ORIGINAL ARTICLE

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Effects of *Bifidobacterium animalis* ssp. *lactis* 420 on gastrointestinal inflammation induced by a nonsteroidal anti-inflammatory drug: A randomized, placebo-controlled, double-blind clinical trial

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Aims: Use of nonsteroidal anti-inflammatory drugs (NSAIDs) can cause damage to the gastric and duodenal mucosa. Some probiotics have proven useful in ameliorating the harmful side-effects of NSAIDs. Our aim was to evaluate whether oral administration of *Bifidobacterium animalis* ssp. *lactis* 420 (B420) can attenuate the increase of calprotectin excretion into faeces induced by intake of diclofenac sustained-release tablets.

Methods: A double-blind, parallel-group, placebo-controlled and randomized clinical study was performed in 50 healthy male and female volunteers aged 20–40 years, in Finland. Study participation consisted of 4 phases: run-in, intervention with B420 or placebo, B420 or placebo + NSAID treatment, and follow-up. The primary outcome was the concentration of calprotectin in faeces. Secondary outcomes were haemoglobin and microbial DNA in faeces and blood haemoglobin levels.

Results: Intake of diclofenac increased the faecal excretion of calprotectin in both groups. The observed increases were 48.19 ± 61.55 μg/g faeces (mean ± standard deviation) in the B420 group and 31.30 ± 39.56 μg/g in the placebo group (difference estimate 16.90; 95% confidence interval: −14.00, 47.77; P = .276). There were no significant differences between the treatment groups in changes of faecal or blood haemoglobin. Faecal *B. lactis* DNA was much more abundant in the B420 group compared to the placebo group (ANOVA estimate for treatment difference 0.85×10^9/g faeces; 95% confidence interval: 0.50×10^9, 1.21×10^9; P < .0001).

Conclusions: Short-term administration of the probiotic B420 did not protect the healthy adult study participants from diclofenac-induced gastrointestinal inflammation as determined by analysis of faecal calprotectin levels.

KEYWORDS
clinical trial, gastrointestinal inflammation, nonsteroidal anti-inflammatory drug, probiotic

1 | INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat pain and inflammation, but their usefulness is limited by their propensity to cause damage to the upper gastrointestinal tract and to induce gastric and duodenal mucosal erosions and ulcers that may result in gastric or intestinal bleeding.[1,2] NSAIDs damage the upper gastrointestinal tract by inhibiting the activity of cyclooxygenases (COX-1 and COX-2), which leads to a shortage of prostaglandins[1] that act as cytoprotectants in the upper gastrointestinal tract. Prostaglandin deficiency increases gastric acid secretion, decreases mucus formation and mucosal blood flow and increases permeability of the epithelium. NSAIDs can cause mitochondrial dysfunction and apoptosis of the gut epithelial cells and production of reactive oxygen species, damaging the tight junctions of the gut epithelium. The increased permeability of the gastrointestinal epithelium allows the translocation of bile acids and bacterial molecules such as lipopolysaccharide from the gut lumen to the bloodstream and tissues. This activates the innate immune surveillance system via Toll-like receptors and up-regulates the inflammasome, causing cytokine secretion, inflammation and mucosal injury.[3]

The gut microbiota may have a role in the intestinal damage induced by NSAIDs.[4] Germ-free rats were protected from indomethacin-induced small intestinal damage.[5] In addition, gnotobiotic rats mono-associated with *Bifidobacterium* or *Lactobacillus* spp. did not develop ulcers when exposed to an NSAID, whereas gnotobiotic rats mono-associated with *Eubacterium limosum* or *Escherichia coli* developed ileal ulcers after NSAID administration.[6] Some studies have shown that co-administration of antimicrobial drugs can inhibit NSAID-induced small intestinal damage.[6–9] Several probiotic strains have been found to alleviate the NSAID-induced intestinal damage. In rats, a 1-week treatment with *Lacticaseibacillus casei* Shirota attenuated indomethacin-induced small intestinal damage and suppressed neutrophil infiltration in histological preparations and inflammatory cytokine expression in intestinal tissue.[10] A clinical trial with healthy volunteers receiving indomethacin showed that a probiotic mixture called VSL#3, containing *L. casei*, *Lactiplantibacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium infantis* and *Streptococcus thermophilus* decreased the faecal excretion of calprotectin compared to placebo.[11] Calprotectin is a neutrophil-specific protein that is commonly employed as a quantitative measure of neutrophil flux into the intestine, and thus serves as a noninvasive biomarker of intestinal inflammation. Furthermore, *L. casei* was found to reduce the number of mucosal breaks in the intestine of patients on chronic low-dose aspirin undergoing repeated capsule endoscopy in a small randomized pilot study.[12]

Bifidobacterium animalis ssp. *lactis* 420 (B420) has been reported to induce COX-1 expression in Caco-2 cells in vitro,[13] which might counteract the NSAID-induced COX inhibition. In addition, the integrity of tight junctions was improved in Caco-2 cells treated with B420.[13,14] In rats, treatment with B420 reduced the indomethacin-induced increase in gastric mucosal permeability and also reduced the

What is already known about this subject

- Nonsteroidal anti-inflammatory drugs (NSAIDs) cause gastrointestinal damage that may be influenced by gut microbiota.
- Prior to this study, it was unclear whether probiotics could be used to prevent the damage.

What this study adds

- *Bifidobacterium animalis* ssp. *lactis* 420 is safe to use during NSAID administration but it does not alleviate the NSAID-induced inflammation in the gastrointestinal tract in healthy adults.

incidence of severe indomethacin-induced lesions.[15] These results led us to hypothesise that B420 could be used during and after NSAID treatment to potentially protect the gastrointestinal tract from NSAID-induced harm.

2 | METHODS

2.1 | Ethical statement

The Ethics Committee of the Hospital District of Southwest Finland approved the study protocol and the National Agency for Medicines (Competent Authority for regulating pharmaceuticals in Finland) was notified before the start of the study. The study was performed in accordance with the guidelines of Good Clinical Practice and the Declaration of Helsinki. All participants provided written informed consent before any study related procedures were undertaken. The study was registered in the European Clinical Trials Database with the code 2005–005796-15, but as a phase I trial, it is not publicly available.

2.2 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[16,17]

2.3 | Participants and study design

This was a single-centre, double-blind, parallel-group, placebo-controlled and randomized clinical trial performed in 50 healthy, nonsmoking male and female volunteers aged 20–40 years.

Study participation consisted of 4 phases. After the screening visit, a run-in phase of 14 to 20 days was started, during which the consumption of concomitant treatments, i.e. pharmaceuticals, herbal remedies, trace elements, health products, vitamins, fibre-enriched products, and functional foods and dietary supplements containing probiotics was prohibited. The second phase was an intervention phase, during which half of the study population received capsules containing the probiotic B420 (DSM 22089) and the other half received corresponding placebo capsules for 14 days. In the third phase, the probiotic/placebo was co-administered for another 14 days together with the NSAID, diclofenac. Study participation was concluded with a 2-week follow-up phase for monitoring of possible adverse events (AE), recovery of faecal inflammatory markers, and for the presence of B420 DNA in the faeces. At the end of each study phase, faecal samples were collected for determination of calprotectin and haemoglobin levels and for the isolation of microbial DNA. Blood haemoglobin levels were also monitored. The study included 4 visits to the study centre, and the duration of the study participation was approximately 8 weeks for each volunteer.

Participants were instructed to consume 2 capsules daily with 0.1 g (equivalent to 10^{11} colony forming units, CFU) of lyophilized B420 per capsule or placebo capsules containing maltodextrin. As this was a proof-of-principle study, a fairly high dose of the probiotic was used. The participants consumed 1 capsule in the morning at breakfast and 1 in the evening with approximately 200 mL of a liquid dairy product of their choice, with approximately 12 hours between the 2 doses. Both products were produced by Danisco, were labelled similarly and were identical in taste and appearance. Diclofenac 75-mg tablets (Voltaren Retard, manufactured by Novartis) were taken twice daily together with the B420 or placebo product. Compliance with product intake was monitored with participant diaries that were checked at all study visits. The diclofenac dosage used in this study is the recommended dosage for pain.

2.4 | Inclusion and exclusion criteria

The participants had to fulfil the following criteria: signed informed consent obtained; good general health ascertained by detailed medical history; clinical examination; electrocardiogram (ECG) and laboratory determinations; showing no signs of clinically significant pathology; males and females aged 20–40 years; body weight at least 56 kg; body mass index 20–29 kg/m^2; pulse rate between 50 and 90 beats/min; systolic blood pressure 95–150 mmHg; diastolic blood pressure 55–90 mmHg; and reliable contraception for women of childbearing potential.

A participant was excluded if they had any of the following: doubtful availability to complete the study; poor peripheral venous access; over 30 grams daily consumption of fibre; frequency of defecation < twice a week; excessive alcohol consumption or inability to refrain from alcohol consumption for the duration of the study; suspected current use of illicit drugs; daily use of nicotine-containing products; use of caffeine exceeding 600 mg/d; pregnancy or breastfeeding; history of hypersensitivity or allergies to the study products; participation in another clinical trial or donation of blood within 60 days before the screening visit; severe lactose intolerance or milk protein allergy or other intolerance of milk products; or clinically relevant abnormality in 12-lead ECG. Use of any medications that might influence the gastrointestinal tract was prohibited during the study, e.g. NSAIDs, antacids, iron supplementation, H2-receptor antagonists, proton-pump inhibitors, laxatives, antibiotics, oral corticosteroids and selective serotonin reuptake inhibitors.

2.5 | Faecal sample processing

At the end of each study phase, faecal samples were obtained for the determination of faecal dry matter, calprotectin, haemoglobin and microbial DNA levels. In total, 191 faecal samples were collected, of which 188 samples were analysed, omitting 3 samples from drop-out participants at run-in. Faecal samples were frozen as soon as possible at −20°C and kept frozen until analysis. For dry matter determination, approximately 1 g of faecal sample was weighed and dried at 105°C for 16–18 hours, cooled to room temperature (RT) in an exicator and reweighed.

2.6 | Determination of calprotectin in faeces

Calprotectin concentrations were measured with a commercial enzyme-linked immunosorbent assay kit (PhilCal Test, NovaTec Immundiagnostica GmbH, Dietzenbach, Germany) according to the manufacturer's manual from the soluble fraction of the faeces. First, 0.1 g of faeces was weighed, and 5 mL of extraction buffer was added. The tube was shaken vigorously for 30 seconds and then for 30 minutes at 1000 rpm at RT after which 1.5 mL of the homogenate was centrifuged at 10 000 g for 20 minutes at RT. The supernatant was stored frozen for later assays. Samples were diluted at 1:1.25–1:80, and 50 μL aliquots of standards, controls and samples were added into microtitre plate wells precoated with polyclonal rabbit antibodies specific for calprotectin. The plate was shaken on a horizontal shaker for 45 minutes at RT. The wells were then emptied by aspiration and washed 5 times with washing buffer. Then, 50 μL of anti-calprotectin antibody conjugated with alkaline phosphatase was added into the wells. After 45 minutes incubation, the washing steps were repeated and 100 μL of substrate solution was added into the wells and incubated at RT for 20–25 minutes in the dark. The enzyme reaction was stopped by adding 100 μL of 1 M NaOH stop solution and the absorbance was measured at a wavelength of 405 nm with a spectrophotometer. The concentrations of the calibration standards were plotted *vs.* their absorbance at 405 nm. The absorbance of each sample was determined with at least 2 different dilutions. The concentrations of the samples were calculated using a second-degree polynomial equation, and the results were expressed as μg/g of fresh weight of the sample.

2.7 | Determination of haemoglobin in faeces and blood

Haemoglobin in faeces was determined from 20–30 mg of a well-mixed faecal sample as described previously.[18] Following several extraction and purification steps, 200 µL of aqueous phase from the final step was transferred into a black 96-microwell plate and measured (excitation wavelength 399 nm; emission wavelength 614 nm) with a Fluoroskan Ascent instrument (Thermo Labsystems, Philadelphia, PA, USA). Porcine haemoglobin (H4131, Sigma-Aldrich, St. Louis, MO, USA) in Drabkin's reagent (D5941, Sigma-Aldrich) was used as a reference standard.

Blood haemoglobin was analysed at baseline and at the end of each study phase at a local hospital laboratory (Tykslab, Turku, Finland) with standard clinical laboratory methods after 10 hours fasting before sampling.

2.8 | Analysis of microbial DNA in faeces by quantitative polymerase chain reaction

B. lactis DNA was analysed in the faecal samples with a detection limit of 1000 bacteria per g of faeces. DNA was extracted and purified as described previously.[19] Faecal levels of total bifidobacteria and B. lactis DNA were determined with quantitative polymerase chain reaction (qPCR) as described previously.[20] In addition, the levels of Lactobacillus spp., Roseburia spp., Veillonella spp., Faecalibacterium prausnitzii, sulfate reducers, Collinsella aerofaciens, Blautia coccoides-Eubacterium rectale group, Bacteroidetes and Atopobium spp. DNA were analysed with similar methods. The qPCR primers and their references are listed in Table 1.

2.9 | Statistical analysis

All statistical analyses were done at 4Pharma Ltd, using SAS software version 8.2 (SAS Institute Inc., Cary, NC, USA).

2.9.1 | Sample size

The sample size was derived from a power calculation based on the assumed group difference in the change from baseline to the end of the NSAID treatment phase in calprotectin concentrations in faeces. Faecal calprotectin concentration data from 28 young

TABLE 1 Quantitative polymerase chain reaction primers used in the study with references

Bacteria	Primers	Probe	Reference
Atopobium spp.	FW 5′-ACCGCTTTCAGCAGGGA-3′		38
	REV 5′-ACGCCCAATGAATCCGGAT-3′		
Bacteroidetes	FW 5′-GGCGACCGGCGCACGGG-3′		20
	REV 5′-GRCCTTCCTCTCAGAACCC -3′		
Bifidobacterium spp.	FW 5′-CCTGGTAGTCCACGCCGTAA-3′	ATCCAGCATCCACCG	39
	REV 5′-CAGGCGGGATGCTTAACG-3′		
Blautia coccoides-Eubacterium rectale group	FW 5′-CGGTACCTGACTAAGAAGC-3′		38
	REV 5′-AGTTT(C/T)ATTCTTGCGAACG-3′		
Collinsella aerofaciens	FW 5′-CCCGACGGGAGGGGGAT-3′		40
	REV 5′-CTTCTGCAGGTACAGTCTTGA-3′		
Sulfate-reducing bacteria	FW 5′-GGCGCTGAAATGACCATGAT-3′	TTCGTGCCCGCCCG	41
	REV 5′-GGCCGTAACCGTCCTTGAA-3′		
Domain bacteria	FW 5′-TCCTACGGGAGGCAGCAGT-3′	CGTATTACCGCGGCTGCTGGCAC	42
	REV 5′-GGACTACCAGGGTATCTAATCCTGTT-3′		
Faecalibacterium (Fusobacterium) prausnitzii	FW 5′-CCCTTCAGTGCCGCAGT-3′		38
	REV 5′-GTCGCAGGATGTCAAGAC-3′		
Lactobacillus spp.	FW 5′-TGGAAACAGRTGCTAATACCG-3′		43
	REV 5′-GTCCATTGTGGAAGATTCCC-3′		
Roseburia spp.	FW 5′- GCATGACCTGGTGTGAA −3′		44
	REV 5′-TTGGGCCGTGTCTCAG-3′		
Veillonella spp.	FW 5′-A(C/T)CAACCTGCCCTTCAGA-3′		38
	REV 5′-CGTCCCGATTAACAGAGCTT-3′		
Bifidobacterium animalis group	FW 5′-ACCAACCTGCCCTGTGCACCG-3′		20
	REV 5′-CCATCACCCCGCCAACAAGCT-3′		

FW, forward primer; REV, reverse primer.

adult participants from a comparable study[21] were used in the calculation. In that study, healthy male and female volunteers took diclofenac slow-release 75-mg tablets twice daily for 2 weeks. The mean (standard deviation) increase in faecal calprotectin concentration induced by diclofenac was 60.7 (49.9) µg/g. Assuming 80% power ($\beta = 0.20$) and a 2-sided type I error rate of 5% ($\alpha = 0.05$), a 40.4 µg/g difference between the treatment group means was predicted to be detected with 25 participants per treatment arm.

2.9.2 | Primary efficacy measurements

Calprotectin concentrations in faeces were tabulated with descriptive statistics by treatment group and study phase. In addition, the mean concentration-time profiles were illustrated graphically for both treatment groups. A repeated measures analysis of variance (RMANOVA) model appropriate for a 2-treatment parallel group design was used to analyse the changes from baseline in calprotectin concentrations in faeces. The model included fixed effects of treatment group and study phase and treatment-by-phase interaction. The difference between the treatment groups (B420 vs. placebo) at the end of the NSAID treatment phase and a 95% confidence interval (CI) for the difference was estimated from the RMANOVA model using contrast estimates.

In addition, recovery and the possible difference in recovery between the treatment groups from the end of the NSAID treatment phase to the end of the follow-up phase was estimated from the RMANOVA model. This analysis was planned as a secondary efficacy analysis, and the same model was employed as with the primary efficacy variable.

As a sensitivity analysis, calprotectin concentrations were tabulated and compared (summary statistics and RMANOVA) without 2 outlying participants.

2.9.3 | Secondary efficacy measurements

Haemoglobin and microbial DNA in faeces and blood haemoglobin levels were analysed using similar summaries and RMANOVA models as described above for the primary efficacy variable. Reductions in blood haemoglobin levels below the lower limit of the reference range were summarized with descriptive statistics and analysed with Fisher's exact test.

2.9.4 | Adverse events

AEs were coded with the Medical Dictionary for Regulatory Activities (MedDRA) and tabulated by treatment group, system organ class and preferred term.

3 | RESULTS

3.1 | Recruitment, enrolment and participant flow

The first participant was screened on 8 March 2006 and the last participant completed the study on 26 June 2006. Of 66 screened participants 50 were included (Figure 1). The most common reasons for exclusion were low body weight ($n = 4$), elevated blood pressure ($n = 3$), concomitant medication ($n = 2$) and excessive use of caffeine ($n = 2$).

Three participants discontinued the study prior to the start of the NSAID treatment phase. The reasons for discontinuation were an unsuitable schedule of the study ($n = 2$) and positive faecal haemoglobin test results ($n = 1$). The mean duration of product intake was 28.3 days for B420 or placebo product and 14.1 days for NSAID in both B420 and placebo groups.

3.2 | Baseline health and demographics

Demographic information of the participants is presented in Table 2. All participants had normal findings on physical examination and ECG. Two participants in the B420 group and 3 participants in the placebo group were irregular users of nicotine. Two participants (8%) in the B420 group and 9 participants (36%) in the placebo group reported fibre consumption of 10–20 g/d while 23 participants (92%) in the B420 group and 16 participants (64%) in the placebo group reported fibre consumption of 20–30 g/d.

Several out-of-range laboratory values were observed in the screening haematology and plasma chemistry assessments, but none of them were considered clinically significant. Three participants in both groups had *Helicobacter pylori* antibody titres above the upper limit of the reference range, suggesting presence of a chronic *H. pylori* infection.

3.3 | Primary outcomes

3.3.1 | Calprotectin in faeces

Calprotectin concentrations in faeces were determined at the end of each study phase to monitor intestinal inflammation (Figure 2). Excretion of calprotectin increased in both groups during the NSAID phase (mean change from baseline to the end of the NSAID phase ± standard deviation in the B420 group was 48.19 ± 61.55 µg/g faeces and in the placebo group 31.30 ± 39.56 µg/g faeces). No statistically significant difference in calprotectin excretion was observed between the placebo and B420 groups at the end of the NSAID phase (estimate 16.90; 95% CI: −14.00, 47.77; $P = .276$). The range of individual calprotectin concentration values was large in both groups, i.e. 2–341 µg/g faeces in the B420 group and 1–164 µg/g faeces in the placebo group. Two participants in the B420 group had markedly
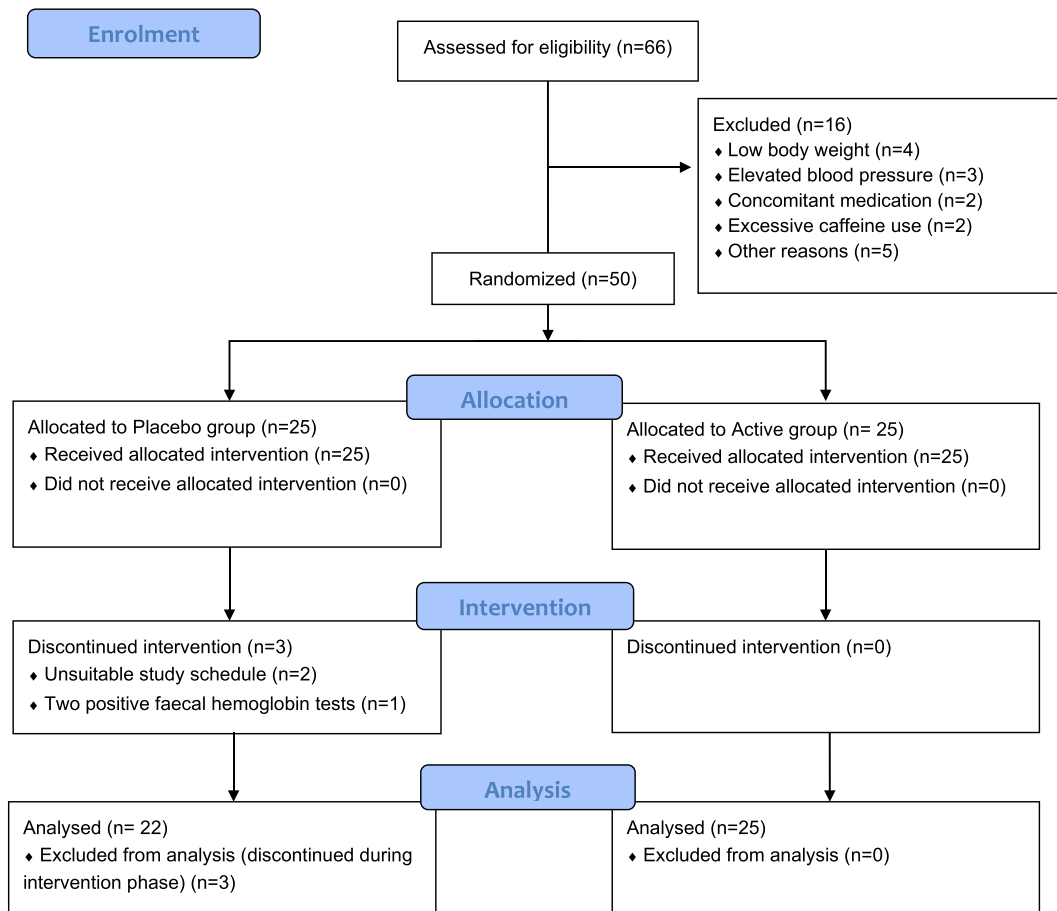
FIGURE 1 Consort flow diagram of the study

TABLE 2 Subject demographics and blood haemoglobin levels at baseline

	B420 group ($n = 25$)	Placebo group ($n = 25$)	Total ($n = 50$)
Age (y), mean ± SD	27.3 ± 5.0	27.6 ± 5.9	27.5 ± 5.4
Male, n (%)	11 (44%)	12 (48%)	23 (46%)
Ethnic origin Caucasian, n	25	25	50
Weight (kg), mean ± SD	70.2 ± 9.4	71.3 ± 10.3	70.8 ± 9.8
Height (cm), mean ± SD	172.9 ± 7.9	175.4 ± 8.1	174.1 ± 8.0
BMI (kg/m^2), mean ± SD	23.5 ± 2.5	23.1 ± 2.2	23.3 ± 2.3
B-Hb (g/L), mean ± SD	139.1 ± 11.4	142.0 ± 13.0	140.6 ± 12.1

SD, standard deviation; B420, *Bifidobacterium lactis* 420; BMI, body mass index; B-Hb, blood haemoglobin.

high calprotectin concentrations at the end of the NSAID phase and at the follow-up visit. Therefore, a sensitivity analysis of treatment and visit effects was performed with these 2 participants excluded. This did not change the outcome: no statistically significant group difference was found in changes from baseline in calprotectin concentrations (estimate 4.56; 95% CI: −18.94, 28.05; $P = .698$). Calprotectin concentrations in faeces decreased after the end of the NSAID treatment phase, indicating recovery of intestinal inflammation in both groups (B420 group estimate −29.34; 95% CI: −48.84, −9.83; $P = .004$; placebo group estimate −35.87; 95% CI: −56.66, −15.08; $P = .001$).

3.3.2 | Gastrointestinal symptoms

Gastrointestinal symptoms were coded according to MedDRA and are here reported separately from other AEs. Four participants in the B420 group and 5 participants in the placebo group reported gastrointestinal symptoms during the B420/placebo phase, and 15 participants in both groups had gastrointestinal-related symptoms during the NSAID phase (Table 3). In the follow-up phase, 2 participants in each treatment group reported gastrointestinal symptoms. No distinct patterns were observed for group differences in the occurrence of gastrointestinal-related AEs.

3.4 | Secondary outcomes

3.4.1 | Haemoglobin in faeces

As a secondary outcome, the amount of haemoglobin excreted in faeces was analysed at the end of each study phase (Table 4). No statistically significant differences b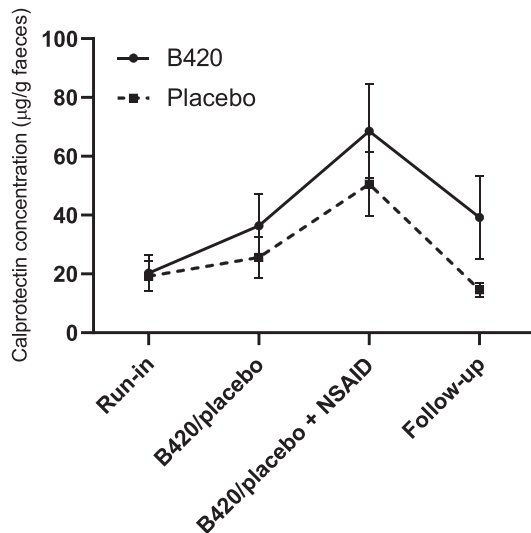etween the treatment groups were found in changes from the run-in phase to the NSAID phase (estimate 5.03; 95% CI: −2.14, 12.20; $P = .165$).

3.4.2 | *B. lactis* and bacterial DNA quantity in faeces

The quantity of *B. lactis* in faeces was analysed at the end of each study phase (Figure 3). The mean quantity ± standard error of *B. lactis* DNA in the B420 group was $1.39 \pm 0.18 \times 10^9$ /g faeces at the end of the B420 phase and $0.89 \pm 0.16 \times 10^9$/g faeces at the end of the NSAID phase. In the placebo group, the quantity of *B. lactis* DNA was $0.047 \pm 0.032 \times 10^9$/g faeces and $0.022 \pm 0.11 \times 10^9$/g faeces, at the end of the placebo and NSAID phases, respectively. The ANOVA estimate for treatment difference in the end of the NSAID phase was 0.85×10^9; 95% CI: 0.50×10^9, 1.21×10^9, $P < .0001$. The more abundant occurrence of *B. lactis* in faeces of the B420 group compared to placebo group indicated good product compliance. The other bacteria quantitated were *Lactobacillus* spp., *Roseburia* spp., *Veillonella* spp., *Faecalibacterium prausnitzii*, sulfate reducers, *Collinsella aerofaciens*, *Blautia coccoides–Eubacterium rectale* group, *Bacteroidetes* and *Atopobium* spp. No differences were found in any of these investigated bacterial quantities between the B420 and placebo groups (see Figure S1 for the qPCR results for each bacterial genus or species).

3.4.3 | Blood haemoglobin and faeces dry matter

Changes in blood haemoglobin concentrations from the run-in phase until the end of the NSAID phase were not significantly different

FIGURE 2 Calprotectin concentration in faeces (μg/g) as mean ± SE for the *Bifidobacterium lactis* 420 (B420) group (solid line; $n = 25$) and placebo group (dashed line; $n = 22$) for different study phases: run-in, intervention with either B420/placebo, intervention (B420/placebo) + NSAID and follow-up. NSAID, nonsteroidal anti-inflammatory drug

TABLE 3 Gastrointestinal symptoms during study phases 2 (B420/placebo), 3 (B420/placebo + nonsteroidal anti-inflammatory drug) and 4 (follow-up), summarized as event counts (subject counts). Symptoms and signs were classified according to System Organ Class as defined in the Medical Dictionary for Regulatory Activities

Symptom	B420 group			Placebo group			Total		
Phase	2	3	4	2	3	4	2	3	4
Abdominal pain	1 (1)	11 (8)	1 (1)	8 (3)	11 (8)	1 (1)	9 (4)	22 (16)	2 (2)
Diarrhoea	-	5 (4)	2 (2)	5 (3)	5 (5)	4 (1)	5 (3)	10 (9)	6 (3)
Nausea	2 (2)	2 (2)	-	-	3 (2)	5 (1)	2 (2)	5 (4)	5 (1)
Constipation	1 (1)	-	-	-	1 (1)	-	1 (1)	1 (1)	-
Dyspepsia	1 (1)	3 (2)	-	-	-	-	1 (1)	3 (2)	-
Vomiting	1 (1)	1 (1)	-	-	2 (2)	-	1 (1)	3 (3)	-
Gastrointestinal haemorrhage	-	2 (2)	-	1 (1)	5 (5)	-	1 (1)	7 (7)	-
Flatulence	-	2 (2)	-	-	-	-	-	2 (2)	-
Aphthous stomatitis	-	1 (1)	-	-	-	-	-	1 (1)	-
Haematochezia	-	1 (1)	-	-	-	-	-	1 (1)	-
Rectal haemorrhage	-	1 (1)	-	-	-	-	-	1 (1)	-
Abdominal pain, upper	-	-	-	-	1 (1)	-	-	1 (1)	-
Toothache	-	-	-	-	1 (1)	-	-	1 (1)	-
Total	6 (4)	29 (15)	3 (2)	14 (5)	29 (15)	10 (2)	20 (9)	58 (30)	13 (4)

B420, *Bifidobacterium lactis* 420.

TABLE 4 Haemoglobin in faeces (mg/g faeces)

Treatment group	Phase	n	Mean	SD	SE	Min	Median	Max
B420	Run-in	25	0.96	0.67	0.13	0.2	1.0	2.7
	B420	25	1.32	0.93	0.19	0.2	1.4	4.1
	B420 + NSAID	25	4.91	13.84	2.77	0.3	1.7	70.3
	Follow-up	25	1.89	1.43	0.29	0.2	1.8	7.1
Placebo	Run-in	22	3.47	9.74	2.08	0.3	1.0	45.9
	Placebo	22	1.11	0.90	0.19	0.3	0.8	3.5
	Placebo + NSAID	22	2.38	2.59	0.55	0.4	1.4	11.4
	Follow-up	22	1.74	1.18	0.25	0.3	1.4	4.2

SD, standard deviation; SE, standard error; B420, *Bifidobacterium lactis* 420; NSAID, nonsteroidal anti-inflammatory drug.
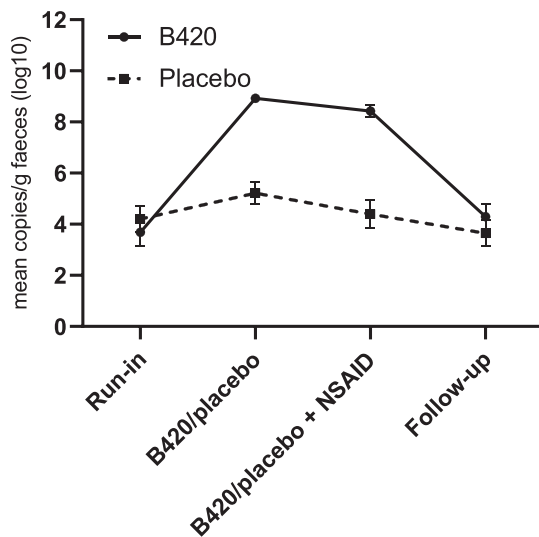
FIGURE 3 Mean quantity of *Bifidobacterium lactis* as $\log_{10}$ copies of DNA/g faeces ± SE for the *B. lactis* 420 (B420) group (solid line; $n = 25$) and placebo group (dashed line; $n = 22$) for different study phases: run-in, intervention with either B420/placebo, intervention (B420/placebo) + NSAID and follow-up. NSAID, nonsteroidal anti-inflammatory drug
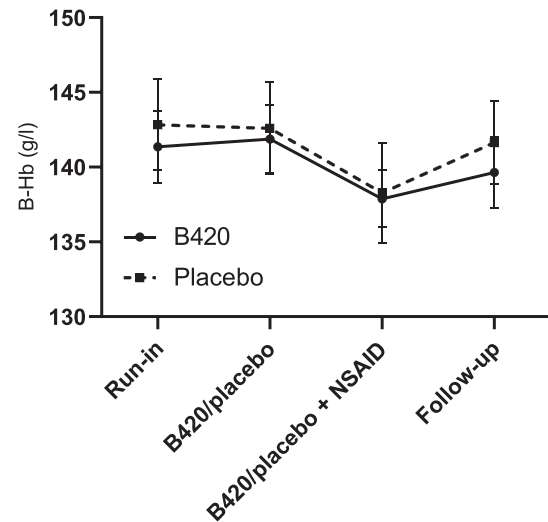
FIGURE 4 Blood haemoglobin concentrations (g/L) as mean ± SE for the *Bifidobacterium lactis* 420 (B420) group (solid line; $n = 25$) and the placebo group (dashed line; $n = 22$) in the different phases of the study: run-in, intervention with either B420/placebo, intervention (B420/placebo) + NSAID and follow-up. B-Hb, blood haemoglobin; NSAID, nonsteroidal anti-inflammatory drug

(estimate 1.07; 95% CI: −2.64, 4.77; $P = .565$) between the B420 (−3.48 g/L) and the placebo (−4.55 g/L) groups (Figure 4). The blood haemoglobin values tended to decrease in both groups during the NSAID treatment phase. Faecal dry matter excretion remained stable during the study, with no statistically significant differences between the groups at the end of NSAID phase (estimate 0.51; 95% CI: −2.84, 3.85; $P = .763$; Table S1).

3.5 | Adverse events

Altogether 243 AEs were recorded in 45 participants during the study (Table 5). The most common AE was headache. Out of the 105 AEs that were regarded as related to the study products, 82 were mild in intensity, 21 were moderate and 2 were considered severe. Both severe AEs were reported in the B420 group. One participant

reported severe abdominal pain lasting for 5 hours, starting 1.5 hours after study product (B420) intake on the fourth day of the B420 phase. Another participant had severe abdominal pain for 2 hours, starting 11 hours after study product intake (B420 + diclofenac) on the third day of the NSAID phase. Both events were resolved without medical intervention. No serious adverse events or other significant AEs occurred during the study.

4 | DISCUSSION

Faecal excretion of calprotectin is commonly used as a biomarker of intestinal inflammation in Crohn's disease and in the diagnostics and follow-up of ulcerative colitis.[22] It has also been utilized in clinical trials as an objective and quantitative biomarker of intestinal inflammation.[23] Faecal excretion of calprotectin may also be increased in

TABLE 5 The total number and the most common adverse events (AEs) during the study phases: 1 (run-in), 2 (B420/placebo), 3 (B420/placebo + nonsteroidal anti-inflammatory drug) and 4 (follow-up) showing event counts (subject counts)

Phase	B420 group				Placebo group				Total			
	1	2	3	4	1	2	3	4	1	2	3	4
Total AE	28 (12)	21 (11)	42 (18)	18 (10)	34 (18)	39 (14)	37 (16)	24 (8)	62 (30)	60 (25)	79 (34)	42 (18)
Headache	10 (6)	10 (6)	3 (3)	8 (5)	10 (8)	12 (7)	2 (2)	4 (3)	20 (14)	22 (13)	5 (5)	12 (8)
Abdominal pain	-	1 (1)	11 (8)	1 (1)	5 (2)	8 (3)	11 (8)	1 (1)	5 (2)	9 (4)	22 (16)	2 (2)
Diarrhoea	-	-	5 (4)	2 (2)	-	5 (3)	5 (5)	4 (1)	-	5 (3)	10(9)	6(3)

B420, *Bifidobacterium lactis* 420.

people with metabolic syndrome[24,25] and obesity.[26] Still, the day-to-day variation of faecal calprotectin excretion is considerable.[27] The present study employed a 4-phase, parallel-group, placebo-controlled design. All participants were exposed to a 14-day course of 75-mg sustained-release diclofenac tablets twice daily causing a considerable increase in their faecal excretion of calprotectin. Half of the study participants received the B420 test product and the NSAID, and the other half received placebo capsules and the NSAID. No statistically significant difference between the groups was detected in calprotectin excretion when B420 or placebo was co-administered with diclofenac. To our knowledge, this is the first clinical trial to investigate the effect of a probiotic on diclofenac-induced intestinal inflammation in healthy adults. Previous reports exist on the effects of probiotics on enteropathy induced by other NSAIDs such as indomethacin and aspirin.[11,12]

In a clinical trial with 20 healthy participants exposed to indomethacin, a multistrain probiotic product called VSL#3 was administered before and during indomethacin treatment, and was reported to reduce faecal calprotectin levels compared to placebo.[11] Compared to our study, the dose of NSAID was lower, with indomethacin administered at 50 mg/d (recommended dose range 50–150 mg/d), and it was only administered for 4 days, whereas in our study, 150 mg/d of diclofenac (recommended dose usually 100–150 mg/d) was administered for 14 days. It is possible that the multi-strain product given at the high dose of 900 billion CFU/d is more effective than the lower dose B420 in the prevention of NSAID-evoked enteropathy, or the shorter duration and/or lower dose of indomethacin may have been a less severe challenge to induce inflammation in the intestine than the course of diclofenac employed in our study. Another clinical trial used capsule endoscopy to evaluate *L. casei* as a treatment for low-dose aspirin-associated small bowel injury[12] and found 3-month treatment with *L. casei* to have efficacy. The study recruited participants with unexplained iron deficiency anaemia while using 100 mg of aspirin and 20 mg of omeprazole (proton-pump inhibitor) daily and showed that the number of mucosal breaks in the small bowel was reduced in *L. casei* treated participants compared to the control group.

Several clinical studies have shown positive effects of probiotics on calprotectin levels in various paediatric and adult patient populations. A recent study by De Loera Rodriguez *et al.* showed that *B. lactis* Bi-07 and *L. acidophilus* NCFM together with blue agave inulin decreased faecal excretion of calprotectin in patients with cervical

cancer.[28] Nine months of the probiotic treatment (*L. acidophilus*, *L. bulgaricus* and *B. lactis*) after restorative proctocolectomy reduced the number of patients with pouchitis, i.e. inflammation of the intestinal mucosa of the small intestine, and decreased faecal calprotectin levels.[29] Another study, however, did not find the probiotics *L. plantarum* 299 and *B. infantis* Cure21 effective in reducing faecal calprotectin in patients with poor ileal pouch function.[30] *L. rhamnosus* GG administration reduced faecal calprotectin and partially restored intestinal microbiota in children with cystic fibrosis.[31] Furthermore, administration of *Bifidobacterium breve* PS12929 and *Ligilactobacillus salivarius* PS12934 decreased faecal calprotectin excretion in low birth weight preterm infants.[32] Apparently, probiotics may have efficacy in reducing calprotectin excretion in some disease conditions where the normal gut microbiota is perturbed. There is evidence that patients with cystic fibrosis,[33] cervical cancer[34] and restorative proctocolectomy[35] may all have some form of dysbiosis in their gut microbiota. The participants in the present study were young, nonobese, healthy adults, and it may be that administration of a probiotic is not effective in reducing NSAID-induced faecal calprotectin excretion in this population with an assumedly normal and balanced gut microbiota.

The present results demonstrate that B420 was well tolerated. No accumulation of treatment emergent AEs or particular gastrointestinal symptoms in either of the treatment groups was evident. In this study, B420 did not seem to cause any disturbance to the analysed bacterial groups in faeces, representing the major inhabitants of the normal human gut microbiota, i.e. Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes phyla.[36] In a previous study with obese study participants, consumption of B420 did increase the relative levels of *Lactobacillus* and *Akkermansia* spp. in faeces after 6 months of intervention.[37] It may be that a longer intervention is needed to see changes in the faecal microbiota, or the microbiota of a healthy nonobese young adult is more resistant to modification with probiotic administration than the microbiota of an overweight participant.

In summary, this randomized, placebo-controlled, double-blind clinical study with healthy young adults did not find any significant effect of short-term consumption of the probiotic B420 on diclofenac-induced faecal calprotectin excretion or faecal or blood haemoglobin levels. However, administration of the probiotic together with an NSAID was safe and did not cause additional AEs to the

participants compared to placebo. It would be of interest to investigate the possible effects of B420 intake in participants with a dysbiotic microbiota who are using NSAIDs regularly because of e.g. chronic pain. In addition, more direct methods of investigation, such as endoscopy, could be used to study the effects of probiotic intervention on NSAID-induced gastrointestinal tract damage.

COMPETING INTERESTS

A.C.O., S.D.F., S.M.M. and K.T. are employees of Danisco Sweeteners Oy, IFF Health & Biosciences (Kantvik, Finland). IFF produces and markets *B. animalis* ssp. *lactis* 420. M.K., M.S. and V.L.L. were employees of the University of Turku at the time of clinical study conduct. The University of Turku (CRST) was contracted by Danisco Sweeteners Oy to carry out the clinical trial.

CONTRIBUTORS

Mäkelä S.M.: writing—original draft preparation. Forssten S.D.: writing—review and editing, formal analysis of qPCR data. Kailajärvi M., Langén V.L. and Scheinin M.: conducting a research and investigation process, writing—review and editing. Tiihonen K. and Ouwehand A.C.: writing—review and editing, conceptualization, supervision, project administration and resources.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.