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## **Beneficial effects of *Lactobacillus acidophilus* and *Bifidobacterium lactis*, alone or in combination with a prebiotic, on the partially suppressed immune system of mice with psychological chronic stress**

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**Introduction:** Stress-induced immunologic alterations have been considered as a major cause of increased risk for immune-related diseases, such as cancer, autoimmune disorders, food allergy, alteration of mucosal barrier function and infection susceptibility. Stressors trigger physiological responses involving the hypothalamic–pituitary–adrenal (HPA) axis, which is closely associated with the immune system. It is a well-known fact that some prebiotics and probiotics may play a role in maintaining a balanced immune system, improving disease resistance and general health status, which may be especially relevant when there is an immunocompromised status.

**Objective:** The aim of this study was to evaluate the influence of a mix of two probiotic strains, alone or in combination with a prebiotic, on the partially suppressed immune response exhibited by chronically stressed mice.

**Diets and animals:** A prebiotic source, Synergy 1 (Beneo HP, Orafiti) and a mix of probiotic strains, *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* B107 (Danisco) were tested. Two experimental diets were used: a standard AIN-93M diet with 5% cellulose (Control diet); and the same diet containing 5% Synergy 1 (as a unique source of fibre). Probiotics were given at a dose of  $1 \times 10^8$  cfu/d of each strain in the drinking water. Sixty Balb/c female 7-weeks old mice (Charles River España) were distributed into five experimental groups: Sham, Control and Probiotic (PRO), Prebiotic (PRE) and Synbiotic (SYN) ( $n = 12$ ).

**Experimental design:** Mice were fed with the experimental diets and probiotic solutions during 39 days. One week after the nutritional intervention was initiated, mice were subcutaneously sensitized with ovalbumin (OVA), and received a booster 21 days later. The following day, the water avoidance stress protocol was applied to the mice. The chronic psychological stressor was based on the natural panic that mice feel to water; thus, animals were placed on a very small water-surrounded-platform, 1.5 h per day during 10 days. Then, mice were sacrificed and three kinds of parameters were measured: (1) morphohistological parameters such as small intestine length and body, small intestine, intestinal mucosa; caecum, spleen and thymus weights; (2) biochemical parameters such as serum corticosterone level and maltase and alkaline phosphatase (AP) activities from intestinal mucosa (biochemical markers for intestinal function or intestinal damage, respectively); and (3) immunological parameters such as, serum OVA-specific antibody production, splenic NK cell activity number of spleen cells and the expression of the surface spleen cell markers CD45, CD3, CD4, CD8, CD49b and CD45R/B220.

**Results:** PRE and SYN were able to revert significantly ( $P < 0.05$ ) the stress-induced thymus weight loss and increase the caecum size of 25% with respect to control, as well as counteract the increase in the AP activity caused by stress. The three treatments increased the OVA-specific secondary response, although the effect of both PRE and SYN was double than the one from PRO ( $P < 0.05$ ). In addition to that, SYN seemed to increase the *Helper/Tcytotoxic* ratio, whereas PRO seemed to increase the T cell/B cell ratio. In addition, PRO was able to increase the NK cell activity, as well as the percentage of splenic NK cells (CD49b+ cells) significantly ( $P < 0.05$ ) with respect to both control and prebiotic treated groups.

**Conclusions:** Although the mix of *L. acidophilus* NCFM and *B. lactis* B107 exhibited some beneficial effects, the prebiotic Synergy 1 seemed to perform better than the probiotic mixture in this experimental model, and reversed most of the pernicious effects induced by psychological stress. Both technologies may be useful to maintain an adequate healthy balance in a partially immunosuppressed status, such as the one induced by psychological stress.