An investigation into the feasibility and effect of dietary resistant starch supplementation in HIV-positive adults in India.

By

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ABBREVIATIONS

ADR	adverse drug reaction
AE	adverse event
AIDS	acquired immune deficiency syndrome
AIIMS	All India Institute of Medical Sciences
ART	antiretroviral therapy
ASHM	Australasian Society for HIV Medicine
BMI	body mass index
CONSORT	Consolidated Standards of Reporting Trials
CROI	Conference on Retroviruses and Opportunistic Infections
EED	environmental enteric dysfunction
ELISA	enzyme-linked immunosorbent assay
FFQ	food frequency questionnaire
FOS	fructo-oligosaccharide
GALT	gut-associated lymphoid tissue
GI	gastrointestinal
GIT	gastrointestinal tract
GOS	galacto-oligosaccharide
GRAS	Generally Recognized as Safe
HAMS	high amylose maize starch
HIV	human immunodeficiency virus
IAS	International AIDS Society
ICTC	integrated counselling and testing centre
IEC	institutional ethics committee
IgA	Immunoglobulin A
IL	interleukin
ITT	intention to treat

LMICs	low-to-middle-income countries
ME	mixed-effect
MSMs	men who have sex with men
NIHCR	National Institute for Health and Care Research (United Kingdom)
NNRT	non-nucleoside reverse transcriptase
NRT	nucleoside reverse transcriptase
OI	opportunistic infection
OTU	operational taxonomic unit
PI	Principal Investigator
PICF	Participant Information and Consent Form
QIIME	Quantitative Insights into Microbial Ecology
QPE	qualitative process evaluation
RCT	randomised controlled trial
RS	resistant starch
SCFA	short-chain fatty acid
SEM	standard error of the mean
SIV	simian immunodeficiency virus
TasP	treatment as prevention
Th17	T helper 17
TLE	tenofovir–lamivudine–efavirenz
UNAIDS	Joint United Nations Programme on HIV/AIDS
U=U	undetectable = untransmittable
VU	viraemic untreated

DECLARATION

I, Elissa Mortimer, certify that this thesis:

1. does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university;
2. and the research within will not be submitted for any other future degree or diploma without the permission of Flinders University; and
3. to the best of my knowledge and belief, does not contain any material previously published or written by another person except where due reference is made in the text.

A professional editor was used for the purpose of formatting, grammar and style; did not alter or improve the substantive content or conceptual organisation of the thesis; and did not alter errors in primary sources, including graphs, tables, direct quotes and translation.

Signed:

Date: 12th July 2023

Orientation to the thesis

As this thesis is written by the doctoral researcher in the third person, the terminology 'doctoral researcher' is used where required. This thesis is written by the doctoral researcher, Elissa Mortimer, who conceptualised, designed and conducted the study in Bhubaneswar, India. The doctoral researcher implemented the study from commencement until completion of final data collection through both in-person and remote arrangements. She established the study in-person by documenting workflows, training staff and commencing participant recruitment during her three-week field visit to the study site in February–March 2020. Thereafter, she managed the study remotely from Australia. Unless indicated otherwise, the doctoral researcher undertook and completed all tasks and activities for this study, including study design, ethics approvals, study implementation and management, data collection, data analysis and thesis writing and preparation.

PUBLICATIONS BY STUDENT DURING PERIOD OF CANDIDATURE

Peer-reviewed journal articles

1. Alpers DH, Young GP, Tran CD, **Mortimer EK**, Gopalsamy GL, Krebs NF, Manary MJ, Ramakrishna BS, Binder HJ, Brown IL, Miller LV. **Drug-development concepts as guides for optimizing clinical trials of supplemental zinc for populations at risk of deficiency or diarrhea.** *Nutrition Reviews* 2017, 75(3).
2. Gopalsamy GL, **Mortimer EK**, Greenfield P, Bird AR, Young GP, Christophersen CT. **Resistant Starch Is Actively Fermented by Infant Faecal Microbiota and Increases Microbial Diversity.** *Nutrients* 2019, 11(6), 1345.
3. Wang Y*, **Mortimer EK***, Katundu KGH*, Kalanga N, Leong LEX, Gopalsamy GL, Christophersen CT, Richard AC, Shivasami A, Abell GCJ, Young GP, Rogers GB. **The Capacity of the Fecal Microbiota from Malawian Infants to Ferment Resistant Starch.** *Frontiers in Microbiology* 2019, 10(1459). (* Joint first authors)
4. Balamurugan R, Pugazhendhi S, Balachander GM, Dharmalingam T, **Mortimer EK**, Gopalsamy GL, Woodman RJ, Meng R, Alpers D, Manary M, Binder HJ, Brown IL, Young GP, Ramakrishna BS. **Effect of Native and Acetylated Dietary Resistant Starches on Intestinal Fermentative Capacity of Normal and Stunted Children in Southern India**. *Int. J. Environ. Res. Public Health* 2019, 16(20), 3922.
5. De Zylva R, **Mortimer E**, Miller E, Tsourtos G, Lawn S, Wilson C, Karnon J, Woodman R, Ward P. **Efficacy of mindfulness and goal setting interventions for increasing resilience and reducing smoking in lower socio-economic groups: randomised controlled trial protocol.** *Addiction Science & Clinical Practice* 2023, 18(7).

Conference posters

1. **Mortimer EK**, Young GP, Rogers GB, Ward PR. **Effect of oral prebiotic consumption on the gastrointestinal microbiota and immune markers in Human Immunodeficiency Virus (HIV) and Simian Immunodeficiency Virus (SIV) infection: a systematic review.** *2020 Joint Australasian HIV&AIDS and Sexual Health Conferences:VIRTUAL,* 16–20 November 2020.
2. **Mortimer E,** Miller E, Woodman R, Ward P. **The Resilience Interventions for Smoking Cessation Study: A Randomized Controlled Trial Protocol.** *Association of Pacific Rim Universities (APRU) Global Health Conference 2021 – Global Urban Health*, 16–18 November 2021.

3. **Mortimer E**, Tsourtos G, De Zylva R, Ward, P. **The Resilience Interventions for Smoking Cessation ('RISC') study: Increasing resilience and reducing smoking for lower socio-economic groups.** *International Conference on Mindfulness Asia-Pacific*, Melbourne, 15–18 November 2022.
4. **Mortimer EK**, Venugopal G, Agrawal S, Biswal R, Srinivisan N, Ramesh V, Young GP, Rogers G, Ramadass B. **A capacity building initiative to develop research leadership and knowledge transfer between India and Australia as part of a study examining the effect of a dietary prebiotic in people living with HIV.** *The Global Health Network Collections. The Global Health Network Conference Proceedings 2022***,** Cape Town, November 2022. URL: https://tghncollections.pubpub.org/pub/4kbbw2up

DEDICATION

This work is dedicated to my Mum, Rosalind Mortimer, and my daughters, Anjali and Rosa Thapa, who have been by my side throughout the highs and lows of my doctoral journey. Your love sustains me, always.

To my amazing study participants, without whom none of this would have been possible.

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ABSTRACT

Globally, human immunodeficiency virus (HIV) continues to cause disease and loss of life, with 1.5 million people becoming infected in 2021 and 650 000 deaths attributed to AIDS-related illness. In developing countries, HIV disease is likely worsened by a gut malabsorption syndrome caused by unsafe water and inadequate sanitation, compromising health, education, economic and societal outcomes.

The gut microbiota refers to bacteria which live throughout the gastrointestinal (GI) system. HIV disease and antiretroviral therapy (ART) both result in gut microbiota changes. This dysbiosis results in aberrant gastrointestinal function. Correcting these effects will improve morbidity and likely improve adherence to ART, potentially reducing population viral load and decreasing transmission. Dietary supplementation with resistant starch (RS) derived from maize is a low-cost intervention that provides clinical benefit in several gut inflammatory conditions by increasing the production of short-chain fatty acids (SCFAs), improving gut integrity, and reducing bacterial translocation.

The objective of the work described here was to determine the feasibility and effect of RS supplementation in people living with HIV in India, a low-to-middle-income country (LMIC) where malnutrition arising from environmental enteric dysfunction (EED) exacerbates morbidity from HIV and other co-morbid conditions. In addition to an exploratory study of feasibility, it was hypothesised that RS would be fermented in the large intestine by gut microbiota, resulting in increased intestinal SCFAs, and that an associated decrease in pH would be observed. These changes to the colonic luminal environment were postulated to favour gut microbiota with beneficial functions, providing a selective advantage to these bacteria and correcting dysbiosis. This thesis represents an original contribution to knowledge, being the first study using a sole RS supplement in an HIV-positive population.

The feasibility assessment demonstrated that RS is a safe, well-tolerated and acceptable intervention in this population, providing a basis for intervention studies with HIV-positive populations in other settings. While this study did not produce evidence of any effect of RS supplementation on primary or secondary outcome measures, a trend was observed in CD4+ T cell response to RS supplementation that warrants further investigation. These results will inform the design of future studies.

SYNOPSIS

This thesis examines the feasibility and effect of dietary RS supplementation in people living with HIV in India. It aims to address whether a randomised controlled trial (RCT) utilising RS supplementation is feasible in terms of logistics, participant acceptability and tolerability of the intervention. It also considers whether RS supplementation influences outcome measures indicative of fermentation and others associated with improved gut health and HIV immunity.

The gut microbiota consists predominantly of bacterial populations, residing throughout the gastrointestinal tract (GIT), which exert a considerable impact on host physiology and health outcomes[1-3]. HIV and ART both result in gut microbiota changes and aberrant GI function[4-14]. Since the gut microbiota plays an important role in the maturation of gut-associated lymphatic tissue (GALT), secretion of immunoglobulin A (IgA) and production of antimicrobial peptides, its disruption by HIV infection and ART compromises these essential immune functions[15]. Although many of the biochemical cascades that are affected by changes to the gut microbiota in human disease are yet to be elucidated[16-18], adjunct therapies that ameliorate changes in gut microbiota characteristics might provide benefit by reducing GI symptoms of HIV and the side effects of ART[19-21]. Such benefits could promote adherence to ART[19], potentially resulting in reduced morbidity for people living with HIV and a reduced population viral load, with associated decreases in onward HIV transmission risk.

Residents of LMICs frequently suffer from undernutrition and micronutrient deficiency. These conditions arise as a result of food insecurity and frequent GI infections due to suboptimal water and sanitation infrastructure[22, 23]. High levels of exposure to environmental pathogens contribute to the common development of EED, an inflammatory gut pathology syndrome[24, 25]. EED has previously been described as 'tropical sprue', 'environmental enteropathy' and 'tropical malabsorption'[22, 23, 26].

RS is a component of dietary starch found in plant-derived foods. It resists digestion by amylase and protease in the upper GI tract and reaches the large intestine (colon) intact, providing an energy source for certain commensal bacterial clades[27, 28]. RS is classified into five subtypes: RS1, RS2, RS3, RS4 and RS5, as defined by the nature and properties of the RS granule and the methods of production that render them so[29]. RS is a candidate prebiotic[30-32], which, with further evidence, will likely meet the prebiotic definition of: '*a substrate that is selectively utilized by host microorganisms conferring a health benefit*'[33,p.491]. Reviews of previous studies of RS, have reported that it is a preferred substrate for fermentation by the colonic gut microbiota[34, 35]. Beneficial outcomes associated with the by-products of fermentation of RS and other fermentable carbohydrates, are increasingly being shown to confer clinical benefit to the human host[33, 36, 37]. For example, in vitro and in vivo studies have

shown that RS fermentation increases production of butyrate, which provides a major energy source for colonic epithelial cells, contributing to their growth and differentiation[38-44]. The increased concentration of SCFAs in the colonic lumen resulting from RS fermentation[44-47] is associated with a decrease in luminal pH[38, 48] and may also lead to favourable changes in gut microbiota populations[32, 49, 50]. Other reported benefits of RS in animal and/or human models include reduction of cancer risk[51-53]; improvement in insulin sensitivity and postprandial glycaemic response[54-59]; increased fluid re-absorption in the colon with reduced duration of diarrhea[60, 61]; increased mineral absorption[62]; and increased total faecal output accompanied by easier defecation[38]. Although some studies have investigated the effect of dietary prebiotic interventions on gut health (via changes to the colonic luminal environment) and immune measures in people living with HIV[9, 63-69], none have investigated the effect of a sole dietary RS intervention in this population. This represents a knowledge gap of potential relevance to global health efforts in the ongoing fight against HIV/AIDS.

In addition to assessing feasibility, this study was originally intended to determine efficacy of RS supplementation compared to a comparator starch containing negligible quantities of RS. However, as will be addressed by this thesis, COVID-19 interrupted supply chains at the study site, requiring an alternate 'control' starch to be used. Post-hoc analyses indicated that the comparator starch contained a substantial quantity of RS. This led to part of the thesis being reconceptualised as a dose-response trial, and the aim of determining *efficacy* was amended to determining *effect*.

Based on the above, this study explored two overarching research questions:

1. Are RS supplementation studies with HIV-positive populations in India feasible?
2. What is the treatment effect of dietary RS supplementation on the colonic luminal environment and HIV-related immunity in this population?

These two research questions will be addressed using different methodological approaches. A mixed methods approach using qualitative data (from participants and the researcher) will be complemented by quantitative data (self-report tool of participants' gastrointestinal symptoms) to determine whether the proposed supplementation approach is feasible. Assessing the physiological effect of supplementation will be addressed by testing a series of specific hypotheses, as outlined below:

1. That dietary RS supplementation is:
    a. associated with an increase in faecal SCFA concentrations;
    b. associated with a decrease in faecal pH;
    c. associated with changes to microbial abundance and diversity in faecal samples;

d. associated with the following changes to two HIV-related immune measures:
    i. increased concentration of CD4 + T cells in blood;
    ii. decreased HIV viral load in blood.

In summary, this study set out to use a mixed-methods approach to determine feasibility and effect within an RCT study design. Commencing in February 2020, participants were recruited from a tertiary teaching hospital and research facility, the All India Institute of Medical Sciences (AIIMS), Bhubaneswar, India. They consumed a high-RS or moderate-RS dietary supplement for 14-day periods, the order of which was randomised. RS was supplemented to the habitual diet in study foods (wholemeal roti flatbread) which contained either 61.75g of RS/day for the high-RS periods or 8.25g of RS/day for the moderate-RS periods. Supplementation periods were separated by a 'washout' period of normal diet during which participants were instructed to consume their habitual diet with no restrictions. This design reduced the likelihood of a carryover effect, which can occur when the response to a particular treatment is influenced by the previous application of a treatment[70].

Feasibility was determined according to Thabane et al.'s typology for conducting feasibility and pilot studies[71]. This typology informed the CONSORT (Consolidated Standards of Reporting Trials) guideline for randomised pilot and feasibility trials and focused on participant recruitment and retention; adherence and tolerability to protocol and intervention; the practicalities of study resourcing and management; and treatment effect[72, 73]. Data to assess feasibility according to this typology were collected from interviews with participants, field notes recorded by the doctoral researcher and a validated self-report tool focused on GI effects experienced by participants.

To determine treatment effect, faecal samples were collected for the primary outcome measures of pH, SCFA concentrations, and alpha and beta diversity of gut microbiota. Venous blood was collected for the secondary outcome measures of HIV viral load and circulating levels of CD4+ T cells, the global marker of HIV disease progression. The effect of interventions was examined both within and between participants. Due to the typical high variability in gut microbiota between individuals, participants acted as their own controls, with data collected at baseline and at the end of normal diet periods used for this purpose. Both univariate and mixed-effect (ME) multi-level regression analyses were undertaken to determine treatment effects.

Feasibility analysis indicated that protocol adherence by study staff and participants was high. Recruitment pathways utilised for this study were effective. Study foods containing moderate and high doses of RS were well-tolerated in this population, with intervention adherence being

high. Participants who completed the study complied with the requirements for survey completion, blood and stool sampling, and study food consumption. Overall, the feasibility assessment confirmed that RS supplementation studies with HIV-positive populations are feasible.

The examination of the effect of RS supplementation in this population, indicated that there was no significant effect on primary or secondary outcome measures. Future studies could be conducted with an increased sample size to determine effect/efficacy of dietary RS supplementation on pH, SCFA concentration and gut microbial populations of faecal samples and on CD4+ T cell concentration and HIV viral load of blood samples. Among other modifications, controlling for habitual dietary RS intake and using a control starch with zero/negligible RS content will be important for future trials.

This trial was prospectively registered with the Australian New Zealand Clinical Trials Registry (ANZCTR), Trial Id: ACTRN12619000739112.

INTRODUCTION

In the study described in this thesis, the doctoral researcher set out to determine whether RS supplementation studies (1) are feasible and (2) result in a treatment effect in HIV-positive populations living in India, an LMIC. This study builds on previous studies of the impact of HIV and ART on gut microbiota[8-10, 12, 13] and how consumption of a dietary supplement with prebiotic effects, impacts on health[37].

The study was undertaken for a Doctorate of Public Health. Its focus is therefore the application of findings for the prevention of ill-health and the management of chronic conditions at a population level. The results will be explored in terms of how future studies examining dietary supplementation with RS could be expanded and scaled to a population level, with a particular focus on LMICs. It is within this context that dietary RS offers significant advantages because of known benefits in both disease states and the maintenance of health[74, 75], along with attributes such as cost-effectiveness, not requiring a transportation and storage cold-chain, and being acceptable to the general population[19, 76, 77]. These characteristics position RS as an ideal dietary fortificant of staple foods if further evidence supporting a health benefit is confirmed.

In this chapter, the detailed background to this thesis will be set out, including the rationale for conducting an RS supplementation study in an HIV-positive population. It will explore why it was of particular interest to conduct the study in India.

Clinical context

Global burden of disease of HIV

In 2021, 1.5 million people acquired HIV globally and 650,000 deaths were attributed to AIDS-related illness[78]. Key sub-populations that are over-represented in the HIV pandemic include men who have sex with men (MSMs); sex workers and their clients; people who inject drugs; transgender people; and sexual partners of these populations[78]. In LMICs, females in heterosexual relationships are also over-represented[78], creating a risk for unborn children if ART use is not commenced early or adherence to ART is not maintained. Globally, of the 38.4 million people living with HIV, an estimated 28.7 million were accessing ART in 2021[78].

While the prevalence of HIV is still highest in eastern and southern Africa, with 20.6 million people living with HIV in 2021, countries in the Asia Pacific region are also significantly affected, with 6 million people living with HIV[78]. In India, there were an estimated 2.4 million HIV-positive people in 2021, 1.9 million of whom were aware of their HIV status[79]. Of these,

1.6 million were taking ART and 1.3 million were virally suppressed, defined as having an HIV viral load <200 copies per mL of venous blood[79, 80].

Natural history and symptomatology of HIV

The physiological and immune aspects of HIV disease will be discussed in the following section, providing a justification for this study to examine the potential benefit for RS in HIV-positive populations.

HIV disease is caused by the human immunodeficiency virus, a lentivirus which causes progressive disease after unusually long periods (months or years) of subclinical infection[81, 82]. HIV is transmitted when an uninfected person is exposed to the blood or other bodily fluids (e.g., semen, breastmilk) of an HIV-infected person[78]. This transmission can occur through sexual intercourse, the sharing of injecting equipment, blood transfusions where the blood has not been adequately screened for HIV, perinatally during pregnancy or birth, and via breastfeeding[78].

The natural history of HIV is characterised by chronic depletion of CD4+ T cells during viral replication[83]. CD4+ T cells are central to cell-mediated immunity via their role in coordinating the immune response, by stimulating macrophages, B lymphocytes and CD8 T lymphocytes to fight infection[84]. CD4+ T cell count is used globally as the agreed standard of immune function in HIV and, together with HIV viral load, is used to indicate HIV disease progression[85, 86]. A decline in CD4 + T cell concentration in blood and tissue results in increased susceptibility of the host to opportunistic infections (OIs). These OIs are the hallmark of progressive HIV disease and define acquired immune deficiency syndrome (AIDS)[83].

During the initial viraemic stage, HIV RNA is detected in plasma, leading to the seroconversion stage when HIV antibodies are detectable in the newly infected person, usually between three and six months after exposure to the virus[83]. This primary HIV infection stage is typified by replication of HIV within infected cells, with resultant elevated levels of circulating virus. In about 50% of cases, this period is associated with viraemic symptoms typical of a febrile illness, including joint and muscle pain, fatigue, diarrhea, swollen lymph nodes, sore throat and rash[83]. The development of HIV-specific antibodies enables the body to achieve a ‘steady state’ or viral load set point which influences patient morbidity and onward transmission risk[87, 88]. During this phase, individuals are commonly asymptomatic for several years. The ongoing destruction of CD4+ T cells ultimately leads to susceptibility to pathogens and the development of OIs and other complications of AIDS, including nephrotic syndrome, cancer and dementia, eventually presenting a high risk of mortality if not managed with ART[83].

Effect of HIV infection on gastrointestinal physiology and microbiota

Initial HIV infection is commonly established in the gut, targeting intestinal CD4+ T cells in the GALT (**Figure 1**)[89]. The GALT is the collection of lymphoid tissues that protect the body from translocation of microbes from the gut lumen into systemic circulation, and includes Peyer's Patches and isolated lymphoid follicles[90]. The initial localisation of HIV reservoirs in the GALT is true regardless of the route of transmission – be it sexual, vertical (mother to child) or intravenously, such as via injecting drug use[91]. HIV-infected CD4+ T cells in the GALT then lead to HIV infection into other cellular compartments, during both initial and chronic infection[92, 93]. The normal function of the GALT involves priming and differentiation of adaptive immune cells[94]. Disruption of this normal function may therefore compromise the maturation of immune cells and associated sequelae.

Figure 1 illustrates the impact of immune disruption on inflammation of the mucosal lining of the GIT and impairment of the epithelial barrier[95, 96]. The assault by HIV on the gastrointestinal system occurs as T helper 17 (Th17) cells, generated from CD4+ T cells, are systematically depleted[97-99]. One of the primary homeostatic functions of the Th17 pathway is preservation of the epithelial lining of the gut[100]. The mechanistic pathway leading to inflammation of the mucosal lining involves mucosal epithelial cells responding directly to the envelope glycoprotein of HIV-1 by upregulating inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-8 (IL-8)[101, 102]. Activation of the kynurenine pathway of tryptophan catabolism is also understood to lead to weakening of the epithelial barrier during HIV infection, via the indoleamine 2,3-dioxygenase 1 (IDO1) pathway[103-106]. This results in impaired barrier function in the tight junctions of the gut mucosa[101]. The resultant increased permeability of the gut, in combination with the ongoing depletion of Th17 CD4+ T cells, results in increased rates of translocation of bacteria from the lumen across the epithelial barrier, referred to as bacterial (or microbial) translocation[97, 107]. Translocation of HIV virus across the epithelium also occurs[101]. The increased circulation of bacteria and virus in blood and lymphatic systems is thought to further activate the systemic immune system, leading to and exacerbating morbidity in HIV-positive people, including those on ART[89, 91, 107, 108]. There is some evidence from murine models that disruption of the gut immune barrier also contributes to dysbiosis of the gut microbiota via disturbance of microbial pattern recognition sensors[103, 109, 110].

A greater dominance of species with pathogenic potential (e.g., *Pseudomonas aeruginosa* and *Candida albicans*) and reduced levels of commensal bacteria with beneficial functions, such as *Bifidobacteria* and *Lactobacillus,* have been reported in HIV-positive populations[8-10, 111]. HIV infection has also been observed to cause enrichment of Enterobacteriaceae and *Prevotella* species in HIV-positive populations with decreases in *Bacteroides* species[112-114].

This resultant dysbiosis in the gut lumen interferes with control of homeostatic immune responses by the microbiota and further compromises mucosal barrier function[113, 115]. Several peer-reviewed articles have observed that HIV infection is associated with a reduction in microbial diversity in faecal samples, which is not restored by ART[111, 112]. With these impacts of HIV on gut microbiota, interventions which reduce dysbiosis are warranted[19].

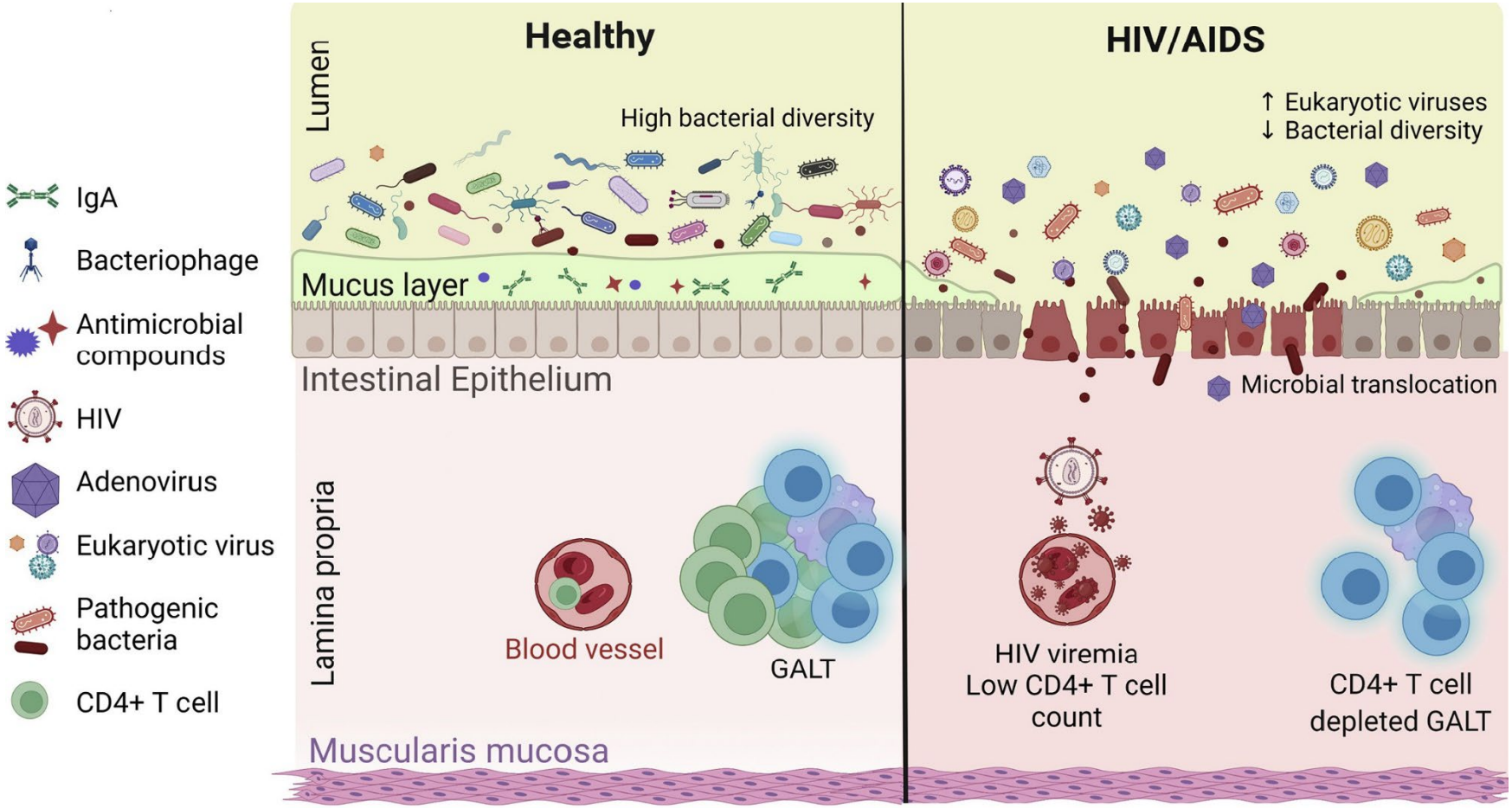

Figure 1: Bacterial, viral and immune interactions in the gut lumen, epithelium, lamina propria and GALT in healthy and HIV disease models[89].

ART in the management of HIV infection

Although ART is a life-saving treatment, it does cause side effects that impact on the quality of life of HIV-positive populations. The effects of ART on physiology, immunity and gut microbiota are explored in the following sections.

Since the approval of the nucleoside reverse transcriptase (NRT) inhibitor, zidovudine, for the treatment of HIV in 1987, four other classes of antiretroviral drugs have been developed to treat HIV:

- non-nucleoside reverse transcriptase (NNRT) inhibitors;
- protease inhibitors;
- integrase inhibitors (INSTI); and
- entry/fusion inhibitors[83].

While ART has been hugely successful in extending life expectancy by preventing progression from HIV infection to AIDS, the immune system is limited to a partial restoration only[116].

Treatment as prevention

In terms of the importance of improving ART adherence to decrease HIV transmission, a pivotal event occurred in 2008 when the 'Swiss Statement' was released[117]. This statement asserted that HIV is not transmissible during condomless sexual intercourse between an HIV-positive person with an undetectable viral load and an HIV-negative individual[117] and was made on the basis of resounding evidence in support of the 'Undetectable = Untransmittable' (U=U) claim[118-120]. Although there is yet to be a globally consistent adoption of the U=U claim in other HIV risk contexts, such as breastfeeding[121, 122], public health advocates have been calling for broader adoption of the treatment as prevention (TasP) model, shown to be markedly effective in reducing onwards transmission of HIV as well as reducing stigma and discrimination[123, 124].

Effect of ART on gastrointestinal physiology and microbiota

Despite the rapid improvement of ART for HIV over the past 40 years, even patients who are virally suppressed owing to consistent ART use can still experience symptoms of HIV generated in the GALT and mucosal lining of the gut[125]. Following HIV infection, the gastrointestinal mucosa continues to act as a reservoir of HIV RNA, despite the success or failure of viral suppression in patients on ART, as well as in people living with HIV who have never used ART (ART-naïve)[126]. Some authors, however, have reported that almost complete restoration of mucosal immune systems is evident when ART is initiated early[92].

Following acute initial infection with HIV and perturbation of the gut microbiota, ART regimens may at least partially restore the gut microbiota towards a profile more similar to that of HIV-negative populations[14]. However, in terms of this re-shaping of the gut microbiota, other studies have observed that individuals on ART often resemble ART-naïve populations rather than HIV-negative controls[13]. A study analysing faecal microbiota following 12 months of ART showed that microbiome diversity in HIV-infected patients was not restored even when ART successfully suppressed HIV viraemia[12]. The implication of this is that gut microbiota alterations initiated by HIV infection could contribute to the development of co-morbidities that occur and endure even when ART suppresses HIV viraemia[13]. Some studies have indicated that ART use is associated with a gut microbiota that is less able to be influenced by strategies to restore the gut microbiota. For example, the gut microbiota of HIV-positive people taking ART was shown to be less changeable following a dietary prebiotic supplement than it was in ART-naïve individuals[64].

With a diverse suite of ART regimens available, different regimens have been associated with variable responses in gut microbiota composition[14]. This variability relates to different mechanisms of action between regimens in their capacity to reduce proviral DNA[14, 127]. For example, integrase inhibitor-ART regimens such as raltegravir, have been associated with levels of systemic inflammation, bacterial translocation and microbial diversity similar to HIV-negative populations, and typically show reduced dysbiosis of the gut microbiota, likely leading to fewer HIV-related complications[14]. One of the ART regimens frequently used in first-line management of newly diagnosed HIV-positive patients, tenofovir–lamivudine–efavirenz (TLE), is comprised of two NRT inhibitors (tenofovir and lamivudine) with an NNRT inhibitor (efavirenz)[128]. TLE is the dominant first-line treatment regimen in many LMICs[129, 130]. This was the case in the study examined in this thesis, where all study participants were taking TLE, a regimen that has been shown to partially restore alpha-diversity of bacterial populations in the gut[14]. However, when compared to individuals on other types of ART, such as ritonavir-based protease inhibitor regimens, NRT–NNRT regimens have been associated with significantly lower levels of gut microbiota alpha diversity[12].

The effect of ART on gastrointestinal microbiota and physiology leads to clinical side effects which influence treatment adherence[131-135]. For example, diarrhea is highly prevalent in HIV-positive populations, impacting on nutritional status and ART adherence[19, 136]. In a meta-analysis, Al-Dakkak et al. found that patients with clinical effects described as adverse events (AEs) were less likely to be adherent with ART compared to patients not experiencing AEs[134]. Of the AEs reported, several affected the gastrointestinal system – including nausea, which was significantly associated with reduced adherence (OR 0.574; 95% CI: 0.427–0.772)[134]. A study which examined adverse drug reactions (ADRs) for specific ART regimens reported that

46% of total ADRs were in participants taking the TLE regimen[137]. Further to this, tenofovir-based regimens, such as TLE, were associated with increased likelihood of grade 1–4 diarrhea compared to placebo[138]. Treatment adherence is of paramount importance for both the individual in terms of HIV-related morbidity[139], and the broader population, because of an increased onward transmission risk when viral replication is not suppressed[134, 140]. Given the widespread use of first-line ART regimens in LMIC[129, 130] and the considerable gastrointestinal morbidity which they cause[135], there is a strong case for adjunct treatments to reduce these side effects[19].

Environmental enteric dysfunction – implications for HIV and ART

EED adds a further complication affecting gut physiology, microbiota balance and ART effectiveness in HIV-positive populations[141, 142]. Owing to suboptimal water and sanitation infrastructure, LMIC residents are chronically exposed to environmental pathogens which weaken the mucosal barrier of the gut, resulting in malabsorption[22, 143]. The resultant repeated infections of the gut are the hallmark of EED[144-146] and are associated with the following symptoms:

- compromised gut integrity;
- macronutrient undernutrition and micronutrient deficiencies;
- bacterial translocation;
- dysbiosis; and
- inflammation[22, 23, 25, 143, 144, 147, 148].

The effect on immune-compromised individuals, such as people living with HIV, is particularly dire, considering the compromised Th17 pathway described above[4, 97, 100]. Based on the reported detrimental effect of EED on oral vaccine efficacy[26, 144, 149], it is also likely that EED-related malabsorption impacts ART efficacy in HIV-positive populations. The triple-burden on gut health of HIV infection, ART use and co-morbidity with EED provides a strong justification for examining the effect of dietary RS supplementation in an HIV-positive population residing in an LMIC.

Understanding the knowledge gap

In addition to the public health concerns regarding reduced ART adherence and efficacy, it is essential to consider how pharmacological management of HIV symptoms and ART side effects affects the quality of life for people living with HIV and how adjunct treatments might improve this[132]. The broad adoption of ART has led to reduced morbidity and mortality for HIV-positive people[116, 150, 151]. People living with HIV attain a similar life expectancy as the general population, albeit with significant co-morbidity occurring as a result of HIV infection and the

medications required to manage it[152, 153]. An elevated level of polypharmacy, defined as the use of five medications or more for chronic conditions[154, 155], has been noted in this population[156] and has been shown to impact on adherence to ART and other medications[152, 153, 157]. This provides further justification for considering non-pharmacological adjunct therapies to improve the gut microbiota and intestinal barrier function in HIV-positive populations[19, 158]. Possible adjunct therapies currently being researched include dietary probiotics, synbiotics and prebiotics[19, 159].

Although some studies have tested the effect of dietary prebiotics or synbiotics in HIV-positive populations, none have considered the effect of a sole RS intervention. Very few studies have examined the effect of prebiotics in people living with HIV in LMICs, where EED is assumed to be highly prevalent. Nor have any studies considered the feasibility of conducting RCTs using RS supplementation in an HIV-positive population. This study therefore addresses the gap in the evidence base regarding the feasibility and effect of sole RS interventions in people living with HIV in populations residing in LMICs.

RS and prebiotics as adjunct therapy

A prebiotic is defined as *'a substrate that is selectively utilized by host microorganisms conferring a health benefit'* [33 p.491]. Although yet to be formally defined as a prebiotic under the current definition, RS may be categorised as a prebiotic in the future with additional evidence[33, 160].

The rationale supporting RS as a dietary supplement in an HIV-positive population is the observed effects of RS and prebiotic supplementation in other populations in both healthy and diseased states. One of these effects is increased SCFA production as a by-product of colonic fermentation[38, 41, 64]. SCFAs, defined as having six or fewer carbon atoms, are produced by microbial fermentation of indigestible carbohydrates in the colon[161, 162]. The major SCFAs produced by this process are acetic acid, propionic acid and butyric acid, all of which provide an energy source for colonocytes and hepatocytes[162]. These metabolites have an important impact on host metabolism via their role in the regulation of appetite[163, 164], insulin sensitivity and energy expenditure[165].

Amongst multiple beneficial effects[166], SCFAs can reduce gut inflammation[167], with one of the SCFAs, butyrate, known to be particularly beneficial owing to its role in enhancing intestinal barrier function and mucosal immunity[39, 166, 168]. As HIV infection and replication in the GALT compromises intestinal barrier function and mucosal immunity[4, 102, 169], it is hypothesised that increasing production of butyrate through dietary supplementation with RS will attenuate the epithelial lining damage and associated dysbiosis caused by HIV replication in the GALT[39, 64]. There is also a likely benefit to gut health from RS supplementation based on the observed

role of prebiotics in selectively stimulating the growth of bacteria with beneficial functions in the colon, such as *Bifidobacteria* and *Lactobacilli* [33, 170]. Since the gut microbiota has a key role in regulating immune function[19], interventions that reduce dysbiosis through the stimulation of bacteria with beneficial functions may provide benefit by modulating innate and acquired immunity in the host[19]. Although mechanisms of action of RS and prebiotics via microbiota-mediated pathways are yet to be comprehensively delineated, various authors have proposed that prebiotics, and fermentable carbohydrates more generally, might provide benefit, based on evidence from lentiviral models[9, 19, 63, 64, 171].

The rationale underpinning the anticipated benefit of dietary RS supplementation is also based on the effects observed for prebiotics, such as fructo-oligosaccharide (FOS), galacto-oligosaccharide (GOS) and inulin in people living with HIV. These include:

- attenuation of HIV-associated dysbiosis[9, 64, 67];
- changes in production of butyrate and associated increases in abundance of *Faecalibacterium* and *Lachnospira* [64];
- decline in bacterial translocation, as evidenced by a reduction in soluble CD14 (sCD14)[9, 64, 172];
- reduction in bacterial DNA concentrations in plasma[63];
- increase in CD4+ T cells[63]; and
- improved natural killer cell activity[9].

Study rationale

Choice of intervention

The role of various types of RS in providing an energy source for large intestinal bacteria, and associated benefits, has been studied previously[173, 174]. RS has been shown to be a preferred energy source of butyrate-producing bacteria, such as those from the phylum Firmicutes and the order Clostridiales, as well as *Eubacterium, Roseburia*, *Anaerostipes*, *Clostridium*, *Ruminococcus*, *Coprococcus* and *Butyrivibrio* species and those in the families *Veillonellaceae* and *Thermoanaerobacterales* [39, 41]. The growth of butyrogenic bacteria is considered to confer a significant health advantage, since butyrate is a preferred energy source for colonic epithelial cells[34, 35, 39, 175, 176]. More broadly, SCFAs generated by fermentation of RS in the large intestine provide benefit to the human host by regulating innate immune cell activity and positively affecting defense mechanisms[177].

A further potential benefit of RS supplementation is a reduced osmotic pressure on the gut compared to oligosaccharide-based prebiotics such as FOS and GOS[178, 179]. This osmotic pressure, in conjunction with the rapidity of fermentation of FOS and GOS, results in

abdominal distension, both in disease states such as Irritable Bowel Syndrome, as well as in healthy individuals[180, 181]. Owing to the known effect of fructose on abdominal symptoms[182] and the high prevalence of fructose intolerance in both adults and children[183], FOS and GOS supplementation is not generally recommended by gastroenterologists and dietitians. Owing to its slower fermentability, RS has been shown to result in lower levels of total gas production, thus improving its tolerability profile compared to oligosaccharides[184]. RS has also been shown to have favourable effects on laxation in terms of faecal bulk and transit time[40, 185]. RS was therefore chosen as the interventional agent for this thesis owing to its anticipated effect and tolerability profile.

High Amylose Maize Starch (HAMS) is a source of RS that has been used in food manufacturing for decades, as it improves the development of a crisp texture in baked products and adds fibre without the negative organoleptic properties associated with increasing fibre content[186-188]. The US Food and Drug Administration previously certified HAMS with GRAS (Generally Recognized as Safe) status, determining it to be safe for human consumption[186, 189, 190]. HAMS contains approximately 42% RS[191, 192] and is an RS2-type derived from selectively bred maize starch varietals[46, 160]. This selective breeding increases the amylose-to-amylopectin ratio, which encourages RS formation during the cooling process[29, 160, 193]. RS2 starches are defined by their tightly packed amylose structure that forms a crystallised molecule which resists digestion because of its granular configuration[29, 194]. Food sources of RS2 include high-amylose corn, as well as green bananas, potatoes and legumes[29]. Hylon VII, a non-genetically modified HAMS product used in this study, is one of various types of HAMS which have been used in the commercial baking industry[195].

Overall, the safety[196], cost-effectiveness and field-friendly characteristics of RS[197] provided ample justification supporting it as the interventional agent in this thesis. Indeed, dietary RS supplements have previously been used in studies with children and adults in LMICs and were found to be well tolerated[49, 198, 199]. Studies into the effect of starches with prebiotic effects in people living with HIV are warranted[159, 200, 201], particularly in LMICs where the additional impact of EED on gut health is high.

Choice of population

The site chosen for this study was Bhubaneswar, India, owing to the higher burden of EED, compared to high-income countries[202]. Many issues present barriers to health for HIV-positive populations in LMICs – including food insecurity and poverty, along with their intersectionality with other social determinants of health, such as stigma and discrimination, gender, access to health services, language and literacy[203]. In many LMICs, such as India, there is also often a higher prevalence of HIV disease and more barriers to ART adherence, such as cost, distance

to health services, and fears around the disclosure of HIV status to family members and employers[204]. Controlling transmission rates of HIV in LMICs is particularly important given the enormity of the total population and the hard-to-reach sub-populations at risk of infection, including people who inject drugs, men who have sex with men, sex workers, migrants and itinerant workers such as truck drivers.

Study aims and overarching rationale for chosen outcome measures

The following study aims were formulated to address the gap in the literature identified above:

1. to determine whether an RS supplementation study with HIV-positive people in India is feasible; and
2. to determine whether RS supplementation is associated with treatment effect(s) relating to gut health and HIV-related immunity.

Study Aim 1 – Feasibility

To address Study Aim 1, feasibility was assessed according to the typology defined by Thabane et al.[71]. This typology describes four reasons for conducting pilot studies and assessing feasibility, as summarised below:

1. **Process:** Assesses the practicality of the tasks that need to take place as part of a future main study.
2. **Resources:** Assesses considerations such as time, budget and other resources.
3. **Management:** Addresses staffing and data management issues with a view to optimising identified problems and bottlenecks.
4. **Scientific:** Assesses the safety of the intervention, determines dosage levels and response, and estimates treatment effect and its variance[71].

Based on the literature and the importance of delineating objectives for feasibility studies[73], this study will use the following tools to assess feasibility:

- Qualitative semi-structured interviews to explore barriers and enablers to participation in the study. This maps to the Process category of the Thabane typology.
- Qualitative process evaluation (QPE), a term coined by Molloy et al. (2022), to be based on field notes taken by the doctoral researcher examining how the study could be improved for future trials[205-208]. The field notes include reflections of the doctoral researcher both while she was in 'the field' during her three-week stay in India and while she was remotely managing the RCT from Australia. This approach to embedding of qualitative data collection within RCT designs has been utilised

extensively by other health researchers[209-211]. Data collected using this method map to the Process, Resources and Management categories of the Thabane typology.

- Signs and Symptoms Checklist for HIV-gastrointestinal subset (SSC-HIVrev)[212]. A validated tool to quantify gastrointestinal side effects reported by participants. This maps to the Scientific category of Thabane et al.[71].

The assessment of treatment effect, included within Thabane's Scientific category, will be examined in detail via the treatment effect sub-study of this thesis.

Study Aim 2 – Treatment effect

To address Study Aim 2, the primary outcome measures of faecal concentrations of pH and four SCFAs – acetate, butyrate, propionate and valerate – were chosen as a basis to determine (1) whether intestinal fermentation of RS occurred in an HIV-positive population; and (2) whether RS supplementation resulted in increased concentrations of SCFAs. As evidence of a direct effect of fermentation, pH was chosen as the primary outcome that informed the sample size calculation[38]. The rationale for utilising these SCFAs as primary outcome measures was that these metabolites of fermentation indicate that RS fermentation has occurred[30, 46], are associated with decreases in faecal pH[38, 213] and are implicated in the hypothesised mechanism of action[39, 64]. If the hypothesised increase in SCFAs and decrease in pH is observed, then this indicates that fermentation of RS occurs in people living with HIV, as evidenced in other populations. SCFAs are detectable in the intestinal lumen, faecal samples and circulatory system[162]. Faecal SCFAs and pH assays provide a convenient and cost-effective method of determining if colonic fermentation has occurred and have been used frequently in dietary supplementation studies using non-digestible carbohydrates[38, 40, 213, 214].

Diversity of gut microbial populations as measured by alpha diversity (including relative abundance) and beta diversity were also included as primary outcome measures. These terms are defined as follows:

- Alpha diversity: measures diversity *within* samples to answer the following two questions:
  - How many microbes?
  - How are the microbes balanced to each other?
- Beta diversity: measures differences between samples to answer the question:
  - How different is the microbial composition in one environment compared to another?[215]

Blood HIV viral load and circulating CD4+ T cell concentration were included as secondary outcome measures to determine treatment effects relating to HIV-related immunity. Lower

CD4+ T cell count equates to a higher level of immune compromise and a more significant degree of disease progression whereas a higher viral load indicates HIV disease progression[83].

Rationale for the mixed-methods approach

In terms of the epistemological alignment of this mixed-methods study and how the qualitative data will be interpreted to determine meaning, a realist pragmatic approach was utilised[216, 217]. Pragmatic approaches offer an alternative to both theory-driven (theory-first) and grounded theory (theory after) approaches[217-219]. This encourages consideration of the different types of data in relation to each other, such as the qualitative findings being considered in light of the quantitative data, and vice versa, to specifically consider what it is about the RS intervention that works and for whom, in what circumstances, in what respects, and why[216]. Noting that the feasibility assessment is based on both qualitative (participant interviews and field notes) and quantitative (validated tool for gastrointestinal effects) data and that the treatment effect is assessed by quantitative data, this moving back and forth between different types of data using a pragmatic approach is essential to determine justification for future larger-scale studies.

In relation to evaluations that assess the practical nature of studies and whether the logistical and process aspects are able to be carried out, there is consistent acknowledgement of their importance[216, 220]. Based on the 'Outcome = Mechanism + Context' formula, an outcome comprises an understanding of not just the mechanism but also the process or context within which the outcome was observed[221, 222]. For example, without careful consideration of the context, it is not possible to discern if a lack of hypothesised outcome resulted from the intervention itself or from a failure in the *delivery* of the intervention[208, 223]. The importance of process evaluations in effective public health research justifies the focus on assessing feasibility in this study[224-226].

Significant original contribution to knowledge

The significant original contributions to knowledge resulting from this doctorate are as follows:

- This study determines the feasibility and treatment effect of a sole RS dietary supplement in an HIV-positive population in India, an LMIC with an assumed high prevalence of EED. An intervention of this type has not been tested to date in this population, making this the first study of its kind.
- Given the unanticipated interruption of COVID-19 on the delivery of the study, this study also adds significant and original knowledge about the delivery of RCTs

within the context of lockdowns and the risk management required to minimise exposure to COVID-19 for this immune-compromised participant cohort.

- This study describes challenges relating to remote management of a dietary supplementation RCT, which will inform future public health research.

This research study was justified because of:

- the beneficial effects of RS observed in other populations[35, 38, 40, 60, 61, 227-230]; and
- the gap in sole RS supplementation studies in HIV-positive populations where EED is assumed to be endemic.

The findings of this study will contribute to:

- the growing body of research in the functional food area, with a specific application in people living with HIV. Since the burden of HIV disease disproportionately affects LMIC populations[78], which also have higher rates of EED[25], research on the potential benefit of functional food ingredients is justified;
- the development of public health policy, management and practice by enhancing TasP approaches for HIV. This is based on the importance of adjunct therapies to facilitate ART adherence in this population[19, 20];
- the development of approaches for feasibility testing of studies using dietary RS supplementation;
- the evidence base regarding RS content of habitual diets, HIV immune response to RS supplementation, and gastrointestinal tolerance of RS supplementation in this population;
- understanding of the barriers and enablers to management of a complex RCT during the COVID-19 pandemic, which will inform future trials, increasing efficiencies by articulating what worked and what did not; and
- understanding the experience of the participant cohort, including factors that motivated participation and sustained study involvement. It provides insight as to how the eligibility criteria enabled the selection of participants and associated factors that impacted on recruitment rates. It reports findings relating to recruitment pathways which can be generalised to future studies – not only those focused on HIV-positive populations, but also those with other populations in the context of India and other LMICs.

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LITERATURE REVIEWS

Background to the literature reviews

Two systematic reviews of the literature were undertaken to inform the current study. These were focused on determining the current evidence base and associated knowledge gaps in relation to two issues: the (1) feasibility and (2) treatment effect of studies examining RS and dietary prebiotic interventions (administered either alone/with other prebiotics or with a probiotic as a 'synbiotic'[†]) in HIV-positive populations. The rationale for conducting two separate literature reviews relates to the divergence of the two areas in terms of their outcome measures. Furthermore, evidence relating to the treatment effect outcomes are informed by non-human mammalian models such as simian immunodeficiency virus (SIV), whereas the feasibility literature was focused on studies with human participants only. The two search strategies did return some of the same articles, reflecting the high proportion of pilot/feasibility studies in this emerging field.

Feasibility Literature Review

Aim

The aim of the literature review examining feasibility was to analyse methods and findings from studies that administered dietary prebiotic, synbiotic or interventions containing RS, in HIV-positive populations that examined any measures of feasibility. Studies were included if they were described as 'feasibility' or 'pilot' studies, terms that are considered interchangeable within this thesis, as described below. Studies that focused on efficacy/effect only, without any feasibility objectives, were excluded.

[†] Probiotics are defined as *'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host'* [231]. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature reviews Gastroenterology & hepatology. 2014;11(8):506-14., p.506. A prebiotic is defined as *'a substrate that is selectively utilized by host microorganisms conferring a health benefit'* [33]. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Ibid. 2017;14:491-502., p.491. A synbiotic is a combination of a probiotic and prebiotic and is defined as *'a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host'* [232]. Swanson KS, Gibson GR, Hutkins R, Reimer RA, Reid G, Verbeke K, et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. Ibid. 2020;17(11):687-701., p.687.

Terminology

Many authors have broached the overlap in terminology relating to 'pilot' and 'feasibility' studies[71, 233-237]. Although these terms are frequently used interchangeably, some authors argue that they are distinct and should be treated as such[233-235]. It is generally agreed that both terms refer to studies undertaken in preparation for larger RCTs, which will be sufficiently powered to determine the effect and efficacy of an intervention[235]. Some authors consider that a distinction exists for the term 'pilot', used to indicate small-scale studies designed to test the function and connection of various study components to inform future larger-scale RCTs[234, 235, 238]. 'Feasibility' studies are often described as enabling (1) an assessment of parameters such as participant compliance; and (2) an estimate of effect sizes required for design of the main study[234]. The UK National Institute for Health and Care Research (NIHCR) reflects the broad definitions previously stated by other authors[233-236]:

> ***Feasibility Studies*** *are pieces of research done before a main study in order to answer the question 'Can this study be done?'. They are used to estimate important parameters that are needed to design the main study.*[238, n.p.]
>
> ***Pilot studies*** *are a smaller version of the main study used to test whether the components of the main study can all work together. It is focused on the processes of the main study, for example to ensure that recruitment, randomisation, treatment, and follow-up assessments all run smoothly.*[238, n.p.]

The work of Eldridge et al. in establishing agreed definitions of these terms informed the development of a CONSORT extension method for reporting pilot and feasibility studies specifically[73]. These authors assert that 'pilot trial' is interchangeable with 'feasibility study'[73]. This thesis incorporates elements of both proof-of-concept and randomised feasibility study approaches[73]. Proof-of-concept studies are considered a type of feasibility study[73] in that they focus on assessing the feasibility of future research with an interventional agent[239] – in the case of this thesis, RS as HAMS. Although pilot and feasibility studies are not usually intended to be statistically powered to determine efficacy or effect[236], some authors have implemented these studies for this purpose[240]. There is general agreement that, whether statistically powered or not, outcome measures from feasibility and pilot studies can still be used to inform sample size calculations for future studies[234, 236].

Scope

While many studies assess feasibility based on a limited number of quantitative outcome measures – such as recruitment/retention, safety, and adherence/tolerability measures – other approaches are also used[73]. For example, in order to capture data focused on where

refinements are required ahead of future trials, qualitative aspects (sometimes described as process evaluations) are also incorporated into feasibility studies[73, 241-244]. Qualitative approaches can either be incorporated within pilot studies that are focused on estimating effect size[240] or reported independently[243, 244]. In addition to quantitative measures of feasibility, the literature was therefore also examined to identify studies that used process-related measures. This review will examine all aspects of feasibility assessments reported in articles identified by the below selection criteria and will report on both methods and findings to inform the current thesis.

Method

Three databases used frequently in the public health field (PubMed, Scopus and Web of Science) were searched for articles according to the following selection criteria.

Inclusion criteria

- Articles examining dietary RS or prebiotic interventions (either as a sole agent or with a probiotic as a 'synbiotic') in an HIV-positive population which were described as a 'feasibility study/trial' or 'pilot study/trial' and/or included a 'feasibility' analysis (defined as reporting on factors included in Thabane et al.'s typology[71]);
- Any geographic location; and
- Primary experimental research papers published in peer-reviewed journals.

The search was restricted to English-language publications owing to the costs associated with translation and a limited overall budget for the study. The publication date was not restricted in order to increase the sensitivity of the search and increase the numbers of potentially available documents.

Exclusion criteria

- Studies using a sole probiotic intervention;
- Review papers;
- Protocol papers;
- Non-human studies; and
- Studies focused on efficacy/effectiveness only without any feasibility objectives.

The specified search terms are provided below:

PubMed – ((“human immunodeficiency virus” OR “HIV”) AND (prebiotic* OR “resistant starch*” OR synbiotic*) AND diet* AND (feasibility OR pilot))

Scopus – TITLE-ABS-KEY (((“human immunodeficiency virus” OR “hiv”) AND (prebiotic* OR “resistant starch*” OR synbiotic*) AND diet* AND (feasibility OR pilot)))

Web of Science – ((“human immunodeficiency virus” OR “HIV”) AND (prebiotic* OR “resistant starch*” OR synbiotic*) AND diet* AND (feasibility OR pilot))

The screening process involved reading article titles and abstracts to identify if eligibility criteria were met. The eligibility shortlisting process involved reading the full text of each article. Following the screening and eligibility shortlisting process, the doctoral researcher and one other public health colleague conducted independent assessments of the methodological validity of articles to determine if they should be included in the review. Standardised critical appraisal instruments from the Joanna Briggs Institute Meta-Analysis of Statistics Assessment and Review Instrument (JBI-MAStARI) were used for this purpose[245]. If overall appraisal according to the JBI-MAStARI instrument met the *Include* criteria, articles were incorporated into the literature review.

This literature review adopted the typology for feasibility and pilot studies proposed by Thabane et al.[71], which has been applied by other authors such as Tickle-Degnen[236]. In the below review, feasibility findings from articles returned by the search strategy will be assigned to the four categories of the Thabane typology – Process, Resources, Management and Scientific[71].

Results

The PRISMA diagram provided at **Figure 2** shows the article selection process and outcome. Six studies were included in the Feasibility Literature Review.

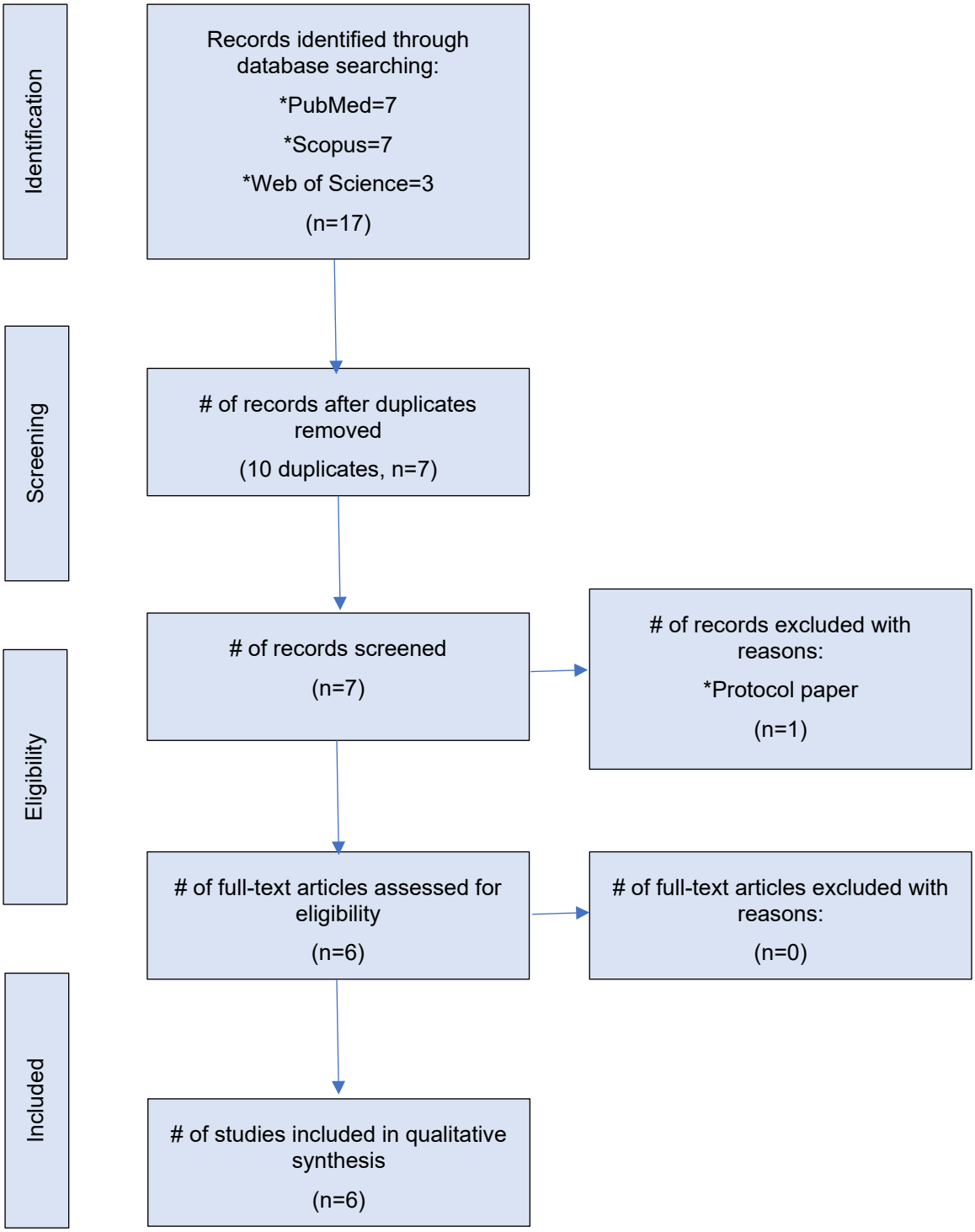

Figure 2: PRISMA diagram: HIV and dietary RS/prebiotic interventions – Feasibility Literature Review.

Table 1 summarises the key aspects of the papers. One study utilised an intervention containing only prebiotics, comprised of long-chain FOS and short-chain GOS with pectin hydrolysate-derived acidic oligosaccharides[9]. Remaining articles used prebiotic interventions in conjunction with other interventional agents. Most used FOS and GOS prebiotics combined with probiotics or amino acids[9, 63-66]. One used a combination of betaglucan, inulin, pectin and RS with four strains of probiotics[68]. Most were conducted in high-income or 'developed' countries[9, 64-66, 68], with the exception of one which was conducted in Mexico[63]. Three studies came from the same research group in Spain[64-66].

Table 1: Data extraction table: HIV and dietary resistant starch/prebiotic interventions – Feasibility Literature Review.

Author	Year	Journal	Study design	Setting	Population including ART status and sample size of participants (n)	Mean age (SD), years unless otherwise specified	Sample type	Intervention-dosage per day, prebiotic type	Study duration
Gonzalez-Hernandez et al.	2012	*Nutrition Journal*	DB RCT	Mexico	ART naïve adults: Probiotic group: 5 Synbiotic group: 5 Prebiotic group: 5 Placebo group: 5 Total: 20	Adults: Probiotic: 28 (6) Synbiotic: 26 (7) Prebiotic: 27 (6) Placebo: 30 (8)	Faeces	10g of agavins from *Agave tequilana* (FOS); Synbiotic as combination of *Lactobacillus* and *Bifidobacterium* and *Agave tequilana*	16 weeks
Gori et al.	2011	*Nature*	DB RCT	Italy	HIV subtype 1, ART naïve adults Total: 57	38.3 (9.5)	Faeces	15g OR 30g mixture containing sc-GOS, lc-FOS, pectin hydrolysate-derived acidic oligosaccharides	12 weeks
Sainz et al.	2020	*Nutrients*	DB RCT	Spain	Vertically infected children, on ART: 24 HIV-ve siblings as controls: 11 Total: 35	Controls: 10 (4.4) Placebo: 13.8 (3.6) Intervention group: 10 (3.4)	Faeces	21.1g PMT25341 containing 4g lc-FOS, 3.3g sc-GOS plus EPA, DHA, GLA, L-Glutamine, L-Arginine, *Saccharomyces boulardii*, AM3 and Vitamin D	4 weeks
Schunter et al.	2012	*BMC Complementary and Alternative Medicine*	DB RCT	USA	Adults (female) on ART Total: 33	Fibre-only group: 48.8 (6.1) Synbiotic group: 46.4 (8.0)	Faeces	Synbiotic 2000 containing 4 probiotic bacteria plus 4 nondigestible dietary fibres at 2.5g each: betaglucan, inulin, pectin and resistant starch	4 weeks
Serrano-Villar et al.	2016	*Mucosal Immunology*	RCT	Spain	Adults, 4 study groups: VU: 12 IR: 15 INR: 8 HIV-ve: 9 Total: 44	VU: 34 (33–35) IR: 48 (51–53) INR: 40 (33–48) HIV-ve: 47 (31–60) (median (IQR))	Faeces	20g mixture including 5g of sc-GOS, 10g lc-FOS, 5g of glutamine	6 weeks
Serrano-Villar et al.	2018	*Clinical Infectious Diseases*	DB RCT	Spain	ART naïve adults Total: 78	Intervention: 36 (8) Placebo: 38 (14)	Faeces	PMT25341 containing 6g lc-FOS, 5g sc-GOS plus EPA, DHA, GLA, L-Glutamine, L-Arginine, *Saccharomyces boulardii*, AM3 and Vitamin D	48 weeks

DB RCT: Double Blind Randomised Controlled Trial, RCT: Randomised Controlled Trial, ART: Antiretroviral Therapy, lc-FOS: long-chain Fructo-Oligosaccharide, sc-GOS: short-chain Galacto-Oligosaccharide, VU: HIV+ve Viraemic Untreated, IR: HIV+ve ART-treated virally suppressed immunological responders (CD4+ T cells>=350cells/mm^3), INR: HIV+ve ART-treated virally suppressed immunological non-responders (CD4+ T cells<350cells/mm^3), EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid, GLA: Gamma-linolenic acid, AM3: Inmunoferon.

Overall alignment of the feasibility assessments reported in these articles with the Thabane typology is presented in **Table 2**. In summary, five of the six studies reported on outcomes from the Process category, which typically covered participant recruitment and retention rates as well as adherence to the study intervention[63-66, 68]. All six studies reported on safety outcomes which fall under the Scientific category[9, 63-66, 68]. None of the articles were solely focused on feasibility assessment, with all primarily reporting on effect size. The six articles addressed feasibility outcomes briefly, in a sentence or two, and did not reflect in detail on how the process for the study could be improved for future trials, focusing more on the outcomes measuring the effect of the dietary intervention(s). None reported on aspects that related to Thabane's Resources or Management categories.

Table 2: Reporting of feasibility assessments of 6 studies in alignment with the typology by Thabane et al.[71].

Article (author, year)	Category/Purpose of feasibility assessment [71]			
	Process	Resources	Management	Scientific
González-Hernández et al., 2012	✓	×	×	✓
Gori et al., 2011	×	×	×	✓
Sainz et al., 2020	✓	×	×	✓
Schunter et al., 2012	✓	×	×	✓
Serrano-Villar et al., 2016	✓	×	×	✓
Serrano-Villar et al., 2018	✓	×	×	✓

Process

The assessment of process indicators for feasibility relies on a determination of the basic aspects of enabling the study to be functional, such as recruitment and retention rates, adherence with protocol and intervention, how the eligibility criteria were applied in practice, the practical nature of study tools and if they successfully enabled the requisite data to be collected[71].

Recruitment and retention

In relation to recruitment, the six studies included in the review reported recruitment and retention levels at either the anticipated level or better. These data are presented in **Table 3**. The small number of participants reflects the pilot or proof-of-concept nature of these studies. Retention rates appeared to be reflective of the total duration of the study – as seen in the 2018 Serrano-Villar et al. study, which reported high rates of withdrawal, possibly resulting from the 48-week study duration[65]. The mean duration of the remaining five studies was 8.4 weeks, with a range from four to 16 weeks.

Table 3: Recruitment and retention data for studies included in the Feasibility Literature Review.

Article (author, year)	Participants screened for eligibility (n)	Participants randomised (n)	Participants completed (n)
González-Hernández et al., 2012	22	20	20
Gori et al., 2011	72	57	ITT=57 PP=47
Sainz et al., 2020	Controls=11 Placebo=12 Intervention=12	Controls=11 Placebo=12 Intervention=12	Controls=11 Placebo=11 Intervention=11
Schunter et al., 2012	38	33	27
Serrano-Villar et al., 2016	95	60	44
Serrano-Villar et al., 2018	101	78	ITT=59 PP=35

ITT: intention to treat; PP: per protocol.

Adherence

Participant adherence or compliance with study protocol and interventions is routinely reported in clinical trials, including feasibility and pilot studies. Adherence is typically defined as *'x% of consenting participants who complete their randomly allocated treatment with a minimum of (benchmark) adherence'* [237, p.556]. It was common in this literature for there to be no mention of benchmark values and how these were defined with regard to participant adherence. Adherence can also be measured as it relates to clinician or researcher adherence to the study protocol[237]. In this literature review, adherence was focused on participant adherence.

As shown in **Table 4**, Gonzalez-Hernandez et al. reported that participant adherence to the synbiotic intervention in their study was 98%[63]. Details regarding how adherence data were collected was not reported in this article. In their 2016 study, Serrano-Villar et al. also reported high mean adherence rates of greater than 85%, although the method for deriving this figure was not detailed by the authors and no range was included[64]. While some studies relied on participant self-reported adherence with dietary interventions[68], others used the collection of unused study supplements, either in conjunction with participant-reported adherence data[65] or without it[66]. Whereas Sainz et al. reported the method of monitoring adherence but not the findings[66], Schunter et al. reported the serves of the dietary intervention consumed per week for members of two study groups (fibre-only and synbiotic)[68]. In this study, participants were expected to consume one serve per day of the dietary intervention. The adherence data showed that members of the fibre-only group consumed a mean intake of 5.3 serves per week (SD 4.1) compared to the synbiotic group, with a mean intake of 6.1 serves per week (SD 5.4)[68]. In terms of reliability of the options for measuring adherence, Schunter et al. commented that reliance on self-reported adherence data was a limitation of their study, especially considering that full compliance was routinely reported among their study participants[68].

Although Gori et al. did not specifically report on the methods used to assess adherence, they reported that ten participants did not complete the intervention period, discontinuing for reasons including lost to follow-up, withdrawing consent, and AEs[9]. The use of qualitative approaches to further explore these data might have helped explain issues with adherence. In their 2018 study, Serrano-Villar et al. observed that 43.8% of participants in the intervention group and 33.3% in the placebo group reported adhering to the nutritional intervention at a rate lower than 50%[65]. Reasons cited for this suboptimal adherence included bad taste and nausea[65]. A total of 20 participants were excluded from the analysis because of poor adherence to either the intervention (n=14) or the placebo (n=6)[65]. Given that the total number randomised in this study was 78, the large number excluded from analysis was likely a factor in the authors presenting data for both intention-to-treat (ITT) and on-treatment (per protocol)

analyses in the article[65]. This highlights the key role of intervention adherence in informing statistical approaches and enabling or limiting conclusions about effect.

In summary, three of the six articles identified by the search strategy reported a method for assessing adherence[65, 66, 68] and five articles reported levels of adherence[63-66, 68]. None of the studies commented on adherence to the protocol more broadly with regard to the provision of stool or blood samples or the completion of survey tools.

Table 4: Adherence data reported in studies included in the Feasibility Literature Review.

Article (author, year)	Participants randomised (n)	Method for assessing adherence	Adherence rates reported (% unless otherwise indicated)
González-Hernández et al., 2012	20	Not stated	98
Gori et al., 2011	57	Not stated	Intervention not completed: n=10
Sainz et al., 2020	Controls=11 Placebo=12 Intervention=12	Collection of unused supplements	Not stated
Schunter et al., 2012	33	Participant self-report	Mean serves/week: Fibre-only group: 5.3 Synbiotic group: 6.1
Serrano-Villar et al., 2016	60	Not stated	>85
Serrano-Villar et al., 2018	78	Participant self-report and collection of unused supplements	<50% adherence to intervention: Placebo: 33.3% Active group: 43.8%

Resources

According to the Thabane typology[71], the resources aspect of feasibility assessment relates to budgetary and temporal capacity considerations, such as whether recruitment can be managed successfully within the study site(s), support from collaborating institutions, and if the study requirements can be met within the anticipated timeframe. None of the studies included in the literature review commented on aspects relating to the Resources category.

Management

This category describes human and data management systems, such as the use of unique identifiers for each participant and matching these across different data sources, whether data collection tools provided appropriate levels of variability, and the challenges faced by study staff[71]. As with the Resources category, none of the studies included in the literature review commented on aspects relating to the Management category.

Scientific

This category describes the safety of the intervention, analysis of dosage and response, and estimates of effect size.

Tolerability

Although tolerability is not specifically mentioned in Thabane's typology, it might be conceptualised to fit under either the Process or Scientific category, as it has a direct impact on adherence but is also closely related to safety. For the purpose of this thesis, tolerability is conceptualised as part of the Scientific category, owing to its impacts on safety and the identification of AEs. Tolerability assessment is an important aspect of trials using prebiotic and RS interventions, given that changes in gastrointestinal symptoms have been reported[38, 246-248]. Validated Quality of Life tools are commonly used to assess tolerability. For example, Gonzalez-Hernandez et al. utilised a validated tool (IBS-QoL) to monitor tolerability by recording gastrointestinal symptoms during the study period[63]. This study reported increases in symptoms described as AEs – including flatulence, diarrhea and abdominal distension – which were not statistically significant[63].

Gori et al. used a GI symptom tool which measured seven symptoms and asked participants to rate their seriousness, resulting in a total GI score[9]. These scores were compared both within individual participants over time and between intervention and control groups[9]. Significant differences were observed within participants receiving the larger dose (30g/day) of the dietary intervention, with the total GI score being significantly higher at the 12-week timepoint compared to baseline[9]. The GI complaints which featured most prominently were

flatulence and abdominal distension[9]. There was a trend of higher reporting of GI complaints for the intervention groups compared to the control group but this was not significant[9]. Noting how tolerability can impact on retention of study participants, it was reported that four participants withdrew due to these AEs[9].

In their study, Schunter et al. reported that research staff contacted participants by phone after a week of consuming the dietary intervention to assess any issues and give encouragement where an increase in gas/flatulence was reported[68]. Sainz et al. did not report on any tolerability methods or findings[66]. Serrano-Villar et al. reported that one participant in the intervention arm and two in the placebo arm withdrew due to gastrointestinal symptoms[64]. In this study, mild gastrointestinal symptoms, such as diarrhoea/loose stools and bloating/flatulence, were more frequently cited in the intervention versus placebo arm but this was not statistically significant[64]. Noting that safety and tolerability were primary outcomes in their 2018 study, Serrano-Villar et al. used a 4-point scale to measure changes in gastrointestinal symptoms[65]. In this study, the authors reported that no serious AEs were reported[65].

Palatability is an important component of tolerability that is frequently overlooked[249] and the lack of reporting of this outcome in the studies included in the literature review, was noted. A detailed analysis of palatability was undertaken by the doctoral researcher to inform the choice of study food and is provided in Appendix 4.

Safety

Some studies utilised biochemical measures, such as liver and renal function, to assess the safety of the dietary intervention[9]. Gonzalez-Hernandez et al. required participants to record their body temperature and report to the researcher if any symptoms of infection were apparent[63]. These participants were also monitored by clinical examination in conjunction with laboratory testing every month[63]. During the 16 weeks of this study, the authors reported no serious AEs; nor were any clinically significant changes noted as safety outcome measures[63]. Although Schunter et al. did not describe the method for assessing safety, the Participant Flow Diagram indicates that, of the six participants lost to follow-up, four withdrew due to antibiotic use and a further two withdrew without providing any reason[68]. Safety is not specifically mentioned in the Sainz et al. article. Other authors have been more explicit in their reporting of safety outcomes, with Gori et al. stating that no clinically relevant changes in liver and renal function were reported and no serious AEs were identified[9]. Although not reporting any biochemical measures used to assess safety, Serrano-Villar et al. reported no serious AEs in either of their studies included in this review[64, 65].

Summary

Overall, in relation to feasibility assessment, the literature review suggests that some but not all studies identified by the search strategy reported on safety, adherence and tolerability. According to the Thabane typology, the most frequent reason for feasibility assessment in the papers identified was Process or Scientific (**Table 2**). While the Resources and Management categories include important measures of feasibility, none of the included studies commented on them. Although studies identified in this literature review included details in terms of the method utilised for implementing all aspects of the trial, there was no stated objective of reporting how these methods worked in practice and how they could be improved for a future study. Such considerations could fit under the Resources category (given its focus on the capacity of study sites and the impact on recruitment, as well as the suitability of timeframes) or the Management category (given its focus on the challenges faced by study staff).

Discussion

While useful, the Thabane typology does not include all possible aspects that might be included in a feasibility assessment. For example, cultural acceptability is not specified but might fit under the Process category, as it potentially impacts on refusal and adherence rates[250, 251]. There are other typologies for assessing feasibility which could have been applied in this thesis[233, 252, 253]. However, the advantage of utilising the Thabane typology relates to alignment with the CONSORT extension statement for randomised pilot and feasibility trials, ensuring that the consistency of reporting and the management of bias ultimately benefit the content area overall[254, 255]. This is an important approach which contributes to knowledge management and transfer in this area[254, 255]. Indeed, the rationale for the publication of the CONSORT extension statement was that reporting and methodological conduct of these types of trials had historically lacked consistency[71, 72, 256, 257]. More generally, there is a call for articles reporting on clinical trial outcomes to be more rigorous[258], as well as accessible, in order to encourage knowledge transfer into clinical and public health policy and practice approaches[259, 260]. Opportunities to make reporting more stringent in this field will be useful in clarifying terminology relating to both study design (‘feasibility’/‘pilot’) and measures, since terms such as ‘adherence’/‘compliance’ and ‘tolerability’/‘acceptability’ are sometimes used interchangeably, creating complexity where simpler approaches would be valuable.

Conclusion

Although the selection of articles returned by the search terms was limited, the gap identified from this review is that studies of dietary prebiotic interventions in HIV-positive populations have not assessed feasibility with regard to the Resources and Management categories.

Feasibility studies are intended to determine where refinements to study methodologies should be adopted to enhance future efficacy studies[252]. In addition to measures of recruitment, retention, adherence, tolerability and safety, being able to draw on findings from a procedural perspective will be advantageous to the success of future trials[73]. That approach will therefore be pursued in this thesis.

Efficacy and Effect Literature Review

Aim

The aim of this review was to analyse findings from interventional trials that administered dietary prebiotics, synbiotics or RS interventions to mammals with a lentivirus infection.

Method

This literature review examined the association between dietary RS or prebiotic interventions with the following primary outcome measures:

- Changes to the colonic luminal environment as determined by the following measures in faecal samples:
    - faecal acetate, butyrate, propionate and valerate concentrations;
    - faecal pH; and
    - relative abundance and diversity of gastrointestinal microbiota in faecal samples.

Papers were included if they addressed any of the primary outcome measures. Findings relating to CD4+ T cells are also reported in this literature review if they were included in the identified papers.

Medline (Ovid), Scopus and Web of Science databases were used to identify articles for the literature review. Medline is the commercial version of PubMed, utilising the same database, and was preferred to PubMed in this instance because it enabled a more sophisticated search strategy using Medical Subject Headings (MeSH terms). Search terms were applied to the full historical dataset of Medline dating back to 1964. The three databases were searched for articles according to the following selection criteria.

Inclusion criteria

- Articles examining dietary RS or prebiotic supplementation (either as a sole agent or with a probiotic as a ‘synbiotic’) in which measures of any of the primary outcomes were reported;

- Primary experimental research papers or review papers with meta-analysis published in peer-reviewed journals; and
- All in vivo models in which study participants were infected with HIV or a related lentivirus, such as SIV.

As this literature review was focused on reviewing efficacy and effect sizes, review papers were only selected if they included meta-analysis. Any review articles with no meta-analysis were deemed to be introducing duplication of the primary research papers identified by the search strategy and were not included.

The search was restricted to English-language publications only, owing to the costs associated with translation and a limited overall budget for the study. Geographic location and publication date were not restricted in order to increase the sensitivity of the search and increase the numbers of potentially available documents.

Exclusion criteria

- Studies using a sole probiotic intervention;
- Studies relating to human milk oligosaccharides (HMOs). HMOs describes a group of naturally occurring prebiotics found in breast milk;
- Studies using the term 'oligosaccharide' to describe a component of the virus envelope in discussions of vaccine development;
- Review papers with no meta-analysis;
- Protocol papers; and
- In vitro studies, such as those using cell models.

The specified search terms are provided below:

Medline search strategy

Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations, Daily and Versions <1946 to March 31, 2023>

1 hiv/ or hiv-1/ or hiv-2/ 107044
2 hiv infections/ or acquired immunodeficiency syndrome/ or aids-related opportunistic infections/ or hiv enteropathy/ or hiv wasting syndrome/ or lentivirus/ or SIV/ or FIV.mp. 308183
3 (human immunodeficiency virus or HIV* or acquired immunodeficiency syndrome or PLWHA or PLWHIV or SIV or FIV).tw,kw. 385938
4 or/1-3 458762
5 Prebiotics/ or (prebiotic* or pre-biotic*).tw,kw. 13333
6 (resistant starch* or synbiotic* or oligosaccharide* or ((Non-digest* or nondigest*) adj4 (starch or food or diet* or component*))).tw,kw. 42420
7 oligosaccharides/ or oligosaccharides, branched-chain/ 27138
8 (fructooligosaccharide* or galactooligosaccharide* or maltooligosaccharide* or dextrin* or resistant maltodextrin* or resistant starch based derivative* or inulin or lactulose).tw,kw. 17598

9 (Diet* or Digest* or Gut or Intestin* or Gastro* or gastric* or GIT or Colon* or enteric* or enteral* or fecal* or faecal*).tw,kw. 2378295
10 (Microbiome* or Microbiota or Microorganism* or micro-organism* or Bacteria* or Microbe* or Microbial or Microflora or flora* or “pH” or “SCFA” or acetate or butyrate or propionate or valerate).tw,kw. 1761863
11 5 or 6 or 7 or 8 76028
12 9 or 10 3801734
13 4 and 11 and 12 169
14 limit 13 to english language 166

Scopus search syntax

TITLE-ABS-KEY(((“human immunodeficiency virus” OR hiv* OR “acquired immunodeficiency syndrome” OR plwha OR plwhiv OR lentivirus OR SIV OR FIV) AND (prebiotic* OR “pre-biotic*” OR “resistant starch*” OR synbiotic* OR oligosaccharide* OR ((“Non-digest*” OR nondigest*) W/4 (starch OR food OR diet* OR component*)) OR fructooligosaccharide* OR galactooligosaccharide* OR maltooligosaccharide* OR dextrin* OR “resistant maltodextrin*” OR “resistant starch based derivative*” or inulin or lactulose) AND (diet* OR digest* OR gut OR intestin* OR gastro* OR gastric* OR git OR colon* OR enteric* OR enteral* OR fecal* OR faecal* OR microbiome* OR microbiota OR microorganism* OR “micro-organism*” OR bacteria* OR microbe* OR microbial OR microflora OR flora* OR “pH” OR “SCFA” OR acetate OR butyrate OR propionate OR valerate))) AND (LIMIT-TO (LANGUAGE,“English”))

Web of Science search syntax

((“human immunodeficiency virus” OR hiv* OR “acquired immunodeficiency syndrome” OR plwha OR plwhiv) AND (prebiotic* OR “pre-biotic*” OR “resistant starch*” OR synbiotic* OR oligosaccharide* OR ((“Non-digest*” OR nondigest*) NEAR/4 (starch OR food OR diet* OR component*)) OR fructooligosaccharide* OR galactooligosaccharide* OR maltooligosaccharide* OR dextrin* OR resistant maltodextrin* OR resistant starch based derivative* or inulin or lactulose) AND (diet* OR digest* OR gut OR intestin* OR gastro* OR gastric* OR git OR colon* OR enteric* OR enteral* OR fecal* OR faecal* OR microbiome* OR microbiota OR microorganism* OR “micro-organism*” OR bacteria* OR microbe* OR microbial OR microflora OR flora* OR “pH” OR “SCFA” OR acetate OR butyrate OR propionate OR valerate)) **(Topic)**

Refined by: LANGUAGES: (ENGLISH)

Timespan: All years. **Indexes:** SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC.

Given its infancy and the current interest in this research area, an additional search for unpublished studies/grey literature using the simplified search terms ‘HIV AND (prebiotics OR synbiotics OR resistant starch)’ was applied to the following repositories:

- Pan African Clinical Trials Registry (PACTR);
- ISRCTN Register (UK);
- Clinicaltrials.gov (US National Institutes of Health);
- ProQuest Dissertations & Theses;
- CORE (Digital Institutional Repository) – UK. Limit Field to ‘Medicine’;
- Networked Digital Library of Theses and Dissertations (NDLTD);
- WorldCat (US);
- TROVE (National Library of Australia);

- Theses Canada (National Library of Canada);
- EThOS (the UK's national thesis service);
- Networked Digital Library of Theses and Dissertations; and
- NZResearch.org.

The screening process involved reading article abstracts to identify if eligibility criteria were met. The eligibility shortlisting process involved reading the full text of each article. Following the screening and eligibility shortlisting process, the doctoral researcher and one other public health colleague independently assessed the articles for methodological validity prior to inclusion in the review. This was done using standardised critical appraisal instruments from the Joanna Briggs Institute Meta-Analysis of Statistics Assessment and Review Instrument (JBI-MAStARI), provided as Appendix 1[245]. Overall appraisal for all shortlisted articles according to the JBI-MAStARI instrument was *Include.* A hand search of the reference lists of papers retrieved by this search and short-listing process was also undertaken to identify other relevant articles.

Results

The PRISMA diagram provided at **Figure 3** shows the article selection process and outcome. Nine studies were included in the Efficacy and Effect Literature Review. **Table 5** summarises the key aspects of those papers.

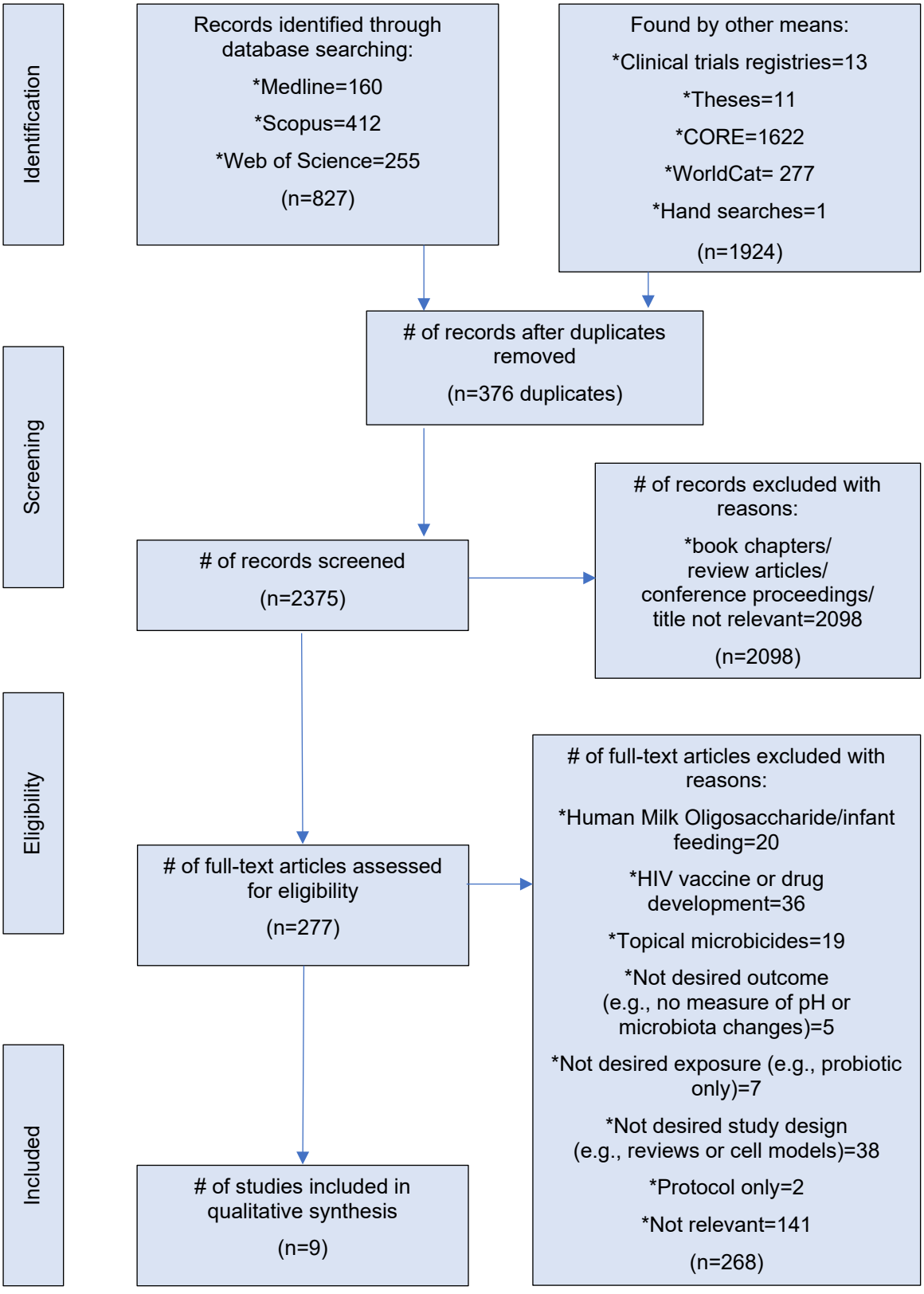

Figure 3: PRISMA diagram: HIV and dietary resistant starch/prebiotic interventions – Efficacy and Effect Literature Review.

Table 5: Data extraction table: HIV and dietary resistant starch/prebiotic interventions – Efficacy and Effect Literature Review.

Author	Year	Journal	Study design	Setting	Population including ART status, study group allocation and sample size of participants (n)	Mean age (SD), years unless otherwise specified	Sample type	Sequencing method	Mean CD4+ (cells/mm^3) (SD) per group at baseline unless otherwise specified	Dosage per day, prebiotic type	Study duration
Deusch et al.	2018	*AIDS*	RCT	Spain	4 study groups: VU: 5 IR: 7 INR: 4 HIV-ve: 6 Total: 22	Ages not reported Inclusion criteria was Adults: 18+	Faeces	16S rRNA and shotgun proteomics	VU: 558 (432–646) IR: 291 (230–324) INR: 561 (426–794) (median (P25–P75))*	20g mixture including 5g of sc-GOS, 10g lc-FOS, 5g of glutamine	6 weeks
Gonzalez-Hernandez et al.	2012	*Nutrition Journal*	DB RCT	Mexico	ART naïve: Synbiotic group: 5 Prebiotic group: 5 Probiotic group: 5 Placebo group: 5 Total: 20	Adults: Synbiotic: 26 (7) Prebiotic: 27 (6) Probiotic: 28 (6) Placebo: 30 (8)	Faeces	16S rRNA	Synbiotic: 620 Prebiotic: 533 Probiotic: 754 Placebo: 542	Synbiotic: combination of *Lactobacillu*s and *Bifidobacterium* and 10g *Agave tequilana* (FOS); Prebiotic:10g *Agave tequilana.* Probiotic: above stated bacterial strains only.	16 weeks
Gori et al.	2011	*Nature*	DB RCT	Italy	HIV subtype 1, ART naïve Control: 19 15g/d: 19 30g/d: 19 Total: 57	Adults: 38.3 (9.5)	Faeces	RT qPCR	Total ppt: 520 (161) Control: 502 (149) 15g/day: 536 (173) 30g/day: 519 (166)	15g OR 30g mixture containing sc-GOS, lc-FOS, pectin hydrolysate-derived acidic oligosaccharides	12 weeks
Jimenez-Hernandez et al.	2019	*Nutrients*	RCT	Spain	4 study groups: VU: 12 IR: 18 INR: 9 HIV-ve: 14 Total: 53	Adults: 18+	Saliva and faeces	16S rRNA	VU: 521 (385–738) IR: 561 (426–667) INR: 271 (204–321) (median (P25–P75))	20g mixture including 5g of sc-GOS, 10g lc-FOS, 5g of glutamine	6 weeks

Author	Year	Journal	Study design	Setting	Population including ART status, study group allocation and sample size of participants (n)	Mean age (SD), years unless otherwise specified	Sample type	Sequencing method	Mean CD4+ (cells/mm^3) (SD) per group at baseline unless otherwise specified	Dosage per day, prebiotic type	Study duration
Klatt et al.	2013	*Journal of Clinical Investigation*	RCT	USA	SIV+ve pig-tail macaques, ART naïve at baseline Total: 11	Primate	Faeces	16S rRNA	Between 20 and 1000 (broad range of CD4+ T cells is typical in this primate model)	Synbiotic with inulin as prebiotic, probiotic described as 'VSL#3' which contains 8 bacterial strains. ART also commenced during trial	60 days
Sainz et al.	2020	*Nutrients*	DB RCT	Spain	Vertically infected, on ART: 24 HIV-ve siblings as controls: 11 Total: 35	Children: Controls: 10 (4.4) Placebo: 13.8 (3.6), Intervention group: 10 (3.4)	Faeces	16S rRNA	Placebo: 556 (453–754), Intervention: 852 (617–1182) (median (P25–P75))	21.1g PMT25341 containing 4g lc-FOS, 3.3g sc-GOS plus EPA, DHA, GLA, L-Glutamine, L-Arginine, saccharomyces boulardii, AM3 and Vitamin D	4 weeks
Schunter et al.	2012	*BMC Complementary and Alternative Medicine*	DB RCT	USA	Females on ART Fibre only: 17 Synbiotic: 16 Total: 33	Adults: Fibre only group: 48.8 (6.1) Synbiotic group: 46.4 (8.0)	Faeces	16S rRNA	Synbiotic group: 685 +/– 249 Fibre only group: 588 (309)	Synbiotic 2000 containing 4 probiotic bacteria plus 4 nondigestible dietary fibres at 2.5g each: betaglucan, inulin, pectin, and resistant starch	4 weeks

Author	Year	Journal	Study design	Setting	Population including ART status, study group allocation and sample size of participants (n)	Mean age (SD), years unless otherwise specified	Sample type	Sequencing method	Mean CD4+ (cells/mm^3) (SD) per group at baseline unless otherwise specified	Dosage per day, prebiotic type	Study duration
Serrano-Villar et al.	2016	*Mucosal Immunology*	RCT	Spain	4 study groups: VU: 12 IR: 15 INR: 8 HIV-ve: 9 Total: 44	Adults: VU: 34 (33–35) IR: 48 (51–53) INR: 40 (33–48) HIV-ve: 47 (31–60) (median (IQR))	Faeces	16S rRNA	VU: 558 (432–646) IR: 291 (230–324) INR: 561 (426–794) HIV -ve: 762 (653–878) (median (P25–P75))	20g mixture including 5g of sc-GOS, 10g lc-FOS, 5g of glutamine	6 weeks
Serrano-Villar et al.	2018	*Clinical Infectious Diseases*	DB RCT	Spain	ART naïve Total: 78	Adults: Intervention: 36 (8) Placebo: 38 (14)	Faeces	16S rRNA	226 (117–283)	PMT25341 containing 6g lc-FOS, 5g sc-GOS plus EPA, DHA, GLA, L-Glutamine, L-Arginine, *saccharomyces boulardii*, AM3 and Vitamin D	48 weeks

DB RCT: Double Blind Randomised Controlled Trial, ART: Antiretroviral Therapy, lc-FOS: long-chain Fructo-Oligosaccharide, sc-GOS: short-chain Galacto-Oligosaccharide, VU: HIV+ve Viraemic Untreated, IR: HIV+ve ART-treated virally suppressed immunological responders (CD4+ T cells>=350cells/mm^3), INR: HIV+ve ART-treated virally suppressed immunological non-responders (CD4+ T cells<350cells/mm^3), EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid, GLA: Gamma-linolenic acid, AM3: Inmunoferon, P25–P75: 25th–75th percentile.

* This study used a subset of data from the 2016 Serrano-Villar study and cited that paper for participant characteristics.

General characteristics of the study population

One study considered an SIV-model in pigtail macaques[171]; the remaining eight examined HIV models. Seven studies were with an adult human population[9, 63-65, 67-69], while one study was with children[66]. Of the human studies, mean participant age ranged from 10 years to 48 years. Four studies included only ART-naïve participants[9, 63, 65, 171]. Mean duration of ART in the remaining studies was 6.1–8.9 years. The child study did not report on duration of ART use but the inclusion criteria required a minimum of six months on ART[66].

In terms of prebiotic duration, dosage and type, interventions ranged from four to 48 weeks and the majority (78%) utilised a combination of FOS/GOS with other components such as probiotics or amino acids[9, 63-67, 69]. Prebiotic dosage was variable, ranging from 10 to 30g/day in the human studies, while the SIV study utilised 200mg/day. Only one study used RS, applying a 2.5g/day dose as a component of a formulation containing inulin, betaglucan and pectin along with four probiotics[68]. The pigtail macaque paper described the use of inulin in combination with probiotics[171]. Overall, the emphasis on oligosaccharide interventions reflects the broader literature in which oligosaccharides dominate, with fewer studies utilising RS.

Faecal SCFA

Only one article measured SCFA content of faecal samples[64]. This study observed that the SCFA profile at baseline differed between HIV-positive participants and those who were HIV-negative (Adonis p=0.019)[64], with this difference characterised by elevated levels of propionate (p=0.05) and lower levels of acetate (p=0.036)[64]. This study also reported that the prebiotic intervention was associated with increased levels of butyrate in HIV-positive participants not taking ART (p=0.05)[64]. Butyrate abundance was found to positively correlate with butyrogenic bacteria, including in species shown to prosper when RS is available as an energy source, as noted in the Introduction chapter of this thesis, such as *Roseburia faecis* (p=0.0003) and *Ruminococcus torques* (p=0.0093)[64].

Faecal pH

None of the identified articles included faecal pH as an outcome measure.

Relative abundance and diversity of gastrointestinal microbiota

All articles included at least one measure of faecal microbial abundance/diversity. Eight articles measured bacteria in faecal samples[9, 63-68, 171], while one measured bacteria in both saliva and faecal samples[69]. Four papers reported an increase in diversity of bacterial taxa following the dietary intervention[9, 64, 67, 69]. Although the 2018 Serrano-Villar et al. paper did not report significant changes in alpha or beta diversity in the intervention group compared to the placebo, a linear discriminant effect size (LEfSe) analysis highlighted that intervention group participants had an enrichment of

Lachnospiraceae and Victivallaceae bacteria[65]. In addition, a reduction in *Blautia* species compared with placebo was noted[65]. Interestingly, *Blautia* has been associated with the consumption of animal-source proteins, fast food, processed meat, soft drinks and sugar[261]. Such associations have been shown to increase energy harvest from the diet and increase risk for immune-mediated inflammatory disorders and obesity[262, 263]. Depletion of *Blautia* in this instance might suggest a protective effect of the dietary intervention, especially considering that increased consumption of plant-based foods has been associated with lower abundance of pathobionts, including those implicated in inflammatory processes[261]. It is also important to consider changes to gut microbiota that have been reported in sub-populations disproportionately affected by HIV. For example, pathogenic changes in the gut microbiome have been noted in HIV-negative MSM populations, with the suggestion that these changes increase susceptibility to HIV[264]. The macaque study found only modest (and not significant) changes in the composition of faecal microbiota in those treated with the synbiotic preparation plus ART compared with macaques treated with ART alone[171]. The Gori et al. study reported a significant increase in bifidobacteria *compared to baseline* after 12 weeks of intervention in both the 15g/day and 30g/day dosage groups (p=0.007 and p=0.01 respectively)[9]. The levels of bifidobacteria were also significantly higher at week 12 for the 15g/day and 30g/day dosage groups when *compared to the control group* (p=0.014 and p=0.007 respectively)[9].

There was little support for the hypothesis that prebiotics or synbiotics modulate gut dysbiosis in the Gonzalez-Hernandez et al. study[63]. A non-significant reduction in relative abundance of bacteria exhibiting beneficial (*Bifidobacterium*) and pathogenic (*Clostridium*) functions was observed in the synbiotic group (p=0.082 and p=0.091, respectively)[63]. An increased level of *Bifidobacterium* and *Clostridium* in faecal samples was observed for the prebiotic group but these were also at a non-significant level (data not shown in article)[63]. The study by Schunter et al. showed that the synbiotic intervention resulted in changes in two species in faecal samples (*Lactiplantibacillus plantarum* (p=0.001) and *Pediococcus pentosaceus* (p=0.036))[68]. However, this study only assayed for the probiotic bacteria which were supplemented in the synbiotic intervention product[68].

The one paper examining a paediatric cohort reported that the baseline gut microbiota of HIV-positive children on ART was less diverse than non-HIV negative controls but only on beta diversity measures (Adonis p=0.042), not alpha diversity[66]. The statistical difference in beta diversity was no longer present after the nutritional intervention (Adonis p=0.272), suggesting that the dietary intervention may play a role in the amelioration of gut microbiota dysbiosis in HIV-infected participants[66]. The 2016 Serrano-Villar et al. paper reported differences in bacterial abundance after the prebiotic intervention in the viraemic untreated (VU) group only, with changes to Firmicutes and Actinobacteria noted (data not shown in article)[64]. One study reported a decrease in bacteria with pathogenic potential (*Eubacterium rectale/Clostridium coccoides* cluster) following the dietary intervention when compared to baseline in both 15g/day and 30g/day groups (p=0.035 and p=0.05, respectively)[9]. These authors

also reported a decrease in the pathogenic *Clostridium lituseburense/Clostridium histolyticum* group levels with 30g/day prebiotic supplementation when compared to baseline (p=0.009)[9]. This group includes the potentially pathogenic *Clostridium perfringens* and *Clostridium difficile* species[9]. A significant decrease in these species was also observed when compared to the control group for both dosages (15g/day: p=0.011; 30g/day: p=<0.001)[9].

Some of the articles identified in this review observed that the gut microbiota of participants taking ART was found to be more resilient, being less perturbed by the prebiotic interventions[64, 67, 69]. This might suggest that gut microbiota modulated by the selective pressures of ART are more resilient to interventions associated with prebiotics. For example, Serrano-Villar et al. observed significant changes to the gut microbiota in only one of their HIV-positive groups, comprising participants who were not taking ART, the VU group[64]. Using a LEfSe analysis, species abundance differed after the prebiotic treatment in the VU group with an increase in Firmicutes (*Faecalibacterium, Catenibacterium, Blautia, Eubacterium*) and *Actinobacteria* (*Collinsella* and *Corinebacterium*)[64]. Following the dietary intervention, and compared with HIV negative controls, VU group members showed a diminished HIV-associated dysbiosis and increased growth of beneficial bacteria which was not observed in HIV-positive participants who were on ART[64]. In a follow-on article to the 2016 Serrano-Villar study, Deusch et al. added a shotgun proteomics step to further determine changes to the active fraction of the microbiota, where 'active fraction' was defined as *'bacteria that are actively synthesizing proteins when examining the metaproteome of bacteria from faeces'* [67, p.1230]. The Deusch et al. paper reported that, compared to the study groups in which participants were taking ART, the VU group displayed the greatest change in richness of the active fraction of the microbiota following the dietary intervention (p<=0.029)[67]. Similarly, Jimenez-Hernandez et al. noted that the effect of prebiotics was greater in participants from the VU group, with an enrichment in commensal bacteria of saliva samples while bacteria with pathogenic potential were depleted[69]. These observations add further weight to the theory that ART treatment may be associated with a more resilient microbiota and that potentially beneficial effects of prebiotics may be more significant in individuals before ART commencement.

CD4+ T cell count

Gori et al. reported that CD4+ T cells did not change with the 12-week regimen of prebiotics, either between groups or within groups over time[9]. However, they did observe a significant decline in T cell activation, as measured by %CD4+ CD25+ T cells[9]. Gonzalez-Hernandez et al. found the highest increase in CD4+ T cells compared to baseline in the synbiotic group (p=0.05)[63]. While Klatt et al. reported that CD4+ T cells increased in the antiretroviral plus synbiotic group, it also increased in the antiretroviral-only group and there was no significant difference between groups[171]. These authors observed an almost twofold increase in the frequency of CD4+ T cells in the colon of macaques

receiving the intervention compared with those receiving antiretrovirals alone (p=0.0061)[171]. Schunter et al. reported no changes to CD4+ T cells with either the synbiotic or prebiotic-alone intervention (p=0.862)[68]. Similarly, Serrano Villar et al. (2018) observed no changes in participants consuming the dietary intervention containing long chain-FOS and short chain-GOS, when compared to placebo participants for mean CD4+ T cell count (278 vs 250 cells/μL, p=0.474). Jimenez-Hernandez et al., however, observed a decrease in T cell activation markers following the prebiotic intervention (p=0.031, adj.fdr p value (Benjamini–Hochberg method)=0.0115)[69]. Neither the Deusch et al. nor the Sainz et al. studies considered CD4+ T cells as an outcome measure, being focused primarily on gut microbiota changes.

Discussion

The effect of prebiotic interventions on gastrointestinal microbiota in these nine studies is variable and may have been influenced by ART status and dosing parameters, such as amount, duration and intervention type. Only two of these studies utilised prebiotic interventions with no additional products (such as probiotics or amino acids) and, even then, there was a difference between studies in the type of prebiotic used. In the prebiotic intervention studies, only the Gonzalez-Hernandez et al. study included an arm that utilised one type of prebiotic alone (FOS from *Agave tequilana*)[63]. This makes discerning a likely candidate that exerts the effect on the microbiota and immune measures difficult. Of note was the published paper that did report changes to SCFA after prebiotic supplementation[64] and those that reported changes to the microbiota and HIV immune markers in a desirable direction and with a strong degree of significance[9, 64].

The identified studies showed that there may be differences in the resilience of the microbiota according to participant characteristics, including ART status[64, 67, 171]. The idea that microbiota changes might be influenced more readily in participants who are not receiving ART is intriguing but should be considered with caution since the Gonzalez-Hernandez et al. study did not observe significant microbiota changes in their group of treatment-naïve individuals[63]. Similarly, in the pilot phase of the Klatt et al. study, changes in microbiota were not evident when no antiretrovirals were administered[171]. Following initial SIV-infection, Klatt et al. reported no substantial alterations in the microbiota of the macaques, consistent with other studies in this population[265]. This may suggest that the gut microbiota of these animals is more stable compared to humans and could explain the lack of microbiota changes following the prebiotic intervention.

The implications of the 2016 Serrano-Villar article for future study design are of particular relevance[64]. Many countries have adopted revised treatment schedules as recommended by UNAIDS, in which ART commences as soon as possible after diagnosis[78, 266]. Prior to this change in approach, ART was started when CD4+ cells reached a cut-off value of significant immune compromise[267]. However, in many LMICs, the approach of using CD4+ cut-offs for commencement of ART is still applied[268]. Given

the possibility of increased resilience of gut microbiota after ART commencement, future studies need to consider the optimal timing for prebiotic adjunct therapy with regard to co-administration with ART.

Limitations of literature review

Owing to similarities in disease states between HIV and SIV and the frequent use of non-human primates in research, the literature review included any lentivirus infection in mammals. A limitation of this literature review is that human studies often rely on proxy measures, such as faecal samples, to determine gut microbiota outcomes, owing to the advantage of avoiding invasive sampling procedures such as intestinal biopsy. This reliance on faecal samples results in microbiota data that do not reflect bacteria which adhere to the intestinal mucosa or are translocated across the gut epithelium[8, 111]. Non-human mammal models, such as the pigtail macaques used by Klatt et al.[171], provide data derived from tissue samples, assisting the determination of more sensitive microbiota outcomes[8]. However, while it is noted that similarities exist between SIV and HIV, some authors have reported that the gut dysbiosis which occurs in HIV infection is not consistently observed in SIV infection[269]. These factors suggest that caution should be applied in the translation of results from one model to another[269].

There was a lack of consistency in outcome measures for gut microbiota and immune marker changes in the nine papers included here. Given the relative infancy of research into prebiotics in HIV, there has yet to be agreement on standardised markers of effect. This limits the generalisation and application of findings and a more harmonised approach to outcome measures would be beneficial. The impact for this literature review was that meta-analysis was not possible due to the high level of dissimilarity in outcome measures between articles.

As identified by the overlap of articles in the two literature reviews, many of these studies were pilot studies; as such, they were often underpowered to detect differences in effect. Studies examining changes to gut microbiota require large sample sizes owing to the high degree of variability normally observed between an individual's gut microbiota and the large order of magnitude of bacterial populations[270].

Conclusion

Overall, the findings of this review suggest that the application of prebiotic treatments, either alone or with probiotics, is yet to be elucidated thoroughly in an HIV-positive population. Some evidence does exist for a beneficial effect of prebiotics on gut microbiota and immune markers[9, 64], as well as evidence for changes to SCFA output known to be associated with benefit[64]. Effects may be influenced by ART status and dosing parameters such as amount, duration and prebiotic type. There have been no studies to date using a sole RS dietary intervention in an HIV-positive population. Considering the changes related to RS which have been observed via in vitro and in vivo studies with diverse populations[32, 38, 52, 53, 213, 229, 246, 271], there is a knowledge gap regarding the effect of RS

supplementation in HIV-positive people. Finally, only one study identified in this review was conducted in an LMIC (Mexico)[63]. It is therefore relevant to respond to this research gap in an LMIC context where a high prevalence of EED is assumed among the general population.

METHODS

This chapter will first provide a description of the methods which were used for the study overall. It will then provide detailed descriptions of the specific methods used to determine (1) feasibility and (2) treatment effect.

Definition of terms

study 1:	First crossover period, days 1–56
study 2:	Second crossover period, days 57–112
absolute baseline:	Day 0
study 1 baseline:	Day 0
study 2 baseline:	Day 56
HAMS:	High Amylose Maize Starch. The brand used was Hylon VII, a non-genetically modified maize starch produced by Ingredion Incorporated, New Jersey, USA
Cornstarch:	The brand used was Ruchi Cornstarch, India, purchased from Big Bazaar supermarket chain, Bhubaneswar
Diet A:	Normal diet plus Cornstarch supplemented at 55g/day (equivalent to 8.25g RS/day) for 14 days
Diet B:	Normal diet plus HAMS, supplemented at 95g/day (equivalent to 61.75g RS/day) for 14 days
Normal diet:	The 14-day interval between two dietary supplementation periods in which participants consumed their normal diet only. This is also referred to as the 'washout period' in this thesis.

Study site

Based on prior research undertaken by the doctoral researcher in India, an arrangement was negotiated with AIIMS, Bhubaneswar, to be the study site for participant recruitment.

Bhubaneswar is the capital of Odisha state, situated in East India, with the state's coastline extending almost 500 kilometres along the Bay of Bengal. Bhubaneswar is situated approximately 70 kilometres inland. The most recent census, conducted in 2011, reported a total population of 843,402 for the Bhubaneswar city region, of whom 53% were male[272]. Recent estimates indicate that the population grew to 1.258 million in 2023[273].

The most recent estimates for Odisha state indicated that there was a total population of 49,000 people in the 15–49 year age bracket who were living with HIV in 2020[274]. People who were newly diagnosed with HIV in 2019 and managed by the AIIMS, Bhubaneswar integrated

counselling and testing centre (ICTC) clinic are represented on the map shown in **Figure 4**, in which each case is represented by a location point. Each location point symbol (◯) indicates a newly diagnosed case of HIV. Fill colour indicates cases per district.

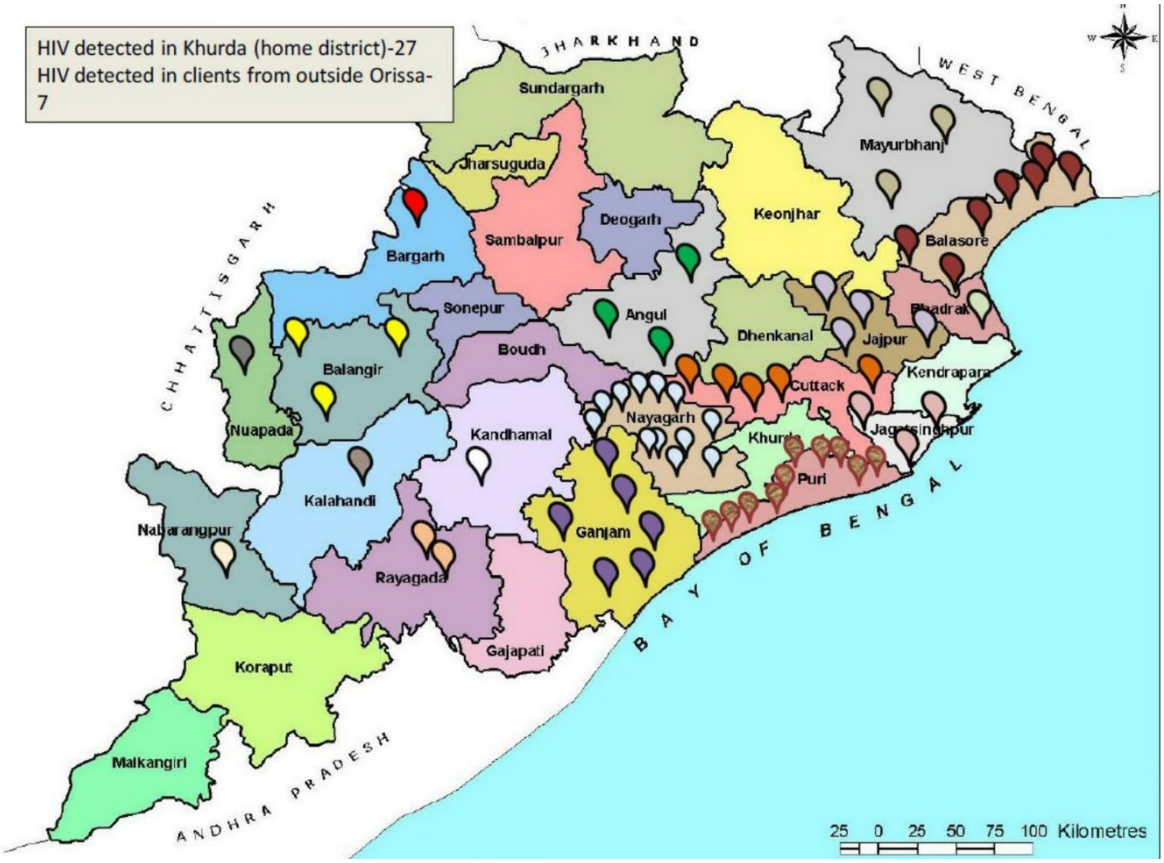

Figure 4: Newly diagnosed cases of HIV identified in 2019 and managed by AIIMS, Bhubaneswar. Source: AIIMS, Bhubaneswar ICTC clinic.

: Each point indicates one newly diagnosed case of HIV identified in 2019.

Study design

This study used a participant-blinded, two-arm double crossover during the study period of 112 days. HIV-positive adults living within a 30-kilometre radius of AIIMS, Bhubaneswar who met the study eligibility criteria were recruited. Details of the recruitment process are presented below under the heading 'Participant recruitment'. Originally, the study intended to use a single crossover design of 56 days. Four of the participants were enrolled on this basis before COVID-19 disruptions led to the change from a single to double crossover design to utilise the participant cohort as efficiently as possible. The study intervention required participants to consume two different types of rotis (Indian flatbread), which were supplemented with either HAMS or Cornstarch. The order of these dietary supplementation periods differed between participants, based on randomisation group allocation. Supplementation periods were separated by a washout period in which participants consumed their normal diet with no dietary intervention, during which the effect of the prior intervention period was assumed to washout or disappear.

In summary, the trial design involved two 14-day intervention periods *per crossover*, where each intervention period was followed by a 14-day period of Normal diet. This pattern was then repeated for the double crossover, with randomisation being undertaken again for the second crossover. The 14-day feeding periods were informed by a prior study undertaken by the doctoral researcher in non-HIV positive populations which used 14-day feeding periods[213] and on the study which informed the sample size calculation, which used 21-day feeding periods[38]. The 14-day period was preferred because the dose of HAMS was high in comparison to other studies and tolerability was uncertain in an HIV-positive population. This design is summarised in **Figure 5**, where 'S1'–'S9' indicates the 'Sample timepoint ID' at which stool and blood were collected. This terminology is used in the Results chapter when presenting data.

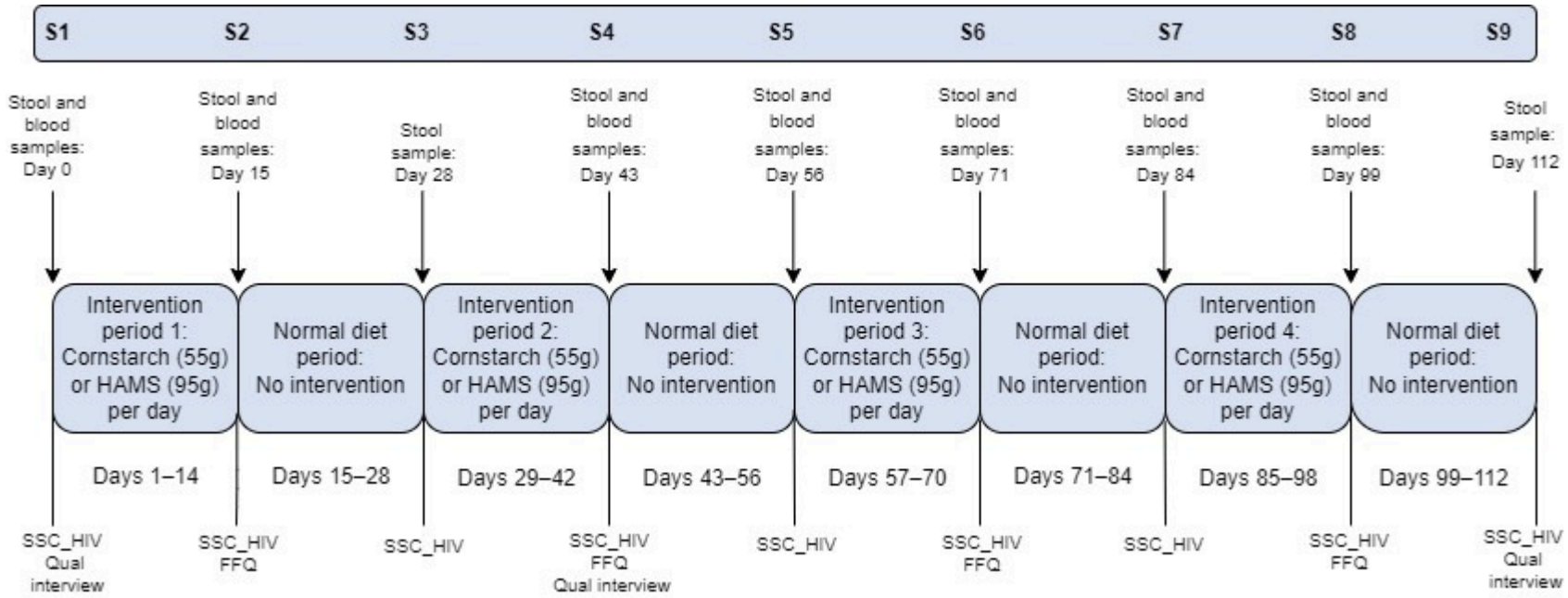

Figure 5: Study design graphic showing supplementation and Normal diet periods and sample timepoints for survey completion and sample collection.

HAMS: High Amylose Maize Starch; SSC_HIV: Revised Signs and Symptoms Checklist for HIV self-report tool; FFQ: Food Frequency Questionnaire.

Ethics

This study was approved by the:

- Southern Adelaide Clinical Human Research Ethics Committee (SAC HREC) on 29 March 2019, reference number 345.18, HREC reference number HREC/19/SAC/39; and
- AIIMS, Bhubaneswar, Institutional Ethics Committee (IEC) on 16 September 2019, reference number T/EMF/Biochem/19/04, Committee registration number ECR/534/Inst/OD/2014/RR-17.

This study was conducted in accordance with the Declaration of Helsinki[275]. Full IEC approvals are attached in Appendices 2 and 3.

In addition to securing ethics approval from both the primary research organisation (Flinders University) and the AIIMS IEC, the following guidelines for working with vulnerable populations were reviewed and adhered to throughout the study:

- Research & Evaluation statement of the National AIDS Control Organisation of India (NACO). NACO works closely with and consults HIV-positive and key at-risk populations in development of policy and programs. http://naco.gov.in/research-evaluation-0; and
- Indian Council of Medical Research (ICMR) 2017, National Ethical Guidelines for Biomedical and Health Research Involving Human Participants, https://main.icmr.nic.in/sites/default/files/guidelines/ICMR_Ethical_Guidelines_2017.pdf.

Amendments to the original ethics application were submitted in January 2020 and April 2020 and were approved in March 2020 and May 2020, respectively. The May 2020 amendment enabled significant changes to be incorporated to circumvent COVID-19-related disruptions. This included the change of study design from a single crossover to double crossover so that existing participants could continue in the study for longer, and further changes so that participants did not have to attend the AIIMS hospital facility in person.

Nature and delivery of study intervention

Roti was chosen as the study food based on the following factors:

- advice of the Indian study team regarding culturally appropriate foods which could be consumed over the course of the study;
- avoidance of ingredients which influence the gut microbiota, such as high-fat products and sugars[276-278]; and

- results of sensory evaluation/palatability testing, provided as Appendix 4.

HAMS (in the form of Hylon VII) was chosen as the primary intervention RS based on the following properties:

- high and quantifiable RS content;
- non-genetically modified; and
- favourable organoleptic profile[279].

Cornstarch (in the form of Ruchi Cornstarch) was chosen as the alternative intervention starch based on the following factors:

- Although the original intention was that a control starch containing zero or minimal RS would be used, global supply chain disruptions from COVID-19 meant that the control starch chosen for this purpose (Amioca, Ingredion Incorporated) was not available when the doctoral researcher travelled to India to commence the study. As a result, a substitute was required from the local commercial market at the study site.
- At the commencement of the study, Ruchi Cornstarch was assumed to have a low/marginal quantity of RS of approximately 0.1%.‡

In terms of ensuring dosage, two members of the study team, including the doctoral researcher, weighed portions of each of the intervention starches (HAMS and Cornstarch) into ziplock plastic bags which were labelled 'A' for Cornstarch and 'B' for HAMS. The dosage of HAMS was intended to supplement the diet with 40g of RS per day during the HAMS intervention period, based on a clinical trial which used a similar dose of 39g/day in adult participants with no AEs reported[38]. The dosage was chosen to be at the higher end of the range used in dietary RS supplementation studies[230] so that the effects in this cohort would be more likely to be observable. Based on the manufacturer's advice that the RS content of Hylon VII was ~42.5%, the amount supplemented to the diet was 95g/day, which was assumed to contain 40g of RS and 55g of digestible starch. The amount of Ruchi Cornstarch supplemented to the diet was 55g/day, the dosage of which was based on matching the digestible starch portion of the HAMS. This was based on the initial assumption that the Ruchi Cornstarch contained only 0–1% RS and that almost the entire fraction would therefore be digestible starch.

‡ To verify this, RS assays were performed on the Ruchi Cornstarch. These assays showed that Ruchi Cornstarch actually contained 15% RS, which equates to a supplemented dose of 8.25g RS per day.

These packets of starches were then provided to the study team member responsible for the preparation of rotis. During each participant's feeding period, rotis were prepared fresh every morning by the staff member and wrapped in aluminium foil which was labelled with 'A' or 'B' to reflect the supplemented starch.

The ingredients and method for study food preparation are described in **Table 6**.

Table 6: Ingredients for study foods.

Ingredient	Quantity (g)	
	Roti A	**Roti B**
HAMS (as Hylon VII)	0	95
Cornstarch (as Ruchi Cornstarch)	55	0
Wholewheat ('atta') flour	200	200
Salt	5	5
Water	250	250
Nutrient composition		
Supplemented RS (from HAMS or Cornstarch)	8.25	61.75
Total carbohydrate (%)[280]	57.8	57.8
Protein (%)[280]	12.7	12.7
Fat (%)[280]	7.1	7.1

Roti preparation method

Mix all ingredients together to form the dough. Knead the dough and then roll it out to a flat disc shape. Heat groundnut oil in a roti pan. Once heated, place the roti on the pan and add extra groundnut oil around the edges. Cook for 2–3 minutes, turn over the roti, and continue to cook for a further 2–3 minutes. Remove from the pan. Transfer to packaging labelled 'A' for Cornstarch and 'B' for HAMS.

The field worker collected the labelled study food parcels each day during the supplementation periods and delivered them to the homes of participants. Participants were instructed to consume the full serve of study food (two rotis) every day during the 14-day supplementation periods and otherwise consume their normal diet. The field worker observed participants consuming the rotis each day, with the exception of a few occasions when the COVID-19 pandemic restricted the movements of study staff. If participants did not consume the entire serve, the field worker was instructed to weigh any leftover portion to calculate the amount consumed. During COVID-19 lockdowns, participants were provided with packets of either starch A or B, corresponding to their next supplementation period(s), and instructed to incorporate the full contents of one packet into a food or dish of their preference and consume the entire serve in one sitting.

Eligibility criteria

Inclusion criteria

- HIV-positive adults aged 18 years or over on ART;
- Blood CD4+ T cell count more than 200 cells/mm^3; and
- No antibiotic use within the previous six weeks.

Exclusion criteria

- Gastrointestinal conditions such as Crohn's disease or ulcerative colitis;
- Current participation in other research studies; and
- Pregnant or breastfeeding.

The CONSORT diagram depicting participant recruitment is provided below as **Figure 6**.

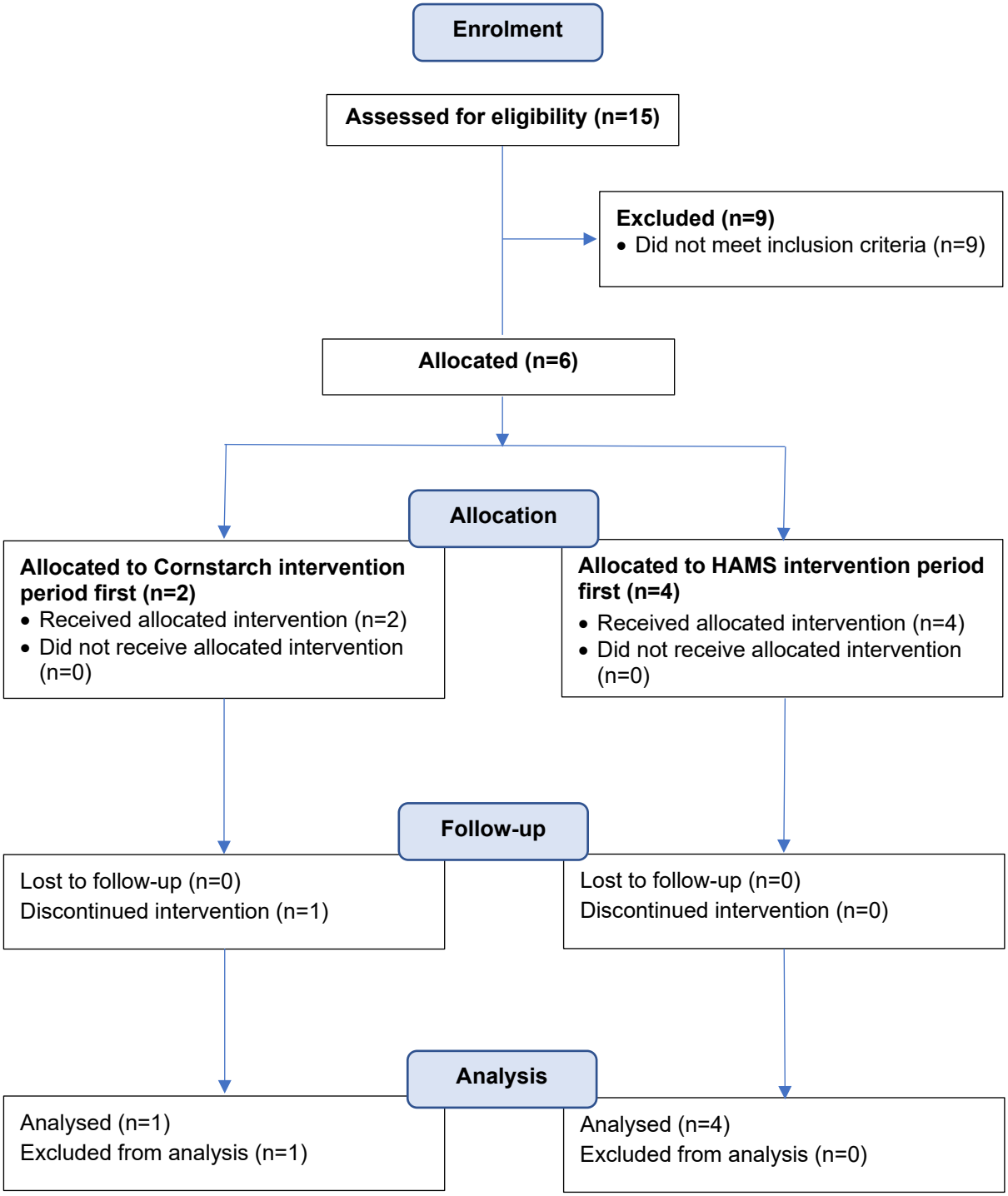

Figure 6: CONSORT diagram summarising participant enrolment and completion status.

Participant recruitment

Participant recruitment was open from February to May 2020. At the commencement of recruitment on 28 February 2020, there were no restrictions on movements owing to COVID-19. A total of four participants were recruited and commenced in the clinical trial between 28 February and 12 March 2020. Due to the COVID-19 pandemic, the Government of India imposed section 144 of the Constitution on 17 March 2020, restricting gatherings, markets and public transport and closing the borders of individual states. Due to these lockdowns, recruitment to research studies was prohibited by AIIMS, Bhubaneswar, on 17 March 2020. The doctoral researcher discussed the ramifications of this with her supervisory team in Australia and the Indian Principal Investigator (PI) who hosted this RCT at the study site in his role as Head of the Department of Biochemistry at AIIMS, Bhubaneswar. As a result, the study design was amended from a single crossover with an eight-week duration to a double crossover with a 16-week duration, to keep the existing participants for a longer period and create further opportunities for data collection and maximising statistical power. Fortunately, the Indian PI was able to secure authorisation from AIIMS, Bhubaneswar, for the field worker to travel to participants' homes during most of the lockdown period. Restrictions on recruitment were then eased in May 2020, allowing a further two participants to be recruited and commence in the study. The last participant completed the study and provided their final data on 2 September 2020. Participant 2 withdrew from the study, resulting in a total sample of n=5 participants who completed the study.

The primary method for recruitment of participants was via the active patient list of the AIIMS tertiary hospital outpatient ICTC clinic. This was supported by a snowballing recruitment strategy which encouraged participants to refer members of their personal networks to the research team. A rolling recruitment strategy was utilised whereby individuals were started in the study as soon as they consented. To ensure confidentiality for patients of the ICTC clinic, the first contact with potential participants was made by the ICTC counsellor who had interacted with the patient previously. This ensured that no identifying details were made available to the study team unless a person indicated that they were interested and agreed to be contacted. It was decided that a catchment area extending to a 30-kilometre radius around AIIMS would be applied to ensure that the field worker was able to deliver study foods to participants every day, as well as collect stool samples and administer survey tools. Using this criteria, ICTC staff (counsellors), contacted active patients residing in this catchment area by phone, describing the study and the commitment required from participants. If the patient was interested and agreed to be contacted by the research team, ICTC counsellors provided the patient's phone number to the research team. The field worker then contacted patients to set up a time for them to attend the offices of the study team at AIIMS. During clinic time, ICTC

counsellors also invited people living with HIV to consider joining the study when they were attending for appointments. If they were interested, ICTC counsellors contacted the study team, who then met privately with the interested person on the same day. Guided by the Intake Questionnaire Form (Appendix 5), the first meeting checked that the patient met the eligibility criteria; explained the study via the Participant Information and Consent Form (PICF); and sought consent. Following consent, the participant was supplied with a stool collection kit comprising plastic bags, wooden spatulas and screw-lid containers, as well as soap and a travel allowance of 100 rupees. Instructions for collecting stool were also provided to the participant in hard-copy form (Appendix 6) and verbally explained by the field worker at this first meeting.

Negotiation of consent

The PICF was translated by a member of the study team into Oriya, the local language of Odisha state. Potential participants were provided with the PICF in hard copy during the first meeting with the study team. The field worker then explained each aspect of the PICF verbally and prompted the person if they had any questions. This verbal explanation was important as some study participants were illiterate and not able to read the PICF. If the person consented to join the study, they were asked to sign the PICF with either their name or a fingerprint and this was witnessed and signed either by their support person or by a study team member when a support person was not present. For participants who joined the study before the COVID-19 disruption, the field worker provided an updated PICF to re-negotiate consent when ethics approval allowed the study to be modified from a single to double crossover design. All three PICFs are provided as Appendices 7, 8 and 9 as follows:

- original PICF for the single crossover study;
- updated PICF for existing participants to change from the single to double crossover study; and
- PICF for new participants joining the double crossover study.

Randomisation of starch order, allocation concealment and single blinding

Immediately following enrolment and consent, the order of starch consumption was randomised for the first crossover period (days 0–56) and again for the second crossover period (days 57–112). The method for randomisation utilised the ‘RAND’ function in Microsoft Excel, which is based on the Mersenne-Twister algorithm[281]. The randomisation allocation was concealed from participants and – because the study foods were similar in serve size, appearance and texture – it was easy to blind participants to which starch they were

consuming at a given time. Allocation concealment was addressed by arranging for a team member other than the doctoral researcher to perform the randomisation process and, since a rolling recruitment strategy was used, ensuring that only this member of the study team had access to the randomisation allocation codes[282]. This staff member was not directly involved in participant recruitment or management.

Sample size calculation

The primary outcome measure (pH) determined the sample size estimate. Assuming an effect size of 0.4 based on prior data[38] for a paired crossover, a sample size of 20 participants was required to determine a significant change in pH which could be attributed to the RS intervention. This assumed a correlation between the four measures (pre and post each treatment) for each participant of r=0.6 and a corresponding variance inflation factor=(1–0.6)/4=0.1[283]. An approach to sample size determination for pilot trials in the context of knowing standardised effect sizes, further informed the calculation[284]. This approach suggested that, for a full RCT designed with 90% power and two-sided 5% significance, the pilot trial sample size based on a standardised effect size of ~0.5 should be 15[284]. When the change was made from a single to double crossover design, the revised sample size calculation indicated that the same effect size of 0.4 could be detected with 80% power using n=10. This was an advantage which justified the change from a single to double crossover design. When the impact of COVID-19 on recruitment was fully realised, a further revision to the calculation revealed that with the n=6 participants who were enrolled, the study had 80% power to detect an effect size of 0.55.

Laboratory resources

Assays to test study assumptions and determine primary and secondary outcome data were performed by the doctoral researcher, the wider study team based in Australia or India, collaborative partners outside of the study team or commercial third-party providers. A summary of these assays and the relevant laboratory providers is provided in **Table 7**.

Table 7: Laboratory resources used in study assays.

Assay	Laboratory provider
Faecal pH	Study team – India
Faecal SCFA	Collaborative partners – India; and Commercial laboratory provider – Australia
Faecal absolute and relative abundance of bacterial species	Commercial laboratory provider – India
Blood CD4+ T cell concentration	Commercial laboratory provider – India
Plasma HIV viral load	Commercial laboratory provider – India
Faecal calprotectin	Study team – India
RS content of starches	Study team – India; and Doctoral researcher with study team – Australia
RS content of faecal samples	Study team – India

Addressing the study assumptions – methods

A number of assumptions were made prior to commencement of the study, as follows:

1. that the primary intervention starch (HAMS as Hylon VII) would have an RS content of ~42g/100g of starch;
2. that the control starch (Cornstarch as Ruchi Cornstarch) would have a zero/negligible RS content of 0–1g/100g of starch;
3. that dietary RS supplementation with study foods would result in RS intake above the normal dietary intake (measured by the Food Frequency Questionnaire (FFQ) and calculation of average daily RS intake);
4. that the RS content of faecal samples would increase during the RS supplementation periods (measured by the Megazyme-KRAPRS assay kit adapted for faecal samples);
5. that a dose-response effect could be established with increasing concentrations of HAMS inocula added to donor faecal samples (measured by the Megazyme-KRAPRS assay kit adapted for faecal samples); and
6. that gut inflammation of all participants would be above the normal range (measured by faecal calprotectin concentration).

RS, digestible starch and total starch fractions of starches

To address study assumptions (1) and (2) relating to the RS content of study starches, assays of HAMS and Cornstarch were undertaken using a rapid RS assay, the Megazyme K-RAPRS kit (Megazyme, Ireland). Based on the original Megazyme assay kit, K-RSTAR, the K-RAPRS method more closely resembles the human digestive process and small intestinal transit time by reducing the incubation period to four hours. The K-RSTAR method requires an incubation

time of 16 hours. Megazyme advises that the K-RSTAR kit is preferable when a high content of RS4 is anticipated in samples. However, as this study supplemented RS2 and did not anticipate high levels of RS4, the K-RAPRS kit was used because it was much quicker and saved time[285, 286].

An assay to measure the RS fraction of both starches to determine RS, digestible starch and total starch fractions was undertaken in Australia using the Megazyme K-RAPRS kit with the following modification: Samples were mixed by agitation using a serological pipette instead of a magnetic stirring bar, owing to the availability of only one magnetic stirrer at the time of the assay. To verify that similar results were achieved using either method, one control 100mg sample was mixed with a magnetic stirrer.

Digestion of complex carbohydrates into digestible starch was performed as steps iv to xii of the *'Hydrolysis and solubilisation of digestible (non-resistant) starch'* section of the protocol. The resulting supernatant was used to measure the amount of digestible starch according to the *'Measurement of Digestible (Non-Resistant) Starch'* section of the protocol, while the remaining suspension containing RS was further digested using Amyloglucosidase (AMG) as per the *'Measurement of Resistant Starch'* section of the protocol.

As the assay differed based on the anticipated content of RS, different steps were followed for the measurement of RS in HAMS and Cornstarch. As HAMS was anticipated to have an RS content greater than the 10% cut-off value stated in the Megazyme protocol, 100mg samples of HAMS were transferred into 2 x 100mL flasks. Volumes were adjusted to 100mL with distilled water and mixed well. Aliquots of ~2mL were then centrifuged at 13,000 rpm for 5 minutes. Then, a 0.1mL aliquot of each sample was used for RS measurement.

For the Cornstarch which was anticipated to contain less than 10% RS, 0.1mL aliquots of the *undiluted* sample was used.

RS was then measured as per steps vi–vii of the *'Measurement of Resistant Starch'* section of the protocol, with measurements taken in duplicate and a mean value calculated. Optical density (OD) measurement at 510nm was calculated using a Cary 300 UV-Visible spectrophotometer (Agilent).

Starch fractions were then calculated based on the Mega-Calc Data Calculator spreadsheet, which uses the formula shown in **Figure 7**.

Resistant Starch (g/100g)

$$= \Delta_A \times F \times \frac{EV}{0.1} \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180}$$

$$= \Delta_A \times F \times \frac{EV}{W} \times 0.90$$

Digestible Starch (g/100g)

$$= \Delta_A \times F \times \frac{EV}{0.1} \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180}$$

$$= \Delta_A \times F \times \frac{EV}{W} \times 0.90$$

Total Starch (g/100g) = Resistant Starch + Digestible Starch

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where:

ΔA	= absorbance of sample solution read against reagent blank.
F	= factor to convert absorbance values to μg glucose (100 μg glucose divided by the absorbance value obtained for 100 μg of glucose).
EV	= sample extraction volume (10.3 mL or 100 mL).
0.1	= volume of sample analysed.
1000	= conversion from μg to mg.
100/W	= conversion to 100 mg sample.
W	= sample weight in mg.
162/180	= factor to convert from free glucose, as determined, to anhydroglucose, as occurs in starch.

Figure 7: Mega-Calc Data Calculator formula.

Following the above confirmation of the starch content, calculation of the average daily supplementation of RS from both the study foods was undertaken.

Dietary intake of RS

To address study assumption (3), *that dietary RS supplementation with study foods would result in RS intake above the normal dietary intake (measured by FFQ and calculation of average daily RS intake)*, dietary RS intake was quantified, based on the RS content of HAMS and Cornstarch rotis as well as an analysis of the RS content of the normal diet consumed during the study period.

FFQ

An FFQ asking respondents to indicate the frequency and quantity of consumption of dietary items during the preceding 14-day period was designed by the doctoral researcher to determine dietary intake of RS. An FFQ validated for use in a southern Indian population was the most representative tool available for adaption[287]. In order to adapt this FFQ for use in the study population, a small group of local experts (n=3) was requested to participate in a face validity task that asked if they understood what was meant by each item on the original FFQ and to describe each food or beverage item[288]. For the foods they were familiar with, they were then asked to indicate which of those foods were commonly consumed in Bhubaneswar and to suggest others that were commonly eaten locally. In recruiting these experts, individuals who represented a variety of demographics, including younger (mid-20s) and older (mid-40s) females and males with different educational and professional backgrounds, were involved. This method of determining 'face' or 'expert' validity was important to ensure that the dietary items included in the revised FFQ were suitable for the study population[289]. The approach of engaging local experts to assess the suitability of a tool has been used in studies where the vetting or provision of feedback by users or administers of an intervention has been an important step[288-290]. This process led to the removal of some items from the original FFQ and the addition of foods and beverages typically consumed in Bhubaneshwar.

Following a training by the doctoral researcher, the FFQ was administered by the field worker with each study participant. The FFQ was administered at four timepoints, each corresponding to the day after a dietary supplementation period at days 15, 43, 71 and 99. This corresponds to timepoints S2, S4, S6 and S8. The FFQ, which is provided as Appendix 10, used a consumption scale of 'Never or less than once', '1–3 times', '4–6 times', '7–10 times', 'More than 10 times', '1 time per day', '2 times per day' and '3 + times per day' for 50 dietary items.

Although the primary rationale for the FFQ was to quantify dietary sources of RS other than the study foods and therefore to be administered after supplementation periods, the omission

of the baseline timepoint to determine typical RS consumption was compromised due to logistic issues. To address this, the FFQ was administered to study participants between four and eight weeks after completion of the double crossover intervention to determine habitual dietary intake. As the field worker was no longer available to assist, staff who were trained and experienced in the administration of dietary intake surveys completed this data collection. To enable this, the doctoral researcher conducted a workshop with these staff via Zoom to agree on standardised measurements and serving sizes to be used to quantify food intake. Assumptions of serving vessel quantities and other serve sizes were constructed based on these discussions between the Indian study team and the doctoral researcher, who previously lived and worked in South Asia for three years.

These standards were then used during administration of the FFQ to elicit accurate portion size estimates[287, 291]. These values are provided below in **Table 8**.

Table 8: Capacity of typical Indian measuring vessels.

Vessel/Item	Serving size
Serving vessel	
Tablespoon	20ml
Cup	200ml
Bowl	300ml
Glass	400ml
Plate	400g
Dietary item	
Chapatti/Paratha	1 bowl = 2 pieces of chapatti
Idli	1 bowl = 4 pieces 1 piece = 20g
Vada	1 bowl = 5 pieces 1 piece = 20g
Biscuits	1 bowl = 5 biscuits 1 piece = 15g
Normal roti (i.e., not supplemented with RS)	1 piece = 50g
Chapatti	1 piece = 50g
Indian banana	1 piece = 100g
Pitha	1 bowl = 3 pieces 1 piece = 100g

A method for quantifying RS intake from the FFQ data was then required. Given the relative infancy of research into RS and the lack of inclusion of RS content in standard food composition tables, a method for quantifying intake relied on values reported in research articles. While there are various articles reporting the RS content of single ingredients and composite foods, there is frequent disagreement regarding the RS content of commonly consumed foods, such as white rice, stemming from the method used for quantification[292-294]. This disagreement was problematic within the current study, as white rice represented a significant proportion of total dietary intake in this population. However, given that RS content of rice is generally agreed to be quite low, using mean values of published RS content was considered to be a suitable approach by the doctoral researcher.

In a 2020 paper, RS content of foods commonly consumed in the United States was reported based on data pooled from studies analysing RS content with in vivo or in vitro methods but excluding methods using heating before enzyme hydrolysis or an incubation temperature of 100°C as this reflects only RS3 content[29]. Similarly, articles that applied preparation steps not commonly involved in human consumption – for example, the addition of acid, enzymes such as pullanase, or extrusion processes – were excluded[29]. Given that this article reported foods consumed in the United States and was therefore of limited use for typical Indian foods consumed by study participants, other references were drawn on, as indicated in **Table 9**. Some of these articles provided incomplete details of the RS assay method used or relied on methods which had not been validated in the ileostomy model and were not equivalent with method AOAC 2002.02. However, one paper using a Megazyme kit specifically based on AOAC 2002.02, was particularly useful for its examination of typical Indian dishes containing legumes[295].

Table 10 shows the RS content for dietary items which either comprised a large portion of the typical dietary intake or contained moderate–high levels of RS. Where possible, the criteria applied in selecting articles for this purpose was that the method for RS quantitation had been validated in the ileostomy model. On occasion, either this method was not followed or it was not possible to determine if it was followed. Where more than one value for the same dietary items was reported in the literature, a mean of these values was used. In the case of some dietary items, generation of mean values had already been published, such as in the Patterson et al. article[29].

Table 9: RS content of dietary items used to calculate RS intake with reference details.

Dietary item	RS g/100g food	Source/citation of reference RS value
STUDY FOOD (Roti A): Wheat roti with Cornstarch	14	Based on laboratory assay of Cornstarch using Megazyme K-RAPRS kit
STUDY FOOD (Roti B): Wheat roti with HAMS	61	Based on laboratory assay of HAMS using Megazyme K-RAPRS kit
Idli	4.20	296,297
Vada	0.42	295
Dosa	5.72	296
Chapatti/Paratha	2.20	296
Roti-maize (makka)	1.87	297
Whole makka roasted or steamed	39.4	29, 298
Pitha (with jaggery and coconut)	1.67	299-301
Upma (all types)	3.13	296
Corn flakes/Rice flakes	1.83	302
White boiled rice	0.20	29
Brown rice	0.10	29, 303
Flattened/Beaten rice (chuda)	0.60	29, 304
Vegetable fried rice	1.40	29, 305
Biryani	1.40	29
Sambar/Other dhal curries	0.82	295
Erussery/Pumpkin, salt, tamarind and lentil curry	5.45	306
Whole gram curry	1.21	295
Any potato dish	0.70	29
Raw/Green banana	2.80	29
Yellow (ripe) banana	1.80	29
Chickpeas (chola), any preparation	2.10	29
Roasted channa (chickpeas)	2.10	29
Biscuits (sweet, cream, etc.)	1.10	29, 307

While there were reported values for some Indian composite foods typically eaten by participants, such as vada[295] and idli[297,296], for others, such as pitha, RS values were not available. As these composite foods were most often store-bought, this required the calculation of RS content based on local recipes provided by nutrition research staff from the Indian collaborating organisation. Although the Patterson et al. article was useful, as it provided multiple values for RS content of typically consumed foods[29], there were large differences with values reported in other articles for the same composite foods, such as chapatti[296, 308]. Estimates of RS content were utilised for foods which either required calculation of RS content based on raw ingredients (e.g., jaggery pitha; see **Table 10**) or required a mean RS content value to be derived from different studies (e.g., chapatti).

Table 10: Ingredients and calculation of RS content for composite dish, jaggery pitha.

Jaggery pitha ingredients	**Volume (g)**	**RS content g/100g and source reference**	**RS content of each ingredient (g)**
Jaggery	270	0	0
Water	375	0	0
Sugar	50	0	0
Rice flour	300	3 [299]	9
Wheat flour	200	3.15 [300]	6.3
Coconut	100	0.04 [301]	0.04
Salt	0.5	0	0
Total	**~920**		**15.34**
Makes 9 pitha, serve size ~100g each			1.67

Jaggery pitha is a steamed food made from rice flour and black gram batter, stuffed with jaggery/sugar and coconut.

Daily RS intake

The dietary intake data from the FFQ was then used to calculate average daily RS intake by deriving the RS content multiplier of each food item from the RS content values (RS g/100g food) listed in **Table 10**, as follows:

$$\frac{\text{RS (g/100g) of food}}{100}$$

In applying this formula to the example of white rice which has an RS content of 0.2g/100g (Patterson et al, 2020), the above formula produces an RS content multiplier as follows:

$$0.2/100=0.002$$

The dietary analysis for the four RS supplementation periods was then converted into RS intake in grams for each food by multiplying the quantity of food for each 14-day period by the RS content multiplier. For example, if a participant ate 500g of rice over the 14-day period and the RS content multiplier of white rice is 0.002, then the amount of RS consumed was:

$$500 * 0.002=1\text{g of RS}$$

This resulted in RS intake from each food for the 14-day period. Total RS intake for the 14-day period was then calculated by adding RS intake for all foods together. This value was divided by 14 to result in an average daily RS intake for each participant for each of the four supplementation periods.

RS content of faecal samples

In a post-hoc analysis, the Megazyme K-RAPRS assay was used to determine RS concentrations in stool stored at ultra-freezing temperatures (-80° Centigrade) with samples provided from (1) study participants to determine the residual amount of RS remaining following gut transit as a verification step to confirm adherence to study foods and (2) a single HIV-negative donor to establish a standard curve of the dose-response effect.

Several modifications were applied to the Megazyme K-RAPRS assay to adapt it for use with stool samples as recommended by the kit manufacturer: First, stool samples were freeze-dried and then milled to pass a 0.5mm screen. Next, samples were dissolved in deionised water and free glucose was determined using GOPOD reagent. As per the Megazyme guidance, no free glucose was found in stool samples collected in this study so the method outlined above using the K-RAPRS kit for starches was followed.

Dose-response effect

To verify if a dose-response effect could be demonstrated, stool samples with a low detectable amount of RS from a single HIV-negative donor were spiked with increasing amounts of HAMS. 20g samples of stool were spiked with 1g, 2g, 5g and 10g of HAMS and then assayed for total RS according to the K-RAPRS protocol with the above modification for faecal samples.

Faecal calprotectin

The rationale for including faecal calprotectin concentration was to test the study assumption that gut inflammation reflecting EED would be present in this population at commencement of the study. Although it was not originally intended to measure calprotectin at subsequent timepoints, the unexpected baseline values led to calprotectin being assayed at the remainder of the study timepoints, to enable examination of the dynamic responsiveness of calprotectin and the performance of the assay.

Calprotectin was measured in faecal samples collected at two-weekly intervals from baseline over the 9 timepoints of the study as follows: using the Epitope Diagnostics Inc EDI Quantitative Faecal Calprotectin ELISA Kit, 50–100mg aliquots of thawed stool were measured into weighed tubes and weights were recorded. Extraction buffer was then added as 39 parts of stool volume and vortexed to dissolve the sample. The sample was centrifuged at 3000G for 5 minutes. 0.15mL of clear supernatant was then transferred to a clean tube with 1.2ml of extraction buffer followed by gentle vortexing.

Following reagent preparation according to the EDI Quantitative ELISA Kit instructions, the assay was undertaken as follows: Using a microwell plate, 50µL of assay buffer was added to each well to coat the well evenly. 50µL of standard or extracted participant stool sample was then added. The plate was protected from light and rotated on an ELISA plate shaker for 1 hour at 450rpm. Immediately prior to the end of the incubation time, the proportional amount of tracer antibody for the assay was prepared by dilution. Following washing, 100µL of the diluted tracer was added to each well. The shaking step detailed above was then repeated for a period of 45 minutes. Following this step, 100µL of ELISA HRP substrate was added into each of the wells. The plate was then covered with foil again and incubated at room temperature for 12 minutes. After gentle shaking of the plate to ensure the even distribution of colour, absorbance was read at 620nm. 100µL of ELISA stop solution was then added to each well, followed by gentle mixing. Absorbance was read again at 450nm with reference filter at 620nm. Absorbance measurements were recorded in duplicate for each well.

Average absorbance was calculated for pairs of duplicate results. The average of the level 1 standard (0 ng/mL) was then subtracted from the average of all other readings in order to

arrive at the corrected absorbance. Log-log paper was used to generate a standard curve by plotting the corrected absorbance of all standard levels on the ordinate against the standard concentration on the abscissa. The standard curve was then used to read calprotectin concentrations by referring to the respective corrected absorbance.

Method 1: Are RS supplementation studies in this population feasible?

Qualitative semi-structured interviews

The data collection tools and method of analysis to assess feasibility according to Thabane et al.'s Process, Resources, Management and Scientific categories (2010) are detailed as follows.

Data collection

This study utilised both theory-driven and grounded theory approaches in the semi-structured interview method[219]. The interview questions were designed to explore the following key areas and are provided in detail in Appendix 11:

1. Barriers and enablers to participation in the study (example questions: What factors led you to agree to participate in this study? What do you think will be difficult about participating in the study? What do you think will be easy about participating in the study?).
2. The role that trust played in motivating participants to join the study (example questions: Did the involvement of an Australian University with AIIMS-Bhubaneswar influence your decision about participation? How confident do you feel in the research team and what factors have influenced this?). The literature on trust informed this line of questioning which encouraged acknowledgement of the inherent power dynamic in the researcher–participant relationship, similar to the dynamic described for the clinician–patient relationship, with a particular focus on LMICs and disadvantaged populations[309]. Noted also was the inherent vulnerability that is present in health-seeking populations[309-311].

In this participant cohort, none of the participants were fluent in English. Noting that the doctoral researcher was not fluent in Oriya or Hindi (another commonly used language within the participant cohort), an interview guide was designed by the doctoral researcher and translated into Oriya language and script independently by two members of the research team. To assess validity, these research team members then back-translated each other's translation and, where there was divergence, finalised the translation in consultation with the

doctoral researcher, who provided clarification regarding intended meaning[312]. Interviews were then conducted by the field worker, who was fluent in Oriya.

During the field visit, one-on-one training was provided to the field worker by the doctoral researcher to ensure that the style of interviewing was appropriate for the study. This included the importance of open-ended questions and avoiding prompts which might lead the participant to respond in a particular way. The training also provided strategies for clarifying meaning where there was ambiguity in participant responses. Following each participant interview during her field visit, the doctoral researcher discussed the completed interviews with the field worker in person to try to identify areas where encouragement or critical feedback could enhance future interview processes. Once the doctoral researcher returned to Australia, she continued this interaction with the field worker after each interview via video calls using WhatsApp.

Interviews were conducted at timepoints S1, S4 and S9, as indicated in the study design graphic in **Figure 5**. The day 0 interviews were conducted in the presence of the doctoral researcher for three of the participants (Participants 1, 2 and 3); the remainder of the qualitative interviews were conducted with only the field worker and the participant present. Participants were asked to allow for at least 30 minutes to complete the interview. The baseline interviews at the day 0 timepoint were conducted in the offices of the research team on the AIIMS, Bhubaneswar campus. Subsequent interviews were conducted at participants' homes by the field worker on days 43 and 112. During the interview, the field worker took handwritten notes of the participant responses in Oriya script. These were then translated into English by another member of the research team, in consultation with the field worker to ensure that the intended meaning of participants was reflected.

Data analysis

Using a thematic analysis approach, these data were compiled and then disassembled to determine key themes[313, 314]. Disassembly involved separating the data and grouping similar data together, through the process of coding[313, 315]. Noting the relatively small size of the dataset, a manual approach was utilised for coding, rather than a software-based approach such as NVivo[316]. This involved identifying phrases and words which were used often during interviews and using different coloured highlighters to mark these on the translated versions of the interview transcripts. During this task, the doctoral researcher maintained a log of phrases and words under broad conceptual headings – such as 'will power', 'health benefit' and 'trust' – which emerged from the data, with the intention of testing the headings as themes and determining the levels of similarity between the words and phrases under each heading[219]. This process of developing themes also involved identifying connections between the

conceptual headings/categories and theoretical categories identified in the literature on trust in healthcare[219]. This approach to analysis recognised that while the two key areas of interest were declared before the analysis as (1) barriers and enablers to participation in the study and (2) the role of trust in motivating participants to join the study, an inductive approach led to participant responses shaping the findings[219, 317].

Following the disassembly task, reassembly was performed to create themes according to the inductive method, involving putting the codes into context with each other[219, 313, 318].

Qualitative process evaluation

Data collection

Field notes have been used by researchers focused on ethnographic qualitative research as well as by clinical triallists aiming to improve study design via the reflections of researchers[208, 319, 320]. Detailed field notes were kept by the doctoral researcher during the study period, including when she was at the field site in India and also when managing the study remotely from Australia. The field notes collected when the doctoral researcher was in Australia were based on communications with the Indian study team, including through phone calls, emails and instant messaging via WhatsApp.

Handwritten or typed field notes were documented each day during the field visit. Intending to apply an *a priori*[321] approach to the categorisation of field note content, the doctoral researcher established the following topics prior to commencement of the study:

- perspectives and preferences of members of the Indian study team about how to implement the study protocol and specific methods;
- refinements required to methods and processes during the planning, recruitment and trial delivery stages, including protocol, budgetary, staffing and logistics. Anticipated examples included refinements to the FFQ tool, the ingredients and method for roti preparation, and pilot study food testing;
- observations of systems and processes utilised to get things done at AIIMS, Bhubaneswar – for example, approval for expenditure, accessing resources from other teams in terms of staffing, lab equipment, and accessing endorsements and authorisations from the Chief Executive; and
- lists of action items to follow up in subsequent days, including points of discussion to raise with the Indian team and the primary supervisor of the doctoral study in Australia during regular calls.

Data analysis

Field notes were analysed thematically[206] utilising an inductive approach. This method of analysis both relied on and acknowledged the researcher's perspective when it came to the interpretation of field notes that were primarily descriptive in nature[322, 323]. To verify this interpretation and the themes generated, the analysis stage included a consultation phase in which the Indian team was asked to provide feedback so that the doctoral researcher could arrive at an agreed interpretation of the qualitative data. This triangulation process was essential to verify the validity of interpretations and resultant themes[324], particularly given the divergence in cultural background between the doctoral researcher and both study participants and staff.

Sign and Symptom Checklist (SSC-HIVrev) – gastrointestinal subset

Data collection

The SSC-HIVrev (gastrointestinal subset) tool comprises a subset of a 64-item self-report checklist which has been validated in HIV-positive populations[212, 325]. The eight items reflect gastrointestinal symptoms and ask participants to rank the intensity of any symptoms on the day of survey completion on a scale of Mild, Moderate and Severe. The tool is provided at Appendix 12.

Data analysis

Owing to the limitations of the small sample size, a descriptive analysis approach was taken with the SSC-HIVrev data. The maximum number of reported signs and symptoms and the maximum severity of reported signs and symptoms were calculated for baseline and each of the three study periods (Cornstarch, HAMS and Normal diet).

The data described above were incorporated into an assessment of the following themes:

- the feasibility and acceptability of the study food intervention; and
- the feasibility and acceptability of the study design and procedures[253].

Method 2: Was there a treatment effect from RS supplementation?

Faecal samples

Faecal sample collection

Once-daily stool samples were collected by participants during the RS supplementation periods as outlined above under the 'Participant recruitment' section. Participants were requested to provide one stool sample each day during these periods, rather than samples collected over a full 24-hour period. Participants then contacted the field worker via WhatsApp

to arrange collection of the sample as soon as possible. The field worker transported the sample to the laboratory in a container filled with ice. Aliquots weighing 0.5g were then taken and stored in labelled cryovials at –80ºC within a four-hour period of the sample being produced by the participant.

Faecal pH measurement

Thawed faecal samples were diluted in a 1:10 dilution with saline, then homogenised. Samples were then centrifuged at 13,000g, 4ºC, for 10 minutes. Change in stool pH was measured by assaying the supernatant of samples using a Mettler-Toledo pH meter on a scale of 1–14.

Faecal SCFA measurement

SCFA assays were performed twice. On the first occasion, assays were undertaken in India by a laboratory with which the Indian PI had an existing collaborative partnership. Since this first set of results included multiple nonsensical values, remaining aliquots of faecal samples were imported to Australia and the assays were repeated by CSIRO, Health and Biosecurity. The CSIRO results are presented in the Results chapter. Methods employed by the different laboratories are described below.

Indian laboratory method

Butyrate, acetate, propionate and valerate were measured using a gas chromatograph fitted with a flame ionisation detector as follows: qualitative and quantitative analysis of the SCFA composition was undertaken using the Exactive Plus Orbitrap high-resolution mass spectrometer and Ultimate 3000 high-performance liquid chromatography (Thermo Scientific, USA). LC-MS grade methanol, water, formic acid, acetonitrile, and acetone were obtained from Fisher Scientific (USA). In 2mL centrifuge tubes, 0.5g aliquots of thawed faecal sample were mixed with 1.5 mL of a 1:1 dilution of methanol with water. The solution was centrifuged for 10 minutes at 4°C at a speed of 10000 rpm to remove solid particles. The solution was then filtered in a 0.22µM syringe filter to remove additional particles. 0.5mL of filtrate was aspirated and transferred to DP ID vials (Cat#C4000-1W, Thermo Scientific, USA) for mass spectrometry. A Hypersil BDS C18 (250 mm × 2.1 mm, 5 µm; Thermo Scientific, USA) column was used to separate the compounds in the solution. The mobile phase utilised a 1:1 solution of methanol: water with 0.1% formic acid. Sample flow rate was set at 3µL/Min with a column temperature of 30°C with pressure maintained at 700 bar. Molecules were then ionized through an electrospray ionization mechanism at a 3eV potential and were sourced through nitrogen gas into an orbitrap chamber. This sample was run for a duration of 15 minutes. The ions were detected in both positive and negative polarity with a scan range of 50–750 m/z. To calculate the concentration of SCFAs present in the faecal samples, mass peak intensities

were compared with the standard curve which was made by running 5 different concentrations of each of the SCFAs.

CSIRO laboratory method

Samples were diluted with 6mL of 1.68mM heptanoic acid internal standard solution mixed and centrifuged. A portion of the supernatant was hydrolysed with phosphoric acid, filtered and analysed by gas chromatography using an Agilent GC (7890A). SCFA concentrations were determined against a prepared GC calibration standard mixture containing known amounts of SCFA components.

Faecal absolute and relative abundance of bacterial species

Sample preparation

Samples were prepared by adding ~0.15g of thawed stool to an empty weighed 1.5mL Eppendorf tube. After weighing the tube to calculate the pellet weight, 500µL of cold 1 × PBS was added to the tube followed by mixing the suspension by vortex. The samples were then spun on a centrifuge at 13,000rpm for 20 minutes at 4°C, after which the supernatant was transferred to a 2mL tube and stored at –80°C.

DNA extraction

DNA extraction was performed using a MoBIO PowerLyzer PowerSoil 96 well DNA isolation kit (Mo Bio Laboratories). DNA concentrations were quantified fluorometrically with a Quant-iT dsDNA Assay kit (Life Technologies).

16S rRNA gene amplicon sequencing

Bacterial communities were determined using next-generation sequencing (NGS) approaches with gene amplicon sequencing of the V4 hypervariable region of bacterial 16S rRNA, as described by Choo et al. (2015). Sequencing using an Illumina MiSeq platform was performed by Clevergene, Bengaluru (Bangalore), India. Paired-end 16S rRNA gene amplicon sequence reads were analysed using the Quantitative Insights into Microbial Ecology (QIIME2) software [326], as previously described[327]. Taxonomy was assigned based on the SILVA 16S rRNA database (v138) clustered at 99% sequence identity. All samples were subsampled to a depth of 5,050 reads for downstream analysis. The gene sequence datasets will be publicly available from the National Center for Biotechnology Information (NCBI) Sequence Read Archive immediately upon publication of the study manuscript in a peer-reviewed journal in late 2023/early 2024.

Blood samples

Sample collection

2 × 2mL of venous blood was collected at the timepoints indicated in **Figure 5**. At each timepoint, one 2mL sample from each participant was stored at room temperature between 18ºC and 22ºC (for CD4 count). The other 2mL sample was transported at room temperature and then refrigerated immediately upon arrival at the laboratory (for HIV viral load). Blood assays were performed within two hours of collection. Two timepoints for collection of blood following Normal diet periods (S3, S9) were removed, following advice from the ICTC counsellors that the original aim of sampling blood at nine timepoints would be too onerous for the participant cohort.

Blood CD4+ T cell concentration

2mL samples of whole blood were utilised in a single platform flow cytometry assay to determine absolute and percentage values of CD4+ T cells as follows: Whole blood samples were analysed using the BD FACSCanto II automated six-colour modules system with BD Multitest six-colour TBNK reagent and BD Trucount tubes by BD Biosciences. Samples were added to the BD Trucount tube, which contained a lyophilised pellet with a known count of fluorescent beads. Following staining of the samples with the BD Multitest six-colour TBNK reagent, the automated system enumerated CD4 as absolute counts (cells/μL) and percentage (of total absolute lymphocyte count measured by CD45).

Plasma HIV viral load

Whole blood samples were centrifuged for 20 minutes at 1000 G. The plasma fraction was then decanted and used to determine HIV viral load. Viral RNA was extracted using the Roche High Pure Nucleic Acid kit (Cat. No. 11858874001) with a sample volume of 200μL for a final elution volume of 50μL. HIV viral load was measured using the Quantiplus HIV-1 RT-PCR Kit from Huwel Life Sciences, India, which is based on Taqman probes. The RealTime PCR step was set up with the internal control provided in the Huwel kit, as per the specifications in **Table 11**.

Table 11: Specifications for RealTime PCR (Huwel kit).

Components	Volume per reaction (µL) (for final volume of 26µL)
Huwel HIV Ready Mix	15.0
Huwel RT Enzyme	1.0
Huwel IC Mix	1.0
Extracted RNA/QS/PW	10.0

The assay was performed as per the Quantiplus protocol and using the Bio-Rad CFX 96 instrument (Bio-Rad Laboratories).

Statistical analysis

Descriptive and univariate analysis

Baseline/Day 0 samples acted as each individual participant's own control. The use of Normal (or washout) diet periods between the starch feeding periods in this study created multiple baselines which varied between participants based on randomisation group allocation. The analysis approach for this study therefore considered the below factors, which influenced the choice of baseline for each analysis:

1. the different randomisation group allocation which determined the *group baseline*;
2. the two distinct but related sub-studies (first and second crossovers), which influenced the *study baseline*; and
3. the *absolute baseline* or day 0 of the study before participants consumed any supplementary starches.

The analysis described here was focused on the study and absolute baselines summarised in 2 and 3 above.

Although the absolute baseline of day 0 and the end of each Normal diet period did not differ between participants, the timepoints following each dietary intervention period did differ, based on randomisation group allocation. Consequently, summary variables were created for data collected at the timepoints following each intervention period. This ensured that randomisation order differences were factored in by reflecting combined data collected at the end of the two interventions, regardless of the order in which they occurred. The outcome measures and their corresponding study timepoint ID were as follows:

For all participants:

- 'Baseline': day 0, S1 timepoint; and
- 'Post-Normal': day 28 (S3), day 56 (S5), day 84 (S7), day 112 (S9).

For participants in Randomisation cohort A:[§]

- 'Post-Cornstarch': day 15 (S2) and day 71 (S6); and
- 'Post-HAMS': day 43 (S4) and day 99 (S8).

For participants in Randomisation cohort B:[††]

- 'Post-Cornstarch': day 43 (S4) and day 99 (S8); and
- 'Post-HAMS': day 15 (S2) and day 71 (S6).

Mean values for each participant were calculated from the data collected at each timepoint for these summary variables. Descriptive and univariate analyses were then performed to determine the effect of RS supplementation by study period on the outcome variables described above.

Microbiota analysis

Bacterial relative abundances were used to assess microbial alpha diversity and composition (beta diversity). QIIME2 (v2.2019.4) was used to compute alpha diversity measures of microbial richness (operational taxonomic units (OTUs)), evenness (Pielou's evenness) and diversity (Faith's phylogenetic diversity), as well as weighted UniFrac distances to assess microbiota composition [326]. Normality of data distribution was assessed using the Shapiro–Wilk test. Between the study periods of HAMS, Cornstarch and Normal diet, intergroup differences in alpha diversity measures and bacterial relative abundances were determined using the Friedman test, as well as the Wilcoxon test for post-hoc comparisons. Intergroup differences in microbiota composition were determined using a permutational analysis of variance (PERMANOVA) model. This model assumed the parameters' permutation of residuals under a reduced model and a type III sum of squares (Primer-E v.7; Primer-E Ltd., Plymouth, United Kingdom) [328]. Due to the compositional nature of the microbiome data which

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[§] *Randomisation cohort A* feeding order: Cornstarch – Normal diet – HAMS – Normal diet – Cornstarch – Normal diet – HAMS – Normal diet.
[††] *Randomisation cohort B* feeding order: HAMS – Normal diet – Cornstarch – Normal diet – HAMS – Normal diet – Cornstarch – Normal diet.

had a non-normal distribution, common statistical methods such as ANOVA were not appropriate for composition analysis[329]. A trajectory graph of microbiota composition changes within individual participants across each timepoint was generated using the *ecotraj* package (v0.0.1) in R statistical software. Intergroup differences in measures of fermentation outcomes were assessed using Wilcoxon rank-sum tests (GraphPad Prism version 7.00).

Linear mixed-effect model

A linear ME model was used to determine the effects of the different starches, both within individual participants and between participants. The fixed effects were the two dietary starches or 'treatments', as well as the two crossover periods. Carryover effects were added to the ME model via the creation of two coefficients, 'carry1' and 'carry2', which reflected the difference in values for each outcome measure at the beginning of each treatment period. Multilevel regressions were performed using StataCorp, USA, version 17.0. The significance of all statistical analyses was determined based on a p-value of <0.05.

RESULTS

Following a description of the overview of study participants, this chapter will address findings relating to the study assumptions. Results will then be presented in relation to:

1. **The feasibility assessment.** This will address study hypothesis 1, that the feasibility assessment will indicate that future studies with a larger sample size are feasible.
2. **The determination of treatment effect.** This will address study hypothesis 2, that dietary RS supplementation will result in fermentation of RS by gut bacteria in the large intestine, leading to increased SCFA production and a decrease in pH that will positively influence the gut microbiota and HIV-related immunity.

Participant characteristics

As outlined in the Synopsis, a total of six participants were recruited; one withdrew on day 3, shortly after commencing in the study, and provided only one blood sample. The remaining participants (n=5) completed the full 112 days of the study. Baseline characteristics of all participants are provided in **Table 12**.

Table 12: Baseline characteristics of study participants.

Participant ID	1	2	3	4	5	6	All participants (male; n (%)) n=6	Randomisation cohort A* (male; n (%)) n=2	Randomisation cohort B** (male; n (%)) n=4
Sex (M/F)	M	F	M	M	M	M	5 (83.3)	1 (50)	4 (100)
Date of enrolment	22/02/2020	27/02/2020	3/03/2020	11/03/2020	8/05/2020	13/05/2020	**Summary values for 3 subgroups: (median; (IQR))***		
Age at enrolment	19 years	50 years, 7 months	51 years, 11 months	49 years, 8 months	34 years	37 years, 1 month	43 years and 5 months (15 years, 7 months)	Min: 43 years, 5 months Max: 50 years, 7 months	41 years and 10 months (19 years, 12 months)
Months living with HIV	1	20	20	30	12	3	16 (14.75)	Min: 3 Max: 20	16 (13.25)
Months of ART use	1	19	20	30	11	3	15 (14.75)	Min: 3 Max: 20	15.5 (14)
Height (m)	1.67	1.38	1.70	1.68	1.65	1.58	1.66 (0.08)	Min: 1.38 Max: 1.58	1.68 (0.02)
Weight (kg)	44	45	53	54	59	45	49 (8.75)	Min: 45 Max: 45	53.50 (4.50)
BMI	15.8	23.6	18.3	19.1	21.7	18.0	18.7 (2.98)	Min: 18 Max: 23.6	18.7 (2.08)
CD4+ T cells (cells/µL)	442.73	819.77	230.88	406.53	440.00	271.37	423.27 (136.89)	Min: 371.37 Max: 819.77	423.27 (78.07)
Qualitative HIV viral load (RNA)	SAMPLE LYSED	SAMPLE MISSING	NOT DETECTED	NOT DETECTED	NOT DETECTED	NOT DETECTED			
ART drug regimen	TLE	TLE	TLE	TLE	TLE	TLE			
Randomisation cohort	B	A	B	B	B	A			

* *Randomisation cohort A* starch feeding order: Cornstarch – Normal diet – HAMS – Normal diet – Cornstarch – Normal diet – HAMS – Normal diet.
** *Randomisation cohort B* starch feeding order: HAMS – Normal diet – Cornstarch – Normal diet – HAMS – Normal diet – Cornstarch – Normal diet.
*** Summary values are presented for the *Randomisation cohort A* subgroup as minimum and maximum values since the sample included only two participants.

BMI: Body Mass Index; ART: Antiretroviral Therapy; TLE: Tenofovir–Lamivudine–Efavirenz.

Alignment of findings with study assumptions

To address the assumptions which were made prior to commencement of the study (see the Methods chapter), the following data are presented below:

- RS content of intervention starch (HAMS as Hylon VII);
- RS content of control starch (Cornstarch as Ruchi Cornstarch);
- average daily RS consumption;
- RS content of faecal samples by study period;
- dose-response effect of donor stool spiked with increasing concentration of HAMS; and
- calprotectin as a measure of gut inflammation.

Outcomes relating to the testing of these assumptions are reported below.

RS, digestible starch and total starch fractions of starches

Results summarising the resistant, digestible and total starch fractions of the two intervention starches are provided in **Table 13**.

Table 13: Total, digestible and resistant starch content of study starches.

Starch source	Total starch (g/100g of starch source)	Digestible starch (g/100g of starch source)	RS (g/100g of starch source)	RS fraction (%) of total starch*
Cornflour (Ruchi Cornflour)	93.4	79.5	14.0	15.0
HAMS (Hylon VII)	94.2	33.0**	61.2	65.0

* These values were derived as follows: [RS(g/100g) divided by total starch(g/100g)] multiplied by 100.
** Based on one absorbance reading only (0.3945), as the other reading (0.7075) was deemed an outlier and not reliable.

Although this assay aimed to test the assumption that the Ruchi Cornstarch contained only 0–<1% RS, **Table 13** shows that the actual amount was 15% RS. This led to the reconceptualisation of this study as a dose-response trial rather than a control trial. See the Discussion chapter for further comment.

Dietary intake of RS

To determine dietary intake of RS during the study, the RS content of study foods was first calculated followed by an analysis of the dietary intake of RS from the habitual diet. Results are provided below.

RS content of study foods

Based on the above results for RS content of the two study starches, it was then possible to calculate the total RS content of the two different types of roti.

Since 55g of Ruchi was fed daily and the RS content as per the above assay was 15%, then:

> **55 × (15/100) = 8.25g of supplemented RS provided daily from roti A during Cornstarch supplementation periods.**

Since 95g of HAMS was fed daily and the RS content as per the above assay was 65%, then:

> **95 × (65/100) = 61.75g of supplemented RS provided daily from roti B during HAMS supplementation periods.**

These values are summarised in **Table 14**.

Table 14: Quantity and source of RS contained in study foods.

Study food	Type of supplemented starch	Total supplemented RS per serve (g)
Roti A	Ruchi Cornstarch	8.25
Roti B	Hylon VII	61.75

Food Frequency Questionnaire

The FFQ analysis showed that, aside from the study foods of wholewheat rotis supplemented with the intervention starches, dietary items that contributed the highest RS to the diet were as follows:

- whole roasted corn (typically bought as a snack from roadside vendors);
- lentils used in curries and daal. Often this was split chickpeas but other times it was described by participants as black or yellow lentils;
- roasted chickpeas;
- potato dishes. Typical consumption patterns of potato were described by participants as curries containing cooked potato. Another local dietary item containing potato that was included in the FFQ was sandwiches with cooked and cooled potato with mayonnaise. This item was not consumed by any participants in this cohort;
- bananas. Typically, these were ripe bananas but two participants (Participants 1 and 5) also reported eating 'raw' green bananas; and
- white boiled rice also contributed to the RS content of the habitual diet, primarily because of the quantity and frequency of consumption.

Daily RS intake

Table 15 demonstrates that the HAMS intervention periods resulted in higher levels of overall RS consumption compared to Baseline, Cornstarch supplementation and Normal diet in all participants. However, the data show that the total amount of RS consumed during the Cornstarch supplementation was almost always less than the amount consumed at Baseline. This was a surprising outcome and shows the limitations of not controlling the normal dietary intake of RS during the study period. It reflects the relatively modest amount of RS supplemented during the Cornstarch periods and also highlights the elevated amount of RS in the typical diet of this population which primarily relies on plant-derived foods such as pulses, grains, fruits and vegetables rather than animal-source proteins[330, 331].

Table 15: Mean RS consumption per day (grams) at different study timepoints.

	Timepoint*				
	Baseline	**S2**	**S4**	**S6**	**S8**
Participant 1	27.64	83.47	14.20	63.07	17.61
Participant 3	41.52	63.10	15.21	62.59	11.46
Participant 4	38.63	67.01	11.64	65.54	15.95
Participant 5	32.51	82.52	8.49	77.51	20.99
Participant 6	28.47	31.00	91.83	24.91	63.65
Mean	33.75				
SD	6.14				

*Red highlighting indicates data collected at Post-HAMS timepoints and blue highlighting indicates data collected at Post-Cornstarch timepoints, according to the randomisation allocation of participants

RS content of faecal samples

Based on the assumption that not all RS will be broken down by bacteria in the large intestine[38], RS was expected to be higher in faecal samples following RS supplementation. Originally, the doctoral researcher expected RS content of faecal samples to be higher after HAMS and Cornstarch supplementation periods compared to after Normal diet periods. However, based on the above results showing the actual quantity of RS consumed by participants in the different study periods (**Table 15**), the revised hypothesis was that RS content of faecal samples would be higher immediately following HAMS supplementation compared to Baseline, Normal diet and Cornstarch periods. These results are shown below in **Figure 8** and **Figure 9**.

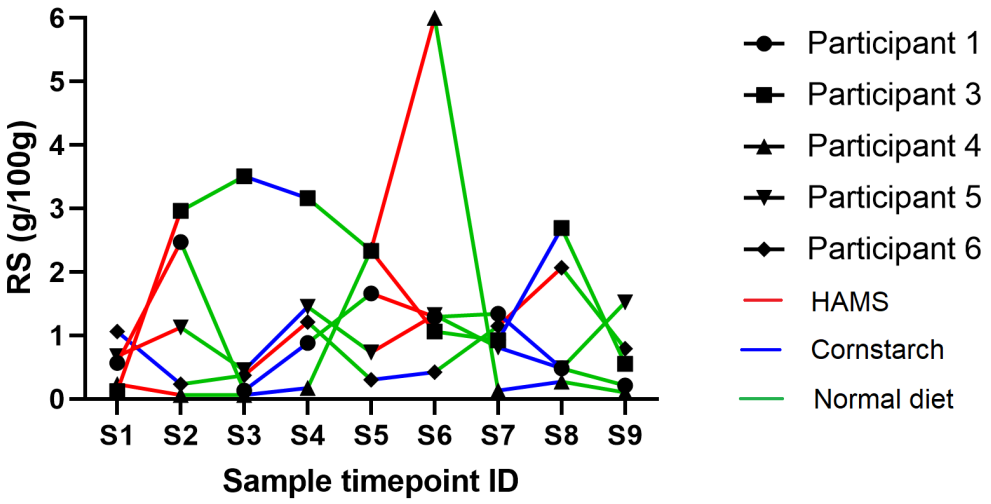


Figure 8: Faecal RS concentration at 9 sample timepoints (n=5).

Figure 8 indicates variable results that do not consistently accord with the hypothesised outcomes described above. There is some evidence for an increase in faecal RS concentration following HAMS supplementation; however, the opposite is also seen, with decreases in faecal RS concentration following HAMS supplementation on some occasions.

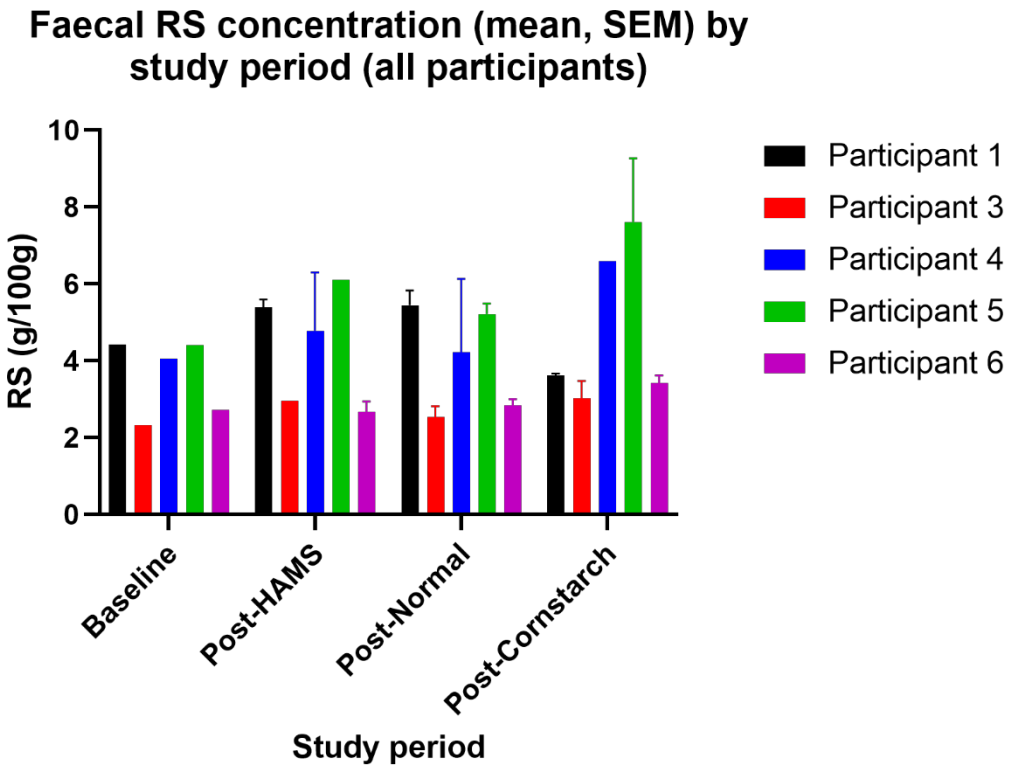


Figure 9: Faecal RS concentration (mean, SEM) by study period (n=5).

Figure 9 also indicates variable results that do not accord with the hypothesised outcomes. For example, the highest faecal RS concentrations for Participants 4 and 5 were observed after the Cornstarch supplementation periods. Only Participant 1 appears to follow the anticipated trend of elevated faecal RS concentration following HAMS supplementation as compared to Baseline, Post-Normal and Post-Cornstarch periods. However, on closer inspection, while the mean value for Participant 1 at the Post-HAMS timepoints is 5.38g/100g, faecal RS concentration was further elevated to 5.44g/100g at the Post-Normal timepoints. Overall, when considering both **Figure 8** and **Figure 9**, it is clear that the high degree of variability within participants reduces the generalisability of any trends. This suggests limitations associated with the composite measures of Post-Normal, Post-HAMS and Post-Cornstarch. Indeed, the SEM bars in **Figure 9** show this high degree of variability, particularly in Participants 4 and 5.

Dose-response effect

As described in the Methods chapter, donor stool samples from an HIV-negative volunteer were spiked with HAMS in increasing volumes, as shown in **Table 16** and then assayed for RS content. It was expected that a proportional increase in the RS content of samples would be observed with the increasing volumetric spiking of RS. Data recorded in **Table 16** show that, while increasing the dose of HAMS resulted in a linear increase as expected, this was not mathematically proportional with the degree of spiking.

Table 16: RS content of donor stool samples spiked with increasing volumes of RS.

Sample type	Actual RS (g/20g of stool*)	Calculated** RS (g/100g of stool)
Baseline (unspiked)	0.75	3.75
1g of HAMS	6.78	33.9
1g of HAMS (duplicate)	5.66	28.3
2g of HAMS	9.04	45.2
2g of HAMS (duplicate)	8.79	43.95
5g of HAMS	10.56	52.8
5g of HAMS (duplicate)	10.90	54.5
10g of HAMS	12.60	63.00
10g of HAMS (duplicate)	13.89	69.45

* Stool samples were from a single HIV-negative donor who did not consume a special diet but consumed a habitual diet which was low in RS. The stool sample had a low detectable amount of RS.

** Derived by multiplying Actual RS g/20g stool by 5.

Faecal calprotectin

The Quantitative Faecal Calprotectin ELISA Kit used in this study assigned a normal calprotectin level as under 43.2µg/gram[332]. This is broadly consistent with published articles which note a cut-off value for normal calprotectin of 50µg/gram[333]. These values are primarily derived from studies undertaken with European populations[333]. **Figure 10** shows faecal calprotectin values across the 9 study timepoints for all participants.

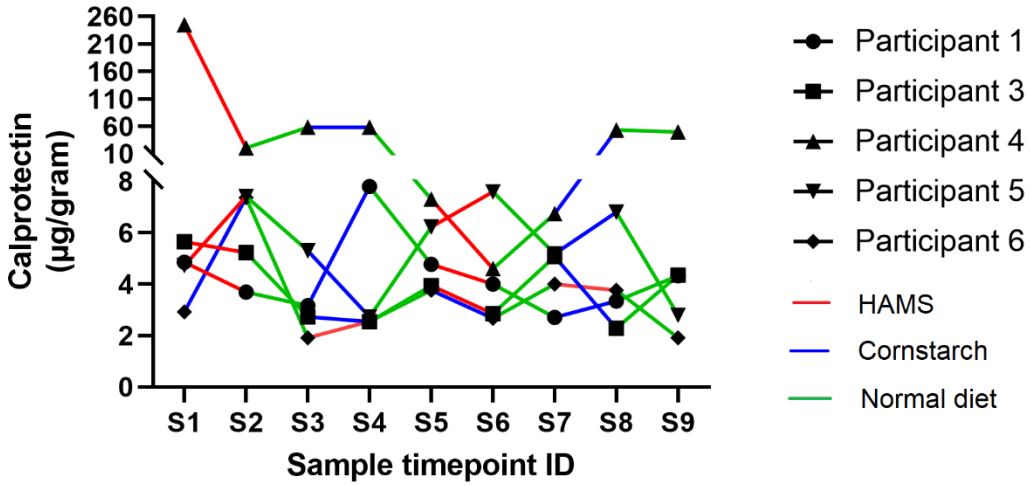


Figure 10: Faecal calprotectin concentration at 9 sample timepoints (n=5).

Contrary to the study assumption, **Figure 10** illustrates that four/five participants had a normal calprotectin level at Baseline. In addition to normal levels at Baseline, Participants 1, 3, 5 and 6 had faecal calprotectin values in the normal range at all 9 timepoints of the study. Participant 4, however, had a high baseline calprotectin value of 245.1µg/gram, which decreased dramatically after the first RS supplementation period to a normal value of 20.30µg/gram. This participant received HAMS for the first supplementation period. **Figure 10** shows the calprotectin values for Participant 4 then increased to 58.21µg/gram following the first Normal diet period. The calprotectin value then reaches 49.5µg/gram at the end of the study. It is important to note that faecal calprotectin values for Participant 4 were vastly reduced over the course of the study and reached a value in the normal reference range compared to baseline levels (even if slightly above the normal cut-off point stated for the Epitope kit). This finding warrants further investigation into the effect of RS supplementation in populations with gut inflammation as indicated by elevated faecal calprotectin levels, although it does contradict the findings of other studies which found that faecal calprotectin increased after RS supplementation ($p=0.003$)[49].

Figure 11 provides further support for the beneficial effect of RS supplementation in reducing faecal calprotectin levels in Participant 4. This representation of the data shows that there was a more marked reduction in faecal calprotectin following HAMS supplementation compared to Cornstarch.

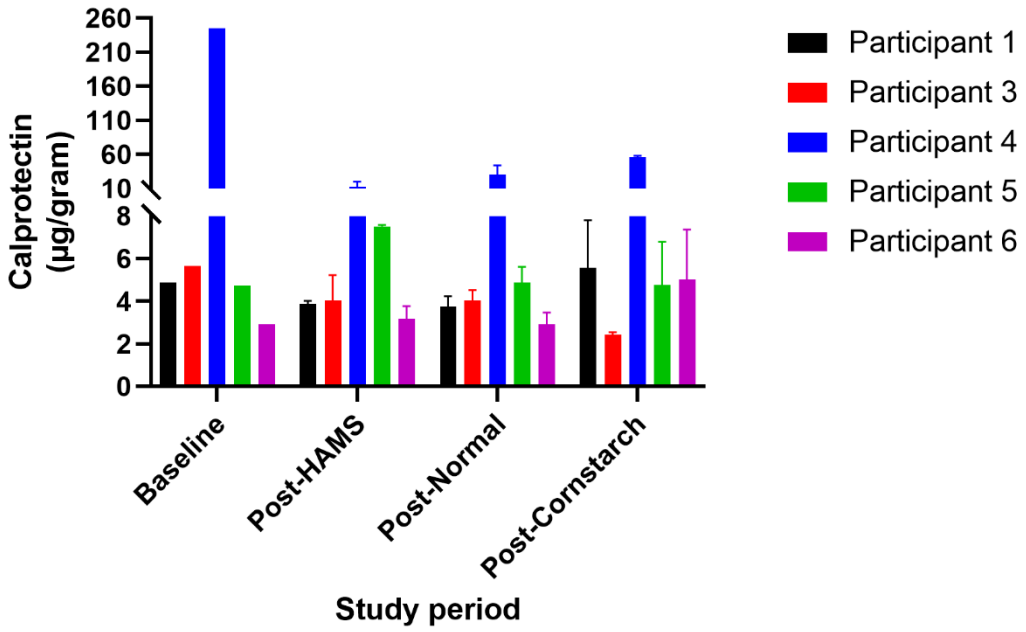


Figure 11: Faecal calprotectin concentration (mean, SEM) by study period (n=5).

Results 1: Are RS supplementation studies in this population feasible?

Feasibility was assessed according to the Thabane et al. typology[71], which describes four reasons for conducting pilot studies: Process, Resources, Management and Scientific. These reasons will be used as headings where relevant under each of the data collection methods employed to assess feasibility.

Although the study's second hypothesis, examining the treatment effect of the RS intervention, utilised a traditional per protocol analysis, the first study objective utilised data provided by all participants in order to capture the qualitative data provided by Participant 2, who withdrew. Overall, this could be described as a modified per protocol approach.

Qualitative semi-structured interviews

Process

The Process category primarily addresses recruitment, retention and adherence to study protocol[71]. The semi-structured interview data provide insights from study participants regarding barriers and enablers in these areas.

Questions asked during semi-structured interviews at the day 0 timepoint were different from those asked at the day 43 and day 112 timepoints. Questions and results are grouped according to timepoint below. The most frequently cited themes quoted by participants at each interview timepoint are summarised in **Table 17**, along with their frequency of mention categorised into: '4+ mentions', '2–3 mentions' and '1 mention'.

Table 17: Semi-structured interview questions by timepoint and frequency of mention of themes by participants*

	4+ mentions	2–3 mentions		1 mention	
Day 0					
1. What factors led you to agree to participate in this study?	For improvement in health	Access to study food	Motivation provided by ICTC staff	Curiosity	Motivated by own personal determination
2. How did the staff involved in recruitment and consent help you make your decision about participating?	Motivation provided by ICTC staff			Motivation provided by friend (participant)	
3. Did the involvement of an Australian university with AIIMS-Bhubaneswar influence your decision about participation?	Not influenced by Australian team member	Motivated by own personal determination		Yes, perception of endorsement	
4. How confident do you feel in the research team and what factors have influenced this?	High confidence	Hopeful/ Have faith		Curiosity	Expect that participating will be easy
5. What do you think will be difficult about participating in the study?	Not anticipating any difficulty				
6. What do you think will be easy about participating in the study?	Expect that participating will be easy				
7. What benefits, if any, do you believe the study will bring to you?	Overall health improvement				
Day 43					
1. What was difficult about participating in the study?	No difficulties			Stress related to other life factors	
2. What was easy about participating in the study?		Overall, it was easy		Field worker was helpful	
3. What benefits do you feel the study brought you?	Overall health improvement	Improved energy		Less weakness	
4. Did you ever think about leaving the study?	Never				
5. If yes, what were the reasons you wanted to leave the study?	n/a				
6. What made you stay in the study?	Overall health improvement			Support from field worker	
7. Did the research team motivate you to stay in the study?	Yes				
8. If yes, how did they motivate you?	Motivated by own personal determination			Field worker encouraged me	
Day 112					
1. What was difficult about participating in the study?	No difficulties				
2. What was easy about participating in the study?	Overall, it was easy				
3. What benefits do you feel the study brought you?	Overall health improvement			Improved energy	
4. Did you ever think about leaving the study?	Never				
5. If yes, what were the reasons you wanted to leave the study?	n/a				
6. What made you stay in the study?		For improvement in health	Support from field worker		
7. Did the research team motivate you to stay in the study?	Yes				
8. If yes, how did they motivate you?	Field worker encouraged me	Motivated by own personal determination		Motivated but did not pressure me	

*(Day 0, n=6; Day 43 and Day 112, n=5).

The following section uses headings of frequently identified themes at each timepoint (4+ mentions or 2–3 mentions) to explore the findings, along with supporting quotes from participants. Themes are identified in this section with *blue italics.*

Day 0 – Themes

At the day 0 timepoint, interviews were conducted with six participants. Data from all respondents were included in the analysis.

For improvement in health/Overall health improvement

Participants expressed similar sentiments in response to the first two questions, which asked them to describe (1) factors that led them to agree to join the study and (2) how the staff involved in recruitment and consent helped them make the decision. The most common factor that led patients to join the study was *improvement in health*. This was also a frequent response to question 7 at day 0, which was 'What benefits, if any, do you believe the study will bring to you?' Participants were focused on the potential benefit of the study to their health and this influenced their decision to participate:

> *My whole life, I have always been interested in ways to improve my health, especially since being diagnosed with HIV.* (Participant 3)

> *This study gives me a great chance to do something to improve my health so I was motivated to join.* (Participant 5)

Motivation provided by ICTC staff

Four of six participants stated that referral by the ICTC counsellor had influenced them to join the study at day 0. This reflects the primary recruitment process whereby ICTC counsellors engaged with patients either via phone or during clinical consultations to describe the study, referring patients to the research team if they were interested:

> *Sarita-Mam [ICTC counsellor] has always provided useful advice to me so I was motivated to join because of her recommendation of this study.* (Participant 3)

> *Ashwin-Sir and Sarita-Mam suggested I talk to the researchers about the study and, because I know them well, it made me interested to hear more about the study.* (Participant 3)

Access to study food

This theme emerged as a motivating factor at the day 0 timepoint, being cited by two participants as an important factor. Although data on the socio-demographic profile of

participants were not collected, it might be implied that participants were from a low-SES background since they were accessing HIV care via a government rather than private hospital. In this context, the access to study food can be understood as a factor motivating participants to join the study:

> *Being provided with food also motivated me to join the study.* (Participant 1)

High confidence

In response to question 4 regarding perceived levels of confidence in the research team, two-thirds of participants expressed that they had *high confidence* in the study. In terms of related themes which supported the generally positive nature in which participants perceived the study at day 0, one-third of participants stated that they were *hopeful/have faith* in response to question 4:

> *I am feeling confident about this study and am hopeful it will be of benefit.* (Participant 4)

Not influenced by Australian team member

The majority of participants (five out of six) stated that the involvement of the Australian university (question 3) had no influence on their decision to participate. One participant stated this, along with confirmation of their own desire to improve their health:

> *It is not related to Australian University. I'm doing it for my own health.* (Participant 1)

One participant reported that because the Australian researcher was involved, he perceived a level of endorsement of the study that positively influenced his decision to participate:

> *Seeing the Australian researcher made me think that something good will happen to me.* (Participant 3)

The doctoral researcher was present for the day 0 interviews for three of the study participants (Participants 1, 2 and 3). When considering answers to question 3 for all participants, no difference in themes was detected based on whether the doctoral researcher was present or not.

Motivated by own personal determination

This theme arose in response to questions 1 and 3 at the day 0 timepoint. It was exemplified by the following quote:

> *I am motivated to take part for myself, by my own strength and determination.* (Participant 3)

Expect that participating will be easy/Not anticipating any difficulty

There was a general consensus among participants at the day 0 timepoint that they were confident in the research team and that the study would not present any difficulties and would be easy. For example, the following perspective was offered in response to questions 5 and 6:

> *I have no reason to believe that the study will be difficult. So far, everything has been very easy.* (Participant 5)

Day 43 – Themes

Following the withdrawal of Participant 2, five participants completed interviews at the day 43 timepoint and were included in the analysis. The questions at this timepoint were designed to examine barriers and enablers to continuation in the study.

No difficulties/Overall it was easy

At the day 43 timepoint, four out of five participants reported *no difficulties* with their participation, with three out of five reporting that *overall, it was easy*. The following quotes summarise impressions reported by participants at this timepoint:

> *I am still finding no problems in participating.* (Participant 4)

> *Participating in this study is easy. I am not experiencing any difficulty at all!* (Participant 3)

Overall health improvement/Improved energy

Connecting back to the perspectives of participants at day 0 in which they described feelings that were coded as *Expect that participating will be easy/Not anticipating any difficulty*, the majority of participants reported overall health improvement in response to question 3 ('What benefits do you feel the study brought you?'). A related theme of *improved energy* was also frequently reported by participants in response to this question:

> *Somehow I feel that I have more energy.* (Participant 3)

Similarly, in response to question 6 ('What made you stay in the study?'), four out of five participants provided responses that fitted the theme of *Overall health improvement*. Examples of these included:

> *I feel that my health is improving. Every day I feel strong.* (Participant 1)

> *I feel that I am doing something that is really benefiting my health.* (Participant 4)

Never (thought about leaving the study)

All five participants reported that they *never thought about leaving the study* in response to question 4 ('Did you ever think about leaving the study?'). Supporting quotes from participants included the following:

> *No, I have never considered leaving the study. I think I am lucky to be involved!* (Participant 1)

> *An opportunity to join a study like this does not come along very often so I never thought about leaving the study.* (Participant 5)

Motivated by own personal determination

This theme arose again among a majority of participants at day 43, underpinning an overt determination to continue in the study which was expressed by all participants. When reflecting on the role of the research team in motivating them to stay in the study, participants were inclined to speak of their own determination before elaborating on the motivation provided by the field worker:

> *I tend to rely on my own personal will to get things done in life. Sure, the field worker encouraged me but most of the motivation came from me.* (Participant 6)

Day 112 – Themes

Five participants completed interviews at the day 112 timepoint.

No difficulties/Overall it was easy

Similar to the responses at day 43, all participants reported that they experienced *no difficulties* during the study and that, *overall, it was easy*:

> *I did not experience any difficulties right up until the end!* (Participant 6)

Overall health improvement/Improved energy

As a similar reflection of overall satisfaction among the participant cohort, all participants reported an *overall health improvement* at the day 112 timepoint. One participant also reported *improved energy* as a benefit:

> *I do think I am healthier now than at the beginning of the study and I have more energy too.* (Participant 1)

Never (thought about leaving the study)

Comparable to the responses at the day 43 timepoint, all participants reported that they *never* thought about leaving the study in response to question 4 at the day 112 timepoint. The following insights were offered by respondents:

> *No, I still never had any thoughts about leaving the study. Glad I was chosen to participate!* (Participant 3)

> *I never thoughts about leaving the study. I am grateful to have been involved.* (Participant 1)

Field worker encouraged me

Three participants commented on the importance of the field worker's role in motivating them in response to question 6 ('What made you stay in the study?'):

> *The field worker kept me focused and motivated me to finish the study.* (Participant 1)

Furthermore, in response to question 8 regarding factors motivating participants to persevere in the study, the most frequent responses was *field worker encouraged me*, with one participant elaborating that:

> *The field worker did encourage me but it was always appropriate and never with pressure.* (Participant 5)

Other responses included:

> *I appreciated the encouragement from the field worker. It did help to keep me going.* (Participant 4)

> *Satya [field worker) was good at keeping me motivated.* (Participant 6)

Motivated by own personal determination

Similar to day 43, the *motivated by own personal determination* theme appeared frequently when respondents were asked to comment on factors affecting their motivation to stay in the study, such as in the following quote:

> *I never doubted my ability to finish the study because my inner strength and determination to do so were strong.* (Participant 3)

Comparison across timepoints

In connecting the findings of the semi-structured interviews to the focus on participant recruitment and retention, it was clear that recruitment was positively influenced by ICTC staff

who took a leading role in promoting the study to their patient group. It was not possible to discern further detail from the interview data around whether this effect on recruitment was influenced by a trust or power dynamic between patient and staff. However, the responses suggested that ICTC staff were respected by the patient group and the recommendation of the study by these staff was a positive endorsement that encouraged participation.

Other findings that specifically related to recruitment included that the study was perceived as a chance for *health improvement* by participants. Furthermore, all participants expressed that they expected that *participating would be easy* and commonly expressed that *personal determination* enabled their participation in the study, with half of all respondents citing this as a motivating factor at day 0. Other factors listed which influenced participants to join the study included *curiosity* and *motivation by a friend* (who was also a participant in the study). The following quotes highlight these findings:

> *I was curious to see for myself if there was a benefit.* (Participant 4)
>
> *My friend suggested I also join the study as he thought it was benefiting him.* (Participant 3)

The qualitative interview data indicated that retention in the study was influenced by multiple factors. The most frequently cited factors affecting retention were personal determination, anticipation of health benefit, and encouragement/motivation from the field worker. The experience of the study being easy was also a key factor affecting retention.

Although no specific questions about adherence to the protocol were included in the interview guide, several of the questions asked at days 43 and 112 elicited responses about adherence, such as factors which made the study easy or difficult and those assessing contemplation of withdrawing from the study. Overall, the consistency at which participants reported that the study was easy suggests that maintaining adherence was not perceived as difficult. Participants also consistently reported that they never considered withdrawing from the study, which indicates a strong level of commitment and implies that protocol adherence was high. Consideration should be given to the possible influence of a reporting bias in this context, where participants might have felt inclined to provide the 'preferred answer' perceived to be expected by the interviewer. However, the interpretation of findings about protocol adherence is supported by other findings in the Process category, such as those from the QPE analysis, which found that the full serve of study foods was consumed by participants on each occasion. There were only three occasions when this 'consumption under observation' could not be undertaken because of COVID-19 disruptions. Furthermore, all participants who completed the study provided stool and blood samples at the timepoints stipulated by the study design and completed all survey instruments when required. This provides further confirmation that

both protocol and intervention adherence were high amongst this cohort. No differentiation was made by participants in their perceived benefit of the different rotis (roti A and roti B), with interview responses suggesting that participants believed they experienced a health benefit throughout the whole duration of the study rather than during different periods of the study.

Qualitative process evaluation

Field note data collected for the QPE assessment were used to determine feasibility in the Process, Resources and Management categories[71]. These findings are explored below under the relevant headings, with excerpts from the doctoral researcher's field notes provided in italics under the date of the entry.

Process

The excerpts of the doctoral researcher's field notes illustrate the breadth of content which focused on the Process category of the feasibility assessment, from logistical matters to those relating to recruitment processes[71].

Recruitment

The doctoral researcher recorded the following field notes which related to participant recruitment:

> 28 February 2020
>
> *I came across two issues today which may impact on participant recruitment and retention.*
>
> *The first was expressed by patients referred by the ICTC counsellors who fit the eligibility criteria and were contacted by the study team. A husband and wife couple who are both HIV-positive declined to be involved as they were concerned that the delivery of study foods to their home would risk the confidentiality of their HIV status and that they 'did not want the rest of the village to find out'. Even despite reassurances that the field worker would not disclose their HIV status, they determined that the risk was too great and that there would be 'too many questions' from their neighbours. Since it is not logistically possible for participants to attend AIIMS every day to collect their study foods because of the cost and inconvenience of travel, the regularity of home visits may be a barrier to participation for some members of the target group.*

> *The second issue was raised by Satya with Swayambara, who then raised it with me. Satya suggested that it would be problematic for females to join the study because they may anticipate suspicion amongst their neighbours about the reason for him attending their home every day to deliver the study foods. Swayambara pointed out that this was a potential barrier to participation by females as they may fear that neighbours would suspect that Satya was an intimate partner. This potentially represents a significant risk to female participant recruitment.*

Although the doctoral researcher was familiar with the impact of stigma and discrimination in HIV-positive populations through her previous employment roles in blood-borne virus policy and funding, she did not anticipate these barriers to recruitment.

Further to these points raised by study staff involved in recruitment, the doctoral researcher noted the following in her field note record of her preliminary discussions with the two ICTC counsellors:

> 25 February 2020
>
> *Today the two ICTC counsellors, Sarita and Ashwin, advised me that there was an adult patient population of ~400 people living with HIV on the ICTC register. The counsellors noted that many of these patients were lost to follow-up, having changed contact details without advising the clinic, or had not attended appointments. Further noting that co-morbidity records on the register were not up to date, plus the frequent use of antibiotics in India, the potential number of participants was likely to be much lower once eligibility criteria were applied. Eventually, we agreed that Sarita and Ashwin would contact any adult patients within a 30km radius of AIIMS and invite them to attend the clinic for more information.*

While it was essential that the study be practical to deliver, the 30km restriction on recruitment did limit the number of participants. However, adhering closely to this criterion became integral in the second month of recruitment, when COVID-19 restrictions meant that the field worker had to attend participants' homes for survey and sample collection as well as study food delivery.

The doctoral researcher noted a high level of interest among the target population when the study first commenced recruitment:

28 February 2020

It was exciting today to hear further from Sarita and Ashwin regarding recruitment to the study. Several members of the target population or their family members enquired about participating in the study. The most frequent reason for not meeting the eligibility criteria was a co-morbid condition affecting the gastrointestinal system or antibiotic use in the previous 6 weeks. This illustrates the importance of ensuring that co-morbidities which might affect recruitment outcomes are factored into sample size targets, particularly in the LMIC context where rates of co-morbidity amongst people living with HIV are high.

An assessment of eligibility criteria is recommended in Thabane et al.'s Process category, to determine if the criteria functioned effectively and were appropriate, being neither too restrictive nor too inclusive[71]. As indicated in the field notes, participants were excluded for appropriate reasons in that the co-morbidities, such as abdominal tuberculosis and stomach cancer, would have interfered with interpretations of results. Eligibility criteria also restricted participation in people who had taken antibiotics in the previous six weeks. In the field of microbiota research, these criteria are important to apply since both have a significant effect on gut physiology and/or gut microbiota. It would therefore be prudent for future studies to factor in rates of antibiotic use and co-morbid GIT conditions on recruitment targets. For the current study, because recruitment via AIIMS ICTC was the only option available, these restrictions on recruitment had to be accommodated. The doctoral researcher reflected that recruitment via the ART clinic would likely have increased the number of individuals who could be recruited according to the eligibility criteria, since clients from this service are usually past their initial period of seroconversion and are less likely to be on antibiotics related to their initial diagnosis. The field notes also indicated that all participants who met the eligibility criteria did consent to join the study.

Study food compliance and protocol adherence

To determine compliance with study food consumption, the field worker observed participants eating study foods in order to weigh and record any uneaten portion. On the days when the field worker observed participants eating study foods, all participants consumed the entire serve. There was therefore no requirement for the field worker to weigh any unconsumed portions.

The COVID-19 disruption meant that the field worker could not always deliver study foods and observe their consumption, as was originally intended. On these occasions, packets of the relevant starch were provided to participants in advance and they were instructed to

incorporate the contents into home-prepared meals and consume the entire portion themselves. Study food compliance relied on participant self-reported data on these occasions. Participants consistently reported consuming the whole serve on the required days. Overall, the field notes provided the following insights regarding the high level of compliance with study foods:

> 6 March 2020
>
> *It has been so encouraging to hear from Satya that the study foods are being consumed in their entirety every day! Some reflection on this based on conversations I have had with Satya and Bala [Indian PI] include the convenience of the study in terms of the field worker delivering study food to participants in their homes. By providing the study foods in this way, we have removed all barriers to compliance which relate to time and cost of transport. Of course, other important factors relate to the LMIC-context and associated food insecurity of the general population. I also found a reference relating to the elevated levels of poverty in the HIV-positive target population in India and the resultant food insecurity[334].*

In field notes about adherence to other aspects of the study protocol, it was noted that participants consistently provided blood and stool samples on the required days and completed surveys when requested. In fact, COVID-19 exerted more of an influence on adherence than on participant willingness by, in some cases, interfering with exact dates for survey administration:

> 19 March 2020
>
> *Today I discussed approaches to managing study logistics in light of the COVID-19 lockdowns just announced by the Government of India. The risk to the study from the lockdowns is that Satya may not be able to travel to participants' homes to deliver study food, collect faecal samples and administer surveys. An idea for the future might be to translate the hard-copy survey tools for online delivery via mobile phone with software programs such as RedCap or Qualtrics. A barrier to this approach is mobile phone access as well as literacy. Sometimes in India, families share a mobile phone. If participants themselves do not have their own mobile phone or cannot read, then this introduces issues relating to confidentiality if online administration of surveys was to be pursued. This is worth thinking about for future trials although disturbances such as COVID-19 lockdowns will still interfere with sample collection and study food delivery since these rely on a physical visit by the field*

worker. When I designed the study, I intentionally included surveys on the days that samples would be collected to increase efficiency. It also made sense as sample collection days are at the end of a study period such as supplementation periods or normal diet periods.

Stool collection and storage

The doctoral researcher noted that the instructions explaining the stool collection method to participants were adequate. Future acceptability testing could be undertaken with a smaller cohort to further refine the instructions. Anecdotal data collected by the field worker and doctoral researcher indicated that participants did not experience any difficulty following the instructions. If anything, there was a need to impress upon participants and the field worker the importance of the sample being stored in freezing conditions as soon as possible after being produced. The doctoral researcher's below note highlights this point:

28 February 2020

Today, I experienced the discomfort of having to keep pestering Satya as to the whereabout of Participant 1's latest stool sample! As Satya and this participant were conversing in Oriya language, I could not determine if the participant had brought his stool sample in or not. I discreetly asked Satya and I understood his nodded reply to mean that the participant did have this sample with him. When their conversation continued and the participant did not provide the sample, I realised I had to ask again in order to ensure the sample was stored in the freezer without further delay from the ongoing conversation! Afterwards, I discussed this with Satya and Bala to ensure that everyone understood the importance of the samples being refrigerated without delay. This is particularly important as there is also a delay in time from the stool being passed to it being delivered to AIIMS.

The findings regarding time for stool samples to be stored in frozen temperatures were important to understand from a feasibility perspective so that approaches for future studies could be improved and the optimal duration requirements could be met in order to enable the accuracy of gut microbiota results[335].

Participant withdrawal

It was noted that Participant 2 withdrew on day 3 of her involvement in the study and that the reason reported to the field worker was that she felt unwell with gastric pain and attributed these symptoms to the study foods. The field notes summarising this incident are provided

below under the Scientific heading since it relates to Adverse Events (AEs) however, it also impacts on participant retention and is therefore noted here.

Data collection tools

In terms of the suitability of the data collection tools considered under the Process category[71, 236], the field notes included the following observations:

> 26 February 2020
>
> *Anecdotal feedback from participants and Satya about the Food Frequency Questionnaire indicated that this tool included too many items and that, even when used at 4 timepoints, it required too much time to complete. Alternative approaches could be considered for future trials. It will be important to manage the response burden carefully with the specificity required for the RS dietary intake measurement.*
>
> *SSC_HIVrev was ideal as a data collection tool as it contained only 8 measures and, even when repeated at the 9 timepoints, it required only 1–2 minutes to complete on each occasion.*

While not being able to be reflected in the doctoral study because of the prior ethics approval of all data collection tools and limitations on any further delays to timelines, these findings will be useful for the design of future dietary supplementation and analysis studies with this target group.

Resources

QPE was the only tool used to collect data for the Resources category and addresses time and resource issues that should be addressed prior to a larger main study[71].

Equipment

A resourcing issue noted by the doctoral researcher as a potential risk related to the stadiometer and scales which were required to record each participant's height and weight at enrolment. This was captured in the field notes as follows:

> 28 February 2020
>
> *With recruitment of the first participant today, it became clear that, depending on the day of recruitment, the stadiometer and scales available in the clinic room next door to the ICTC clinic might not be readily available. Sarita and I quickly realised this when it came time to record height and weight of our first participant and there was another (non-HIV) clinic being run next door in the*

room with the equipment! We were able to negotiate access to the equipment, but the arrangement did interfere with the recruitment process as often a consultation was being conducted in the outpatient room so the doctoral researcher and field worker had to wait with the participant or interrupt the consultation to use the equipment. This disruption appeared to be accommodated without concern but for future trials with a larger number of participants, a stadiometer and scales should be included in the initial budget and located in the research offices. This will also better accommodate participant confidentiality concerns which must be considered a high priority.

Other issues relating to equipment and resourcing included the freezer storage which was required for stool samples from this study. Owing to the COVID-19 disruption, there was a relatively small number of samples requiring storage. For future larger trials, a dedicated freezer would be beneficial to allow storage of more samples. Below is the field note excerpt which summarises how the limited freezer space was managed by the doctoral researcher:

20 February 2020

Discussions with the Indian team today revealed that the freezer space available for this study is a shared freezer where samples are stored from various studies. Easily identifiable labelling of vials for blood and stool samples is therefore essential. The code needs to make clear the study, participant number and sample (timepoint) number as well as allowing for duplicate or triplicate samples to be indicated. The following nomenclature is proposed: 'FLRS_P1_S1' where the following apply:

- *'FLRS' refers to the study title: **F**linders **U**niversity **R**esistant **S**tarch study;*
- *'P1' refers to Participant 1;*
- *'S1' refers to sample timepoint 1, to be collected at day 0. Sample timepoints are detailed in the study design graphic.*

If more than 1 vial is collected from each participant at a given timepoint, the following nomenclature can be added to indicate this:

- *'FLRS_P1_S1_1',*
- *'FLRS_P1_S1_2' etc*

Action: Discuss with Satya tomorrow and sit together to label all vials.

Changes to budget to meet transport requirements

The field notes of the doctoral researcher described a change to the budget requested by the Indian PI. A mode of transport was required to allow the field worker to travel to the participant homes to deliver study food and collect data and samples for the study. The previous arrangement, whereby the Indian team had access to a vehicle, was no longer in place. Consequently, the Indian PI proposed moving funds from one budget line to another for the purchase of a scooter. In effect, the budget line related to the staffing costs for the field worker were used for this purpose. The field worker had been employed to also work for another study and so his time on the current study was provided as an in-kind contribution. The field notes provide further context:

> 13 February 2020
>
> *Hopefully Bala's revised approach to the budget was the correct decision to support today. I was asked to sign-off on the transfer of funds from one budget line to another to enable the purchase of a scooter. I can see that it is essential to have transport for the field worker so that he can deliver the study foods to participants and collect samples. Now I am just hopeful that the approval allows the scooter to be available as soon as possible!*

Access to laboratory facilities

In terms of sufficiency of other resources for the successful implementation of the study[71, 236], the doctoral researcher noted that the lack of dedicated laboratory facilities for all assays limited the efficiency of the study. This became apparent when the results of the SCFA assays performed by a laboratory in India returned nonsensical values:

> 3 March 2021
>
> *I finally received the SCFA results yesterday from Giri [Postdoctoral Research Fellow, Department of Biochemistry, AIIMS]. These were performed by a lab at AIIMS but not Bala's Department of Biochemistry lab. I discussed these results with Graeme [member of the doctoral supervisory team], who agreed that the acetate values in particular did not make any sense whatsoever. The acetate values from the AIIMS lab ranged from 0.02 to 2.04 nanomoles/gram of faeces and were the lowest in concentration compared to the other three SCFAs assayed (butyrate, propionate and valerate). I looked up the literature to verify that acetate usually represents the highest concentration of any of the SCFA in faeces[336, 337] and are usually in the order of 60–100 µmoles/gram[228].*

Since the lab was not under the direct control of the Indian PI and the results suggested errors in the assays, one of the only solutions was to bring the remaining faecal samples to Australia to repeat the SCFA assays. This introduced a significant cost pressure into the budget and the doctoral researcher was fortunate to be able to access emergency funds to cover this.

Communication with participants

The primary mode of communication with study participants was WhatsApp, a direct messaging platform featuring end-to-end encryption, which enabled achievement of the study objectives by ensuring participant confidentiality while facilitating communication between participants and the field worker. Consideration for future larger trials should be to ensure that field workers and participants are aware of the expectations around hours of availability of this communication method. In this study, the field worker was comfortable to make himself available out-of-hours as required, but resourcing considerations in larger trials need to factor in the budgetary implications of the field worker's availability. This is particularly relevant to aspects such as stool collection, the timing of which cannot be predicted. Direct messaging via WhatsApp also provided a communication channel for participants to reach study staff in case of any AEs. Although it is not expected that dietary RS supplementation would result in AEs, study participants may seek reassurance in relation to some of the possible side effects, such as increased flatulence. The importance of a reliable digital communication method was particularly important given the COVID-19 disruptions to mobility and transport which occurred during the study, as reflected in the following field note entry:

> 14 April 2020
>
> *I had to laugh today as Satya was telling me how busy he had been over the past week with receiving WhatsApp messages from first thing in the morning until early evening about stool sample collection! Fortunately, he was in good spirits about it and also relayed to me how several participants had expressed gratitude that they could reach him at any time via WhatsApp.*

Quantitative data collection via FFQ

The Resources category also addresses '*length of time to fill out all the study forms'* [71, p.4]. The doctoral researcher's field notes included the following reflections on suitability of the FFQ and SSC-HIVrev tools used in this study:

> 5 March 2020
>
> *Anecdotal feedback from participants and Satya about the Food Frequency Questionnaire indicated that this tool included too many items and that, even*

when used at 4 timepoints, it required too much time to complete. Alternative approaches could be considered for future trials. It will be important to manage the response burden carefully with the specificity required for the RS dietary intake measurement.

SSC_HIVrev, however, was ideal as a data collection tool as it contained only 8 measures and, even when repeated at the 9 timepoints, it required only 2 minutes to complete on each occasion.

Ethics and staffing for recruitment

The resourcing implications of relying so heavily on the ICTC team for recruitment also need to be considered for future trials. Although there is overlap with Thabane et al.'s Resources and Management categories for this finding, it will be addressed here, under Resources.

Field notes revealed that ethics timelines were unable to be predicted and this impacted directly on recruitment approaches:

20 February 2020

Despite being advised that recruitment pathways via the Bhubaneswar ART clinic could be utilised in time for participant recruitment, it has unfolded since I arrived that the ethics approval will need to be undertaken afresh, rather than endorsement of the existing ethics clearance that I had received from AIIMS. It has been frustrating to be made aware of this so late in the piece but there is little that can be done now other than to utilise the recruitment pathway via the AIIMS ICTC pathway. Luckily, I met with the ICTC counsellors today, Sarita and Ashwin, who let me know that they can help, even though they are busy every day with clinic. Considering that this assistance will be an in-kind contribution, I will keep this in mind for future planning to ensure that research does not hinder clinical service resources.

Management

QPE was the only tool used to collect data for Thabane et al.'s Management category, which addresses *'potential human and data management problems'* [71, p.4].

Staffing for qualitative data collection via semi-structured interviews

In terms of considering the capacity and expertise of staffing resources[71, 236], the QPE revealed that the field worker did not invest adequate time in clarifying meaning and encouraging participants to elaborate on answers during the qualitative semi-structured interviews. This was highlighted in the field notes below:

1 March 2020

Today I was disappointed to read the translation of the first qualitative interviews that Satya had undertaken. The detail I was hoping would be uncovered just wasn't there. It is clear that Satya feels uncomfortable with this aspect of his role and he actually asked me if the semi-structured interview aspect of the study could be removed. I have made a time to discuss this with Bala tomorrow.

Additional resources need to be considered for future trials, either to allow dedicated training of field workers in qualitative interviewing techniques or to ensure that the recruitment of field workers selects candidates who have significant qualitative research experience. An important approach will be to educate field workers and other staff on the importance of all of the study components, as illustrated by the next stage of the resolution of this issue:

2 March 2020

Based on my conversation with Bala and Satya today, I am feeling more optimistic that the qualitative data collection might improve. Firstly, I met with Bala and relayed to him my concerns at the feedback that I had received from Satya and also tabled the transcript of the first interview for us to discuss. Bala agreed that this needed to be resolved and that he would have a discussion with Satya before having a meeting with both of us. When we all met, it was clear that Satya had a better understanding of the purpose of the qualitative data collection. I then confirmed the reasons for collecting interview data. Satya committed to continuing with the interviews, as required. Let's see what comes out of the next interview.

Managing participant milestones

Relating to the chosen approach to manage participant milestones throughout the study, the doctoral researcher developed an Excel spreadsheet that mapped out data collection and study period dates (for both RS supplementation and Normal diet periods) for the duration of the study. This was important to ensure that there was an agreed mechanism for managing milestones when the doctoral researcher returned to Australia. The simplicity of this spreadsheet and the familiarity of study staff with Excel were advantages which made this method acceptable. The approach using Excel could be adapted for a larger trial or, alternatively, a software program such as RedCap, which enables the automation of milestone reminders, could be used. Noting the above-stated considerations about mobile phone access and confidentiality concerns, RedCap could also be used in the context of a larger multi-site

trial to automate survey distribution to participants via email or SMS and allocate staff resourcing to collect blood and faecal samples and deliver study food. A brief field note reflected this possibility:

> 4 March 2020
>
> *I feel confident with the system I set up on Excel for tracking milestones for each participant. I have also made sure that Satya has a hard copy of the spreadsheet indicating these milestones. Since participant numbers are likely to be modest, this system should be adequate. I can however see the benefit of using an automated system for future larger-scale studies which enables the automatic distribution of surveys to participants by SMS. This would increase both efficiency and accuracy. Qualtrics would be suitable or RedCap which I am less familiar with but understand to be ideal for this situation.*

Scientific

The field notes collected for the QPE were useful in assessing feasibility as per Thabane et al.'s Scientific category, which *'deals with the assessment of treatment safety, dose, response, effect and variance of the effect'* [71, p. 4].

Safety

The withdrawal of Participant 2 from the study was summarised in the field notes of the doctoral researcher as follows:

> 3 March 2020
>
> *Participant 2 reported gastric pain to Satya today along with her desire to withdraw from the study. She attributed these symptoms to the study foods. Satya then advised me he had instructed this participant to contact him if she 'felt any pain after consuming the study foods'. Satya had not been asked to communicate this to study participants. Considering that gut pain is not a commonly cited side effect of RS, I feel that this direct suggestion from Satya might have influenced Participant 2's decision to withdraw from the study. I discussed this with Bala and we agreed that a direct communication with Satya was required to clarify information to be exchanged with participants and how best to provide information in a neutral way. As his manager, Bala led this communication with Satya. I was not invited to be present for the discussion.*

Sign and Symptom Checklist (SSC-HIVrev) – gastrointestinal subset

Scientific

In addition to the QPE findings uncovered by the field note data, Thabane et al.'s Scientific category was also assessed with data collected by the SSC-HIVrev tool. When considered with the findings of the examination of treatment effect, these findings provide a comprehensive summary of feasibility related to Thabane et al.'s (2010) Scientific category.

Number of reported signs and symptoms

Participants rarely reported gastrointestinal signs or symptoms during the study period. Across all timepoints, only one participant reported a gastrointestinal symptom on one occasion, with one other participant reporting a symptom on more than one occasion. **Table 18** shows that the most frequently cited gastrointestinal symptom was *Gas/Bloating*, which was reported on four occasions. *Loose stools* and *Abdominal pain* were reported at a lesser frequency, on two and one occasions, respectively. All other signs and symptoms included in the SSC-HIVrev tool were never reported during the study.

Table 18: Total count of self-reported gastrointestinal signs and symptoms at 9 timepoints (n=5).

Frequency of reported signs and symptoms	Baseline	Post-Cornstarch	Post-Normal	Post-HAMS	All timepoints combined
Loose stools	1	0	0	1	2
Diarrhoea	0	0	0	0	0
Gas/Bloating	0	2	2	0	4
Abdominal pain	1	0	0	0	1
Nausea	0	0	0	0	0
Vomiting	0	0	0	0	0
Lack of appetite	0	0	0	0	0
Constipation	0	0	0	0	0
Total	**2**	**2**	**2**	**1**	**7**

Severity of reported signs and symptoms.

In terms of the severity of reported signs and symptoms, **Table 19** highlights that the symptom reported by participants as most severe during the study was *Gas/Bloating*, followed by *Loose stools* and *Abdominal pain*. Symptoms were more likely to be reported with a higher magnitude of severity following Normal diet periods than for either of the starch intervention periods. This indicates that neither Cornstarch nor HAMS caused any worsening of gastrointestinal signs and symptoms compared to Normal diet.

Table 19: Total severity of self-reported gastrointestinal signs and symptoms at 9 timepoints for 5 participants.

Severity of reported signs and symptoms	Baseline	Cornstarch	Normal	HAMS	All timepoints
Loose stools	1	0	0	1	2
Diarrhoea	0	0	0	0	0
Gas/Bloating	0	2	4	0	6
Abdominal pain	1	0	0	0	1
Nausea	0	0	0	0	0
Vomiting	0	0	0	0	0
Lack of appetite	0	0	0	0	0
Constipation	0	0	0	0	0
Total	**2**	**2**	**4**	**1**	**9**

Results 2: Was there a treatment effect from RS supplementation?

A per protocol analysis was undertaken to determine treatment effect, including all participants who completed the study (n=5). This analysis addressed study hypothesis 2, *that dietary RS supplementation will result in fermentation of RS by gut bacteria in the large intestine, leading to increased SCFA production and a decrease in pH that will positively influence the gut microbiota and HIV-related immunity.*

To ensure that interaction effects of outcome variables were accounted for (as well as carryover and/or treatment by period effects), a linear ME model analysis was undertaken. The results are summarised below, followed by results of the descriptive analysis for outcome variables.

Linear ME model

A linear ME model was developed to determine the intervention/treatment effect on the outcome variables included in this study. To consider the effects of factors which were additional to the intervention/treatment, two further models were developed:

- a model that assesses the treatment by period interaction effect (to determine if the effects of treatments were different in the two crossovers); and
- a model that assesses (and adjusts for) carryover effects (to determine if changes that accumulated during the intervention periods affected results in subsequent intervention periods).

Both models utilised an ME regression model and included adjustments for baseline values (day 0).

Assumptions

The linear ME models assumed that:

- each participant was measured at the beginning and end of each 'treatment', where 'treatment' is defined as one of the four dietary intervention periods (Cornstarch 'Diet A' and HAMS 'Diet B') in the double crossover; and
- the correlation between pH measurements within participants is approximately r=0.6, based on a dietary RS supplementation study [38].

The residual intraclass correlation (ICC) to test this assumption showed that there was no correlation between pH measurements within participants (2.25^{13}, std error=0, 95% conf interval=2.25^{13}–2.25^{13}), suggesting that pH is not consistent within each participant.

The ME analysis produced the following results:

1. No treatment effects (Diet A vs Normal diet, Diet B vs Normal diet or Diet A vs Diet B).
2. No treatment X period interactions.
3. No carryover effects.

Contrast, p values and 95% confidence intervals are presented in **Table 20**.

Table 20: Mixed-effect model results for all comparisons relating to *Diet A, **Diet B and ***Normal diet.

Outcome variable and units of measurement	Contrast	p>z	95% CI
pH			
Diet A vs Diet B	–0.044	0.874	–0.593–0.504
Normal diet vs Diet A	–0.207	0.392	–0.682–0.267
Normal diet vs Diet B	–0.163	0.501	–0.638–0.312
Acetate (µmoles/gram)			
Diet A vs Diet B	–9.089	0.332	–27.463–9.285
Normal diet vs Diet A	3.818	0.638	–12.095–19.730
Normal diet vs Diet B	12.907	0.112	–3.006–28.819
Propionate (µmoles/gram)			
Diet A vs Diet B	–11.055	0.185	–27.398–5.288
Normal diet vs Diet A	–0.786	0.913	–14.940–13.368
Normal diet vs Diet B	10.269	0.155	–3.885–24.422
Butyrate (µmoles/gram)			
Diet A vs Diet B	–2.166	0.796	–18.593–14.262
Normal diet vs Diet A	–0.328	0.964	–14.555–13.898
Normal diet vs Diet B	1.838	0.800	–12.389–16.064
Valerate (µmoles/gram)			
Diet A vs Diet B	–0.125	0.923	–2.656–2.406
Normal diet vs Diet A	–0.036	0.975	–2.228–2.157
Normal diet vs Diet B	0.089	0.936	–2.103–2.282
Calprotectin (µg/gram)			
Diet A vs Diet B	–5.982	0.279	–16.811–4.848
Normal diet vs Diet A	–4.206	0.379	–13.584–5.173
Normal diet vs Diet B	1.776	0.711	–7.603–11.154
CD4+ T cells (cells/mm^3)			
Diet A vs Diet B	–53.100	0.407	–178.640–72.439
Normal diet vs Diet A	–40.733	0.408	–137.237–55.771
Normal diet vs Diet B	12.367	0.837	–105.312–130.046
HIV viral load (copies/mL)			
Diet A vs Diet B	–12.434	0.720	–80.349–55.480
Normal diet vs Diet A	–6.024	0.838	–63.674–51.625
Normal diet vs Diet B	6.410	0.833	–53.015–65.835

*Diet A consisted of 14 days of 8.25g/day RS supplemented in roti A with Cornstarch.
**Diet B consisted of 14 days of 61.75g/day RS supplemented in roti B with HAMS
***Normal diet consisted of 14 days of the participant's habitual diet

Given the lack of significance in any of the comparisons included in the ME analysis, the following section explores the findings of the descriptive analysis of the outcome variables included in the ME analysis, with the aim of identifying trends of interest which might justify and inform future larger studies.

Faecal pH

A key primary outcome of this study to determine treatment effect was pH, as it provides an indication of whether fermentation has occurred. This outcome measure endpoint is readily measured as long as the rules of collection are followed.

pH values for each participant are plotted by timepoint in **Figure 12** with colour-coding indicating the order and type of starch consumption for each participant, according to randomisation.

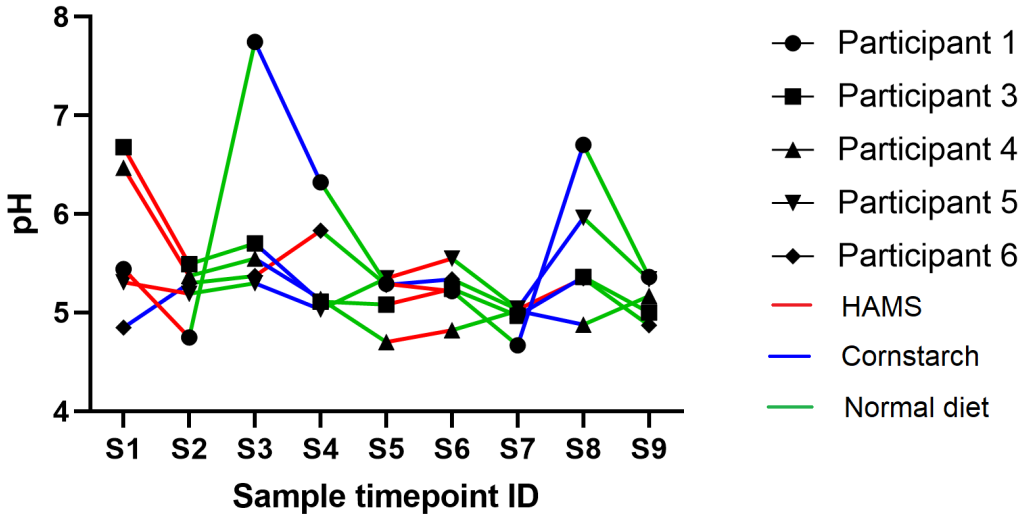


Figure 12: Faecal pH at 9 sample timepoints (n=5).

Based on the literature and the known effect of RS fermentation in human and animal models, it was expected that faecal pH would decrease following RS supplementation, reflecting the increased production of SCFA produced during fermentation. **Figure 12** shows that, with the exception of Participant 6, who received the Cornstarch supplementation first, a trend to decreased pH after the first feeding period was evident in the four participants who received the HAMS supplement first. The trend supports the hypothesised changes to pH with a decrease in pH following HAMS supplementation, then an increase in pH following the Normal diet period, with a decrease again after Cornstarch supplementation. The pH values reported for the second crossover (timepoints S5–S9) do not consistently support the hypothesis, with the results contradicting the expected outcome and pH values increasing after both HAMS and Cornstarch supplementation, suggesting a less acidic colonic environment.

pH values were then analysed according to study period using the stated approach to production of summary variables outlined in the Methods chapter, under the heading: *Descriptive and univariate analysis*. Values are plotted below in Figure **13**.

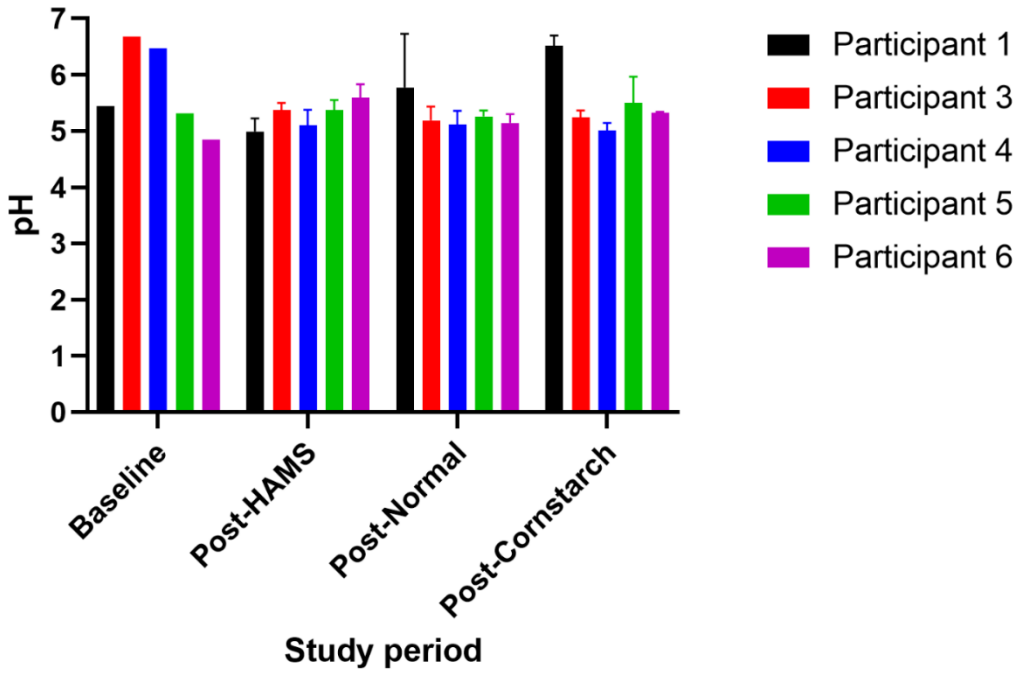


Figure 13: Faecal pH (mean, SEM) by study period (n=5).

These results provide some support for study hypothesis 2, but there are overt inconsistencies. While 60% (three out of five) of participants displayed a reduction in pH at the Post-HAMS timepoint compared to Baseline (Participants 1, 3 and 4), two participants showed an increase in pH after HAMS supplementation (Participants 5 and 6). When compared to both Baseline and Post-Normal pH values, Post-Cornstarch values for pH decreased as anticipated only in Participant 4.

Faecal SCFA

As per the study hypothesis, it was expected that SCFA production would increase during fermentation. The four key SCFAs of most interest in RS fermentation studies (acetate, butyrate, propionate and valerate) were assayed and are reported here.

Faecal acetate concentrations were plotted by study timepoint for each participant in **Figure 14**.

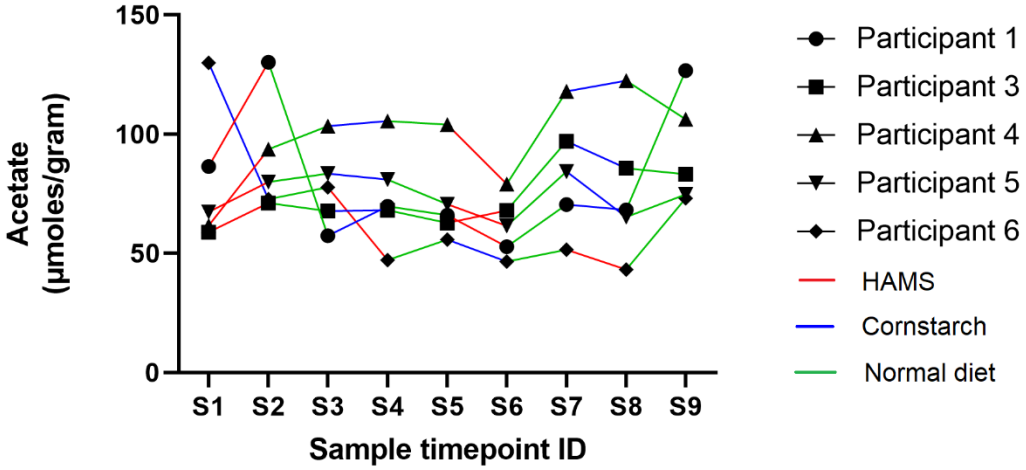


Figure 14: Faecal acetate concentration at 9 sample timepoints (n=5).

Although an initial increase in acetate levels is evident for the four participants who undertook HAMS supplementation first (Participants 1, 3, 4 and 5), the opposite effect is seen in all other timepoints immediately following HAMS supplementation. The treatment effect hypothesis suggests that the Normal diet period would be associated with a decrease in acetate following either HAMS or Cornstarch supplementation, but this is not apparent in the data, as shown in **Figure 14**.

Mean acetate values with SEM for each participant were calculated and graphically represented by study period in **Figure 15**.

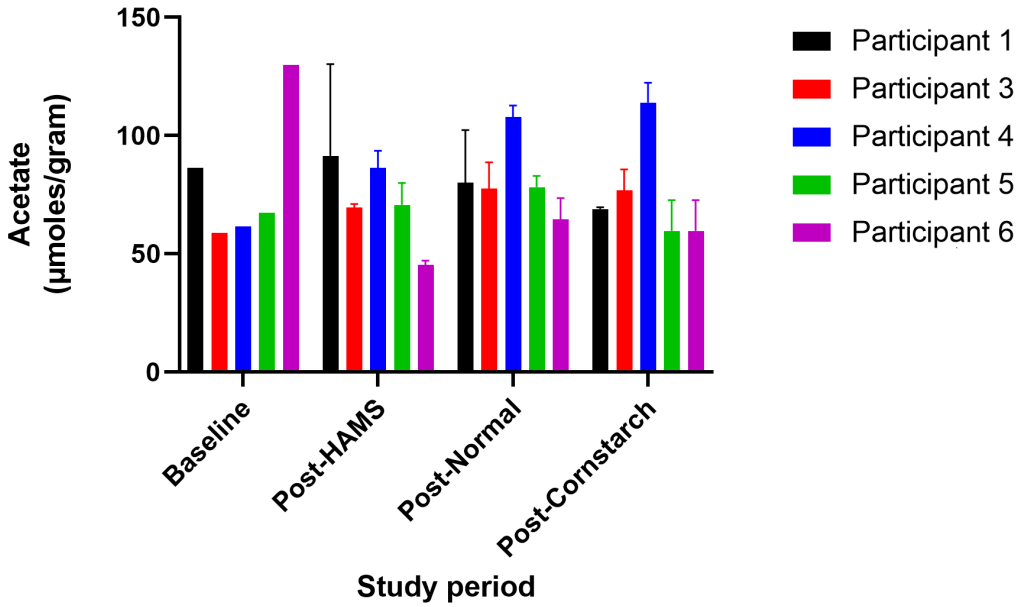


Figure 15: Faecal acetate concentration (mean, SEM) by study period (n=5).

The data presented in **Figure 15** does not support the study hypothesis of an increase in SCFA production following RS supplementation periods. While there is some support for the hypothesis in Participants 3, 4 and 5, this is only true when comparing Post-HAMS increases to Baseline. This trend to increase acetate levels after any RS supplementation is only upheld when comparing Post-Cornstarch vs Baseline, and Post-Cornstarch vs Post-Normal, for Participant 4.

Increased faecal butyrate concentration is considered one of the most relevant outcomes indicating a possible clinical benefit, owing to the role of butyrate as a preferred substrate for colonic epithelial cells as well as its involvement in many biochemical pathways in the large intestine[39].

Faecal butyrate concentrations were plotted by study timepoint for each participant in **Figure 16**.

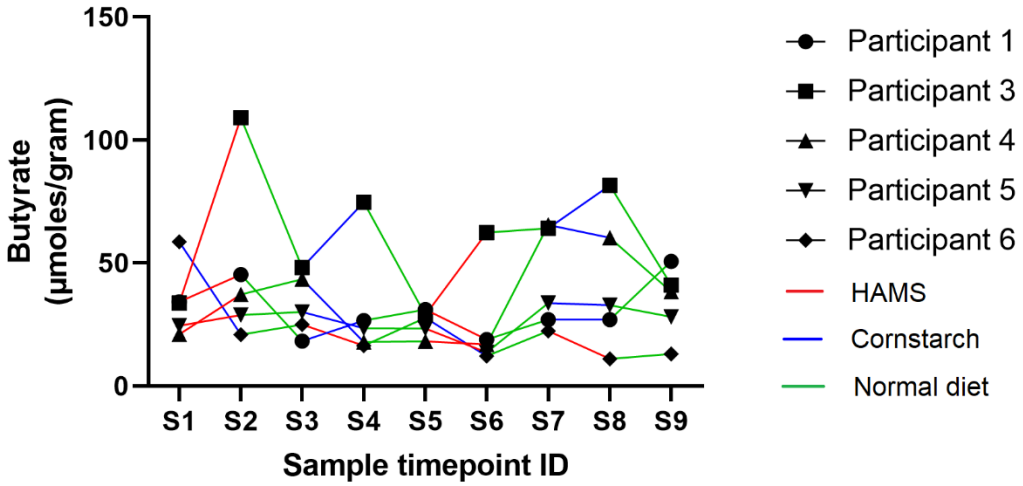


Figure 16: Faecal butyrate concentration at 9 sample timepoints (n=5).

In total, there are 45 timepoints at which SCFA data were collected (5 participants with 9 faecal collection timepoints each). Five of these were baseline values, 10 of these were after HAMS supplementation periods, with a further 10 after Cornstarch supplementation periods. The remaining 20 were after Normal diet periods. **Figure 16** shows that the hypothesised trend to increased levels of butyrate following RS supplementation was evident on 6 out of 10 occasions (60%) following HAMS supplementation and 3 out of 10 occasions (30%) following Cornstarch supplementation. The anticipated decrease in butyrate following Normal diet periods was observed on 6 out of 20 occasions (30%).

Mean butyrate values with SEM for each participant were calculated and graphically represented by study period in **Figure 17**.

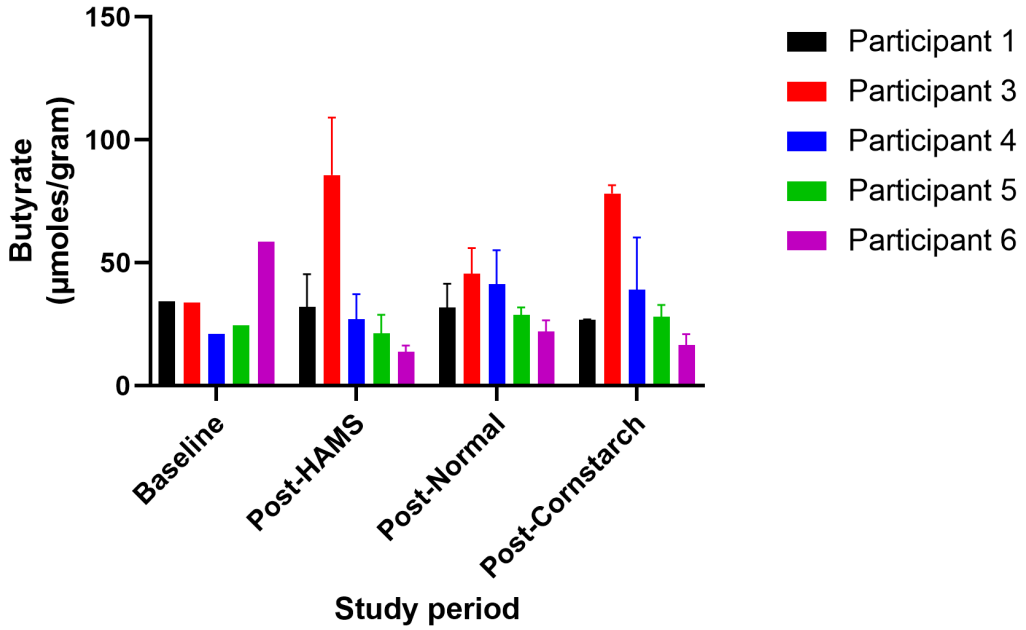


Figure 17: Faecal butyrate concentration (mean, SEM) by study period (n=5).

Figure 17 indicates support for the study hypothesis in terms of increases in faecal butyrate concentration following HAMS supplementation when compared to Baseline in Participants 3 and 4. When comparing Post-Cornstarch concentrations of butyrate, the values also support the study hypothesis for Participants 3, 4 and 5. Overall, only Participant 3 displays the expected trend of increased concentrations after HAMS and Cornstarch supplementation when compared to both Baseline and Post-Normal values.

Faecal propionate concentrations were plotted by study timepoint for each participant in **Figure 18**.

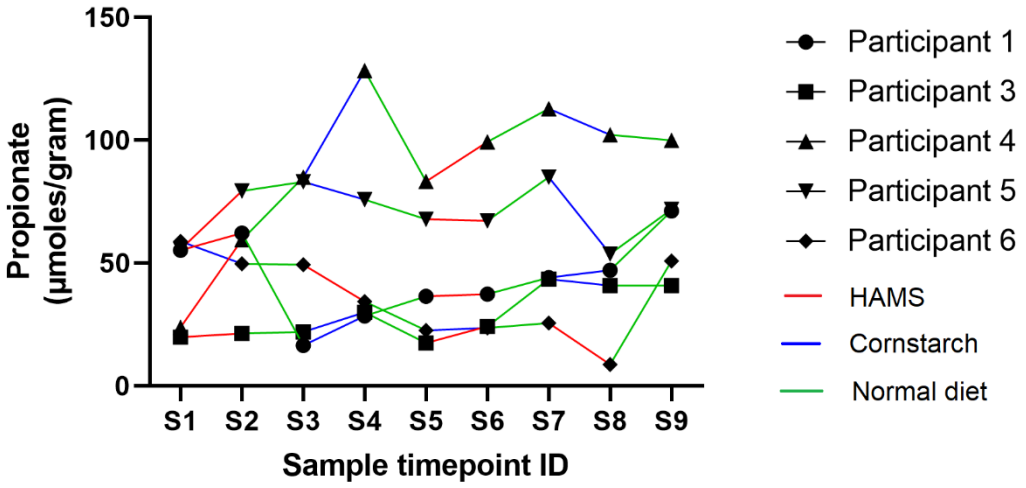


Figure 18: Faecal propionate concentration at 9 sample timepoints (n=5).

As for the trends observed for acetate and butyrate, faecal propionate levels increased compared to Baseline for all participants who received HAMS supplementation first, shown by the red lines from S1 to S2.

Mean propionate values with SEM for each participant were calculated and graphically represented by study period in **Figure 19**.

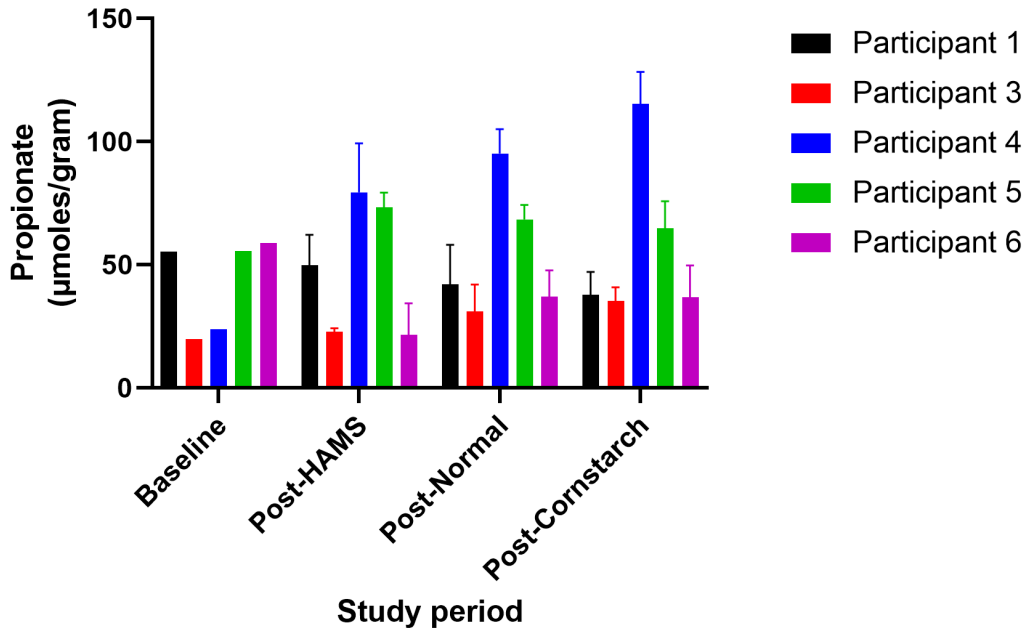


Figure 19: Faecal propionate concentration (mean, SEM) by study period (n=5).

Whilst the data in **Figure 19** shows some support for the hypothesis predicting an increase in SCFA concentrations such as propionate following RS supplementation, this is only apparent for Participants 3, 4 and 5 following HAMS supplementation when compared to Baseline and also for Participants 4 and 5 following Cornstarch supplementation when compared to Post-Normal values.

Faecal valerate concentrations were plotted by study timepoint for each participant in **Figure 20**.

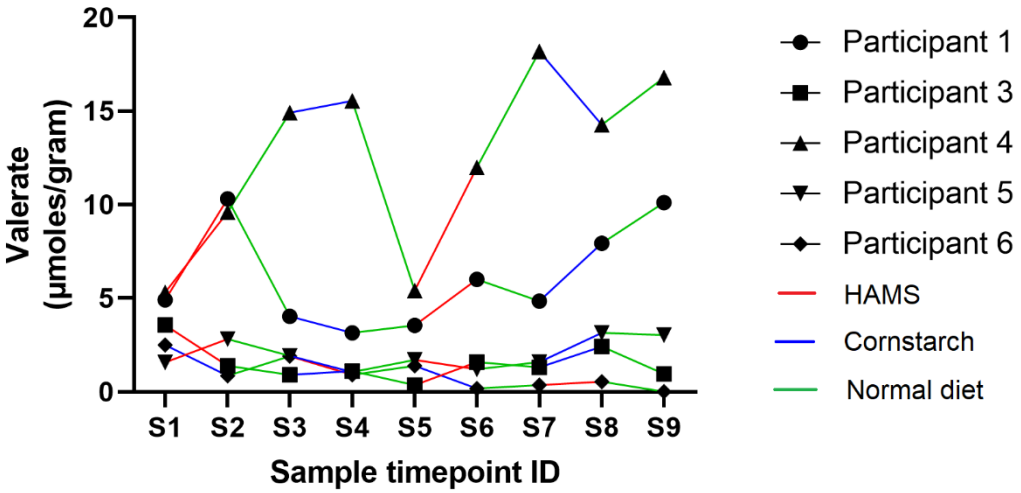


Figure 20: Faecal valerate concentration at 9 sample timepoints (n=5).

Faecal valerate increased in concentration in 7 out of 10 timepoints following HAMS supplementation and 5 out of 10 timepoints following Cornstarch supplementation.

Mean valerate values with SEM for each participant were calculated and graphically represented by study period in **Figure 21**.

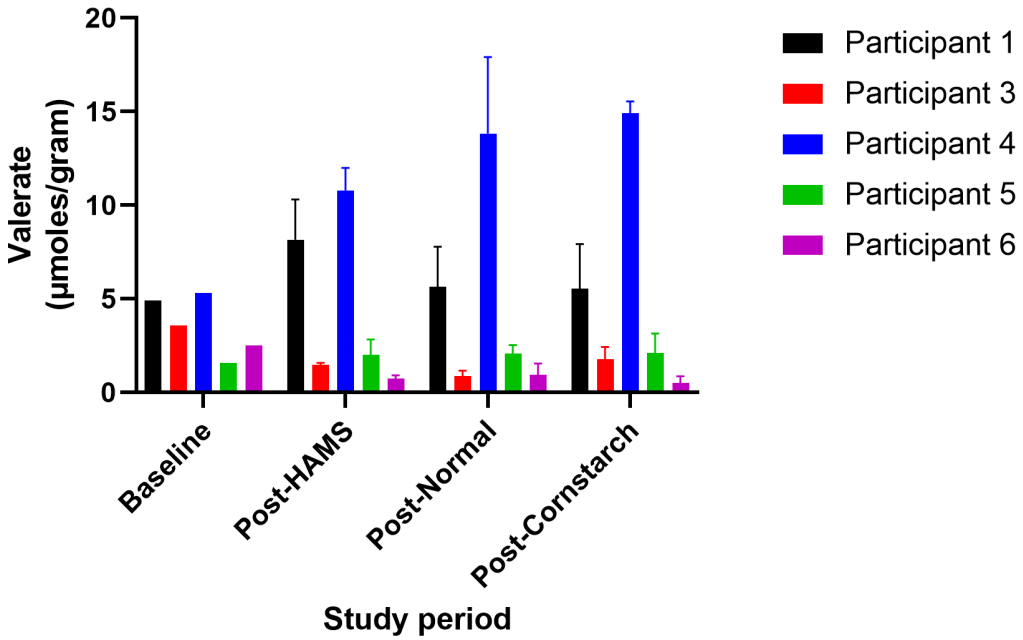


Figure 21: Faecal valerate concentration (mean, SEM) by study period (n=5).

Similar to the findings for the other three SCFAs, **Figure 21** shows that faecal valerate data also inconsistently supported the study hypothesis for an increase after any RS supplementation compared to Baseline and Normal diet periods.

Blood CD4+ T cell count

Based on a 2011 study, the reference range for CD4+ T cells in Indian adults is 381–1565 cells/μL for males and 447–1846 cells/μL for females[338]. A higher level of CD4+ T cells reflects improved HIV-related immunity.

The analysis of CD4+ T cell count changes by study period showed that a majority of participants demonstrated an increase in CD4+ T cells following the HAMS supplementation period (four out of five) (**Figure 22**). Three of these participants also displayed a decrease in CD4+ T cells after the Normal diet period followed by an increase after the Cornstarch supplementation period (Participants 3, 4 and 5). Although Participant 6 did not display an increase in CD4+ T cells after the HAMS supplementation period, the data did, however, show a modest increase after the Cornstarch supplementation period.

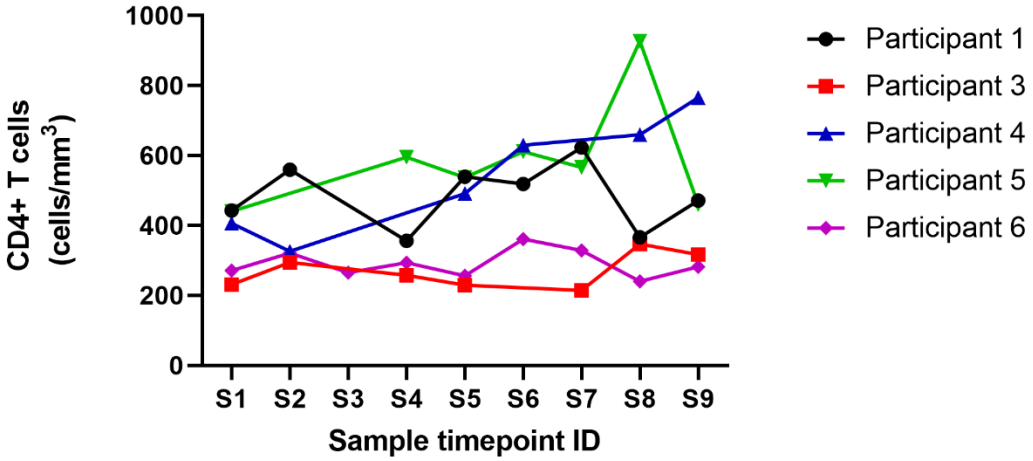


Figure 22: Venous blood CD4+ T cell concentration at 9 sample timepoints (n=5).

Mean CD4+ T cell values for all study participants by study period were also calculated and graphically represented in **Figure 23**.

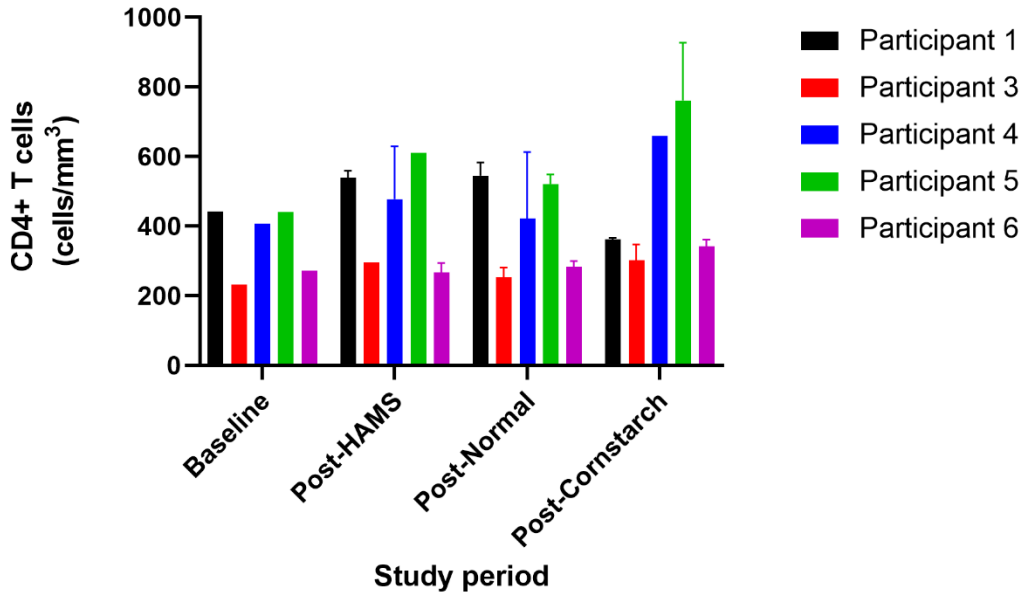


Figure 23: Venous blood CD4+ T cell concentration (mean, SEM) by study period (n=5).

The trend described above, which was noted for 9 sample timepoints, is also evident in **Figure 23** above. Participants 3, 4 and 5 are seen to follow the trend of increasing CD4+ T cell counts after both HAMS and Cornstarch supplementation periods, when compared to Baseline. They also follow the hypothesised trend of decreasing after Normal diet periods.

Plasma HIV viral load

Three of five participants had HIV viral load values of zero throughout the whole study and one participant had values of zero for half of their observations. This result is consistent with effective ART in this population. Owing to COVID-19 disruptions and lysed blood samples, HIV viral load data for some timepoints was missing. Univariate analysis of this data was therefore not undertaken because there was too little variability in the data to pick up any meaningful trends, but, for completeness, HIV viral load data were included in the ME model. This ME model analysis showed no significant results for HIV viral load as discussed above.

Faecal microbiota

The structure of the gut microbial community was measured at baseline, after starch interventions and after normal diet washout periods. Where required to distinguish between the different washout periods in study 1 and study 2, the following nomenclature is used in the figures below: after Cornstarch referred to as 'Aftercorn'; after HAMS referred to as 'AfterHAMS'; after normal diet washout period for study 1 ('Afternorm'); and after normal diet washout period for study 2 ('Afternorm2') combined. In the figures included in this section, Participant ID is denoted by P1, P3, P4, P5 and P6. Sample timepoint ID is denoted by S1-S9.

Core microbiota of the study population

The core human microbiome has been defined as: 'the set of genes present in a given habitat in all or the vast majority of humans'[339,p.806] and can be specific to functional areas such as the gastrointestinal system or the whole body[339]. The core microbiota (determined based on presence of the bacterial taxa in at least 50% of samples at an average relative abundance of >1%[339, 340]) of the five participants was plotted based on their baseline faecal microbiome, as shown in **Figure 24**.

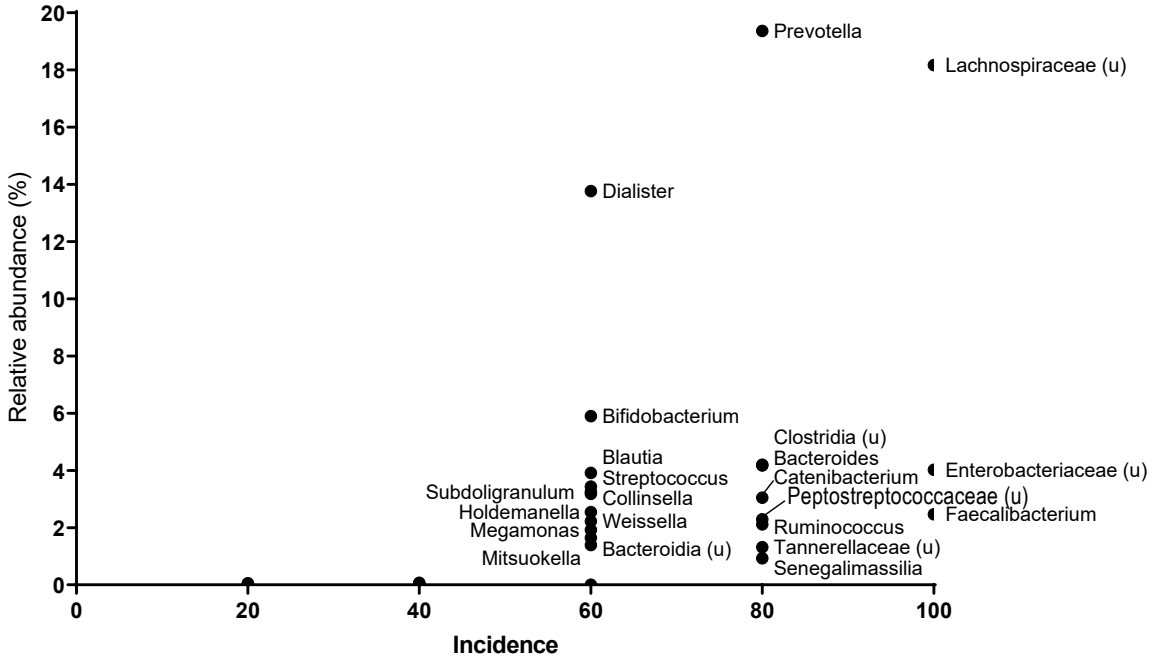


Figure 24: Core microbiota of baseline faecal samples (n=5).

Incidence indicates the proportion of individuals who satisfies the criteria of relative abundance >1% for the specific bacterial taxa indicated. '(u)', denoting 'unknown', is used where the genera information of an amplicon sequence variant could not be obtained. When this occurs, the lowest phylogenetic information followed by '(u)' is indicated.

Figure 24 highlights that this participant cohort displayed gut microbiota enriched with *Prevotella*. Other commensal gut bacteria, including *Faecalibacterium* and *Ruminococcus*, were present in all participants, while *Bifidobacterium* was present in four participants. Specifically, the mean relative abundance of *Bifidobacterium*, *Ruminococcus* and *Faecalibacterium* in the participants in this study are ~6%, 2% and 3%, respectively.

Alpha diversity

The OTU plot in **Figure 25** shows the inter-individual differences in gut microbiome between participants, indicating how many bacterial species of each taxa are present in the sample. The variability in taxa shown in the OTU plot is as expected, noting the vast differences that are expected between individuals. This figure also shows the dominance of *Prevotella* in all participants, with the exception of Participant 3, who had a greater dominance of Lachnospiraceae.

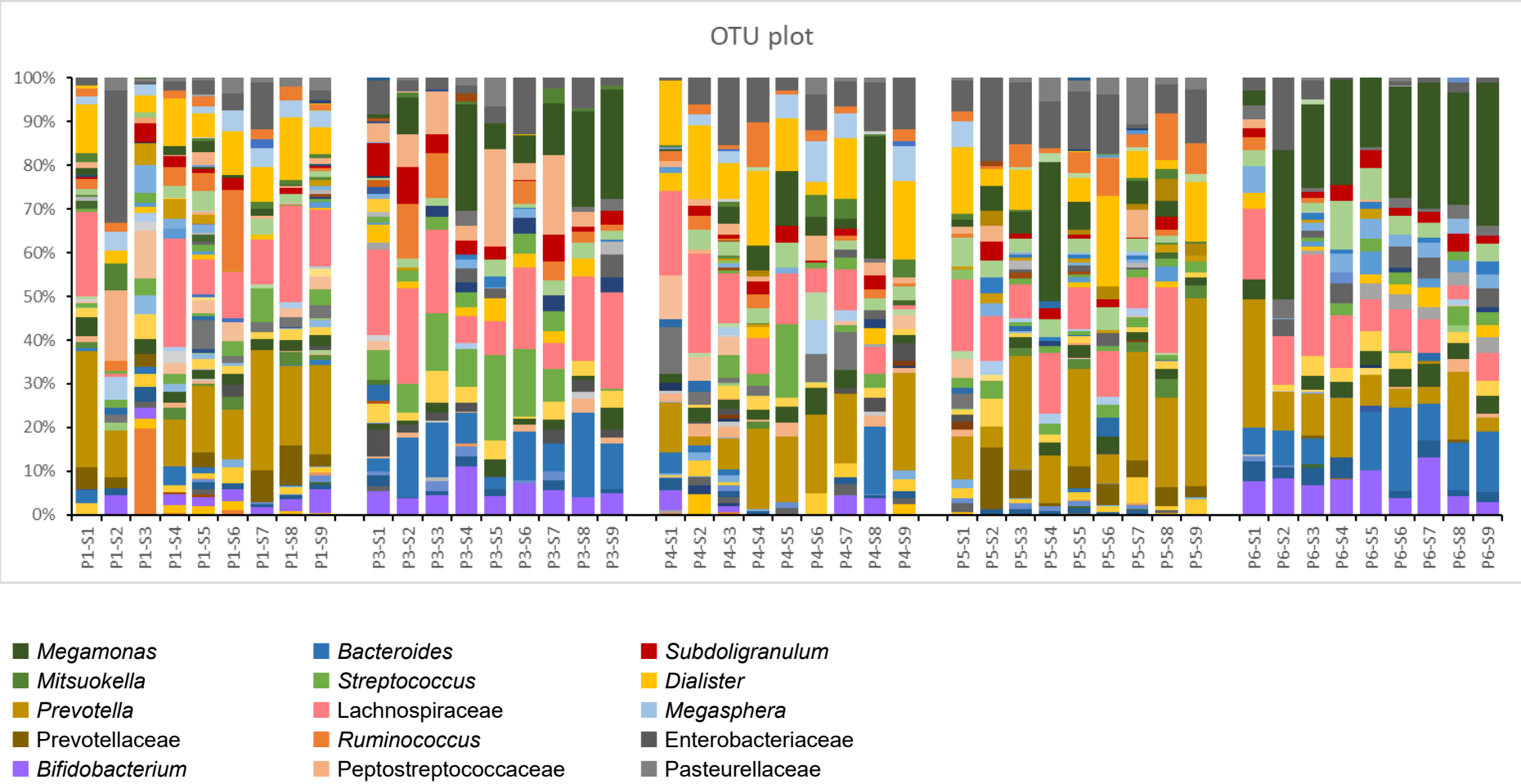
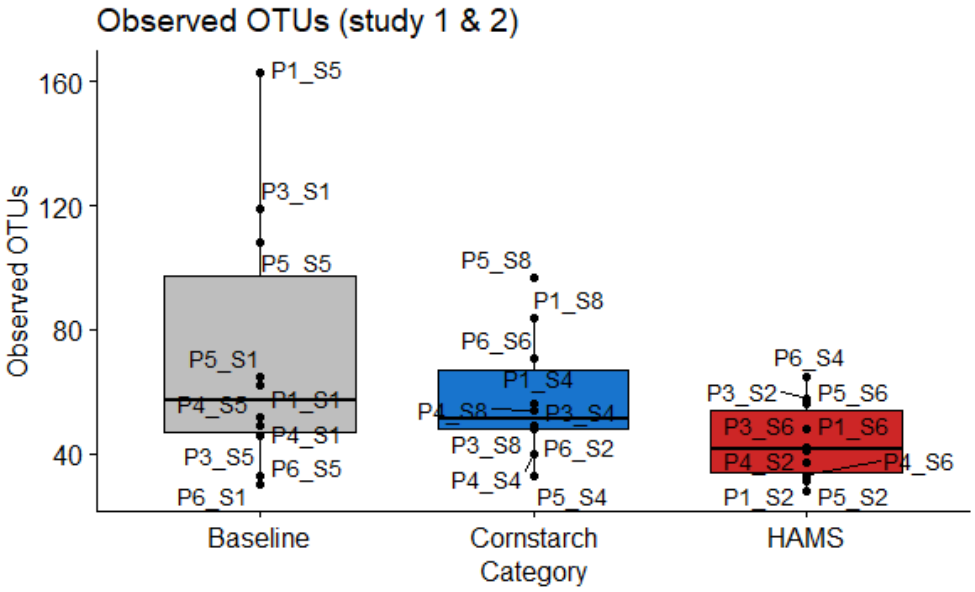


Figure 25: OTU plot for Participants 1, 3, 4, 5 and 6 (n=5).
(P1, P3, P4, P5 and P6: Participant ID. S1-S9: Sample timepoint ID.)

The structure of the gut microbial community was determined based on alpha diversity measures of microbial richness, evenness and diversity, as assessed by observed OTUs (**Figure 26**), Faith's phylogenetic diversity (**Figure 27**), and Pielou's evenness (**Figure 28**). The below analyses utilise the study 1 baseline and study 2 baseline, not the absolute baseline of day 0.

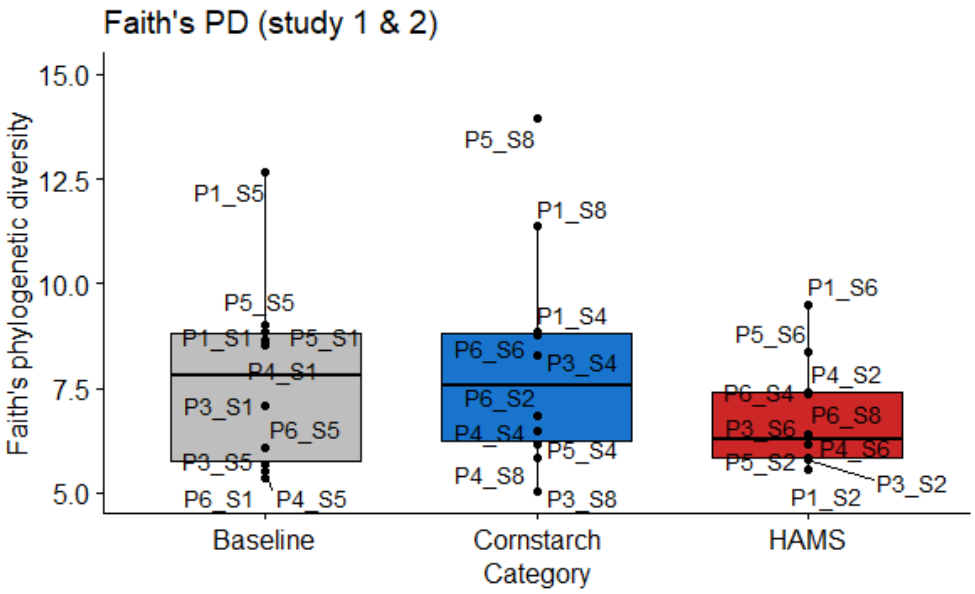
While there were no significant changes observed for microbial richness and diversity between the groups (Friedman test, $p>0.05$), the analysis showed a trend of larger decreases in microbial richness and diversity following HAMS when compared to the change observed following Cornstarch. Microbial evenness was not significantly altered by HAMS or Cornstarch supplementation when compared to that at baseline ($p=0.343$ and 0.058, respectively). However, within-group variations in microbial evenness (**Figure 28**) were observed following either HAMS or Cornstarch supplementation. Based on these observations, the following was concluded: Pairwise comparison between the groups indicated no significant differences in the structure of the microbial community following the Cornstarch or HAMS diet, although microbial richness and diversity trended lower with the HAMS diet.



Friedman test, p=0.182

Pairwise comparison	Individual p value	FDR p
Baseline vs HAMS	0.0578	0.1733
Baseline vs Cornstarch	0.3428	0.3428
HAMS vs Cornstarch	0.3428	0.3428

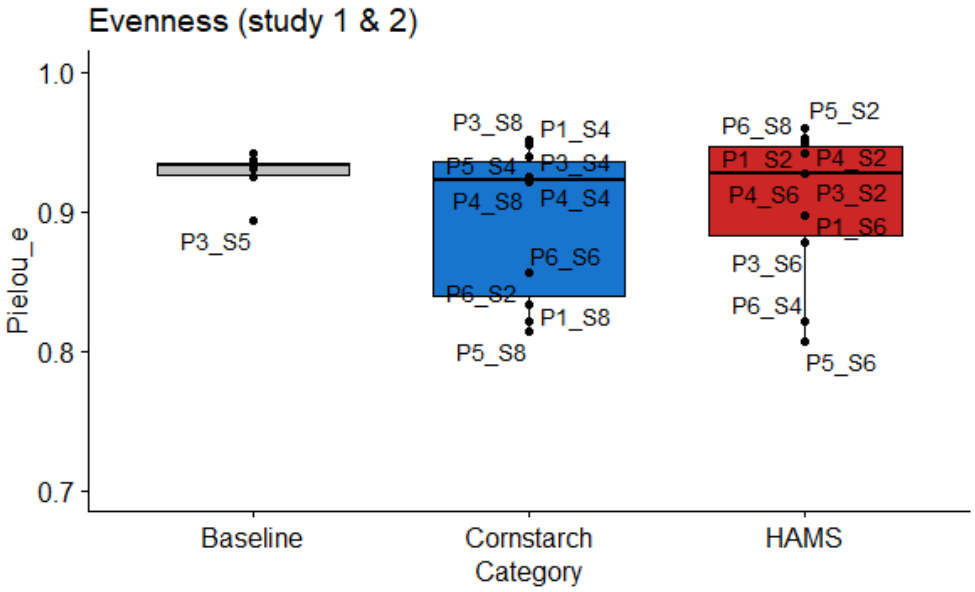
Figure 26: Microbial richness based on alpha diversity as measured by OTUs and associated Friedman test examining pairwise comparison.
(P1, P3, P4, P5 and P6: Participant ID. S1-S9: Sample timepoint ID.)



Friedman test, p=0.367

Pairwise comparison	Individual p value	FDR p
Baseline vs HAMS	0.2059	0.3089
Baseline vs Cornstarch	0.7518	0.7518
HAMS vs Cornstarch	0.1138	0.3089

Figure 27: Phylogenetic diversity based on alpha diversity as measured by Faith's phylogenetic diversity and associated Friedman test examining pairwise comparison.
(P1, P3, P4, P5 and P6: Participant ID. S1-S9: Sample timepoint ID.)



Friedman test, p=0.182

Pairwise comparison	Individual p value	FDR p
Baseline vs HAMS	0.3428	0.3428
Baseline vs Cornstarch	0.0578	0.1733
HAMS vs Cornstarch	0.3428	0.3428

Figure 28: Microbial evenness based on alpha diversity as measured by Pielou's evenness (Pielou_e) and associated Friedman test examining pairwise comparison and within-group variation. (P1, P3, P4, P5 and P6: Participant ID. S1-S9: Sample timepoint ID.)

Changes in faecal microbiota composition across the study period

Microbiota composition was not significantly altered by the HAMS (PERMANOVA p=0.40) or Cornstarch diet intervention (p=0.19), when compared to their respective microbiota composition baseline (study 1 and study 2 baselines). The trajectory graph (**Figure 29**) shown below depicts the distances between samples (calculated based on their microbiota composition as measured by weighted UniFrac distances), reflecting the changes in faecal microbiota composition from one timepoint to another across the study period. Samples were ordinated on a multidimensional scaling plot. Larger distances from one sample to another represent larger microbiota differences between the two samples. Samples were clustered according to participants rather than to timepoints, suggesting that the microbiota composition differed primarily between participants.

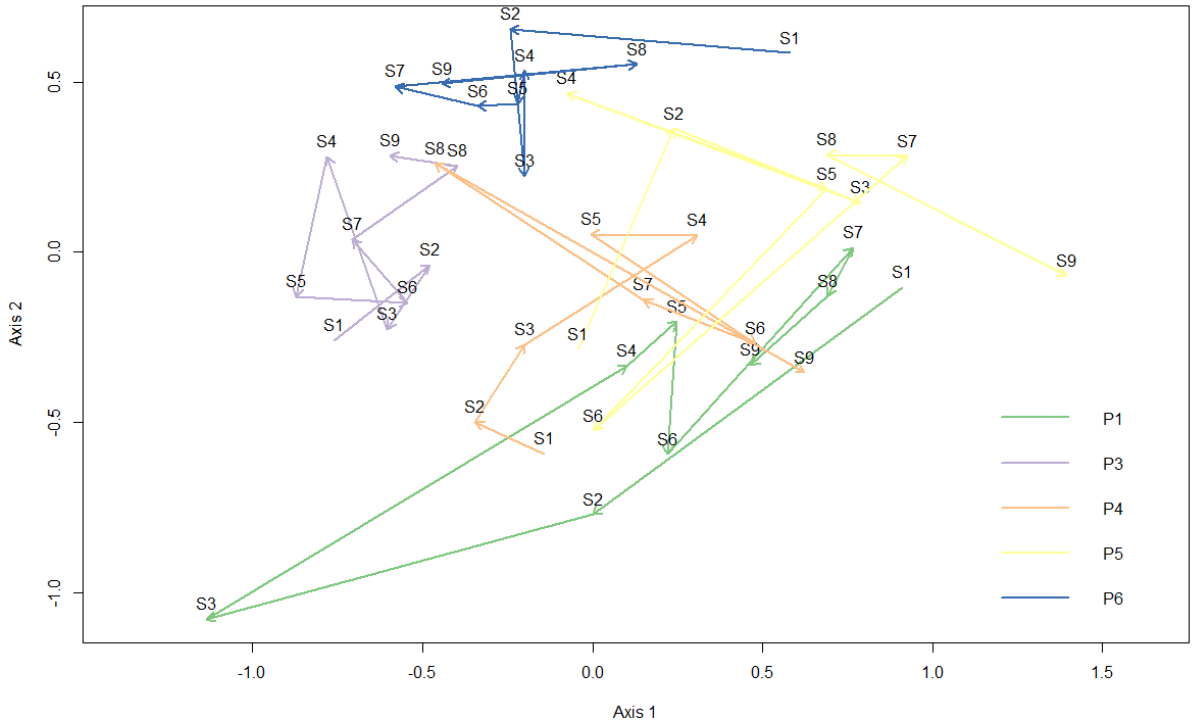


Figure 29: Trajectory graph of faecal microbiota composition.
(P1, P3, P4, P5 and P6: Participant ID. S1-S9: Sample timepoint ID.)

Microbiota composition at baseline vs after two weeks of Normal diet

To test the study assumption that the Normal diet (or washout) periods would result in the microbiome composition returning to a value which was the same or similar as the absolute baseline of day 0, an analysis was undertaken comparing the microbiome composition at absolute baseline (S1) compared to the end of each 14-day period of Normal diet or washout periods (S3, S5, S7 and S9).

The microbiota composition at all timepoints corresponding to the end of 14 days of Normal diet or washout periods (S3, S5, S7 and S9) did not significantly differ from those at absolute baseline (S1) (PERMANOVA, Pseudo-F=0.716, p=0.847; ANOSIM, R=–0.069, p=0.811). Additionally, the magnitude of microbiota composition alteration between the initial composition at baseline (S1) and at the end of each two weeks of Normal diet (S3, S5, S7 and S9) was similar among each Baseline–Normal diet comparison (Friedman test, p=0.18), as shown in **Table 21** and **Figure 30**.

This analysis confirmed that there were no significant microbiome compositional differences between the study 1 baseline (S1) and study 2 baseline (S5).

Table 21: PERMANOVA analysis of microbiota composition at baseline (study 1 or study 2 baseline) vs after 2 weeks of Normal diet.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Ti	4	4735.4	1183.8	0.71643	0.8473	9906
Res	20	33049	1652.4			
Total	24	37784				

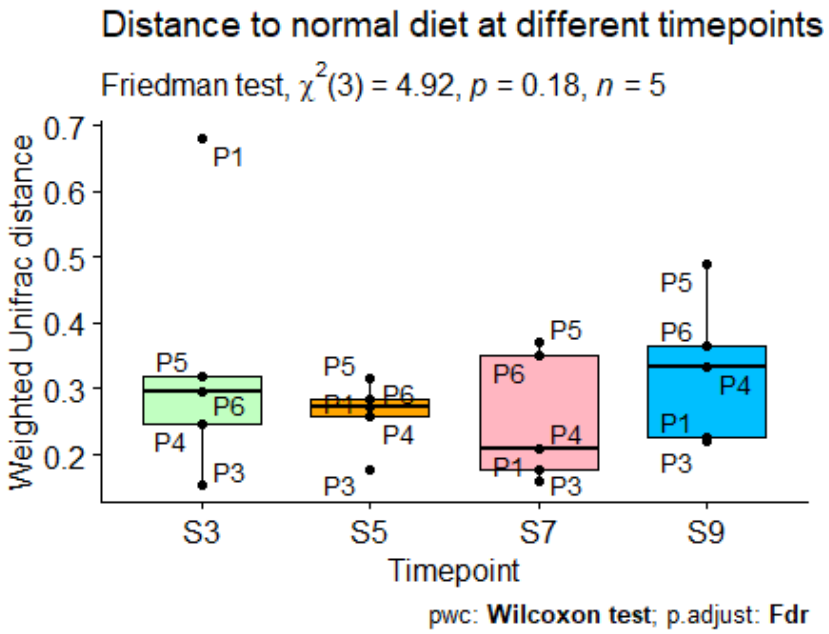


Figure 30: Changes in faecal microbiota composition across the study period measured by weighted UniFrac distance to Normal diet at different timepoints, as indicated by different colours for sample timepoints S3, S5, S7 and S9.
(P1, P3, P4, P5 and P6: Participant ID. S1-S9: Sample timepoint ID.)

Microbiota composition changes based on treatment groups

PERMANOVA analysis presented in **Table 22** indicated that there was no significant difference in the microbiota composition between groups (Baseline, after 14 days of Cornstarch, HAMS or Normal diet) (PERMANOVA, Pseudo-F=0.628, p=0.883). As expected, pairwise comparisons (**Table 23**) indicated that there were no significant differences in microbiota composition between each of the groups (p>0.189). Baselines included in this analysis are relative to the study number (i.e., the study 1 baseline was used for study 1 data and the study 2 baseline was used for study 2 data).

Table 22: PERMANOVA analysis with pairwise comparison of microbiota changes based on treatment groups, using the study 1 and study 2 baselines.

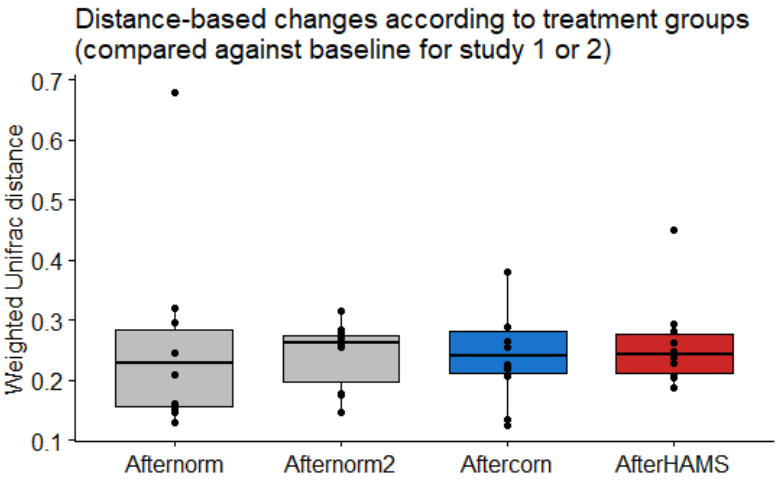
Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Ra	3	4728.4	1576.1	0.6282	0.8834	9913
Pa(Ra)	16	42373	2648.3	3.0572	0.0001	9839
Res	25	21656	866.25			
Total	44	68757				

Table 23: PERMANOVA analysis of microbiota changes based on treatment groups.

Groups	t	P(perm)	Unique perms
Baseline, HAMS	1.0089	0.4023	252
Baseline, Normal	0.97176	0.4521	252
Baseline, Cornstarch	1.19	0.1891	252
HAMS, Normal	0.57682	0.9137	252
HAMS, Cornstarch	0.77044	0.7047	126
Normal, Cornstarch	0.63588	0.8936	252

Magnitude of change against the respective study baseline

Figure 31 shows that the magnitude of microbiota composition alteration did not significantly differ between the groups (Baseline – HAMS vs Baseline – Cornstarch vs Baseline – First Normal diet vs Baseline Second Normal diet) (Friedman test, p=0.709). All comparisons for magnitude were based on the baseline composition for study 1 (day 0) or study 2 (day 56). Noting that a larger weighted UniFrac distance means a larger magnitude of change resulting from the respective intervention, **Figure 31** below shows that, for the combined studies 1 and 2, HAMS and Cornstarch both resulted in larger changes, compared to those observed following the first Normal diet (washout) period.



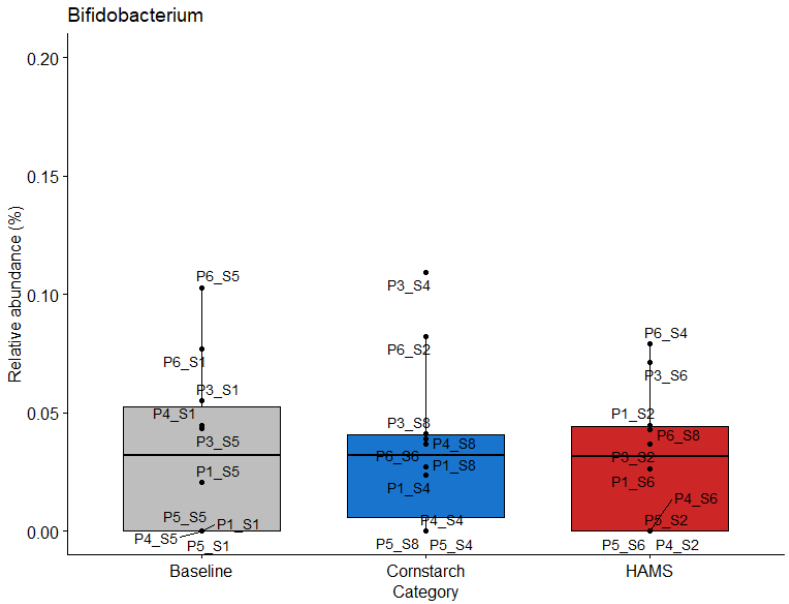
Friedman test, p=0.709

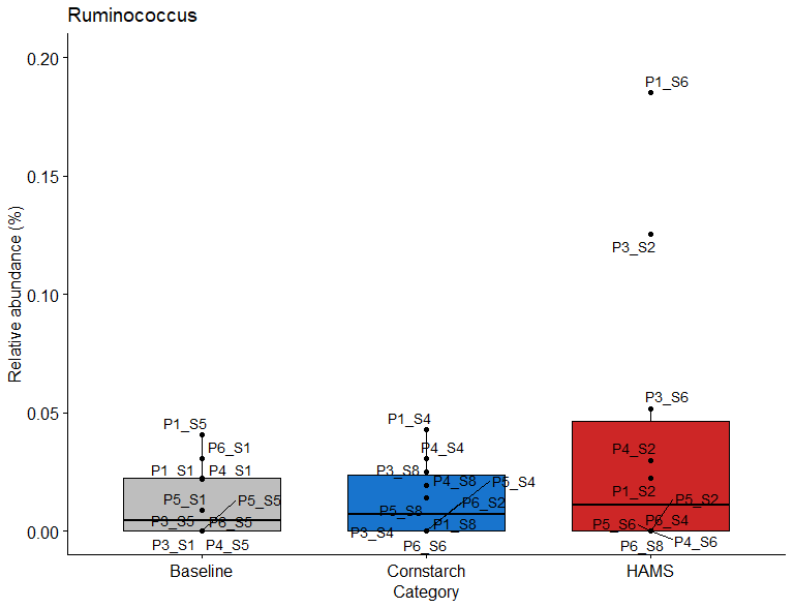
Figure 31: Boxplots showing distance-based change in weighted UniFrac distance for (L–R) total study, study 1 and study 2.

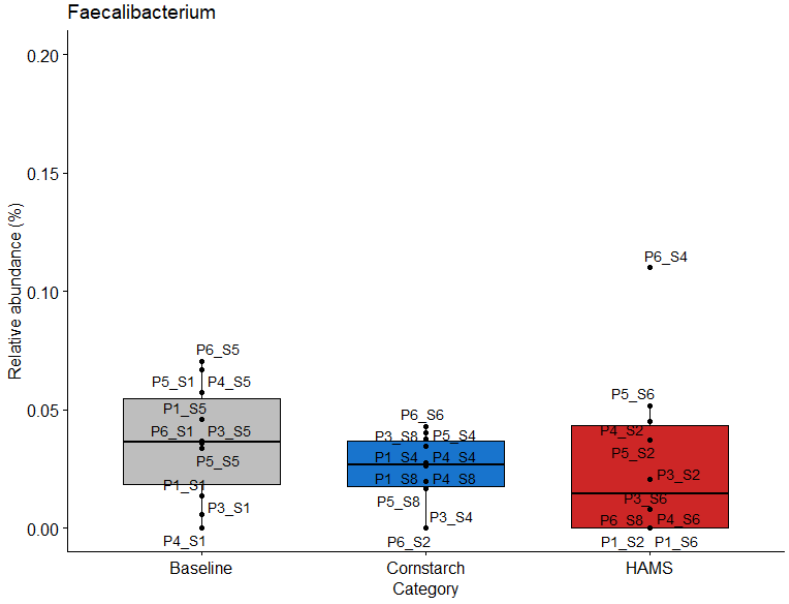
Grey boxes represent Normal diet period; blue box represents Cornstarch diet period; red box represents HAMS diet period.

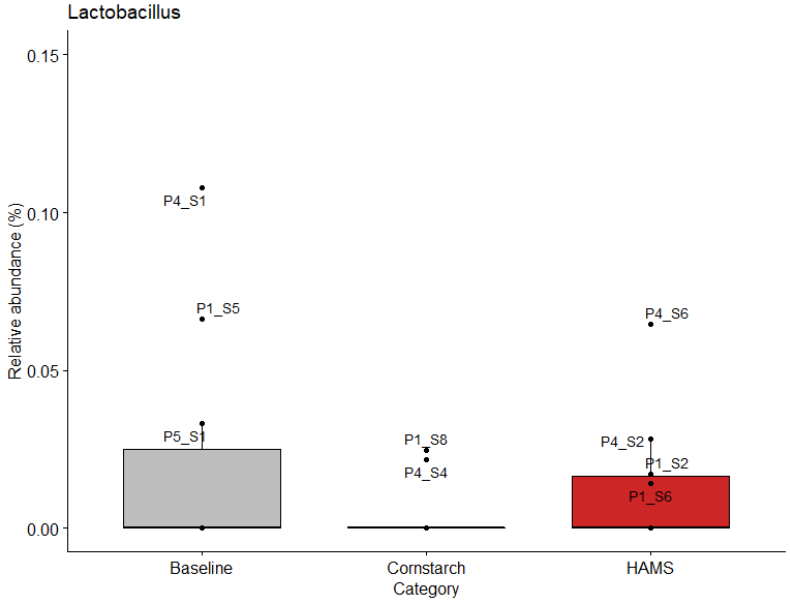
Relative abundance of potential taxa implicated in RS metabolism

A secondary analysis was undertaken to determine changes in the relative abundance of specific bacterial taxa that are implicated in RS metabolism. Based on the lack of significant changes found in the microbiota analysis, it was not expected that this further analysis would produce any significant results but it was undertaken to definitively address this research question. As illustrated in **Figure 32**, there were no significant differences in the relative abundances of these bacterial taxa between the groups (Friedman test, $p > 0.05$).


Bifidobacterium
Relative abundance (%)
0.20
0.15
0.10
0.05
0.00
Baseline
Cornstarch
HAMS
Category


Ruminococcus
Relative abundance (%)
0.20
0.15
0.10
0.05
0.00
Baseline
Cornstarch
HAMS
Category


Faecalibacterium
Relative abundance (%)
0.20
0.15
0.10
0.05
0.00
Baseline
Cornstarch
HAMS
Category


Lactobacillus
Relative abundance (%)
0.15
0.10
0.05
0.00
Baseline
Cornstarch
HAMS
Category

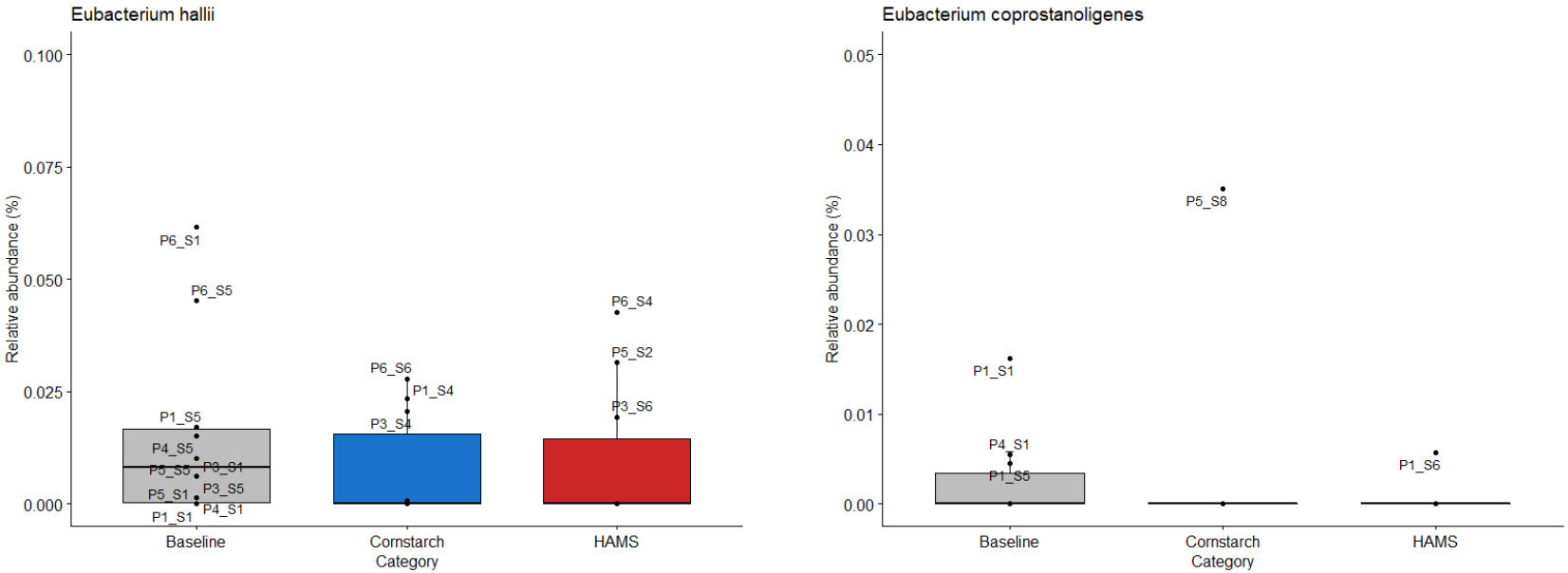


Figure 32: Relative abundance (%) of potential taxa implicated in RS metabolism.
Grey box represents Normal diet period; blue box represents Cornstarch diet period; red box represents HAMS diet period.
(P1, P3, P4, P5 and P6: Participant ID. S1-S9: Sample timepoint ID.)

DISCUSSION

This chapter will examine the findings of the study in relation to the literature and the original study objectives. It will explore the strengths and limitations of the study and the application of the findings to public health policy, management and practice.

Executive summary of findings

As indicated in the Feasibility Literature Review, dietary intervention studies of a similar nature with an HIV-positive population have been successful in recruiting and retaining participants and achieving adequate adherence to protocol and intervention. The current study is contextually different as it utilised a sole RS dietary intervention, was conducted in an LMIC, was disturbed by the COVID-19 pandemic, and was managed remotely for some of its duration. In addressing the first aim of this study which related to feasibility (*to determine whether an RS supplementation study with HIV-positive people in India is feasible*), the literature review showed that there are various typologies against which feasibility can be assessed. Significant overlap exists and the framework published by Thabane et al.[71] was used in this thesis since it informed the CONSORT extension for the reporting of feasibility and pilot studies[73, 235]. The application of this typology to published studies that utilised RS and prebiotic interventions in HIV-positive populations indicated that, while data relating to the Process and Scientific categories were frequently reported, factors affecting feasibility in the Resources and Management categories were overlooked.

The Efficacy and Effect Literature Review, which informed the second aim of the study (*to determine whether RS supplementation is associated with treatment effect(s) relating to gut health and HIV-related immunity*), highlighted that prebiotic dietary supplementation resulted in increased diversity of the gut microbiota in some studies[9, 64, 67, 69]. The literature was primarily focused on FOS and GOS prebiotic preparations, either alone or in combination with probiotics or amino acids. Studies which used the same type of RS as the current study are particularly informative, although only one was conducted with HIV-positive populations and used a small dose of 2.5g of RS/day[68]. This confirmed the knowledge gap addressed by this thesis as the first of its kind to investigate the effect of sole RS2 dietary supplementation in an HIV-positive population. The literature examining the effect of RS in HIV-negative populations and animal models provides evidence of a breadth of benefits, sometimes using the same outcome measures as used in this thesis: faecal pH, faecal SCFA concentration, and absolute and relative abundance of gut bacteria measured in faecal samples[38, 41, 64, 214, 341].

The doctoral researcher conducted a 112-day double-crossover trial in India. A key finding of the feasibility assessment undertaken as part of this trial was that high-dose RS

supplementation was well tolerated in this population. Overall, few gastrointestinal signs and symptoms were reported by participants. This was particularly noteworthy since the dose of the main intervention starch, HAMS, was high (61.75g/day) compared to other studies supplementing RS in adult populations[68, 230, 342]. Observed consumption of study foods confirmed that participants adhered to the intervention, consuming the entire serve on all occasions when observation was not disrupted by COVID-19.

The results of the semi-structured interviews with participants indicated that individuals who were approached to join the study were motivated to participate because of the prospect of improving their health and because of referral by ICTC counsellors. Participants reported having a high degree of commitment to the study and were able to maintain their participation over an extended period based on their own personal will, with support from the field worker. None of the participants expressed negative factors associated with the involvement of the Australian researcher, with most stating that it did not affect their decision to participate. One participant stated that they felt the involvement of the Australian researcher provided a level of endorsement and positively influenced their decision to join the study.

Findings of the treatment effect study showed inconsistent changes in faecal pH and SCFA concentrations. Although changes to these outcome measures did not reach significance according to the linear ME model analysis, a trend was observed in the four participants who were randomised to HAMS in the first supplementation period. For these participants, concentration of the four SCFAs increased from the baseline timepoint (S1) to the timepoint immediately following HAMS supplementation (S2) on the majority of occasions. There was also an associated decrease in faecal pH observed at S2 compared to baseline in these four participants. The hypothesised decrease in faecal pH was based on the expected increase in SCFA production during fermentation of RS, as informed by clinical trials and confirmed by a systematic review with meta-analysis published in 2017[230]. The COVID-19 limitations on participant recruitment resulted in randomisation allocation being unequal across the small sample, which needs to be considered when interpreting results.

In addition to the ME model analysis showing no significant effect of RS supplementation on pH, acetate, butyrate, propionate, valerate and bacterial alpha and beta diversity, no significant effects were reported for CD4+ T cells or HIV viral load for either of the RS treatments. This finding was consistent when comparing effects of the starches to each other (Diet A vs Diet B) and effects of each starch to the Normal diet periods (Diet A vs Normal diet, Diet B vs Normal diet). There were no treatment by period interactions and no carryover effects from previous RS supplementation periods. However, carryover effects of the study intervention may still occur, despite adjusting for these effects statistically[214]. This may partially explain the variability of individual response in this study.

Despite the high dose of RS, the effect of dietary RS supplementation on the gut microbiota was difficult to discern, being impacted by the limited sample size resulting from COVID-19 disruptions to recruitment. This disruption also interfered with supply of the control starch to the study site, resulting in a locally available starch being used as the comparator starch. Assays performed after study commencement revealed that this starch contained 15% RS. Since the intervention starch, HAMS, contained 65% RS, the study was reconceptualised as a dose-response trial. Dose-response studies of RS have been identified in the literature as essential to extend this research topic[342, 343].

With regard to observed trends, the magnitude of microbiota composition alteration (when compared to Baseline) was similar following HAMS and Cornstarch supplementation. These results suggest that supplementation of RS for 14 days may not be sufficient to significantly alter the gut microbial community. The trajectory graph (**Figure 29**) suggested that the magnitude of microbiota changes across the study period differed between participants. These differences may be attributed to several host factors, including colonisation resistance of the baseline microbial community whereby the composition of the microbiota itself influences susceptibility to microbiota changes/colonisation[344].

Although no significant differences in CD4+ T cell levels were observed with either starch, the analysis showed that a majority of participants demonstrated an increase in CD4+ T cells following HAMS supplementation periods when compared to Cornstarch and Baseline. Despite the lack of significant findings, the trends observed in this study as they relate to the effect of RS on SCFA and CD4 +T cell levels provide a rationale for future studies with an increased sample size, since treatment effects may have been obscured by the small number of participants.

Re-visit of significant original contribution to knowledge

This study examined a sole RS dietary supplementation intervention in an HIV-positive population residing in an LMIC where EED was assumed to be highly prevalent. This thesis observed that the habitual diet of this population was found to be high in RS, as determined by the results of the dietary analysis. Given that a reliance on plant-based diets is typical in many LMICs, levels of RS supplementation in future studies need to be designed with this in mind. Consideration should be given to controlling diets for intake of RS from sources other than the study foods, to improve future studies. Although approaches to controlling habitual diets does add complexity, controlling macronutrient (carbohydrate, protein, fat) and non-starch polysaccharide intakes have been employed successfully in other RS supplementation studies[38]. This thesis also confirmed that, as observed in other studies where plant-based

foods dominate the habitual diet, the gut microbiota of the study population was dominated by *Prevotella* species (**Figure 24**)[345-347].

In terms of original contributions to knowledge of the treatment effect study, the observed trend in CD4 + T cell responsiveness to RS supplementation warrants further investigation, despite not reaching statistical significance. This study is also the first to report the effect of a sole dietary RS supplement on butyrate production in this population. Given the contribution of butyrate to colonic health[39, 166, 168, 176], this study's consideration of how this global health priority population may benefit from increased butyrate production, is significant. The variable responses to RS supplementation that were observed for the four SCFAs included in this study would benefit from further examination. The delivery of such future studies will benefit from the findings of the feasibility study, in terms of refining methods and processes.

Although the change of the study design from a single to double crossover did partially offset the reduction in statistical power because of COVID-19 restrictions, the small number of participants (n=5) meant that the generalisability of study findings is constrained. However, within the context of the feasibility assessment, findings of this study can be generalised to other hard-to-reach populations, as they examine how to access members of this population as well as retain them in a clinical trial. This research also considers governance and procedural requirements for working with collaborating institutes in India and how best to navigate ethics and funding considerations. This study therefore makes an economic, social and cultural contribution by recommending approaches to the successful partnering of research institutions and the sharing of expertise between India and Australia. Indeed, in order to solve global problems, there is a need to continue to collaborate internationally. This approach must factor in the potential for future restrictions on international travel caused by disruptions like COVID-19 and how best to apply digital technologies to manage studies remotely. In this way, the findings of this study are highly generalisable and inform research in the post-COVID global context.

Strengths

Level of RS supplementation

The original intention for the daily dose of RS to be supplemented in this study was based on prior studies conducted by the doctoral researcher[49, 199, 213], a study using RS as part of a composite dietary supplement in an HIV-positive population[68], and other studies supplementing RS in non-HIV positive populations[230]. The dosage of RS was designed to ensure that adequate levels were supplemented to the habitual diet to increase the likelihood of an observable effect. As the study foods represented a known dose of RS per day and the

dietary intake of participants was otherwise uncontrolled, the FFQ tool was used to determine how much RS was consumed in the habitual diet.

Study design

Prebiotic and synbiotic preparations used in previous studies have been highly heterogeneous, including multiple ingredients in composite preparations, thus rendering it difficult to determine which ingredient was the cause of any observed effect[348]. A strength of the current study is that only one type of nutritional intervention (RS) was tested.

Criticisms of previous studies in this area assert that exploratory study designs which lack predefined primary outcomes linked to sample size calculations have limited the usefulness of findings[348]. A strength of this study is that the primary and secondary outcomes were determined and defined prior to study commencement. The chosen primary outcome of pH was used to determine sample size prior to recruitment of the first participant and was based on the outcomes of a study using a 39g/day dose of RS in a different population (healthy Australians)[38].

Published studies examining the effect of prebiotics or synbiotics in HIV-positive populations have thus far been conducted in high-income countries, primarily Spain and Italy[9, 64, 66, 67, 69, 349]. An exception is the study by Gonzalez-Hernandez et al., which was conducted in Mexico[63], and that by Santos et al. in Brazil[20]. The doctoral researcher deliberately chose to conduct this study in an LMIC setting to examine the effect of RS in a context where EED was likely to be highly prevalent. A further rationale was that the populations of LMICs have the most to gain from RS supplementation efforts, given that the cost of RS is exceedingly cheap and that large-scale fortification approaches could be undertaken to benefit both the HIV-positive and broader population. This translational aspect serves as a foundation to effective global health research. Given the growing interest in the functional food area, it is likely that functional foods and staple food fortification will become more commonplace, based on the success of fortification approaches for conditions such as iodine and folate deficiencies. The opportunity to improve population health based on the known benefits of RS is enormous and this thesis makes an important contribution to confirming safety and tolerability of RS in HIV-positive populations.

The crossover design utilised in this study was a key strength since variability is reduced when each participant acts as their own control[342]. This is particularly important in studies of the gut microbiome since a large degree of inter-individual variation exists[270]. This was the primary rationale for originally choosing a single crossover design. Significant variation between individuals is observed in microbial abundance and in the overall functional capacity of the gut microbiome and is influenced by factors such as diet, host genetics, age and medication

use[270]. Given this variability, gut microbiota studies are enhanced by collecting longitudinal data which accounts for some of these factors[270]. Although the duration of the current study did not span years as others have done[270], it did extend over three months. This is a further strength of the study design, albeit one that was an unexpected outcome of the COVID-19 disruption which prompted the modification from a single to double crossover.

Washout period and controlling for carryover effects

One approach to avoiding carryover effects is to include an adequate washout period[213, 350]. In this thesis, this consisted of a 14-day period between starch interventions in which participants consumed their habitual diet with no dietary supplementation, so that outcome measures could be returned to baseline values. This 'Normal diet' washout period was based on prior studies undertaken by the doctoral researcher[49, 198, 199, 271]. There is no agreed standard for the duration of a washout period for dietary intervention studies which supplement RS or prebiotics. Some studies use no washout period[38], whereas others have used washout periods of four weeks[229, 342, 351] or three weeks[352]. In addition to the washout period, in order to adjust the linear ME model for postulated carryover effects, these effects were incorporated into the model. The revised ME model therefore included adjustments for the following effects: treatment/intervention (type of starch), sequence (order of starches), and carryover (effect of previous intervention period). This approach is a further strength of the study, resulting in the ME analysis representing the effect of the intervention alone.

QPE method

The QPE method allowed the doctoral researcher to use the reflections and insights recorded in field notes to assess how the study could be improved for future trials. The role of field notes as part of the QPE method was unlike the traditional approach to quantitative data analysis, which usually occurs once all data have been collected[323]. In this study, qualitative data were analysed throughout the data collection period, enabling methods and processes to be assessed and refined based on the daily experience of trialling workflows and operating procedures with the Indian team[323]. The qualitative analysis of field notes encouraged the doctoral researcher to consider her own biases and assumptions and to develop alternative perspectives on how methods could reflect the preferences of the Indian team, using a pragmatic paradigm approach. This paradigm enables more than one reality or world view to empirically address 'real-world' research questions[211, 217]. In the current study, this encompassed perspectives of the doctoral researcher, members of the Indian study team, and study participants to elucidate understanding as it related to feasibility.

Limitations

COVID-19 impact

The COVID-19 pandemic represented a unique challenge within which to conduct clinical research[353]. The CONSERVE 2021 (CONSORT and SPIRIT Extension for RCTs Revised in Extenuating Circumstance) statement was designed to provide guidance for reporting clinical trials where important modifications to the study resulted from extenuating circumstances, such as COVID-19[354]. Here, the change from single to double crossover of the current study meets the definition of 'important modification' referred to in the CONSERVE statement, where 'important' refers to *'the subset of modifications that could have a potentially meaningful effect on the study objectives or research question, ethical acceptability (including benefits and harms to participants), internal validity, generalizability, feasibility, or analytical methods and statistical power'* [354, p.261]. Although COVID-19 created limitations for this study in terms of recruitment, study design and statistical power, the CONSERVE 2021 statement provides a method for reporting on these important modifications and will be utilised by the doctoral researcher in future peer-reviewed publications.

A particular limitation relating to the COVID-19 disruption was the effect on randomisation. As recruitment was paused during the study period, the total sample size only reached six participants. The randomisation allocation was uneven with four out of six participants being randomised to the same schedule (HAMS-Cornstarch-HAMS-Cornstarch) with the remaining two randomised to Cornstarch-HAMS-Cornstarch-HAMS. Had there been the opportunity to recruit more participants, this randomisation would have eventually evened out to equal numbers of participants in each of the randomisation schedules. This is noted as a limitation of this thesis. A further limitation relating to the sample size was the effect on the semi-structured interview data. Since the sample size was limited to only five participants, the study was grossly underpowered and it is unlikely that saturation of themes was reached. Consequently, this data, along with data from the treatment effect study should be interpreted with adequate caution.

ART adherence of participants

The participant selection bias in which people were preferentially recruited if they were compliant with ART, meant that the level of ART adherence was high throughout the study, as it was at baseline. The recommendation to only include participants who were ART-compliant came from the ICTC counsellors, who suggested that this would ensure that participants would fulfil the study requirements. It is also relevant to note that social desirability

reporting bias may have influenced responses, resulting in participants consistently reporting that ART adherence was high, as has been observed in other studies with HIV-positive populations[355]. However, HIV viral load results also confirmed that ART adherence was high in participants, as discussed in the Results chapter. This highlights the importance of using biochemical markers to support self-reported adherence rates[356]. Future studies could focus on recruiting a broader cross-section of participants and measuring ART adherence throughout the study to determine if RS supplementation has an effect.

Translation of study documents

The translation of the interview guide from English into Oriya was identified as a possible limitation, given that the level of fluency in the Oriya language differed between research team members and that the doctoral researcher had no familiarity whatsoever. Translation of the interview tool utilised two members of the research team and translation of interview responses used back-translation methods to ensure a rigorous process[312, 357]. While back-translation methods are useful in partially addressing this limitation, some authors have described the additional complexity of translations involving languages, such as Oriyan, which are not based on the Latin-script alphabet[358].

Resourcing and logistics

Limitations were also identified as they related to resourcing and logistics. The study was conducted on a limited budget, which meant that the qualitative interviews were administered by the sole field worker, sometimes in the presence of the Australian doctoral researcher but often not. A social desirability response bias might therefore have been anticipated, influencing participants to provide a favourable reply to the researcher conducting the interview since he was also the field worker[359, 360]. Ideally, these interviews would have been led by an independent staff member with no direct involvement in the study to improve objectivity of responses regarding the research team. Alternatively, responses could have been collected anonymously by electronic survey, thus encouraging participants to provide more critical feedback.

Food Frequency Questionnaire

The primary objective of the assessment of dietary intake was to enable calculation of the habitual RS intake of participants. When considered with the amount of RS supplemented in the diet via the study foods, this provided an overall intake of RS and addressed criticisms by some authors of dietary supplementation studies that do not quantify dietary intake[66]. However, several limitations of this method were identified based on the literature, as outlined below.

Although FFQs have been utilised with Indian populations, to be locally specific, FFQs need to reflect the prevailing food culture – even down to the local geographic area's food culture, rather than just the country level[287, 361-364]. At the time of implementation of the study, the published literature did not include any FFQ tools which had been validated for use in a population from East India or Odisha state specifically. An appropriate method of deriving an FFQ food list recommends adapting existing tools with input from local experts[365]. This led to an FFQ tool validated for use in the South Indian population[287] being adapted for this study with input from local experts, as described in the Methods chapter of this thesis. However, the lack of a validated FFQ for the Odisha region was a limitation of the study.

In deciding how to present the FFQ in relation to including composite foods or individual ingredients, the literature cautions that having too many or too few items can interfere with validity[366]. The FFQ used for this study therefore utilised a composite food-based approach in which prepared or 'composite' foods were included (e.g., whole gram curry), rather than individual ingredients alone (e.g. green gram lentils, ghee, turmeric, ginger, garlic, red chillies, onion). It was anticipated that this would assist participants to accurately recall their intake, even if they did not prepare the food. Despite this approach and attempts to limit the number of items on the recommendation of Sudha et al.[366], feedback from the field worker and some participants was that the list of items was too extensive, resulting in the FFQ taking a long time to administer. An associated limitation was the paucity of published values for the RS content of composite foods consumed by participants, requiring RS content to sometimes be derived from the individual ingredients of composite foods. Noting the high degree of variability in ingredients used in different preparations of the same composite food, this represents a limitation of this thesis.

Other potential limitations of the FFQ include: possible overestimation of RS intake because of the reliance on pooled RS data from the literature, as has been shown for other dietary components[367]; and the collection of dietary intake data using an FFQ tool is also subject to limitations related to accuracy of recall and to underreporting[287, 365]. The literature suggests that a valid biologic marker should therefore be used to determine the validity of the reported dietary intake data[368]. Accordingly, an assessment of the RS content of stool was included to verify RS intake determined by the FFQ. However, these results had their own limitations, as seen in the high degree of variability in **Figure 9**, which does not support the anticipated trend of increased faecal RS content following RS supplementation periods.

Recruitment pathways

There was a high level of interest in the study among the target population. However, the presence of co-morbid conditions affecting the GI tract precluded participation of some

individuals – as did recent antibiotic use, which was listed as an exclusion criterion because of the associated perturbation of the microbiome. Antibiotic use excluded three newly diagnosed HIV-positive individuals owing to the standard protocol in India to initiate antibiotics in HIV seroconverters to reduce the risk of pneumocystis pneumonia.

Recruitment pathways which utilised referral by ICTC staff were effective, although this did introduce a selection bias. Noteworthy were the limitations of this recruitment method, as discussed below. The feasibility analysis indicated that recruiting HIV-positive participants from their routine ART clinic, rather than relying on only the outpatient ICTC clinic of a tertiary hospital, should be pursued in future studies. Several individuals from the outpatient ICTC clinic who were interested in joining the study were not eligible because they had significant gastrointestinal co-morbidities, reflecting their higher level of clinical care they required from the tertiary hospital. In addition, the outpatient ICTC clinic was the first point of contact with HIV services for newly diagnosed people. This further restricted eligibility to join the study due to the routine commencement of antibiotics immediately following HIV diagnosis. Despite these barriers to recruitment, the outpatient ICTC clinic counsellors at AIIMS proved to be well respected among the population cohort and were able to motivate their patients to consider joining the study. Future studies would benefit from ensuring that referral pathways via both ART clinics and outpatient ICTC clinics are in place to maximise recruitment. This will be particularly useful when scaling up in other geographic contexts (*intervention replication*) and, ultimately, to a population level if interventions are shown to be effective in specific settings (*dissemination research*)[369].

The findings of the qualitative study highlighted how recruitment outcomes were impacted by reluctance of some members of the HIV-positive population to participate in the study over fears of disclosure of HIV status and the requirement for the field worker to visit participants in their homes. These findings were valuable as they related not only to HIV stigma and discrimination but also to the cultural context in which the study was being delivered, reflecting the status of women and traditional approaches to marriage. In terms of the implication of these findings on participant recruitment, future studies with this population should consider enabling participants to attend an associated health service for access to study foods and sample collection. As the current study was delivered during COVID-19 lockdowns, the requirement for home attendance by the field worker was essential and the doctoral researcher therefore had to persevere with this impact on recruitment.

Ethics

Although ethics approval was secured from the host institution of the doctoral researcher, as well as AIIMS, in time for the commencement of recruitment, the timeline for approval from

the ART clinic was delayed. As the ART clinic is not part of AIIMS, it had no formal arrangement to recognise the AIIMS ethics approval. When it became apparent that recruitment via the ART clinic would not be possible, the ICTC staff were engaged in the recruitment role at short notice. Longer lead times will need to be factored in to ensure that ethics processes are streamlined in the future. Fortunately, ICTC staff were able to accommodate recruitment activities for the current study into their clinic schedule. Future approaches should consider a more strategic rather than reactive approach to resource inputs for recruitment.

EED prevalence

Given the evidence described above that HIV and EED both contribute to gut inflammation, a baseline level of inflammation was assumed to be present in the study population. Although there is limited and contradictory evidence for the effect of RS on gut inflammation[49, 370, 371], some studies have reported findings which suggest that, in murine models, RS may be beneficial in reducing gut inflammation via the modulation of key species of the microbiota[370, 372, 373]. This led the doctoral researcher to consider the possible effect of RS supplementation on gut inflammation and faecal calprotectin was used to determine this. Faecal calprotectin is used as a clinical marker of gut inflammation in many disease models[374, 375]. It is considered a marker of neutrophilic intestinal inflammation owing to its secretion from activated neutrophils in the intestinal mucosa during periods of active inflammation[374-376]. Alternative methods for determining gut inflammation are more invasive and costly – for example, jejunal biopsy or dual sugar absorption tests such as the lactulose–mannitol test, which requires study participants to ingest doses of each carbohydrate solution and then undergo urine collection and calculation of absorption[26]. Although some authors have previously reported that elevated calprotectin is not indicative of EED[377], many studies employ it to determine gut inflammation relating to EED[378, 379].

Based on the literature, it was anticipated that the baseline measurement of calprotectin would be high in this population. This assumption was based on reported prevalence of EED in settings where water and sanitation infrastructure are suboptimal, including India[22, 26, 377], and on the elevated levels of calprotectin reported in HIV-positive populations[380, 381]. Although the literature indicates that the prevalence of EED has decreased in India in recent years[23], it also suggests that it is still the leading cause of malabsorption[202, 382]. This was a primary assumption of the study and partially underpinned the choice of population.

This study showed that, other than in Participant 4, faecal calprotectin levels in participants were largely in the normal reference range (<50ug/g) and often below 10µg/g. Although the small sample size limits generalisations about EED prevalence, it is noted that the one

participant with elevated calprotectin at baseline represents 20% of the study population. The consistently low levels of faecal calprotectin in remaining participants suggest that either this population had very low levels of inflammation, there was an error with the calprotectin assay kit, or the laboratory process was flawed. Ideally, a comparator cohort would have been included, comprising an HIV-negative population from the study site, to determine whether this population displayed normal faecal calprotectin levels. Future studies examining EED in this population should also consider other field-friendly measures to determine the presence of enteric inflammation[383, 384]. For example, the lactulose–mannitol test could be used. There is evidence that this method for determining intestinal permeability has been used effectively in HIV-positive populations in an LMIC setting (Brazil)[20]. Prior studies using this method in paediatric LMIC populations have indicated that this test reliably reflects EED and could be considered for future studies[385].

Safety and participant withdrawal

The consumption of RS and other low-digestible carbohydrates is generally considered to be associated with side effects of flatus and bloating[248]. Following the circumstances surrounding the withdrawal of Participant 2 (see Results chapter), it was concluded that a useful modification would be to ensure that any potential side effects be communicated to study participants in an objective way and be based on current evidence.

It would also have been beneficial to have had a withdrawal questionnaire in place to ensure that the field worker collected agreed data at the time of participant withdrawal. Unfortunately, as Participant 2 communicated her reasons for withdrawal to the field worker during a home visit, no further data were collected about the circumstances of the withdrawal.

Determining RS content of starches and study foods

The RS content of the comparator starch was assumed to be negligible since there was no evidence of it being selectively bred or having undergone processing to increase amylose content[386]. However, on testing, it was shown to contain significant amounts of RS, although still less than HAMS. As the RS content of the comparator starch was not determined until after dietary supplementation had started, the timing of the starch assay was a distinct limitation.

Although some types of RS2 may be susceptible to degradation when exposed to the high temperatures used in food preparation[177], HAMS was chosen for this study as it retains its granular configuration even when exposed to high temperatures and other processing techniques[279]. It was therefore assumed that the roti preparation method would not significantly affect RS content[279]. In hindsight, an appropriate strategy to verify this would have

been to assay the study foods for RS content, as well as the starches, as other studies have done[38].

RS laboratory assay method

The various methods which have been trialled for quantification of RS are intended to replicate the in vivo processing of RS in the human gut[387]. Results vary according to variables such as the preparation and pre-treatment of samples, the utilisation of enzymes, and the duration and type of incubation[29]. With respect to mimicking the in vivo steps, quantitation methods were historically validated against the ileostomy method[388, 389]. Following the publication of a direct RS quantitation method in 1986[390], various updated methods have also been validated in the ileostomy model[391-393]. An assay kit based on this methodology (AOAC 2002.02) is commonly used to determine RS in foods[394]. This assay accurately measures the sum of RS1, RS2 and RS3 but does not produce reliable estimates of RS4, owing to underestimation[387]. Methods for the specific measurement of RS5 in foods are yet to be elucidated[29].

To determine the RS content of the two intervention starches and faecal samples, a modified version of AOAC 2002.02 was used in this thesis. The K-RAPRS assay removes the requirement for the long incubation period of 16 hours in the original AOAC 2002.02 method, and replaces it with an incubation period of 4 hours with pancreatic α-amylase/amyloglucosidase[285]. This modification more closely mimics the human small intestinal transit time and reduces assay time[286], making this part of the analysis more efficient.

Although the K-RAPRS method reduced laboratory time, a limitation was that this assay is not typically used in other studies. This limits comparability. Furthermore, given that the COVID-19 disruption to supply chains meant relying on a comparator starch that contained RS, another limitation of the assay was the assumption that the Cornstarch contained less than 10% RS. This assumption determined the assay steps undertaken, according to the Megazyme method, leading to an undiluted aliquot of the Cornstarch being used. This was in contrast to the method for starches with more than 10% RS, involving dilution of the starch in distilled water and centrifugation as described on page 66.

Other laboratory results

As addressed in the Results chapter, the uncertainty about reliability of some of the laboratory results was a limitation of the current study, adding cost pressures and time delays. For example, the acetate results derived from the first round of SCFA assays were considered unreliable because, of the four SCFAs measured (acetate, valerate, propionate and butyrate), acetate values were lowest. This contradicts published data which indicate that acetate is the most abundant SCFA in the colon and should comprise at least 50% of total SCFAs present

in faecal samples[395, 396]. This brought the remainder of the SCFA results into question and it was decided that the remaining faecal samples would be brought to Australia to repeat these assays. Despite the significant funding required for this importation due to the need for a cold chain during transportation, and the requirement for quarantine approvals, funds were secured for this purpose. The results generated by the secondary analysis of faecal samples undertaken in Australia aligned with the expectations of the literature.

This highlights the difficulty of collaborations with in-kind providers who are outside the direct influence of the study team, as it reduces the ability to ensure that appropriate quality assurance measures are in place. As the assays were initially performed as an in-kind arrangement by an AIIMS laboratory that was not under direct control of the Indian PI, it was difficult to ask for the assays to be repeated. This limitation of the study had a significant impact on budget and timelines.

Importance of controlling for dietary intake of RS

The dietary intake analysis showed higher levels of overall RS consumption during the HAMS intervention periods compared to Baseline, Cornstarch and Normal diet periods in all participants. However, the analysis also showed that the total amount of RS consumed during Cornstarch supplementation was almost always less than the amount consumed at Baseline. This shows the limitations of not controlling for RS intake and highlights the significant amount of RS in the habitual diet of this population, which primarily consists of plant-derived foods such as pulses, grains, fruits and vegetables[330, 331]. The dietary analysis showed that RS consumption in the habitual diet of this cohort (27.6–41.5g/day) is much higher than in Western countries such as Australia (3.4–9.4g/day[397]) and the United States of America (2.8–7.9g/day[398]). This reflects the emphasis on plant-based diets in many LMICs and the dominance of animal-source fats and proteins in Western diets[173, 227]. This study is one of few conducted in LMICs where the habitual intake of RS is high. Most studies examining dietary supplementation with indigestible carbohydrates such as RS, have been conducted in high-income countries where the population typically consumes lower levels of RS. This is important when interpreting the findings of the current study as it has limited the ability to measure effects of raising RS intake. A preferred study design would be to control for dietary RS consumption during the study.

Sample collection, transport and storage

Despite the enormous interest in and analysis of gut microbial populations of faecal samples, there remain no standardised protocols for the collection and storage of stool for this purpose. The ideal time to storage in ultra-freezing conditions is provided as a guideline only and

several caveats surround transportation options which rely on the use of stabilising solutions[335]. Nor is there any reference microbiome that could be used to analyse deviance from the reference, allowing reflection on steps in the sampling, storage, transport and analysis workflow that might have hindered quality[399]. Indeed, microbiome composition has been shown to be impacted by variations in transport and storage workflows between different laboratories[400]. Owing to the remote management of the study during some of its operation, the doctoral researcher was not always present to facilitate quality assurance methods in terms of collection, transport and time to storage. Although standard operating procedures were developed and communicated to the Indian team as part of a training, a limitation of this thesis is that quality control was hampered by remote management. Improvement of the collection, transport and storage workflows is particularly important to ensure that faecal samples avoid the potential interference of post-collection bias in bacterial community composition[335]. Alternatively, consideration could be given to using storage tubes for stool which contain a stabilising agent. The OMNIgene.GUT kit is an example of a stool preservation solution found to result in the least alteration of microbial populations when compared to rapid freezing to –80ºC[335].

Noting the challenge of freezing stool samples immediately after collection in resource-limited settings and the impact on gut microbial measures, it is also important to acknowledge the impact on pH and SCFA[401]. Although approaches using stool preservation solutions such as OMNIgene will help manage the influence of artefactual microbial fermentation on SCFA levels (and therefore pH)[402], other alternatives could be considered for future studies, such as assay of plasma SCFAs[162, 403]. Barriers to using plasma rather than faecal samples includes diminished plasma responsiveness since the majority of SCFAs are utilised by colonocytes and hepatocytes after their initial production, resulting in a reduced and/or delayed effect on plasma levels[214]. Other concerns relate to blood sampling methods such as needle-aversion amongst participants and the need for trained phlebotomists in the study team.

Another limitation that arose during the study related to transport of blood samples. Some of the blood samples collected to determine CD4+ T cells and HIV viral load, lysed between collection and assay. Although this often occurs with blood samples, it was a limitation resulting in missing data in the current thesis.

Finally, a limitation was identified in terms of the disagreement in faecal sample collection methods for determining excreted RS and reporting periods for dietary intake of RS. Despite RS intake being described on a per day basis, the faecal RS concentrations presented in Figures 8 and 9 were based on one stool sample per day rather than a complete 24-hour collection. Noting that it is common for people to pass more than one stool per day, there is a lack of alignment in the reported dietary intake and excretion periods. A preferred approach

would have been to collect faecal samples over a 24-hour period which, despite introducing further burden to participants, would have enabled congruence and comparability in reporting periods. This lack of alignment may explain the observed variability in faecal RS concentrations.

Using proxy measures

SCFA concentration of faecal samples was used in this study as a proxy measure of SCFA production. However, without measuring concentrations in the large intestine lumen, faecal samples only provide an indication of SCFA production as they are influenced by the degree of uptake of SCFA by the gut mucosa[64].

Although stool is an accepted proxy measure for the colonic luminal environment, including the gut microbiota, the extent to which it precisely represents bacterial communities in the colon is also variable[404]. For example, a study comparing the composition of stool samples with intestinal biopsy samples observed that the bacterial signatures were different[405]. This was observed in both human and murine models. Gut microbiota adhere to mucosal surfaces in vivo and can therefore be either reduced in number in faecal samples or elevated in number on mucosal surfaces, distorting accurate representation of the luminal environment depending on which sample type is utilised[406].

Other methods that could be applied in future studies include rectal swabs[111] or intestinal biopsies, which have been used by other researchers, particularly in SIV models[98, 171]. This is an obvious advantage of using other mammalian models, such as non-human primates; however, significant differences between the gut microbiota have been noted in SIV and HIV models, presenting a further limitation[269].

Semi-structured interview tool

Noting the reflections from the QPE analysis in the Results chapter, the success of this tool was hampered by the discomfort of the field worker in qualitative interview techniques. Although the depth of detail in interviews did improve after the issue was raised with the Indian PI, there was still scope to improve the interviewing method to garner more granular insights from participants. Reflection on the semi-structured interview process also brings into question the feedback mechanism used to assist the field worker improve his interview technique. This mechanism involved a meeting between the doctoral researcher and field worker after each interview and relied on the field worker to provide an honest summary of each interview. In these feedback meetings, the field worker routinely reported that everything had gone well, leaving the doctoral researcher to wait until the translation was available to follow-up with the field worker in more detail. Another limitation of the semi-structured interview tool was that it

did not specifically ask about barriers and enablers to adherence to study foods or compliance with both survey and sample collection requirements. Dedicated questions would have provided insights into factors affecting the high degree of adherence and compliance reported in this study.

Data analysis

The creation of the summary variables 'Post-HAMS', 'Post-Cornstarch' and 'Post-Normal' was intended to enable the comparison of data collected at the end of intervention and washout periods, regardless of the order in which the interventions occurred owing to different randomisation schedules. However, these summary variables were problematic because of a large degree of variability within the data collected for each variable and a small number of data points. This is reflected in the standard error of the means shown in the associated figures (see Results chapter). This limits the generalisability of trends. As an alternative approach, data collected at each timepoint for individual participants were plotted as line graphs. The visual representation of the data from this study was important in terms of identifying trends, since the results of the ME model indicated no significant effects.

Blinding

Two starches were chosen for consumption by participants. Given the crossover design of the study and the importance of not biasing participant responses to survey and interview tools, the starch order was single-blinded so that participants were not aware of which starch they were consuming. This blinding of participants but not investigators has been used in other studies examining the tolerability of RS[180]. Participant blinding was particularly relevant for responses to the validated tool on gastrointestinal signs and symptoms and the semi-structured interview, to increase objectivity of responses. Ideally, the study would have been double-blinded to both investigators and participants but, with the constraints of the limited team and resources, this was difficult to achieve. Some of these constraints stemmed from the matching of the digestible starch fraction of the comparator starch (Cornstarch) to the digestible starch fraction of the HAMS, which meant that the actual quantity of starches was visibly different to the eye, rendering blinding more difficult. This could have been managed by ensuring that the food preparation team member was the only one to see the packets of starch added during the cooking. Logistically, however, this was difficult, especially given the COVID-19 context and the fact that the study foods were prepared by the wife of the Indian PI. To manage this, the importance of survey techniques which would not influence participant responses, was emphasised to the field worker, including avoiding leading questions which might bias results[407].

Public health management, policy and practice implications

This section considers the appropriateness of the feasibility and effect study designs used in this thesis and considers how public health and medical research outcomes can benefit from reflections of its delivery in practical terms. This chapter also considers how future studies examining dietary supplementation with RS could be expanded and scaled, with a particular focus on LMICs.

As is the case for other topics, feasibility and pilot studies of HIV and prebiotics have tended to focus on recruitment, retention and safety, as well as intervention adherence. The lack of evidence around what worked and what did not in relation to time and resource issues, as well as human and data management questions, is driving inefficiency in research, leading to each study team having to reinvent the wheel[71, 256]. The original contribution that this thesis provides is a granular examination of the cultural and local context and how this impacted methods and processes. This detail was primarily provided via the field notes of the doctoral researcher in the QPE analysis and addressed all categories of Thabane's typology, including those previously overlooked in the literature: Resources and Management.

This thesis sits within a broader variety of study designs undertaken to examine similar research questions. By using a feasibility study design within a dose-response trial, it raises the question of how to weigh the benefit of objective confirmation of effect/efficacy in clinical trials if it is not feasible to deliver the intervention. It occupies the nexus between public health and medical research by shedding light on the value of two studies in one. In doing so, it encourages consideration of where there is most value and utility in research: proving that an intervention is efficacious before determining if it is feasible or proving feasibility first before determining efficacy[252, 253]. From a bird's eye view, this thesis showed that RS supplementation was more feasible than effective in this population. However, the areas where the feasibility study suggested that changes were required (e.g., recruitment pathways), impacted on interpretation of the outcomes of the effect study. Consequently, the overall advantage of this study design was in conducting both studies in parallel, enabling the realist pragmatic approach to modify study processes during delivery.

The tension between studies examining feasibility versus those examining efficacy has led some authors to highlight the interdependency of evidence-based practice and practice-based evidence in the translation of health interventions[408]. This catch-22 suggests that the best way to approach this interdependency may be to conduct a pilot or feasibility trial as a starting point, with a smaller sample size than that required to determine efficacy[252]. Such approaches enable public health research to propose future distal translation opportunities (e.g., improved adherence to ART) if immediate and more proximal translation opportunities are supported

(e.g., reduced gastrointestinal symptoms). In a practical sense, this approach encourages evidence generation that addresses intermediate rather than final outcomes, and allows smaller sample sizes to be used, which, despite reduced statistical power, enable insights into treatment effects and may inform future sample size calculations[252].

Within the opportunities for future trials described by Rychetnik et al., consideration should be given to tailoring approaches used in the current study to local contexts[369]. Hawe et al. assert that, despite the common belief that standardisation between studies and cohorts is essential to the integrity of RCTs, complex interventions can benefit from the tailoring of intervention delivery whilst still achieving defensible methodological rigour[409]. While standardising the process and function of an RCT between sites, adaptation of the intervention to the local context is essential and appropriate[409]. Consider, for example, undertaking a similar study in sub-Saharan Africa, where the study foods used in this thesis – rotis – do not form part of the habitual diet. Tailoring the study to include culturally specific study foods in different geographic contexts could therefore optimise its delivery, despite it not being a pure replication of the current study. The important aspect is to ensure that study foods are equal in terms of the dosage of the interventional agent, RS. Future studies would then match the digestible starch fractions between intervention and control starches, as was attempted in this study. An improvement would be that the proportion of RS in each starch would be known beforehand and study foods themselves would be assayed for RS content, addressing the limitations of the current study.

Hawe et al. argue that adapting studies to suit different contexts does not equate to compromising study integrity[409]. In this regard, the theory of change or hypothesised mechanism of action overrides the specifics of the delivery of the interventional agent. Controlling for habitual dietary intake of RS is therefore essential when planning for the study to be replicated across diverse sites. Failure to do so may mean that the habitual RS intake obscures observable effects of RS supplementation, since RS intake will be more variable when different geographic and dietary contexts are at play.

To explore this concern about the pitfalls of not tailoring studies to local contexts, Hawe et al. asks the question:

> *Could one of the reasons for the interventions not working be that the components have been overly standardised?*[409, p.1561]

This is where the tension between fidelity and adaptation presents itself[410], challenging the traditional approach of RCTs which have tended to adhere to a more historical definition of rigour[411]. It is adaptations such as modifying study foods to cultural and geographic contexts that will allow more flexible approaches to standardisation, which need to be considered if

future studies are conducted at different study sites as part of intervention replication or dissemination research[369].

This tailoring of a future RCT to different study sites would also allow the cultural and gender barriers to recruitment observed in this study, to be addressed. For example, as described in the Results chapter, visits by the field worker to participants' homes to deliver study foods, collect biologic samples, and administer surveys, proved to be a barrier in the current study. The impact of cultured gender norms had been overlooked when this aspect of the study was designed. Future studies should consider if study foods can be provided in advance to allow collection by study participants rather than delivery. The impact on adherence of providing multiple days of study foods to participants would need to be considered, especially where other members of the family would have access to the study foods. Another approach to reduce the burden of home visits on study participants includes the administration of surveys via mobile phones, with a link provided to participants by SMS via apps such as SurveyMonkey, Qualtrics or RedCap. This would, however, require participants to be literate, which, in the context of LMIC settings, may be problematic. Applying a flexible approach to study logistics at each study site according to local cultural sensitivities, would achieve the advantage described by Hawe et al., where adaptability results in more successful RCT delivery[409]. This also connects to the increasing focus on the localisation of health interventions being pursued by United Nations' agencies and other development partners working in global health[412].

This thesis provides evidence regarding the acceptability of RS and adherence to RS supplementation at high doses in a key global health priority population. Given that the feasibility assessment indicates that high-dose RS supplementation studies are feasible in HIV-positive populations, appropriately adapted intervention replication[369] trials could be pursued in order to reach conclusions regarding RS effect in HIV-populations in different geographic contexts.

Knowledge transfer

Noting the importance of ensuring that clinical trial reporting be used to inform future research by aligning with minimal standards, it is essential to consider the role of knowledge translation and management in this context[73, 255, 258, 259]. It is also apparent that historical approaches to the dissemination of public health evidence and interventions have been criticised for their lack of relevance and for barriers to implementation among both practitioners and members of affected/target populations[413].

With regard to advocating for adjunct therapies to enhance TasP approaches by lessening the side effects of ART, political and clinical support will be required to disrupt the business-as-

usual model. Clinical practitioners providing services within the HIV care cascade[414] could logically be expected to adopt innovative approaches based on results of muti-site efficacy trials. Models of delivery will need to delineate how the supply and administration of adjunct therapies such as prebiotics would be delivered to the target group in order to convince HIV clinicians to adopt and support this approach. If RS supplementation is deemed to be efficacious and effective in improving gut health and HIV immunity in future trials, national and jurisdictional policymakers will first need to be convinced to endorse this new clinical adjunct therapy within the existing cascade of care for HIV. To influence governmental policymakers, key stakeholder organisations such as UNAIDS and WHO should be engaged in the conversation early, with a view to identifying change champions who might ultimately support the adoption of prebiotic adjunct therapies at the global policy level. Other stakeholders who might also be engaged are those that influence global policy, including HIV research centres. Considering the amenability of RS to large-scale food fortification approaches, the confirmed health benefits of RS in many populations should be verified in future research and emphasised in communication with key stakeholders. Key nutrition organisations such as the Global Alliance for Improved Nutrition (GAIN), which leads food fortification approaches, could also be engaged in preliminary discussions. Starch manufacturers should continue to be involved in RS research and kept abreast of the outcomes of future studies. They may ultimately be engaged in refining starch product formulations to best meet required specifications in terms of RS/digestible starch/total starch fractions, as well as other attributes such as shelf-life and granule size, to assist industrial fortification approaches.

The foundation of the global HIV response has been led by affected populations, including HIV-positive populations and members of at-risk populations. Without endorsement and adoption by these populations, any intervention will fail. To ensure support, it is essential to utilise knowledge dissemination opportunities via HIV-specific forums. All efforts should therefore be made for this research to be presented at regional and global HIV conferences. The systematic review of the efficacy and effect literature undertaken for this thesis was presented at the 2020 Joint Australasian HIV&AIDS and Sexual Health Conferences. An abstract summarising the key findings of the thesis was then submitted for the 2023 International AIDS Society (IAS) conference. Although this abstract was not accepted, another opportunity to disseminate the findings will be pursued at the 2024 conference organised by the Australasian Society for HIV Medicine (ASHM). As ASHM is the peak body for the HIV response in Australia, the dissemination of findings via ASHM forums and networks will provide an opportunity to develop community support and political backing. Abstracts will also be submitted to the 2024 IAS conference and to the Conference on Retroviruses and Opportunistic Infections (CROI). These two international conferences are the most important

on the global HIV research agenda, attracting the most delegates from the clinical, policy and research response, as well as affected communities. Submitting abstracts will, at least, ensure that the selection panel is kept up to date with this research area and, at best, make this research accessible to a wider population if it is accepted for presentation as a poster or oral paper.

With regard to publication of the study in peer-reviewed journals, close attention will be paid to the recommendations for reporting of randomised pilot and feasibility studies according to the CONSORT extension statement[73] as well as the CONSERVE 2021 statement[354], acknowledging the COVID-19 context in which this study was delivered. It will be advantageous to aim for publication in HIV/AIDS-specific journals since this is where the clinical and policy actors of the HIV response are most accessible. Submission of manuscripts reflecting subsets of the study findings will also be pursued. For example, a manuscript examining the use of calprotectin to determine gut inflammation arising from EED in this population could be submitted to a gastroenterology or global health journal; a manuscript reporting results of the FFQ dietary analysis could be published in a nutrition and dietetic journal; a manuscript examining the baseline and post-intervention response in gut microbial populations could be published in a microbiology journal; and a manuscript examining the attributes and effect of the RS intervention used in this study could be published in the *International Journal of Probiotics & Prebiotics*.

Conclusions

This thesis contributes valuable feasibility data regarding the high-dose RS intervention and importantly, verifies that RS is a safe, tolerable and acceptable dietary intervention in this population. This finding provides a basis for intervention studies with HIV-positive populations in other settings. The comprehensive assessment of feasibility undertaken for this study addressed not only Process and Scientific aspects but also Resources and Management categories of the Thabane typology[71], addressing this gap identified during the literature review. This thesis highlights findings that can be applied in future dietary supplementation studies with HIV-positive populations in LMIC, specifically.

This thesis does not provide evidence of any statistically significant effect of RS supplementation on primary outcome measures observed in faecal samples (pH, acetate, butyrate, propionate, valerate, microbial abundance and diversity). Furthermore, it does not provide evidence of any statistically significant effect of RS supplementation on secondary outcome measures observed in blood samples (CD4+ T cells and HIV viral load). It does, however, show trends which support the hypothesis that RS supplementation would be associated with an increase in faecal SCFA concentrations and a reduction in faecal pH,

indicative of RS fermentation. The other promising result of the effect study was the change in CD4+ T cells. Although not statistically significant, this trend did show that the mean CD4+ T cell concentration of blood samples increased following RS supplementation in a majority of participants, suggesting a beneficial effect of RS on HIV-related immunity that warrants further investigation. Overall, the findings of the study accord with conclusions reached in a recent systematic review and meta-analysis, which reported that evidence for the efficacy of prebiotics and synbiotics in HIV-positive populations is currently variable[159].

This thesis has shown that there is a pressing demand for a therapeutic approach to manage GI consequences such as altered gut epithelial barrier function and dysbiosis arising from HIV infection, particularly in the context of ART use and EED[19, 159]. The challenge is to identify an adjunct therapy that does not increase the existing polypharmacy present in this population, whilst still resulting in measurable improvements in biomarkers of intestinal health, reduced GI symptoms and, ultimately, better ART adherence. Dietary prebiotics and other fermentable starches, such as RS, may play an important role in the future therapeutic landscape of HIV. These functional food components may be particularly advantageous in LMICs, where the burden of EED on gut health, nutritional status and immune outcomes is high.

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APPENDIX 1:
Joanna Briggs Institute Meta-Analysis of Statistics Assessment and Review Instrument (JBI-MAStARI) – Methodological quality appraisal instrument

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APPENDIX 2:
Ethics approval – Flinders University

Office for Research

Flinders Medical Centre
Ward 6C, Room 6A219
Flinders Drive, Bedford Park SA 5042
Tel: (08) 8204 6453
E: Health.SALHNOfficeforResearch@sa.gov.au

Government of South Australia
SA Health
Southern Adelaide Local Health Network

Final Approval for Ethics Application

29 March 2019

Professor Paul Ward
College of Medicine and Public Health
Flinders Medical Centre

Dear Professor Ward,

OFR Number: 345.18
HREC reference number: HREC/19/SAC/39
Project title: What is the effect of a dietary resistant starch intervention on the colonic luminal environment and HIV-related immunity and is a feeding trial feasible in HIV-positive adults in India?
Chief Investigator: Professor Paul Ward

Ethics Approval Period: 29 March 2019 – 29 March 2022

The Southern Adelaide Clinical Human Research Ethics Committee (SAC HREC) (EC00188) have reviewed and provided approval for this application which meets the requirements of the *National Statement on Ethical Conduct in Human Research (2007, updated 2018).*

You are reminded that this letter constitutes **Ethics** approval only. **Ethics approval is one aspect of the research governance process**.

You must not commence this research project at any SA Health sites listed in the application until a Site Specific Assessment (SSA), or Access Request for data or tissue form, has been approved by the Chief Executive or delegate of each site.

The below documents have been reviewed and approved:

Document	Version	Date
Human research ethics application	AU/1/4A4A312	15 March 2019
Protocol	4	15 March 2019
Participant information sheet and consent form	3	13 March 2019
Food frequency questionnaire (FFQ)	1	8 February 2019
Symptom tool	2	8 February 2019
Interview guide	1	29 January 2019
Literature review	1	29 January 2019

Terms and Conditions of Ethics Approval:

SALHN has recently introduced site monitoring of authorised studies. This approval/authorisation is subject to participation in this monitoring process. You will be notified in advance if your site has been selected for an inspection
It is essential that researchers adhere to the conditions below and with the *National Statement chapter 5.5.*

Final ethics approval is granted subject to the researcher agreeing to meet the following terms and conditions:

1. This ethical approval is subject to approval from Institutional Ethics Committee of the All India Institute of Medical Sciences.
2. If University personnel are involved in this project, the Principal Investigator should notify the University before commencing their research to ensure compliance with University requirements including any insurance and indemnification requirements.
3. Compliance with the *National Statement on Ethical Conduct in Human Research (2007, updated 2018)* & the *Australian Code for the Responsible Conduct of Research (2018).*
4. To immediately report to SAC HREC anything that may change the ethics or scientific integrity of the project.
5. Report Significant Adverse events (SAE's) as per SAE requirements available at our website.
6. Submit an annual report on each anniversary of the date of final approval and in the correct template from the SAC HREC website.
7. Confidentiality of research participants MUST be maintained at all times.
8. A copy of the signed consent form must be given to the participant unless the project is an audit.
9. Any reports or publications derived from the research should be submitted to the Committee at the completion of the project.
10. All requests for access to medical records at any SALHN site must be accompanied by this approval email.
11. To regularly review the SAC HREC website and comply with all submission requirements, as they change from time to time.
12. Once your research project has concluded, any new product/procedure/intervention cannot be conducted in the SALHN as standard practice without the approval of the SALHN New Medical Products and Standardisation Committee or the SALHN New Health Technology and Clinical Practice Innovation Committee (as applicable). Please refer to the relevant committee link on the SALHN intranet for further information.

For any queries about this matter, please contact The Office for Research on (08) 8204 6453 or via email to Health.SALHNOfficeforResearch@sa.gov.au

Yours sincerely,

Professor Bill Heddle
Chair
Southern Adelaide Clinical Human Research Ethics Committee

APPENDIX 3:
Ethics approval – AIIMS, Bhubaneswar

INSTITUTIONAL ETHICS COMMITTEE
Registration No: ECR/534/Inst/OD/2014/RR-17)

All India Institute of Medical Sciences Bhubaneswar
Level 3 Academic Block, AIIMS Bhubaneswar (At Sijua)
Bhubaneswar 751019, Odisha
Email: iec.aiimsbbsr@gmail.com **Phone:** 0674-2476083

Date: September 16, 2019

Dr Balamurugan Ramadass
Assistant Professor,
Department of Biochemistry,
AIIMS, Bhubaneswar

Ref Number: T/EMF/Biochem/19/04

Subject: What is the effect of a dietary resistant starch intervention on the colonic luminal environment and HIV-related immunity and is a feeding trial feasible in HIV-positive adults in India?

Dear Dr Balamurugan Ramadass,

This is regarding your above-mentioned project proposal which was discussed in the Institutional Ethics Committee, AIIMS Bhubaneswar meeting held on July 13, 2019 (Saturday) and your subsequent letter dated September 14, 2019 responding to queries raised during IEC meeting.

The study is approved from ethical angle prospectively with effect from **September 16, 2019** till the entire period of the conduct of study according to the study duration mentioned in the protocol under direct responsibility of Dr Balamurugan Ramadass, Principal Investigator.

As a Principal Investigator, you are responsible for following requirements as applicable for the present protocol.

1. All co-investigators must be kept informed of the status of the project.
2. Obtain HMSC approval before initiation of the study and inform IEC after the approval.
3. No significant change to the protocol should be made and implemented without prior intimation and approval of the IEC
4. IEC should be reported about all Serious Adverse Events (SAEs) occurring during the study.
5. Only approved informed consent form and participant information sheet to be used for enrolment of the participants. All consent forms and other documents must be archived safely with PI for IEC audit
6. A six-monthly study progress report of the project must be submitted to IEC
7. It is hereby confirmed that neither you nor any of the study team members have participated in the voting/ decision making process of Institute Ethics Committee of AIIMS Bhubaneswar related to this study.

Member who attended the meeting and voted in favour:

1. Dr Suresh Chandra Dash *(Chairperson)* 2. Dr Manaswini Mangaraj 3. Mr. Santanu Sarangi 4. Dr Sandip Mishra 5. Dr Rituparna Maiti 6. Dr S. Manwar Ali 7. Dr Bikram Kishore Behera 8. Dr Amit Ghosh.

Chairperson
(IEC AIIMS Bhubaneswar)

APPENDIX 4:
Summary report –Sensory evaluation/palatability testing of study foods

Background

The quantity of the primary intervention starch (HAMS) to be consumed by participants every day during the 14-day supplementation periods was 95 grams. This represented a large quantity to add to a food or drink without significantly altering the organoleptic properties and making it unpalatable. Sweetened rice pudding was proposed by the Indian team as a possible vehicle for the HAMS supplement however, this option was considered problematic because high-fat foods are known to have a pro-inflammatory effect through lipid metabolism[1,2] and change the proportions of commensal bacteria residing in the gut[3,4]. This was considered likely to interfere with interpretation of microbiota results because many of the microbe-mediated processes that could provide benefit through HAMS supplementation, would compete with the pro-inflammatory effects from the high fat content. There was also concern about using a study food that was high in refined sugar as this would favour microorganisms with a preference for this energy source and make trends associated with RS supplementation difficult to discern[5,6]. Rotis were therefore deemed preferable as the study food, providing additional advantages because they did not require refrigeration and were easy to package and transport by the field worker who visited participant's homes on a motorised scooter. This choice of study food also easily allowed the Ruchi Cornstarch (substitute control starch), to be supplemented in rotis which appeared and tasted similar to the HAMS rotis, assisting with single blinding of participants. Although it was later determined that the Ruchi Cornstarch contained 15% RS and was therefore not a true control starch, rotis still provided the benefit of concealing any differences between roti A (Cornstarch) and roti B (HAMS) in the re-conceptualised dose-response study.

In February 2020, a sensory evaluation/palatability testing sub-study was conducted using three variations of HAMS-supplemented rotis, which were being considered for use in the main study.

Method

Four participants, comprising staff from the AIIMS Department of Biochemistry, consumed the following three study foods:

- Option 1: White flour rotis – 2-piece serve;
- Option 2: Wholemeal flour rotis – 2-piece serve; and
- Option 3: Wholemeal flour rotis – 3-piece serve

A 7-point hedonic Likert scale (1=like a lot, 2=like moderately, 3=like a little,4=neither like nor dislike, 5=dislike a little, 6=dislike moderately, 7=dislike a lot) was used to determine ranking of lowest to highest scores for the following sensory characteristics, where lower scores signified greater likeability:

1. *Appearance;*
2. *Smell;*
3. *Taste;*
4. *Aftertaste;*
5. *Consistency; and*
6. *Overall impression.*

This scale and approach has been used previously in similar palatability testing studies[7,8].

Testing was completed within a one-hour period. Likert scale scores for each study food were recorded by participants on a data entry form which also provided space for participants to record any qualitative remarks for each study food option. Participants were blinded to the order of the study foods they were testing. Water was encouraged to be consumed between the testing of the three study foods.

Data entry forms with Likert scale scores from all four participants were collected at the end of the study. Median likeability values for each sensory characteristic were then derived, tabulated (Table 1A) and represented visually (Figures 1A-3A).

Results

As shown in Table 1A and Figures 1A-3A, the majority of sensory characteristics had median rating scores ≤4. The exception was the *Overall impression* characteristic for Option 3 (Wholemeal flour rotis – 3-piece serve) which had a median rating score of 5. Option 2 (Wholemeal flour rotis – 2-piece serve) was ranked most favourably on *Appearance* (median score 2), *Taste* (median score 1), *Aftertaste* (median score 2), *Consistency* (median score 2) and *Overall impression* (median score 1). There was no difference in the median score of 2 for *Smell* between the three study food options.

Table 1A: Median score for Likert scale scores recorded by participants (n=4)

	Likert scale median score (n=4)		
Sensory characteristics	**Option 1**	**Option 2**	**Option 3**
Appearance	3	2	3
Smell	2	2	2
Taste	2	1	3
Aftertaste	3	2	3
Consistency	3	2	4
Overall impression	3	1	5
Total	**16**	**10**	**20**

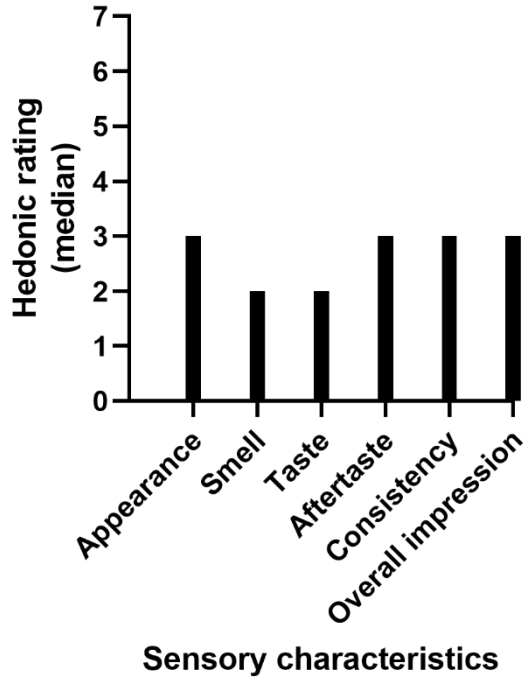

Figure 1A: Median hedonic ratings (n=4) – **Option 1: White flour rotis – 2-piece serve**.

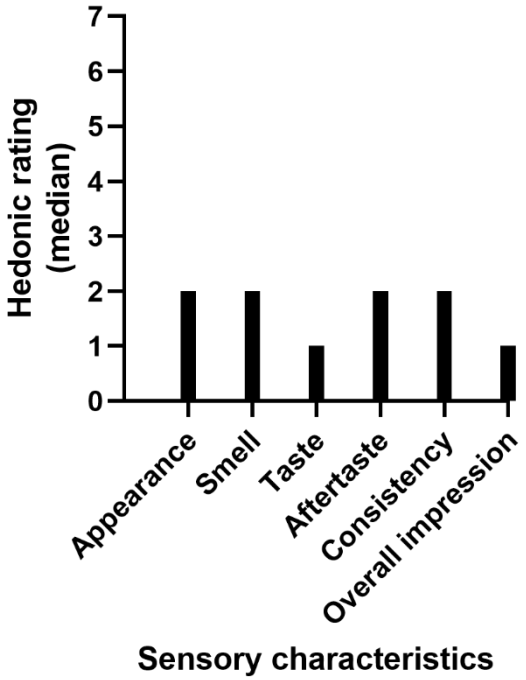

Figure 2A: Median hedonic ratings (n=4) – **Option 2: Wholemeal flour rotis – 2-piece serve**.

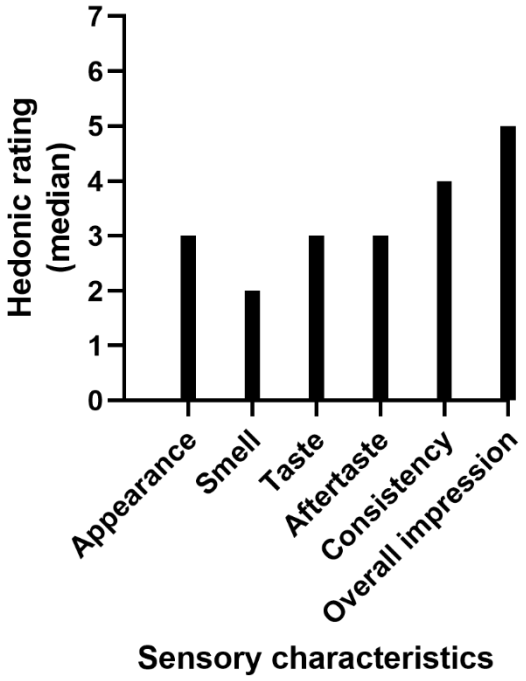

Figure 3A: Median hedonic ratings (n=4) – **Option 3: Wholemeal flour rotis – 3-piece serve**.

The total median hedonic scores from Table 1A (above) are illustrated with their associated ranking of likeability results in Table 2A (below), where the lowest hedonic score indicates the greatest likeability:

Table 2A: Total hedonic score and likeability ranking for three study food options.

Total hedonic score	Study food option	Likeability ranking
10	Option 2: Wholemeal flour rotis – 2-piece serve	1
16	Option 1: White flour rotis – 2 piece serve	2
20	Option 3: Wholemeal flour rotis – 3 piece serve	3

Qualitative data was provided on a few occasions only, with some participants indicating that the size of the portion of Option 3 was problematic, with comments stating: '*serve size was overly large*' and '*could not consume the entire serve in one sitting'*.

Conclusion

Option 2 was therefore chosen as the study food for the main study.

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APPENDIX 5:
Intake Questionnaire Form

Patient Name	
DOB	
Gender	
Study Code	
Date of enrolment and consent	
Height (cm)	
Weight (kg)	
Phone number	
Address	
Education	
Occupation	
SES	

SES Score	
Year of HIV diagnosis	
Year of commencement of ART	
ART regimen drug(s)	
CD4	
Viral load	
Antibiotic use in last 6 weeks?	
Any other gastrointestinal disease present (non-HIV related)?	
Participating in other interventional studies at present?	
Pregnant or breastfeeding?	

APPENDIX 6:
Instructions for stool collection

Instructions for stool collection

Using western toilet

- Wear the gloves provided to you
- Open up the bag fully.
- If there is a seat on the toilet, lift up the seat then place the bottom of the bag in the toilet with the top edges of the bag wrapped around the edges of the toilet bowl. Put down the seat of the toilet to keep the bag in place.
- Sit on the toilet seat and pass your stool into the bag.
- Using the ice-cream stick provided, scoop enough stool to fill the container provided to at least half-way.
- Secure the lid on the container.
- Dispose of the ice-cream stick and the bag.
- Remove the gloves and dispose of them.
- Wash your hands with soap.
- Contact the Field worker to collect the stool.

Outside or squat toilet

- Wear the gloves provided to you
- Open up the bag fully. Place the bag on the ground or on the bottom of the toilet pan if you are using a squat toilet.
- Pass your stool into the bag.
- Using the ice-cream stick provided, scoop enough stool to fill the container provided to at least half-way.
- Secure the lid on the container.
- Dispose of the ice-cream stick and the bag.
- Remove the gloves and dispose of them.
- Wash your hands with soap.
- Contact the Field worker to collect the stool.

APPENDIX 7:
Original Participant Information and Consent Form – single crossover study

Flinders University of South Australia and All India Institute of Medical Sciences (AIIMS) – Bhubaneswar.

Participant Information Sheet/Consent Form

Interventional Study – *Adult providing own consent*

Bhubaneswar, India

Title	What is the effect of a dietary resistant starch intervention on the colonic luminal environment and HIV-related immunity and is a feeding trial feasible in HIV-positive adults in India?
Short Title	Resistant starch in HIV-positive adults in India.
Project Sponsor	Flinders University
Coordinating Principal Investigator/ Principal Investigator	Professor Paul Ward – Chief Investigator Assistant Professor Balamurugan Ramadass – Principal Investigator-India (PI-India) Ms Elissa Mortimer – Study Coordinator and Principal Investigator.
Associate Investigator(s)	Professor Geraint Rogers Professor Graeme Young Professor BS Ramakrishna
Location where recruitment will occur	Bhubaneswar, India

Part 1 What does my participation involve?

1 Introduction

You are invited to take part in this research project. The research project is testing a treatment for symptoms of the gut which may be useful for people living with HIV. A food additive made from the maize plant called High Amylose Maize Starch or 'HAMS,' will be added to the normal diet because it is high in a nutrient called 'resistant starch.' This study is being done in India because in countries where the water and sewerage system is not that good, people who live there come into contact every day with bacteria that can lead to repeated infections of the gut. Research has shown that resistant starch can be of benefit when this happens. This Participant Information Sheet/Consent Form (PICF) tells you about the research project. It explains the tests and treatments involved. Knowing what is involved will help you decide if you want to take part in the research. Please read this information carefully. Ask questions about anything that you don't understand or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend or your local doctor.

Participation in this research is voluntary. If you don't wish to take part, you don't have to. You will receive the best possible care whether or not you take part.

If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:

- Understand what you have read
- Consent to take part in the research project
- Consent to have the tests and treatments that are described
- Consent to the use of your personal and health information as described.

You will be given a copy of this PICF to keep.

2 What is the purpose of this research?

Aim (1) To see what happens when HAMS is added to the diet by measuring changes in:

a. your stool;
b. your blood;
c. symptoms in your gut.

Aim (2) To explore the things that might make a study with HAMS easy or difficult for participants.

Significance: HIV disease and Anti-Retroviral Therapy (ART) for HIV can both cause gut problems in the stomach and intestines. New ways of managing these problems will be helpful to make patients feel better and help them to stay on their ART. This will decrease the chance of HIV infection being passed on to other people.

Dietary starch comes from plants like cereals and potatoes and provides a source of energy for the body. It is an important part of the human diet. Instead of being digested in the stomach and small intestine, resistant starch reaches the large intestine and is used by the bacteria that live there in a process called fermentation. This is thought to be good for the body. So far, studies with resistant starch have happened with children and adults in countries including India, Australia, England and the United States of America. Some studies have tested resistant starch mixed with other special food ingredients in HIV-positive participants but no studies have tested what happens when resistant starch by itself is added to the diet in HIV-positive people.

Resistant starch is considered very safe for consumption by humans and is found in the normal diet in foods like potatoes and bananas. Resistant starch as an ingredient has been used by bakers for many years because it makes bakery foods crusty. One example of resistant starch is HAMS which will be used in this study. HAMS has been given GRAS status (Generally

Recognized As Safe) by the United States of America Food and Drug Administration. This means it is allowed to be added to foods being eaten by humans. It is of natural origin from maize plants which have not been genetically modified.

The results of this study will be used by the study coordinator, Ms Elissa Mortimer, to obtain a Doctorate of Public Health degree. Ms Mortimer developed the idea for this study which is funded by Flinders University of South Australia with All India Institute of Medical Sciences (AIIMS) Bhubaneswar as the research partner.

3 What does participation in this research involve?

You will be participating in a 'cross-over' dietary study. In a cross-over study, different groups each have different treatments in turn. The treatments in this study are two different types of starch: HAMS and cornstarch. You will be asked to eat foods which have HAMS or cornstarch added to them. Cornstarch is digested by your body in the stomach and small intestines in the usual way that food is digested. Instead of being digested in the stomach and small intestine, HAMS reaches the large intestine where it is used as a food by bacteria that normally live there. These bacteria can do beneficial things in the human body which is why we are interested in studying the effect of HAMS on them. HAMS and cornstarch do not change the taste of food so you may not notice any difference compared to normal food.

The order in which participants consume the two starches will be chosen by chance (random). This is to try to make sure the groups are the same. There will be a break between the HAMS and cornstarch foods so that your body adjusts back to normal before you start the other starch. You will not know if you are consuming resistant starch or cornstarch. This research project has been designed in this way to make sure that participants give the most accurate responses to the questionnaires and that the researchers interpret the results in a fair way.

Initial steps

The research staff will explain the study to you using this PICF document. If you decide to participate, the research staff will ask you to sign the consent form. They will then ask you some questions in the screening step. If you meet the below and exclusion criteria, you will be able to participate in the study:

Inclusion criteria

1. HIV-positive adults aged 18 years or over on ART
2. CD4+ T cell count more than 200 cells/mm3
3. No antibiotics within last 6 weeks

Exclusion criteria

1. Other gut problems such as Crohn's Disease or Ulcerative Colitis.
2. Current participation in other research studies
3. Pregnant or breastfeeding.

This is a study with two parts: the first is the dietary part, the second is the interview or focus group part where the research team will ask participants the things that make this study easy or difficult. These two parts of the study will happen at the same time, during the 8 weeks that participants will be involved in the study.

Participants will be asked to give a stool and blood sample at day 0 and to complete a questionnaire about any gut symptoms so the researchers can see how these measurements change once the starches are added to the normal diet. Participants will be randomly put into one of two groups. The first group will eat HAMS added to their usual diet for 2 weeks then only their usual diet for 2 weeks then cornstarch added to their diet for the final 2 weeks. The second group will do this in the reverse order by eating the cornstarch for 2 weeks then the normal diet for 2 weeks then the HAMS for 2 weeks. This is shown in the below **Figure 1**.

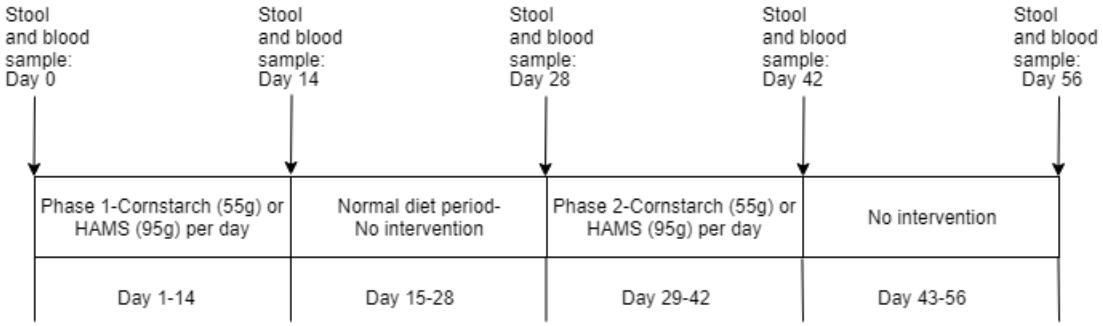


Figure 1: Study timing.

Participants will be provided daily with the study food containing HAMS or cornstarch. HAMS and cornstarch are a similar texture to wheat flour and do not have a strong taste. The study coordinator will test different study foods with a small group of 5 participants first to work out which one tastes best and can be eaten for the study period. This testing will be done with the first 5 participants who are enrolled.

Participants will be asked to provide stool and blood samples on the days shown in Figure 1. There will be a total of 5 days when these samples will be collected. The research team will teach participants how to collect the stool samples and will then collect these samples from participants. Samples will then be frozen and transported to the laboratory for storage. The blood samples will be taken by a health worker employed by the ART clinic. Samples will be transported by the research team to the laboratory in vials labelled with the participant identifier code rather than their name.

There are no additional costs associated with participating in this research project, nor will you be paid. All of the study food and the cost of stool and blood tests required as part of the research project will be provided to you free of charge. The usual way you access drugs for your HIV such as ART will not be affected.

You may be reimbursed for any reasonable travel costs associated with the research study.

4 What do I have to do?

- You will be asked to eat the whole serve of the study food. The study food will be either flatbread or sweetened rice pudding with HAMS or cornstarch added with the other ingredients. The total amount of time during which you will consume study foods is 4 weeks in total. The total amount of time you will participate in the study is 8 weeks:
    - **Phase 1**: 2 weeks eating study foods containing HAMS or cornstarch
    - **Normal diet:** 2 weeks eating your normal diet
    - **Phase 2**: 2 weeks eating study foods containing the starch you did not eat in Phase 1.
    - You will then return to your normal diet. After another 2 weeks, your stool and blood will be collected for the last time.
- In total, 5 stool samples and 5 blood samples will be collected over the total 8 weeks.
- On collection days you will also be asked to complete the questionnaire which will ask about any side effects you may be experiencing. This will take 5-10 minutes to complete. Study staff can help you.
- On two days (days 14 and 42), you will also be asked to complete a brief questionnaire about what you have been eating and drinking recently. This should take 10-15 minutes to complete. Study staff can help you.
- You will also be asked to participate in two interviews for the qualitative part of the study. You can choose if you prefer to have an interview by yourself or join 5 other

participants for a focus group discussion. Both of these will be with a research staff member plus interpreter. The two interviews/focus groups will be held on:
    - Day 0 immediately before phase 1 begins;
    - Day 42 at the end of phase 2.

  The interviews and focus groups will be recorded (audio only) so that the discussion can be written down afterwards. The only people who will access these audio recordings are the interpreter and two members of the research team. The recordings will be kept in a locked filing cabinet when not in use.

  After the interviews and focus groups are completed, participants will be invited to check the summary report written by the research team. The reports will be written in a way that keeps the identity of participants anonymous.
- You will continue to take your ART and any other medications that you normally take. If however, you are required to take antibiotics, you may be required to leave the study as this will affect the results in an unwanted way.
- There are no other lifestyle or dietary changes you need to make.
- Participants will be checked on by research staff on the sample collection days which happen every 2 weeks and with phone calls between the sample collection days. This will provide a chance for you to report any side effects to the research team. Participants will also be provided with the phone number of a research team member who can be contacted if there are any issues in between phone call and sample days. The research team member will report any issues to the study coordinator. They will then decide on the best way to solve the problem. This may require consultation with your HIV doctor.

5 Other relevant information about the research project

This study will be conducted in Bhubaneswar only. It will be conducted by researchers from the All India Institute of Medical Sciences-Bhubaneswar and Flinders University of South Australia. Approximately 30 adults from Bhubaneswar will participate in the study.

6 Do I have to take part in this research project?

Participation in any research project is voluntary. If you do not wish to take part, you do not have to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage. If you do decide to take part, you will be given this PICF to sign and you will be given a copy to keep. Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with the Bhubaneswar ART clinic.

7 What are the alternatives to participation?

You do not have to take part in this research project to receive treatment at the ART clinic. Other options are available; these include continuing your usual medication and care regimen for HIV and any other conditions you may have. A member of the research team will discuss these options with you before you decide whether or not to take part in this research project. You can also discuss the options with your local doctor.

8 What are the possible benefits of taking part?

We cannot guarantee or promise that you will receive any benefits from this research; however, possible benefits may include:

- A benefit to your body especially your intestines and the bacteria that live there. This will likely only last as long as you are eating the HAMS;
- A benefit to your HIV disease;
- Being provided with the study foods for 28 days;
- Contributing to the research about gut health, HIV and resistant starch.

There may be no clear benefit felt or noticed by you from your participation in this research.

9 What are the possible risks and disadvantages of taking part?

Medical treatments often cause side effects. You may have none, some or all of the effects listed below, and they may be mild, moderate or severe. If you have any of these side effects, or are worried about them, talk with a member of the research team. This team will also be looking out for side effects. There may be side effects that the researchers do not expect or do not know about yet. Tell a research team member or your HIV doctor immediately about any new or unusual symptoms that you get.

Many side effects go away shortly after treatment ends. However, sometimes side effects can be serious, long lasting or permanent. This is very unlikely to occur after eating HAMS. If a severe side effect or reaction occurs, the research team may need to stop your participation in the study. The research team and your HIV doctor will discuss the best way of managing any side effects with you.

Possible side effects:

- flatulence, bloating, gas, increased frequency of passing stool, increased size and weight of stool.

If you experience these side effects they will almost certainly go away once you stop consuming the HAMS. If they cause you significant discomfort, you are free to stop your participation in the study.

The effects of high doses of HAMS on the unborn child and on the newborn baby are not known. Because HAMS has been certified with Generally Recognized as Safe (GRAS) status from the United States Government, it is very unlikely that it could harm the growing baby or mother. However, since eating large amounts of HAMS can change what happens in the gut normally, pregnant women should not participate in this study. Women who may be pregnant will be asked to do a simple pregnancy test with a small sample of their urine before they start the study.

Having a blood sample taken may cause some discomfort, bruising, minor infection or bleeding. If this happens, it can be easily treated. You should raise this with the research team if it becomes an issue. The blood sample will be taken in the same way as the ones you have for your normal HIV care at the clinic.

10 What will happen to my test samples?

The collection of your stool and blood will allow the researchers to test the effect of the HAMS on the health of your gut and if there is any effect on your HIV disease. The laboratory will use the following tests:

<u>Stool</u>

- pH (a measure of how acidic your stool sample is)
- short chain fatty acids (a measure of fermentation in your large intestine)
- the number and type of bacteria present in your large intestine
- calprotectin which measures if your gut is inflamed
- total, digestible and resistant starch (the different types of starch present in your stool).

<u>Blood</u>

- CD4+ T cell count (the measure of your immune response to HIV infection)
- HIV viral load (how much HIV is present)

The containers for your stool and blood samples will be labelled with only your participant identifier code to protect your confidentiality. The only researchers able to re-identify your samples from the code is by referring to the code key which will be kept in a password-

protected document on a password protected computer by the study coordinator and Principal Investigator-India, Dr. Ramadass.

- Any leftover supply of your blood and stool will be stored for future tests. Blood and stool samples will be stored at the AIIMS laboratory of the Principal Investigator-India, Dr Ramadass, to be used within the next 5 years for other tests to see how resistant starch changes your body. For example, blood samples may be used to test if the resistant starch made your gut wall stronger. Stool samples may be used to test if resistant starch changed the bacteria that live in your gut. These extra tests can be done once the results of the first tests are known.

By providing consent, you are giving permission for the collection and analysis of your blood and stool and storage of any leftover samples for use in future studies even if they are not related to this study.

11 What if new information arises during this research project?

Sometimes during the course of a research project, new information becomes available about the treatment that is being studied. If this happens, the research team will tell you about it and discuss with you whether you want to continue in the research project. If you decide to withdraw, the research team will make arrangements for your regular health care to continue. If you decide to continue in the study, you will be asked to sign an updated consent form. Also, on receiving new information, the research team might consider it to be in your best interests to withdraw you from the research project. If this happens, he/ she will explain the reasons and arrange for your regular health care to continue.

12 Can I have other treatments during this research project?

There is no restriction on taking other treatment or medications during the study period other than antibiotics which will require you to withdraw from the study. Please discuss with the research team if you need to start a course of antibiotics.

13 What if I withdraw from this research project?

If you decide to withdraw from the study, please notify a member of the research team before you withdraw. This notice will allow that person or the research supervisor to discuss any health risks or special requirements about withdrawing. If you do withdraw your consent during the research project, the research team will not collect additional personal information from you, although personal information already collected will be retained to ensure that the results of the research project can be measured properly and to comply with law. You should be aware that data collected by the sponsor up to the time you withdraw will form part of the research project results. If you do not want them to do this, you must tell them before you join the research project.

14 Could this research project be stopped unexpectedly?

This research project may be stopped unexpectedly for a variety of reasons. These may include reasons such as:

- Unacceptable side effects

15 What happens when the research project ends?

A summary report of the study results will be provided back to participants within 6 months of the completion of the laboratory analysis.

Part 2 How is the research project being conducted?

16 What will happen to information about me?

Following your participation in the study, documents containing the results will be stored in a secure filing cabinet in the office of the study coordinator in Australia or on a password-protected computer in the case of soft copies. Study data will be stored away ('archived') 2 years after publication of the study results are published in a report. Summary data may be used in future reports or funding proposals. By signing the consent form you consent to the research team collecting and using personal information about you for the research project. Any information obtained in connection with this research project that can identify you will remain confidential. Your information will only be used for the purpose of this research project and it will only be provided to other people with your permission, except as required by law.

Information about you may be obtained from your health records held at this and other health services for the purpose of this research. By signing the consent form you agree to the study team accessing health records if they are relevant to your participation in this research project.

Your health records and any information obtained during the research project are subject to inspection (for the purpose of checking the procedures and the data) by the relevant authorities and authorised representatives of Flinders University of South Australia, or as required by law. By signing the Consent Form, you authorise release of, or access to, this confidential information to the relevant study personnel and regulatory authorities as noted above.

It is anticipated that the results of this research project will be published and/or presented in a variety of forums. In any publication and/or presentation, information will be provided in such a way that you cannot be identified, except with your permission. Reporting will be about the whole group of participants together or by using participant's confidential identifier codes only.

Information about your participation in this research project may be recorded in your health records.

In accordance with relevant Indian privacy and other relevant laws, you have the right to request access to your information collected and stored by the research team for this study. You also have the right to request that any information with which you disagree be corrected. Please contact the study team member named at the end of this document if you would like to access your information.

Any information obtained for the purpose of this research project and for the future research described in Section 16 that can identify you will be treated as confidential and securely stored. It will be disclosed only with your permission, or as required by law.

17 Complaints and compensation

If you suffer any injuries or complications as a result of this research project, you should contact the study team as soon as possible and you will be assisted with arranging appropriate medical treatment.

18 Who is organising and funding the research?

This research project is being conducted by Ms Elissa Mortimer on behalf of Flinders University under the direction of Dr Balamurugan Ramadass from the All India Institute of Medical Sciences-Bhubaneswar.

Flinders University and All India Institute of Medical Sciences-Bhubaneswar may benefit financially from this research project if, for example, the project assists these institutions to obtain approval for a new therapy.

By taking part in this research project you agree that samples of your blood or stool (or results from analysing your blood or stool) may be provided to Flinders University or All India Institute of Medical Sciences-Bhubaneswar and that these institutions may directly or indirectly benefit financially from your samples or from what is learnt from analysis of your samples.

You will not benefit financially from your involvement in this research project even if, for example, your samples (or what is learnt from analysis of your samples) proves to be of commercial value to Flinders University or All India Institute of Medical Sciences-Bhubaneswar.

In addition, if what is learnt from this research leads to discoveries that are of commercial value to Flinders University or All India Institute of Medical Sciences-Bhubaneswar, the research team or their institutions, there will be no financial benefit to you or your family from these discoveries.

No member of the research team will receive a personal financial benefit from your involvement in this research project (other than their ordinary wages).

19 Who has reviewed the research project?

All research administered from Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). The ethical aspects of this research project have been approved by the HREC of Southern Adelaide Clinical Human Research Ethics Committee and the Institute Ethics Committee (IEC) of the All India Institute of Medical Sciences-Bhubaneswar.

This project will be carried out according to the Australian *National Statement on Ethical Conduct in Human Research (2007)*. This statement has been developed to protect the interests of people who agree to participate in human research studies.

20 Further information and who to contact

The person you may need to contact will depend on what your question is about. If you want any further information concerning this project or if you have any medical problems which may be related to your involvement in the project (for example, any side effects), you can contact any of the following people:

Clinical contact person

Name	Dr Balamurugan Ramadass
Position	Assistant Professor
Telephone	(+91) 8547805341
Email	balaramadass1@gmail.com

Complaints contacts:

Name	Dr Balamurugan Ramadass
Position	Assistant Professor
Telephone	(+91) 8547805341
Email	balaramadass1@gmail.com

Name	Ms Elissa Mortimer
Position	Study Coordinator and Principal Investigator
Telephone	+61 415216907
Email	elissa.mortimer@flinders.edu.au

Name	Southern Adelaide Local Health Network
Position	Director, Office for Research
Telephone	8204 6453
Email	Health.SALHNOfficeforResearch@sa.gov.au

Reviewing HREC approving this research and HREC Executive Officer details

Reviewing HREC name	All India Institute of Medical Sciences (AIIMS) – Bhubaneswar IEC
HREC Executive Officer	Member Secretary, Arvind Kumar Singh
Telephone	TBA
Email	arvind28aug@gmail.com

Consent Form – *Adult providing own consent*

Title	What is the effect of a dietary resistant starch intervention on the colonic luminal environment and HIV-related immunity and is a feeding trial feasible in HIV-positive adults in India?
Short Title	Resistant starch in HIV-positive adults in India.
Project Sponsor	Flinders University
Coordinating Principal Investigator/ Principal Investigator	Professor Paul Ward – Chief Investigator Assistant Professor Balamurugan Ramadass- Principal Investigator-India (PI-India) Ms. Elissa Mortimer-Study Coordinator and Principal Investigator
Associate Investigator(s)	Professor Geraint Rogers Professor Graeme Young Professor BS Ramakrishna
Location of recruitment	Bhubaneswar, India

Declaration by Participant

I have read the Participant Information Sheet or someone has read it to me in a language that I understand.

I understand the purposes, procedures and risks of the research described in the project.

I give permission for my doctors, other health professionals, hospitals or laboratories outside this hospital to release information to Flinders University of South Australia and All India Institute of Medical Sciences-Bhubaneswar, concerning my disease and treatment for the purposes of this project. I understand that such information will remain confidential.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the study without affecting my future health care.

I understand that I will be given a signed copy of this document to keep.

Name of Participant (please print) _____

Signature _____ Date _____

Thumbprint

Name of Witness* to Participant's Signature (please print) _____

Signature _____ Date _____

* Witness is not to be the investigator, a member of the study team or their delegate. In the event that an interpreter is used, the interpreter may not act as a witness to the consent process. Witness must be 18 years or older.

Declaration by Senior Researcher†

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Senior Researcher† (please print) _____

Signature _____ Date _____

† A senior member of the research team (Study Coordinator or Principal Investigator) must provide the explanation of, and information concerning, the research project.

Note: All parties signing the consent section must date their own signature.

I also consent to providing blood and stool samples and the storage and use of these samples, as described in the relevant section of the Participant Information Sheet, for:

- This specific research project
- Other research that is closely related to this research project
- Any future research.

Name of Participant (please print) _____

Signature _____ Date _____

Thumbprint

Name of Witness* to Participant's Signature (please print) _____

Signature _____ Date _____

* Witness is not to be the investigator, a member of the study team or their delegate. In the event that an interpreter is used, the interpreter may not act as a witness to the consent process. Witness must be 18 years or older.

Declaration by Senior Researcher†

Name of Senior Researcher† (please print) _____

Signature _____ Date _____

† A senior member of the research team (Study Coordinator or Principal Investigator) must provide the explanation of and information concerning the research project.

Note: All parties signing the consent section must date their own signature.

Form for Withdrawal of Participation – *Adult providing own consent*

Title	What is the effect of a dietary resistant starch intervention on the colonic luminal environment and HIV-related immunity and is a feeding trial feasible in HIV-positive adults in India?
Short Title	Resistant starch in HIV-positive adults in India.
Project Sponsor	Flinders University
Coordinating Principal Investigator/ Principal Investigator	Professor Paul Ward – Chief Investigator Assistant Professor Balamurugan Ramadass-Principal Investigator-India (PI-India) Ms. Elissa Mortimer – Study Coordinator and Principal Investigator
Associate Investigator(s)	Professor Geraint Rogers Professor Graeme Young Professor BS Ramakrishna
Location of recruitment	Bhubaneswar, India

Declaration by Participant

I wish to withdraw from participation in the above research project and understand that such withdrawal will not affect my routine treatment, my relationship with those treating me or my relationship with All India Institute of Medical Sciences-Bhubaneswar, ART Clinic Bhubaneswar or Flinders University of South Australia.

Name of Participant (please print) _____

Signature _____ Date _____

Thumbprint

Circumstances of withdrawal:

Declaration by Senior Researcher[†]

I have given a verbal explanation of the implications of withdrawal from the research project and I believe that the participant has understood that explanation.

Name of Senior Researcher[†]
(please print) _____

Signature _____ Date _____

[†] A senior member of the research team (Study Coordinator or Principal Investigator) must provide the explanation of and information concerning withdrawal from the research project.

Note: All parties signing the consent section must date their own signature.

APPENDIX 8:
Updated Participant Information and Consent Form – change from single to double crossover study (for existing participants)

Flinders University of South Australia and All India Institute of Medical Sciences (AIIMS) – Bhubaneswar.

Participant Information Sheet/Consent Form

Interventional Study – *Adult providing own consent*

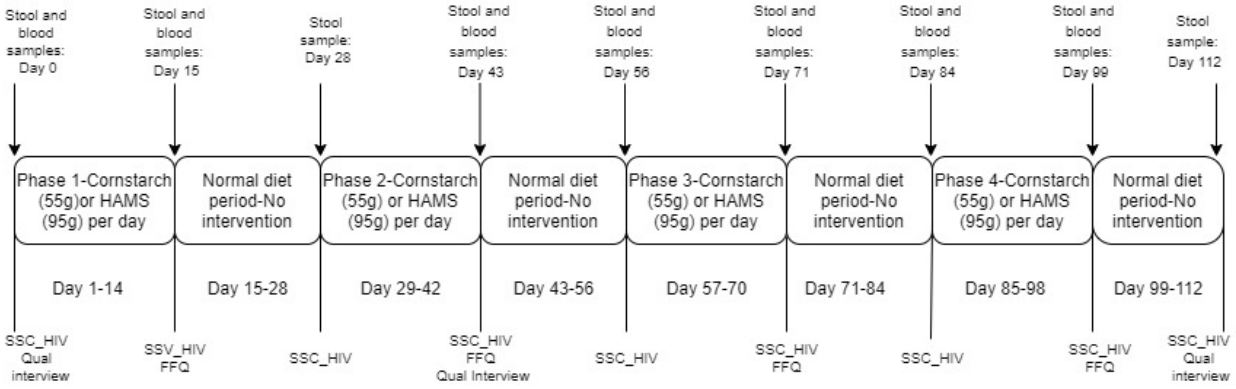
Bhubaneswar, India

Title	What is the effect of a dietary resistant starch intervention on the colonic luminal environment and HIV-related immunity and is a feeding trial feasible in HIV-positive adults in India?
Short Title	Resistant starch in HIV-positive adults in India.
Project Sponsor	Flinders University
Coordinating Principal Investigator/ Principal Investigator	Professor Paul Ward-Chief Investigator Assistant Professor Balamurugan Ramadass-Principal Investigator-India (PI-India) Ms Elissa Mortimer-Study Coordinator and Principal Investigator.
Associate Investigator(s)	Professor Geraint Rogers Professor Graeme Young Professor BS Ramakrishna
Location where recruitment will occur	Bhubaneswar, India

Part 1 What does my participation involve?

1 Introduction

You are invited to continue in this research project for a further 8 weeks. The research project is testing a treatment for symptoms of the gut which may be useful for people living with HIV. A food additive made from the maize plant called High Amylose Maize Starch or 'HAMS,' will be added to the normal diet because it is high in a nutrient called 'resistant starch.' All of the details which you agreed to in the first consent form have stayed the same except that the research team wants you to repeat the same study a second time. The total study details are included in the diagram below:



You are asked to sign the consent section so that we can be sure that you fully understand the addition to the original study. By signing it you are telling us that you:

- Understand what you have read
- Consent to continue to take part in the research project
- Consent to have the tests and treatments that are described
- Consent to the use of your personal and health information as described.

You will be given a copy of this PICF to keep.

2 What do I have to do?

- Just like last time, you will be asked to eat the whole serve of the study food (rotis). The rotis will have HAMS or cornstarch added with the other ingredients. The total amount of time during which you will consume study foods is an additional 4 weeks in total. The total amount of time you will participate in the study is an additional 8 weeks:
    - **Phase 3**: 2 weeks eating study foods containing HAMS or cornstarch
    - **Normal diet:** 2 weeks eating your normal diet
    - **Phase 4**: 2 weeks eating study foods containing the starch you did not eat in Phase 1.
    - You will then return to your normal diet. After another 2 weeks, your stool will be collected for the last time.
- You will continue to take your ART and any other medications that you normally take. If however, you are required to take antibiotics, you may be required to leave the study as this will affect the results in an unwanted way.
- There are no other lifestyle or dietary changes you need to make.

Further information and who to contact

The person you may need to contact will depend on what your question is about. If you want any further information concerning this project or if you have any medical problems which may be related to your involvement in the project (for example, any side effects), you can contact any of the following people:

Clinical contact person

Name	Dr Balamurugan Ramadass
Position	Assistant Professor
Telephone	(+91) 8547805341
Email	balaramadass1@gmail.com

Complaints contacts:

Name	Dr Balamurugan Ramadass
Position	Assistant Professor
Telephone	(+91) 8547805341
Email	balaramadass1@gmail.com

Name	Ms Elissa Mortimer
Position	Study Coordinator and Principal Investigator
Telephone	+61 415216907
Email	elissa.mortimer@flinders.edu.au

Name	Southern Adelaide Local Health Network
Position	Director, Office for Research
Telephone	+ 61 8 8204 6453
Email	Health.SALHNOfficeforResearch@sa.gov.au

Reviewing HREC approving this research and HREC Executive Officer details

Reviewing HREC name	All India Institute of Medical Sciences (AIIMS) – Bhubaneswar IEC
HREC Executive Officer	Member Secretary, Arvind Kumar Singh
Telephone	(+91) 96549 05213
Email	arvind28aug@gmail.com

Consent Form – *Adult providing own consent*

Title	What is the effect of a dietary resistant starch intervention on the colonic luminal environment and HIV-related immunity and is a feeding trial feasible in HIV-positive adults in India?
Short Title	Resistant starch in HIV-positive adults in India.
Project Sponsor	Flinders University
Coordinating Principal Investigator/ Principal Investigator	Professor Paul Ward – Chief Investigator Assistant Professor Balamurugan Ramadass-Principal Investigator-India (PI-India) Ms. Elissa Mortimer-Study Coordinator and Principal Investigator
Associate Investigator(s)	Professor Geraint Rogers Professor Graeme Young Professor BS Ramakrishna
Location of recruitment	Bhubaneswar, India

Declaration by Participant

I have read the Participant Information Sheet or someone has read it to me in a language that I understand.

I understand the purposes, procedures and risks of the research described in the project.

I give permission for my doctors, other health professionals, hospitals or laboratories outside this hospital to release information to Flinders University of South Australia and All India Institute of Medical Sciences-Bhubaneswar, concerning my disease and treatment for the purposes of this project. I understand that such information will remain confidential.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the study without affecting my future health care.

I understand that I will be given a signed copy of this document to keep.

Name of Participant (please print) _____

Signature _____ Date _____

Thumbprint

Name of Witness* to Participant's Signature (please print) _____

Signature _____ Date _____

* Witness is not to be the investigator, a member of the study team or their delegate. In the event that an interpreter is used, the interpreter may not act as a witness to the consent process. Witness must be 18 years or older.

Declaration by Senior Researcher†

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Senior Researcher† (please print) _____

Signature _____ Date _____

† A senior member of the research team (Study Coordinator or Principal Investigator) must provide the explanation of, and information concerning, the research project.

Note: All parties signing the consent section must date their own signature.

I also consent to providing blood and stool samples and the storage and use of these samples, as described in the relevant section of the Participant Information Sheet, for:

- This specific research project
- Other research that is closely related to this research project
- Any future research.

Name of Participant (please print) _____

Signature _____ Date _____

Thumbprint

Name of Witness* to Participant's Signature (please print) _____

Signature _____ Date _____

* Witness is not to be the investigator, a member of the study team or their delegate. In the event that an interpreter is used, the interpreter may not act as a witness to the consent process. Witness must be 18 years or older.

Declaration by Senior Researcher†

Name of Senior Researcher† (please print) _____

Signature _____ Date _____

† A senior member of the research team (Study Coordinator or Principal Investigator) must provide the explanation of and information concerning the research project.

Note: All parties signing the consent section must date their own signature.

APPENDIX 9:
Participant Information and Consent Form for new participants joining the double crossover study

Flinders University of South Australia and All India Institute of Medical Sciences (AIIMS) – Bhubaneswar.

Participant Information Sheet/Consent Form

Interventional Study – *Adult providing own consent*

Bhubaneswar, India

Title	What is the effect of a dietary resistant starch intervention on the colonic luminal environment and HIV-related immunity and is a feeding trial feasible in HIV-positive adults in India?
Short Title	Resistant starch in HIV-positive adults in India.
Project Sponsor	Flinders University
Coordinating Principal Investigator/ Principal Investigator	Professor Paul Ward-Chief Investigator Assistant Professor Balamurugan Ramadass-Principal Investigator-India (PI-India) Ms Elissa Mortimer-Study Coordinator and Principal Investigator
Associate Investigator(s)	Professor Geraint Rogers Professor Graeme Young Professor BS Ramakrishna
Location where recruitment will occur	Bhubaneswar, India

Part 1 What does my participation involve?

1 Introduction

You are invited to take part in this research project. The research project is testing a treatment for symptoms of the gut which may be useful for people living with HIV. A food additive made from the maize plant called High Amylose Maize Starch or 'HAMS,' will be added to the normal diet because it is high in a nutrient called 'resistant starch.' This study is being done in India because in countries where the water and sewerage system is not that good, people who live there come into contact every day with bacteria that can lead to repeated infections of the gut. Research has shown that resistant starch can be of benefit when this happens. This Participant Information Sheet/Consent Form (PICF) tells you about the research project. It explains the tests and treatments involved. Knowing what is involved will help you decide if you want to take part in the research. Please read this information carefully. Ask questions about anything that you don't understand or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend or your local doctor.

Participation in this research is voluntary. If you don't wish to take part, you don't have to. You will receive the best possible care whether or not you take part.

If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:

- Understand what you have read
- Consent to take part in the research project
- Consent to have the tests and treatments that are described
- Consent to the use of your personal and health information as described.

You will be given a copy of this PICF to keep.

2 What is the purpose of this research?

Aim (1) To see what happens when HAMS is added to the diet by measuring changes in:

a. your stool;
b. your blood;
c. symptoms in your gut.

Aim (2) To explore the things that might make a study with HAMS easy or difficult for participants.

Significance: HIV disease and Anti-Retroviral Therapy (ART) for HIV can both cause gut problems in the stomach and intestines. New ways of managing these problems will be helpful to make patients feel better and help them to stay on their ART. This will decrease the chance of HIV infection being passed on to other people.

Dietary starch comes from plants like cereals and potatoes and provides a source of energy for the body. It is an important part of the human diet. Instead of being digested in the stomach and small intestine, resistant starch reaches the large intestine and is used by the bacteria that live there in a process called fermentation. This is thought to be good for the body. So far, studies with resistant starch have happened with children and adults in countries including India, Australia, England and the United States of America. Some studies have tested resistant starch mixed with other special food ingredients in HIV-positive participants but no studies have tested what happens when resistant starch by itself is added to the diet in HIV-positive people.

Resistant starch is considered very safe for consumption by humans and is found in the normal diet in foods like potatoes and bananas. Resistant starch as an ingredient has been used by bakers for many years because it makes bakery foods crusty. One example of resistant starch is HAMS which will be used in this study. HAMS has been given GRAS status (Generally

Recognized As Safe) by the United States of America Food and Drug Administration. This means it is allowed to be added to foods being eaten by humans. It is of natural origin from maize plants which have not been genetically modified.

The results of this study will be used by the study coordinator, Ms Elissa Mortimer, to obtain a Doctorate of Public Health degree. Ms Mortimer developed the idea for this study which is funded by Flinders University of South Australia with All India Institute of Medical Sciences (AIIMS) Bhubaneswar as the research partner.

3 What does participation in this research involve?

You will be participating in a 'double cross-over' dietary study. In a double cross-over study, different groups each have different treatments in turn separated by a period of normal diet. Following this, the whole process is then repeated. The treatments in this study are two different types of starch: HAMS and cornstarch. You will be asked to eat foods which have HAMS or cornstarch added to them. Cornstarch is digested by your body in the stomach and small intestines in the usual way that food is digested. Instead of being digested in the stomach and small intestine, HAMS reaches the large intestine where it is used as a food by bacteria that normally live there. These bacteria can do beneficial things in the human body which is why we are interested in studying the effect of HAMS on them. HAMS and cornstarch do not change the taste of food so you may not notice any difference compared to normal food.

The order in which participants consume the two starches will be chosen by chance (random). This is to try to make sure the groups are the same. There will be a break between the HAMS and cornstarch foods so that your body adjusts back to normal before you start the other starch. You will not know if you are consuming resistant starch or cornstarch. This research project has been designed in this way to make sure that participants give the most accurate responses to the questionnaires and that the researchers interpret the results in a fair way.

Initial steps

The research staff will explain the study to you using this PICF document. If you decide to participate, the research staff will ask you to sign the consent form. They will then ask you some questions in the screening step. If you meet the below and exclusion criteria, you will be able to participate in the study:

Inclusion criteria

1. HIV-positive adults aged 18 years or over on ART
2. CD4+ T cell count more than 200 cells/mm3
3. No antibiotics within last 6 weeks

Exclusion criteria

1. Other gut problems such as Crohn's Disease or Ulcerative Colitis.
2. Current participation in other research studies
3. Pregnant or breastfeeding.

This is a study with two parts: the first is the dietary part, the second is the interview or focus group part where the research team will ask participants the things that make this study easy or difficult. These two parts of the study will happen at the same time, during the 16 weeks that participants will be involved in the study.

Participants will be asked to give a stool and blood sample at day 0 and to complete a questionnaire about any gut symptoms so the researchers can see how these measurements change once the starches are added to the normal diet. Participants will be randomly put into one of two groups. The first group will eat HAMS added to their usual diet for 2 weeks then only their usual diet for 2 weeks then cornstarch added to their diet for the final 2 weeks. The second group will do this in the reverse order by eating the cornstarch for 2 weeks then the

normal diet for 2 weeks then the HAMS for 2 weeks. Following this, the whole process is then repeated. This is shown in the below Figure 1.

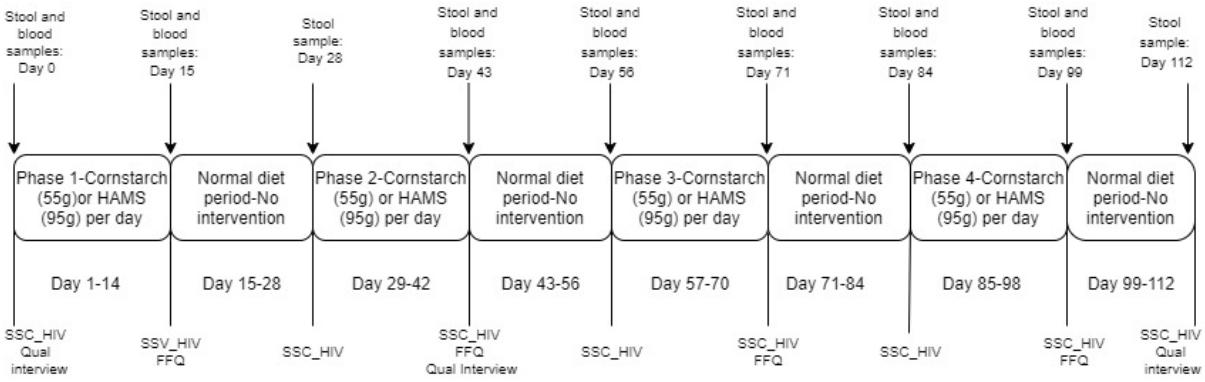


Figure 1: Study timing.

Participants will be provided daily with the study food containing HAMS or cornstarch. HAMS and cornstarch are a similar texture to wheat flour and do not have a strong taste. The study coordinator will test different study foods with a small group of 5 study staff first to work out which one tastes best and can be eaten for the study period.

Participants will be asked to provide stool and blood samples on the days shown in Figure 1. The research team will teach participants how to collect the stool samples and will then collect these samples from participants. Samples will then be frozen and transported to the laboratory for storage. The blood samples will be taken by a health worker employed by the ART clinic. Samples will be transported by the research team to the laboratory in vials labelled with the participant identifier code rather than their name.

There are no additional costs associated with participating in this research project, nor will you be paid. All of the study food and the cost of stool and blood tests required as part of the research project will be provided to you free of charge. The usual way you access drugs for your HIV such as ART will not be affected.

You may be reimbursed for any reasonable travel costs associated with the research study.

4 What do I have to do?

- You will be asked to eat the whole serve of the study food. The study food will be roti with HAMS or cornstarch added with the other ingredients. The total amount of time during which you will consume study foods is 8 weeks in total. The total amount of time you will participate in the study is 16 weeks. The details are provided in Figure 1.
- In total, 9 stool samples and 7 blood samples will be collected over the total 16 weeks.
- On collection days you will also be asked to complete the questionnaire which will ask about any side effects you may be experiencing. This will take 5-10 minutes to complete. Study staff can help you.
- On three days (days 15, 43 and 99), you will also be asked to complete a brief questionnaire about what you have been eating and drinking recently. This should take 10-15 minutes to complete. Study staff can help you.
- You will also be asked to participate in three interviews for the qualitative part of the study. You can choose if you prefer to have an interview by yourself or join 5 other participants for a focus group discussion. Both of these will be with a research staff member plus interpreter. The three interviews/focus groups will be held on:
    - Day 0 immediately before phase 1 begins;
    - Day 43; and
    - Day 112.

The interviews and focus groups will be recorded (audio only) so that the discussion can be written down afterwards. The only people who will access these audio recordings are the interpreter and two members of the research team. The recordings will be kept in a locked filing cabinet when not in use.
After the interviews and focus groups are completed, participants will be invited to check the summary report written by the research team. The reports will be written in a way that keeps the identity of participants anonymous.

- You will continue to take your ART and any other medications that you normally take. If however, you are required to take antibiotics, you may be required to leave the study as this will affect the results in an unwanted way.
- There are no other lifestyle or dietary changes you need to make.
- Participants will be checked on by research staff on the sample collection days which happen every 2 weeks and with phone calls between the sample collection days. This will provide a chance for you to report any side effects to the research team. Participants will also be provided with the phone number of a research team member who can be contacted if there are any issues in between phone call and sample days. The research team member will report any issues to the study coordinator. They will then decide on the best way to solve the problem. This may require consultation with your HIV doctor.

5 Other relevant information about the research project

This study will be conducted in Bhubaneswar only. It will be conducted by researchers from the All India Institute of Medical Sciences-Bhubaneswar and Flinders University of South Australia. Up to 30 adults from Bhubaneswar will participate in the study.

6 Do I have to take part in this research project?

Participation in any research project is voluntary. If you do not wish to take part, you do not have to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage. If you do decide to take part, you will be given this PICF to sign and you will be given a copy to keep. Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with the Bhubaneswar ART clinic.

7 What are the alternatives to participation?

You do not have to take part in this research project to receive treatment at the ART clinic. Other options are available; these include continuing your usual medication and care regimen for HIV and any other conditions you may have. A member of the research team will discuss these options with you before you decide whether or not to take part in this research project. You can also discuss the options with your local doctor.

8 What are the possible benefits of taking part?

We cannot guarantee or promise that you will receive any benefits from this research; however, possible benefits may include:

- A benefit to your body especially your intestines and the bacteria that live there. This will likely only last as long as you are eating the HAMS;
- A benefit to your HIV disease;
- Being provided with the study foods for 56 days;
- Contributing to the research about gut health, HIV and resistant starch.

There may be no clear benefit felt or noticed by you from your participation in this research.

9 What are the possible risks and disadvantages of taking part?

Medical treatments often cause side effects. You may have none, some or all of the effects listed below, and they may be mild, moderate or severe. If you have any of these side effects, or are worried about them, talk with a member of the research team. This team will also be looking out for side effects. There may be side effects that the researchers do not expect or do not know about yet. Tell a research team member or your HIV doctor immediately about any new or unusual symptoms that you get.

Many side effects go away shortly after treatment ends. However, sometimes side effects can be serious, long lasting or permanent. This is very unlikely to occur after eating HAMS. If a severe side effect or reaction occurs, the research team may need to stop your participation in the study. The research team and your HIV doctor will discuss the best way of managing any side effects with you.

Possible side effects:

- flatulence, bloating, gas, increased frequency of passing stool, increased size and weight of stool.

If you experience these side effects they will almost certainly go away once you stop consuming the HAMS. If they cause you significant discomfort, you are free to stop your participation in the study.

The effects of high doses of HAMS on the unborn child and on the newborn baby are not known. Because HAMS has been certified with Generally Recognized as Safe (GRAS) status from the United States Government, it is very unlikely that it could harm the growing baby or mother. However, since eating large amounts of HAMS can change what happens in the gut normally, pregnant women should not participate in this study. Women who may be pregnant will be asked to do a simple pregnancy test with a small sample of their urine before they start the study.

Having a blood sample taken may cause some discomfort, bruising, minor infection or bleeding. If this happens, it can be easily treated. You should raise this with the research team if it becomes an issue. The blood sample will be taken in the same way as the ones you have for your normal HIV care at the clinic.

10 What will happen to my test samples?

The collection of your stool and blood will allow the researchers to test the effect of the HAMS on the health of your gut and if there is any effect on your HIV disease. The laboratory will use the following tests:

Stool

- pH (a measure of how acidic your stool sample is)
- short chain fatty acids (a measure of fermentation in your large intestine)
- the number and type of bacteria present in your large intestine
- calprotectin which measures if your gut is inflamed
- total, digestible and resistant starch (the different types of starch present in your stool).

Blood

- CD4+ T cell count (the measure of your immune response to HIV infection)
- HIV viral load (how much HIV is present)

The containers for your stool and blood samples will be labelled with only your participant identifier code to protect your confidentiality. The only researchers able to re-identify your samples from the code is by referring to the code key which will be kept in a password-protected document on a password protected computer by the study coordinator and Principal Investigator-India, Dr. Ramadass.

- Any leftover supply of your blood and stool will be stored for future tests. Blood and stool samples will be stored at the AIIMS laboratory of the Principal Investigator-India, Dr Ramadass, to be used within the next 5 years for other tests to see how resistant starch changes your body. For example, blood samples may be used to test if the resistant starch made your gut wall stronger. Stool samples may be used to test if resistant starch changed the bacteria that live in your gut. These extra tests can be done once the results of the first tests are known.

By providing consent, you are giving permission for the collection and analysis of your blood and stool and storage of any leftover samples for use in future studies even if they are not related to this study.

11 What if new information arises during this research project?

Sometimes during the course of a research project, new information becomes available about the treatment that is being studied. If this happens, the research team will tell you about it and discuss with you whether you want to continue in the research project. If you decide to withdraw, the research team will make arrangements for your regular health care to continue. If you decide to continue in the study, you will be asked to sign an updated consent form. Also, on receiving new information, the research team might consider it to be in your best interests to withdraw you from the research project. If this happens, he/ she will explain the reasons and arrange for your regular health care to continue.

12 Can I have other treatments during this research project?

There is no restriction on taking other treatment or medications during the study period other than antibiotics which will require you to withdraw from the study. Please discuss with the research team if you need to start a course of antibiotics.

13 What if I withdraw from this research project?

If you decide to withdraw from the study, please notify a member of the research team before you withdraw. This notice will allow that person or the research supervisor to discuss any health risks or special requirements about withdrawing. If you do withdraw your consent during the research project, the research team will not collect additional personal information from you, although personal information already collected will be retained to ensure that the results of the research project can be measured properly and to comply with law. You should be aware that data collected by the sponsor up to the time you withdraw will form part of the research project results. If you do not want them to do this, you must tell them before you join the research project.

14 Could this research project be stopped unexpectedly?

This research project may be stopped unexpectedly for a variety of reasons. These may include reasons such as:

- Unacceptable side effects

15 What happens when the research project ends?

A summary report of the study results will be provided back to participants within 6 months of the completion of the laboratory analysis.

Part 2 How is the research project being conducted?

16 What will happen to information about me?

Following your participation in the study, documents containing the results will be stored in a secure filing cabinet in the office of the study coordinator in Australia or on a password-protected computer in the case of soft copies. Study data will be stored away ('archived') 2 years after publication of the study results are published in a report. Summary data may be used in future reports or funding proposals. By signing the consent form you consent to the research team collecting and using personal information about you for the research project. Any information obtained in connection with this research project that can identify you will remain confidential. Your information will only be used for the purpose of this research project and it will only be provided to other people with your permission, except as required by law.

Information about you may be obtained from your health records held at this and other health services for the purpose of this research. By signing the consent form you agree to the study team accessing health records if they are relevant to your participation in this research project.

Your health records and any information obtained during the research project are subject to inspection (for the purpose of checking the procedures and the data) by the relevant authorities and authorised representatives of Flinders University of South Australia, or as required by law. By signing the Consent Form, you authorise release of, or access to, this confidential information to the relevant study personnel and regulatory authorities as noted above.

It is anticipated that the results of this research project will be published and/or presented in a variety of forums. In any publication and/or presentation, information will be provided in such a way that you cannot be identified, except with your permission. Reporting will be about the whole group of participants together or by using participant's confidential identifier codes only.

Information about your participation in this research project may be recorded in your health records.

In accordance with relevant Indian privacy and other relevant laws, you have the right to request access to your information collected and stored by the research team for this study. You also have the right to request that any information with which you disagree be corrected. Please contact the study team member named at the end of this document if you would like to access your information.

Any information obtained for the purpose of this research project and for the future research described in Section 16 that can identify you will be treated as confidential and securely stored. It will be disclosed only with your permission, or as required by law.

17 Complaints and compensation

If you suffer any injuries or complications as a result of this research project, you should contact the study team as soon as possible and you will be assisted with arranging appropriate medical treatment.

18 Who is organising and funding the research?

This research project is being conducted by Ms Elissa Mortimer on behalf of Flinders University under the direction of Dr Balamurugan Ramadass from the All India Institute of Medical Sciences-Bhubaneswar.

Flinders University and All India Institute of Medical Sciences-Bhubaneswar may benefit financially from this research project if, for example, the project assists these institutions to obtain approval for a new therapy.

By taking part in this research project you agree that samples of your blood or stool (or results from analysing your blood or stool) may be provided to Flinders University or All India Institute of Medical Sciences-Bhubaneswar and that these institutions may directly or indirectly benefit financially from your samples or from what is learnt from analysis of your samples.

You will not benefit financially from your involvement in this research project even if, for example, your samples (or what is learnt from analysis of your samples) proves to be of commercial value to Flinders University or All India Institute of Medical Sciences-Bhubaneswar.

In addition, if what is learnt from this research leads to discoveries that are of commercial value to Flinders University or All India Institute of Medical Sciences-Bhubaneswar, the research team or their institutions, there will be no financial benefit to you or your family from these discoveries.

No member of the research team will receive a personal financial benefit from your involvement in this research project (other than their ordinary wages).

19 Who has reviewed the research project?

All research administered from Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). The ethical aspects of this research project have been approved by the HREC of Southern Adelaide Clinical Human Research Ethics Committee and the Institute Ethics Committee (IEC) of the All India Institute of Medical Sciences-Bhubaneswar.

This project will be carried out according to the Australian *National Statement on Ethical Conduct in Human Research (2007)*. This statement has been developed to protect the interests of people who agree to participate in human research studies.

20 Further information and who to contact

The person you may need to contact will depend on what your question is about. If you want any further information concerning this project or if you have any medical problems which may be related to your involvement in the project (for example, any side effects), you can contact any of the following people:

Clinical contact person

Name	Dr Balamurugan Ramadass
Position	Assistant Professor
Telephone	(+91) 8547805341
Email	balaramadass1@gmail.com

Complaints contacts:

Name	Dr Balamurugan Ramadass
Position	Assistant Professor
Telephone	(+91) 8547805341
Email	balaramadass1@gmail.com

Name	Ms Elissa Mortimer
Position	Study Coordinator and Principal Investigator
Telephone	+61 415216907
Email	elissa.mortimer@flinders.edu.au

Name	Southern Adelaide Local Health Network
Position	Director, Office for Research
Telephone	8204 6453
Email	Health.SALHNOfficeforResearch@sa.gov.au

Reviewing HREC approving this research and HREC Executive Officer details

Reviewing HREC name	All India Institute of Medical Sciences (AIIMS) – Bhubaneswar IEC
HREC Executive Officer	Member Secretary, Arvind Kumar Singh
Telephone	(+91) 96549 05213
Email	arvind28aug@gmail.com

Consent Form – *Adult providing own consent*

Title	What is the effect of a dietary resistant starch intervention on the colonic luminal environment and HIV-related immunity and is a feeding trial feasible in HIV-positive adults in India?
Short Title	Resistant starch in HIV-positive adults in India.
Project Sponsor	Flinders University
Coordinating Principal Investigator/ Principal Investigator	Professor Paul Ward – Chief Investigator Assistant Professor Balamurugan Ramadass- Principal Investigator-India (PI-India) Ms. Elissa Mortimer-Study Coordinator and Principal Investigator
Associate Investigator(s)	Professor Geraint Rogers Professor Graeme Young Professor BS Ramakrishna
Location of recruitment	Bhubaneswar, India

Declaration by Participant

I have read the Participant Information Sheet or someone has read it to me in a language that I understand.

I understand the purposes, procedures and risks of the research described in the project.

I give permission for my doctors, other health professionals, hospitals or laboratories outside this hospital to release information to Flinders University of South Australia and All India Institute of Medical Sciences-Bhubaneswar, concerning my disease and treatment for the purposes of this project. I understand that such information will remain confidential.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the study without affecting my future health care.

I understand that I will be given a signed copy of this document to keep.

Name of Participant (please print) _____

Signature _____ Date _____

Thumbprint

Name of Witness* to Participant's Signature (please print) _____

Signature _____ Date _____

* Witness is not to be the investigator, a member of the study team or their delegate. In the event that an interpreter is used, the interpreter may not act as a witness to the consent process. Witness must be 18 years or older.

Declaration by Senior Researcher†

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Senior Researcher† (please print) _____

Signature _____ Date _____

† A senior member of the research team (Study Coordinator or Principal Investigator) must provide the explanation of, and information concerning, the research project.

Note: All parties signing the consent section must date their own signature.

I also consent to providing blood and stool samples and the storage and use of these samples, as described in the relevant section of the Participant Information Sheet, for:

- This specific research project
- Other research that is closely related to this research project
- Any future research.

Name of Participant (please print) _____

Signature _____ Date _____

Thumbprint

Name of Witness* to Participant's Signature (please print) _____

Signature _____ Date _____

* Witness is not to be the investigator, a member of the study team or their delegate. In the event that an interpreter is used, the interpreter may not act as a witness to the consent process. Witness must be 18 years or older.

Declaration by Senior Researcher†

Name of Senior Researcher† (please print) _____

Signature _____ Date _____

† A senior member of the research team (Study Coordinator or Principal Investigator) must provide the explanation of and information concerning the research project.

Note: All parties signing the consent section must date their own signature.

Form for Withdrawal of Participation – *Adult providing own consent*

Title	What is the effect of a dietary resistant starch intervention on the colonic luminal environment and HIV-related immunity and is a feeding trial feasible in HIV-positive adults in India?
Short Title	Resistant starch in HIV-positive adults in India.
Project Sponsor	Flinders University
Coordinating Principal Investigator/ Principal Investigator	Professor Paul Ward – Chief Investigator Assistant Professor Balamurugan Ramadass-Principal Investigator-India (PI-India) Ms. Elissa Mortimer-Study Coordinator and Principal Investigator
Associate Investigator(s)	Professor Geraint Rogers Professor Graeme Young Professor BS Ramakrishna
Location of recruitment	Bhubaneswar, India

Declaration by Participant

I wish to withdraw from participation in the above research project and understand that such withdrawal will not affect my routine treatment, my relationship with those treating me or my relationship with All India Institute of Medical Sciences-Bhubaneswar, ART Clinic Bhubaneswar or Flinders University of South Australia.

Name of Participant (please print) _____

Signature _____ Date _____

Thumbprint

Circumstances of withdrawal:

Declaration by Senior Researcher†

I have given a verbal explanation of the implications of withdrawal from the research project and I believe that the participant has understood that explanation.

Name of Senior Researcher†
(please print) _____

Signature _____ Date _____

† A senior member of the research team (Study Coordinator or Principal Investigator) must provide the explanation of and information concerning withdrawal from the research project.

Note: All parties signing the consent section must date their own signature.

APPENDIX 10:
Food Frequency Questionnaire

	Average times consumed – last 14 days								
Food/Drink items	**Never or less than once**	**1–3 times**	**4–6 times**	**7-10 times**	**More than 10 times**	**1 time per day**	**2 times per day**	**3+ times per day**	**Amount (e.g. half cup, cup, bowl)**
Idli									
Vada									
Dosa									
Chapatti/paratha									
Roti-wheat									
Roti-maize (makka)									
Whole makka roasted or steamed									
Puri/bhatura									
Any pitha									
Upma (all types)									
Bread-white									
Bread-wholemeal									
Bread-multigrain or seeded									
Corn flakes/rice flakes									
White boiled rice									
Brown rice									
Flattened/beaten rice (chuda)									
Kanji/pazhamhkanji									
Vegetable fried rice									
Biriyani									
Sambar/Other dhal curries									
Erussery/Pumpkin, salt, tamarind and lentil curry									
Whole gram curry									
Amarakka/beans – broad bean with coconut dry fried side dish									
Any potato dish									
Sweet potato									
Snake gourd/bitter gourd/other gourds									
Raw/Green banana									
Yellow (ripe) banana									
Plantain flower/stem preparations									

	Average times consumed – last 14 days								
Food/Drink items	**Never or less than once**	**1–3 times**	**4–6 times**	**7–10 times**	**More than 10 times**	**1 time per day**	**2 times per day**	**3+ times per day**	**Amount (e.g. half cup, cup, bowl)**
Unniappam – Fried banana pitha									
Banana roast – Battered fried green banana/plantain									
Mango									
Dried sour mango-Ambula									
Mango pickle									
Papad fried									
Nuts – specify type									
Potato chips									
Chick peas-Chola-Any preparation.									
Kidney beans-Rajma									
Oats-Any-Daliya									
Green peas									
Roasted channa									
Rye crackers-Khandbahale									
Plain crackers									
Biscuits (sweet, cream, etc.)									
Kheer (rice pudding)									
Sandwiches with cooked and cooled potato and mayonnaise									
Pokhala-Fermented brown rice plus water									
Mandia – Fermented alcoholic beverage									

Adapted from Iqbal, R., Ajayan, K., Bharathi, A. V., Zhang, X., Islam, S., Soman, C. R., Merchant, A. T. (2009). Refinement and validation of an FFQ developed to estimate macro- and micronutrient intakes in a south Indian population. *Public Health Nutr,* 12(1);12–18.

APPENDIX 11:
Interview guide – semi-structured interviews

HIV and resistant starch in India study

Ms Elissa Mortimer

Interview guide for use in semi-structured individual interviews.

Day 0 data collection

1. What factors led you to agree to participate in this study?

2. How did the staff involved in recruitment and consent help you make your decision about participating?

3. Did the involvement of an Australian University with AIIMS-Bhubaneswar influence your decision about participation?

4. How confident do you feel in the research team and what factors have influenced this?

5. What do you think will be difficult about participating in the study?

6. What do you think will be easy about participating in the study?

7. What benefits, if any, do you believe the study will bring to you?

Day 42 data collection

1. What was difficult about participating in the study?

2. What was easy about participating in the study?

3. What benefits do you feel the study brought you?

4. Did you ever think about leaving the study?

5. If yes, what were the reasons you wanted to leave the study?

6. What made you stay in the study?

7. Did the research team motivate you to stay in the study?

8. If yes, how did they motivate you?

Day 112 data collection

1. What was difficult about participating in the study?

2. What was easy about participating in the study?

3. What benefits do you feel the study brought you?

4. Did you ever think about leaving the study?

5. If yes, what were the reasons you wanted to leave the study?

6. What made you stay in the study?

7. Did the research team motivate you to stay in the study?

8. If yes, how did they motivate you?

APPENDIX 12:
Revised Sign and Symptom Checklist for HIV (SSC-HIVrev)

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