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Impact of IDP® on Enhancing Immune Response to the Influenza Vaccine: A Double-Blind, Randomized, Placebo Controlled, Pilot Study

Rachel Page[1,*], Yongsijia Wei[2], Cheryl Gammon[2], Judy Thomas[3], Tasnima Akter[4], Katharine Helen Adam[5], Rodney Claycomb[5], Colin Roger Ogle[5] and Kay Rutherfurd-Markwick[2]

[1]School of Health Sciences, College of Health, Massey University, PO Box 756, Wellington 6140, New Zealand
[2]School of Health Sciences, College of Health, Massey University, Private Bag 102904, North Shore, Auckland 0745, New Zealand
[3]Department of Exercise Sciences, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand
[4]Centre for Addiction Research, Faculty of Medical and Health Sciences, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand
[5]Quantec Ltd, Waikato Innovation Park, P.O. Box 9466, Hamilton 3240, New Zealand

[*]**Corresponding Author:** Rachel Page, School of Health Sciences, College of Health, Massey University, PO Box 756, Wellington 6140, New Zealand, Tel.: ++64 275 350 615 , E-mail: r.a.page@massey.ac.nz

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Abstract

Background: Immune Defense Proteins (IDP) is a complex of milk proteins that has been shown to have anti-inflammatory activity and provide protection from infection. The effect of IDP on enhancing immune response after an influenza vaccine in humans has not been shown and is the objective of this study.

Methods: An 8-week, double-blinded, randomized, placebo-controlled pilot study was conducted. Fifty three participants were randomized to three study arms, consuming either high dose IDP (200 mg), standard dose IDP (50 mg) or placebo. Serum samples were collected prior to influenza vaccination (at 4 weeks) and 2 and 4 weeks postvaccination. Levels of influenza-specific Ig were measured in serum using enzyme linked immunosorbent assay (ELISA). Proportion of individuals achieving a 4-fold or greater increase in antibody levels, the geometric mean antibody levels after influenza vaccination and effect size of IDP dosage were determined.

Results: Statistically significant difference was observed in geometric mean influenza-specific antibody levels between high dose IDP and placebo arms at 4 weeks postvaccination. High dose IDP participants exhibited a sustained 4-fold increase in antibody levels 4 weeks postvaccination. The high dose IDP provides a medium effect size trending towards large effect size at 2 and 4 weeks postvaccination compared to participants that consumed the placebo.

Conclusion: This study has shown that with increasing dose of IDP supplementation a greater effect in improving immune response postvaccination occurs. Consumption of IDP may improve protective immunity which would be especially beneficial to at risk populations.

Trial Registration: https://www.anzctr.org.au/TrialSearch.aspx. Identifier ACTRN12622000462785

Keywords: Immune defense proteins; milk bioactive whey; Antiviral; influenza vaccine; immune response

Introduction

Influenza is an upper respiratory infection caused by influenza viruses and most infections are mild and self limiting [1]. The influenza virus can have a greater impact on more vulnerable groups such as those with compromised immune systems, of older age or very young, pregnant and those with chronic medical conditions [1].

Globally, an average of 700,000 people die each year from seasonal influenza [2]. In temperate countries such as New Zealand, influenza epidemics most likely occur in the winter months. For New Zealand the influenza season occurs between the months of April and October and on average there are approximately 500 deaths per year due to influenza and pneumonia [3].

Influenza vaccines are the best method for reducing numbers contracting influenza and hence decreasing number of infections, hospitalizations and death [1,2]. However, influenza viruses can evolve rapidly. Each year the World Health Organization, Global Influenza Surveillance and Response System [4] recommends which influenza strains are best included in the vaccine for the following year. This recommendation is based on worldwide influenza surveillance data and identification of a strain most likely to circulate during the coming influenza season. This can be challenging because the strains chosen for the vaccine may have slightly changed their antigenic profile which will then escape the body's immune response [5] and hence reduce the effectiveness of the vaccine.

One way of improving immune function and response to the influenza vaccine is via dietary supplementation with whole foods or extracts. In the past 40 years there have been a number of randomized control trials (RCTs) examining the effect of vitamins [6,7], minerals [8], vitamins and minerals [9], nutritional supplements or formulas [10-15], prebiotics [16-23] and probiotics [22-34] on antibody responses to the influenza vaccine. Results from these trials have been equivocal.

The majority of RCTs that have been performed have included people over 50 years of age [7-17, 20, 24-27, 29-31, 34] or included 50 years or over in their age range for recruitment [6, 18, 19, 22, 23, 28, 32, 33]. The use of the 50 and over age group is likely due to the knowledge that the immune system gradually declines with age; a process classically termed as immunosenescence, which is characterised by changes in both the humoral and cell mediated arms of the immune system. However, more recent research has shown that obesity [35] and both chronic stress and inflammation can also cause and accelerate immunosenescence even in the young [36], meaning the term should be redefined towards immune system dysfunction irrespective of age, and that younger individuals may also benefit from dietary supplements which enhance the immune system.

In the elderly, cell mediated immunity is decreased, with lower T cell numbers and reduced T cell proliferation. Humoral immunity is also negatively impacted with reduced primary responses to new antigens and reduced specificity of the antibodies that are produced. Together these negative changes contribute to the influenza vaccine being even less efficient in the elderly, with some studies indicating the level of protection may be as low as 30% in this population [37]. This low efficacy contributes to the increased morbidity and mortality of influenza in the elderly cohort compared to younger populations. Obese individuals have also been shown to exhibit low responses to vaccinations [35,36]. Therefore, any supplementation that can improve the

effectiveness of the influenza vaccine especially in the elderly or younger individuals suffering from immunosenescence would be valuable in improving health outcomes.

The bioactive whey protein ingredient called immune defense protein (IDP®) is a complex of milk-derived bioactive whey proteins. Recently the effect of IDP on immune function was examined in mice [38]. It was shown that medium and high dose IDP significantly enhanced sheep red blood cell-induced delayed type hypersensitivity and improved the phagocytic ability of monocyte-macrophage cells. These results demonstrated that IDP was immune enhancing. Lactoferrin, one of the key proteins in IDP, was also tested and showed similar results to IDP [38].

Lactoferrin has been well studied for its antiviral activity and is known to reduce infection by a number of viruses including human influenza virus, HIV, Epstein-Barr virus (the cause of Mononucleosis otherwise known as glandular fever), and HSV-1 and -2 (herpes simplex virus, responsible for cold sores and genital herpes) [39-43]. Another key protein in IDP is lactoperoxidase, a protein present in milk and other exocrine secretions of mammals, such as tears and saliva. Peroxidases, such as lactoperoxidases, catalyse peroxide-dependent oxidation of halides (e.g., thiocyanate, iodide, bromide, etc.) that can react with and kill microbes [44].

Many supplements focus on a single molecule, such as lactoferrin or β-1,3/1,6-glucan. Beta-glucans are water soluble fibre from the cell walls of bacteria, fungi, yeasts, and some plants. In the past 18 years there has been numerous clinical studies [see review 45] that have investigated the potential health benefits of β-glucan supplementation [45]. It has been shown to date that β-glucans have immunomodulatory effects with a wide range of immunological activities [45].

One of the features of IDP, that differentiates it from other supplements, is that it contains over 50 proteins. The effect of IDP on enhancing immune response to influenza vaccine in humans has not been previously investigated.

The objectives of this study were to examine if IDP could improve immune responses after an individual is administered the influenza vaccine by conducting a randomized double blind placebo control trial. The immunoenhancing effect of IDP was examined as in previous studies [12,15] by determining the proportion of individuals achieving a 4-fold or greater increase in antibody levels and measuring the geometric mean antibody levels after influenza vaccination.

Materials and Methods

Materials

Commercial samples of IDP (Supplement A and B) and placebo (Supplement C) were prepared and supplied by Quantec Ltd, Hamilton, New Zealand. Supplement A contained 11.5% instantized whole milk powder, 76.1% instantized skim milk powder, 2% dextrose anhydrous, 10% IDP, and 0.4% Monk fruit extract. Supplement B contained 12.5% instantized whole milk powder, 82.6% instantized skim milk powder, 2% dextrose anhydrous, 2.5% IDP, and 0.4% Monk fruit extract. The placebo (Supplement C) contained 12.8% instantized whole milk powder, 84.8% instantized skim milk powder, 2% dextrose anhydrous, and 0.4% Monk fruit extract.

Study Design

A double-blinded randomized placebo-controlled pilot study was performed to examine the effect of standard dosage (50 mg) IDP and high dosage (200 mg) IDP daily on immune response after administration of the influenza vaccine. This study was conducted over two years (2022 -2023). The study was approved by the Health and Disability Ethics Committees (HDEC 21/-CEN/233) and the trial was registered with the Australian New Zealand Clinical Trial Registry (ANZCTR, registration number: ACTRN12622000462785; Registered on 24 March 2022). Written informed consent was obtained from the participant before

enrolment into the study.

Sample Size

The sample size for this study follows the rules of thumb for the size of a pilot study and fits within sample size range from 12 to 35 individuals per arm [46-48] with medium to large effect size [46,48,49].

Study Population

Individuals were recruited using posters, research newsletter, social media platforms and Trialfacts, a company that aids in recruitment for clinical trials. Participants were recruited during April 2022 to September 2022 and April 2023 to July 2023. Inclusion criteria included: 25-65 years of age; male or female; healthy; able to read and speak English. Exclusion criteria included: Lactose intolerance; known or suspected allergy to dairy products, starch, gluten or artificial sweeteners; participation in another clinical trial; pregnancy or currently lactating; having already had the influenza vaccination in the current year; having had a recent cold; having any chronic inflammatory conditions.

After eligibility for the study was confirmed (screening using a general health questionnaire) and participants consented to be part of the study, participants were randomized to either placebo (Supplement C), 50 mg IDP (Supplement B) or 200 mg IDP (Supplement A) arms of the study. During the first period of recruitment (2022), randomisation to placebo and two arms of treatment (Supplement A and B) occurred. For the second recruitment period (2023) randomisation was for placebo and one treatment arm (Supplement A). Block randomisation, to attain equal sample size in each arm, was performed by a person external to the research group. Participants and researchers working with the participants were blinded to which supplement the participant was receiving.

Study Protocol

Participants were required to attend Massey University Nutrition Research Facility, located at the Albany campus, Massey University, New Zealand for six visits over a 9 week period. The first visit was induction into the trial and was 1-7 days before the 8 week trial started. For Visit 1, baseline measures of height, weight and blood pressure were performed. Participants were provided with sachets (2 g powder/sachet) for the first four weeks of consumption (supplement consumed daily). They had to consume the supplement with first meal of the day and were instructed not to mix it with hot drinks. The participants were also provided with a daily consumption and general health diary which had to be completed for each day for their first four weeks of the trial. This enabled identification of any problems or adverse effects associated with consumption of the supplement.

Visit 2 was 7 days (week 1 of the trial), Visit 3 was 14 days (week 2 of the trial) and Visit 4 was 28 days (4 weeks of the trial). In week 4 participants received an influenza vaccine and their consumption and general health diaries were collected. Participants were provided with supplement sachets for a further 4 weeks of daily consumption after vaccination and were provided another diary for noting any adverse effects over the next four weeks of the trial. The fifth visit at day 42 was 2 weeks postvaccination and Visit 6 at day 56 was 4 weeks postvaccination and the end of the trial. The diaries were collected during Visit 6.

At 4 weeks the participants received the influenza vaccine. For the first period of recruitment (April – September 2022) participants received the influenza vaccination (Afluria® Quad-A/Victoria/2570/2019 (H1N1) pdm09-like virus (A/Victoria/2570/2019 IVR-215, A/Darwin/9/2021 (H3N2)-like virus (A/Darwin/9/2021 IVR-227), B/Austria/1359417/2021-like virus (B/Austria/1359417/2021 BVR-26), B/Phuket/3073/2013-like virus (B/Phuket/3073/2013 BVR-1B). For the second period of recruitment (April – July 2023) participants received the influenza vaccine (Afluria® Quad- A/Sydney/5/2021 (H1N1)pdm09-like virus; A/Darwin/9/2021 (H3N2)-like virus; B/Austria/1359417/2021 (B/Victoria lineage)-like virus; and B/Phuket/3073/2013 (B/Yamagata lineage)-like virus).

Blood samples (2x 6 ml) were collected prior to initiation of treatment (Visit 1 – 0 days) and Visits 2-6 (days 7, 14, 28, 42 and 56) of the trial. The serum or plasma obtained from the two blood samples collected were aliquoted into smaller samples and frozen in -80°C freezer in the Sport and Exercise Science Laboratory, Albany campus, Massey University, New Zealand.

Levels of influenza-specific Ig were measured in the serum sample and determined using an optimised in-house enzyme-linked immunosorbent assay (ELISA) using a sourced antibody (Invitrogen; Goat anti-Human IgG, IgM, IgA (H+L) Secondary Antibody, HRP catalogue number A18847; Thermo Fisher Scientific, Auckland). The ELISA assay was linear ($R^2 > 0.996$) in the standard range used (0 - 2000 units/mL) and was validated by assessing dilutional linearity, intra- and inter-assay precision (< 5% and < 10% respectively), specificity through use of negative and positive controls, and limit of detection (10 units/mL) [50]. Briefly, wells of a 96-well microtitre plate (Nunc, Roskilde, Denmark) were coated with influenza vaccine (100 ng/well) diluted in carbonate coating buffer (pH 9.6) at 4°Cfor 18h. The plate was washed six times with wash buffer (PBS (pH 7.2) containing 0.05% Tween 20), before suitably prediluted (Dilution buffer: wash buffer with 0.1% skim milk powder) standards and test samples were added to each well and incubated at room temperature for 1h. After washing three times with wash buffer, diluted secondary antibody (1/20,000), was added to each well and incubated for 1h at room temperature. Following further washing, substrate (TMB, Thermo Fisher Scientific, Auckland) was added to each well and incubated for 30min at room temperature prior to acidification (1N HCl) to stop the reaction. The plate was read on a plate reader (Bio Tek Synergy 2), at 450nm. The highest standard was prepared using a 1/20,000 dilution of hyperimmune serum and assigned an arbitrary value of 2000 units/mL.

Statistical Analysis

Statistical analysis was performed using software Stata version 15.1. Descriptive analyses were performed to determine the baseline characteristics of study participants across different study arms using the Chi-squared test for categorical and ANOVA test for continuous variables. Following previous literature [12,15] antibody levels are reported as a geometric mean. The Wilcoxon signed-ranks test was used to investigate the differences within groups [antibody responses at day 42 (2 weeks postvaccination) and 56 (4 weeks postvaccination) compared with day 28 (prevaccination - baseline) was measured for each study arm] and the Mann-Whitney U-test was performed to determine the changes between groups (the antibody response of study arms A and B compared with study arm C was measured at day 42 and 56). The proportion of participants with at least a 2-fold or 4-fold antibody response after influenza vaccination in each study group at days 42 and 56 was calculated using binomial point estimates and 95% confidence intervals. Differences were considered statistically significant at $p < 0.05$ level. The effect size was calculated for Supplement A (High dose IDP) and B (Standard dose IDP) study arm compared to Supplement C (Placebo) study arm on days 42 (2 weeks postvaccination) and 56 (4 weeks postvaccination). Cohen's d was used to measure effect size, which quantifies the mean difference between the two groups for assessing the magnitude of the effect.

Results

Study Cohort

As shown in Fig 1, 216 people were screened for eligibility, with 67.6% excluded due to not meeting the inclusion criteria and 5.6% declining to be part of the study. The 70 participants enrolled in the study received placebo (Supplement C) or IDP at 50 mg standard dose (Supplement B) or a high dose of 200 mg (Supplement A) and 75.7% of the randomized participants completed the study (Figure 1). Of the 24.3% eligible participants that were randomized and did not complete the study, they either did not attend Visit 1, a blood sample could not be collected or their health worsened. The recruitment of participants covered two time periods. For the first recruitment period in 2022, thirty two participants completed the 8 week trial for the three arms of treatment. After the first recruitment period, a preliminary analysis of the effect of IDP consumption on immune response following injection of the influenza vaccine was performed by researchers external to the study. Little difference was observed in the antibody response results between treatment arms B and C but there was an observable difference between arms A and C.

Due to the difficulties in recruitment experienced within the first recruitment period, the decision was made to only include two treatment arms (one being the placebo) for the second period of recruitment in 2023 to ensure the sample size for the treatment arms [46-48]would be achieved. The researchers external to the study, who knew which Supplement code was assigned to placebo, low and high IDP dose, recommended that Supplements C and A (and not Supplement B) be utilized for the second recruitment period. This is reflected in the total number of participants for the study: Supplement A (n=21), Supplement C (n=21) and Supplement B (n=11).

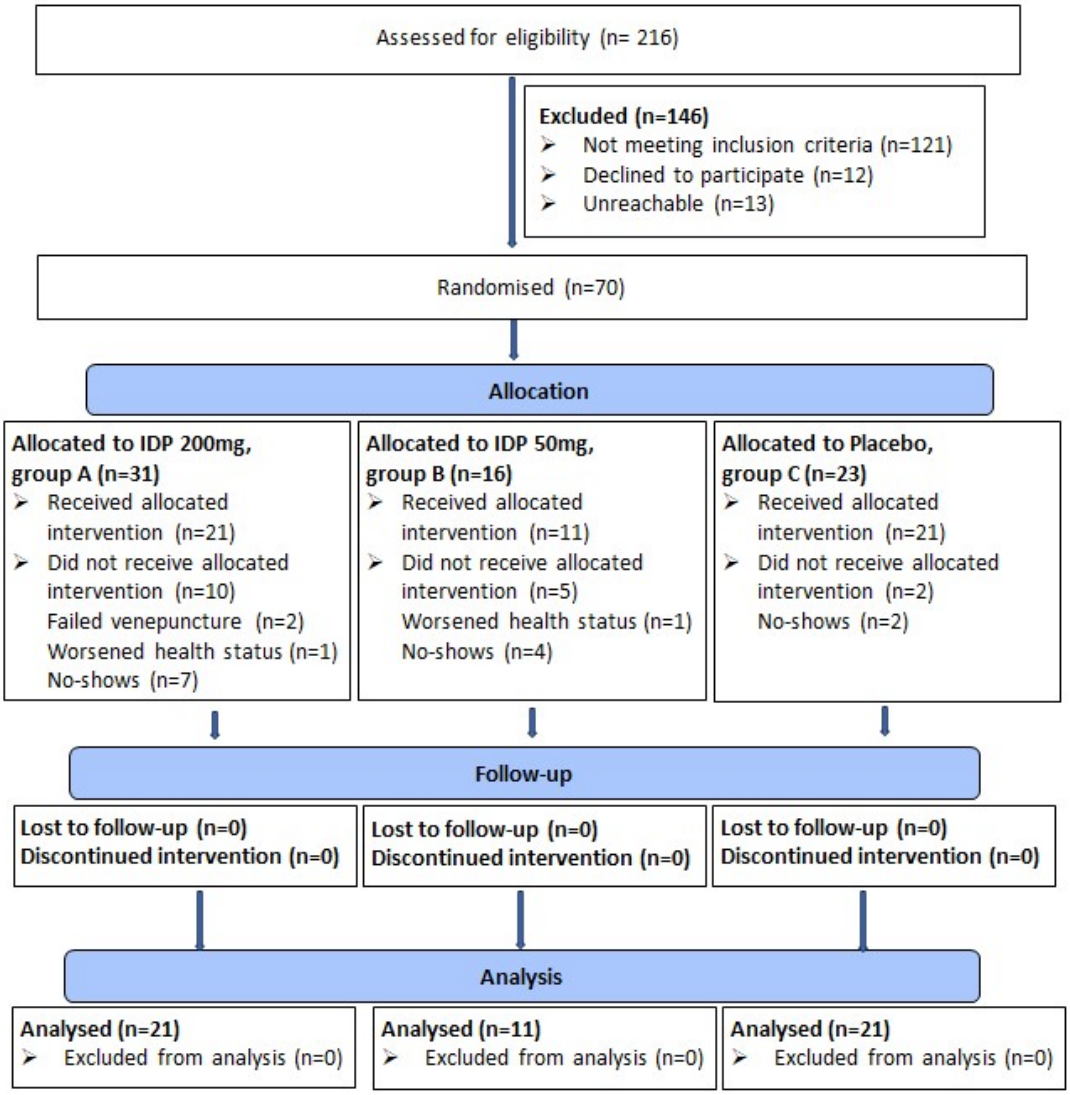

Figure 1: CONSORT flow diagram for the 8 week trial. The diagram shows number of recruited volunteers and participant numbers included in the data analysis.

Baseline characteristics of the study cohort are shown in Table 1. There were no statistically significant differences for age and sex between the study arms. The first meal for the participants was breakfast, which meant there were no shift workers in the cohort. The powdered supplement was consumed in a variety of ways ranging from putting it in their cereal or yoghurt to just swallowing it. There were no reported adverse effects from consuming the supplement or the placebo.

Table 1: Baseline Characteristics of Study Cohort

Characteristics	Treatment arm		
	A (n=21)	**B (n=11)**	**C (n=21)**
Age (mean, SD)	45.21 (11.34)	50.26 (12.17)	44.07 (10.41)
Sex (%)			
Male	47.62 (10)	45.45 (5)	33.33 (7)
Female	52.38 (11)	54.55 (6)	66.67 (14)
Ethnicity (%)			
Asian	38.10 (8)	27.27 (3)	19.05 (4)
European	47.62 (10)	63.64 (7)	71.43 (15)
Other	14.29 (3)	9.09 (1)	9.52 (2)
BMI (mean, SD)	28.01 (6.46)	27.30 (4.47)	28.79 (7.66)
Influenza vaccine before (%)			
Yes	61.90 (13)	63.64 (7)	80.95 (17)
No	23.81 (5)	9.09 (1)	9.52 (2)
Unknown	14.29 (3)	27.27 (3)	9.52 (2)

A = study arm that consumed Supplement A high dose IDP; B = study arm that consumed Supplement B standard dose IDP; C = study arm that consumed Supplement C placebo.

SD = standard deviation; BMI = Body mass index. Ethnicity: other represents Filipino-European (1), MELAA (1), Māori (1), Middle Eastern (1), and Pacific (2). Influenza vaccine "yes" means the participant had received the influenza vaccine in previous years

Effect of IDP on antibody responses to Influenza vaccine

The mean antibody levels in each study arm following 28 days (baseline), 42 days (2 weeks after receiving the influenza vaccine) and 56 days (4 weeks after receiving the influenza vaccine) consumption of the supplement are shown in Table 2. For all three study arms, there was a significant difference within each group between baseline and 42 days, and baseline and 56 days. At 2 weeks postvaccination, there was a statistically significant difference observed between study arm A (high dose 200 mg IDP) and study arm C (Placebo).

Table 3 shows the fold changes in antibody responses over the 4 weeks after the influenza vaccine was administered. Supplement A and B induced greater than 2-fold antibody responses in a larger percentage of participants than Supplement C, though the differences were statistically insignificant. However, a statistically significant difference was observed between the 4-fold increase or greater in immune response 2 weeks and 4 weeks post vaccination in the proportion of participants consuming high dose IDP compared to placebo. For participants consuming the high dose IDP (Supplement A), antibody levels were maintained at a greater than 2-fold increase and 4-fold increase 4 weeks after administration of the influenza vaccine.

Table 4 shows the effect of high dose (200 mg) IDP supplementation on antibody responses against the influenza vaccine 2 and 4 weeks after administration of the vaccine. High dose IDP provided a medium effect size trending towards large effect size at 2 and 4 weeks postvaccination when compared to participants that consumed the placebo (Supplement C).

Table 2: Mean influenza specific antibody levels in each treatment group at baseline (28 days) and 42 days (2 weeks after vaccination) and 56 days (4 weeks after vaccination)

Study arm	Mean[1] antibody levelsunits/mL			Mean difference at 42 days		Mean difference at 56 days	
				Baseline	Compared with group C	Baseline	Compared with group C
	Baseline(28 days)	42 days	56 days	p[2]	p[3]	p[2]	p[3]
A (n=21)	179.37	1100.88	941.40	<0.001*	0.042*	<0.001*	0.084
B (n=11)	189.82	968.79	807.59	0.003*	0.108	0.003*	0.171
C (n=21)	212.10	726.63	623.19	<0.001*	-	<0.001*	-

[1] = Geometric mean; [2]=Wilcoxon signed-rank test; [3]=Mann-Whitney U-test.

28 days baseline – is representative of the antibody levels for the first 4 weeks of the trial; 42 days represents antibody levels 2 weeks after injection of influenza vaccine; 56 days represents antibody levels 4 weeks after influenza vaccine injection. *statistically significant = p<0.05.

Table 3: Proportion of participants with at least a 2-fold or 4-fold antibody response after influenza vaccination in each treatment group at 42 days (2 weeks postvaccination) and 56 days (4 weeks postvaccination) compared with 28 days (baseline – prevaccination)

	Study arm; % of participants (and 95% CI)				
Fold increase	**A**	**p[1]**	**B**	**p[2]**	**C**
At 42 days					
At least 2-fold	76.19 (52.83, 91.78)	0.72	81.82 (48.22, 97.71)	0.52	71.43 (47.82, 88.71)
At least 4-fold	61.90 (38.43, 81.89)	<0.05	45.45 (16.74, 76.62)	0.21	23.81 (8.21, 47.16)
At 56 days					
At least 2-fold	76.19 (52.83, 91.78)	0.10	63.64 (30.79, 89.07)	0.54	52.38 (29.78, 74.28)
At least 4-fold	57.14 (34.02, 78.18)	<0.05	36.36 (10.92, 69.20)	0.28	19.05 (5.44, 41.90)

CI = confidence interval. [1]= p-value comparing supplement group A (High dose IDP) and group C (Placebo); [2]= p-value comparing supplement group B (Standard dose IDP) and group C (Placebo).

Table 4: Effect size (based on mean comparison) compared with Supplement C

	A (n=21)
At 42 days	
Cohen's d (95% CI)	0.74 (0.11, 1.36)
At 56 days	
Cohen's d (95% CI)	0.71 (0.08, 1.32)

CI = Confidence Intervals; 42 days = 2 weeks postvaccination; 56 days= 4 weeks postvaccination; A: Supplement A high dose 200 mg IDP; Supplement C is Placebo. Cohen's d () measures effect size. *Standardised effect size:* Extra small (<0.1); Small (0.1≤ <0.3); Medium (0.3 ≤ <0.7); Large (≥0.7) [45,47]

Discussion

For this randomized control trial, healthy adults consumed IDP daily for 8 weeks, 4 weeks before vaccination and 4 weeks after vaccination. Increased influenza specific antibody responses for low and high dose IDP group participants were observed at 2 weeks after influenza vaccination. Approximately sixty percent of participants consuming the high dose IDP exhibited a 4-fold sustained increase in antibody levels 4 weeks postvaccination. Standard dose IDP supplementation had 82% of participants with 2-fold increase in their antibody levels at 2 weeks, and showed a decline to 64% in proportion of participants with 2-fold increase in their antibody levels from 2 to 4 weeks postvaccination. The placebo group had 71% of participants with 2-fold increase in their antibody levels at 2 weeks and showed a decline to 52% in proportion of participants with 2-fold increase in their antibody levels from 2 to 4 weeks postvaccination.

The persistence of influenza antibodies after vaccination is an important determinant of susceptibility to future infection. Studies indicate the most rapid decline in influenza antibodies occurs 2-6 months after peak levels are achieved, with a slower decline in the following 6 months [51]. Hsu and colleagues [51] showed only 25% and 14% of individuals had titres that are considered protective (>1/40) 6 months and 1 year post the influenza antibody peak, respectively. The rate of decline appeared to be faster in older individuals [51], therefore placing them at greater risk of influenza infection.

Modelling to estimate levels of protection against the influenza virus based on data from the hemagglutination assay (HI) shows that as the titre increases, the protection increases in a logarithmic manner [52]. A titre of 1/40 is considered to reduce the risk of contracting influenza by 50% in susceptible populations [53], however, modelling indicates a titre of 1/100 only achieves around 80-90% protection [52]. Similarly, meta-analysis data has shown that the efficacy of the influenza vaccine in adults (18-65y) is only 59 % [54]. Therefore, unlike common childhood vaccinations such as MMR (measles, mumps and rubella) and polio, which have an effectiveness of over 90% from a single vaccination and around 99% after a second vaccination [55,56], the poor efficacy of the influenza vaccine means that the higher the antibody response, the better the level of protection likely achieved.

Several studies have shown an improved antibody response or serological protection after influenza vaccination with participants consuming over the trial period either a combination of micronutrients [9], nutritional supplementation, [10,11,13-15], prebiotic supplementation [14-16,19,21,23], probiotic supplementation [23,25,27,30,32,33], a combination of prebiotic and probiotic supplementation [16] or neutraceutical formulation [18].

A systematic review and meta analysis of 12 RCTs (papers published up to July 2017) showed that prebiotics/probiotics supplementation enhanced the hemagglutination inhibition (HI) antibody titres in all influenza A/H1N1, A/H3N2, and B strains (20%, 19.5%, and 13.6% increases in HI antibody titres, respectively) [23]. These studies indicated prebiotics/probiotics could improve the efficacy of the influenza vaccine however further studies were needed due to the observed heterogeneity (the strain, doses, and duration of prebiotics/probiotics supplementation differed) of the studies.

A RCT by Langkemp-Henken and colleagues (2004) showed that 87% of the elderly subjects (65 years or older) receiving a nutritional supplement containing antioxidants, zinc, selenium, fermentable oligosaccharides, and structured triacylglycerol for183 days, achieved a fourfold or greater increase in serum antibody titre to the A/Beijing component of the influenza vaccine (P=0.012) at day 57 (postvaccination) [13]. In 2006, Langkemp-Henken and colleagues performed another RCT [14] using the same nutritional supplement used in the 2004 RCT [13], however the cohort was a more frail population. For the H1N1 (A/-Caledonia) component of the influenza vaccine, the percentage of participants with HI antibody titres greater than 100 (protective titre in older people [51-53]) at 42 days postvaccination was higher in the intervention group than the control group (P=0.047). Both RCT studies [13,14] have indicated that their specific nutritional supplement (contained vitamins, minerals,

zinc, selenium, proteins, fructo-oligosaccharides and structured triacylglycerol) improved vaccine response to the influenza vaccine for the elderly. One study found that the long term (6 months) oral supplementation of trace elements (zinc and selenium) increased the HI antibody titre to the H3N2 component of the influenza vaccine whereas the vitamin supplementation (vitamin C, E, and β-carotene) had significantly lower antibody titres ($p<0.05$) for the institutionalized older people (n=725) in the study [9].

It is difficult to compare these studies to our study and the impact of each supplement on immune responses after influenza vaccination as the antibody levels and titres are expressed differently. Our study has shown an improved immune response (increase in influenza specific antibody levels) and a sustained increase in antibody levels 4 weeks after influenza vaccination, for participants consuming high dose 200 mg of IDP. These results indicate great potential for high dose IDP consumption having a beneficial effect and providing better protective immunity.

We are the first study to examine impact by determining effect size, a measure showing how meaningful the relationship is between two variables, in this case, supplement dose and antibody responses postvaccination. Our study had a small sample size but still showed that high dose IDP supplementation had medium effect trending to large effect in regard to improving the immune response against the influenza vaccine in healthy subjects aged between 25-65 years old. This medium effect was sustained 4 weeks postvaccination.

A limitation of this study was difficulty in recruitment of participants and needing to reduce the study arms in the second recruitment period to increase sample size in Supplement A and placebo treatment arms. Using the preliminary results from first recruitment period, it was determined based on Whitehead and colleagues [48] stepped rules of thumb with assumption that medium effect size would be achieved with the high dose 200 mg IDP treatment arm, that a further 10 participants would be required in recruitment for Supplement A. Due to the small participant number in this study, for Supplement B, further research is required to examine the effectiveness of 50 mg IDP supplementation in improving the efficacy of the influenza vaccine.

Dysfunction of the immune system (immunosenescence), means that it is more difficult to fight infection if you are obese, suffer from chronic inflammation or stress or as you age [36], leaving those affected at greater risk. This pilot study has provided encouraging results showing daily consumption of IDP at high dose (200 mg) can lead to continued high influenza antibody responses postvaccination, indicating the likelihood of improved protective immunity especially in those most at risk.

Conclusion

IDP is an ideal ingredient for nutritional and dietary supplements and as an ingredient to enhance the benefits of foods. Investigation of the immunomodulatory effect of IDP has shown that consumption of high dose 200 mg IDP has a medium impact on improving immune response against the influenza vaccine and provides a sustained increase in antibody response 4 weeks post influenza vaccination. This study has shown that with increasing dose of IDP supplementation a greater effect in improving immune response postvaccination occurs. This beneficial effect may induce better protective immunity. Further studies are required to confirm these findings.

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Ethics Statement

A double-blinded randomized placebo-controlled pilot study was performed to examine the effect of IDP on immune response after administration of the influenza vaccine. This study was approved by the New Zealand Health and Disability Ethics Committees (HDEC 21/CEN/233), a Ministerial committee (established under section 87 of the Pae Ora (Healthy Futures) Act 2022). The role of HDEC is to check that the benefits of health and disability research meets or exceeds ethical standards. The randomized control trial was registered with the Australian New Zealand Clinical Trial Registry (ANZCTR, registration number: ACTRN12622000462785). Written informed consent was obtained from participants prior to their enrolment into the study.

Disclosure Statement

Rodney Claycomb, Colin Ogle and Katharine Adam are employees of Quantec Ltd, Waikato Innovation Park, New Zealand, the study sponsor. Rodney Claycomb, Colin Ogle and Katharine Adam were not involved in the interpretation of results and did not influence the outcomes at any stage of this clinical study. Quantec Ltd, Waikato Innovation Park, New Zealand provided Supplements A, B and C for the study.

All authors have declared that they have no other conflict of interest that could have appeared to influence the work reported in this paper.

Authors Contribution

Rachel Page: Conceptualization, Methodology, Formal Analysis, Writing original draft, Writing - Review & Editing, Visualization, Project administration, Funding acquisition; Kay Rutherfurd-Marckwick: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Writing - Review & Editing, Visualization, Supervision; Project administration, Funding acquisition; Colin Ogle: Conceptualization, Writing - Review & Editing, Visualization; Rodney Claycomb: Conceptualization, Writing - Review & Editing; Katharine Adam: Conceptulization, Writing - Review & Editing, Visualization; Judy Thomas: Validation, Investigation, Writing - Review & Editing, Supervision, Project administration; Cheryl Gammon: Investigation, Writing - Review & Editing, Project administration; Tasnima Akter: Formal analysis, Data curation, Writing - Review & Editing; Yongsijia Wei: Investigation, Writing - Review & Editing. All authors read and approved the final manuscript.

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