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RESEARCH ARTICLE

Probiotic-enriched foods and dietary supplement containing SYNBIO positively affects bowel habits in healthy adults: an assessment using standard statistical analysis and Support Vector Machines

Stefania Silvi[1,2]*, M. Cristina Verdenelli[1,2]*, Cinzia Cecchini[1,2], M. Magdalena Coman[1,2], M. Simonetta Bernabei[3], Jessica Rosati[3], Renato De Leone[3], Carla Orpianesi[1,2], and Alberto Cresci[1,2]

[1]*School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy,* [2]*Synbiotec S.r.l., Camerino, Italy, and* [3]*School of Science and Technology, University of Camerino, Camerino, Italy*

Abstract

A randomised, double-blind, placebo-controlled, parallel group study assessed in healthy adults how daily consumption of the probiotic combination SYNBIO®, administered in probiotic-enriched foods or in a dietary supplement, affected bowel habits. Primary and secondary outcomes gave the overall assessment of bowel well-being, while a Psychological General Well-Being Index compiled by participants estimated the health-related quality of life as well as the gastrointestinal tolerance determined with the Gastrointestinal Symptom Rating Scale. Support Vector Machine models for classification problems were used to validate the total outcomes on bowel well-being. SYNBIO® consumption improved bowel habits of volunteers consuming the probiotic foods or capsules, while the same effects were not registered in the control groups. The recovery of probiotic bacteria from the faeces of a cohort of 100 subjects for each supplemented group showed the persistence of strains in the gastrointestinal tract.

Keywords

L. paracasei IMC 502, *L. rhamnosus* IMC 501, probiotic dietary supplement, probiotic functional foods, Support Vector Machines

History

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Introduction

The addition of live microorganisms (probiotics) to foods and dietary supplements to confer health benefits has become widespread in recent years, garnering increasing interest from the scientific community and the general public. This is due to the increasing attention and attraction of people to functional foods containing probiotics and prebiotics (Annunziata & Vecchio, 2013; Ares et al., 2009; Figueroa-González et al., 2011; Marette et al., 2010; Reid, 2008). In fact, such is the interest in these products that regulatory bodies have also focused on the development and marketing of probiotic products. Recently, EU decision makers adopted regulations concerning nutrition and health claims for foods, with particular attention to ensuring that claims made on food labels (included probiotic-containing foods) in the EU are clear and substantiated by scientific evidence (European Food Safety Authority, 2009). Furthermore, countries such as Canada, India and Japan are providing or developing guidelines that would require probiotic strains to be studied in clinical trials (Chonan, 2011; Gokhale & Nadkarni, 2007; Health Canada, 2009).

In recent years, numerous studies have examined the use of probiotic-enriched foods (Coman et al., 2012; Cruz et al., 2009a, b; Granato et al., 2010), as the possibility of obtaining beneficial effects from probiotics simply by adding them to the daily diet, potentially in a wide range of foods, offers promising commercial avenues. Different categories of food have been studied for addition of probiotic strains, from widely used dairy products to other foods that may be less used or not yet appreciated (Martins et al., 2013).

Fermented milk containing well-known probiotic strains has been used to relieve constipation in women and children, improving defecation frequency as well as stool condition and consistency (Tabbers et al., 2011; Yang et al., 2008). A recent review on probiotics and bowel habits indicated that short-term probiotic supplementation decreased intestinal transit time with consistently greater effects in constipated adults (Miller & Ouwehand, 2013).

Our previous studies found that a (1:1) combination of the probiotic bacterial strains *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502® administered in functional foods persisted in the intestinal tract of test subjects, promoting natural regularity and intestinal well-being (Verdenelli et al., 2009, 2011). Moreover, probiotics in capsules offer promising potential for particular conditions and for some categories of people.

This study designed to determine whether daily consumption of our SYNBIO® strain formulation confers beneficial effects on bowel habits, compared administration in foods and in capsules and used a larger healthy adult population than our previous studies. As food samples are highly heterogeneous and contain many different biologically active components that could complicate understanding of the effects on bowel habit parameters, we developed a standardised method of administration to avoid these food matrix effects.

*These authors contributed equally to this study.

Correspondence: Stefania Silvi, School of Biosciences and Veterinary Medicine, University of Camerino, Via Gentile III da Varano, 62032 Camerino, Italy. Tel: +39 (0)737402707. Fax: +39 (0)737402418. E-mail: stefania.silvi@unicam.it

Verification of the beneficial effects of SYNBIO® probiotic-enriched foods and dietary supplement capsule should enable us to offer consumers a varied choice of matrices to match their preferences.

Methods

Trial design

The study was a randomised, double-blind, placebo-controlled, parallel group study assessing the effect of daily consumption of SYNBIO® (SYNBIOTEC Srl, Camerino, Italy), a mixture 1:1 of *L. rhamnosus* IMC 501® (SYNBIOTEC Srl) and *L. paracasei* IMC 502® (SYNBIOTEC Srl), by probiotic-enriched foods or by dietary supplement on the bowel habits of healthy adults. The subjects were randomly assigned to one of four parallel groups, in a 1:1:1:1 ratio, to receive either probiotic-enriched foods, capsules or the respective placebo. For the allocation of the participants, a computer-generated list of random numbers was used. The experimental groups are the following: probiotic foods group (PFG), probiotic foods control group (cPFG), capsule group (CG) and capsules control group (cCG).

Study products

Six different food products were used as carriers for delivering probiotic bacterial strains: yoghurt – produced and provided by a local dairy producer; ''ricotta'' cheese and ''mozzarella'' cheese – produced and provided by two local cheese factories specialised in the production of these particular kinds of cheeses; chocolate – produced and provided by a local chocolate maker; chocolate mousse – produced and provided by a local pastry maker; and ice-cream – produced and provided by a local ice-cream shop.

All the products were enriched with the SYNBIO® mixture during their normal production process, as previously reported (Verdenelli et al., 2009), directly on production site and after a careful analysis of the best method of inoculum for each specific product. Each product was tested with different concentrations of bacterial strains to reach the best inoculum concentration that allowed a value of approximately 10^9 CFU/g in the final food product and during its shelf-life (Coman et al., 2012). The capsules containing approximately 10^9 CFU/capsule of SYNBIO® were prepared by the School of Sciences of the Drug and Health Products, University of Camerino, Italy.

The placebos allocated to the food control group were the same food carriers devoid of the test probiotics and they were produced and provided by the same producers of the foods with probiotics. The placebos allocated to the cCG were identical capsules containing pure maltodextrin instead of probiotics. Maltodextrin is completely digested before entering the colon, and it does not affect the intestinal microflora. The placebo capsules were produced and provided by the same Institution of the probiotic capsules.

Subjects

Eligible participants were all healthy adults aged 18–65 years, and they were recruited mostly from the University personnel and students *via* e-mail and word-of-mouth advertisements.

Inclusion criteria were healthy persons (chronic diseases controlled with proper medications were allowed), age and acceptance of the study protocol. Antibiotics were not allowed two months before the intervention. The lactose-intolerant people were excluded. Background information was collected from the volunteers through screening interviews and included questions regarding general health, medications and manner of living. All the subjects gave their informed consent to participate. The followed procedures were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 1983.

Intervention

The study was performed with a four-week run-in period followed by a 12-week intervention period. During the run-in period, subjects were asked to discontinue the consumption of laxatives, dietary fibre supplements and probiotics. The participant received a list of all probiotic products offered by the market and were not allowed to consume any of them. A part from that, they were instructed to continue their normal diet and lifestyle. During the intervention period, the subjects of one group (PFG) consumed one or more probiotic-enriched food daily. The subjects were instructed to consume during the day one or more probiotic-enriched food portions of approximately 80–120 g (about 10^9 CFU per serving). They were free to consume their favourite foods among those provided, at least one probiotic-enriched product per day. Furthermore, the subjects were also instructed to consume the whole variety of six foods within the week. The investigators weekly delivered a refrigerated bag containing the different food products or the capsules to each volunteer at a distribution site placed in the University.

The use of the study products was recorded daily, and the records were checked by the investigators at each visit. Volunteer compliance was determined by verbal assessment by the investigators. The amount of product returned was recorded to confirm compliance. The subjects of second group (CG) took one capsule in the morning at breakfast (about 10^9 CFU/capsule).

Outcome measures

Bowell well-being

The study was designed for one primary outcome: the overall assessment of bowel well-being. Primary outcome measures were intestinal regularity and stool volume. These parameters were self-evaluated by subjects at the end of the intervention by questions on ''change in the numbers of times of defecation per day as stool frequency'' and ''change in the number of eggs (large size) that correspond to the volume by visual estimation''. The people were asked to record if in the last three months their bowel well-being in terms of intestinal regularity and stool volume has remained the same, improved or worsened compared to the period before beginning the consumption of the probiotic products and also the degree of change on a combined scale leading to a 10-point Likert scale (−5, 0, +5). Secondary outcome measures were also investigated: ease at defecation, bloating, constipation, abdominal pain, intestinal cramps, feeling of incomplete defecation, incontinence and halitosis (also the change of these individual bowel habits was assessed with the same combined Likert scale). Stool consistency was defined by the Bristol Stool Form Scale (Lewis & Heaton, 1997).

Health-related quality of life

Health-related quality of life (HRQoL) of subjects was assessed by self-administration of Psychological General Well-Being Index (PGWBI) (Dupuy, 1984) that is a general questionnaire measuring psychological well-being and distress and is composed of 22 items, which constitute six dimensions (anxiety, depression, self-control, positive well-being, general health and vitality).

The multidimensional scores can be summarised to provide a global score. The score ranges from 0 (worst) to 100 (best).

Gastrointestinal tolerance

Gastrointestinal tolerance was determined with the Gastrointestinal Symptom Rating Scale (GSRS) (Revicki et al.,

1998) based on 15 items, each rated on a seven-point Likert scale from no discomfort (score of 1) to very severe discomfort (score of 7). All the 15 items of the questionnaire have been evaluated and intolerance was defined as a symptom score of two or higher on the GSRS.

Sample size

The calculated sample size was based on the assumption that the primary outcome measures, such as intestinal regularity and stool volume, would be increased at least 20% in the groups receiving probiotics in comparison with a control. To detect such a difference with a two-tailed test significance level of 0.05 and a power of 90%, a total of 1072 subjects was enrolled. However, some subjects withdrew prematurely and were not replaced and a total of 862 subjects participated to the study.

Randomization

Subjects were randomly allocated to one of the four treatment groups using computer-generated randomization lists. The study coordinator at the investigative site enrolled and assigned subjects to treatment groups. Study products were labelled with sequential subject identification numbers and were provided to the investigative site by the producers.

Blinding

The study was conducted using double-blinding method: subjects and investigators remained blinded to the treatment assignments until data analyses were completed. Study products were delivered to the investigative site. Identical boxes, each containing the six food products, were labelled only with the lot number and subject identification codes and delivered to the investigative sites. Furthermore, capsules were identical in appearance, texture, taste and smell.

Recovery of probiotic strains from faecal samples

This analysis was carried out in order to confirm intestinal transit survival of probiotics. Faecal samples from a cohort of 100 subjects from each group (50 male and 50 female) were handed to the investigator after run-in (day 0) and at the end of 12 weeks of probiotic supplementation. The faecal samples analysis consisted in the enumeration of vancomycin and gentamicin-resistant lactobacilli and in the recovery of the probiotic strains identified by RAPD method as previously described (Verdenelli et al., 2009).

Statistical methods

Comparisons of the baseline characteristics of group subjects and of the mean absolute changes in the bowel habits frequency score, in stool consistency and in the PGWBI across the treatment and control groups were performed using the Four Multiple Comparisons test, with significance level of $p < 0.05$.

The same tests were applied also to compare the evaluation of gastrointestinal symptom score according to GSRS, used in our case to define the tolerance of the probiotics. The Student's t test was applied to the microbiological analysis results.

Support vector machines

To support and validate the outcomes coming from the study, we take advantage of an additional approach to the data using Support Vector Machines (SVM). This tool consists of a class of ''learning machines'', which have recently been introduced for solving pattern recognition and function estimation problems and have become a subject of intensive study because of their successful applications in the fields of economics, engineering, science and sociology. The SVM method is based on learn models that use a training set of known data and are able to well generalise to unseen data (for the correct use of the model, various specific parameters need to be set). We applied the basics of SVM for classification problems to point out if there are substantial differences, first, between people assuming probiotics and people in the control groups and second between the modality of administration and to select a subset of relevant features to use in model construction for people assuming probiotics (De Cosmis & De Leone, 2012; De Leone, 2010).

Results

Participant flow

Figure 1 describes the flow of subjects through the protocol. From the 1072 contacted subjects, 914 were included in this study and a total of 862 were randomised. Of the subjects, 14 did not complete the entire 12-week double-blind period for personal reasons. Therefore, 848 subjects were included in the statistical analysis. No subject withdrawals were related to the study product. Among subjects who completed the study, compliance with the study products was 100% in each study group.

Baseline characteristics of subjects

The comparison of the baseline characteristics of the 848 volunteers who completed the study is listed in Table 1. Subjects were well balanced over the four groups with respect to baseline characteristic such as age, gender, height and weight. In fact, there were no significant differences neither within the treated and the respective control group nor between the two different treated groups ($p > 0.05$; by Four Multiple Comparison test).

Bowel well-being

Primary outcomes, such as intestinal regularity and stool volume, are listed in Table 2. Changes in intestinal regularity and stool volume score showed significantly ($p < 0.05$) higher values in the capsules group (CG) and PFG compared with the respective control groups over the 12 weeks of intervention. No significant differences were presented between the two probiotic supplemented groups ($p > 0.05$).

The subjects using the probiotics in both formulations also presented significant higher values ($p < 0.05$) in the positive scale of score regarding other bowel habits such as ease at defecation, bloating, constipation and feeling of incomplete defecation compared to the subjects of the respective control groups (Table 2). The same bowel habits were not significantly different if compared between the two probiotic supplemented groups. Abdominal pain, intestinal cramps, incontinence and halitosis showed no significant difference neither within treated groups and respective controls nor between the two probiotic-treated groups. Stool consistency was generally evaluated of type 3 or 4 and presented significant difference both between the treated groups and their respective controls and between the two probiotic-treated groups.

Health-related quality of life

HRQoL was estimated as PGWBI global score resulting mean values of 81.7 and 82.1 for the probiotic-supplemented groups (CG and PFG, respectively) and 73.3 and 73.4 for the respective control groups (cCG and cPFG, respectively) considering an evaluated range from 0 to 100 (best). The subjects who had the probiotic supplementations (in both the ways) showed a significant higher PGWBI global score ($p < 0.05$).

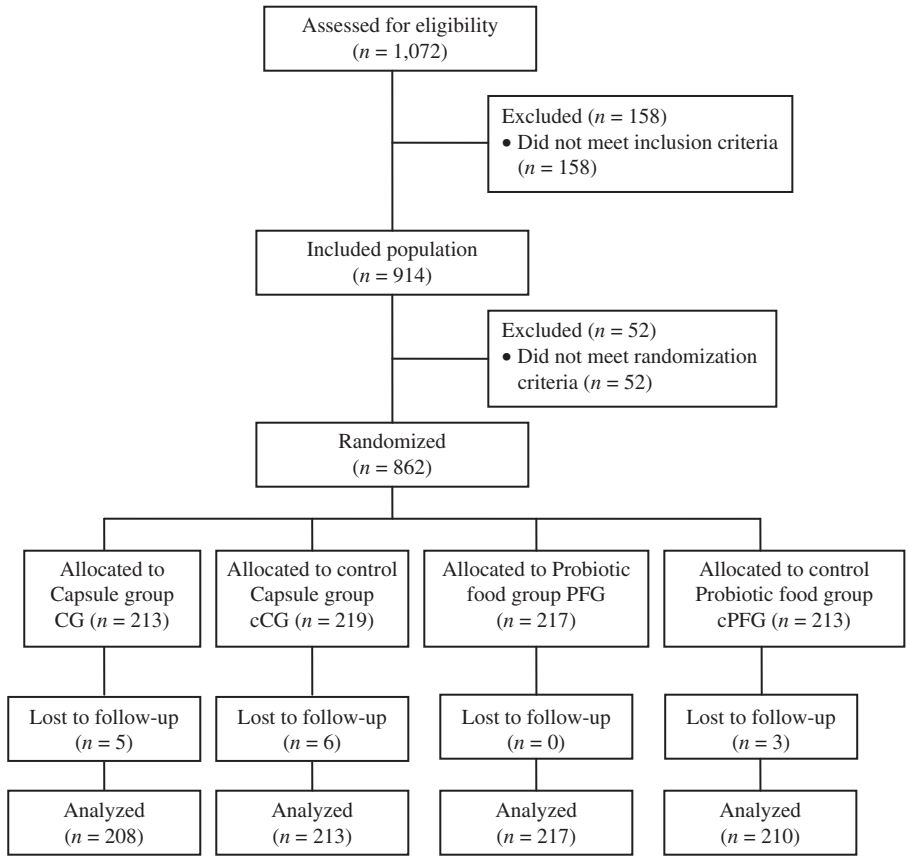

Figure 1. CONSORT subjects flow diagram. CG, capsules group; cCG, control capsules group, PFG, probiotic foods group; and cPFG, control probiotic foods group.

Table 1. Baseline characteristics of subjects: comparison between the four experimental groups.

	Capsules group (CG) ($n = 208$)	Control capsules group (cCG) ($n = 213$)	Probiotic foods group (PFG) ($n = 217$)	Control probiotic foods group (cPFG) ($n = 210$)	p[a]
Age, year (mean ± confidence limits)	44.1 ± 1.4	44.0 ± 1.2	45.0 ± 0.9	44.0 ± 0.9	>0.05
Male	48.6 ± 1.4	48.1 ± 1.1	48.1 ± 0.8	47.8 ± 0.9	>0.05
Female	40.7 ± 1.2	40.8 ± 1.0	42.3 ± 0.8	41.2 ± 0.7	>0.05
Gender					
Male *n* (%)	92 (44)	96 (45)	100 (46)	90 (43)	
Female *n* (%)	116 (56)	117 (55)	117 (54)	120 (57)	
Height (m) (mean ± confidence limits)	1.73 ± 1.3	1.72 ± 2.3	1.73 ± 0.9	1.73 ± 1.1	>0.05
Weight (kg) (mean ± confidence limits)	69.4 ± 1.3	65.8 ± 1.4	68.6 ± 1.2	68.3 ± 1.3	>0.05

[a]The significance level is of $p < 0.05$ by Four Multiple Comparisons test.

Gastrointestinal tolerance

The GSRS based on 15 items revealed a median value of 1 (no discomfort), for each symptom and for each treated group as well as in the respective control. This showed the absence of intolerance of the probiotic treatment in both of groups. However, in all groups (treated and control), the maximum score assessed was 5 (moderately severe discomfort), and there was no significant difference between the probiotic groups and their respective controls (Table 3).

Computational experiments by SVMs

To support the outcomes coming from the bowel habits questionnaire, the HRQoL considering the PGWBI global score and the evaluation of gastrointestinal tolerance based on the GSRS, SVMs models for the classification problem have been used in three different experiments considering the whole data set. In what follows, we will indicate with A and B the input data sets, where A is the set containing data from people assuming probiotics with capsule and the respective placebo (CG group+cCG group) and B the set containing people assuming probiotics with foods and the respective placebo (PFG group+cPFG group). In the first experiment, the input data sets were joined, in order to build the set referred as (A+B), and the 80% of (A+B) was used as the training set, that is to train the SVM model, and the remaining 20% of (A+B) was used to test the performances of the SVM model to discriminate between the use or not of probiotics (assumed with capsules or through food). We recall that in order to build a model, it is necessary to define the kernel function and a set of parameters.

Table 4 displays the training and the testing sets prediction accuracy (the second row in the table contains the percentages, while the last row contains the percentage of correctly predicted values upon the whole). The testing error is the minimum found after numerous changes of the parameters of the SVMs model. This experiment points out substantial differences between people who had probiotics and people who had not, regardless the method of administration.

Table 2. Mean absolute changes in bowel habits frequency score, stool consistency and global score of the Psychological General Well-Being Index (PGWBI) questionnaire over the 12 weeks of supplementation period.

Bowel habits[a]	Capsules group (CG) ($n=208$) (mean ± confidence limits)	Control capsules group (cCG) ($n=213$) (mean ± confidence limits)	Probiotic foods group (PFG) ($n=217$) (mean ± confidence limits)	Control probiotic foods group (cPFG) ($n=210$) (mean ± confidence limits)	CG versus PFG p
Intestinal regularity	3.3 ± 0.2^b	1.0 ± 0.1	3.5 ± 0.2^b	0.9 ± 0.1	NS
Stool volume	2.7 ± 0.2^b	0.8 ± 0.1	2.7 ± 0.2^b	0.7 ± 0.1	NS
Ease at defecation	2.6 ± 0.2^b	0.6 ± 0.1	2.5 ± 0.2^b	0.8 ± 0.1	NS
Bloating	1.7 ± 0.2^b	0.3 ± 0.1	1.5 ± 0.2^b	0.4 ± 0.1	NS
Constipation	1.3 ± 0.3^b	0.2 ± 0.1	1.0 ± 0.3^b	0.2 ± 0.1	NS
Abdominal pain	0.4 ± 0.2	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	NS
Intestinal cramps	0.5 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	NS
Feeling of incomplete defecation	1.3 ± 0.2^b	0.2 ± 0.1	1.1 ± 0.2^b	0.2 ± 0.1	NS
Incontinence	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	NS
Halitosis	0.5 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	NS
Stool consistency[c]					
Type	3 ± 0.1^b	4 ± 0.1	4 ± 0.1^b	3 ± 0.1	S
PGWBI[d]					
Global score	81.7 ± 2.0^b	73.3 ± 0.2	82.1 ± 1.5^b	73.4 ± 0.4	NS

[a]Changes in bowel habits were assessed with a ten-combined Likert scale (−5, 0, +5);
[b]Significantly different from the respective control group (Four Multiple Comparison test, $p<0.05$)
[c]The assessment has been obtained following the Bristol Stool chart
[d]The global score ranged from 0 to 100 (best)
NS: not significantly different (Four Multiple Comparison test, $p>0.05$).
S: significantly different (Four Multiple Comparison test, $p<0.05$).

Table 3. Gastrointestinal symptom score according to Gastrointestinal Symptom Rating Scale (GSRS) in the four experimental groups (median value and range).

	Capsules group (CG) ($n=208$)		Control capsules group (cCG) ($n=213$)		Probiotic foods group (PFG) ($n=217$)		Control probiotic foods group (cPFG) ($n=210$)	
Symptom[a]	Median	Range	Median	Range	Median	Range	Median	Range
Stomach ache or pain	1	1–4	1	1–5	1	1–4	1	1–5
Heartburn	1	1–3	1	1–4	1	1–4	1	1–5
Acid reflux	1	1–2	1	1–3	1	1–2	1	1–3
Hunger pains stomach/belly	1	1–5	1	1–5	1	1–5	1	1–5
Nausea	1	1–1	1	1–5	1	1–3	1	1–3
Rumbling stomach/belly	1	1–4	1	1–5	1	1–5	1	1–4
Bloated stomach/belly	1	1–3	1	1–5	1	1–5	1	1–5
Burping	1	1–4	1	1–5	1	1–4	1	1–4
Passing gas or flatus	1	1–5	1	1–5	1	1–4	1	1–4
Constipation	1	1–5	1	1–5	1	1–2	1	1–5
Diarrhoea	1	1–4	1	1–4	1	1–3	1	1–2
Loose stools	1	1–3	1	1–4	1	1–2	1	1–5
Hard stools	1	1–5	1	1–2	1	1–5	1	1–5
Urgent need (bowel movement)	1	1–5	1	1–5	1	1–4	1	1–5
Feeling of not completely emptying	1	1–5	1	1–3	1	1–4	1	1–4

[a]Gastrointestinal symptoms were assessed with a seven-point Likert scale from no discomfort (1) to very severe discomfort (7) using GSRS questionnaire.

Table 4. Basic classification in the computational experiments and the corresponding prediction accuracy.

Training set	Testing set
80% (A+B)	20% (A+B)
96.9%	95.85%
658/679	162/169

A=data from people assuming probiotics with capsule and the respective placebo group.
B=data from people assuming probiotics with foods and the respective placebo group.

The second experiment used the 80% of A as the training set. After calculating the best values of the parameters using the remaining 20% of A, the resulting model was used to discriminate the data in the set B. The results of this second experiment are listed in Table 5, where the same notation introduced above is used. Table 5 shows that there are substantial differences between the sets A and B; in fact, the prediction accuracy for the Testing Set 1 is larger than the accuracy for the Testing Set 2.

The last experiment was completely analogous to the second one, but the roles of sets A and B were inverted. This means that the 80% of B was used as the training set, and the testing sets were the remaining 20% of B and the set A. The second part of Table 5 confirms differences between the input data sets A and B.

Table 5. Prediction accuracy of B and A testing set.

Training set	Testing set 1	Testing set 2
Testing set B		
80% A	20% A	B
99.40%	92.85%	84.30%
335/337	78/84	360/427
Testing set A		
80% B	20% B	A
98.53%	96.47%	86.93%
337/342	82/85	366/421

A = data from people assuming probiotics with capsule and the respective placebo group.
B = data from people assuming probiotics with foods and the respective placebo group.

Table 6. Prediction accuracy of B and A testing set with feature selection.

Training set	Testing set 1	Testing set 2
Testing set B		
80% A	20% A	B
94.06%	100%	82.20%
317/337	84/84	351/427
Testing set A		
80% B	20% B	A
94.44%	100%	88.83%
323/342	85/85	374/421

A = data from people assuming probiotics with capsule and the respective placebo group.
B = data from people assuming probiotics with foods and the respective placebo group.

Two additional experiments were performed for features selection, that is, to identify those features that better characterise the sets A and B, respectively. In the first one, the training set was 80% of A. With fixed parameters for the model, we used Simulated Annealing (Kirkpatrick et al., 1983) to determine the six features that maximise the prediction accuracy on the testing set, constituted by the remaining 20% of A. Repeating this experiment with different model parameters, the best values, i.e. the parameters that minimise the prediction error, and the corresponding six features were selected. The quality of the final model was tested on the second testing set B. Table 6 contains the training and testing sets with the relative prediction accuracy. The selected features were: easy at defecation, bloating, stool consistency, PGWBI (global score), burping and feeling of not completely emptying.

Table 6 shows that the prediction accuracy on the testing set 2 (set B) using only six of the 27 available features is comparable with the prediction accuracy of B using all the features (see Table 5 for comparison). Another important observation is related to the features that mainly emerged from the analysis (that is changing the parameters). They are as follows: easy at defecation, feeling of not completely defecation and loose stools. These three features were selected as the most relevant in all experiments and can be considered as the ones which let to better distinguish between people assuming probiotics with capsule and the respective placebo.

In a new experiment, the roles of A and B were inverted, and the corresponding prediction accuracy is displayed in Table 6. In this case, the selected features were as follows: intestinal regularity, stool volume, abdominal pain, intestinal cramp, halitosis and PGWBI (global score).

In this experiment, removing some of the available features reduces the prediction error on the testing set 2 (set A) (see Table 5 for comparison). This means that some features are able to characterise the phenomenon under examination more than others. Moreover, when changing the parameters, it can be observed that the most present features are intestinal regularity and PGWBI (global score). These are the features that let to better distinguish between people assuming probiotics with foods and the respective placebo.

Recovery of probiotic strains from faecal samples

Faecal samples were collected from a cohort of 100 subjects for each experimental group at the first day after the run-in and after 12 weeks of probiotic supplementation. Specimens were analysed, and the *Lactobacillus* spp. was enumerated and assessed by RAPD to detect the presence of the probiotic strains. Prior to probiotic supplementation, it was found that all the subjects randomised in the four experimental group harboured lactobacilli in a range of values from $8.9 \times 10^4 \pm 1.2 \times 10^5$ CFU/g of faeces (PFG) to $1.9 \times 10^5 \pm 1.4 \times 10^5$ CFU/g of faeces (CG) (Table 7). No strains were recovered, which had RAPD fingerprints corresponding to *L. rhamnosus* IMC 501® and *L. paracasei* IMC 502®. After 12 weeks of probiotic supplementation, the *Lactobacillus* counts as CFU/g of faeces remained almost stable in the two control groups ($2.2 \times 10^5 \pm 1.4 \times 10^5$ for cCG and $1.4 \times 10^5 \pm 1.1 \times 10^5$ for cPFG), while in the probiotic groups, the *Lactobacillus* counts significantly increased over the 12 weeks ($p < 0.05$) at a value of $2.4 \times 10^7 \pm 5.2 \times 10^6$ CFU/g of faeces and $1.9 \times 10^7 \pm 6.3 \times 10^6$ CFU/g of faeces in CG and PFG groups, respectively.

Lactobacillus rhamnosus IMC 501® and *L. paracasei* IMC 502® were detected in the faecal samples of subjects belonging to both supplemented probiotic groups with a mean frequency of about the 75% for *L. rhamnosus* IMC 501® and the 83.5 % for *L. paracasei* IMC 502® (Table 7).

Discussion

In this study, the ability of foods and a dietary supplement containing SYNBIO® to affect bowel habits was investigated in healthy adults volunteers.

The literature documents the positive effects of probiotics on several gastrointestinal disorders (Ciorba, 2012; Marteau et al., 2001; Ng et al., 2009), whereas only a few studies have addressed the positive effects of probiotics on healthy people (Kim et al., 2013; Larsen et al., 2006; Olivares et al., 2006; Verdenelli et al., 2011), mainly because of the difficulty of assessing the effects of a probiotic treatment on people free of significant health problems. This hurdle needs to be overcome, as it would be very important to demonstrate the effectiveness of probiotics in improving the well-being of healthy subjects, who form the vast majority of consumers of functional products (Annunziata & Vecchio, 2013).

Bowel habits can be perceived in different ways, and a universal definition of the cut-off between normality and disease has yet to be established (Bassotti et al., 2004; Higgins & Johanson, 2004; Sandler & Drossmann, 1987). This study evaluated bowel habits in subjects who perceived themselves as normal regarding these functions. This criterion of self-perceived normalcy cannot be considered arbitrary, as well-being is essentially a matter of self-perception.

We were interested in furthering inquiry begun in a previous study of ours that yielded significant results on the ability of SYNBIO® to improve intestinal regularity and faecal volume in a population of healthy adults (Verdenelli et al., 2011). To eliminate some limits of the previous study, the number of adult subjects has

Table 7. Total vancomycin- and gentamicin-resistant *Lactobacillus* count and recovery of *L. rhamnosus* IMC 501® and *L. paracasei* IMC 502® in faecal samples of subjects at day 0 (the first day after the run-in) and after 12 weeks of probiotic supplementation.

	Lactobacillus spp CFU/g of faeces (means ± sd)		N° of positive subjects for the recovery of *L. rhamnosus* IMC501®/cohort subjects		No. of positive subjects for the recovery of *L. paracasei* IMC502®/cohort subjects	
Day of sampling	Day 0[a]	After12 weeks[b]	Day 0	After12 weeks	Day 0	After12 weeks
Capsules group (CG)	$1.9 \times 10^5 \pm 1.4 \times 10^5$	$2.4 \times 10^7 \pm 5.2 \times 10^{6c}$	0/100	76/100	0/100	85/100
Control capsules group (cCG)	$9.8 \times 10^4 \pm 1.1 \times 10^5$	$2.2 \times 10^5 \pm 1.4 \times 10^5$	0/100	0/100	0/100	0/100
Probiotic foods group (PFG)	$8.9 \times 10^4 \pm 1.2 \times 10^5$	$1.9 \times 10^7 \pm 6.3 \times 10^{6c}$	0/100	74/100	0/100	82/100
Control probiotic foods group (cPFG)	$1.2 \times 10^5 \pm 1.3 \times 10^5$	$1.4 \times 10^5 \pm 1.1 \times 10^5$	0/100	0/100	0/100	0/100

[a]Day 0: the first day after the run-in.
[b]After 12 weeks of probiotic supplementation.
[c]Significantly different from the day 0 ($p < 0.05$ by Student t test).

been increased and both probiotic-enriched foods and capsules have been used as carriers.

Interest in probiotics has been growing over the years, with the number of products containing probiotic bacteria increasing significantly from North America to Asia, and Eastern Europe to Western Europe (Heller, 2009). It is estimated that the probiotic industry holds about a 10% share of the global functional food market (Starling, 2009), with 10 billion euros in sales in 2008, and that the global market for probiotic supplements such as pills, caplets and capsules in 2008 alone was worth approximately $1.5 billion (Heller, 2009). Therefore, products that contain probiotic bacteria are of considerable and growing economic importance.

Probiotic bacteria were initially incorporated into yogurt products. Today, however, numerous foods are employed or are being developed as delivery vehicles for probiotics. In recent years, new consumer demand has prompted research on novel probiotic foods such as beverages, cookies, ice-cream, dairy dessert, sausages and others (Caramia & Silvi, 2011; Coman et al., 2012; Martins et al., 2013). This study examined six foods as well as capsules filled with the same SYNBIO® blend to satisfy the varying preferences of consumers.

Confirming the 2011 report by Verdenelli et al., this study found that consumption of SYNBIO® probiotic-enriched foods and dietary supplement produced improved intestinal regularity, according to the results of a validated questionnaire on bowel habits filled out by healthy volunteers enrolled in the human study, who normally do not suffer from gastrointestinal diseases. The same effects were not registered in the control groups. In fact, subjects who consumed the SYNBIO® foods or supplement capsules reported significantly higher mean score values than control group subjects for parameters such as intestinal regularity, stool volume, ease of defecation and less frequent bloating, constipation and sensation of incomplete defecation. It is generally thought that optimal bowel function consists of large stools, more frequent defecation and more rapid transit rates (Slavin & Marlett, 1980; Vuksan et al., 2008). These are considered measures of optimal bowel function because they presumably prevent prolonged residence of residue in the colon and promote ease of defecation, while at the same time, permit adequate small bowel digestion and absorption and large bowel fluid and electrolyte re-absorption.

The questionnaire results were also confirmed by the use of SVM, which allowed us to draw other interesting conclusions. First of all, the SVMs model pointed out a sharp division between subjects given probiotics and those in the control groups, regardless of the mode of administration. In fact, the prediction accuracy of classification results, listed in Table 4, were remarkably good. Table 5 lists differences between delivery of the probiotics through food and through capsules: the prediction errors are different for the testing sets 1 and 2. Finally, the feature selection experiment made it possible to define the features that better characterise subjects who received probiotics through food and those who received them in capsules. Ease of defecation, a sensation of incomplete defecation and loose stools seemed to be the most significant features for people receiving probiotics in capsules, while intestinal regularity and the PGWBI global score emerged as the prominent ones for the group receiving probiotics through food.

In general, the increase in faecal volume (weight) is frequently associated with reduced intestinal transit time of food. In our study, not only stool volume but also its consistency was significantly different between the probiotic group and controls. It should be kept in mind, however, that this feature is also affected by the carrier of supplementation. The stool type in subjects receiving the probiotic foods differed significantly from that in subjects who received the capsule form. The food matrix affected the stool consistency, nevertheless the types 3 and 4 represent the ideal stool consistency/shape, in particular type 4, which is easy to defecate. It is noteworthy that all participants who consumed probiotics reported a sensation of bowel well-being as ''feeling good''. This condition was strongly highlighted by the PGWBI global scores for both probiotic supplemented groups, which were significantly higher than those of control groups. The SVM singled out the PGWBI global score as one of the most present features, defining it a characterizing feature for the probiotic consumption groups.

In addition, SYNBIO® supplementation is safe, as test subjects reported no adverse events when providing information for the gastrointestinal symptom score, calculated using the GSRS (Revicki et al., 1998).

The persistence of probiotics in the gastrointestinal tract is an important parameter for assessing their effectiveness. Saxelin et al. (2010) showed that capsules, yoghurt and cheese were good vehicles for the administration of a probiotic combination of four bacteria strains, verifying the persistence of the strains in the gastrointestinal tract of a generally healthy population. Using the SYNBIO® probiotic combination, Verdenelli et al. (2009) and Martarelli et al. (2011) reported recovery of two strains administered in food and in a dietary supplement, respectively, in two different healthy populations. In this study, the recovery of probiotic bacteria from the faeces of a cohort of 100 subjects for each supplemented group demonstrated the persistence of strains in the gastrointestinal tract and indicated that both methods of administration, food matrix and capsule, yielded high quantities of the two strains. A parallel study by Coman et al. (2012) confirmed that all the probiotic foods used in our study were of great potential as vehicles for probiotic cultures and offered the additional advantage of being foods for all age groups. At the

same time, the Coman et al. (2012) study demonstrated that the food matrices do not affect the viability and functionality of the probiotic combination.

Conclusions

To our knowledge, this study is the first to compare the effects of two different types of probiotic carriers (foods and a dietary supplement) on bowel habits in a large healthy population. The results of this study demonstrated no significant differences in the two carriers of probiotics, as both gave positive significant effects on bowel habits of subjects who received the probiotics, compared to the control population. There are varying opinions about the choice between probiotics from natural foods or from supplements. Some point out that it is difficult to know exactly what is contained in a probiotic supplement, since supplements are not subjected to the rigorous testing that medications or foods must undergo. Others deem probiotic foods healthier than capsules or powders, because foods contain other beneficial nutrients that supplements do not.

The results of this study are of both scientific and commercial interest, since they indicate that the consumer may safely choose between our probiotic foods and capsules, which have proven persistence in the gastrointestinal tract and the same beneficial effects on the bowel habits of a healthy adult population.

Declaration of interest

The authors declare no conflict of interest.

References

Annunziata A, Vecchio R. 2013. Consumer perception of functional foods: a conjoint analysis with probiotics. Food Qual Prefer 28: 348–355.

Ares G, Giménez A, Gambaro A. 2009. Consumer perceived healthiness and willingness to try functional milk desserts: influence of ingredient name and health claim. Food Qual Pref 20:50–56.

Bassotti G, Bellini M, Pucciani F, Bocchini R, Bove A, Alduini P, Battaglia E, Bruzzi P. 2004. An extended assessment of bowel habits in a general population. World J Gastroenterol 10:713–716.

Caramia G, Silvi S. 2011. Probiotics: from the ancient wisdom to the actual therapeutical and nutraceutical perspective. In: Malago JJ, Koninkx JFJG, Marinsek-Logar R, editors. Probiotic bacteria and enteric infections. New York: Springer. p 3–37.

Chonan O. 2011. FOSHU Japanese regulation for probiotic foods. In: Takeda Y, editor. Probiotic foods in health and diseases. Boca Raton (FL): Sciences publishers CRC Press Enfield. p 33–40.

Ciorba MA. 2012. A gastroenterologist's guide to probiotics. Clin Gastroenterol Hepatol 10:960–968.

Coman MM, Cecchini C, Verdenelli MC, Silvi S, Orpianesi C, Cresci A. 2012. Functional foods as carriers for SYNBIO®, a probiotic bacteria combination. Int J Food Microbiol 157:346–352.

Cruz AG, Antunes AEC, Pilleggi ALOPS, Faria JAF, Saad SMI. 2009a. Ice cream as probiotic food carrier. Food Res Int 42: 1233–1239.

Cruz AG, Buriti FCA, Souza CHB, Faria JAF, Saad SMI. 2009b. Probiotic cheese: health benefits, technological and stability aspects. Trends Food Sci Technol 20:344–354.

De Cosmis S, De Leone R. 2012. Support vector machine for robust regression. In: COMTESSA, editor. Proceedings of the CTW 2012 11th Cologne-Twenty Work-shop on Graph and Combinatorial Optimization. Munich, Germany. p 96–99.

De Leone R. 2010. Support vector regression for time series analysis. In: Hu B, Morash K, Lickl S, Siegle M, editors. Operations research proceedings 2010. Berlin: Springer. p 33–38.

Dupuy HJ. 1984. The psychological general well-being (PGWB) index. In: Wenger N, Mattson ME, Furberg CF, Elinson J, editors. Assessment of quality of life in clinical trials of cardiovascular therapies. New York: Le Jacq Publishing. p 170–184.

European Food Safety Authority. 2009. Briefing document for member states and European Commission on the evaluation of Article 13.1 health claim. Parma, Italy, 11 November 2009. EFSA J 7:1386–1396.

Figueroa-González I, Quijano G, Ramírez G, Cruz-Guerrero A. 2011. Probiotics and prebiotics-perspectives and challenges. J Sci Food Agr 91:1341–1348.

Gokhale G, Nadkarni A. 2007. Probiotic food market in India soon be regulated. India Law J. Available at: http:www.indialawjournal.com/volume3/issue_1/article_by_aditi.html. Accessed on 2007.

Granato G, Branco GF, Nazzaro F, Cruz AG, Favia JAF. 2010. Functional foods and nondairy probiotic food development: trends, concepts, and products. Compr Rev Food Sci Food Saf 9:292–302.

Health Canada. 2009. Guidance document – the use of probiotic microorganisms in food, Food Directorate, Health products and food branch Health Canada, April 2009. Available at: http://www.hc-scgc.ca/n-an/alt_formats/hpfb-dgpsa/pdf/legislation/probiotics_guidance-orientation_probiotiques-eng.pdf.

Heller L. 2009. Danisco breaks down probiotics market, Nutra Ingredients, USA (2009). Available at: www.nutraingredients-usa.com/content/view/print/245558.

Higgins PR, Johanson JF. 2004. Epidemiology of constipation in North America: a systematic review. Am J Gastroenterol 99:750–759.

Kim SW, Suda W, Kim S, Oshima K, Fukuda S, Ohno H, Morita H, Hattori M. 2013. Robustness of gut microbiota of healthy adults in response to probiotic intervention revealed by high-throughput pyrosequencing. DNA Res Adv Access 4:1–13.

Kirkpatrick S, Gelatt CD, Vecchi MP. 1983. Optimization by simulated annealing. Science 220:671–680.

Larsen CN, Nielsen S, Kaestel P, Brockmann E, Bennedsen M, Christensen HR, Eskesen DC, et al. 2006. Dose-response study of probiotic bacteria *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Lactobacillus paracasei* subsp. *paracasei* CRL-341 in healthy young adults. Eur J Clin Nutr 60:1284–1293.

Lewis SJ, Heaton KW. 1997. Stool form scale as a useful guide to intestinal transit time. Scand J Gastroenterol 32:920–924.

Marette S, Roosen J, Blanchemanche S, Feinblatt-Mélèze E. 2010. Functional food, uncertainty and consumers choice: a lab experiment with enriched yoghurts for lowering cholesterol. Food Policy 35: 419–428.

Martarelli D, Verdenelli MC, Scuri S, Cocchioni M, Silvi S, Cecchini C, Pompei P. 2011. Effect of a probiotic intake on oxidant and antioxidant parameters in plasma of athletes during intense exercise training. Curr Microbiol 62:1689–1696.

Marteau PR, de Vrese M, Cellier CJ, Schrezenmeir J. 2001. Protection from gastrointestinal diseases with the use of probiotics. Am J Clin Nutr 73:430S–436S.

Martins EMF, Ramos AM, Vanzela ESL, Stringheta PC, de Oliveira Pinto CL, Martins JM. 2013. Products of vegetable origin: a new alternative for the consumption of probiotic bacteria. Food Res Int 51:764–770.

Miller LE, Ouwehand AC. 2013. Probiotic supplementation decreases intestinal transit time: meta-analysis of randomized controlled trials. World J Gastroenterol 19:4718–4725.

Ng SC, Hart AL, Kamm MA, Stagg AJ, Knight SC. 2009. Mechanisms of action of probiotics: recent advances. Inflamm Bowel Dis 15:300–310.

Olivares M, Diaz-Ropero MA, Gomez N, Lara-Villoslada F, Sierra S, Maldonado JA, Martín R, et al. 2006. Oral administration of two probiotic strains, *Lactobacillu gasseri* CECT5714 and *Lactobacillus coryniformis* CECT5711, enhances the intestinal function of healthy adults. Int J Food Microbiol 107:104–111.

Reid G. 2008. Probiotics and prebiotics – progress and challenges. Int Dairy J 18:969–975.

Revicki DA, Wood M, Wiklund I, Crawley J. 1998. Reliability and validity of the gastrointestinal symptom rating scale in patients with gastroesophageal reflux disease. Qual Life Res 7:75–83.

Sandler RS, Drossmann DA. 1987. Bowel habits in young adults not seeking health care. Dig Dis Sci 32:841–845.

Saxelin M, Lassing A, Karjalainen H, Tynkkynen S, Surakka A, Vapaatalo H, Järvenpää S, et al. 2010. Persistence of probiotic strains in the gastrointestinal tract when administered as capsules, yoghurt, or cheese. Int J Food Microbiol 144:293–300.

Slavin JL, Marlett JA. 1980. Influence of refined cellulose on bowel function and calcium and magnesium balance. Am J Clin Nutr 33: 1932–1939.

Starling S. 2009. ''Alcimed'' study, probiotics must meet Europe's new health claim laws head on Nutra Ingredients, 2009. Available at: www.nutraingedients.com/content/view/print/242071.

Tabbers MM, Chmielewska A, Roseboom MG, Crastes N, Perrin C, Reitsma JB, Norbruis O, et al. 2011. Fermented milk containing *Bifidobacterium lactis* DN-173 010 in childhood constipation: a randomized, double-blind, controlled trial. Pediatrics 127: 1392–1399.

Verdenelli MC, Ghelfi F, Silvi S, Orpianesi C, Cecchini C, Cresci A. 2009. Probiotic properties of *Lactobacillus rhamnosus* and *Lactobacillus paracasei* isolated from human faeces. Eur J Nutr 48: 355–363.

Verdenelli MC, Silvi S, Cecchini C, Orpianesi C, Cresci A. 2011. Influence of a combination of two potential probiotic strains, *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502® on bowel habits of healthy adults. Lett Appl Microbiol 52: 596–602.

Vuksan V, Jenkins AL, Rogovik AL, Sievenpiper JL, Jovanovski E. 2008. Using cereal to increase dietary fiber intake to the recommended level and the effect of fiber on bowel function in healthy persons consuming North American diets. Am J Clin Nutr 88:1256–1262.

Yang YX, He M, Hu G, Wei J, Pages P, Yang XH, Bourdu-Naturel S. 2008. Effect of a fermented milk containing *Bifidobacterium lactis* DN-173010 on Chinese constipated women. World J Gastroenterol 14: 6237–6243.