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Brief running head: *L. helveticus* Lafti® L10 modulates immunity in elite athletes

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ABSTRACT

In order to test the influence of probiotic supplementation on humoral immune response a double-blind placebo-controlled trial was conducted. Thirty athletes (24 males and 6 females, females: VO_2max 38.2 ± 4.9 ml/kg/min, age 23.2 ± 1.4 years; males: VO_2max 57.5 ± 9.2 ml/kg/min, age 24.0 ± 2.4 years, mean ± SD) were randomized either to the probiotic group (*L. helveticus* Lafti® L10, 2 x 10^{10} CFU), or to the placebo group. Serum and saliva samples were collected at the baseline and after 14 weeks. Total and specific anti-bacterial antibody levels of IgM, IgG and IgA classes were determined towards different bacteria in the serum, while in saliva total and specific anti-bacterial IgA levels were examined. Total IgM was elevated in both probiotic (18%, 15 to 20%; mean, 90% confidence interval; p=0.02) and placebo group (35%, 22 to 47%; p=0.02), without observed differences in changes between the groups. No significant changes in IgM levels specific for tested bacteria were found. Total IgG level was constant in both groups. A significant (16%, -2.8 to 35%, p=0.04) reduction of anti - *Enterococcus faecalis* IgG was noted in the placebo group, in comparation to the probiotic group. There was a substantial decrease in total IgA level in the placebo group, when measured either in serum (15%, 12 to 18%, p=0.04) or in saliva (35%, -1.4 to 53%, p=0.03). The significantly reduced levels of serum anti-LAB IgA antibodies in the placebo group compared to the probiotic group were detected for *L. rhamnosus* LA68 for (24%, 5.8 to 42%, p=0.02) and for *L. rhamnosus* LB64 for (15%, 2.7 to 27%, p=0.02). Probiotic administration could have beneficial effects on systemic humoral and mucosal immune responses.

Key words: probiotics, salivary IgA, immunoglobulins, immune system

INTRODUCTION

Due to the competitive nature of professional sport, elite athletes are constantly in need to push boundaries, which is a difficult task, especially in times of rapidly increasing global population. Strenuous exercise leads to physical stress, which has an impact on the individuals' immune system. While moderate exercise has a beneficial effect on the immune system, compared to a sedentary lifestyle, excessive amounts of prolonged high-intensity exercise can impair immune function, leading to higher risk of upper respiratory tract infections (URTI) (37). URTI occurs in the period of strenuous exercise, particularly during winter months (18), thus negatively influencing athletes' training and consequently impairing performance during competitions.

Mucosal immunity impairment has been suggested to be a key risk factor for higher URTI incidence in elite athletes (37). Secretory IgA is reported to play a multifunctional role in mucosal immunity, including host protection by neutralizing bacterial, viral and fungal antigens and modulation of epithelial cells (9, 21). It is generally considered that salivary IgA level decreases in response to high intensity exercise, especially if it lasts over longer periods of time (>6 months) (14). Nevertheless, certain discrete dietary changes could compensate for the detrimental effects of strenuous exercise on mucosal immunity (14). Recent studies suggested that probiotic supplementation could help better mucosal immunity maintenance, or even induce its enhancement (15, 31, 36, 38).

As part of immune modulation due to probiotic consumption, systemic humoral immune responses could be induced as well. Several studies confirmed that immunoglobulins, main mediators of humoral immunity, were influenced by oral probiotic administration (21, 28, 29, 32). In addition, enhancement of specific humoral response would be of special interest for

professional athletes in terms of prevention of bacterial infections and minimization of their detrimental impact on training and performance.

The probiotic strain *Lactobacillus helveticus* Lafti® L10 was previously reported to have inherent immunity enhancing properties and nonpathogenic nature in animal studies (30). This data was corroborated with human trials: an enhancement of antigen stimulated IFN-γ production after a month of daily intake *L. helveticus* Lafti® L10 in dose of 2 x 10^{10} CFU was reported in a cohort of fatigued athletes suffering recurrent viral infections (8). Moreover, supplementation with *L. helveticus* Lafti® L10 reduced the duration of URTI episodes and increased CD4+/CD8+ (T helper/T suppressor) cells ratio in a cohort of elite athletes (Marinkovic et al, submitted results). While there is an emerging amount of evidence that probiotics could modulate mucosal immune system, data regarding the influence of probiotics on professional athletes' humoral immunity is rather scarce. Therefore, the aim of this study was to test the effects of *Lactobacillus helveticus* Lafti® L10 supplementation not only on the total antibody levels, but also on specific anti-bacterial antibody levels in serum and saliva.

METHODS

Experimental Approach to the Problem

The study included a randomized, double-blind, placebo-controlled parallel-groups design. The athletes were randomly allocated to the probiotic (n=15) or the placebo group (n=15), taking into account maximal aerobic capacity (determined by cardiopulmonary testing). All the participants finished the study.

We have tested both serum and salivary antibody reactivity towards several species/strains of *Lactobacillus,* as well as three clinical isolates - two of Gram-negative bacteria *E. coli* and *Proteus mirabillis,* and a Gram-positive *Enterococcus feacalis*. Moreover, total salivary IgA, as well as total IgA, IgG and IgM antibodies in serum were determined.

The supplementation started in the middle of January, in winter, and lasted for 14 weeks. The experimental group received the probiotic capsules of *Lactobacillus helveticus* Lafti® L10 ($2x10^{10}$ CFU) daily for 14 weeks. Each capsule contained $1x10^{10}$ colony forming units (CFU) of *Lactobacillus helveticus* Lafti® L10, so subjects were instructed to take 2 capsules per day. The control group received also 2 placebo capsules daily, which were identical in taste and appearance as probiotic capsules. The placebo capsules contained 1% magnesium stearate and 99% maltodextrin and the probiotic capsules contained 72.2% of the bacterial mass, 26.7% maltodextrin and 1% magnesium stearate. Capsules were composed of hydroxypropylmethylcellulose (HPMC) and covered by titanium dioxide (TiO_2). Both probiotic and placebo capsules were kept in a refrigerator (2˚ C to 8˚ C).

The athletes were asked to return the remaining capsules when coming to the final testing after the intervention. The researchers counted the remained capsules; the compliance in the probiotic group was 95.2% and in the placebo group was 94.8% (p=0.74).

Both athletes and the study team were blinded to the intervention until the statistical analyses were finished.

Subjects

A total of 30 of elite athletes were involved in the trial: 24 men (VO_{2max} ranged from 49.5 to 82.0 ml/kg/min) and 6 women (VO_{2max} ranged from 45.0 to 57.0 ml/kg/min), aged 18-28 years, non-smokers, with training >11 hr/week. Professional athletes from several different sports (badminton, triathlon, bicycling, athletics, karate, kayaking and judo) participated in the study. Exclusion criteria were: sensitivity to the ingredients of probiotics, the use of probiotics and antibiotics a month before the beginning of the study, recent surgical intervention and/or the presence of chronic diseases (immune, neurological, renal, pulmonary etc.).

Athletes were asked to take capsules after breakfast, in order to ensure the compliance. Furthermore, subjects were required to refrain from supplements which are intended for promotion of immune system (*e.g*): *Echinacea*, caffeine, *Ginseng panax*, propolis, multivitamins and multiminerals. Moreover, the participants were asked to hold a steady training regimen, diet, without consuming yogurt and fermented milk products.

All the experimental procedures in the current study followed the guidelines laid down in Declaration of Helsinki. The study was approved by the Ethics Committee of Sports Medicine Association of Serbia. Subjects were informed of the benefits and risks of the investigation prior to signing an informed consent approved by the committee.

Procedures

Training loads and maximal aerobic capacity determination

Athletes were required to report their training loads weekly, filling in the standard short form of International Physical Activity Questionnaire (IPAQ; http://www.ipaq.ki.se/downloads.htm).

Training loads in metabolic equivalents (MET-hr/week) were counted on the basis of completed questionnaires, according to Ainsworth (1). Maximum oxygen consumption was determined by a graded cardiopulmonary test on a treadmill (Quark b2-Cosmed). The exercise intensity was progressively increased, while oxygen and CO_2 concentration of the inhaled and exhaled air were measured. A test was considered maximal if participants achieved 90% or more of predicted maximal heart rate for age and gender, a plateau in oxygen consumption was reached despite increased workload, a respiratory exchange ratio was greater than 1.00, and subjects reached volitional exhaustion.

The training loads (MET-hr/week) didn't differ between the groups (Table 1). Maximal aerobic capacity did not change during the study (data not shown).

Table 1 around here

Serum and saliva samples collection

Samples were collected prior to the cardiopulmonary testing. Blood samples (10 mL per serum tube) were taken out of the antecubital vein. Whole unstimulated saliva was collected in a glass tube for 2 min, after sitting quietly for a few minutes, leaning forward, with their heads tilted (4). All the samples were collected twice: before the study and after the study, at the same time (between 9:30 and 10:30 AM), in order to avoid diurnal changes. Serum and saliva were separated by centrifugation 1500 x g, 15 min and 3000 x g, 10 min, respectively and stored frozen at -20 °C until analysis.

Total salivary IgA determination

Saliva samples were diluted 1000 x and then analyzed for IgA concentrations by using a commercial enzyme-linked immunosorbent assay (IBL®, Hamburg, Germany). Samples were

determined in duplicates; the intra-assay coefficient of variation was 10%. Salivary flow (mL/min) was determined by conversion of the amount of saliva in grams to milliliters, assuming that saliva density is 1 mg/mL, and division by time collection (2 minutes). SIgA secretion rate (µg/min) was obtained by multiplying the absolute sIgA concentration (µg/mL) with saliva flow rate (mL/min) (4). Salivary protein concentration was determined by Lowry method (24).

Total serum antibody assessment

Frozen serum samples were sent to a certified human diagnostics laboratory (Laboratorija Beograd, Belgrade, Serbia) and immunoturbidimetric method (Roshe Hitachi) was used for the quantification of total IgA, IgG and IgM in serum samples.

Bacterial strains and growth conditions

In this study, several *Lactobacillus* species used were: *L. helveticus* Lafti® L10, *L. plantarum* WCFS1, *L. rhamnosus* LA68, *L. rhamnosus* LB64 and *L. acidophilus* ViVag, all the strains were grown in MRS medium (Institute of Virology, Vaccines and Sera, "Torlak", Belgrade, Serbia), without shaking, at 37 °C. Overnight cultures were centrifuged 3000 x g 10 min at RT, washed twice with PBS and counted using a hemocytometer. After the optical density of $1x10^8$ of *L. helveticus* Lafti® L10 was determined all other bacteria were diluted to the same optical density. Clinical isolates of *E. coli, P. mirabillis,* and *E. faecalis* were grown in Nutrient broth (Institute of Virology, Vaccines and Sera, "Torlak", Belgrade, Serbia) and overnight cultures were also diluted to the same optical density. Prior to usage all bacterial species were frozen once at -20 °C.

Anti-bacterial ELISA

Anti-bacterial ELISA was essentially done as previously described (34) with minor modifications. MaxiSorp plates (Nunc A/S, Denmark) were filled with 50 µl/well of bacterial suspension. The plates were centrifuged at 1500 x g for 10 min, and the supernatant liquid was decanted. The plates were left for two hours at 50°C in order to dry. The plates were blocked with 200 µL/well 2% BSA/PBS at 37°C for 1h, washed three times with PBS and sera was added at an appropriate dilution. For the analysis of bacteria specific IgG and IgM the sera was diluted 400 x, and for specific IgA sera was diluted 50 x. Salivary IgA specific for bacteria was determined at a 4 x dilution. Sera or saliva was incubated for 2 h at 37°C, washed with PBS and secondary antibodies used were Monoclonal anti-Human IgG (Fc specific) Biotin conjugate, 2000 times diluted, Anti-Human IgM (µ- chain specific) Biotin conjugate, 2500 times diluted, Anti-Human IgA (α- chain specific), 2000 times diluted; all from Sigma Aldrich (St. Louis, MO, USA). All secondary antibodies were incubated for 1 h at RT. After washing with PBS, Streptavidin-HRP (Biolegend, San Diego, CA) diluted 2000 times was added and incubated for 1 h at RT. Colored substrate used was SigmaFast OPD (St. Louis, MO, USA), and the reaction was developed for 10 min. The absorbance was read at 492 nm and at 620 nm and the latter was substracted from the former. Each sample was done in duplicate, and intra-assay coefficients of variation were below or equal to 10% for all of the performed bacterial ELISAs.

Statistical analysis

All statistical analyses were performed with GraphPad Prism software. The normality of the data was checked by Wilk-Shapiro test. The differences in the change scores between the groups were assessed by an unpaired T-test. Spearman correlation was used for checking the correlation

between different antibody subclasses. The results are expressed as mean value and standard deviation (SD). P <0.05 was considered significant.

RESULTS

Total salivary IgA level

There was a significant (35%, -1.4 to 53%; mean, 90% confidence interval; p=0.03) reduction in total salivary IgA concentration in the placebo group (-28%, -38 to -20%, p=0.02) in comparation to the probiotic group (-8.7%, -15 to 1.7%, p=0.34), Fig 1. No significant changes were observed for protein concentration (1.5%, -4.7 to 10%, p=0.85), salivary flow (-9.2% (-99 to 76%), p=0.80) and salivary IgA secretion rate (3.5%, -25 to 34%, p=0.65) between the groups.

Figure 1 around here

Analysis of total IgG, IgA and IgM antibodies levels in serum

Mean difference between change scores in the groups was not significant for total IgG (0.09%, -10 to 5.0%, p=0.99) and IgM level (-27%, -68 to -15%, p=0.14), as shown in Figure 2a and 2c. However, level of total IgM increased in both probiotic (18% (15 to 20%), p=0.02) and placebo group (35%, 22 to 47%, p=0.02). On the other hand, there was a significant 15% (12 to 18%, p=0.04) decrease in total IgA level in the placebo group (-8.0%, -10 to -2.2%, p=0.03), when compared to the probiotic group (0.48%, -0.45 to 1.4%, p=0.77), Fig. 2b.

Figure 2 around here

Analysis of specific anti-bacterial serum IgG levels

The levels of serum IgG specific for different lactic acid bacteria (LAB) species and for selected pathogenic bacteria are shown in Table 2. Statistical analysis showed no difference in the groups for any of the LAB species used. A significant 16% (-2.8 to 35%, p=0.04) reduction over the course of study was noted for anti - *Enterococcus faecalis* IgG, since it decreased insignificantly by 2.0% (-4.4 to 10%, p=0.88) in the probiotic group, but significantly in the placebo group by 10% (-25 to -8.5%, p=0.02).

Table 2 around here

Analysis of specific anti-bacterial serum IgM levels

No significant differences in reactivity towards LAB species were detected between the groups. This was also the case with clinical isolates of pathogenic bacteria (Table 3).

Table 3 around here

Analysis of specific anti-bacterial serum IgA levels

No significant differences were detected between individual groups of sera, but statistically significant changes with time were detected in the placebo group (Table 4). The reduced levels of anti-LAB antibodies in the placebo group in comparison to the probiotic group were detected for *L. rhamnosus* LA68 for 24% (5.8 to 42%, p=0.02) for and for *L. rhamnosus* LB64 for 15% (2.7 to 27%, p=0.02). In the case of clinical isolates of pathogenic bacteria, no differences in antibody levels were detected in different time points.

Table 4 around here

Lactobacillus specific salivary IgA

No statistically significant differences in specific IgA level antibodies towards any of tested LAB species between the groups were observed (Fig. 3).

Figure 3 around here

DISCUSSION

The most important finding of this study was preservation of total salivary IgA level in the group supplemented with Lafti® L10. Given the fact that mucosal surface is the first line of defense against different pathogens, this finding might have a practical application in terms of prevention of URTIs during strenuous exercise in elite athletes. Our result is in accordance with literature data concerning highly active individuals (15, 36), and other immunologically susceptible populations, such as elderly and the children (31, 38). However, there are some studies showing the lack of positive impact on mucosal immunity (10, 16). This fact is of no surprise, since the effects of probiotics are strongly dose and strain dependent.

In line with salivary IgA level maintenance, a trend of systemic IgA level preservation occurred. However, we can't be sure that there is a direct connection between these two findings, since the production of secretory and serum IgA are regulated differently and located in different compartments. Consequently, the mechanisms by which mucosal and humoral immunological responses could be influenced by probiotic consumption are probably different (11, 23). Namely, the majority of adult human plasma cells produce IgA antibodies (22, 33), which exists as two subclasses, IgA1 and IgA2. The former, predominant in the human serum, is generated by B

cells in the bone marrow and peripheral lymphoid organs (11, 23). On the other hand, IgA2 is predominant in mucosal secretions (26) and is produced partly by B1 peritoneal cells (25%) and partly by B2 cells from mucosal associated lymphoid tissues (25).

Even though all individuals who participated in the study are young adults and elite athletes, individual differences, as in any other human population are vast, and this is also the case for levels of specific anti-bacterial antibodies. Apart from the diversity of each individual's genetic background, there are also differences in the histories of antigen encounter, which ultimately shape antibody repertoire. An interesting finding was that although serum IgA levels specific for *L. rhamnosus* LA68 and *L. rhamnosus* LB64 were reduced in the control group, salivary IgA was not lowered towards any of the LAB species tested. Yet the analysis of IgA levels specific for LAB either in saliva or serum showed no difference in the probiotic group. Moreover, the reduction in specific anti-LAB IgA levels might be explained by a certain level of cross-reactivity between LAB species, since all the participants were told to restrain from fermented milk products and other probiotic supplements. Therefore, the consumption of *Lactobacilli* results in the maintenance of certain anti-*Lactobacillus* IgA antibody levels, which is a relatively specific effect, as we didn't observe the change in the antibody levels specific for other bacteria tested. Finally, it might be concluded that specific salivary IgA is just a poor indicator of specific intestinal IgA response, as reported by previous studies (13, 29).

Nevertheless, the mechanism by which probiotics could induce salivary IgA remains elusive, since the sIgA-mediated immunity is very complex. Some new findings suggest that the generation of mucosal IgA+ B-cells is both T-cell dependent and T-cell independent, but their relative contributions are still unclear (6). Mucosal IgA+ cells migrate out of the gut mucosa to

the circulation, arriving to the local mucosal immune tissues, like the salivary glands. There they produce IgA, secreted into the salivary gland duct as sIgA (7, 21, 35).

IgM antibody class is the first immunoglobulin in serum to elevate in concentration, generally within 1–2 weeks (28). It is the most cross-reactive antibody class, as it represents the first line of defense against pathogens. No changes in the levels of antibacterial specific IgM levels were found, which was to be expected due to low IgM specificity.

Interestingly, total IgM antibody levels were significantly increased in both probiotic and placebo groups. Previous studies about the effects of exercise reported that the greatest effect of acute exercise on humoral response was an increase in serum IgM levels (27), although other authors reported no change (17) or even a decrease (19). Different mechanisms were proposed to explain this increase in IgM, including the nonspecific interaction between sympathetic neural system and immunity and antigen stimulation by larger amounts of microorganisms entering the body during the intensive training (27). In addition, the observed reduction of IgA and an increase of IgM which was observed in the placebo group is also found in IgA-deficient individuals, where the lack of IgA is compensated by the increase of IgM (5).

On the other hand, the IgG antibody class is considered to be a true and main indicator of antigen encounter. It is the most specific antibody class and provides immune memory (28). A significant 16% reduction during the study was noted for anti - *Enterococcus faecalis* IgG. It appears that supplementation with Lafti® L10 helped in maintaining the adequate antigen-specific response against a Gram-positive uropathogenic strain *Enterococcus faecalis*, but not against G-negative *Proteus mirabilis* or *Escherichia coli*. These findings indicate that supplementation with Lafti® can enhance specific, but not generalized immune activation.

Therefore, a future study should include testing of specific responses to a greater number of antigens, especially those of common infective agents causing URTI, like influenza.

Several trials conducted in athletes showed the ability of some strains to reduce the incidence of URTIs (10), the severity of symptoms (39), and shorten the duration of an URTI episode (40). Lafti® showed the potential to reduce the duration of an URTI episode and decrease the number of respiratory symptoms (Marinkovic et al, submitted results).

Similar enhancement of specific humoral response were observed against *Haemophilus influenza* (32), *Cholera* (29), enterotoxigenic *Escherichia coli* (28). However, to our knowledge, this is the first study to examine specific humoral responses upon probiotic supplementation in professional athletes.

Apparently, circulating sera IgG is critical for defense against URTI (12, 20), but there are some contrasting findings concerning its response to prolonged exercise (37). It is reported that serum level of both total IgG and IgG subclasses are significantly lower in swimmers in comparation to sedentary controls (27). However, total IgG didn't change during the period of supplementation in both probiotic and placebo groups. Similar results were reported by Gleeson (15). In fact, this could be expected, since total levels of immunoglobulins are less likely to respond to dietary changes, except in some extremes (HIV infections, severe malnutrition) (3). Conversely, there are studies showing probiotics might affect circulating antibodies counts: probiotic supplementation of critically ill patients resulted in a substantial increase of systemic IgA or IgG concentrations (2, 38).

Correlation found for IgG, IgM and IgA levels in different individuals was trivial, which is also in connection with the specificity of these different antibody classes.

In conclusion, we suggest that *L. helveticus* Lafti® L10 supplementation could be an appropriate dietary aid in humoral and mucosal immunity maintenance, which is critical for URTI prevention in elite athletes. Further investigations should elucidate the mechanisms of the interactions between *L. helveticus* and immunity.

Practical application

The current study indicates that probiotic supplementation restores mucosal and humoral immunity impairment caused by intense training during winter months. Apparently, respiratory illness occurs typically in the period of heavy exercise, particularly during winter months (18). In that manner, every training disruption during preparations for forthcoming sport competitions may result in performance impairment. In addition, humoral and especially mucosal immunity plays a crucial role in the defense against pathogen translocation. Hence, our findings might have a practical implication, in the sense of prevention, or the reduction of length and severity of URTI episodes. Additionally, athletes and their coaches might take *L. helveticus* Lafti® L10 into consideration as an appropriate nutritional supplement, in order to avoid performance impairment due to illness.

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Figure Legend

Figure 1. Levels of total salivary IgA in the probiotic and placebo groups at the baseline and after 14 weeks of supplementation. Results are expressed as mean and standard deviation.

*- $p<0.05$

Figure 2. Total serum antibodies: A) IgG, B) IgA and C) IgM antibody levels in the probiotic and placebo groups at the baseline and after 14 weeks of supplementation. Results are expressed as mean ± standard deviation.

*- $p<0.05$

Figure 3. Specific salivary IgA antibodies: A) anti- *L. plantarum* WCFS1 B) anti-*L. rhamnosus* LA68 and C) anti-*L. helveticus* L10 D) anti-*L. acidophilus* Vivag antibody levels in the probiotic and placebo groups at the baseline and after 14 weeks of supplementation. Results are expressed as mean ± standard deviation.

Table 1. Physical and anthropometric characteristics of the participants

	Probiotic Lafti® L10	*Placebo*	*p value*
Number	15	15	
Males/females	12/3	12/3	
Age (years)	22.5±2.9	23.6±2.9	0.88
VO_2 max (mL/kg/min)	54.3±10.9	55.2±8.2	0.80
BMI	22.9±2.5	22.9±2.5	0.95
Training loads (MET-hr/week)	96±52	97±56	0.94

BMI, Body-Mass Index. Results are expressed as mean ± standard deviation.

Table 2. Specific serum IgG antibodies towards LAB and pathogenic bacteria in serum

	Probiotic Lafti L10			*Placebo*				
	Baseline	*14 weeks*	*Change scores (90% CI)*	*Baseline*	*14 weeks*	*Change scores (90% CI)*	*Mean difference (90% CI)*	*p*
L. plantarum WCSFS1	0.40±0.24	0.51±0.27	29 (20 to 38)	0.61±0.31	0.71±0.37	11 (7.4 to 14.5)	18 (-21 to 58)	0.35
L. rhamnosus LB64	0.42±0.29	0.43±0.24	3.0 (0.30 to 5.8)	0.39±0.29	0.49±0.37	23 (19 to 27)	-20 (-42 to 2.9)	0.09
L. rhamnosus LA68	0.24±0.25	0.27±0.25	7.8 (3.0 to 12)	0.27±0.32	0.31±0.28	22 (17 to 27)	-14 (-45 to 16)	0.35
L. helvetus Lafti L10	0.45±0.27	0.56±0.20	24 (18 to 30)	0.52±0.35	0.61±0.36	41 (34 to 48)	-17 (58 to 24.4)	0.41
E. coli	0.21±0.23	0.17±0.17	-14 (-18 to -11)	0.13±0.09	0.14±0.09	15 (8.7 to 22)	-30 (-65 to 4.5)	0.09
P. mirabilis	0.50±0.48	0.51±0.51	6.4 (2.3 to 10)	0.41±0.34	0.38±0.32	-6.6 (-11 to 8.6)	13 (-7.6 to 34)	0.21
E. faecalis	0.23±0.12	0.21±0.13	-2.0 (-4.4 to 10)	0.32±0.23	0.29±0.20	-10 (-25 to -8.5)	16 (-2.8 to 35)	0.04

Results are expressed as mean ± standard deviations. Change scores in the groups and mean difference in the change scores between the groups are expressed in percents (%).

Table 3. Specific serum IgM antibodies towards LAB and pathogenic bacteria

	Probiotic Lafti® L10			*Placebo*				
	Baseline	*14 weeks*	*Change score (90% CI)*	*Baseline*	*14 weeks*	*Change score (90% CI)*	*Mean difference (90% CI)*	*p*
L. plantarum WCSFS1	0.54±0.22	0.49±0.20	-11 (-7.3 to -2.7)	0.58±0.26	0.56±0.26	-17 (-19 to -16)	-6.8 (-24 to 9.0)	0.41
L. rhamnosus LB64	0.29±0.12	0.28±0.13	-2.9 (-6.6 to 0.80)	0.34±0.20	0.33±0.16	7.8 (3.7-12)	-10 (-37 to 16)	0.42
L. rhamnosus LA68	0.29±0.12	0.26±0.14	-13 (-16 to -10)	0.33±0.21	0.30±0.16	-3.9 (-7.8 to 0.10)	-9.4 (-32 to 13)	0.40
L. helvetus Lafti L10	0.43±0.13	0.39±0.17	-12 (-15 to -9.4)	0.50±0.29	0.48±0.27	1.9 (-2.3 to 6.0)	-13 (-37 to 10)	0.26
E. coli	0.29±0.14	0.25±0.12	-6.9 (-11 to -2.5)	0.31±0.18	0.31±0.16	3.1 (-1.3 to 7.5)	-9.7 (-37 to 17)	0.46
P. mirabilis	0.46±0.16	0.46±0.12	-1.6 (-5.8 to 2.6)	0.55±0.27	0.54±0.28	-1.9 (-5.5-1.6)	0.4 (-24 to 25)	0.97
E. faecalis	0.49±0.16	0.44±0.12	-6.5 (-10 to -2.6)	0.56±0.30	0.54±0.29	-5.3 (-8.1 to -2.5)	-1.2 (-22 to 20)	0.91

Results are expressed as mean ± standard deviations. Change scores in the groups and mean difference in the change scores between the groups are expressed in percents (%).

Table 4. Specific serum IgA antibodies towards LAB and pathogenic bacteria

	Probiotic Lafti ® L10			*Placebo*				
	Baseline	*14 weeks*	*Change score (90%CI)*	*Baseline*	*14 weeks*	*Change score (90%CI)*	*Mean difference (90%CI)*	*p*
L. plantarum WCSFS1	0.16±0.09	0.15±0.09	-3.6 (-6.6 to 0.62)	0.17±0.07	0.15±0.06	-9.8 (-12 to -7.2)	6.2 (-11 to 8.5)	0.47
L. rhamnosus LB64	0.12±0.05	0.11±0.05	-2.8 (-5.3 to -0.33)	0.17±0.11	0.14±0.09	-17 (-19 to -16)	15 (2.7 to 27)	0.02
L. rhamnosus LA68	0.09±0.06	0.08±0.05	-4.3 (-7.6 to -1.1)	0.11±0.07	0.08±0.07	-28 (-31 to -26)	24 (5.8 to 42)	0.02
L. helvetus Lafti L10	0.21±0.08	0.18±0.08	-11 (-14 to -7.4)	0.21±0.06	0.18±0.06	-10 (-13 to -8.4)	-0.26 (-18 to 18)	0.98
E. coli	0.12±0.11	0.09±0.09	-11 (-15 to -7.6)	0.08±0.04	0.07±0.04	1.8 (-2.8 to 6.6)	-13 (-40 to 14)	0.33
P. mirabilis	0.14±0.08	0.14±0.08	-4.6 (-6.4 to -2.5)	0.14±0.05	0.13±0.05	-5.1 (-7.9 to -2.3)	0.5 (16.8-17.8)	0.95
E. faecalis	0.16±0.07	0.15±0.08	-5.8 (-8.1 to -3.5)	0.19±0.10	0.17±0.08	-3.2 (-5.6 to -0.82)	-2.6 (-18 to 13)	0.73

Results are expressed as mean ± standard deviations. Change scores in the groups and mean difference in the change scores between the groups are expressed in percents (%).