Study summaries L. casei 431®

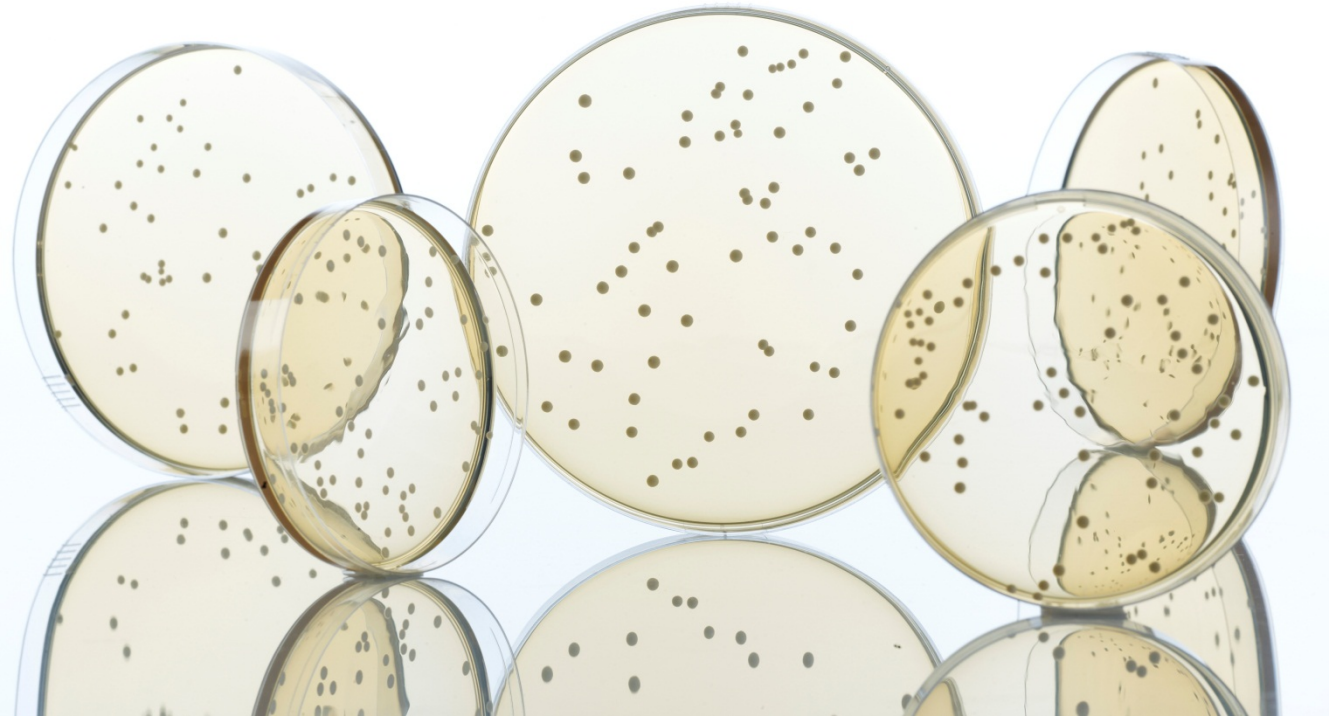
This binder provides you with summaries of selected publications on *Lactobacillus paracasei* subsp. *paracasei* L. casei 431®.

The publications are clinical studies performed in humans documenting the effects in various conditions.

December 2012

Chr. Hansen A/S

Human Health & Nutrition



Chr. Hansen A/S -10-12 Bøge Allé – DK-2970 Hørsholm, Denmark - Phone: +45 45 74 74 74 - Fax: +45 45 74 88 88 - www.chr-hansen.com

Research field: Immune Health
Research subfield: Immune stimulation
Study type: Human study
Probiotic strain: BB-12 and L. casei 431
Dosage CFU/day: 1 billion
Product formulation: Fermented milk (acidified milk) or capsules
Reference number: 1325

Rizzardini, et al. Evaluation of the immune benefits of two probiotic strains Bifidobacterium animalis ssp. lactis, BB-12(R) and Lactobacillus paracasei ssp. paracasei, L. casei 431(R) in an influenza vaccination model: a randomised, double-blind, placebo-controlled study Br.J.Nutr. 2011:1-9

Abstract: The present study investigated the ability of *Bifidobacterium animalis* ssp. *lactis* (BB-12®) and *Lactobacillus paracasei* ssp. *paracasei* (L. casei 431®) to modulate the immune system using a vaccination model in healthy subjects. A randomised, double-blind, placebo-controlled, parallel-group study was conducted in 211 subjects (56 % females, mean age 33·2 (sd 13·1) years). Subjects consumed a minimum of 10^9 colony-forming units of BB-12® (capsule) or L. casei 431® (dairy drink) or a matching placebo once daily for 6 weeks. After 2 weeks, a seasonal influenza vaccination was given. Plasma and saliva samples were collected at baseline and after 6 weeks for the analysis of antibodies, cytokines and innate immune parameters. Changes from baseline in vaccine-specific plasma IgG, IgG1 and IgG3 were significantly greater in both probiotic groups *v.* the corresponding placebo group (L. casei 431®, $P = 0{\cdot}01$ for IgG; $P < 0{\cdot}001$ for remaining comparisons). The number of subjects obtaining a substantial increase in specific IgG (defined as ≥ 2-fold above baseline) was significantly greater in both probiotic groups *v.* placebo (BB-12®, $P < 0{\cdot}001$ for IgG, IgG1 and IgG3; L. casei 431®, $P < 0{\cdot}001$ for IgG1 and IgG3). Significantly greater mean fold increases for vaccine-specific secretory IgA in saliva were observed in both probiotic groups *v.* placebo (BB-12®, $P = 0{\cdot}017$; L. casei 431®, $P = 0{\cdot}035$). Similar results were observed for total antibody concentrations. No differences were found for plasma cytokines or innate immune parameters. Data herein show that supplementation with BB-12® or L. casei 431® may be an effective means to improve immune function by augmenting systemic and mucosal immune responses to challenge.

Chr. Hansen A/S -10-12 Bøge Allé – DK-2970 Hørsholm, Denmark - Phone: +45 45 74 74 74 - Fax: +45 45 74 88 88 - www.chr-hansen.com

Research field: Other
Research subfield: Safety
Study type: Human study
Probiotic strain: B. animalis subsp. lactis BB-12 and L. paracasei subsp. paracasei L. casei 431
Dosage CFU/day: NA
Product formulation: Milk powder
Reference number: 1253

Vlieger, et al. Tolerance and safety of Lactobacillus paracasei ssp. paracasei in combination with Bifidobacterium animalis ssp. lactis in a prebiotic-containing infant formula: a randomised controlled trial. Br.J.Nutr. 2009;102:869-875

Abstract: The addition of probiotics to infant formula has been shown to be an efficient way to increase the number of beneficial bacteria in the intestine in order to promote a gut flora resembling that of breast-fed infants. The objective of the present study was to evaluate the safety and tolerance of a combination of two probiotic strains in early infancy. A group of 126 newborns were randomised to receive a prebiotic-containing starter formula supplemented with Lactobacillus paracasei ssp. paracasei and Bifidobacterium animalis ssp. lactis or the same formula without probiotics for the first 3 months of life. A total of eighty infants continued the study until they were aged 6 months. Growth measurements were taken monthly at healthy baby clinics. Diaries were used to monitor behaviour, infections, use of antibiotics, as well as stool characteristics. Normal growth occurred in all infants and no statistically significant differences were detected between the probiotics group and the control group for gain in weight, length and head circumference. Infants in the probiotics group produced softer and more frequent stools during the first 3 months of life. No differences were found in crying and sleeping hours, number of parent-diagnosed infections, antibiotic use, visits to the general practitioner and number of adverse events. The use of a prebiotic-containing starter formula supplemented with L. paracasei ssp. paracasei and B. animalis ssp. lactis in early infancy is safe, well tolerated and has no adverse effects on growth and infant behaviour.

Chr. Hansen A/S -10-12 Bøge Allé – DK-2970 Hørsholm, Denmark - Phone: +45 45 74 74 74 - Fax: +45 45 74 88 88 - www.chr-hansen.com

Research field: Immune Health
Research subfield: Immune stimulation
Study type: Human study
Probiotic strain: B. animalis subsp. lactis BB-12 and L. paracasei subsp. paracasei L. casei 431
Dosage CFU/day: 0.1 billion, 1 billion, 10 billion, 100 billion
Product formulation: Capsules
Reference number: 0501

Christensen, et al. Immunomodulating potential of supplementation with probiotics: a dose-response study in healthy young adults. FEMS Immunol.Med.Microbiol. 2006;47:380-390

Abstract: Certain probiotic microorganisms have been found beneficial in the treatment of immune-related diseases and may also affect immune function in healthy people. Intervention studies of probiotics in healthy humans are urgently required. Here, the immunomodulating potential of Bifidobacterium animalis ssp. lactis (BB-12) and Lactobacillus paracasei ssp. paracasei (CRL-431) was studied in a double-blind placebo-controlled parallel dose-response trial (n=71) based on five randomly assigned groups of young healthy adults supplemented for 3 weeks with 0, 10(8), 10(9), 10(10) and 10(11) CFU day(-1), respectively, of a mixture of BB-12 and CRL-431. No statistically significant dose-dependent effect was found for phagocytic activity in blood leukocytes, fecal immunoglobulin A (IgA) concentrations or production of interferon-gamma and interleukin-10 in blood cells. When evaluating data according to the amount of viable BB-12 recovered from faeces, the interferon-gamma production in blood cells was significantly reduced. In conclusion, no solid effect on the immune function of young healthy adults supplemented with even high doses of B. animalis ssp. lactis BB-12 and L. paracasei ssp. paracasei CRL-431 was demonstrated in this study.

Chr. Hansen A/S -10-12 Bøge Allé – DK-2970 Hørsholm, Denmark - Phone: +45 45 74 74 74 - Fax: +45 45 74 88 88 - www.chr-hansen.com

Research field: Gastrointestinal Health
Research subfield: Recovery
Study type: Human study
Probiotic strain: B. animalis subsp. lactis BB-12 and L. paracasei subsp. paracasei L. casei 431
Dosage CFU/day: 0.1 billion, 1 billion, 10 billion, 100 billion
Product formulation: Capsules
Reference number: 0500

Larsen, et al. 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. 2006;60(11):1284-1293

Abstract: OBJECTIVE: This study was performed to investigate the dose-response effects of supplementation with Bifidobacterium animalis subsp lactis (BB-12) and Lactobacillus paracasei subsp paracasei (CRL-431) on blood lipids, recovery from feces and bowel habits. Changes of the fecal microflora was analyzed in the 10(10) CFU/day probiotic and placebo group. DESIGN: The study was designed as a randomized, placebo-controlled, double-blinded, parallel dose-response study. SUBJECTS: Healthy young adults (18-40 years) were recruited by advertising in local newspapers. Of the 75 persons enrolled, 71 (46 women, 25 men, mean age 25.6 years (range 18-40 years)) completed the study. INTERVENTION: The volunteers were randomly assigned into five groups receiving either placebo or a mixture of the two probiotics in the concentration of 10(8), 10(9), 10(10) or 10(11) CFU/day in 2 weeks run-in period, 3 weeks intervention and 2 weeks wash-out. Diary reporting bowel habits and well being (abdominal bloating, flatulence and headache) was kept for all 7 weeks and blood lipids, fecal recovery of BB-12 and CRL-431, as well as fecal microflora was tested before, immediately and 2 weeks after intervention. RESULTS: The fecal recovery of BB-12 increased significantly ($P < 0.001$) with increasing dose. In the group receiving 10(11) CFU/day BB-12 was recovered from 13 out of 15 volunteers. CRL-431 was not recovered in any of the fecal samples. Supplementation with probiotics did not change the fecal bacterial composition. A significant linear increase in fecal consistency (looser stool) with increasing probiotic dose ($P = 0.018$) was observed. No overall dose-response effect was found on the blood lipids. High doses of probiotics were well tolerated. CONCLUSION: A dose-related recovery of BB-12 from feces was observed.

Chr. Hansen A/S -10-12 Bøge Allé – DK-2970 Hørsholm, Denmark - Phone: +45 45 74 74 74 - Fax: +45 45 74 88 88 - www.chr-hansen.com

Research field: Immune Health
Research subfield: Immune stimulation
Study type: Human study
Probiotic strain: L. paracasei subsp. paracasei L. casei 431 and L. rhamnosus LGG
Dosage CFU/day: 10 billion
Product formulation: Fermented milk
Reference number: 0462

de Vrese, et al. Probiotic bacteria stimulate virus-specific neutralizing antibodies following a booster polio vaccination. Eur.J.Nutr. 2005;44:406-413

Abstract: BACKGROUND: Orally ingested probiotic bacteria may modulate the immune response and increase antibody titers against enteric infections by bacteria or viruses. Even though positive effects of probiotics on respiratory tract infections have been reported, overall only few studies have examined effects on virus infections concerning organs other than the gastrointestinal tract. AIM OF THE STUDY: It was the aim of the study to investigate whether and how probiotics affect the immune response to a standardized enterovirus challenge (polio) and infections not limited to the gastrointestinal tract in healthy adults. METHODS: In a randomized, controlled and double-blind study 64 volunteers consumed for 5 weeks chemically acidified clotted milk without bacteria or with 10(10)/serving (Lactobacillus rhamnosus) GG or Lactobacillus acidophilus CRL431 added. In the second week subjects were vaccinated orally against polio 1, 2 and 3. Polio virus neutralizing serum activity, the primary parameter, was determined by the standard neutralization test (WHO) before and three times after vaccination. Polio-specific IgA, IgG and IgM were detected by ELISAs. RESULTS: Probiotics increased poliovirus neutralizing antibody titers (NT) and affected the formation of poliovirus-specific IgA and IgG in serum. The maximum increase after immunization was about 2, 2.2, or 4-fold higher, respectively, for NT, IgG or, IgA, in volunteers consuming probiotics instead of placebo. No consistent difference was noted between bacterial strains. CONCLUSIONS: Probiotics induce an immunologic response that may provide enhanced systemic protection of cells from virus infections by increasing production of virus neutralizing antibodies.

Chr. Hansen A/S -10-12 Bøge Allé – DK-2970 Hørsholm, Denmark - Phone: +45 45 74 74 74 - Fax: +45 45 74 88 88 - www.chr-hansen.com

Research field: Gastrointestinal Health
Research subfield: Diarrhea
Study type: Human study
Probiotic strain: L. paracasei subsp. paracasei L. casei 431
Dosage CFU/day: 3.5 - 350 trillion
Product formulation: Fermented milk
Reference number: 0419

Gaon, et al. Effect of Lactobacillus strains and Saccharomyces boulardii on persistent diarrhea in children. Medicina 2003;63:293-298

Abstract: The efficacy of probiotics on persistent diarrhea remains uncertain. The purpose of this study was to evaluate the effect of Lactobacillus sp and Saccharomyces boulardii on persistent diarrhea in children. In a double-blind trial eighty-nine children, aged 6-24 months were randomly distributed to receive pasteurized cow milk containing 2 viable lyophilized strains Lactobacillus casei and Lactobacillus acidophillus strains CERELA, (10(10)-10(12) colony-forming units per g) (n = 30), or lyophilized S. boulardii, (10(10)-10(12) colony forming units per g) (n = 30) or pasteurized cow milk as placebo (n = 29); on each diet 175 g was given twice a day for a 5 day period. Number of depositions, duration of illness and frequency of vomiting were considered. Enteric pathogens were isolated from stools in 40% of the patients, 27% had rotavirus. Lactobacillus and S. boulardii significantly reduced the number of depositions (p

---

Chr. Hansen A/S -10-12 Bøge Allé – DK-2970 Hørsholm, Denmark - Phone: +45 45 74 74 74 - Fax: +45 45 74 88 88 - www.chr-hansen.com

Research field: Gastrointestinal Health
Research subfield: Diarrhea
Study type: Human study
Probiotic strain: L. paracasei subsp. paracasei L. casei 431
Dosage CFU/day: NA
Product formulation: Capsules
Reference number: 0407

Gaon, et al. Effect of Lactobacillus strains (L. casei and L. Acidophillus Strains cerela) on bacterial overgrowth-related chronic diarrhea. Medicina 2002;62:159-163

Abstract: Small bowel bacterial overgrowth and related diarrhea is a condition that frequently accompanies anatomic disorders, surgically created blind loops or strictures with partial small bowel obstruction and although it is often controlled with antimicrobial therapy, alternative treatment may be needed. The aim of this study was to evaluate the efficacy of an oral probiotic preparation of 2 viable lyophilized strains of lactobacilli (1.5 g each) compared with placebo. Twenty two patients with proven overgrowth and chronic diarrhea are described. In random order and double-blind fashion, 2 groups of patients received identical capsules with both Lactobacillus casei and L. acidophillus strains CERELA (12 patients) (LC) and placebo (10 patients) (P) during three consecutive periods of 7 days each followed by a similar three periods of control after withdrawal. At the end of each period the mean daily number of stools, glucose breath H2 test, and symptoms were considered. Lactobacillus were investigated in feces in both groups at day 0 (baseline), on day 21 of treatment with LC and P and on day 21 after withdrawal. Compared with P a significant reduction in mean daily number of stools was achieved with LC (p

---

Chr. Hansen A/S -10-12 Bøge Allé – DK-2970 Hørsholm, Denmark - Phone: +45 45 74 74 74 - Fax: +45 45 74 88 88 - www.chr-hansen.com

Research field: Immune Health
Research subfield: Cancer
Study type: Human study
Probiotic strain: L. paracasei subsp. paracasei L. casei 431
Dosage CFU/day: 22 billion
Product formulation: Fermented milk
Reference number: 0334

Moises, et al. Lactobacillus casei and Lactobacillus acidophilus in the treatment of bladder superficial tumors: follow-up during 36 months. Third communication 1996

Abstract: DESIGN: 20 patients with superficial urinary bladder carcinoma were surgically treated, and then orally administered CRL-431 and Lactobacillus acidophilus for 8 weeks. Tumor recurrence was evaluated for 36 months. RESULTS: Treatment with milk fermented with CRL-431 and Lactobacillus acidophilus minimized tumor recurrence after surgery, and maintained or diminished the tumoral grade.Only 1 relapse was detected. No adverse side-effects, including hepatomegaly, splenomegaly or blood alterations were observed. CRL-431 and Lactobacillus acidophilus fermented milk is recommended as immunopotentiators in tumoral processes.

Chr. Hansen A/S -10-12 Bøge Allé – DK-2970 Hørsholm, Denmark - Phone: +45 45 74 74 74 - Fax: +45 45 74 88 88 - www.chr-hansen.com

Research field: Gastrointestinal Health
Research subfield: Lactose intolerance
Study type: Human study
Probiotic strain: L. paracasei subsp. paracasei L. casei 431
Dosage CFU/day: 2.4 - 240 or 4.8 - 480 billion
Product formulation: Fermented milk
Reference number: 0331

Gaon, et al. Lactose digestion by milk fermented with Lactobacillus acidophilus and Lactobacillus casei of human origin. Medicina 1995;55:237-242

Abstract: 18 persons with lactose intolerance and 12 persons without lactose intolerance consumed either milk fermented with CRL-431 and Lactobacillus acidophilus, or regular unfermented milk. Lactose absorption and orocecal transit time was measured by the breath hydrogen test during 3 hours after ingestion. In lactase deficient subjects, breath hydrogen was significantly lower with consumption of 480 ml of fermented milk compared to regular milk ($P<0.008$). Intestinal orocecal transit was also significantly slower with the fermented milk ($P<0.001$). In lactose tolerant subjects, breath hydrogen and orocecal transit were at similar levels with both milks. Clinical symptoms with 480 ml of fermented milk were significantly less frequent ($P<0.08$). Regular milk caused more frequencies of borborygmi ($P<0.025$), bloating ($P<0.05$), diarrhea ($P<0.05$) and abdominal pain ($P<0.05$). No significant differences were observed with consumption of 240 ml of the milks, except for borborygmi ($P<0.05$). Milk fermented with CRL-431 and Lactobacillus acidophilus enhances lactose digestion, influences orocecal transit time, and diminishes intolerance symptoms in lactose intolerant people.

Chr. Hansen A/S -10-12 Bøge Allé – DK-2970 Hørsholm, Denmark - Phone: +45 45 74 74 74 - Fax: +45 45 74 88 88 - www.chr-hansen.com

Research field: Gastrointestinal Health
Research subfield: Diarrhea / microbiota
Study type: Human study
Probiotic strain: L. paracasei subsp. paracasei L. casei 431
Dosage CFU/day: 10 - 100 billion
Product formulation: Fermented milk
Reference number: 0333

Gonzalez, et al. Biotherapeutic role of fermented milk. Biotherapy 1994:129-134

Abstract: Fermented milk was used as therapy in infantile diarrhoea due to post-gastroenteritis syndrome. This treatment eliminated the disease in 4.0 days (mean value, SD = 2.8; n = 13) and allowed patients to return to free feeding according to their age. The weight percentile variation during treatment with fermented milk (15 days) was higher in the patients showing 3rd degree malnutrition than in other children. Bacteriotherapy can restore faecal flora which has been lowered by diarrhoea. Our results showed that levels higher than 10(6) UFC lactobacilli/g of faeces correlated with a healthy status of the children. Clinical applications of fermented milk with a mixture of Lactobacillus casei and Lactobacillus acidophilus in the prevention of gastrointestinal disorders are possible.

Chr. Hansen A/S -10-12 Bøge Allé – DK-2970 Hørsholm, Denmark - Phone: +45 45 74 74 74 - Fax: +45 45 74 88 88 - www.chr-hansen.com

Research field: Gastrointestinal Health
Research subfield: Diarrhea
Study type: Human study
Probiotic strain: L. paracasei subsp. paracasei L. casei 431
Dosage CFU/day: 0.15 - 1.5 billion
Product formulation: Fermented milk
Reference number: 0296

Gonzalez, et al. Prevention of infantile diarrhoea by fermented milk. Microbiologie - Aliments - Nutrition 1990:349-354

Abstract: Prevention of intestinal diseases through administration of lactobacilli was studied in a double-blind study with 49 apparently healthy children from populations at high risk of developing diarrhoea. Lactobacillus casei and Lactobacillus acidophilus, originally isolated from faeces of healthy children, were used to prepare cultured milk (microorganisms were added to sterile 10% reconstituted milk at final concn. of 107-108 cells/ml). Children received daily for three 15-day periods (with two 15-day periods of no supplementation between) 240 ml of skim milk supplemented with 15 ml of non-cultured or cultured milk. Of the 25 children receiving non-cultured milk, 13 (52%) developed diarrhoeal syndrome during or after the experimental periods. Stool cultures from only 2 of these children gave positive results for lactic flora (>1 Ãƒâ€” 105 c.f.u./g), while unaffected children showed faecal lactobacilli counts >106 c.f.u./g. Of the 24 children given cultured milk, only 4 (17%) developed diarrhoea and in 3 cases this was attributed to antibiotics therapy; in the 4th case stool cultures were negative for lactobacilli. Mean wt increases were 203 and 445 g for children receiving non-cultured and cultured milk resp. The use of cultured milk in prevention of diarrhoea in children of high-risk populations is suggested.

Chr. Hansen A/S -10-12 Bøge Allé – DK-2970 Hørsholm, Denmark - Phone: +45 45 74 74 74 - Fax: +45 45 74 88 88 - www.chr-hansen.com