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Lactobacillus acidophilus NCFM, Inulin, and Oat Bran Reduce TC and LDL-C in Adults with Hypercholesterolaemia

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ABSTRACT

The present study was carried out to examine the effect of daily intake of 20 × 10^9 CFU *Lactobacillus acidophilus* NCFM or 10 g inulin or 10 g beta-glucan (β-glucan) or synbiotic on fasting blood lipid levels in healthy adult men and women with moderately raised total plasma cholesterol (TC). This study was a randomized-controlled intervention in which 30 participants received either 10 g inulin or 10 g β-glucan or 20 × 10^9 CFU *L. acidophilus* NCFM or synbiotic for a period of 8 weeks. Fasting blood samples were collected before the supplementation period (baseline) and at week 8, with a follow-up at week 12. There was a trend for TC values, compared with baseline, to be lower in the probiotic group by 9.31%, (–0.55 mmol/l; P > 0.05), inulin group by 9.58%, (–0.53 mmol/l; P > 0.05), and β-glucan group by 8.55%, (–0.47 mmol/l; P > 0.05) at week 8. There was a trend for LDL-C values, compared with baseline, to be lower in the probiotic group by 9.34% (–0.34 mmol/l; P > 0.05), inulin group by 7.98% (–0.29 mmol/l; P > 0.05), and β-glucan group by 16.08% (–0.41 mmol/l; P > 0.05) at week 8. The changes were statistically insignificant but clinically significant in all groups except synbiotic, as all levels fell into the desirable biochemistry range. There were no statistical and clinical changes in the TC and LDL-C levels in synbiotic groups. There were no statistical and clinical changes in the HDL-C and TG levels in all groups. These data suggest that the intervention supplementation except synbiotics may improve blood lipid profiles, mainly TC and LDL-C.

INTRODUCTION

Cardiovascular disease (CVD) is one of the leading causes of death worldwide. The World Health Organization (WHO) has predicted that CVD will remain the leading cause of global death by 2030. High levels of serum cholesterol and low-density lipoprotein (LDL) cholesterol are major determining factors for CVD. Clinical and epidemiological studies have proved that an increased risk of CVD is highly correlated with hypercholesterolaemia. The risk of heart attack is threefold in people with elevated cholesterol levels compared to those with normal cholesterol levels.

Current recommendations for lowering serum cholesterol levels include dietary management, lifestyle modification, and medications (Stone et al., 2014). The AHA Prevention Guideline also reported that these measures are not always effective in lowering serum cholesterol levels. Some actions, such as cholesterol medication, may have adverse side effects on the patients. Many hypercholesteraemic people seek nonpharmacological approaches and natural products to improve their serum lipid profile. Probiotics, prebiotics, and dietary fibre are some of the most popular choices.

Probiotics, defined as living microorganisms, when administered orally in adequate amounts, may exert health benefits to the host beyond the inherent essential nutrition (Guarner & Schaafsma, 1998). Probiotics are safe for human consumption, and many probiotic products are available in the marketplace. Many studies have been conducted to investigate the role of probiotics as a hypercholesterolaemia lowering agent (Cho & Kim, 2015). In vitro and animal studies have supported the probiotic effect in reducing hypercholesterolaemia (Kim et al., 2017, Das et al., 2016). Probiotics are well recognized for their general health improvements, such as lactose intolerance alleviation, improvement of inflammatory and allergic reactions (Ouwehand, 2007)and the incidence is still rising with no sign of an end to this trend. Reduced exposure to microbial allergens as a result of our hygienic lifestyle has been suggested as one of the possible causes. It has also been suggested that probiotics may provide safe alternative microbial stimulation needed for the developing immune system in infants. This idea is supported by the fact that allergic infants have been observed to have an aberrant intestinal microbiota. They were shown to have more clostridia and fewer bifidobacteria and, in addition, to have an adult-like Bifidobacterium microbiota. Clinical trials have shown that the standard treatment of infants with atopic eczema, extensively hydrolyzed infant formula, can be significantly improved through the addition of Lactobacillus rhamnosus GG or Bifidobacterium lactis Bb-12. It has also been shown possible to halve the incidence of allergy in at-risk infants through administration of L. rhamnosus GG to expecting mothers and subsequently to their infants during the first half-year of life. Many mechanisms have been proposed for these beneficial effects, ranging from improved mucosal barrier function to direct influences on the immune system. However, the exact mode(s, anti-colon cancer effect (Hirayama & Rafter, 2000) and antihypertensive effects (Ayyash et al., 2018). More recently, probiotics have been studied for their cholesterol-lowering effect in human and animal studies (Michael et al., 2017; Ruscica et al., 2019). However, the studies on humans yielded inconsistent results with some showed positive effects, while others had no effect. With conflicting results, human randomized controlled trials may benefit in evaluating the cholesterol-lowering effect of probiotics.

Lactobacillus acidophilus NCFM is a human isolate bacteria used commercially for over 25 years as a probiotic culture (Sanders & Klaenhammer, 2001). The strain can survive in the gastrointestinal tract (Sanders & Klaenhammer, 2001; Sui et al., 2002), adhere to human epithelial cells (Greene & Klaenhammer,

1994), utilizes fructooligosaccharides (Barrangou et al., 2003), modulates the host immune response, and prevent microbial gastroenteritis (Varcoe et al., 2003). Analysis of the NCFM strain genome sequence revealed the presence of two putative bile-salt hydrolases (BSH) genes (Altermann et al., 2005).

The reduction of TC and LDL-C found in blood serum is thought to lower the risk of CHD. Probiotic cultures have been proposed to play a part in reducing cholesterol, although research to date is still equivocal. An in vitro study on *L. acidophilus* NCFM (Gilliland et al., 1985; Gilliland & Walker, 1990) proposed the ability of the strain to remove cholesterol from a laboratory growth medium. NCFM was reported to take up cholesterol in the presence of bile and the absence of oxygen, both conditions present in the colon. The significance of these in vitro studies has been proved in human studies to evaluate cholesterol levels in NCFM consumers.

Prebiotics are the alternative of cofactors to probiotics. Prebiotics are indigestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of one or a limited number of indigenous bacteria, thus promoting the host's health. Prebiotics can reduce cholesterol in many studies. A randomized controlled trial in hamsters treated with inulin resulted in a total reduction of 29% decrease in cholesterol (Delzenne et al., 2019). In a randomized controlled human study, inulin administered to 120 hypercholtoteraemic men had a mean significant reduction in cholesterol by 6.6% (Kietsiriroje et al., 2015).

Dietary fibres, which are neither absorbed nor digested, are subjected to bacterial fermentation in the gastrointestinal tract, thus impacting the composition of indigenous bacteria and their metabolic activities. In the meta-analysis of randomized controlled trials by (Ho et al., 2016), dietary fibres have been shown to exert cholesterol-lowering effects when consumed at recommended levels. In a randomized, double-blind study by study by Wolever et al. (2016), consuming 3 g of β-glucan for four weeks is effective in reducing human LDL-C levels.

Studies combining probiotics and prebiotics have been studied to a limited extent. Synbiotics may improve the survival of the upper GI bacteria, thereby enhancing their beneficial effect on the colon. According to the research conducted by Wang et al. (1999), the combination of resistant starch with probiotics has increased the survival of the probiotics. Remarkably, synbiotics can selectively stimulate growth and activate the limited number of health-promoting bacteria in the GI tract, thus improving the GI tract's microbial balance (Roberfroid, 2000). Prebiotics, probiotics, and synbiotics treatments are still in their infancy but are becoming the mainstream options to reduce hypercholesterolaemia.

MATERIALS AND METHODS

This study was a randomized controlled trial in adults with mild hypercholesterolaemia with fasting serum cholesterol levels ranging from 5.2 to 6.0 mmol/l. This study was a randomized-controlled intervention in which 30 adult participants between 23 to 66 years old (Table 1) received either 10 g inulin as prebiotic or 10 g β-glucan as dietary fibre, or 20×10^9 CFU *L. acidophilus* NCFM as probiotic or a combination of prebiotic and probiotic for the synbiotic effect of statin as a positive control group or diet counselling as a negative control group for a period of 8 weeks. Before supplementation intervention, the groups were comparable in weight, height, BMI, fasting blood glucose (Table 2), and nutritional status (Table 3). Fasting blood samples were collected before the supplementation period (baseline) and at week 8, with a follow-up at week 12. Volunteers were recruited from housing areas in the vicinity of Universiti Malaysia Sabah (UMS) through leaflets and

advertisements in Polyclinic UMS. Participants expressing interest in the study who met the initial screening criteria were invited for a blood screen after fasting 10 to 12 hours before the test. Exclusion criteria included immune-compromised disease, pre-existing medical conditions, recent use of lipid-altering medications such as statins, and pregnancy for female volunteers. The conditions and procedures of the study were reviewed, and written informed consent was obtained from each subject. The Medical Research Ethics Committee of the Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, approved the protocol. The ethics approval code is JEtika 3/14(2). Blood samples were collected from each participant's forearm for serum cholesterol levels on the day of each time frame (baseline, week-8 and week-12). Before the study, each participant was asked to fill out detailed diet records for 3 days (2 weekdays and 1 weekend). Nutrient calculations were performed using The Nutritionist Pro software (First Data Bank) to convert all reported foods and beverages into energy and nutrient intakes. If an analytical value is not available for a nutrient in a food, the values were calculated based on the nutrient content of other nutrients in the same food or on a product ingredient list or estimated the value based on the nutrient content of similar foods. Participants were advised not to change their eating habits throughout the intervention. Participants were instructed to maintain their usual level of activity during the study. They were instructed to note as specific as they could what they eat (the brand and, if possible, the preparation method of the food). The amount of food consumed was measured based on household measurements or grams. Participants were given symptom evaluation surveys to complete once during each intervention. All data were analysed using IBM SPSS Statistics 24 (SPSS Incorporated, USA). The mean differences between 3 or more groups of continuous variables were examined using one-way ANOVA with Tukey's-b post hoc test and Kruskal-Wallis test. The probability value of $P < 0.05$ was considered to be significant. The statistical test used to analyze the effect size was eta-squared (η^2). Size of effect: $0.01 \leq \eta^2 \leq 0.06$ (small), $0.06 \leq \eta^2 \leq 0.14$ (medium), $\eta^2 \geq 0.14$ (large).

Table 1 Socio-demographic characteristics of all volunteers

Characteristics	All (n = 30) N(%)
Sex	
Female	20 (66.7)
Male	10 (33.3)
Ethnic groups	
Kadazandusun	14 (6.7)
Malay	11 (36.7)
Chinese	2 (6.7)
Others	3 (10.0)
Education level	
Primary education	5 (16.7)
Secondary school	11 (36.7)
A-Level/ STPM	4 (13.3)
Bachelor's degree	8 (26.7)
Master's degree	1 (3.3)
PhD	1(3.3)
Income (RM/month)	
< 2,000	18 (60.0)
2,000 – 3,000	2 (6.7)
3,001 – 4,000	3 (10.0)
4,001 – 5,000	2 (6.7)
> 5,000	5 (16.7)
Marital status	
Single	7 (23.3)
Married	22 (73.3)
Widow/ Widower	1 (3.3)

Table 2 Baseline data of anthropometric and fasting blood glucose (FBG) measurements by treatment groups

Treatment group	Prebiotic	Probiotic	Dietary fibre	Synbiotic	Statin (Control)	Diet counselling (Control)
All, n = 30						
N (%)	6	5	5	5	4	5
Weight (kg)	66.7±7.64	69.70±10.72	58.12±13.97	68.66±13.88	71.67±13.43	57.76±11.60
Height (m)	1.56±0.07	1.53±0.02	1.57±0.10	1.56±0.01	1.60±0.56	1.52±0.08
BMI (kg/m2)	27.34±1.23	29.83±4.60	23.03±3.82	28.11±5.21	27.79±3.90	24.64±3.61
FBG	4.83±0.45	4.54±0.36	5.14±0.39	5.07±0.89	5.00±0.85	5.14±0.30

Data presented as Mean±SD.

Table 3 Baseline data of energy and macronutrients on all treatment groups

Treatment group/ Energy/ macronutrients	Prebiotic, N = 6	Probiotic, N = 5	Dietary Fibre, N = 5	Synbiotic, N = 4	Statin, N = 4	Diet Counselling, N = 5	Total mean, N = 29
Energy (kcal/day)	1,359±140	1,834±763	1,281±262	1,910±891	1,027±327	1,372±315	1,445±553
Carb (g/day)	172.2±37.6	220.1±76.9	183.4±27.7	276.7±141.7	151.0±52.3	195.2±36.2	196.7±73.5
Protein (g/day)	63.5±19.7	86.2±50.9	51.2±13.5	67.4±25.4	47.6±10.4	55.7±8.4	61.5±26.9
Fat (g/day)	44.7±10.2	57.5±38.5	39.2±16.5	61.4±37.0	22.6±9.2	43.7±24.6	44.1±25.7
Dietary fibre (g/day)	5.0±1.8	7.8±5.8	7.6±4.9	3.1±2.7	4.1±2.1	3.0±1.5	5.3±3.9
Cholesterol (mg/day)	180.6±103.7	370.3±291.8	210.3±105.8	207.7±189.1	111.8±66.7	182.6±68.4	211.6±165.0
Mono fat (g/day)	8.2±4.2	11.7±7.8	8.1±2.2	13.1±11.5	4.2±1.8	7.9±3.6	8.7±6.0
Poly fat (g/day)	8.2±1.1	9.4±5.4	8.0±3.9	13.2±14.6	4.6±1.6	8.3±4.7	8.4±6.2
Trans fat (g/day)	0.3±0.5	0.4±0.9	1.2±1.9	0.7±1.2	0.0±0.1	0.7±1.3	0.6±1.2
Saturated fat (g/ day)	7.2±4.8	13.8±14.0	10.0±5.7	13.5±11.7	2.8±1.3	7.4±3.3	9.1±8.3

Data presented as Mean±SD.

Table 4 Mean cholesterol level throughout the intervention

Treatment group	Cholesterol, mmol/L			% Difference (Wk 0 – Wk 8)	CI value (95%)	P-value	Eta-squared
	Wk 0	Wk 8	Wk 12				
Pre	n = 6	n = 6	n = 6				
	5.53±0.18	5.00±0.56	4.95±0.53	–9.58	5.33 – 5.72	0.08	0.28
Pro	n = 5	n = 4	n = 4				
	5.69±0.47	5.16±1.16	5.55±0.77	–9.31	5.11 – 6.28	0.63	0.09
DF	n = 5	n = 5	n = 5				
	5.50±0.22	5.03±0.83	4.90±0.70	–8.55	5.24 – 5.77	0.33	0.17
Syn	n = 5	n = 3	n = 4				
	6.45±0.56	6.93±0.33	6.51±0.45	+7.44	5.75 – 7.14	0.40	0.18
Statin	n = 4	n = 4	n = 4				
	5.62±0.28*	4.01±0.49*	5.31±0.90*	–28.65	5.18 – 6.06	**0.01***	0.63
DC	n = 5	n = 5	n = 5				
	5.63±0.21	5.27±0.40	5.12±0.35	–6.40	5.36 – 5.89	0.08	0.34

Data presented as Mean±SD.

Values with the mark (*) in a row for each analysis are significantly ($P < 0.05$) different. Eta-squared was analyzed by comparing means for eta-squared score. Size of effect: $0.01 \leq \eta^2 \leq 0.06$ (small), $0.06 \leq \eta^2 \leq 0.14$ (medium), $\eta^2 \geq 0.14$ (large). $\eta^2 = 0.28$

Table 5 Mean LDL-C level throughout the intervention

Treatment Group	LDL-C, mmol/L			% Difference (Wk0-Wk 8)	CI value (95%)	p-value	Eta-squared
	W0	Wk 8	Wk 12				
Pre	n = 6	n = 6	n = 6				
	3.63±0.22	3.34±0.54	3.29±0.51	–7.98	3.40 – 3.86	0.39	0.12
Pro	n = 5	n = 4	n = 4				
	3.64±0.19	3.30±0.90	3.59±0.55	–9.34	3.41 – 3.88	0.67	0.07
DF	n = 5	n = 5	n = 5				
	2.55±1.39	2.14±1.37	2.16±1.21	–16.08	0.83 – 4.27	0.86	0.02
Syn	n = 5	n = 3	n = 4				
	4.28±0.70	4.53±0.91[a]	4.40±0.72	+5.84	3.41 – 5.15	0.90	0.02
Statin	n = 4	n = 4	n = 4				
	3.93±0.29*	2.31±0.47*	3.25±0.79*	–41.22	3.48 – 4.39	**0.008***	0.66
DC	n = 5	n = 5	n = 5				
	3.07±0.92	3.55±0.27	3.28±0.33	+15.63	1.93 – 4.22	0.46	0.12

Data presented as Mean±SD.

Values with the mark (*) in a row for each analysis are significantly ($p<0.05$) different. Eta-squared was analyzed by comparing means for eta-squared score. Size of effect: $0.01 \leq \eta^2 \leq 0.06$ (small), $0.06 \leq \eta^2 \leq 0.14$ (medium), $\eta^2 \geq 0.14$ (large). $\eta^2 = 0.28$

Table 6 Mean HDL-C level throughout the intervention

Treatment Group	HDL-C, mmol/L			% Difference (W0- Wk 8)	CI Value (95%)	p-value	Eta-squared
	W0	Wk 8	Wk 12				
Pre	n = 6	n = 6	n = 6				
	1.29±0.32	1.14±0.26	1.08±0.32	Negligible	0.96, 1.63	0.45	0.10
Pro	n = 5	n = 4	n = 4				
	1.07±0.28	1.21±0.40	1.18±0.42	+13.10	0.72, 1.42	0.85	0.03
DF	n = 5	n = 5	n = 5				
	1.32±0.29	1.06±0.31	1.03±0.31	−19.70	0.97, 1.68	0.28	0.19
Syn	n = 5	n = 3	n = 4				
	1.01±0.24	1.17±0.47	1.06±0.32	+15.84	0.71, 1.30	0.81	0.05
Statin	n = 4	n = 4	n = 4				
	1.11±0.43	1.08±0.26	1.30±0.50	Negligible	0.42, 1.80	0.73	0.07
DC	n = 5	n = 5	n = 5				
	1.78±1.16	1.27±0.17	1.24±0.14	+28.65	0.34, 3.22	0.40	0.14

Data presented as Mean±SD.

Eta-squared was analyzed by comparing means for eta-squared score. Size of effect: $0.01 \leq \eta^2 \leq 0.06$ (small), $0.06 \leq \eta^2 \leq 0.14$ (medium), $\eta^2 \geq 0.14$ (large). $\eta^2 = 0.28$

Table 7 Mean triglyceride level throughout the intervention

Treatment group	Triglyceride, mmol/L			% Difference (W 0 – Wk 8)	CI value (95%)	p-value	Eta-squared
	Wk0	Wk 8	Wk 12				
Pre	n = 6	n = 6	n = 6				
	1.14±0.56	1.14±0.68	1.40±1.03	Negligible	0.55, 1.73	0.80	0.02
Pro	n = 5	n = 4	n = 4				
	1.81±0.61	1.50±0.42	1.83±0.54	−17.12	1.05, 2.58	0.62	0.08
DF	n = 5	n = 5	n = 5				
	2.27±1.54	2.54±1.25	2.52±1.37	+11.89	0.36, 4.18	0.94	0.01
Syn	n = 5	n = 3	n = 4				
	2.06±1.17	3.28±3.05	2.51±1.78	+21.84	0.61, 3.52	0.70	0.08
Statin	n = 4	n = 4	n = 4				
	1.19±0.42	0.90±0.30	1.03±0.46	−24.37	0.52, 1.85	0.61	0.10
DC	n = 5	n = 5	n = 5				
	1.22±0.38	0.98±0.44	1.02±0.53	−19.67	0.74, 1.70	0.68	0.06

Data presented as Mean±SD.

Eta-squared was analyzed by comparing means for eta-squared score. Size of effect: $0.01 \leq \eta^2 \leq 0.06$ (small), $0.06 \leq \eta^2 \leq 0.14$ (medium), $\eta^2 \geq 0.14$ (large). $\eta^2 = 0.28$

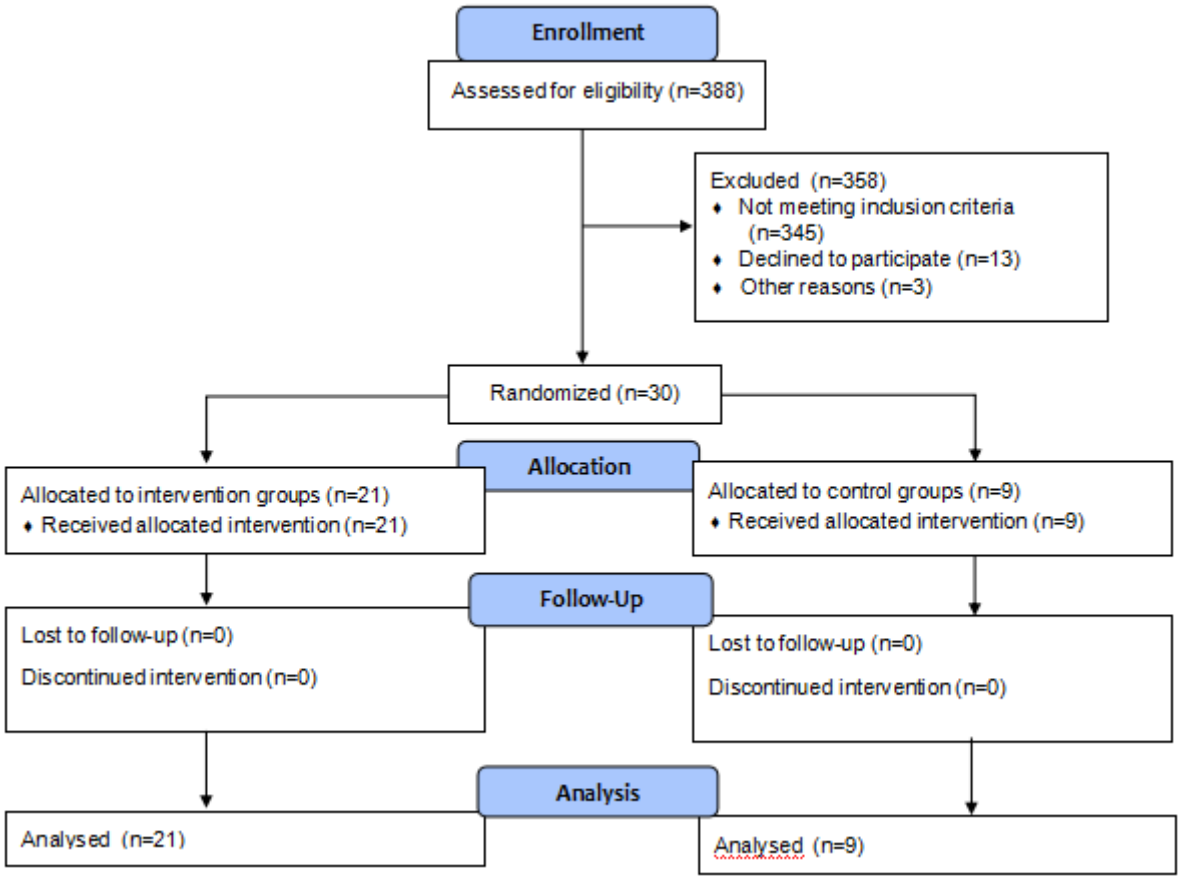

Figure 1 Participants' recruitment flow

RESULTS

Analysis of Participants' Baseline Habitual Diets

The participants' baseline habitual diets (Table 3) revealed a mean caloric intake of 1445 ± 553 kcal/day. The macronutrient breakdown is as follows: Protein: 61.5 ± 26.9 g/day (17% TEI). Fat: 44.1 ± 25.7 g/day (27.47% TEI). Carbohydrate: 196.7 ± 73.5 g/day (54.5% TEI). Dietary fibre (total): 5.3 ± 3.9 g/day. Saturated fat: 9.1 ± 8.3 g/day (5.67% TEI). Monounsaturated fat: 8.7 ± 6.0 g/day (5.42% TEI). Polyunsaturated fat: 8.4 ± 6.2 g/day (5.23% TEI). Trans fat: 0.6 ± 1.2 g/day (0.3% TEI). Dietary cholesterol: 211.6 ± 165.0 mg/day. After the 8-week intervention period, there were no significant differences in dietary intake among the 4 groups.

Analysis of Participants' Blood Lipid Profiles in All Timeline

For the cholesterol category, the trend towards intervention-related reduction was observed in the volunteers in all groups except for the synbiotic ($P > 0.05$) when compared with the statin (positive control) group. Except for the statin control group, all reduction was not statistically significant. Still, it was observed that after the intervention, not only did the cholesterol levels show an improvement, but all subjects except in the synbiotic group and negative control group (diet counselling) had cholesterol levels within the normal range, i.e., < 5.2 mmol/L, thus could be considered as clinically significant. In the prebiotic group, there was a 9.58% (–0.53 mmol/L; $P > 0.05$) reduction in cholesterol at week-8, from 5.53 ± 0.18 mmol/L to 5.00 ± 0.56. After the wash-out period, the cholesterol level maintained its desirable level

at 4.95 ± 0.53, which showed another slight decrease of 1.0% (–0.05 mmol/L; P > 0.05). This observation agrees with results reported by Jackson et al. (1999) that suggested the 8-week daily supplementation of 10 g inulin does not significantly lower cholesterol. However, in this study, it was found that the level of cholesterol entered the healthy/ desirable range, and the effect size (η^2) was 0.28 (large), thus making it clinically significant. In the probiotic group, there was a 9.31% (–0.55 mmol/L; P > 0.05) reduction in cholesterol level after week-8, from 5.69 ± 0.47 to 5.16 ± 1.16, and an increase of 7% (+0.39 mmol/L; P > 0.05) after the wash-out period. The cholesterol level improvement was medium according to the sample size effect score. The concentration fell into the desirable range of below 5.20mmol/l, thus could be considered clinically significant despite not being statistically significant compared to the statin control group. In the dietary fibre group, there was an 8.55% (–0.47 mmol/L; P > 0.05) reduction in cholesterol level after week-8, from 5.50 ± 0.22 to 5.03 ± 0.83 mmol/L. Even after the wash-out period, the improvement could be seen with another slight 2.60% reduction. 10 g/day of oat bran was an efficient therapy for clinically improving cholesterol in this study because there was a significant effect size (0.17), and cholesterol level improved into a desirable range of below 5.2 mmol/l. In the synbiotic group, there was a 7.44% increase in cholesterol (+0.48 mmol/L; P > 0.05) from 6.45 ± 0.56 to 6.93 ± 0.33 mmol/ L. The level reduced to its original level at baseline after the wash-out period. The result is statistically insignificant, as the cholesterol level remained in the 'high' range compared to baseline. The blood sample in the statin group could not be adequately studied due to instrumentation error, thus giving the researcher a questionable result.

Except for synbiotic and negative control (diet counselling group), reduction of LDL-C levels to below 3.4 mmol/L could be seen in all intervention groups. Similar to cholesterol levels, the reduction was seen after the intervention was not statistically significant but could be considered clinically significant as the changes dropped to a more desirable biochemical range. In the prebiotic group, there was a 7.98% (–0.29 mmol/L; P > 0.05) reduction of LDL-C, from 3.63 ± 0.22 mmol/L to 3.34 ± 0.54, and a further 1.50% decrease to 3.29 ± 0.51 after the wash-out period. The result was found to be statistically insignificant. However, after the intervention period, the level of LDL-C improved from the 'borderline high' range in week-8 compared to baseline, and the reduction after wash-out, although small, improved the range to 'above normal.' The effect size score was also found to be medium (0.12). Thus, the improvement in LDL-C level could be suggested as clinically significant. For probiotic group, there was a 9.34% (–0.34 mmol/L; P > 0.05) reduction after week-8, from 3.64 mmol/L to 3.30 mmol/L. Similar to cholesterol, after the wash-out period, the level increased by 8.80% (+0.2 mmo/l; P > 0.05). This result was not statistically significant, but the effect size found was 0.07, suggesting a medium improvement of LDL-C despite the concentration still in the borderline high range after the intervention period. There was a 16.08% (–0.41 mmol/L; P > 0.05) reduction in LDL-C in the dietary fibre group, from 2.55 ± 1.39 to 2.14 ± 1.37 mmol/L after week-8, and the concentration was constant after the wash-out period. The result was not clinically significant because the effect size was small (0.02) and not statistically significant. However, LDL-C concentration was within the healthy range in week-8. There was a 5.84% increase from 4.28 ± 0.70 to 4.53 ± 0.91 mmol/L (+0.25 mmol/L, P > 0.05) for the synbiotic group and a small 2.87% reduction to 4.40 ± 0.72 mmol/L after the wash-out period. The result is statistically insignificant, as the cholesterol level remained in the 'high' range compared to baseline, and the effect size was small.

It was observed that all groups had clinically normal HDL-C levels at baseline, between 1.01 – 1.78 mmol/L and there were no statistically significant changes in HDL-C levels in any timeframe. HDL-C level did not show any significant changes in the prebiotics group in week-8 compared to baseline. The slight

change in HDL-C level (1.29 ± 0.32 to 1.14 ± 0.26 in week-8 and 1.08 ± 0.32 after the wash-out period) is negligible as the mean level of HDL-C remained to be above 1.00 mmol/L, which is the normal and healthy range for HDL-C in healthy humans (National Institutes of Health [NIH], 2002). There was a modest, statistically insignificant 13.10% increase in HDL-C (+0.14 mmol/L, P > 0.05) in the probiotic group after week-8, from 1.07 ± 0.28 to 1.21 ± 0.40 mmol/L, and the concentration was constant after the wash-out period. The concentration of HDL-C in all timelines was maintained in the 'normal' range. For the dietary fibre group, there was a 19.70% (–0.26 mmol/L; P > 0.05) reduction from 1.32 ± 0.29 to 1.06 ± 0.31 mmol/L in week-8 compared to the baseline. The concentration was constant after the wash-out period. However, despite the large effect size found, the reduction was not clinically significant, as HDL-C level remained in the healthy range (over 1.00 mmol/L) after the intervention. In the synbiotic group, there was a 15.84% increase from 1.01 ± 0.24 to 1.17 ± 0.47 mmol/L (+0.16 mmol/L; P > 0.05) after week-8 compared to baseline, and the level reduced to 1.06 ± 0.32 after the wash-out period by 9.40% (–0.11; P > 0.05). The changes are insignificant as the effect size was small. HDL-C levels remained in a healthy biochemical range.

The prebiotic intervention had a neutral effect on triglyceride levels which was in agreement with previous studies (Maki et al., 2012). Triglyceride levels do not show any changes at week-8 and after the wash-out period compared to the baseline level. The levels were identical at all time-frame. The present study could not demonstrate a positive effect from 10 g/day of inulin to decreasing triglyceride levels. Letexier et al. (2003) demonstrated triglyceride reduction efficacy of 10 g/day of inulin only when consumed with a low-fat, high-carbohydrate diet. Ten grams per day of inulin was found to be an efficient therapy to clinically improve cholesterol and LDL-C levels to desirable and healthy levels but had no effect on triglycerides and HDL-C levels in all time frames. The effect size for all the groups was either small or medium, with no significant effect, indicating all treatments did not clinically affect triglyceride levels. Triglyceride levels in the probiotic group had an insignificant decrease by 17.12% (–0.31 mmol/L; P > 0.05) from 1.81 ± 0.61 to 1.50 ± 0.42 in week-8. The level bounced back to its original level at 1.83 ± 0.54, which increased by 22%. Triglyceride level in week-8 was not clinically significant, as the reduction remained at an unhealthy level (NIH, 2002). Triglyceride in the dietary fibre group showed an 11.89% (+0.27 mmol/L; P > 0.05) increase from 2.27 ± 1.54 to 2.54 ± 1.25 mmol/L, and the level remained constant after the wash-out period. The result was clinically and statistically insignificant, as the triglyceride level remained in the 'high' range compared to baseline. Clinical trials have also confirmed that the consumption of β-glucans reduces total cholesterol levels, including LDL-C, without affecting HDL-C and triglyceride levels (Anderson et al., 2009). The increase in triglyceride concentration in the synbiotic group appeared to be big. Still, there were no significant differences statistically and clinically, as the level of triglycerides remained in the high biochemical range. It was seen that after the wash-out period, the concentration of triglyceride decreased to 2.51 ± 1.78 mmol/L, showing a smaller 21.84% change from baseline (–0.45 mmol; P > 0.005) compared with baseline to week-8.

The present study found mild to no abdominal symptoms from consuming the intervention products.

DISCUSSION

Ten grams per day of inulin was found to be an efficient therapy to clinically improve TC and LDL-C levels to desirable and healthy levels but had no effect on triglycerides and HDL-C levels in all time frames. HDL-C had statistically insignificant changes but remained in the healthy range above 1.00 mmol/l. This observation agrees with results reported by Jackson et al. (1999) that suggested the 8-week daily supplementation of 10 g inulin showed a positive effect on lowering cholesterol, although not significant

statistically. Another similar study (10 g/ day in a 12-week intervention) showed that a powdered inulin-based fibre supplement consumption did not significantly change the cholesterol level in volunteers diagnosed with hypercholesterolaemia (Bonsu & Johnson, 2012). However, in this study, it was found that the level of TC entered the healthy/ desirable range, thus making it clinically significant. In several studies, the effect of inulin consumption on HDL-C was either increased modestly or nonsignificant, so the evidence of inulin on HDL-C was found to remain inconclusive. The intervention had a neutral effect on triglyceride levels, which agrees with previous studies (Maki et al., 2012). Beneficial effects of inulin on lipid profile mainly mediated by short-chain fatty acid (SCFA). Butyrate inhibits liver cholesterol synthesis and provides a source of energy for human colon epithelial cells. Acetate may act as a precursor for cholesterol synthesis, while propionate could inhibit hepatic cholesterol synthesis by decreasing the use of acetate as a precursor of cholesterol. Also, inulin-type fructans may contribute to cholesterol reduction by increasing faecal bile acid excretion, reducing intestinal cholesterol absorption, and increasing the expression of 3-hydroxy-3 methylglutaryl-COA reductase (HMG-COA reductase) (Lye et al., 2010).

The present study examined the effects of an 8-week supplementation of *L. acidophilus* NCFM and *Bifidobacterium lactis* Bi-07 mixed powder on serum lipid levels in hypercholesteraemic participants. Based on a previous literature search, this study was the first to evaluate the effects of consumption of *L. acidophilus* NCFM strain on serum lipid concentrations *in vivo* (human trial). As the strain NCFM has never been studied in vivo before this research, no direct comparison could be made for this group. Comparison is made with other *L. acidophilus* subspecies, as in-vitro studies suggested that *L. acidophilus* may be more effective than different types of probiotics in reducing cholesterol (Klaver & Meer, 1993; Lye et al., 2010). Comparison is also made with in vitro and animal studies in place of in vivo studies; 20×10^9 CFUs *L. acidophilus* NCFM was efficient therapeutic to improve TC levels to desirable and healthy levels clinically but had modest effects on triglycerides and HDL-C levels in all time frames. LDL-C level is not clinically and statistically different because the concentration was still borderline high and above normal after the intervention period. In human clinical studies, there is no consensus on the effects of consumption of *Lactobacillus* strains on lipid profile. One of the earliest studies on the *Lactobacillus* strain has shown that 3 weeks of administration of 200 ml milk containing *L. acidophilus* L1 was associated with reducing cholesterol levels in hypercholesterolaemic individuals (Gilliland et al., 1985). However, a more recent study by Hove et al. reported that 12 weeks of intake of milk fermented with *L. helveticus* did not affect serum lipids. As the strain, NCFM has never been studied *in vivo*. This research proved that another subspecies of *L. acidophilus* could be associated with reducing cholesterol levels in hypercholesterolemic individuals. Comparison is made with other *L. acidophilus* subspecies, as studies suggested that *L. acidophilus* may be more effective than different types of probiotics in reducing cholesterol. *L. acidophilus* NCFM strain is often used in clinical trials in combination with *B. lactis* Bi-07 as formulated by the product's manufacturer. However, based on a genetic analysis study by many researchers, only the *L. acidophilus* NCFM strain posseses the bile-salt hydrolase gene, the enzyme needed to deconjugate bile acids via the bile metabolism process, thus aiding in cholesterol removal via fecal (McAuliffe et al., 2005). Increased excretion of bile acids should result in lower serum concentrations, which would decrease the amount of bile acids reaching the liver for secretion back into the intestine through enterohepatic circulation. To replace the excreted bile acids, more would have to be synthesized from cholesterol in the liver. Thus, it has been suggested that in a steady-state situation, deconjugation of bile acids could lead to the reduction of serum

cholesterol by increasing the formation of new bile acids or by reducing cholesterol absorption throughout the intestinal lumen (Pereira & Gibson, 2002). Cholesterol assimilation in vitro was shown by the appearance of cholesterol in the cells during growth which was associated with decreases in cholesterol concentration in the growth medium. This uptake of cholesterol occurred only when the culture was grown anaerobically in the presence of bile. The amount of bile required to enable the cultures to remove cholesterol from the growth medium was not in excess of the levels normally encountered in the human intestine (Gilliland et al., 1985). Thus, the conditions required in the in vitro system for cholesterol uptake by NCFM strain would also be expected to occur in the human intestinal tract. This should enable humans to assimilate at least part of the cholesterol ingested in the diet, thus making it unavailable for absorption into the blood. A similar action could be exerted on endogenous cholesterol in the intestines.

Clinical trials have confirmed that the consumption of β-glucans reduces total cholesterol levels, including LDL-C, without affecting HDL-C and triglyceride levels (Anderson et al., 2009). Despite the slight reduction in LDL-C, the result was not clinically and statistically significant, as the week-8 concentration is within the healthy range. Oat brans can bind with bile acids in the small intestine, therefore removing them from the body and decreasing the bile acid recycling activity. As a result, TC and LDL-C in the blood are reduced, while HDL-C and triglycerides are unaffected (Xie et al., 2015). In addition, oat brans are fermented in the colon into short-chain fatty acids (SCFAs) and gases. When SCFAs enter the circulatory system, they may regulate intestine hormones, inhibit the liver from producing cholesterol, and consequently have a direct cholesterol-lowering effect. Many researchers believe that short SCFAs, particularly propionate, might be involved in lowering cholesterol concentrations in serum and liver either by inhibiting hepatic cholesterogenesis and redistributing cholesterol from plasma to the liver (Pereira & Gibson, 2002; Colburn et al., 2012; Xie et al., 2015).

In the synbiotic group, all parameters showed an increase in concentration in week 8 compared with the baseline value. However, all deals dropped back to baseline concentration after week 12. The increase was statistically and clinically insignificant, as all concentrations remained in the same biochemical range. A study by Taghizadeh et al. (2014) found that no significant effects of the synbiotic food consumption on serum TC, LDL, HDL, and plasma TAC levels ($P > 0.05$) could be observed.

Clinical interpretation of treatment outcomes is essential because it influences clinical decision-making, including patient safety and efficacy. Clinical significance is the practical importance of the treatment effect, whether it has a natural, palpable, or noticeable impact on daily life. In this study, none of the results in any groups but statin therapy (positive control) showed a statistically significant improvement in lipid profiles. However, it does not automatically imply that the interventions given were not clinically effective, as the intervention effect offers more information for clinicians to assess the application of the research finding, including the magnitude and direction of the intervention outcome. According to Page (2014), clinically relevant changes in outcomes are identified (sometimes interchangeably) by several similar terms, including "minimal clinically important differences (MCID)," "clinically meaningful differences (CMD)," and "minimally important changes (MIC)." However, the researcher has a reason to believe that the samples in the synbiotic group had lower stability compared to other intervention groups, thus showing a higher concentration of all lipid profiles than the other groups, especially in week 8. This might be due to more extended storage of the samples at −40°C for baseline samples and week-8 samples. The models had to be rerun using a different chemistry analyser because

our faculty's chemistry analyser broke down in the middle of the intervention, creating another limitation, considering other analyser systems might have a diverse reference range for their lipid control values.

When the results in the statin control group were compared with the other intervention groups, it was found that the intervention groups (except synbiotic) maintained the cholesterol level changes even after the wash-out period ended, making the inclusion of intervention products to be a more viable recommendation for short-term treatment of Hypercholesterolaemia than a statin. It is also suggested that the inclusion of the products could continue improving the cholesterol level in the long run without having to consume cholesterol-lowering drugs (statins).

In the prebiotic group, only 1 volunteer reported mild flatulence during the inulin consumption period, while the others said no side effects. Other volunteers could tolerate inulin very well in their diets. Gastroenterology research by Azpiroz et al., 2017 also reported that inulin intake did not cause any increase in symptoms such as bloating and flatulence or other side effects. Plausibly, any positive response to inulin is related to the adaptation of microbiota activity, as inulin can promote the proliferation of more efficacious microbiota such as bifidobacteria, which can ferment residues using metabolic pathways with the lower gas release (Manichanh et al., 2014). Other pools of colonic microorganisms, particularly sulfate-reducing bacteria, acetogenic bacteria, and methanogenic archaea, consume largely the gases produced by fermentation.

In the dietary fibre group, 1 volunteer reported stomach discomfort from very mild bloating and slightly severe flatulence cases. The same volunteer also reported having mild diarrhoea. Quickly adding a large amount of oat bran has been found to cause digestive stress with symptoms such as diarrhoea, stomach discomfort, bloating, and flatulence (Mälkki et al., 2001). To prevent these side effects, it was recommended to slowly introduce foods like oat bran into your diet over a few weeks.

In probiotic and synbiotic groups, the volunteers experienced virtually no abdominal symptoms and reported a relatively high level of digestive comfort. Volunteers well tolerated the intervention products.

This study has some relevant limitations. The first one is the relatively low number of subjects investigated per treatment group, related to the low participation in the enrolment. Naing et al. (2006) suggested oversampling the calculated sample size required by 10 – 20% to consider the possibility of respondents withdrawing their participation during actual research. However, each arm had exactly 5 volunteers as calculated due to participation, time, and financial limitations. However, the study was sufficiently powered based on the sample size calculation. Secondly, the study was relatively short, so we do not know if the observed effect could be confirmed and improved in the long term. However, from the improvement of the cholesterol and LDL-C levels, we have no reason to doubt that this evidence could be translated to the intervention products. Due to limitations on instrumentation and blood sample availability, not all blood samples could be analysed. In the probiotic group, the limitation was due to the failure to draw blood from the volunteer's vein. Because it is an intrusive procedure, the blood drawing was stopped by the medical laboratory technologist to prevent further pain, which if continued, could lead to further problems such as withdrawal from the study. In the synbiotic group, the lack of blood samples was due to the condition of the blood and the instrumentation. The chemistry analyzer which was used to analyze the blood was found to be faulty, thus it did not print out the results needed. Because several attempts were made that used quite a large volume of blood serum, by the time the researcher sent

it to a private path lab, the volume needed for the analysis was insufficient, thus resulting in missing data. The data gathered in other intervention groups was good. A significant body of research aimed at understanding *L. acidophilus* NCFM at microbiological, genetic, and clinical levels has been conducted over the past 25 years. These studies have provided insight into the probiotic functionality of this strain. Furthermore, this strain has been used successfully in commercial applications, with a minimum of technological hurdles. Confirmation of the strain functionality in lowering cholesterol level will, however, require well-controlled clinical evaluations aimed at appropriate target populations and clinical end points.

CONCLUSION

In conclusion, the study findings suggested that a small lifestyle modification such as the introduction of 10 g oat bran, 20×10^9 CFUs *L. acidophilus* NCFM and *B. lactis* Bi-07, and 10g inulin could have a positive effect on the volunteers. The effect size analyzed in this study showed that the interventions had a medium to large effect on the volunteers' health, which suggested a clinically significant effect on the improvement of lipid profiles in hypercholesterolemic adults. The prebiotic group showed the highest improvement in total cholesterol and LDL-C levels, followed by the probiotic group and dietary fibre group respectively. The study was unable to observe beneficial results in lowering cholesterol levels in synbiotic intervention.

CONFLICT OF INTEREST

The authors declare that they have no competing interests in publishing this article.

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