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Research Article

Metabolism of Wheat Dextrin, Partially Hydrolysed Guar Gum and Inulin by *Bifidobacterium lactis* or *Lactobacillus acidophilus* in an *In Vitro* Gut Model Fermentation System

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Combining the fibres wheat dextrin (WD), partially hydrolysed guar gum (PHGG) and inulin with probiotics *Lactobacillus acidophilus* NCFM (NCFM) or *Bifidobacterium lactis* HN019 (HN019) may enhance bacterial metabolites leading to a healthier gut community. The aim of this study was to determine whether WD, PHGG and inulin or NCFM and HN019 alone generate a more favourable gut bacterial community than when combined. A secondary aim was to assess organic acid production following prebiotics, probiotics and synbiotic fermentation. An *in vitro* gut model batch culture fermentation was run for 72 h. Samples were collected for bacterial enumeration (fluorescent *in situ* hybridisation combined with flow cytometry) and organic acid production (gas chromatography). Inulin and HN019 combination significantly increased bifidobacteria compared to inulin alone. Additionally, a significant increase in lactic acid bacteria, *Bacteroides* and *Clostridium coccoides–Eubacterium rectale* was found in the inulin containing probiotic vessels. The WD and PHGG vessels combined with the probiotic did not show any alteration in bacterial metabolism compared to the dietary fibres alone. In conclusion, synbiotic inulin combined with either HN019 or NCFM may help to enhance bacterial metabolites and cross-feeding to lead to a prolonged elevation in *Bifidobacterium* spp., and lactic acid bacteria.

Keywords: *Bifidobacterium lactis* HN019, Inulin, *Lactobacillus acidophilus* NCFM, Partially hydrolysed guar gum, Prebiotics, Probiotics, Synbiotics, Wheat dextrin

Abbreviations Used: Analysis of variance, ANOVA; Calcium chloride, $CaCl_2$; Colony forming units per millilitre, CFU/mL; Fluorescence *in situ* hybridisation flow-cytometry, FISH-FCM; *Bifidobacterium lactis* HN019, HN019; Dipotassium hydrogen phosphate, K_2HPO_4; Monopotassium phosphate, KH_2PO_4; Magnesium sulfate, $MgSO_4$; Man-Rogosa-Sharpe, MRS; Sodium chloride, NaCl; Sodium bicarbonate, $NaHCO_3$; *Lactobacillus acidophilus* NCFM, NCFM; Optical density, OD; Partially hydrolysed guar gum, PHGG; Statistical package for the social, SPSS; Wheat dextrin, WD

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INTRODUCTION

Alterations in the gut microbiota have been associated with a range of disease states. Research has identified that medication, infection, poor diet, lifestyle and aging can result in a range of pathologies such as obesity, diabetes, inflammatory bowel disease, gastroenteritis and possibly digestive cancers (Gagliardi et al., 2018; Hou et al., 2011; Qin et al., 2012; Turnbaugh et al., 2006). The majority of bacteria in the human body reside in the colon with 10^{12} bacteria/g and dietary intervention is the predominant method used to alter the intestinal ecosystem (Sender et al., 2016).

One well-known intervention is a prebiotic, which is a 'substrate that is selectively utilised by host microorganisms conferring a health benefit' (Gibson et al., 2017). An established prebiotic is inulin which is known for being bifidogenic leading to certain health benefits, for example, improving cardiometabolic inflammation and increasing fat oxidation (Nicola et al., 2018; Van der Beek et al., 2018). Inulin is found in chicory and Jerusalem artichoke and comprises of fructose joined by ß-(2 → 1) linkage (Roberfroid, 2015). Another fermentable dietary fibre is wheat dextrin (WD) which is resistant dextrin made up of non-digestible α-1,2 and α-1-3 linkages. In the diet, it is not fully digested and 76–87% reaches the

colon (Van Den Heuvel et al., 2005; Vermorel et al., 2004). Partially hydrolysed guar gum (PHGG) is a dietary fibre made from controlled hydrolysis of guar gum.

Previous research reported in Pyle et al., (2020, unpublished) found that WD and PHGG significantly stimulated *Bacteroides* and *Clostridium* cluster IX which led to an increase in propionate production. Propionate has been associated with appetite regulation and lowering of energy intake (Chambers et al., 2015). Unlike inulin, WD and PHGG do not increase levels of positive groups like bifidobacteria or lactobacilli which have extensive health benefits including in human trials lowering cholesterol in hypercholesterolaemia participants, an effective treatment for acute diarrhoea in children when combined with oral rehydration therapy, improved immune response in elderly volunteers and able to produce neurotransmitter γ-aminobutyric acid in the gut (Barrett et al., 2012; Gill, 2001; Jones et al., 2012; Simakachorn et al., 2000). The addition of live bifidobacteria or lactobacilli combined with a prebiotic may lead to further health benefits to the host, this is termed a synbiotic. Synbiotics work in two ways: either complementarily or synergistically. The probiotic is specific strain(s) of bacteria with health benefits and a prebiotic either increases indigenous microbiota compounds or helps survival of the probiotic (Kolida and Gibson, 2011).

Synbiotics were first considered by Gibson and Roberfroid (1995). Fewer papers have been published on synbiotics in comparison to prebiotic and probiotics but have increased in popularity with over 140 papers published on synbiotics in 2016 (Krumbeck et al., 2018). The majority of these papers are in rodents or *in vitro*. The few papers conducted in humans mainly found improvements in post-operation infection, lower development of sepsis, reduced stay in hospital and shorter duration of antibiotic use (Kinross et al., 2013; Sawas et al., 2015).

A reduction in inflammation can be beneficial for many disease states such as ulcerative colitis which is chronic inflammation of the gastrointestinal tract. Furrie et al. (2005) found that ulcerative colitis patients had a reduction in inflammation markers such as tumour necrosis factor alpha and interleukin 1 alpha and that epithelial tissue started to regenerate after consumption of Synergy1 (6 g) and *Lactobacillus longum* (2×10^{11} CFU/mL). Metabolic diseases may benefit from consumption of synbiotics with fructo-oligosaccharides (10 g) and *L. salivarius* (2×10^{9} CFU/mL) reducing inflammation, total cholesterol and low-density lipoproteins (Rajkumar et al., 2015). There is also some evidence that a mixture of probiotics (*Streptococcus thermophilus*, *L. bulgaricus* and *Bifidobacterium lactis*) and inulin can diminish diarrhoea (Ringel-Kulka et al., 2015). The majority of these human studies lack adequate controls which lead to inconclusive data on whether the synbiotic is more beneficial than the prebiotic or probiotic alone. Additionally, most of these human studies did not assess the gut microbiota communities after the intervention.

Therefore, the aim of the study was to determine whether the carbohydrates WD, PHGG and inulin or probiotics *L. acidophilus* and *B. lactis* alone generate a more favourable gut bacteria community than when combined. A secondary aim was to assess organic acid production from the prebiotics and probiotics alone and combined.

MATERIALS AND METHODS

Subjects

Three healthy volunteers (30 ± 2 years old) donated faecal samples. The volunteers had no history of gastrointestinal disorders and had not consumed antibiotics in the last three months or prebiotic/probiotic in the last two weeks. Ethical approval was obtained from University of Reading Research Ethics Committee.

Faecal Sample and Incubation Protocols

Participants brought a fresh faecal sample (<3 h) to the laboratory in anaerobic conditions (<1% O_2 and 9–13% CO_2) (AnaeroJarTM 2.5 L and AnaeroGemTM, Thermo Fisher Scientific Oxoid Ltd, Basingstoke, Hampshire, UK). The sample was diluted with phosphate-buffered saline (PBS) 10% (w/v) (pH 7.4) and homogenised (Stomacher 400, Seward, West Sussex, UK) for 2 min at 240 paddle beats per minute. Twelve vessels were inoculated with 15 mL of faecal slurry with a total working volume of 300 mL. These vessels were prepared as follows. Each vessel received 135 mL autoclaved basal medium (Peptone water 2 g, yeast extract 2 g, NaCl 0.1 g, K_2HPO_4 0.04 g, KH_2PO_4 0.04 g, $MgSO_4.7H_2O$ 0.01 g, $CaCl_2.6H_2O$ 0.01 g, $NaHCO_3$ 2 g, Tween 80 2 mL, haemin 0.05 g, Vitamin K 10 μL, L-cysteine HCL 0.5 g and bile salt 0.5 g per litre) (Sigma, St. Louis, MO), and incubated overnight in anaerobic conditions (oxygen-free nitrogen at a rate of 15 mL/min). The following day the prebiotic and probiotic bacteria were added to the vessels as outlined below.

V1 – Blank,
V2 – Wheat dextrin (3.6 g),
V3 – Partially hydrolysed guar gum (3.6 g),
V4 – Inulin (3.6 g),
V5 – *B. lactis* HN019 (6×10^7 CFU/mL),
V6 – *L. acidophilus* NCFM (5×10^7 CFU/mL),
V7 – Wheat dextrin and *B. lactis* HN019 (6×10^7 CFU/mL),
V8 – Partially hydrolysed guar gum and *B. lactis* HN019 (6×10^7 CFU/mL),
V9 – Inulin and *B. lactis* HN019 (6×10^7 CFU/mL),
V10 – Wheat dextrin and *L. acidophilus* NCFM (5×10^7 CFU/mL),
V11 – Partially hydrolysed guar gum and *L. acidophilus* NCFM (5×10^7 CFU/mL) and
V12 – Inulin and *L. acidophilus* NCFM (5×10^7 CFU/mL).

Wheat dextrin (Benefiber, GSK, Warren, New Jersey, USA) is a resistant dextrin containing non-digestible α-1,2 and α-1-3 linkages with a degree of polymerisation between 12 and 25 and average molecular weight 4000–6000 Da (Noack et al., 2013). Partially hydrolysed guar gum (Resource Optifiber, Nestlé Health Science, London, UK) comprises of galactose (α-1-6 bonds) and mannose units (β-1-4 bonds) (Noack et al., 2013). Inulin has an average degree of polymerisation of 12 and is made up of fructose joined by ß-(2-1) linkages (Roberfroid, 2005).

The probiotic *B. lactis* HN019 (Danisco Brazil, Cotia) are anaerobic, Gram-positive, non-spore forming bacteria which are safe for human consumption (GRAS, 2012). The probiotic *L. acidophilus* NCFM (Danisco Brazil, Cotia) is a rod-shaped, non-motile, non-spore forming lactic acid bacteria. *Lactobacillus*

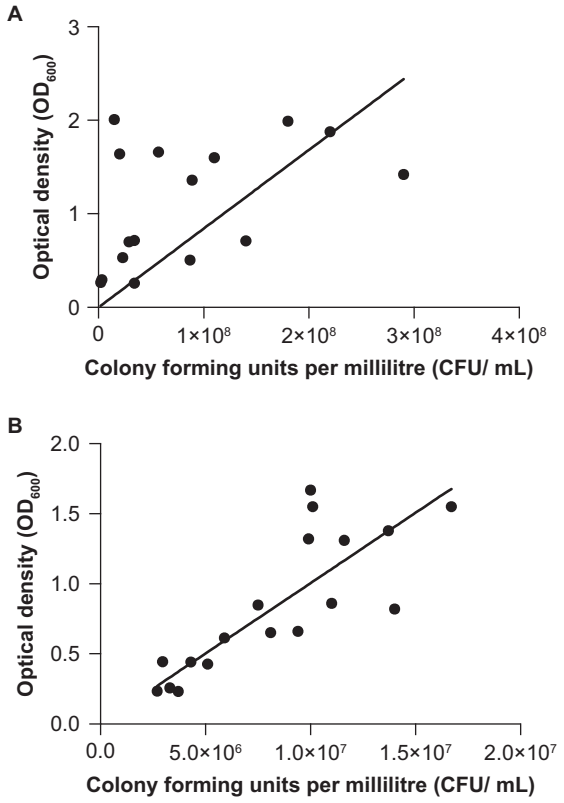

FIGURE 1 | The growth curve of probiotics *B. lactis* HN019 (A) and *L. acidophilus* NCFM (B) correlating the optical density against the colony forming units per millilitre (CFU/mL). The figures show a range of concentrations of probiotic diluted with phosphate-buffered saline grown on de Man-Rogosa-Sharpe for the NCFM strain and de Man-Rogosa-Sharpe supplemented with 0.05% (w/v) cysteine for the HN019 strain after 24 h in anaerobic conditions at 37°C in triplicate.

is safe to consume and is found in a range of dairy products (GRAS, 2010). The HN019 and NCFM strains were freeze dried and stored at −4°C prior to use. The strains were grown in anaerobic conditions at 37°C in de Man-Rogosa-Sharpe (MRS) (Oxoid Ltd, Basingstoke, Hampshire, UK) broth and de Man-Rogosa-Sharpe supplemented with 0.05% (w/v) cysteine (MRS-C, Sigma, St. Louis, MO), respectively. The probiotics were grown at different dilutions in triplicate for 24 h to produce a growth curve of optical density (OD_{600}) against colony forming units per millilitre (CFU/mL) to determine a dilution factor for the batch culture experiment to obtain $5 \times 10^7 \pm 1 \times 10^7$ CFU/mL (Fig. 1). One day prior to inoculating the vessels, strains were grown in the above conditions for 24 h then on the experimental day diluted with PBS according to the growth curve dilution factor and immediately added to the vessels.

Incubation Conditions and Sampling

The vessels were maintained at body temperature (37°C) through a circulating water bath and jacket around the fermenters at a pH between 6.7 and 6.9. They were continuously stirred throughout the experiment.

A 750 µL sample was collected from each vessel after 0, 8, 24, 48 and 72 h. The samples were centrifuged at 1,136 × *g* for 5 min. The resulting pellet was used for bacterial enumeration through fluorescence *in situ* hybridisation flow cytometry (FISH-FCM) and 500 µL of supernatant used to assess organic acid production via gas chromatography. The method used to analyse FISH-FCM and organic acid production are reported in detail elsewhere (Wang et al., 2019). The sequence of the bacterial probes is listed in Table 1.

Statistical Analyses

All statistical analyses used SPSS version 25 (SPSS Inc, Chicago, Ill, USA). The FISH-FCM and organic acid production were analysed using two-way mixed ANOVA to compare different test substrates and time points. Where significant differences were found, a post-hoc analysis was performed using Tukey multiple comparison tests. A paired *t*-test was used to further analyse the difference between vessels at each time point. Statistical analysis was accepted at $P < 0.05$ for all analyses.

RESULTS

Effect of WD in Combination with *B. lactis* HN019 and *L. acidophilus* NCFM on Bacterial Growth

The WD vessels significantly increased total bacterial count by 0.91 $\log_{10}$ at 24 h ($P = 0.046$) (Fig. 2). There was also a significant increase in lactic acid bacteria, (0.42 $\log_{10}$ cells/mL increase, $P = 0.046$), *Bacteroides* (0.9 $\log_{10}$ cells/mL increase, $P \leq 0.035$), *Clostridium coccoides–Eubacterium rectale* (0.9 $\log_{10}$ cells/mL increase, $P \leq 0.035$), *Roseburia* (0.7 $\log_{10}$ cells/mL increase, $P = 0.001$) and *Clostridium* cluster IX (1.7 $\log_{10}$ cells/mL increase, $P = 0.035$). The vessel with HN019 and WD generated higher *Bacteroides* (0.78 $\log_{10}$ cells/mL increase, $P = 0.024$) compared to the WD vessels. This was elevated further in the NCFM and WD vessels with an increase of 1.86 $\log_{10}$ cells/mL ($P = 0.010$). Additionally, in the NCFM and WD vessels there was a significant increase in *C. coccoides–E. rectale* (1.03 $\log_{10}$ cells/mL increase, $P \leq 0.038$) which increased more rapidly in the first 8 h than the WD vessels. The count of *Clostridium* cluster IX (1.39 $\log_{10}$ cells/mL increased, $P = 0.049$) significantly increased in the NCFM and WD vessels but at an overall lower count than the WD vessels.

Effect of Partially Hydrolysed Guar Gum in combination with *B. lactis* HN019 and *L. acidophilus* NCFM on Bacterial Growth

PHGG significantly increased total bacteria at 8 h and 24 h ($P \leq 0.047$), *Bifidobacterium* spp., at 24 h and 72 h ($P \leq 0.032$), *Bacteroides* at 24 h ($P = 0.08$), *C. coccoides–E. rectale* at 48 h ($P = 0.048$), *Atopobium* spp., at 48 h ($P = 0.022$) and *Clostridium* cluster IX at 8 h ($P = 0.034$) (Fig. 2). The HN019 and WD vessels significantly increased at 24 h ($P = 0.044$) at a similar amount to the PHGG vessel. The HN019 and WD vessel also increased in *Bacteroides* ($P = 0.04$) but at a quicker rate (8 h) and higher count (0.3 $\log_{10}$ cells/mL higher) than the PHGG vessels. The NCFM and PHGG vessels significantly increased in *Bifidobacterium* spp., at 8 h ($P = 0.017$) by 0.26 $\log_{10}$ cells/mL more than the PHGG vessels. *Atopobium*

TABLE 1 | Bacterial probe names and DNA sequences used to detect common gut bacterial groups with validation references.

Probe names	Sequences (5′ to 3′)	Target groups	References
Non-Eub	ACTCCTACGGGAGGCAGC		Wallner et al. (1993)
Eub338 I	GCT GCC TCC CGT AGG AGT	Most bacteria	Daims et al. (1999)
Eub338 II	GCA GCC ACC CGT AGG TGT	Planctomycetales	Daims et al. (1999)
Eub338 III	GCT GCC ACC CGT AGG TGT	Verrucomicrobiales	Daims et al. (1999)
Bif164	CAT CCG GCA TTA CCA CCC	Most *Bifidobacterium* spp. and *Parascardovia denticolens*	Langendijk et al. (1995)
Lab158	GGTATTAGCAYCTGTTTCCA	Most *Lactobacillus*, *Leuconostoc* and *Weissella* spp.; *Lactococcus lactis*; all *Vagococcus*, *Pediococcus* and *Paralactobacillus* spp., *Melisococcus*, *Tetragenococcus*, *Catellicoccus*, *Enterococcus*	Harmsen et al. (1999)
Bac303	CCA ATG TGG GGG ACC TT	Most Bacteroidaceae and Prevotellaceae, some Porphyromonadaceae	Manz et al. (1996)
Erec482	GCT TCT TAG TCA RGT ACCG	Most of the *Clostridium coccoides–Eubacterium rectale* group (*Clostridium* clusters XIVa and XIVb)	Manz et al. (1996)
Chis150	TTATGCGGTATTAATCTYCCTTT	Most of the *C. histolyticum* group (*Clostridium* clusters I and II)	Franks et al. (1998)
Rrec584	TCA GAC TTG CCG YAC CGC	*Roseburia* subcluster	Franks et al. (1998)
Prop853	ATT GCG TTA ACT CCG GCAC	Clostridial cluster IX	Walker et al. (2005)
Ato291	GGT CGG TCT CTC AAC CC	*Atopobium, Colinsella, Olsenella and Eggerthella* spp.; *Cryptobacterium curtum*; *M. equigenitalium* and *Mycoplasma elephantis*	Harmsen et al. (2000)
Fprau655	CGCCTACCTCTGCACTAC	*Faecalibacterium prausnitzii* and related sequences	Hold et al. (2003)
DSV687	TAC GGA TTT CAC TCC T	Most Desulfovibrionales and many Desulfuromonales	Devereux et al. (1992)

spp., was significantly elevated in the NCFM and PHGG vessels ($P \leq 0.27$) 0.73 $\log_{10}$ cells/mL higher than the PHGG vessel.

Effect of Inulin in Combination with *B. lactis* HN019 and *L. acidophilus* NCFM on Bacterial Growth

There was a significant increase in *Bifidobacterium* spp., ($P \leq 0.035$) and *Atopobium* spp., ($P \leq 0.015$) in the inulin-containing vessels (Fig. 2). The HN019 and inulin vessels had a significantly higher count of *Bifidobacterium* spp., ($P \leq 0.039$) at 24 h having a 1.76 $\log_{10}$ cells/mL increase. The HN019 and inulin vessels had a 0.23 $\log_{10}$ cells/mL higher amount of *Bifidobacterium* spp., than the inulin vessels alone. The lactic acid bacteria count significantly increased in the HN019 and inulin vessels ($P = 0.049$) and also in the NCFM and inulin vessels ($P = 0.017$). The NCFM and inulin vessels also increased total bacteria ($P = 0.018$), *Bacteroides* ($P \leq 0.015$), *C. coccoides–E. rectale* ($P = 0.026$) and *Atopobium* spp., ($P = 0.022$).

Effect of WD in Combination with *B. lactis* HN019 and *L. acidophilus* NCFM on Organic Acid Production

Acetate production significantly increased at all time points in the WD vessels ($P \leq 0.028$) and HN019 and WD ($P \leq 0.045$) vessels and NCFM and WD vessels ($P \leq 0.037$) (Fig. 3). Butyrate production significantly increased in the WD vessels ($P \leq 0.026$) and NCFM and WD vessels ($P \leq 0.046$). The WD vessels had the highest amount of acetate (increased by 102 mM from baseline) and butyrate (increased by 14.66 mM from baseline) compared to WD with probiotic strains. Propionate production was significantly increased in the WD, HN019 and WD and NCFM and WD vessels with a similar increase from baseline in each 41.6 mM, 54.99 mM and 45.81 mM, respectively.

Effect of Partially Hydrolysed Guar Gum in Combination with *B. lactis* HN019 and *L. acidophilus* NCFM on Organic Acid Production

Acetate was significantly increased in the PHGG vessels in all conditions with PHGG and HN019 significantly increasing at 24 h (increased by 94.75 mM from baseline, $P = 0.021$) until the end of incubation. However, PHGG and PHGG with NCFM vessels increased significantly (increased by 94.75 mM and 92.11 mM from baseline, respectively) ($P \leq 0.039$) and at 48 h until the end of the study (Fig. 3). Butyrate production significantly increased from 24 h ($P \leq 0.027$) until the end of the study at 72 h ($P \leq 0.027$) in all PHGG conditions with very similar production across all PHGG vessels ranging from an increase of 20.43 mM to 27.05 mM. Propionate production significantly increased at 24 h and 72 h in the PHGG vessels (increased by 37.29 mM from baseline, $P \leq 0.046$), 24 h, 48 h and 72 h in the PHGG and NCFM vessels (increased by 48.15 mM from baseline, $P \leq 0.048$) and at 72 h in the PHGG and HN019 vessels (increased by 44.8 mM from baseline, $P = 0.036$).

Effect of Inulin in Combination with *B. lactis* HN019 and *L. acidophilus* NCFM on Organic Acid Production

Inulin-containing vessels significantly increased acetate at 8 h (acetate increased by 49.08 mM from baseline, $P = 0.021$) until the end of the incubation (acetate increased by 78.87 mM from baseline, $P = 0.031$). However, the inulin and probiotic vessels did not show significance until 48 h ($P \leq 0.033$), which persisted until the end of the experiment ($P \leq 0.024$) (Fig. 3). Inulin and HN019 produced the highest amount of acetate with an increase of 107.36 mM compared to baseline. Butyrate production was significantly elevated at 24 h (increased by 26.94 mM compared to baseline, $P = 0.036$) and remained elevated for the duration of the experiment (time point

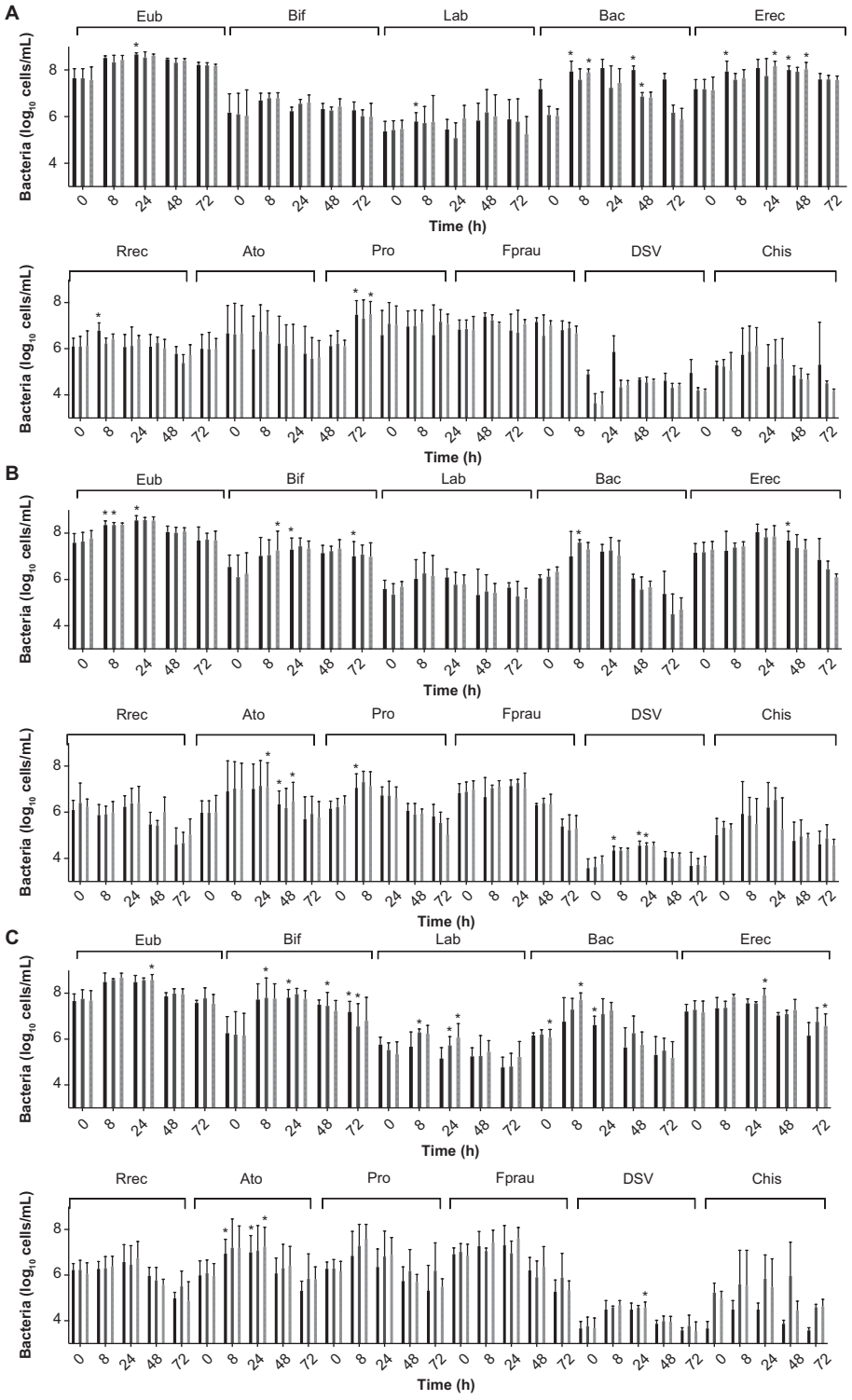

FIGURE 2 | Bacterial enumeration (log_{10} cells/mL) from *in vitro* batch culture after fermenting for 0, 8, 24, 48 and 72 h. The bacteria analysed were total bacteria (Eub), *Bifidobacterium* spp. (Bif), lactic acid bacteria (Lab), *Bacteroidaceae* and *Prevotellaceae* (Bac), Clostridial cluster XIVa and XIVb (Erec), *Roseburia* (Rrec), *Atopobium* spp. (Ato), Clostridial cluster (Pro), *Faecalibacterium prausnitzii* (Fprau), *Desulfovibrionales* and *Desulfuromonales* (DSV) and *Clostridium histolyticum* group (Chis). (A) Highlights the results after fermenting WD (wheat dextrin), WD and HN019 (*B. lactis* HN019) and WD and NCFM (*Lactobacillus acidophilus* NCFM). (B) Shows bacterial counts after fermenting PHGG (partially hydrolysed guar gum), PHGG and HN019 (*B. lactis* HN019) and PHGG and NCFM (*L. acidophilus* NCFM). (C) Displays results after fermenting inulin, inulin and HN019 (*B. lactis* HN019) and inulin and NCFM (*L. acidophilus* NCFM). Data are presented as mean ± SD ($n = 3$) and * shows significant difference ($P \leq 0.05$) compared to the baseline (T0).

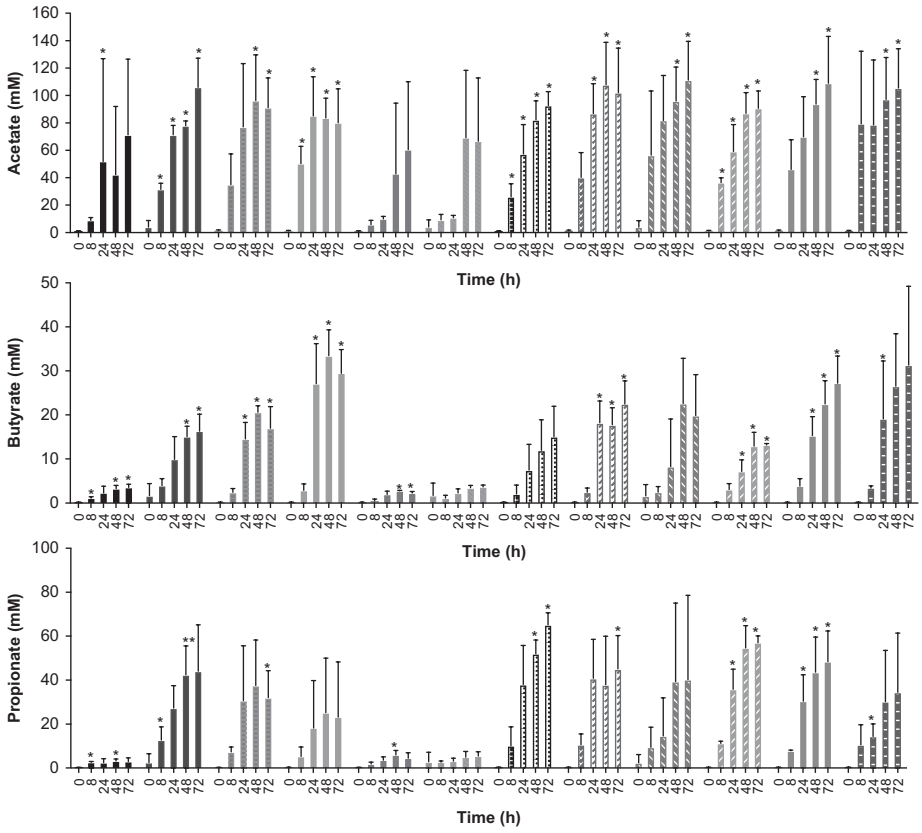

FIGURE 3 | Organic acid production (mM) from *in vitro* batch culture fermentation. From left to right blank, WD (wheat dextrin), PHGG (partially hydrolysed guar gum), inulin, HN019 (*Bifidobacterium lactis* HN019), NCFM (*Lactobacillus acidophilus* NCFM), WD and HN019, PHGG and HN019, inulin and HN019, WD and NCFM, PHGG and NCFM and inulin and NCFM at 0, 8, 24, 48 and 72 h. Organic acids measured were acetate, butyrate and propionate. Data are presented as mean ± SD ($n = 3$) and * shows significant difference ($P \leq 0.05$) compared to the baseline (T0).

48 h had the highest increase in acetate by 33.3 mM from baseline, $P = 0.010$) in the inulin vessels. The only significant increase ($P = 0.049$) in propionate production was in the inulin and NCFM vessels at 24 h with a small increase from baseline of 14.18 mM.

DISCUSSION

Overall, the main findings were that inulin when combined with a probiotic significantly prolonged the increase in bifidobacteria compared to inulin alone and significantly increased lactic acid bacteria, *Bacteroides* and *C. coccoides–E. rectale*. Both WD and PHGG did not have any further benefit in terms of bacterial metabolism profile when combining the dietary fibre with a probiotic.

Inulin is a recognised prebiotic as it reaches the colon intact where it is then selectively fermented principally by bifidobacteria (Roberfroid et al., 1998). However, the degree of degradation depends on the specific strain of bifidobacteria and some strains are able to degrade all chain lengths (DP 12–25). The strain used in the present study is HN019 which has previously been grouped with *B. adolescentis* LMG 10734, which can degrade oligofructose with a low DP but not inulin (Moens et al., 2016).

A recent study showed that HN019 degraded inulin at a slower rate in comparison to fructo-oligosaccharides (partially hydrolysed inulin [DP 2–8]) but was unable to degrade oligosaccharides greater than DP 7, therefore some of the inulin in the current study may have been degraded as the DP was from 2 to 65 (Sims et al., 2014). Similarly, in a study on 60 healthy volunteers with predisposition to constipation were fed *B. lactis* GCL2505 and inulin and there was a significant increase in *B. longum* and *B. adolescentis*, but interestingly not *B. lactis* (Anzawa et al., 2019). This fact was supported by Rossi et al. (2000) showing that the bifidobacterial strains *B. longum*, *B. thermophilus and B. adolescentis* were able to degrade inulin. This demonstrates that other bifidobacteria strains may need to be present in the colon to aid degradation of inulin and allow the HN019 strain to ferment the short chains once released (De Vuyst and Leroy, 2011; Falony et al., 2009; Roberfroid et al., 1998).

This aligns with the current findings as in the inulin vessel *Bifidobacterium* spp., was significantly elevated early in the study and higher than the PHGG and WD vessels, resulting in early production of acetate via fructose-6-phosphate shunt and therefore significantly higher production of butyrate compared to baseline (Rossi et al., 2005). It could also have been due to butyrate-producing bacteria such as *Faecalibacterium prausnitzii, E. rectale–C. coccoides* XIVa and XIVb and *Roseburia*. In this study, production may have been from *E.–C. coccoides* XIVa and XIVb as these were elevated during the study (Duncan et al., 2002; Riviere et al., 2016).

In the presence of inulin and HN019, a delayed increase in *Bifidobacterium* spp., and acetate production occurred compared to the inulin vessel therefore leading to an increase in butyrate

production, but this was found to be insignificant. Therefore, the inulin may determine which bacterial metabolism occurs and a mixture of probiotics is important to increase influence on bacterial communities present.

Neither WD nor PHGG showed any further bacterial metabolism with the addition of HN019 or NCFM and there appeared to be no cross-feeding to enhance bifidobacteria or lactic acid bacteria. However, as mentioned in a previous prebiotic fermentation experiment (Pyle et al., 2020, unpublished), WD and PHGG both significantly increased in *Bacteroides* and *Clostridium* cluster IX, which have been shown to produce propionate, which was elevated in all vessels containing WD and PHGG but not inulin.

Propionate can play a role in appetite regulation by stimulating peptide tyrosine and glucagon-like peptide-1 hormones which lead to a reduction in energy intake and therefore aid weight loss (Psichas et al., 2015). This means that WD and PHGG may be effective for weight management. In 60 overweight volunteers, propionate was delivered by an inulin-propionate ester resulting in weight reduction highlighting its ability for weight management (Chambers et al., 2015). There have been synbiotic randomised, double-blind, placebo-controlled trials (RCT) (Anzawa et al., 2019; Childs et al., 2014; Krumbeck et al., 2018; Min et al., 2012). To our knowledge no other study has combined *L. acidophilus* NCFM and inulin or *B. lactis* HN019 and inulin. However, other RCT have used the above prebiotic and probiotics in different combinations and these have positively impacted common gastrointestinal problems.

The NCFM and HN019 strains combined with polydextrose have been found to shorten transit time in patients suffering from constipation and increase stool frequency in elderly volunteers after consuming NCFM and lactitol (Magro et al., 2014; Ouwehand et al., 2019). This was supported by Waitzberg et al. (2013) as improved transit time, stool consistency and shape were found after consuming fructo-oligosaccharides (FOS) with a mixture of probiotics: *L. paracasei* (Lpc-37), *L. rhamnosus* (HN001), *L. acidophilus* (NCFM) and *B. lactis* (HN019). Additionally, inulin in combination with the probiotics *S. thermophilus*, *L. bulgaricus* and *B. lactis* has been shown to decrease incidence of diarrhoea (Ringel-Kulka et al., 2015). Furthermore, in adults, inulin in the form of a synbiotic can also reduce risk factors (high sensitivity C-reactive protein) for developing cardiovascular diseases and reduce serum insulin concentrations (Asemi et al., 2014).

In children who were receiving antibiotic therapy, the risk of bacterial illness was reduced by 94.3% and an increased energy and weight gain occurred after consuming FOS and *L. acidophilus* (Schrezenmeir et al., 2004). Similar findings were reported in 316 children consuming FOS, *L. acidophilus* and *Bifidobacterium* spp., with a reduction in constipation, days ill and increase in weight gain (Fisberg et al., 2002).

A few studies that have been mentioned earlier have used a single strain as their probiotic intervention. More research is required to fully understand the relationship between each bacterial strain to assess the pathways and cross-feeding of bacteria within the gut to be able to optimise functionality.

CONCLUSION

Overall, the addition of probiotics HN019 and NCFM may help to enhance inulin bacterial metabolism and cross-feeding between bacteria allowing a prolonged increase in *Bifidobacterium* and lactic acid bacteria. However, WD and PHGG may be more optimal alone and may be important in weight management. Therefore, further analysis of inulin and HN019 and inulin and NCFM in a continuous three-stage gut model system will be used to assess the impact of the synbiotic at each stage of the colon.

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