for your next study visit

Week 2: Start date		Day 1:	Day 2:	Day 3:	Day 4:	Day 5:	Day 6:	Day 7:
Foods eaten: Please make one tick for each time a food from a group was eaten on a specific **day**								
1	 Grains & starchy veg							
2	 Legumes							
3	 Nuts and seeds							
4	 Dark green leafy vegetables							
5	 Dark yellow and orange fruit & veg							
6	 Other vegetables							
7	 Other fruits							
8	 Eggs							
9	 Milk and milk products	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other
10	 Meat, poultry, fish							
11	 Mageu							
12	 Traditional beer							
 **Medications**								
Medication 1: Name								
Dosage?								
Why taken?								
Medication 2: Name								
Dosage:								
Why taken?								

If you are experiencing serious symptoms, please contact the study doctor/ follow the adverse event process

Please keep the containers of any medicines you used and bring them with you when you come to the clinic for your next study visit

Week 3: Start date		Day 1:	Day 2:	Day 3:	Day 4:	Day 5:	Day 6:	Day 7:
Foods eaten: Please make one tick for each time a food from a group was eaten on a specific **day**								
1	 Grains & starchy veg							
2	 Legumes							
3	 Nuts and seeds							
4	 Dark green leafy vegetables							
5	 Dark yellow and orange fruit & veg							
6	 Other vegetables							
7	 Other fruits							
8	 Eggs							
9	 Milk and milk products	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other
10	 Meat, poultry, fish							
11	 Mageu							
12	 Traditional beer							
 **Medications**								
Medication 1: Name								
Dosage?								
Why taken?								
Medication 2: Name								
Dosage:								
Why taken?								

If you are experiencing serious symptoms, please contact the study doctor/ follow the adverse event process

Please keep the containers of any medicines you used and bring them with you when you come to the clinic for **your next study visit**

Week 4: Start date		Day 1:	Day 2:	Day 3:	Day 4:	Day 5:	Day 6:	Day 7:
Foods eaten: Please make one tick for each time a food from a group was eaten on a specific **day**								
1	 Grains & starchy veg							
2	 Legumes							
3	 Nuts and seeds							
4	 Dark green leafy vegetables							
5	 Dark yellow and orange fruit & veg							
6	 Other vegetables							
7	 Other fruits							
8	 Eggs							
9	 Milk and milk products	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other
10	 Meat, poultry, fish							
11	 Mageu							
12	 Traditional beer							
 **Medications**								
Medication 1: Name								
Dosage?								
Why taken?								
Medication 2: Name								
Dosage:								
Why taken?								

If you are experiencing serious symptoms, please contact the study doctor/ follow the adverse event process

Please keep the containers of any medicines you used and bring them with you when you come to the clinic for your next study visit

Study Monitoring Sheet: Intervention groups weeks 5,6 and 7

Participant Identification: _____

Protocol: Mageu

Study Doctor: Dr Heather Jaspan

Phone: 0786839806

Week 5: Start date		Day 1:	Day 2:	Day 3:	Day 4:	Day 5:	Day 6:	Day 7:
Mageu: Please circle: Full bottle: drank all Empty or half bottle: Please write reason for not eating all of it								
Foods eaten: Please make one tick for each time a food from a group was eaten on a specific day								
1	Grains & starchy veg							
2	Legumes							
3	Nuts and seeds							
4	Dark green leafy vegetables							
5	Dark yellow & orange fruits & veg							
6	Other vegetables (veg)							
7	Other fruits							
8	Eggs							
9	Milk & milk products	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other
10	Meat, poultry, fish							
11	Traditional beer							
Medications								
Drug 1:								
Dosage:								
Why:								
Drug 2:								
Dosage:								
Why:								

If you are experiencing serious symptoms, please contact the study doctor/ follow the adverse event process
Please keep the containers of any medicines you used and bring them with you when you come to the clinic for your next study visit

Week 6: Start date		Day 1:	Day 2:	Day 3:	Day 4:	Day 5:	Day 6:	Day 7:
Mageu: Please circle: Full bottle: drank all Empty or half bottle: Please write reason for not eating all of it								
Foods eaten: Please make one tick for each time a food from a group was eaten on a specific day								
1	Grains & starchy veg							
2	Legumes							
3	Nuts and seeds							
4	Dark green leafy vegetables							
5	Dark yellow & orange fruits & veg							
6	Other vegetables (veg)							
7	Other fruits							
8	Eggs							
9	Milk & milk products	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other
10	Meat, poultry, fish							
11	Traditional beer							
Medications								
Drug 1:								
Dosage:								
Why:								
Drug 2:								
Dosage:								
Why:								

If you are experiencing serious symptoms, please contact the study doctor/ follow the adverse event process

Please keep the containers of any medicines you used and bring them with you when you come to the clinic for your next study visit

Week 7: Start date		Day 1:	Day 2:	Day 3:	Day 4:	Day 5:	Day 6:	Day 7:
Mageu: Please circle: Full bottle: drank all Empty or half bottle: Please write reason for not eating all of it								
Foods eaten: Please make one tick for each time a food from a group was eaten on a specific day								
1	Grains & starchy veg							
2	Legumes							
3	Nuts and seeds							
4	Dark green leafy vegetables							
5	Dark yellow & orange fruits & veg							
6	Other vegetables (veg)							
7	Other fruits							
8	Eggs							
9	Milk & milk products	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other
10	Meat, poultry, fish							
11	Traditional beer							
Medications								
Drug 1:								
Dosage:								
Why:								
Drug 2:								
Dosage:								
Why:								

If you are experiencing serious symptoms, please contact the study doctor/ follow the adverse event process

Please keep the containers of any medicines you used and bring them with you when you come to the clinic for your next study visit

Study Diary – Fermented Foods: Control Group Booklet Weeks 5,6 and 7

Participant Identification: _____

Protocol: Mageu

Study Doctor: Dr Heather Jaspan

Phone: 0786839806

Week 5: Start date		Day 1:	Day 2:	Day 3:	Day 4:	Day 5:	Day 6:	Day 7:
Foods eaten: Please make one tick for each time a food from a group was eaten on a specific **day**								
1	 Grains & starchy veg							
2	 Legumes							
3	 Nuts and seeds							
4	 Dark green leafy vegetables							
5	 Dark yellow and orange fruit & veg							
6	 Other vegetables							
7	 Other fruits							
8	 Eggs							
9	 Milk and milk products	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other
10	 Meat, poultry, fish							
11	 Mageu							
12	 Traditional beer							
 **Medications**								
Medication 1: Name								
Dosage?								
Why taken?								
Medication 2: Name								
Dosage:								
Why taken?								

If you are experiencing serious symptoms, please contact the study doctor/ follow the adverse event process

Please keep the containers of any medicines you used and bring them with you when you come to the clinic for your next study visit

Week 6: Start date		Day 1:	Day 2:	Day 3:	Day 4:	Day 5:	Day 6:	Day 7:
Foods eaten: Please make one tick for each time a food from a group was eaten on a specific **day**								
1	 Grains & starchy veg							
2	 Legumes							
3	 Nuts and seeds							
4	 Dark green leafy vegetables							
5	 Dark yellow and orange fruit & veg							
6	 Other vegetables							
7	 Other fruits							
8	 Eggs							
9	 Milk and milk products	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other
10	 Meat, poultry, fish							
11	 Mageu							
12	 Traditional beer							
 **Medications**								
Medication 1: Name								
Dosage?								
Why taken?								
Medication 2: Name								
Dosage:								
Why taken?								

If you are experiencing serious symptoms, please contact the study doctor/ follow the adverse event process

Please keep the containers of any medicines you used and bring them with you when you come to the clinic for your next study visit

Week 7: Start date		Day 1:	Day 2:	Day 3:	Day 4:	Day 5:	Day 6:	Day 7:
Foods eaten: Please make one tick for each time a food from a group was eaten on a specific **day**								
1	 Grains & starchy veg							
2	 Legumes							
3	 Nuts and seeds							
4	 Dark green leafy vegetables							
5	 Dark yellow and orange fruit & veg							
6	 Other vegetables							
7	 Other fruits							
8	 Eggs							
9	 Milk and milk products	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other
10	 Meat, poultry, fish							
11	 Mageu							
12	 Traditional beer							
 **Medications**								
Medication 1: Name								
Dosage?								
Why taken?								
Medication 2: Name								
Dosage:								
Why taken?								

If you are experiencing serious symptoms, please contact the study doctor/ follow the adverse event process

Please keep the containers of any medicines you used and bring them with you when you come to the clinic for your next study visit

Study Monitoring Sheet: Intervention groups: Weeks 8, 9 and 10

Participant Identification: _____

Protocol: Mageu

Study Doctor: Dr Heather Jaspan

Phone: 0786839806

Week 8: Start date		Day 1:	Day 2:	Day 3:	Day 4:	Day 5:	Day 6:	Day 7:
Mageu: Please circle: Full bottle: drank all Empty or half bottle: Please write reason for not eating all of it								
Foods eaten: Please make one tick for each time a food from a group was eaten on a specific day								
1	Grains & starchy veg							
2	Legumes							
3	Nuts and seeds							
4	Dark green leafy vegetables							
5	Dark yellow & orange fruits & veg							
6	Other vegetables (veg)							
7	Other fruits							
8	Eggs							
9	Milk & milk products	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other
10	Meat, poultry, fish							
11	Traditional beer							
Medications								
Drug 1:								
Dosage:								
Why:								
Drug 2:								
Dosage:								
Why:								

If you are experiencing serious symptoms, please contact the study doctor/ follow the adverse event process

Please keep the containers of any medicines you used and bring them with you when you come to the clinic for your next study visit

Week 9: Start date		Day 1:	Day 2:	Day 3:	Day 4:	Day 5:	Day 6:	Day 7:
Mageu: Please circle: Full bottle: drank all Empty or half bottle: Please write reason for not eating all of it								
Foods eaten: Please make one tick for each time a food from a group was eaten on a specific day								
1	Grains & starchy veg							
2	Legumes							
3	Nuts and seeds							
4	Dark green leafy vegetables							
5	Dark yellow & orange fruits & veg							
6	Other vegetables (veg)							
7	Other fruits							
8	Eggs							
9	Milk & milk products	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other
10	Meat, poultry, fish							
11	Traditional beer							
Medications								
Drug 1:								
Dosage:								
Why:								
Drug 2:								
Dosage:								
Why:								

If you are experiencing serious symptoms, please contact the study doctor/ follow the adverse event process

Please keep the containers of any medicines you used and bring them with you when you come to the clinic for your next study visit

Week 10: Start date		Day 1:	Day 2:	Day 3:	Day 4:	Day 5:	Day 6:	Day 7:
Mageu: Please circle: Full bottle: drank all Empty or half bottle: Please write reason for not eating all of it								
Foods eaten: Please make one tick for each time a food from a group was eaten on a specific day								
1	Grains & starchy veg							
2	Legumes							
3	Nuts and seeds							
4	Dark green leafy vegetables							
5	Dark yellow & orange fruits & veg							
6	Other vegetables (veg)							
7	Other fruits							
8	Eggs							
9	Milk & milk products	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other
10	Meat, poultry, fish							
11	Traditional beer							
Medications								
Drug 1:								
Dosage:								
Why:								
Drug 2:								
Dosage:								
Why:								

If you are experiencing serious symptoms, please contact the study doctor/ follow the adverse event process

Please keep the containers of any medicines you used and bring them with you when you come to the clinic for your next study visit

Study Diary – Fermented Foods: Control Group Booklet weeks 8,9 and 10

Participant Identification: _____

Protocol: Mageu

Study Doctor: Dr Heather Jaspan

Phone: 0786839806

Week 8: Start date		Day 1:	Day 2:	Day 3:	Day 4:	Day 5:	Day 6:	Day 7:
Foods eaten: Please make one tick for each time a food from a group was eaten on a specific **day**								
1	 Grains & starchy veg							
2	 Legumes							
3	 Nuts and seeds							
4	 Dark green leafy vegetables							
5	 Dark yellow and orange fruit & veg							
6	 Other vegetables							
7	 Other fruits							
8	 Eggs							
9	 Milk and milk products	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other	Amasi Other
10	 Meat, poultry, fish							
11	 Mageu							
12	 Traditional beer							
 **Medications**								
Medication 1: Name								
Dosage?								
Why taken?								
Medication 2: Name								
Dosage:								
Why taken?								

If you are experiencing serious symptoms, please contact the study doctor/ follow the adverse event process
Please **keep the containers of any medicines you used and bring them with you when you come to the clinic for your next study visit**

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

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Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-submissions@uct.ac.za
Website: www.health.uct.ac.za/home/human-research-ethics

31 October 2024

HREC REF: 901/2024

Dr A Happel
Department of Pathology
Division of Immunology-FHS
Email: anna.happel@uct.ac.za
Student: Janine.fredericks@uct.ac.za

Dear Dr Happel

PROJECT TITLE: THE EFFECT OF MAGEU CONSUMPTION ON SYSTEMIC INFLAMMATION AND NUTRITION IN BREASTFEEDING MOTHERS-SUB-STUDY LINKED TO 004/2022- (MSC CANDIDATE-CLINICAL SCIENCES & IMMUNOLOGY-MRS JANINE FREDERICKS)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 November 2025.

Please submit a progress form, using the standardised Annual Report Form (FHS016) or FHS017 if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Mrs Janine Fredericks will also be involved in this study.

Please quote HREC REF 901/2024 in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR MARC LOCKM N
CHAIRPERSON FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637. Institutional Review Board (IRB) number: IRB00001938 NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP),

National Health Research Ethics Council (2024) South African Ethics in Health Research Guidelines: Principles, Processes and Structures, 3rd ed. Department of Health of South Africa. South African Good Clinical Practice Guidelines (SA GCP 2020), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2024) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.