**The relationship between the severity of infection and probiotic treatment on antimicrobial peptide levels.**

An analysis of covariance (ANCOVA) was conducted to determine how severity of symptoms and administration of probiotics are related to the production of antimicrobial peptides in the human gastrointestinal microbiota for 70 to 81-year-old patients suffering from gastrointestinal infections. The ANCOVA passed all diagnostic tests and a significant interaction effect between probiotic administration and the severity of symptoms was found (ANCOVA, F1,76=25.28, P<0.01). A negative correlation was found between the severity of infection (measured in days with symptoms) and the levels of antimicrobial peptides measured, for patients administered the placebo treatment. For this patient group, there was a -1.27 mg/ml reduction in antimicrobial peptide levels for each extra day of symptoms they experienced (figure 1). In contrast to the placebo patient group, patients receiving the probiotic treatment saw a very small increase in antimicrobial peptide levels as the severity of the symptoms increased (figure 1). In patients administered the probiotic treatment, the antimicrobial peptide levels increased by 0.11 mg/ml for each extra day with symptoms before the treatment started. These results show that the administration of the placebo treatment to 70 to 81-year-old patients suffering with gastrointestinal infections reduces the effect of the severity of the infection, which on the placebo treatment decreases the levels of antimicrobial peptides in the human gastrointestinal microbiota. Consequently, these results indicate that probiotic treatments may be an effective way of increasing antimicrobial peptide levels in patients with more severe infection symptoms but not particularly effective for patients with less severe symptoms.

Chart, scatter chart

Description automatically generated

Figure 1. The relationship between the levels of antimicrobial peptides in the human gastrointestinal microbiota and the severity of gastrointestinal infection symptoms when administered placebo and probiotic treatments. The graph is based on data from 80 hospitalised 70 to 81-year-old patients suffering from gastrointestinal infections. Red represents the 40 patients on a probiotic treatment (y=68.51+0.11x) and black represents the other 40 patients on a placebo treatment (y=70.42-1.27x). The two lines are the fitted regression lines from an ANCOVA analysis showing the relationship between antimicrobial peptides and severity of gastrointestinal infection (measured in number of days with symptoms) for the two different treatment groups (placebo or probiotic).

**The effect of diet and probiotics on Shannon diversity of gastrointestinal microbiota.**

A two-way ANOVA was conducted to determine the effects of diet and probiotics on Shannon diversity. The assumptions of the model were checked, and it was found that there was a statistically significant interaction between the effects of diet and probiotic administration on the Shannon diversity of the gastrointestinal microbiota (two-way ANOVA, F1,116=27.16, P<0.01). The effect of probiotic administration to patients on Shannon diversity was different depending on whether the patient was consuming a normal or high fibre diet (figure 2A). Similarly, the effect of diet on the Shannon diversity of patients was different depending on whether they were receiving the probiotic or placebo treatment (figure 2A). A high fibre diet increased the Shannon diversity of the gastrointestinal microbiota more in patients that were receiving the placebo treatment than for those that were being administered probiotics, with an increase in mean Shannon diversity of 1.6 compared to 0.16 in the probiotic group (figure 2B). The administration of probiotics to patients increased the Shannon diversity of those that were consuming a normal diet more than those consuming a high fibre diet, with an increase in mean Shannon diversity of 4.02 compared to 2.58 in the high fibre group (figure 2A). These results suggest that a high fibre diet and probiotic treatment can both be effective methods for increasing Shannon diversity of gastrointestinal microbiota, but each is more effective in the absence of the other.

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Figure 2.

A) A box plot of how the distribution of Shannon diversity of gastrointestinal microbiota is affected by diet and probiotic administration. B) Interaction diagram showing effect of probiotics and diet on mean Shannon diversity. The slopes of the lines illustrate the effect size of moving from the placebo to the probiotic treatment under each dietary condition. Both panels A and B use data from deep amplicon sequencing of stool samples taken from 120 patients with gastrointestinal infections following antibiotic treatment.

**References**

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Your script ran well and was generally well annotated (though a little more detail in places wouldn’t go amiss). This report is really excellent. I would like to have seen a post-hoc Tukey test to disentangle which groups were significantly different in experiment 2. Otherwise very minor comments.

A