ACTIMEL FACT SHEETS

BODY DEFENCES AND IMMUNE SYSTEM

The body has a defense system to prevent external agents to cause harm. This system is composed of elements with different levels of complexity. Those include the physical barriers, as the skin and intestinal mucosa; the biochemical barriers, such as antibacterial peptides in mucous secretions; the microbiota; and the immune system[1].

The whole system works to prevent diseases, but when the first elements are not able to prevent the breach, the immune system needs to act. The Immune Response is the capability of the body to fight infection and disease, while having adequate tolerance to avoid allergy and autoimmune diseases.

THE IMPORTANCE OF THE GASTROINTESTINAL TRACT

The small and large intestines are exchange surfaces for nutrients contained in food. To absorb the needed quantity of micronutrients, the surface is enlarged by villosities and microvillosities. In fact, human gut epithelium has a surface comparable to that of a tennis court[2].

This huge surface implies also bigger exposure to external – and possibly dangerous – agents. For this reason, the defense elements in the gut are many. The intestine harbors about 70% of the body immune cells[3]. Additionally to the underlying immune system, other less specific elements help to protect the body. The mucosa and its mucus limit the transmission of any foreign agent (acting as a physical barrier)[4]. Also, the antimicrobial agents secreted to the lumen help to prevent the proliferation of pathogens (biochemical barrier)[5]. The microbiota, especially abundant in the large intestine[6], also plays a key role in defense by "colonization resistance".

THE MICROBIOTA

In humans, commensal microorganisms are found on all surfaces in contact with the external environment. Those microorganisms are common inhabitants as a result of millenary common evolution. Collectively, all these microorganisms are referred to as the **human microbiota**.

Most of those microorganisms are found in the digestive tract. It is accepted that gut microbiota helps to maintain the host's health by educating the immune system and by keeping unfavorable bacteria at bay. The associated mechanisms of actions have not yet been fully elucidated, however several explanations have been proposed. These include depletion of substrates, competition for receptor sites and generation of a restrictive environment, among others[7].

The effect of regular Actimel® consumption on microbiota and its activity has been clinically assessed [Pawlowska, 2007; Guerin Danan, 1998].

PROBIOTICS AND SURVIVAL

Probiotics are commonly defined as "Live micro-organisms which when administered in adequate amounts confer a health benefit on the host"[8]. Whilst survival in the gastrointestinal tract is not intrinsic in this definition, it is considered as an important criterion in determining whether a probiotic is, in fact, able to exert its beneficial effects.

L. casei CNCM I-1518 is present alive in Actimel® in levels of 10^8 CFU/g or 10^{10} CFU/100g bottle, and several studies have confirmed the survival of the strain through the intestinal tract at levels consistent with the ability to provide a beneficial physiological effect [Rochet, 2008; Rochet, 2006; Oozeer, 2006].

[1]Rote, 2010. "Innate immunity: Inflammation" in "Pathophysiology".
[2]Neish, 2009. "Microbes in Gastrointestinal Health and Disease".
[3]Furness, 1999. "Nutrient Tasting and Signaling Mechanisms in the Gut II. The intestine as a sensory organ: neural, endocrine, and immune responses".
[4]Belley, 1999. "Intestinal mucins in colonization and host defense against pathogens"/ Dai, 2000. "Role of oligosaccharides and glycoconjugates in intestinal host defense".
[5]Ganz, 2000. "Paneth cells - guardians of the gut cell hatchery" Ouellette, 1999. "Mucosal Immunity and Inflammation IV. Paneth cell antimicrobial peptides and the biology of the mucosal barrier".
[6]Xu, 2003. "Honor thy symbionts".
[7]Ducluzeau, 1989. "Ecologie microbienne du tube digestif" / Falk, 1998. "Creating and maintaining the gastrointestinal ecosystem: What we know and need to know from gnotobiology" / Fons, 2000. "Mechanisms of colonisation and colonisation resistance of the digestive tract: Part 2: Bacteria/bacteria interactions"
[8]FAO/WHO expert consultation, 2001. "Report of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria".

ABOUT THE PRODUCT BENEFIT

A selection[9] of the studies assessing the benefits of Actimel® is presented here, with a focus on the broad territory of defenses and immune health. Actimel® has been studied across different age populations.

	Antibiotic-associated diarrhea	Common Infectious Diseases			Immune response	Allergy
			GITI	RTI		
CHILDREN		Prodeus 2016	✓	✓		Giovannini 2007
		Merenstein 2010	✓	✓		
		Sykora 2005	✓			
		Agarwal 2002	✓			
		Agarwal 2001	✓			
		Pedone 2000	✓			
		Pedone 1999	✓			
ADULTS	Alberda 2018	Guillemard 2010b	✓	✓	Ortiz 2008	
		Pereg 2005	✓		Tiollier 2007	
					Meyer 2007	
					Meyer 2006	
					Parra 1 2004	
					Parra 2 2004	
					Marcos 2004	
					Pujol 2000	
					Yoon 1999	
SENIORS	Dietrich 2014	Guillemard 2010a	✓	✓	Boge 2009	
	Lenoir 2014*	Turchet 2003	✓	✓		
	Hickson 2007					
GENERAL POPULATION		Poon 2020**	✓	✓		

GITI: Gastrointestinal Tract Infections, RTI: Respiratory Tract Infections
All clinical trials except for *Lenoir, 2014, nutrieconomy study
**Systematic review and meta-analysis

ANTIBIOTIC-ASSOCIATED DIARRHEA

Another interesting condition for the study of probiotics is *antibiotic-associated diarrhea*. This diarrhea occurs during or shortly after the administration of antibiotic treatment, and it is diagnosed in the absence of other known causes. The incidence varies depending on the population and the type of antibiotic, but it has been reported within a range of 1 to 44%[10]. In hospitalized patients, the presence of diarrhea implies isolation and usually longer stays in the institution, with the corresponding increase in associated costs of management.

COMMON INFECTIOUS DISEASES

Common infectious diseases (CIDs) are defined as upper respiratory tract infections, lower respiratory tract infections, and gastrointestinal tract infections. Decreased incidence or duration of them is a common way to demonstrate the global outcome of improving the body defense system against pathogens.

CIDs commonly occur in general population[11] and more specifically in people under physical or psychological stress[12]. Also, senior population is particularly vulnerable due to the age-associated alterations on the immune system[13]. CIDs are prevalent among children too, and there is strong evidence associating day care attendance with both gastrointestinal and respiratory infections[14].

IMMUNE RESPONSE

The complex process of the immune response involves different actors such as cells, cytokines, antibodies, etc. The variations in these parameters in blood could provide evidence on the effect of an intervention in the systemic immune response.

ALLERGY

Changes in environment, diet, and personal behavior have played a dominant role in the specificity prevalence and severity of allergies. The prevalence of some of them is regarded as epidemic by some authors[15].

[9]This selection is not the result of a systematic review or a scoping study.

[10]Bergogne-Berezin, 2000. "Treatment and prevention of antibiotic associated diarrhea". Graul, 2009. "Lactobacillus and bifidobacteria combinations: a strategy to reduce hospital acquired Clostridium difficile diarrhea incidence and mortality".

[11]Bramley,2002. "Productivity losses related to the common cold".

[12]Yang, 2000. "Stress-induced immunomodulation: impact on immune defenses against infectious disease".

[13]Gavazzi, 2002. "Ageing and infection".

[14]Dales, 2004. "Respiratory illness in children attending daycare".

[15]Platts Mills, 2015. "The allergy epidemics".

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE ESSENTIAL DAIRY & PLANT-BASED

Feasibility of a *Lactobacillus casei* Drink in the Intensive Care Unit for Prevention of Antibiotic Associated Diarrhea and *Clostridium difficile*, a pilot study

Alberda C, Marcushamer S, Hewer T, Journault N, Kutsogiannis D. Nutrients. 2018 Apr; 10(5).

OBJECTIVE

The aim of the present study was to demonstrate the safety and the feasibility of a probiotic drink in Intensive Care Unit (ICU) adult patients for the prevention strategy of any diarrhea associated with antibiotic use, and that caused by *Clostridium difficile*.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

32 ICU patients initiated on antibiotic therapy.

Inclusion criteria

- Age > 18 yo with immediate family able to provide written informed consent.
- Prescribed one or more antibiotics in ICU.
- Functional, intact gastrointestinal tract.
- Anticipated ICU stay of >72h after enrolment.

Exclusion criteria

- Received oral or intravenous antibiotics for > 48h in hospitals.
- Received a course of antibiotics in the past 30 days.
- History of *Clostridium difficile* infection in previous 90 days.
- Poor diagnosis and not anticipated to survive the probiotic treatment period.
- One of the following medical diagnoses: immunosuppression, artificial heart valve, infective endocarditis, rheumatic heart disease, pancreatitis.
- Known to regularly consume probiotics.
- History of milk allergy or intolerance to dairy products.

DESIGN

Pilot, case-controlled trial, not blinded.

16 ICU patients were treated with 100 g of Actimel® twice daily via feeding tube or orally. Probiotic therapy was initiated within 48h of receiving the first antibiotic and lasted during the course of antibiotic therapy + 7 days if staying in ICU, or until discharge.

OUTCOMES

- Safety of probiotic intervention.
- Gastrointestinal tolerance including incidence of diarrhea, nausea and vomiting, or significant gastrointestinal adverse events.
- Occurence of antibiotic-associated diarrhea (AAD) and *Clostridium difficile* infection (CDI).
- Diarrhea was defined as three or more bowel movements per day or greater than 750ml liquid stool. AAD was defined as a diarrhea which continued for more than 3 consecutive days.
- CDI had to be confirmed by stool enzyme immunoassay for enterotoxin A or cytotoxin B produced by *Clostridium difficile* bacteria.

POPULATION ANALYSED

- 32 ICU patients were included in this trial – 16 in probiotics groups, 16 in control group.
- Patients from the control group (no product) matched with respect to age, BMI, and severity of illness.
- Fisher's exact test was used to compare rates of AAD and CDI.
- Test was two-sided at a 5% significance level, and a power of 80%.

MAIN RESULTS

- AAD was documented in 12.5% of the probiotic group (2 patients) and 31.3% in the control group (5 patients) (p-value=0.394).
- 1 patient in the probiotic group developed CDI compared to 3 in the control group (p-value=0.600).
- 3 serious adverse events in the control group, none in the probiotic group.

	Probiotic Intervention (n = 16)	Control group (n=16)	p value
Diarrhea n (%)	11 (68.8)	10 (62.5)	1.000
Antibiotic Associated Diarrhea, n (%)	2 (12.5)	5 (31.3)	0.394
Outcome Diarrhea (Caused by AAD or CDI)	3 (18.7)	7 (44.0)	0.252
ICU Acquired *Clostridium difficile*, n (%)	1 (6.0)	2 (12.5)	0.600

CONCLUSION

A probiotic-containing drink can safely be delivered via feeding tube to ICU patients, and should be considered as a preventive measure for antibiotic-associated diarrhea and *Clostridium difficile* infection.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE ESSENTIAL DAIRY & PLANT-BASED

Fermented Milk Consumption and Common Infections in Children Attending Day-Care Centers: A Randomized Trial

A. Prodeus, V. Niborski, J. Schrezenmeir, A. Gorelov, A. Shcherbina, A. Rumianchev. J. Pediatr. Gastroenterol. Nutr. (JPGN 2016;63: 534–543)

OBJECTIVE

The aim of the study was to investigate the effect of regular consumption of a fermented milk product containing the probiotic *Lactobacillus casei* CNCM I -1518 strain on incidence of common infectious diseases (CIDs) , including respiratory and gastro-intestinal infections, in young children regularly attending a day-care center.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

599 both sex children aged between 3 and 6 years old attending day-care centers 5 days per week in the Moscow area were included in the study. Volunteers were medically healthy.

Exclusion criteria

- Infectious disease within the previous 7 days.
- Currently or recently receiving medical treatment likely to interfere with study parameters (antibiotics, antiseptics, antifungal agents, corticosteroids, vaccines, or anti-histaminic agents).
- Allergy or hypersensitivity to milk proteins, dairy food components or any known food or respiratory allergy.
- Severe evolutive or chronic pathology.
- Current diarrhea or constipation.
- Artificial nutrition, gastrointestinal surgery or any intervention requiring general anesthesia within the 2 months prior to study participation.
- Special medicated diet or eating disorder.

DESIGN

The trial was multi-center, double-blind, randomized, placebo-controlled study with 2 parallel arms. It was conducted in 12 day-care centers of Moscow area from November 2006 to April 2007.

Complete experimental phase was 4 months, comprising 3-month product consumption and a 1-month follow-up period. Participants received either 100 g fermented milk containing *Lactobacillus casei* CNCM I-1518 (Actimel®), or a sweetened flavored non-fermented acidified dairy drink (control) twice a day.

OUTCOMES

Primary outcomes

Number of all common infectious diseases reported during the three months of study products consumption.

The 3 main categories of common infections were defined as:

- Upper respiratory tract infections (URTI): rhinopharyngitis (cold, acute coryza), sore throat, sinusitis, otitis.
- Lower respiratory tract infections (LRTI): acute bronchitis, flu and flu-like illness, pneumonia.
- Gastro intestinal tract infections (GITI): gastroenteritis.

Secondary outcomes

- Number of all common infections reported during the 1-month follow-up phase and total study duration (3-months consumption + 1-month follow-up).
- Number of URTIs, LRTIs, or GITIs or each type of common infectious disease during consumption, follow-up, or total study duration.
- Time to event.
- The global severity of infection.
- The duration of infection.
- Fever.
- Medication.
- Pathogens associated to common infections.
- Duration of sick leave from day-care and parental missed days at work.
- Impact on quality of life.

POPULATION ANALYSED

Among the 599 randomized subjects, 300 received Actimel® product and 299 the control product (Intent-To-Treat (ITT) population). Twenty-one subjects reported major deviations (3.2%). The Per-Protocol (PP) population comprised 578 subjects (292 in Actimel group, 286 in control group).The baseline characteristics of the ITT population were similar between groups in terms of demographics, medical history, current disease and scores of quality of life questionnaire.

Compliance was good and similar for both, Actimel® and control products.

Assessment of the study according to the recommendation of Cochrane collaboration indicated a low risk of bias whatever the dimension considered.

MAIN RESULTS

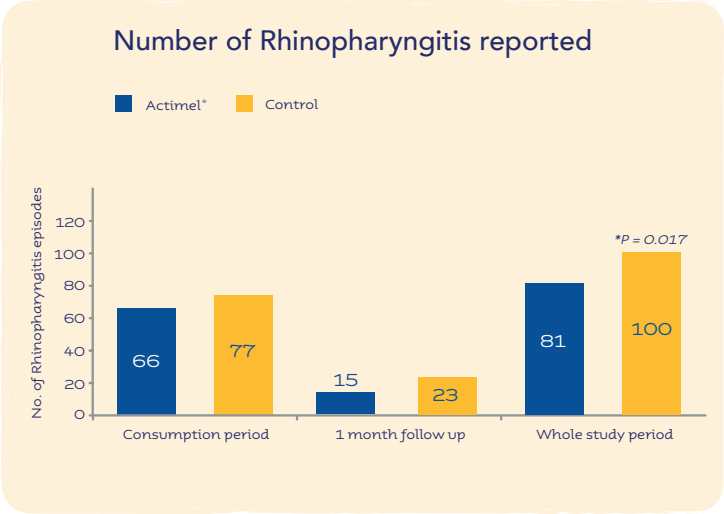
There was no significant difference between Actimel® and control groups for the cumulated number of CIDs over the 3 months of study product consumption in ITT population (primary outcome measure)(Relative Risk RR [95% CI] = 1.06 [0.84; 1.33]). Similar results were obtained for the PP population or when considering other study periods (follow-up and whole study periods).

The majority of all volunteers did not experience any common infection, regardless of study group allocation. The number of common infections in the control was 90% lower compared to what has been assumed in the sample size calculation (3 episodes during the 3 months intervention period) therefore lowering the calculated power of the study.

Overall and for both groups, the majority of all common infections were URTIs (206 episodes, 82%). Of the URTIs reported, most were identified as rhinopharyngitis. Over the whole study period, the adjusted mean rate of rhinopharyngitis was significantly lower in Actimel® group compared to control group 0.217 versus 0.266, RR [95% CI]=0.82 [0.69; 0.96], p=0.017) corresponding to a Risk reduction of 18.45% in favor of the active group. Occurrence (number of subjects with at least one rhinopharyngitis) was also significantly lower during the same period (OR [95% CI]=0.77 [0.62; 0.96]; p=0.021).

No differences were detected between groups in the other secondary outcomes measured.

Overall safety was good in both groups, with less than 5% of the children experiencing one or more adverse events overall.



CONCLUSION

Results from the entire study period may indicate a beneficial effect of Actimel® consumption on CIDs classified as rhinopharyngitis in 3-6 years old children attending day-care centers, which is in line with a reduction of URTIs by this probiotic product found by Merenstein et al. in a randomized controlled trial with similar design and sample size. Actimel® was also well tolerated by this population.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE
ESSENTIAL DAIRY & PLANT-BASED

Commercially available probiotic drinks containing Lactobacillus casei DN-114001 reduce antibiotic-associated diarrhea

Christoph G Dietrich, Tanja Kottmann, Manuela Alavi. World J Gastroenterol 2014 November 14; 20(42): 15837-15844.

OBJECTIVE

The aim of the two studies was to assess the effect of a probiotic drink (Actimel®) containing Lactobacillus casei DN-114001 (International reference: Lactobacillus casei CNCM I -1518) on antibiotic-associated diarrhea.

In the first large scale study, the effect and cost effectiveness of Actimel® use on antibiotic-associated diarrhea (AAD) were assessed.

In the second pilot study, the aim was to compare the effect of two commercially available probiotic drinks (Actimel® and Yakult®) on antibiotic-associated diarrhea.

No primary criteria were defined in either study.

Both studies were performed independently from the manufacturer. They were run in the Internal Medicine department of a primary hospital (Bethlehem hospital in Stolberg/Rhineland, Germany). Probiotic products were provided by the hospital canteen kitchen.

FIRST STUDY

STUDY POPULATION

Eligible participants were 258 senior hospitalized patients under antibiotic treatment. Patients with sepsis, pancreatitis and oncologic diseases and patients on dialysis were excluded.

DESIGN

The study was a monocentric prospective observational cohort study in three hospital wards. On one ward, all patients treated with antibiotics (n=107) were offered 100 g of Actimel® twice a day (Active group) for the duration of antibiotic treatment over a period of 3 months. Patients from the other two wards (n=151) were treated as usual, without any probiotics (Control group).

OUTCOMES

After the study period, charts of patients with informed consent on the three wards were retrospectively analyzed for diarrhea events leading to isolation. Hospital stay duration, as well as incidence, and duration of antibiotic-associated diarrhea were the outcomes measured. Antibiotic-associated diarrhea was defined as at least 3 watery/fluid stools in a 24-h period, considering that one day of diarrhea in hospital leads to patient isolation.

A cost effectiveness of the use of the active product was also calculated, on the basis of the cost of isolating patient vs. the cost of preventative Actimel® intervention.

POPULATION ANALYSED

258 primary care hospital patients treated with antibiotics received either Actimel® (n=107) or no probiotics intervention (n=151).

MAIN RESULTS

Patients receiving Actimel® presented a significantly lower incidence of antibiotic-associated diarrhea compared to the control group (6.5% vs 28.4%, P<0.001). Diarrhea duration was also significantly reduced in the Actimel® group (P=0.015) whereas no significant difference was observed between groups in hospital stay duration.

Endpoint data (mean ± SD or percentage) of the first study

Parameter	Actimel group (ward A5)	Controls (wards A6/A7)	*P* value
Hospital stay (d)	11.2 ± 6.8	12.2 ± 8.3	NS
Diarrhea	6.5%	28.4%	< 0.001
Duration of diarrhea (d)	1.7 ± 1.1	3.1 ± 2.1	0.015

NS: No Significance

The above results were taken as the basis of the calculation of cost-effectiveness of the use of Actimel®. When compared to the cost of isolating patients during antibiotic-associated diarrhea, a prophylactic Actimel® therapy represents a cost advantage of almost 60,000 euros per year in the study hospital setting.

SECOND STUDY

STUDY POPULATION

60 senior hospitalized patients treated with antibiotics from the same wards, consecutively to the first study.

DESIGN

The study was monocentric, prospective, open controlled and randomized. Patients from the three wards treated with antibiotics were randomized to receive either Actimel® (n=30) or Yakult® (n=30) commercial preparation twice daily.

OUTCOMES

All of the patient's charts were analyzed regarding diarrhea during antibiotic treatment. Hospital stay, as well as the incidence and duration of antibiotic-associated diarrhea (same definition as for study 1) and incidence of *Clostridium difficile* associated diarrhea were the outcomes measured.

POPULATION ANALYSED

60 primary care hospital patients treated with antibiotics received either Actimel® (n=30) or Yakult (n=30) commercial preparations twice a day.

MAIN RESULTS

The frequency of diarrhea in the Actimel® group was similar to that seen in the first study (6.7%). Yakult® group showed significantly higher incidence of antibiotic-associated diarrhea (33%), a similar rate to the control group in the first study. The difference between groups was statistically significant (P=0.021). The duration of diarrhea was also shorter in the Actimel® group compared to the Yakult® group (2.5d +/- 0.7 vs 4.4d +/- 2.5), although this difference did not reach statistical significance. The only 3 cases of *Clostridium difficile*-associated diarrhea occurred in the Yakult® group. No significant difference was observed between groups in hospital stay duration.

Endpoint data (mean ± SD or percentage) of the second study

Parameter	Actimel group	Yakult group	*P* value
Hospital stay (d)	10.8 ± 7.7	11.8 ± 7.8	NS
Diarrhea	6.7%	33.3%	0.021
Duration of diarrhea (d)	2.5 ± 0.7	4.4 ± 2.5	NS
CDAD	0.0%	10.0%	NS

NS: No Significance; CDAD: *Clostridium difficile*-associated diarrhea.

CONCLUSION

The first study showed that Actimel® significantly reduced the frequency and duration of antibiotic-associated diarrhea to a degree that was calculated to be cost effective. The second pilot study is the first head-to-head study of two probiotic formulations in AAD prevention. This study confirmed Actimel®'s positive effects and showed its higher efficacy on antibiotic-associated diarrhea as compared to Yakult® product.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE ESSENTIAL DAIRY & PLANT-BASED

Nutrition economic evaluation of a probiotic in the prevention of antibiotic-associated diarrhea

Irene Lenoir-Wijnkoop, Mark J.C. Nuijten, Joyce Craig and Christopher C. Butler. Front. Pharmacol. 5:13. doi: 10.3389/fphar.2014.00013

OBJECTIVE

The aim of this study was to assess the health-economic impact of a preventive nutritional strategy using a fermented milk with probiotic (Actimel®) in the management of antibiotics-associated diarrhea (AAD), and in particular *Clostridium difficile*-associated diarrhea (CDAD), in hospitalized patients older than 65 years in the UK health care setting.

STUDY POPULATION

Elderly hospitalized patients over 65 years of age being treated with antibiotics. The study population for this model is based on those coming from clinical trials which are the clinical input for the model.

DESIGN

Decision analytic modeling methodology was used. A model was constructed to estimate the cost consequences of Actimel® as a preventive intervention in the management of antibiotic-associated diarrhea, from the perspective of the UK National Health Service (NHS) and in line with current National Institute for Health and Clinical Excellence (NICE) guidelines, in comparison to no preventative strategy.

See overleaf for structure of the model used in the analysis

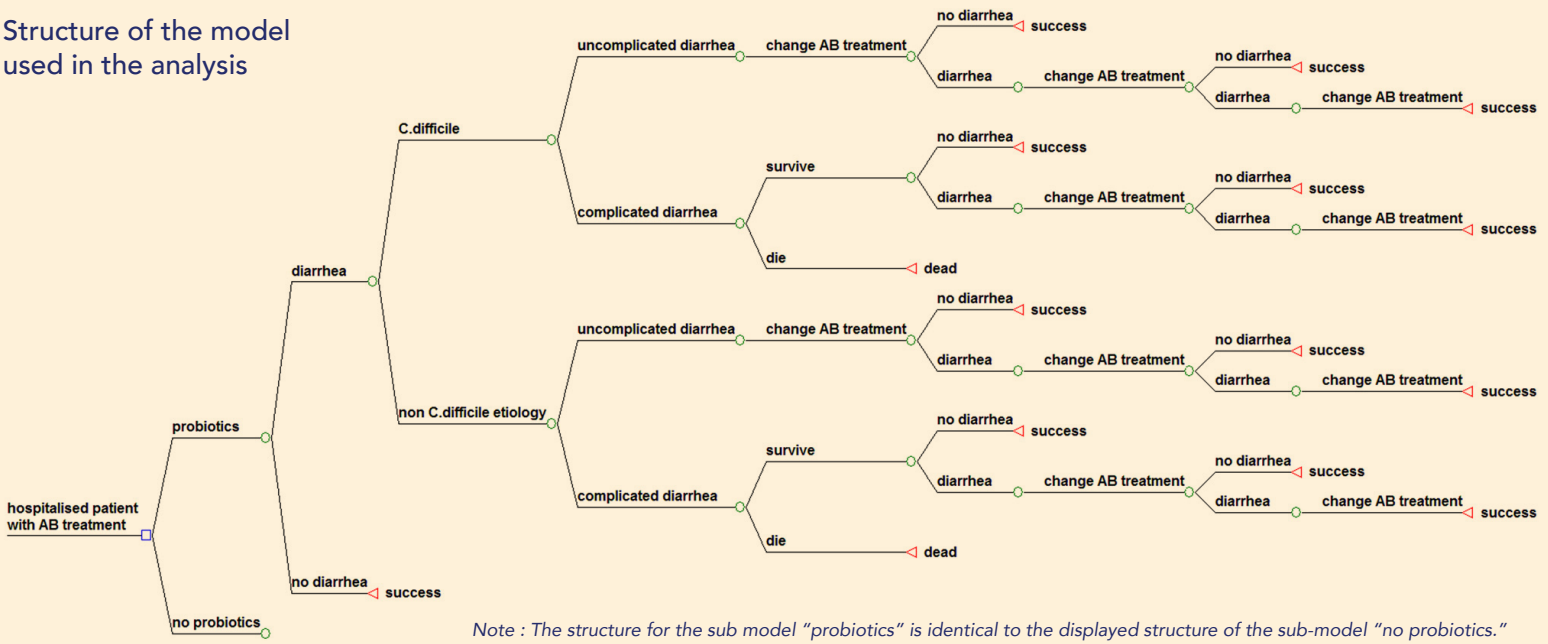
Regarding clinical outcomes, the model extrapolated the efficacy data from the probiotic clinical trials on AAD, and other related clinical events (recurrent diarrhea, complications, and mortality) to calculate overall success rate (proportion of patients fully recovered).

Searches identified only one prospective study conducted in England (Hickson et al., 2007) that investigated the effect of Actimel® on AAD and CDAD. Clinical outcome data were extrapolated from this randomized double blind, placebo controlled trial that presented the same setting as assumed for the model. Treatment choices and cost of management data were calculated and estimated specifically for the UK health care system. Additional estimations due to the absence of robust published data came from a panel of medical specialists practicing in England.

OUTCOMES

Mean cost saving per hospitalized patient over 65 years treated with antibiotics, regardless of whether he/she develops antibiotic-associated diarrhea.

Structure of the model used in the analysis



Note : The structure for the sub model "probiotics" is identical to the displayed structure of the sub-model "no probiotics."

MAIN RESULTS

Actimel® intervention generated estimated mean cost savings of £339 per hospitalized patient over the age of 65 years and treated with antibiotics, regardless of whether he/she develops AAD, compared to no preventive strategy. The cost savings are sensitive to the incidence of AAD and to the proportion of severe patients. A sensitivity analysis based on risk ratio from a most recent Cochrane meta-analysis showed an estimated cost savings of £332 for all forms of AAD.

CONCLUSION

Use of the fermented dairy drink containing the probiotic L. paracasei CNCMI-1518 (Actimel®) to prevent antibiotic-associated diarrhea in older hospitalized patients treated with antibiotics could lead to substantial cost savings.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE ESSENTIAL DAIRY & PLANT-BASED

Effects of a Fermented Dairy Drink Containing *Lacticaseibacillus paracasei* subsp. *paracasei* CNCM I-1518 (*Lactobacillus casei* CNCM I-1518) and the Standard Yogurt Cultures on the Incidence, Duration, and Severity of Common Infectious Diseases: A Systematic Review and Meta-Analysis of Randomized Controlled Trials.

T. Poon, J. Juana, D. Noori, S. Jeansen, A. Pierucci-Lagha, K. Musa-Veloso. Nutrients (2020), 12(11):3443. doi: 10.3390/nu12113443.

OBJECTIVE

The aim of this systematic review and meta-analysis was to assess the effects of the consumption of a fermented dairy drink (FDD) containing *Lacticaseibacillus paracasei* subsp. *paracasei* CNCM I-1518 and the yogurt strains on common infectious diseases (CIDs) in generally healthy children (aged ≥ 2 y.o.) and adults.

Nine randomized controlled trials from eight publications were eligible for inclusion. Effects on CID incidence, duration and severity were investigated.

DANONE NUTRICIA RESEARCH

SYSTEMATIC REVIEW STRATEGY

Studies included were randomized and controlled trials of any duration, that investigated the effects of a FDD containing the *Lacticaseibacillus paracasei* subsp. *paracasei* CNCM I-1518 and the standard yogurt cultures (*L. bulgaricus* and *S. thermophilus)* on CIDs in healthy adults and children ≥ 2 years of age.

Specifically, the trials were included if assessing the incidence, duration, or severity of CIDs, and if the independent effects of the investigational product could be isolated (e.g., the FDD was not co-administered with other bioactives known to affect the incidence, duration, and/or severity of CIDs).

STUDY QUALITY ASSESSMENT

The National Institutes of Health (NIH) tool for the quality assessment of controlled intervention studies was used to assess study quality. Additional confounders were considered to these criteria:

- At baseline, in addition to the general demographic characteristics (e.g., age and gender), (1) presence of CIDs; (2) influenza or rotavirus vaccination status; and (3) medication/supplement use (e.g., proton pump inhibitors) were considered.
- To assess if other interventions were avoided or similar between groups (e.g., similar background treatments), (1) the use of rescue medications/supplements (e.g., for colds, flu, or diarrhea); and (2) the consumption of other probiotic were considered.
- Specific attention was paid on how the outcomes were assessed in the studies, if valid and reliable measures were used. For the incidence, who made the diagnosis was considered. Also, additional criteria was defined on how duration and severity were determined. Based on the overall assessment, the studies were then rated as being of "poor," "fair," or "good" quality.

PUBLICATIONS SELECTED

1120 titles potentially relevant were retrieved in the databases.

After deduplication and inclusion/exclusion criteria application, seven publications finally met all of the inclusion criteria and none of the exclusion criteria. Additionally, one study was identified in the reference list of a selected study, and one publication consisted of a pilot and a confirmatory study.

Thus, the effects of the FDD on CIDs were assessed in a total of nine studies from eight publications.

Of the nine studies, two were conducted in generally healthy children (Merenstein 2010, Prodeus 2016), three were conducted in adults (Guillemard 2010a, Pereg 2005, Tiollier 2007), and four were conducted in the elderly (Boge 2009 – pilot and confirmatory studies, Guillemard 2010b, Turchet 2003).

MAIN RESULTS

Because of the variety of metrics used in the studies, even across a single outcome, several meta-analyses were conducted to assess CID incidence, duration and severity. Also due to the scarcity of data, results were combined across the age groups in order to permit the conduct of meta-analyses.

The incidence of CIDs was assessed in 3 meta-analyses.

In the first one, after combining the results from four studies, the consumption of the FDD was associated with a trend toward a reduced risk in the number of cumulative CIDs compared to placebo (RR (95% CI) = 0.91 (0.82, 1.01); $p = 0.082$) (Figure a).

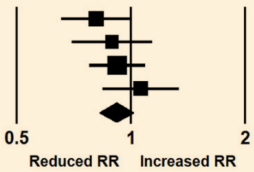
In the second meta-analysis, the consumption of the FDD significantly reduced the mean number of CIDs per subject compared to placebo (−0.09 (−0.15, −0.04); $p = 0.001$) (Figure b).

In the third one, the consumption of the FDD significantly reduced the odds of experiencing ≥1 CID compared to a control (OR = 0.81 (0.66, 0.98); $p = 0.029$) (Figure c).

The consumption of the FDD had no significant effect on CID duration or severity.

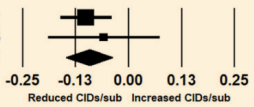
(a)

Study name	Population	FDD	Duration	Risk ratio	Lower limit	Upper limit	p-Value
Merenstein et al. [24]	Children	200 g/d	13 wk	0.810	0.656	1.000	0.050
Guillemard et al. [42]	Elderly	200 g/d	12 wk	0.890	0.697	1.136	0.349
Guillemard et al. [39]	Adults	200 g/d	12 wk	0.920	0.778	1.088	0.329
Prodeus et al. [2]	Children	200 g/d	12 wk	1.060	0.842	1.334	0.619
				0.913	0.823	1.012	0.082



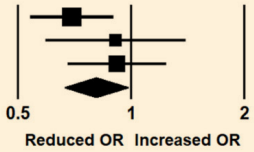
(b)

Study name	Population	FDD	Duration	Difference in means	Variance	Lower limit	Upper limit	p-Value
Guillemard et al. [42]	Elderly	200 g/d	12 wk	-0.100	0.001	-0.160	-0.040	0.001
Guillemard et al. [39]	Adults	200 g/d	12 wk	-0.058	0.004	-0.189	0.073	0.385
				-0.093	0.001	-0.147	-0.038	0.001



(c)

Study name	Population	FDD	Duration	Odds ratio	Lower limit	Upper limit	p-Value
Guillemard et al. [39]	Adults	200 g/d	12 wk	0.695	0.540	0.895	0.005
Turchet et al. [41]	Elderly	200 g/d	3 wk	0.910	0.592	1.397	0.665
Guillemard et al. [42]	Elderly	200 g/d	12 wk	0.918	0.681	1.237	0.574
				0.806	0.664	0.979	0.029



CONCLUSION

The results of this systematic review and meta-analysis contribute to the understanding of the beneficial effects of foods containing the probiotic *L. paracasei* subsp. *paracasei (L. casei)* CNCM I-1518 on CIDs in the general population. Specifically, this study suggests that the consumption of the FDD containing the *L. casei* and the standard yogurt cultures, twice a day, may reduce the incidence of CIDs. Moreover, this systematic review and meta-analysis highlights the needs for standardization of clinical outcomes related to immune function and guidance for confounding factors to be considered.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE ESSENTIAL DAIRY & PLANT-BASED

Effectiveness of probiotics on the duration of illness in healthy children and adults who develop common acute respiratory infectious conditions: a systematic review and meta-analysis.

Sarah King, Julie Glanville, Mary Ellen Sanders, Anita Fitzgerald and Danielle Varley. British Journal of Nutrition (2014), 112, 41–54

OBJECTIVE

The aim of this systematic review was to assess the effect of probiotics on the duration of acute respiratory tract infections in otherwise healthy children and adults. Probiotics under the scope of this review are those which belong to the *Lactobacillus* and *Bifidobacterium* genera.

Although 7 Actimel® studies were selected in this systematic review, this publication results are not specifically on Actimel® but on probiotics in general.

SYSTEMATIC REVIEW STRATEGY

DStudies included were blinded or open-label randomized controlled trials (RCT) of any duration that compared Lactobacillus and/or Bifidobacterium strains (combined or not with other strains) consumed orally with placebo or 'no treatment' in apparently healthy children or adults who developed acute respiratory tract infections (RTI) at some point during the study. 'Acute respiratory infections' were considered to include upper and/or lower RTI, colds or influenza like symptoms. Trials that reported on 'common infectious disease' were also included if RTI were defined within this umbrella term by the trial authors.

To be eligible for inclusion, the trials had to report on a measure of illness duration, such as the length of illness episodes, number of days of illness per person, number of days off sick from day care, school or work, or time without an infection.

Studies conducted in infants (aged 1 year), trained athletes, seriously ill people and institutionalized elderly adults were excluded from this review as they were considered to be immunologically distinct.

Unpublished studies were included in accordance with established approaches to the systematic review process.

Meta-analysis was conducted according to Cochrane collaboration recommendations.

OUTCOMES

Three main outcomes were reported:

- Duration of illness episodes, defined as overall sum of illness episode length in days, divided by the total number of illnesses episodes experienced by the study participants.
- Number of days of illness per person, defined as overall number of days with symptoms divided by the number of individuals with an illness.
- Absenteeism from day care/school/work, defined as number of days absent from day care/school/work divided by the number of participants with at least one illness episode.

PUBLICATIONS SELECTED

3069 records were retrieved in the databases. After deduplication, 1888 remained. Case by case analysis identified 75 as potentially relevant for the objective of this meta-analysis. Inclusion criteria was met by 21 studies, from which 1 was dropped after failing to get further data.

Finally, 20 studies were included in the meta-analysis, 7 of them have been conducted with Actimel® from which two of these were unpublished at the moment of the systematic review.

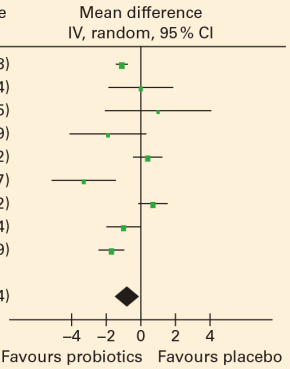
Overall, twelve trials out of twenty were considered to have a 'low' risk of bias and no trials had a 'high' risk of bias.

MAIN RESULTS

The effect of probiotics in duration of illness was assessed in 9 clinical trials, including 4 Actimel® studies[1]. Subjects receiving probiotics had significantly shorter illness episodes than those receiving a placebo by almost a day (weighted mean difference = -0.77, 95% CI : -1.50, -0.04, P=0.04) by almost a day. However there was statistical heterogeneity between studies. An analysis of the six studies out of nine considered to have a 'low' risk of bias (including 3 Actimel® studies) yielded a significant result similar to the overall pooled analysis (weighted mean difference -0.96, 95% CI -1.79, -0.13; P=0·02).

Study or subgroup	Probiotics Mean	SD	Total	Placebo Mean	SD	Total	Weight (%)	Mean difference (95% CI)
Bentley (unpublished results)	5·6	1·3	87	6·7	0·8	98	16·6	−1·10 (−1·42, −0·78)
Berggren *et al.*[5]	7	6·29	121	7	9·73	170	8·2	0·00 (−1·84, 1·84)
Cáceres *et al.*[23]	20·4	14·4	172	19·4	14·8	181	4·3	1·00 (−2·05, 4·05)
de Vrese *et al.*[6]	7	6·29	158	8·9	12·37	153	6·7	−1·90 (−4·09, 0·29)
Guillemard *et al.*[24]	6·9	4·5	213	6·5	4·5	256	14·1	0·40 (−0·42, 1·22)
Guillemard *et al.*[25]	7·7	7·2	120	11	7·7	135	8·2	−3·30 (−5·13, −1·47)
Kloster Smerud *et al.*[27]	5·39	7·89	492	4·69	5·19	564	14·1	0·70 (−0·12, 1·52)
Niborski *et al.*[14]	3	2·04	32	4	2·04	38	13·2	−1·00 (−1·96, −0·04)
Turchet *et al.*[17]	7	3·2	180	8·7	3·7	180	14·7	−1·70 (−2·41, −0·99)
Total (95% CI)			1575			1775	100·0	−0·77 (−1·50, −0·04)

Heterogeneity: $\tau^2 = 0{\cdot}81$; $\chi^2 = 40{\cdot}60$, df = 8 ($P < 0{\cdot}00001$); $I^2 = 80\%$
Test for overall effect: $Z = 2{\cdot}06$ ($P = 0{\cdot}04$)



Mean duration of illness episodes (d). The 'total' is the overall number of illness episodes experienced by the participants (randomised in a 1:1 ratio) in each treatment group. (A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn).

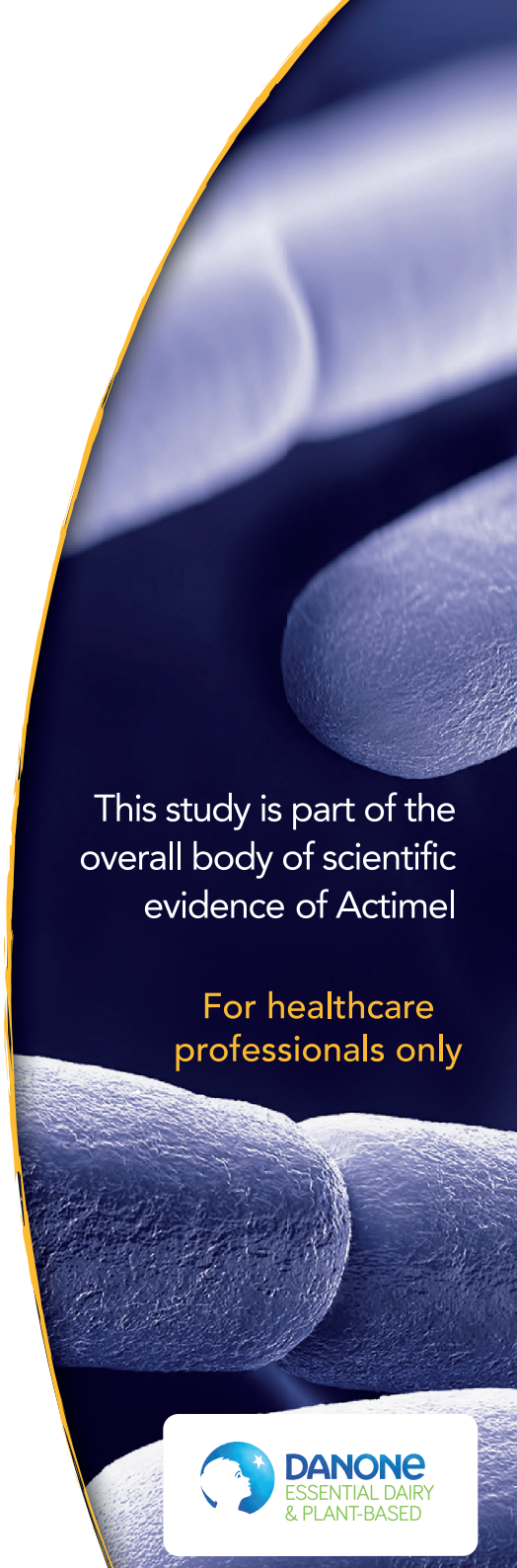
The effect of probiotics on the number of illness days per person was assessed in 10 clinical trials, including 3 Actimel® studies[2]. The results demonstrated that subjects consuming probiotics had significantly fewer days of illness per person than those receiving a placebo (Standard mean difference = -0.31, 95% CI. -0.41, -0.11; P<0.00001). There was no statistical heterogeneity among these studies.

The effect of probiotics on absenteeism due to respiratory infections or common infectious diseases was assessed in 11 clinical trials, including 4 Actimel® studies[3]. Results demonstrated that probiotics significantly reduce the numbers of days absent from daycare/school/work compared to placebo (standardized mean difference -0.17, 95% CI -0.31, -0.03; P=0.02). Significant heterogeneity was also seen among these trials. Additionally a subgroup analysis of these studies conducted on children also showed a significant difference in absenteeism in favor of probiotics (standardised mean difference -0.18, 95% CI -0.34, -0.02: P=0•03). While only eleven trials were included in this analysis, the funnel plot was roughly symmetrical, indicating no publication bias.

[1]Turchet et al., 2003; Niborski et al., 2008 [Unpublished]; Guillemard et al., 2010a; Guillemard et al., 2010b.
[2]Tiollier et al., 2007; Niborski et al., 2008 [Unpublished]; Guillemard et al., 2010b
[3]Niborski et al., 2008 [Unpublished]; Prodeus et al., 2008 [Unpublished]; Guillemard et al., 2010b; Merenstein et al., 2010.

CONCLUSION

This systematic review and meta-analysis provides evidence from a number of well conducted studies that the average duration of RTI episodes, number of day's illness per person and the number of days absent from daycare/school/work are significantly reduced with the use of probiotics (Lactobacillus and Bifidobacterium strains). Although the evidence generated is not focused solely on Actimel® studies, this adds to the pool of evidence in favor of the product.



Use of a fermented dairy probiotic drink containing *Lactobacillus casei* (DN-114 001) to decrease the rate of illness in kids: The drink study. A patient-oriented, double-blind, cluster-randomised, placebo-controlled, clinical trial

Merenstein D, Murphy M, Fokar A, Hernandez RK, Park H, Nsouli H, Sanders ME, Davis BA, Niborski V, Tondu F. Shara NM. Eur J Clin Nutr. 2010 Jul;64(7):685-91. Epub 2010 Mar 10.

OBJECTIVE

The aim of the present study was to evaluate the effect of consumption of a fermented milk containing standard yogurt cultures and *Lactobacillus casei* DN-114 001 (Actimel®) on the incidence of common infectious diseases (CID) and change of behaviour because of illness.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

638 children, aged between 3 and 6 years, attending daycare centre or schools.

Exclusion criteria

- Taking any regular medicines at initiation of study.
- Active respiratory or gastrointestinal infection, or chronic disease.
- Consumption of other probiotic foods or supplements.
- Lactose intolerance and allergy to strawberries (study product flavour).

DESIGN

The trial was randomised, double-blind and controlled in 2 parallel groups.

Participants consumed 200 g per day of either Actimel® (n=314) or a control drink (non fermented milk) (n=324) for 90 consecutive days and were monitored weekly during consumption. The trial was carried out during winter 2006-2007.

Other fermented dairy products were excluded during the whole investigation period.

OUTCOMES

Primary outcomes

- Rate of days with change in activity because of illness (defined as number of days with change of activity divided by number of days in the study) per 100 person days, assessed by parents.
- Incidence rate of CIDs* (defined as number of events divided by number of days in the study) per 100 person days.

Secondary outcomes

- Incidence rate of GITI per 100 person days.
- Incidence rate of URTI per 100 person days.
- Incidence rate of LRTI per 100 person days.

** CIDs were separated into three categories of infections: upper respiratory tract infections (URTI); lower respiratory tract infections (LRTI); and gastrointestinal tract infections (GITI)*

POPULATION ANALYSED

- The analysis was performed on all the evaluable subjects according to the intention-to-treat principle (638 in total, 314 in the Actimel® group and 324 in the control group) and adjusted on household, age and number of drinks consumed.
- Results were expressed as adjusted means and standard errors (SE).
- All statistical tests were two-sided at a 5% significance level.

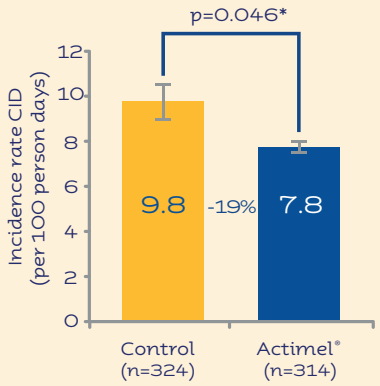
MAIN RESULTS

The rate of days with change of activity because of illness per 100 person days was similar in the Actimel® and control groups (respectively 2.30 ± 0.21 and 2.27 ± 0.21, p=0.91).

The incidence rate of CIDs per 100 person day was 19% lower in the Actimel® group than the control group (respectively, 7.8 ± 0.4 and 9.8 ± 0.7, p=0.046).

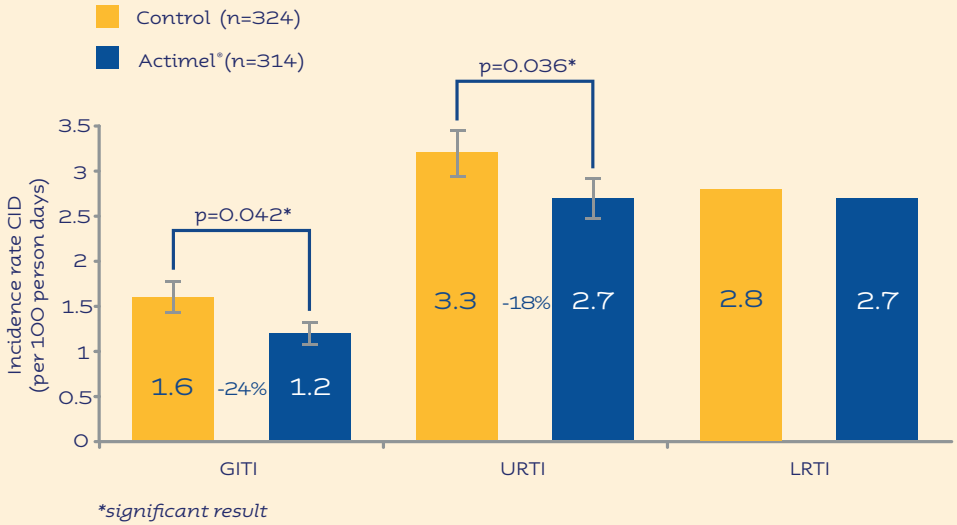
Children who drank Actimel® had a 24% lower incidence rate of gastrointestinal infections (respectively 1.2 ± 0.10 and 1.6 ± 0.15, p=0.042), and a 18% lower incidence rate of upper respiratory tract infections, such as ear infections, sinusitis, pharyngitis and laryngitis, compared to those in the control group (respectively 2.7 ± 0.18 and 3.3 ± 0.20, p=0.036). No difference between groups was found for theincidence rate of LRTI.

Incidence rate of CID
(adjusted means ± SE)



*significant result

Incidence rate of GITI, URTI and LRTI
(adjusted means ± SE)



*significant result

CONCLUSION

This study shows that daily intake of Actimel® showed some promise in reducing overall incidence of illness in kids. However, its effect was primarily on gastrointestinal infections and there were no differences in change of behaviour.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE
ESSENTIAL DAIRY & PLANT-BASED

Consumption of a fermented dairy product containing the probiotic *Lactobacillus casei* DN-114 001 reduces the duration of respiratory infections in the elderly in a randomised controlled trial

Guillemard E, Tondu F, Lacoin F, Schrezenmeir J. Br J Nutr. 2010; 103(1): 58-68. Epub 2009 Sep 14.

OBJECTIVE

The aim of the present study was to evaluate the effect of the consumption of a fermented milk containing *Lactobacillus casei* DN-114001 (Actimel®) on the resistance to common infectious diseases (CID) of the airways and gastrointestinal tract in free-living elderly volunteers.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

1072 free-living elderly volunteers.

Inclusion criteria

- Free living, self-sufficient male and female.
- Body mass index: 17 ≤ BMI ≤ 25kg/m^2.
- Vaccinated against the influenza virus at least 14 days before inclusion.

Exclusion criteria

- Residing in institutions.
- Any current or past severe respiratory, gastrointestinal or metabolic pathology.
- Chronic or iatrogenic immunodeficiency.
- Any progressive or chronic disease.
- Any infection in the last 14 days.
- Laxatives more than twice in the last week.
- Food allergy or intolerance.
- Major surgery with general anaesthesia during the last month or gastrointestinal surgery during the last 3 months.
- Artificial nutrition within the last 2 months.
- Special medicated diets nutritional complements.
- Eating or transit disorders or alcohol abuse.
- Currently receiving or having received in the 4 last weeks drugs likely to interfere with evaluation of the study parameters.

DESIGN

The trial was randomised, double-blind, controlled in 2 parallel groups and multicentric.

Participants were allocated to one of the two parallel groups that received 200 g per day of either Actimel® (n=537) or the control product (non fermented milk) (n=535). The trial was conducted in France for 3 winter months (2006-2007), followed by an additional 1 month's follow-up without product consumption.

Other fermented dairy products were excluded during the whole investigation period.

OUTCOMES

Primary outcomes

- Cumulative number of episodes of all common infectious diseases (CID*).

Secondary outcomes

- Average duration per episode, cumulative duration, severity, occurrence, and time to the first episode of CID and fever associated with CID.

**CID was separated into three categories of infections: upper respiratory tract infections (URTI); lower respiratory tract infections (LRTI); and gastrointestinal tract infections (GITI).*

POPULATION ANALYSED

- The analysis was performed on all the evaluable subjects according to the intention-to-treat principle (1072 in total, 537 in the Actimel® group and 535 in the control group).
- Results were expressed as median and quartiles (Q1–Q3).
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

No statistically significant differences between groups were found for the cumulative number of episodes of CID. The average duration per episode of CID was significantly lower in the Actimel® group than in the control group (respectively 6·5 (4-9) and 8 (5-12) days; p=0·008), as were the cumulative durations of CID (respectively 7 (4-11) and 8 (6-16) days; p=0·009). Similar significant reductions were also observed for the analyses restricted to URTI and rhinopharyngitis (p<0.001 for the four tests).

No significant differences between groups were found for the other secondary outcomes.

	Fermented product					Control product					
	n	Mean	SD	Median	Quartiles (Q1 – Q3)	*n*	Mean	SD	Median	Quartiles (Q1 – Q3)	*p**
All CID											
Average duration per episode (d)	104	7,4	5,6	6,5	4 – 9	111	9,8	7,5	8,0	5 – 12	0,008
Cumulative duration (d)	104	9,1	10,4	7,0	4 – 11	111	12,1	11,4	8,0	6 – 16	0,009
All URTI											
Average duration per episode (d)	61	7,7	7,2	7,0	4 – 8	66	11,0	7,7	8,0	7 – 13	0,0002
Cumulative duration (d)	61	8,5	8,4	7,0	4 – 8	66	11,6	7,9	8,5	7 – 16	0,0003
Rhinopharyngitis											
Average duration per episode (d)	61	7,7	7,2	7,0	4 – 8	58	11,0	8,1	8,0	7 – 13	0,0007
Cumulative duration (d)	61	8,2	8,0	7,0	4 – 8	58	11,5	8,1	8,0	7 – 15	0,0006

Q, quartile ; URTI, upper respiratory tract infections.
**Mann – Whitney test, median and quartiles must be considered as a summary statistic.*

CONCLUSION

This study shows that consumption of the fermented dairy product Actimel® is associated with a significant decrease in the duration of CIDs, especially URTI and rhinopharyngitis. However, there is no demonstrated effect on the incidence of CIDs. This study provides the first evidence that a dairy fermented product containing a probiotic may have a beneficial effect on respiratory infections in the free-living elderly.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE
ESSENTIAL DAIRY & PLANT-BASED

Effects of consumption of a fermented dairy product containing the probiotic *Lactobacillus casei* DN-114 001 on common respiratory and gastro-intestinal infections in shift workers in a randomised controlled trial

Guillemard E, Tanguy J, Flavigny AL, de la Motte S, SchrezenmeirJ. J Am Coll Nut. 2010; 29: 455-468.

OBJECTIVE

The aim of the prospective, single-center, randomised, double-blind and controlled trial was to evaluate the effect of the consumption of a fermented dairy drink on the incidence of respiratory and gastro-intestinal common infectious diseases (CIDs), and on immune functions in healthy shift workers, during the winter of 2006-2007.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

1000 healthy volunteers (male and female aged 18-65 years), working in 2 or 3 shiftwork patterns (including night work).

Inclusion criteria

- Same job position for at least 4.5 months.
- Body Mass Index between 19 and 30kg/m^2.
- No symptoms of CIDs during the 2 weeks before product consumption.

Exclusion criteria

- Part time work (<90%).
- Severe respiratory allergy.
- A history of chronic metabolic/gastro-intestinal disease (except appendectomy).
- Surgery/intervention requiring general anesthesia in the last 4 weeks.
- Administration of systemic/topical treatments likely to interfere with the evaluation parameters in the last 4 weeks.
- Chronic disease requiring antibiotics/ antiseptics/anti-inflammatory medications.
- Special medicated diet.
- Chronic/iatrogenic immunodeficiency.
- Cardiac/renal/respiratory insufficiency.
- Severe evolutive/chronic pathology.
- Pregnancy/breast feeding or lacking effective contraception in females.

DESIGN

The trial was randomised, double-blind, controlled in 2 parallel groups and monocentric.

Participants were randomly allocated to one of two parallel groups and had to consume 200g per day of either Actimel® (n=500) or the control product (non fermented milk) (n=500), during 3 months, followed by a 1-month follow-up period, with no product consumption. Allocated to 2 parallel groups, who consumed 200g per day of either Actimel® (n= 500) or control product (n= 500) for 3 months, followed by a 1-month follow-up, with no product consumption.

Other fermented dairy products were excluded during the whole investigation period.

OUTCOMES

Primary outcomes

- Incidence of CIDs during the 3 months of study product consumption (cumulated number of CIDs reported).

Secondary outcomes

- Occurrence of CID during the 3 months of product consumption (number of subjects having at least 1 CID).
- Immune parameters: Hemogram, C Reactive Protein, NK cells, oxidative burst, and cytokines (TNF-α, IL-8,IFN-α, IFN-β, IFN-γ, IL-10, IL-1, IL-6, IL-12 and CCL-5).

POPULATION ANALYSED

- The analysis was performed on all the evaluable subjects according to the intention-to-treat principle (1000 in total, 500 in the Actimel® group and 500 in the control group).
- For the primary outcome, the primary analysis was performed using a Quasi-Poisson regression model. As this primary model did not fully fit the observed data, especially due to an excess of zeros (based on unexpected low CID frequency), the CID cumulated number was categorized in 3 classes (0: subjects with no CID, 1-2: subjects with a medium number of CID and >2: subjects with more than 2 CIDs, post-hoc analysis). Summary results were expressed as cumulative Odds Ratio (OR) with a 95% confidence interval (CI).
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

Primary outcomes : cumulated number of CIDs

- No statistically significant differences between the groups were observed in the cumulated number of all CIDs, according to the Quasi-Poisson regression model, based on primary analysis.
- A significant product effect was observed on the cumulated number of CIDs in classes according to the categorical post-hoc analyses (OR=0.75, 95% CI [0.59; 0.95], p=0.017), (Figure 1).

Occurrence of CIDs

- A significantly lower occurrence of CIDs was observed in the Actimel® group compared to the control group (43% vs. 51%), (adjusted OR [95% CI] = 0.695 [0.540; 0.896], p=0.005). This corresponds to a lower number of subjects having CID in the Actimel® group (Figure 2).

Immune parameters

- In blood samples collected during the regular visit after 2 months of product consumption (i.e. in the absence of CID), no significant differences between groups were found on the change from baseline of the immune parameters.
- In blood samples collected in cases of CID, the analysis of change from baseline showed a statistically significant higher increase in the Actimel® group compared to the control group in leukocytes (p=0.034) and in NK cells absolute counts (p<0.001).
- No other significant differences between the groups were found on the change from baseline of the other immune parameters in case of CID.

Figure 1: Cumulated number of CIDs by class (cumulative OR, 95% CI)

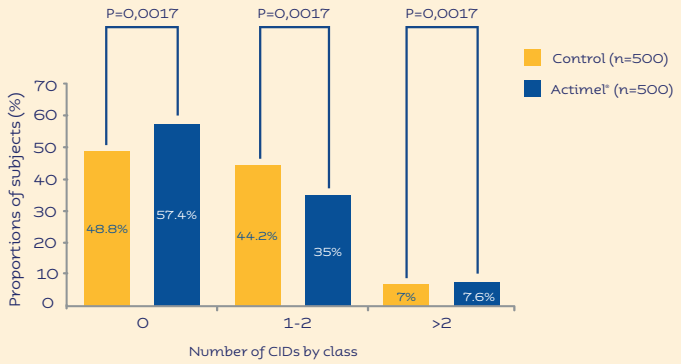
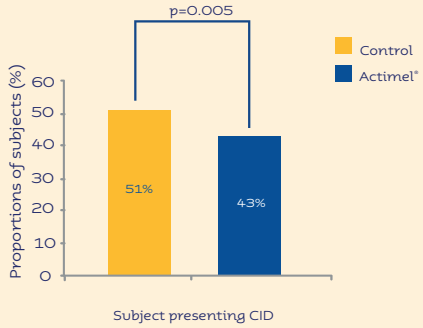


Figure 2: Occurrence of CID



CONCLUSION

This study shows that the daily consumption of Actimel® could improve relevant immune parameters and reduce the incidence of common infections beyond stressed population such as shift-workers.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE ESSENTIAL DAIRY & PLANT-BASED

A probiotic fermented dairy drink improves antibody response to influenza vaccination in the elderly in two randomised controlled trials

Boge T, Rémigy M, Vaudaine S, Tanguy J, Bourdet-Sicard R, Van der Werf S. Vaccine. 2009 Sep 18; 27(41): 5677-84.

OBJECTIVE

The aim of the present study was to investigate the impact of daily consumption of a fermented milk, containing standard yogurt cultures and *Lactobacillus casei* DN-114 001 (Actimel®), on the immune response to influenza vaccination in an elderly population of healthy volunteers over 70 years of age, living in nursing homes.

The 2 trials include 1 pilot study and 1 confirmatory study. The pilot study was on 86 volunteers receiving 200 g per day of either a fermented milk containing standard yogurt cultures and Lactobacillus casei DN-114 001 (Actimel®) or the control product for a period of 7 weeks. After vaccination there were positive tendencies on antibody response for the 3 strains in the Actimel® group. However, the trends were not statistically significant. This pilot study was used to calculate the sample size for the confirmatory study and is not presented in detail in the present summary. The present summary is dedicated to the results of the confirmatory study.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

241 elderly volunteers.

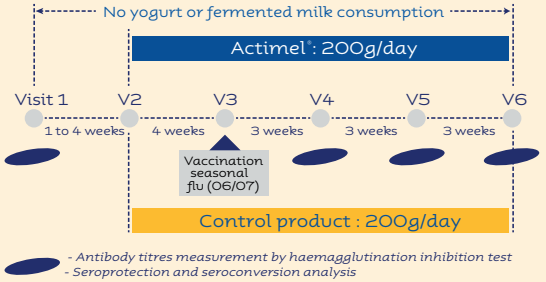
Inclusion criteria

- Healthy volunteers living in nursing homes aged at least 70 years.
- Gerontological Autonomy Iso-Resource Group (AGGIR) score between 2 and 5. The AGGIR score assesses the volunteers' physical and psychological independence on a 6-point scale: a score of 6 represents total autonomy and a score of 1 represents total dependence.

Exclusion criteria

- Serious progressive disease.
- Non-stabilized type I or II diabetes.
- Recent artificial feeding, oral energy, or micronutrient nutritional supplement anorexient or weight-loss medication pathology requiring antibiotic treatment.

Design of the study



DESIGN

The trial was multicentric, double-blind, randomised and controlled.

Subjects were randomly assigned to one of the two parallel groups and received daily 200 g per day of either Actimel® or the control product (non fermented milk) for a period of 13 weeks during a winter vaccination season in 2006–2007.

After the first 4 weeks of consumption, they received a vaccination against seasonal flu (the mix of 3 influenza strains H1N1, H3N2 and B recommended by WHO).

Geometric mean antibody titres (GMT) against the 3 viral strains composing the vaccine (H1N1, H3N2 and B) were measured at several time intervals post-vaccination (3 weeks, 6 weeks, 9 weeks) by the haemagglutination inhibition test.

Other fermented dairy products were excluded during the whole investigation period.

OUTCOMES

Primary outcomes

- Specific serum geometric mean antibody titres against the 3 influenza vaccine strains, measured 3 weeks after vaccination.

Secondary outcomes

- Specific serum geometric mean antibody titres against the 3 vaccine strains, measured 6 weeks and 9 weeks after vaccination.
- Seroconversion rates for each influenza vaccine strain (proportion of subjects achieving a four fold increase in antibody titre after vaccination), measured 3 weeks, 6 weeks, and 9 weeks after vaccination.

POPULATION ANALYSED

- Among the 241 subjects included and randomised, 46 were prematurely withdrawn: 16 due to adverse events (4 in the Actimel® group and 12 in the control group), 2 due to protocol deviations, 20 due to withdrawal of informed consent (8 in the Actimel® and 12 in the control group), and 8 due to other reasons (2 in the Actimel® group and 6 in the control group).
- The analysis was performed on all evaluable subjects according to the intention-to-treat principle (222 in total, 109 in Actimel® group and 113 in control group).
- Results were expressed as geometric mean titres (GMT) ± geometric standard error at the mean (GSEM).
- All statistical tests were two-sided at a 5% significance level.

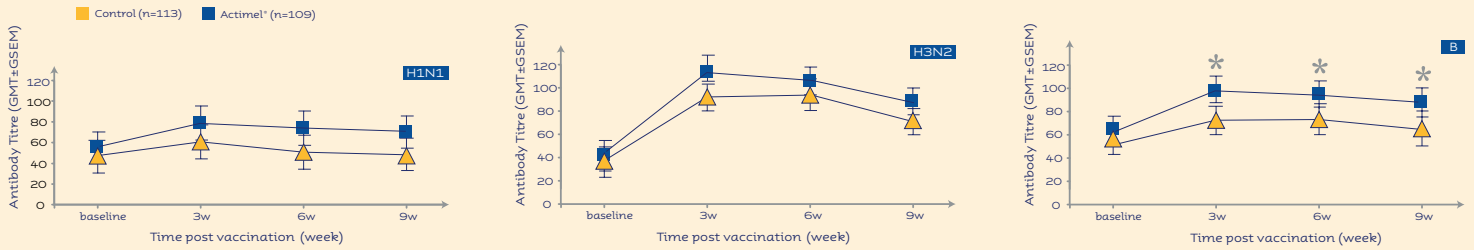
MAIN RESULTS

For B strain, the geometric mean antibody titre was significantly higher during the product consumption period in the Actimel® group compared with the control group (p=0.020) and at all time points post vaccination (3 weeks p=0.029, 6 weeks p=0.027, 9 weeks p=0.025).

The geometric mean antibody titre was also higher but not significant for H1N1 and H3N2 during the product consumption (up to 9 weeks). There were no significant differences between the groups for H3N2 and H1N1 in seroconversion during the product consumption period.

For the B strain, there was a significant effect in favour of the Actimel® group compared with the control group during the product consumption period (overall p=0.002, 3 weeks: p=0.05, 6 weeks: p=0.006 and 9 weeks: p=0.017). 5 months after vaccination, the seroconversion rate was significantly higher in the Actimel® group than in the control group for 2 out of 3 strains (B strain: p=0.016 and H3N2 strain: p=0.031).

Antibody titres at 3, 6 and 9 weeks after vaccination



CONCLUSION

This study shows that daily consumption of Actimel® increased specific antibody responses to influenza vaccination in elderly and may therefore provide a health benefit in this population.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE ESSENTIAL DAIRY & PLANT-BASED

Immunomodulatory effects of the intake of fermented milk with *Lactobacillus casei* DN-114 001 in lactating mothers and their children

Ortiz-Andrellucchi A, Sànchez-Villegas A, Rodríguez-Gallego C, Lemes A, Molero T, Soria A, Peña-Quintana L, Santana M, Ramírez O, García J, Cabrera F, Cobo J, Serra-Majem L. Br J Nutr. 2008 Oct; 100(4): 834-45.

OBJECTIVE

The aim of the present study was to determine the impact of intake of a fermented milk containing standard yogurt cultures and *Lactobacillus casei* DN-114 001 (Actimel®) on the immune system of recently delivered and breast-feeding mothers and on their infants.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

104 healthy pregnant women (18-40 years).

Inclusion criteria

- Low obstetric risk, with a normal delivery at hospital resulting in the birth of a healthy baby.
- Breast-feeding.

Exclusion criteria

- Pre-existing diabetes, hypertension, asthma, allergy and autoimmune, renal, and hepatic diseases.
- Antecedents of viral, bacterial or protozoan infection.
- Multiple pregnancies and high-risk pregnancy.
- Anaemia with haemoglobin below 10.5%.
- Smoking more than 10 cigarettes per day.

DESIGN

The trial was randomised, double-blind and controlled.

Women were randomly allocated after delivery to one of two parallel groups. 59 women received milk fermented with *L. casei* DN-114 001, and 45 received the control product which consisted of the same milk but irradiated (*L. casei* was inactivated and not viable). Both products were administered 3 times a day during a 6-week interval from postnatal day 3 to day 45. The randomisation was stratified on the age group (18-24 or 25-40 years) and among a number of children (primiparous or multiparous).

OUTCOMES

- In mothers' peripheral blood the following parameters were measured:

- T helper 1/T helper 2 profile , determined by IFN-γ and IL-4-producing T cells,
- Lymphocyte subsets,
- Complement components and immunoglobulins.

- In maternal milk, cytokines and IgA were quantified at three stages of breast feeding: colostrum (at day 3, V1), early milk (at day 10, V2), and mature milk (at day 45, V3).
- In infants, anthropometric characteristics, respiratory episodes, gastrointestinal symptoms, dermatitis and allergic episodes, were collected 72 hours after delivery and followed up at 2 months, and at 6 months.

POPULATION ANALYSED

- The analysis was performed on all the evaluable subjects according to the intention-to-treat principle (104 in total, 59 in the Actimel® group and 45 in the control group).
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

In tested mothers, there were a statistically significant increase of NK cells between 10 days and 45 days postpartum (absolute variation=18.5 and -13 cell/µL for the Actimel® and control groups respectively, p=0.026).

In breast milk at 45 days postpartum, a significant drop was observed both in IL-10 (percentage variation= -5.8% and -1.1% for the Actimel® and control groups respectively, p=0.014) and in the pro-inflammatory cytokine TNF-α (percentage variation= -31.7% and -1.6% for Actimel® and control groups respectively, p=0.002).

In infants, probiotic consumption by mothers was associated with a lower incidence of gastrointestinal episodes (29.4% and 54.1% for the Actimel® and control groups respectively, p=0.02) and a lower rate of medication (74.5% and 91.9% for the Actimel® and control group respectively, p=0.037) during the first 2 to 6 month period. The other parameters were not significantly affected.

CONCLUSION

This study shows that the intake of milk fermented with *L. casei* DN-114 001 (Actimel®) during the lactation period modestly contributes to the modulation of the mother's immunological response after delivery and decreases the incidence of gastrointestinal episodes in breast-fed children.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE
ESSENTIAL DAIRY & PLANT-BASED

Modulation of *Lactobacillus casei* in ileal and fecal samples from healthy volunteers after consumption of a fermented milk containing *Lactobacillus casei* DN-114 001^{Rif}

Rochet V, Rigottier-Gois L, Levenez F, Cadiou J, Marteau P, Bresson JL, Goupil-Feuillerat N, Doré J. Can J Microbiol. 2008 Aug; 54(8): 660-7.

OBJECTIVE

The aims of the present study were to investigate the oro-ileal and oro-fecal persistence of amplifiable DNA of a derived strain of *Lactobacillus casei* DN-114 001 when consumed in a fermented milk product (Actimel®) and to investigate the effects of this probiotic on the composition of the intestinal microflora in healthy human subjects.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

7 healthy adults, 6 males, 1 female, aged 22-38 years.

Inclusion criteria

- No history of gastrointestinal disorder.
- No antibiotic treatments for 2 months prior to the study.
- No laxative treatment for the week prior to the study.
- Absence of rifampicin-resistant lactobacilli in stools.

DESIGN

This study consisted of two steps separated by an 8-day washout period.

After a 7-day period of exclusion of fermented dairy products, the subjects ingested one dose of 300mL of Actimel®, and ileal contents were collected. After a washout period of 8 days, the volunteers consumed daily 300mL of Actimel® (10^8 CFU/mL of *L. casei* DN-114 001) for 8 days, and retrieval of the probiotic was analysed in the faeces.

Other fermented dairy products were excluded during the whole investigation period.

OUTCOMES

The study quantified bacteria equivalents of *L. casei* DN-114 001 in ileal and faecal samples and assessed the composition of faecal flora by measuring the mean proportion of 7 phylogenetic groups.

Ileal sampling was performed continuously during 8 hours after ingestion. Faecal sampling was performed before consumption (D7) during ingestion period (D11 and D15) and during the post ingestion period (D18 and D22).

POPULATION ANALYSED

- For ileal results, the analyses was performed on the 4 evaluable subjects according to the intention-to-treat principle (3 subjects were not maintained for data collection because the tube did not go below the jejunum level).
- For faecal results, the analysis was performed on the 7 evaluable subjects according to the intention-to-treat principle.
- Results were expressed as mean ± standard deviation of log of bacterial equivalents of the *L. paracasei* group/mL of ileal fluid or gram of faeces.
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

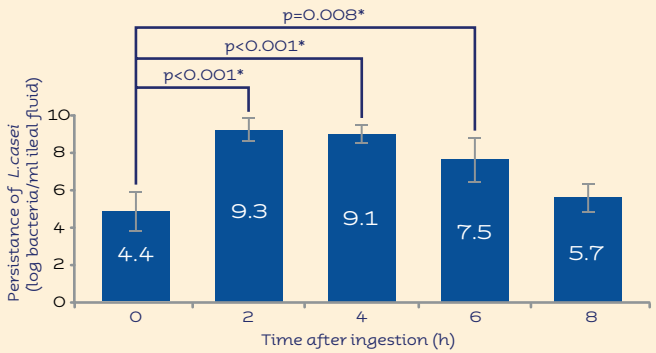
In ileal fluids, 2 and 4 hours after Actimel® was ingested, the *L. paracasei* group, which contains the species of the strain *L. casei* DN-114 001 (expressed in log units) increased considerably and significantly. The mean value at HO was 4.4 ± 1.1; at H2 9.3 ± 0.7; p<0.001; at H4 9.1 ± 0.6, p<0.001. After 6h the mean value decreased but was still statistically different from the base value (H6: 7.5 ± 1.2, p=0.008). Eight hours later, the decrease was more significant (H8: 5.7 ± 0.8).

In faecal samples, after 4 and 8 days of ingestion of Actimel®, a statistically significant increase in *L. casei* DN-114 001 was seen (D7 (baseline): 7.0 ± 1.9; D11: 9.7 ± 0.7, p=0.0046; and D15: 9.7 ± 0.6, p=0.01).

Three days after the end of consumption, a mean decrease of 2 log units was observed; the mean value 7 days after the end of the ingestion period was similar to the base value.

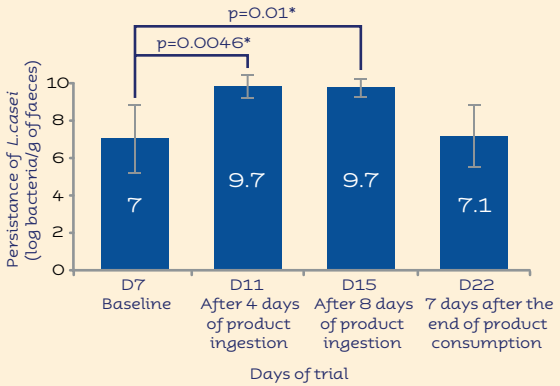
No statistically significant difference was observed in faeces with the proportion of 7 phylogenetic groups between the different steps of the study.

Persistence of *L. casei* DN-114 001 in ileal fluids
(mean ± SD)



*significant result

Persistence of *L. casei* DN-114 001 in faeces
(mean ± SD)



CONCLUSION

This study shows that the ingestion of the probiotic *L. casei* DN-114 001 led to a significant, transient increase of its presence in ileal and faecal samples of healthy human subjects.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE ESSENTIAL DAIRY & PLANT-BASED

Use of probiotic *Lactobacillus* preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial

Hickson M, D'Souza AL, Muthu N, Rogers TR, Want S, Rajkumar C, Bulpitt CJ. BMJ. 2007 Jul 14; 335(7610): 80. Epub 2007 Jun 29.

OBJECTIVE

The aim of the present study was to determine the efficacy of a probiotic drink containing *Lactobacillus casei* for the prevention of any diarrhoea associated with antibiotic use, and that caused by *Clostridium difficile*.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

135 hospitalised patients who were prescribed antibiotics (over 50 years of age).

Exclusion criteria

- Diarrhoea on admission or within the preceding week.
- Recurrent diarrhoea or bowel pathology that could result in diarrhoea.
- Antibiotic use in the previous 4 weeks.
- Severe life threatening illness.
- Immunosuppression.
- Bowel surgery.
- Artificial heart valves.
- History of rheumatic heart disease or infective endocarditis.
- Regular probiotic treatment before admission.
- Lactose intolerance or intolerance to dairy products.

DESIGN

The trial was randomised, double-blind, multicentric and controlled in 2 parallel groups.

Patients were randomly allocated to one of the two parallel groups that received 100 g twice daily of either Actimel® or the control product (long life sterile milkshake) during a course of antibiotics and during one week after the end of the course.

The trial was conducted in 3 London hospitals from November 2002 to January 2005.

OUTCOMES

Primary outcomes

- Occurrence of antibiotic associated diarrhoea during antibiotic course and up to 5 weeks after. Diarrhoea was defined as more than 2 liquid stools a day for more than 3 days and for quantities, exceeding the normal ones, for each patient.

Secondary outcomes

- Occurrence of *C. difficile* infection defined as an episode of diarrhoea combined with the detection of toxins A or B, or both, from a stool sample.

POPULATION ANALYSED

- Among the 135 subjects included and randomised, 22 were lost to follow-up (12 in the Actimel® group and 10 in the control group): 16 for no contact after discharge at home (8 in the Actimel® group, 8 in the control group), 5 due to withdrawal of consent (3 in the Actimel® group, 2 in the control group) and 1 death in the Actimel® group.

- The analysis was performed on all the evaluable subjects according to the intention-to-treat principle (60 in total, 57 in the Actimel® group, and 56 in the control group).
- Fisher's exact tests were used to compare the rates of antibiotic and *C. difficile* associated diarrhoea.
- Logistic regression analysis with removal of non-significant variables was used to investigate the influence of several factors on the occurrence of diarrhoea and estimate the adjusted odds ratio for product effects.
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

A significant reduction in both the incidence of antibiotic associated diarrhoea (P=0.007) and *C. difficile* associated diarrhoea (P=0.001) was found in the probiotic group. After adjustment on concentrations of plasma sodium and albumin, the probiotic treatment reduced the odds for antibiotic associated diarrhoea by 75% (adjusted odds ratio [95% CI]: 0.25 [0.07 ; 0.85]).

Cases of antibiotic associated diarrhoea, and cases that are positive for *C. difficile*

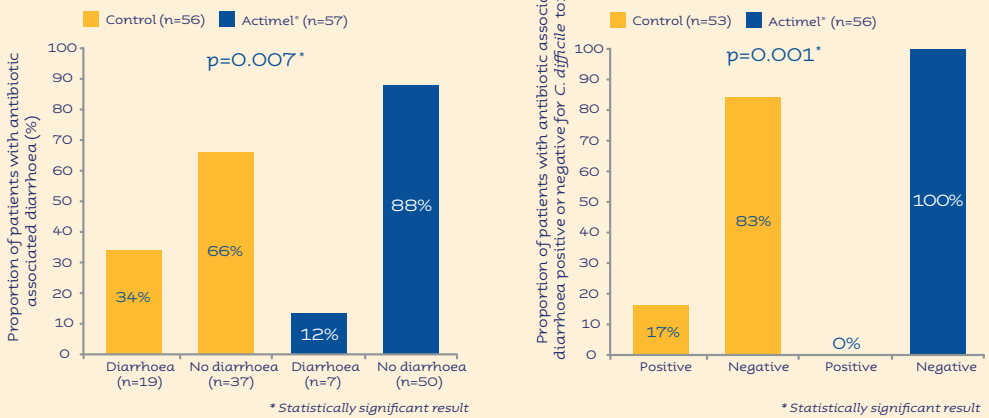


Table: Number of patients and proportion, in brackets, of all patients followed up in hospital and after discharge

	Probiotic	Control	P value*
Diarrhoea			
Yes	7 (12)	19 (34)	0.007
No	50 (88)	37 (66)	
Nb of patients	57†	56†	
C. difficile toxin			
Positive	0	9 (17)	0.001
Negative	56 (100)	44 (83)	
Nb of patients 0	56‡	53‡	

*Fisher's exact test.
† 22/135 patients lost to follow-up or withd rew.
‡ 4/113 patients not tested for C. difficile.

CONCLUSION

This study shows that consumption of Actimel® can reduce the incidence of antibiotic associated diarrhoea and *C. difficile* associated diarrhoea. This has the potential to decrease morbidity, healthcare costs, and mortality if used routinely in patients aged over 50.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE ESSENTIAL DAIRY & PLANT-BASED

Effect of a probiotics supplementation on respiratory infections and immune and hormonal parameters during intense military training

Tiollier E, Chennaoui M, Gomez-Merino D, Drogou C, Filaire E, Guezennec CY. Mil Med. 2007 Sep; 172(9): 1006-11.

OBJECTIVE

The aim of the present study was to investigate the effect of a fermented milk containing standard yogurt cultures and *Lactobacillus casei* DN-114 001 (Actimel®) on respiratory tract infections (RTI) and on mucosal and cellular immune, and hormonal parameters in subjects submitted to a multistressor environment.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

47 young male members of a military officier school (21±0.4 years) in good mental and physical condition.

DESIGN

The trial was randomised, double-blind and controlled.

Subjects underwent 3 weeks of commando training followed by a 5-day combat course (a multistressor environment with heavy, strenuous physical activities and sleep deprivation). They were randomly assigned to one of two parallel groups to consume daily 300 mL of either Actimel® or a non fermented dairy product. RTI and blood parameters were recorded during this period and a week after.

Other fermented dairy products were excluded during the whole investigation period.

OUTCOMES

- Occurrence of RTI: number of RTI episodes, number of days with symptoms, number of symptoms, and number of occurrences of each symptom.
- Quantification of salivary Immunoglobulin A (IgA).
- Quantification of blood cells (each leukocytes and lymphocytes subpopulations).
- Quantification of hormones (cortisol, DHEAS and prolactin).

POPULATION ANALYSED

- Statistical analysis was performed on all the evaluable subjects according to the intention-to-treat principle (47 in total, 24 in the Actimel® group and 23 in the control group).
- Results were expressed as mean ± standard error of the mean.
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

The number of RTI episodes, the mean number of episodes, the mean numbers of days with symptoms, the mean number of symptoms, and the daily mean number of symptoms were not significant different between the two groups. The only significant difference was a greater proportion of rhinopharyngitis in the Actimel® group compared to the control group ($p<0.05$).

For IgA concentrations, there was no significant difference between the two groups at any sampling time. However, there was a decline of IgA concentrations in the control group after the combat course compared with both before and after training ($p<0.01$), whereas IgA values did not change over time in the Actimel® group.

For total and differential leukocytes and lymphocytes subsets, no statistical differences were recorded between the groups, in spite of an observed greater increase in leukocytes, neutrophils and CD19+ concentrations in the control group at the end of the combat course.

For hormones, no significant differences were recorded except on DHEAS: a greater increase in DHEAS was observed in the Actimel® group compared to the control group after the combat course (respectively, 822 ± 140, 502 ± 122, $p=0.047$).

CONCLUSION

This study shows that despite no difference in the number of RTI episodes, the clinical symptomatology showed that the Actimel® and control groups exhibited different profiles with a greater proportion of rhinopharyngitis in the Actimel® group, whereas in the control group symptoms were more evenly distributed. These findings show that in the Actimel® group the infections were mainly confined to the nasopharyngeal area and suggest that Actimel® consumption had prevented the infection from spreading throughout the respiratory tract. In addition, the observed reduced magnitude of changes in several blood subsets, the abolished combat course-induced decline of salivary IgA concentrations, as well as the significantly greater increase of DHEAS concentrations (known to be an immunostimulatory hormone) in the Actimel® group suggest that Actimel® had lessened the negative impact of the training on immune parameters.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE
ESSENTIAL DAIRY & PLANT-BASED

Probiotic, as well as conventional yogurt, can enhance the stimulated production of proinflammatory cytokines

Meyer AL, Elmadfa I, Herbacek I, Micksche M. J Hum Nutr Diet. 2007 Dec; 20(6): 590-8.

OBJECTIVE

The aim of the present study was to determine the effect of a daily intake of a fermented milk containing standard yogurt cultures and 10^8 CFU/mL of *Lactobacillus casei* DN-114 001 (Actimel®) on cytokine production in young healthy women compared to a conventional yogurt.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

33 healthy young women (aged 22-29 years).

Inclusion criteria

- Non-smokers.
- Not pregnant.
- Not suffering from any chronic or contagious disease.

DESIGN

The study was a randomised, controlled trial.

After a 1-week period during which no fermented products were consumed (T1: baseline), subjects were randomly allocated to one of two parallel groups and supplemented daily with standard yogurt or Actimel®. The subjects consumed 100g/day for 2 weeks (T2) and then 200g/day during the 2 following weeks (T3) of the same respective products. These supplementation periods were followed by a 2-week washout period during which no fermented products were consumed (T4). The randomisation was stratified on oral contraceptive use.

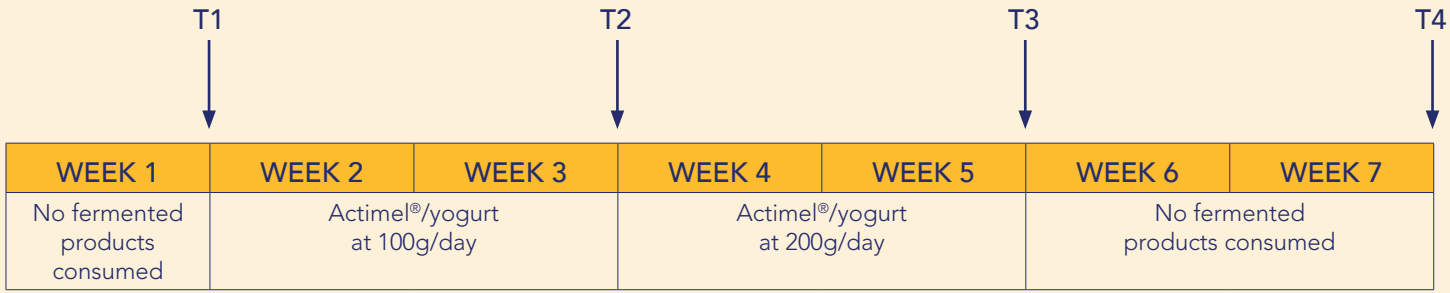
Other fermented dairy products were excluded during the whole investigation period.

OUTCOMES

The production of cytokines (IL-1β, IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10) in the blood culture was measured at the end of every period.

POPULATION ANALYSED

- 33 subjects were analysed (17 in the Actimel® group and 16 in the yogurt group).
- All statistical tests were two-sided at a 5% significance level.



MAIN RESULTS

- The production of TNF-α following stimulation increased significantly after consumption of yogurt or Actimel® (T2 : +63% and +24% compared with the baseline, respectively, $p<0.001$). This production showed further increases after doubling the daily product dose (T3: $p<0.05$ in both groups).
- There was a significantly higher production of IFN-γ in the Actimel® group (T3: +108%, $p<0.05$).
- IL-10 decreased following consumption of Actimel® (T3), but increased significantly after intake cessation (T4: +129%, $p<0.001$).
- No marked effect on the production of IL-1β or IL-6 was induced by the higher consumption of yogurt or Actimel®. IL-2 and IL-4 were virtually undetectable in the samples.
- No statistically significant differences in cytokine responses between the standard yogurt and Actimel® were observed.

CONCLUSION

This study shows that following the consumption of Actimel® or standard yogurt over a period of 4 weeks, an increase in the stimulated production of pro-inflammatory cytokines IL-1β and TNF-α, as well as IFN-γ, the main cytokine from Th1 cells, was observed in young healthy women. Both products were comparable in their effects on the production of these cytokines.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE
ESSENTIAL DAIRY & PLANT-BASED

A randomised prospective double blind controlled trial on effects of long-term consumption of fermented milk containing *Lactobacillus casei* in pre-school children with allergic asthma and/or rhinitis

Giovannini M, Agostoni C, Riva E, Salvini F, Ruscitto A, Zuccotti GV, Radaelli G. Pediatr Res. 2007 Aug; 62(2): 215-20.

OBJECTIVE

The aim of the present study was to examine whether long-term consumption of a fermented milk containing standard yogurt cultures and *Lactobacillus casei* DN-114 001 at 10^8 CFU/mL (Actimel®), may improve the health status of pre-school children suffering from allergic asthma and/or rhinitis.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

196 children aged 2-5 years.

Inclusion criteria

- Inhalants, allergy proved by prick test.
- Diagnosis of allergic asthma and/or rhinitis.

Exclusion criteria

- Cow's milk allergy.
- Lactose intolerance.
- Severe food allergy.
- Other severe chronic diseases.
- Perinatal respiratory problems.
- Antibiotic intake in the 4 weeks preceding the study.

DESIGN

The trial was, multicentric, randomised, double-blind and controlled in 2 parallel groups.

During 12 months, children received daily an oral supplementation of either 100 mL of Actimel® or non fermented milk. Children were visited at the care centres within baseline, after enrolment, and at 3, 6, 9 and 12 months after starting the intervention.

Other fermented dairy products were excluded during the whole investigation period.

OUTCOMES

Primary outcomes

- Time (number of days) free from episodes of asthma and/or rhinitis after the starting intervention, and the total number of episodes and their duration (number of days).

Secondary outcomes

- The number and duration of episodes of diarrhoea or fever.

POPULATION ANALYSED

- Of the 196 randomised subjects, 187 started the study, 11/92 and 18/95 were prematurely withdrawn respectively in the intervention and control groups.
- The analysis was performed on all the evaluable subjects according to the intention-to-treat principle. At baseline and 6 months: 187 subjects in total, 92 in the Actimel® group and 95 in the control group; at 12 months 158 subjects in total, 81 in the Actimel® group and 77 in the control group.
- The effect of the intervention was also assessed with adjustment for potential confounders (age, severity of asthma and rhinitis at baseline).
- Results were expressed as mean, odds ratio or risk ratio, and their 95% confidence interval (CI).
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

Primary outcomes: allergic asthma and/or rhinitis

- Time free from asthma and/or rhinitis episodes: longer in the intervention group than in the control group (mean [95% CI]: 3.5 [2.7 to 4.3] versus 2.1 [1.5 to 2.7] months, p=0.027; adjusted risk ratio [95% CI]: 0.76 [0.56 to 1.03], p=0.082).
- Total number of asthma and/or rhinitis episodes: lower in the intervention than in the control group (median, interquartile range [IQR]: 5 [2 to 9] vs. 7 [4 to 11], unadjusted p=0.036, adjusted p=0.073).
- Mean number of rhinitis episodes: lower in the intervention than in the control group (mean: 3.2 [2.4 to 4.1] vs. 4.8 [3.5 to 6.1], unadjusted p=0.040, adjusted p=0.053).
- Occurrence of rhinitis episodes in the 3–6 months of intervention: lower in the intervention group than in the control group (adjusted odds ratio [95% CI]: 0.39 [0.19 to 0.82], p<0.01).
- No other statistically significant differences between groups were found.

Secondary outcomes: diarrhoea or fever

The mean duration of a single diarrhoea episode (expressed in days) in children with rhinitis was lower in the intervention group than in the control group: (mean [95% CI]: 1.04 [0.55 to 1.53] versus 1.85 [1.32 to 2.38] days, adjusted risk ratio of 0.80 (0.63 to 0.99); p=0.048).

CONCLUSION

This study shows that consumption of fermented milk containing standard yogurt cultures and *Lactobacillus casei* DN-114 001 (Actimel®) may improve the health status of children with allergic rhinitis but not of those with asthma.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE ESSENTIAL DAIRY & PLANT-BASED

Effect of *Lactobacillus casei* DN-114 001 application on the activity of fecal enzymes in children after liver transplantation

Pawlowska J, Klewicka E, Czubkowski P, Motyl I, Jankowska I, Libudzisz Z, Teisseyre M, Gliwicz D, Cukrowska B. Transplant Proc. 2007 Dec; 39(10): 3219-21.

OBJECTIVE

The aim of the present study was to specify the influence of the probiotic strain *Lactobacillus casei* DN-114 001 contained in the fermented milk Actimel® on the activity of faecal enzymes in children with gut microflora imbalance due to liver transplantation.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

25 children after liver transplantation (13 girls, 12 boys) aged 3-17 years.

Inclusion criteria

- Liver transplantation at least 2 years before the beginning of the study.
- No biliary problems.
- No antibiotic treatments.

DESIGN

This prospective study was a double-blind and randomised controlled trial.

Children were randomly allocated to one of two parallel groups and the trial was conducted in two periods. For 2 months 13 children in the Actimel® group were given a daily dose of 10^{10} CFU of the probiotic strain *L. casei* DN-114 001 and the 12 children in the control group received glucose. They were then followed-up for two months.

OUTCOMES

Activities of 3 fecal bacteria enzymes (β-glucuronidase, β-glucosidase, and urease) were measured before product consumption, at the end of product consumption, and after 2 months of follow-up.

POPULATION ANALYSED

- 25 subjects were randomised (13 in the Actimel® group and 12 in the control group).
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

After 2 months' intake of probiotic strain *L. casei* DN-114 001 supplementation, the levels of all 3 enzymes decreased, reaching statistical significance for β-glucuronidase and β-glucosidase. The mean β-glucuronidase activity was 41% lower in the Actimel® group than in the control group, and β-glucosidase activity decreased to mean levels of 71% below the control group. Complete rebound in the 3 enzymes activities was observed 2 months after the end of probiotic supplementation.

CONCLUSION

This study shows that probiotic strain *L. casei* DN-114 001 decreased faecal enzyme activity in liver transplant children, a beneficial effect limited to the period of probiotic intake.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE
ESSENTIAL DAIRY & PLANT-BASED

Daily intake of probiotic as well as conventional yogurt has a stimulating effect on cellular immunity in young healthy women

Meyer AL, Micksche M, Herbacek I, Elmadfa I. Ann Nutr Metab. 2006; 50(3): 282-9.

OBJECTIVE

The aim of the present study was to determine the effect of a daily intake of fermented milk containing standard yogurt cultures and $2x10^8$ CFU/mL of *Lactobacillus casei* DN-114 001 (Actimel®) on cellular immunity in young healthy women compared to a conventional yogurt.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

33 healthy young women (aged 22-29 years).

Inclusion criteria

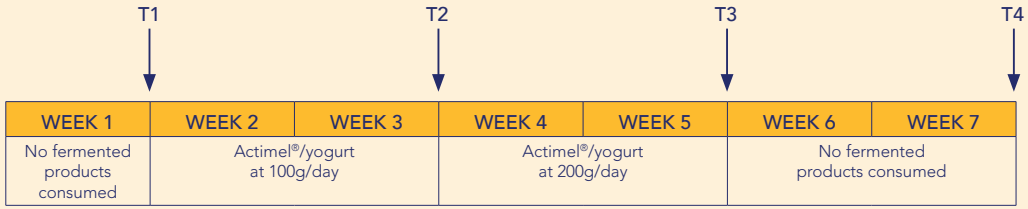
- Non-smokers.
- Not pregnant.
- Not suffering from any chronic or contagious diseases.

DESIGN

The study was a randomised, controlled trial.

After a 1-week period during which no fermented products were consumed (T1: baseline), subjects were randomly allocated to one of two parallel groups and supplemented daily with standard yogurt or Actimel®. The subjects consumed 100g/day for 2 weeks (T2) and then 200g/day during the 2 following weeks (T3) of the same respective products. These supplementation periods were followed by a 2-week washout period during which no fermented products were consumed (T4). The randomisation was stratified on oral contraceptive use.

Other fermented dairy products were excluded during the whole investigation period.



OUTCOMES

- Evaluation of immune status.
- Reaction of T lymphocytes to stimulation.
- Natural cytotoxicity.

POPULATION ANALYSED

- 33 subjects were analysed (17 in the Actimel® group and 16 in the yogurt group).
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

- Regarding the immune status in the Actimel® group , the numbers of cytotoxic T lymphocytes (CD3+CD16+CD56) increased significantly at the end of the first period of supplementation: T2 compared to baseline, respectively 104 ± 40, 136 ± 54,+30.8%; p=0.001. They also showed a significant increase after the wash-out period: T4 compared to baseline respectively 104 ± 40, 104 ± 40, +32.7%; p=0.002.
- There were no major changes for other cell populations, and all remained within the physiological range.
- Regarding the activation of T lymphocytes, the percentage of T cells, CD4+ helper and CD8+ cytotoxic/suppressor increased after 2 weeks of product consumption and maintained this higher level over the entire period of intake. However, the effect was significant only in the standard yogurt group and the observed differences between the groups were not statistically significant.
- Cytotoxic activity also augmented following intake and the effect persisted after subjects stopped consumption. However, there were no significant differences between the Actimel® and standard yogurt groups.

CONCLUSION

This study shows that both, Actimel® and standard yogurt have a stimulating effect on cellular immune functions in young healthy women, although some differences could be observed in the effects of the 2 products on particular immune functions.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE
ESSENTIAL DAIRY & PLANT-BASED

Effects of orally administered *Lactobacillus casei* DN-114 001 on the composition or activities of the dominant faecal microbiota in healthy humans

Rochet V, Rigottier-Gois L, Sutren M, Krementscki MN, Andrieux C, Furet JP, Tailliez P, Levenez F, Mogenet A, Bresson JL, Méance S, Cayuela C, Leplingard A, Doré J. Br J Nutr. 2006 Feb; 95(2): 421-9.

OBJECTIVE

The aims of the present study were to study the orofaecal persistence of *Lactobacillus casei* DN-114 001 following its consumption in a fermented milk product (Actimel®) and to investigate the effects of its consumption on the composition and metabolic activities of the intestinal flora in healthy human subjects.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

12 healthy subjects, 7 women and 5 men, aged 23-44 years.

Inclusion criteria

- No history of digestive pathology.
- No current medication affecting intestinal flora.
- Moderate consumption of fermented milk products (less than 2/week).

DESIGN

This study was divided into 3 steps: a 1-week baseline step (D-7 to D0), a 10-day supplementation step (D0–D10), and a 10-day follow-up step (D10–D20). During the supplementation step, each subject ingested daily 3x100mL of Actimel® (10^8 CFU/mL of *L. casei* DN-114 001).

OUTCOMES

The study assessed the faecal persistence of amplifiable DNA from *L. casei* and measured the composition (in seven groups) of the faecal microbiota. It measured also the bacterial metabolism by faecal enzyme activities, pH, Short Chain Fatty Acid (SCFA), bile acids and neutral steroids production. Analyses of bacterial metabolism were performed before (D0), at the end of (D10), and after the supplementation step (D20).

POPULATION ANALYSED

- The 12 subjects were evaluable. They were all analysed in accordance with the intention-to-treat principle.
- Results were expressed as levels of bacterial equivalents of the *L. paracasei* group/g faeces.
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

L. casei DNA was detected in the faeces of all of the subjects after 10 days supplementation. Its quantification showed a statistically significant 1000-fold increase during the test step compared with initial levels (mean value and range at D0: 6.3 x 10^6; from 3.6 x 10^4 to 2.6 x 10^9; mean value and increase range at D10: 7.9 x 10^9, from 6.3 to 2.5 x 10^6, $p<0.01$). No major modification in either dominant members of the faecal flora or their activities was observed during the trial.

CONCLUSION

This study shows that the short-term consumption of a milk product containing *Lactobacillus casei* DN-114 001 (Actimel®) was accompanied by a high, transient increase in the quantity of this strain in the faeces of all of the subjects without markedly affecting biochemical or bacteriological factors.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE
ESSENTIAL DAIRY & PLANT-BASED

Survival of *Lactobacillus casei* in the human digestive tract after consumption of fermented milk

Oozeer R, Leplingard A, Mater DD, Mogenet A, Michelin R, Seksek I, Marteau P, Doré J, Bresson JL, Corthier G. Appl Environ Microbiol. 2006 Aug; 72(8): 5615-7.

OBJECTIVE

The aim of the present study was to assess, in the human digestive tract, the survival of *Lactobacillus casei* DN-114 001, the probiotic strain contained in Actimel®.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

10 healthy volunteers (8 males and 2 females; aged 22-38 years).

Exclusion criteria

- History of gastrointestinal disorders.
- Antibiotic treatment during the 2 months preceding the study.
- Laxative treatment during the week prior to the study.

DESIGN

The test product consisted of yogurt cultures (*Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus*), supplemented with a rifampicin-resistant spontaneous variant of *L.casei* DN-114 001, to allow the culture of *L. casei* in ileal and fecal samples.

The study consisted of two distinct steps separated by an 8-day washout period.

Step 1: Ileal-survival study of *L. casei* DN-114 001

After 8 days of consuming no fermented dairy products, fasting volunteers were intubated and they ingested a standard meal accompanied with 300 mL of Actimel® and a transit marker. Ileal fluids were collected continuously over the 8 hours following ingestion.

Step 2: Faecal-survival study of *L. casei* DN-114 001

Volunteers consumed daily 3x100 mL of Actimel® (one bottle at each meal) for 8 days, a transit marker was added for the last 3 days. Stools were collected at days 4 and 8 of consumption and 3 and 7 days after the end of consumption.

Other fermented dairy products were excluded during the whole investigation period.

OUTCOMES

The outcome was the recovery of the probiotic strain *L. casei* DN-114 001 from ileal and faecal samples.

POPULATION ANALYSED

- 7 out of 10 subjects consented to intestinal intubation.
- For the ileal-survival study, the analysis was performed on 4 evaluable subjects according to the intention-to-treat principle (3 out of 7 were excluded because the tube failed to go lower than their jejunum).
- For the faecal-survival study, the analysis was performed on 6 evaluable subjects according to the intention-to-treat principle subjects (4 subjects had missing or invalid data).
- Results were presented as mean ± standard error of the mean.
- 4 out of the 7 subjects were enrolled in the ileal-survival study and 6 out of the 10 subjects in the faecal-survival study.
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

Ileal-survival

A peak in the population of *L. casei* DN-114 001 was observed in the ileal fluid during the 3 hours after fermented milk consumption. In contrast, the transit marker persisted longer, reaching a plateau between 2 and 6 hours after ingestion.

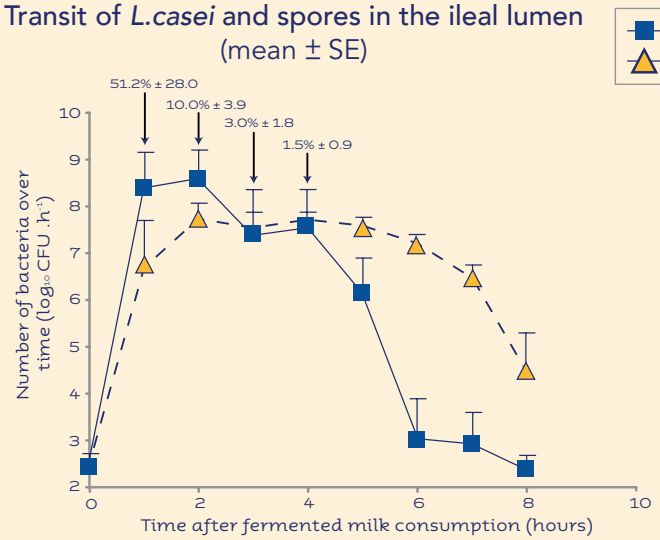
The apparent survival of *L. casei* DN-114 001 in the ileum (based on the ratio between the *L. casei* DN-114 001 organisms and the transit marker) was 51.2%, 10.0% and 3.0% during the first 1, 2, and 3 hours respectively.

The total recovery of *L. casei* DN-114 001 in the ileum over the entire 8-hour period was estimated at 9.2 ± 0.5 $\log_{10}$ CFU, corresponding to around 3.6% ±1.8 of the total ingested quantity.

Faecal-survival study

In stools, a plateau of about 7.6 $\log_{10}$ CFU/g of *L. casei* DN 114 001 was observed from 4 to 7 days following the consumption of Actimel®. This level decreased rapidly after discontinuation. The apparent survival in stools was approximately 28.4% ± 7.0 on day 7 of the experiment.

Transit of *L.casei* and spores in the ileal lumen (mean ± SE)



CONCLUSION

This study shows that the probiotic strain *L. casei* DN-114 001 has the capacity to survive during its transit through the human gut when it is ingested in fermented milk in the course of a meal.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE ESSENTIAL DAIRY & PLANT-BASED

Effects of a specially designed fermented milk product containing probiotic *Lactobacillus casei* DN-114 001 and the eradication of *H. pylori* in children: a prospective randomised double-blind study

Sýkora J, Valecková K, Amlerová J, Siala K, Dedek P, Watkins S, Varvarovská J, Stozický F, Pazdiora P, Schwarz J. J Clin Gastroenterol. 2005 Sep; 39(8): 692-8.

OBJECTIVE

The aim of the present study was to ascertain whether a fermented milk containing standard yogurt cultures and *Lactobacillus casei* DN-114 001 (Actimel®) could improve the eradication rates of triple therapy in symptomatic *Helicobacter pylori* infected children.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

86 symptomatic *H. pylori*-positive children.

Inclusion criteria

- Severe dyspeptic symptoms of more than 3 months duration.
- *H. pylori* infected status.

Exclusion criteria

- Previously treated *H. pylori* infection.
- Gastro-duodenal ulcer, major abdominal surgery or any other diagnosis that would explain dyspeptic symptoms.
- Chronic NSAID intake, antibiotics, bismuth-containing compounds, gastric acid suppressants taken within the previous 8 weeks.
- Known hypersensitivity to components of eradication treatment.

DESIGN

The trial was multicentric, randomised, double-blind and controlled.

Subjects were randomly allocated to one of two parallel groups and received during 7 days either triple therapy (omeprazole, amoxicillin, clarithromycin) and an unfermented pasteurised milk (control group), or the same triple therapy supplemented with Actimel®. Supplementation with 100 mL/day of Actimel® or milk was continued for 1 week after medication. *H. pylori* status was assessed at 4 weeks following therapy, using a specific stool antigen test, the ^{13}C-urea breath test and culture analysis of endoscopic biopsies.

Other fermented dairy products were excluded during the whole investigation period.

OUTCOMES

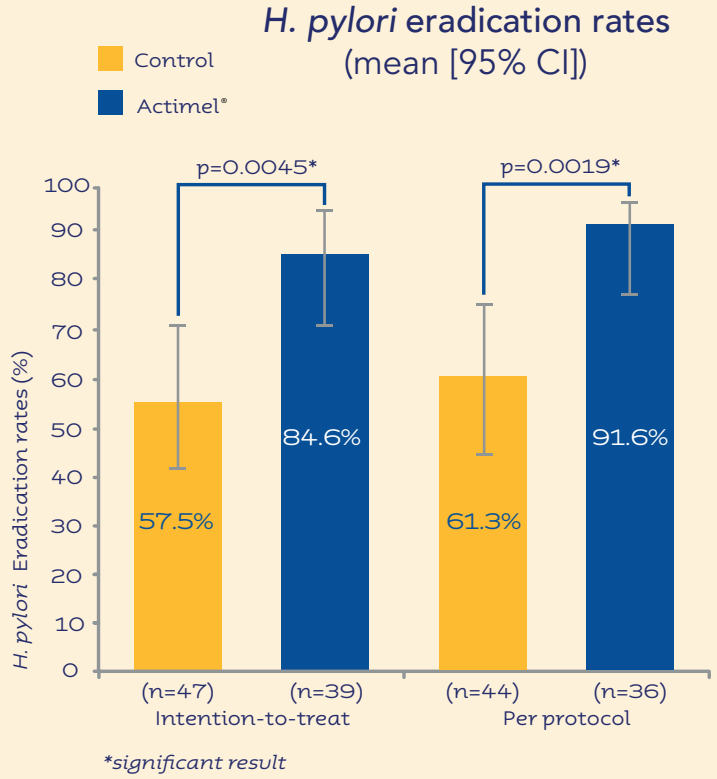
Eradication rate of *H. pylori* (defined as the percentage of patients in whom *H. pylori* infection had been eradicated).

POPULATION ANALYSED

- Among the 86 subjects included, 80 subjects completed the study according to the protocol.
- The analysis was performed on all the evaluable subjects according to the intention-to-treat principle (86 in total, 39 in the Actimel® group and 47 in the control group), and confirmed on the "per protocol" population (80 subjects in total, 36 in the Actimel® group and 44 in the control group).
- Results were expressed as mean rate and their corresponding 95% confidence interval (mean [95% CI]).
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

H. pylori eradication rates were significantly higher in the Actimel® group than in the control group in intention to-treat analysis (respectively 84.6% [71.2%;95.5%], and 57.5% [42.2%;72.3%); p=0.0045). This result was confirmed by the "per protocol" analysis (91.6% [76.9%;98.2%], 61.3% [44.4%;75.0%], p=0.0019).



*significant result

CONCLUSION

This study shows that Actimel® yields a higher eradication rate and confers an enhanced therapeutic benefit on *H. pylori* eradication in children with gastritis on triple therapy.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE
ESSENTIAL DAIRY & PLANT-BASED

The effect of fermented yogurt on the prevention of diarrhea in a healthy adult population

Pereg D, Kimhi O, Tirosh A, Orr N, Kayouf R, Lishner M. Am J Infect Control. 2005 Mar; 33(2): 122-5.

OBJECTIVE

The aim of this study was to assess the effect of a fermented milk containing standard yogurt cultures and *Lactobacillus casei* DN-114 001 (Actimel®) at 10^8 CFU/mL on infectious diarrhoea in new military recruits during basic training.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

541 healthy young male military recruits (average age 18.5 years).

DESIGN

The trial was randomised, single-blind, controlled and monocentric.

Subjects were randomly allocated to one of two groups to receive daily 100 mL (6 days a week for 8 weeks) of Actimel® or a control product (which did not contain live bacteria).

OUTCOMES

Incidence and duration of diarrhoea, defined as the passage of 3 or more loose stools in a 24-hour period.

POPULATION ANALYSED

- Among the 541 enrolled and randomised subjects, 21 out of 275 and 18 out of 266 respectively dropped out of the Actimel® and control groups, due to transfer to other army units.
- The analysis was performed on all the evaluable subjects according to the intention-to-treat principle (502 in total, 254 in the Actimel® group and 248 in the control group).
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

71 participants (14.1%) experienced diarrhoea during the study period. The incidence of diarrhoea in the Actimel® group was lower than in the control group (12.2% and 16.1%, respectively), but not statistically significant (p=0.207). No statistically significant differences between the groups were found in the mean duration of diarrhoea (mean± standard deviation, 3±1.95 and 2.6±1.08 days respectively in the Actimel® and control groups, p=0.276).

CONCLUSION

This study shows that consumption of Actimel® did not demonstrate a statistically significant reduction in the number of diarrheal episodes or their duration among healthy young adults, although a reduction of diarrheal episodes was observed.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE
ESSENTIAL DAIRY & PLANT-BASED

Monocyte function in healthy middle-aged people receiving fermented milk containing *Lactobacillus casei*

Parra D, Martinez De Morentin B, Cobo JM, Mateos A, Martinez JA. J Nutr Health Aging. 2004; 8(4): 208-11.

OBJECTIVE

The aim of the present study was to assess the effects of consumption of fermented milk containing standard yogurt cultures and 10^8-10^{10} CFU/g of *Lactobacillus casei* DN-114 001 (Actimel®) on monocyte activity of middle-aged subjects.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

50 healthy subjects aged 51-58 years.

Exclusion criteria

- Evidence or history of intestinal and/or immune disease.
- Current medication affecting intestinal function and/or immune system.
- Treated dyslipidaemia, obesity (BMI< 30kg/m^2).
- Specific dietary regimen.

DESIGN

The study was a double-blind, randomised and controlled trial.

Subjects were randomly assigned to one of two parallel groups and consumed daily 3x95g of Actimel® or non fermented milk (control), during 8 weeks, after a washout period of 3 weeks.

Other fermented dairy products were excluded during the whole investigation period.

OUTCOMES

The main outcomes were lymphocyte subset counts and the oxidative burst capacity of monocytes before (at day 0) and after 8 weeks of product consumption (at day 56) and leukocyte subsets after 3 weeks of product consumption (at day 21).

POPULATION ANALYSED

- Of the 50 enrolled subjects, 5 were prematurely withdrawn (3 subjects decided to give up and 2 subjects had a cold).
- The analysis was performed on all the evaluable subjects that finished the study, according to the intention-to-treat principle (45 in total, 23 in the Actimel® group and 22 in the control group).
- Results were expressed as mean ± standard deviation.
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

No changes in immune cell proportions were detected in either group between D0 and D56, nor were there any changes in monocyte activity in the control group.

However, in subjects of the Actimel® group the oxidative burst capacity of monocytes increased significantly between D0 and D56 (mean ± standard deviation, change between D0 and D56 = 20.65 ± 8.8 MFI, p=0.029).

Moreover, the increment of burn intensity of monocytes in the Actimel® group was inversely and significantly correlated with the intensity registered at baseline (Pearson's correlation coefficient r=-0.65, p=0.004).

CONCLUSION

This study shows that Actimel® consumption could improve monocyte defence ability in healthy people with low levels of monocyte subsets.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE
ESSENTIAL DAIRY & PLANT-BASED

Daily ingestion of fermented milk containing *Lactobacillus casei* DN-114 001 improves innate-defense capacity in healthy middle-aged people

Parra M, Martínez de Morentin BE, Cobo JM, Mateos A, Martínez JA. J Physiol Biochem. 2004 Jun; 60(2): 85-91.

OBJECTIVE

The aim of the present study was to assess the effects of consumption of a fermented milk containing standard yogurt cultures and *Lactobacillus casei* DN-114 001 (10^8-10^{10} CFU/g) (Actimel®) on the immune response capacity in middle-aged subjects.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

45 healthy subjects aged 51-58 years.

Exclusion criteria

- Evidence or history of endocrine, intestinal and/or immune disease.
- Current medication affecting intestinal function and/or immune system.
- Dyslipemia, obesity (BMI < 30kg/m^2).
- Particular dietary regimen.

DESIGN

The study was a double-blind, randomised and controlled trial.

Subjects were randomly allocated to one of two parallel groups and assigned to consume daily 3x95g of Actimel® or a control product (non fermented milk), during 8 weeks, after a washout period of 3 weeks.

Other fermented dairy products were excluded during the whole investigation period.

OUTCOMES

The main outcomes were lymphocytes subsets and oxidative burst capacity, assessed before (at day 0) and after 8 weeks of product consumption (at day 56) and leukocyte subsets after 3 weeks of product consumption (at day 21).

POPULATION ANALYSED

- The analysis was performed on all the evaluable subjects according to the intention-to-treat principle (45 in total, 23 in the Actimel® group and 22 in the control group).
- Results were expressed as mean ± standard deviation.
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

No changes in immune cell proportions were detected in the Actimel® group nor the control group.

A statistically significant increase in the oxidative burst capacity of monocytes was observed between D0 and D56 in the Actimel® group but not in the control group (mean ± standard deviation, at D0 and D56: 85±22 MFI, 106±29 MFI; p=0.029 in the Actimel® group; 78±20 MFI, 90±25 MFI; p=0.625, in the control group). The tumoricidal activity of NK cells was also improved in the Actimel® group (when the ratio NK:tumoricidal cells was 12:1 (p = 0.042) and 25:1 (p = 0.023)).

CONCLUSION

This study shows that daily intake of Actimel® could be able to activate non-specific defence mechanisms in subjects potentially prone to immunosenescence.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE
ESSENTIAL DAIRY & PLANT-BASED

The effect of milk fermented by yogurt cultures plus *Lactobacillus casei* DN-114 001 on the immune response of subjects under academic examination stress

Marcos A, Wärnberg J, Nova E, Gómez S, Alvarez A, Alvarez R, Mateos JA, Cobo JM. Eur J Nutr. 2004 Dec; 43(6): 381-9.

OBJECTIVE

The aim of the present study was to evaluate the effect of a fermented milk containing standard yogurt cultures and 1x10^8 CFU/mL of *Lactobacillus casei* DN-114 001 (Actimel®) on the immune system of subjects under academic examination stress.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

155 healthy university students aged 18-23 years.

Exclusion criteria

- History of milk allergy or intolerance.
- Atopic symptoms during the study.
- Infection in the previous month before the study or at any time during the study.
- Participation in other nutritional intervention studies during the previous 3 months.

DESIGN

The trial was randomised and controlled.

Students were randomly allocated to one of two parallel groups, receiving 200 mL of Actimel® or 200 mL of semi-skimmed milk daily for 6 weeks (during the 3 weeks prior to the examination period and during the students' 3-week examination period). Assessments were recorded at the start of the study (baseline) and 6 weeks later at the end of the examination period (study end). The total study duration for each subject was 6 weeks.

Other fermented dairy products were excluded during the whole investigation period.

OUTCOMES

Anxiety levels (using the Spielberger state-trait anxiety inventory), routine blood haematological, blood biochemical and immunological (lymphocyte subsets, cytokine production) parameters. Measurements were performed at baseline and at the end of the study (6 weeks later).

POPULATION ANALYSED

- Of the 155 included subjects, 19 did not complete the study mostly because their examinations were rescheduled and 1 student was excluded for illness.
- The analysis was done on all the evaluable subjects according to the intention-to-treat principle. There were 136 subjects in total: 73 in the Actimel® group and 63 in the control group.
- Results were presented as mean ± standard error of the mean.
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

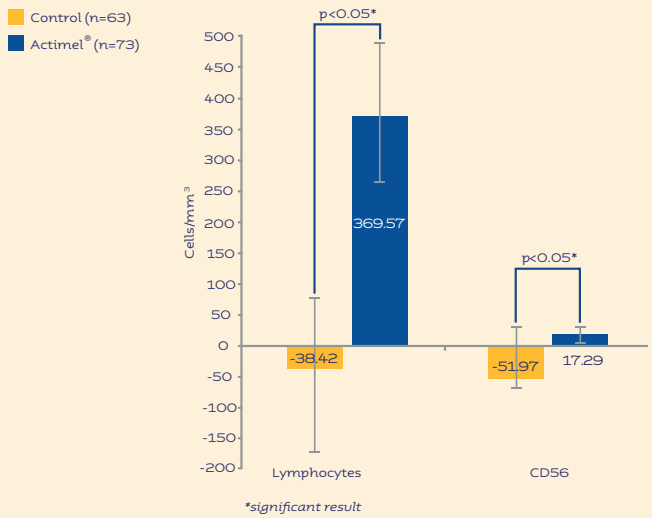
A similar increase in the level of state anxiety (expressed in percentiles) over the 6-week study was observed among all students in both the Actimel® and the control groups. The increase in the Actimel® group group was from 38.31 ± 3.12 to 57.77 ± 3.73, $p<0.05$. In the control group, the rise was from 43.59 ± 4.00 to 65.12 ± 3.71, $p<0.05$.

The mean change in the absolute number of lymphocytes during the 6-week study was significantly different between the Actimel® group and the control group ($p<0.05$). The control group showed a drop of 38.42 ± 116.11 cells/mm^3, whereas the Actimel® group showed a rise of 369.57 ± 112.57 cells/mm^3.

The change in absolute numbers of CD56 cells during the 6-week study was significantly different between the Actimel® group and the control group ($p<0.05$). A decrease was observed in the control group, while cell numbers remained stable in the Actimel® group (respectively, -51.97 ± 21.33 and 17.29 ± 17.27 cells/mm^3).

There were no major changes for other cell populations (immunoglobulins, cytokines), either in phagocytic activity or in oxidative bursts.

Changes in differential lymphocytes during the study (mean ± SE)



CONCLUSION

This study shows that modulation of the immune response altered by psychological stress through consumption of the fermented milk product Actimel®.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE ESSENTIAL DAIRY & PLANT-BASED

Effect of fermented milk containing the probiotic *Lactobacillus casei* DN-114 001 on winter infections in free-living elderly subjects: a randomised, controlled pilot study

Turchet P, Laurenzano M, Auboiron S, Antoine JM. J Nutr Health Aging. 2003; 7(2): 75-7.

OBJECTIVE

The aim of the present study was to evaluate the effect of daily consumption of fermented milk containing standard yogurt cultures and *Lactobacillus casei* DN-114 001 at 10^8 CFU/mL (Actimel®) on the incidence and severity of winter infections (gastrointestinal and respiratory) in free-living elderly people.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

360 free-living subjects over 60 years of age.

Exclusion criteria

- Chronic infection requiring antibiotic therapy more than 3 times per year.
- Immunodepression.
- Regular diarrhoea.
- Colic inflammatory syndrome.
- Chronic anti-inflammatory medication.
- Antibiotic therapy in the previous 3 weeks.
- Allergy.
- History of digestive tract surgery.

DESIGN

The study was a monocentric, randomised, stratified and open pilot trial.

Subjects were randomly allocated to one of the two parallel groups. One received 100 mL of Actimel® twice a day for three weeks. The other group (control group) was given no product. The randomisation was stratified according to the sex gender and vaccinal status. Each group was randomly divided into 3 subgroups (n=60). The study consisted of three consecutive 3-week periods to reduce the risk of an influenza epidemic, hampering the results. All subjects could ingest no more than 2 additional servings of other fermented dairy products per week.

OUTCOMES

The incidence and severity (duration, intensity and maximum temperature) of both respiratory and gastro-intestinal winter infections were assessed and recorded by a physician according to the case report form.

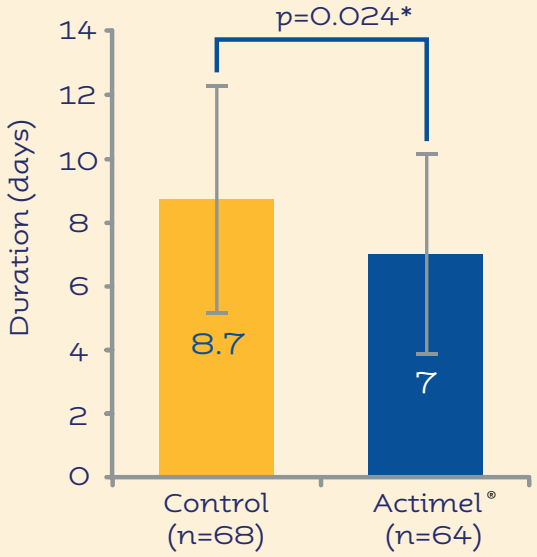
POPULATION ANALYSED

- For incidence, the analysis was performed on all the evaluable subjects according to the intention-to-treat principle (360 in total, 180 in the Actimel® group and 180 in the control group).
- For the duration, the analysis was performed on all the subgroups of infected, evaluable subjects (132 in total, 64 in the Actimel® group and 68 in the control group).
- Results were expressed as proportion or mean ± standard deviation.
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

There were no differences between the Actimel® and the control groups in the incidence of winter infections, either overall or for each infection (Overall incidence was 35.6% for Actimel® and 37.8% for the control group, p=0.66). However, the duration of all infections was significantly lower in the Actimel® group than in the control group (respectively 7.0±3.2 days, 8.7±3.7 days, p=0.024), as was the maximum temperature (respectively 38.3±0.5 °C and 38.5±0.6 °C, p=0.01).

Duration of the winter pathologies
(mean ± SD)



*significant result

CONCLUSION

This study shows that a 3-week supplementation with Actimel® significantly reduces the duration of winter infections in the elderly. No product effect was seen in reducing the incidence of these infections.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE
ESSENTIAL DAIRY
& PLANT-BASED

Feasibility studies to control acute diarrhoea in children by feeding fermented milk preparations Actimel® and Indian Dahi

Agarwal KN, Bhasin SK. Eur J Clin Nutr. 2002 Dec; 56 Suppl 4: S56-9.

OBJECTIVE

The aim of this work was to study feasibility of diarrhoea control in children by feeding fermented milk preparations: Actimel® and Indian Dahi.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

174 children (aged 6 months to 5 years) in hospital or living in the community who suffer from acute diarrhoea.

Inclusion criteria

- Children with 3 loose stools/day without blood/mucus.
- Breast-fed and bottled fed children with or without dehydration.

Exclusion criteria

- Drug-induced diarrhoea.
- Malabsorption syndrome.
- Persistent diarrhoea cases.
- Diarrhoea associated with systemic diseases.
- Antibiotics intake.
- Allergy to dairy products.

DESIGN

The trial was randomised, double-blind and controlled in 2 parallel groups.

Children were allocated to 3 groups and received 3x100 mL daily of fermented milks (2x100 mL for children aged between 6 and 12 months). Group 1 was given a fermented milk (Actimel®) containing *Lactobacillus casei* (DN-114 001), *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Group 2 was given Indian Dahi, containing *Lactococcus lactis*, *Lactococcus lactis cremoris* and *Leuconostoc mesenteroides cremoris*. Group 3 (control) was given ultra-heated yogurt containing no live bacteria. Actimel® was also used as a starter to prepare curd in the community.

Other fermented dairy products were excluded during the whole investigation period.

OUTCOMES

- The primary outcome is control of acute diarrhoea.
- The diarrhoea was considered controlled when the consistency of stools turned semi-solid or solid.

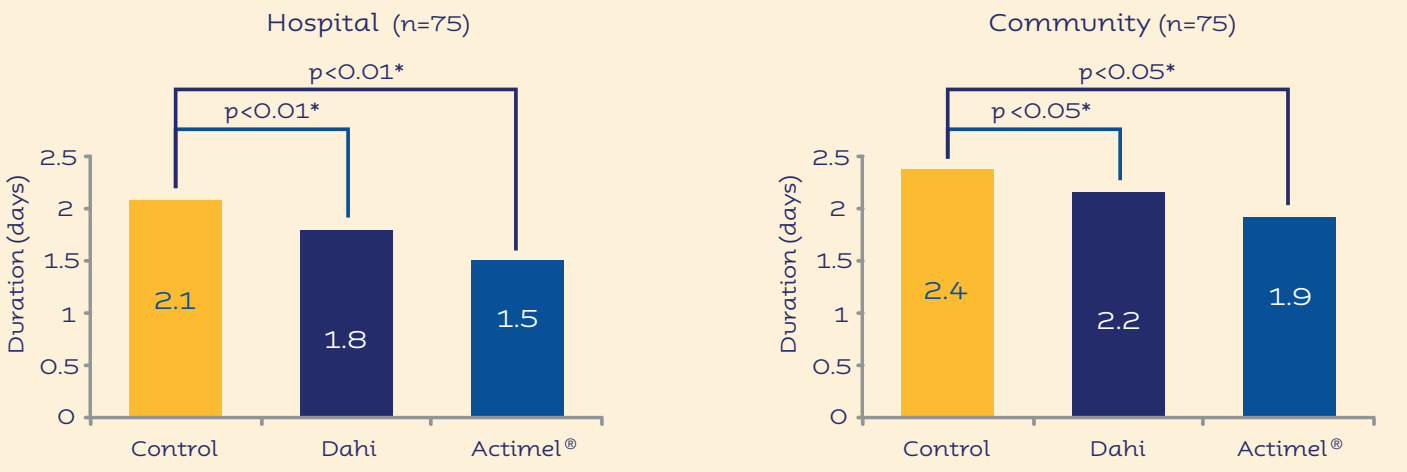
POPULATION ANALYSED

- The analysis was performed on the per protocol population. 150 children out of the 174 enrolled: 24 children dropped out or were lost to follow-up. Of the 150 children analysed, 75 were hospitalised and 75 were living in the community.
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

Administering Dahi and Actimel® to hospitalised children reduced the mean duration of diarrhoea by 0.3 and 0.6 day (P < 0.01), respectively. The corresponding figures in the community children were 0.2 and 0.5 day (P < 0.05), respectively.

Effect of Actimel® & Dahi on duration of acute diarrhoea (mean)



*significant result

CONCLUSION

This study shows that both, Actimel® and Indian Dahi significantly reduced the duration of diarrhoea in children suffering from acute diarrhoea, with Actimel® being the more effective.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE ESSENTIAL DAIRY & PLANT-BASED

Lactobacillus casei in the control of acute diarrhea - a pilot study

Agarwal KN, Bhasin SK, Faridi MM, Mathur M, Gupta S.
Indian Pediatr. 2001 Aug; 38(8): 905-10.

OBJECTIVE

This clinical trial conducted in India aimed to find out the effect of fermented milks on the control of diarrhoea in children.

STUDY POPULATION

75 children (0.5-5 years) with diarrhoea were admitted for study in hospital, while 75 children who suffered from diarrhoea were simultaneously studied in the dispensary of a slum area.

Inclusion criteria

- Children with 3 loose stools/day without blood/mucus.
- Breast-fed and bottle-fed children with or without dehydration.

Exclusion criteria

- Drug-induced diarrhoea.
- Malabsorption syndrome.
- Persistent diarrhoea.
- Diarrhoea associated with other systemic diseases.
- Antibiotics intake.
- Allergy to dairy products.

DESIGN

The trial was randomised, double-blind and controlled in 2 parallel groups.

Children were allocated to 1 of 3 groups and received 3x100 mL of fermented milk daily. Group 1 was given the fermented milk Actimel®, containing *Lactobacillus casei* DN-114 001, *L. bulgaricus* and *Streptococcus thermophilus*. Group 2 was given Indian Dahi, containing *Lactococcus lactis*, *Lactococcus lactis cremoris* and *Leuconostoc mesenteroides cremoris*. Group 3 (control product) was given ultra-heated yogurt containing no live bacteria. These preparations were given along with the rehydration therapy.

OUTCOMES

The main outcome was the number of days required to control diarrhoea. The diarrhoea was considered controlled when the consistency of stools turned semisolid or solid.

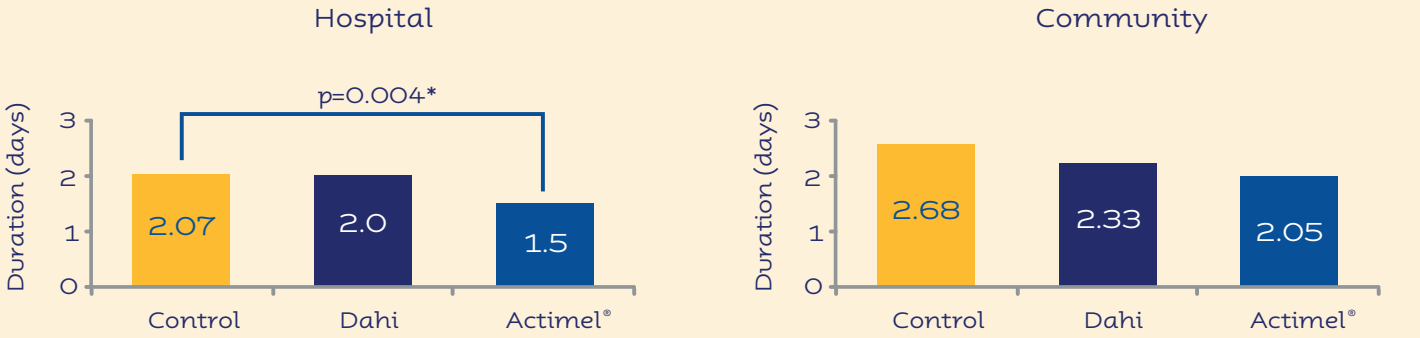
POPULATION ANALYSED

- Per protocol analysis was done on 110 children. Of the 150 children initially recruited 18 were excluded because of infections (15 cases of cholera, 2 cases of *Salmonella*, and one 1 of *E. coli*) and 22 were lost to follow-up.
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

In both the hospital and community studies, diarrhoea was controlled much earlier in the group that received Actimel®. However, the results were statistically significant only in the hospital study (p=0.004). The time required to control diarrhoea was also shortened in the group which received Indian Dahi though Dahi was not as effective as Actimel®.

Effect of Actimel® & Dahi on duration of acute diarrhoea (mean)



*significant result

CONCLUSION

This study shows that Actimel® can reduce the duration of episodes of diarrhoea in children and that it does so more effectively than Dahi.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE ESSENTIAL DAIRY & PLANT-BASED

Multicentric study of the effect of milk fermented by *Lactobacillus casei* on the incidence of diarrhoea

Pedone CA, Arnaud CC, Postaire ER, Bouley CF, Reinert P. Int J Clin Pract. 2000 Nov; 54(9): 568-71.

OBJECTIVE

The aim of this study was to determine if supplementation of healthy children with milk fermented by yogurt cultures and *Lactobacillus casei* strain DN-114 001 (Actimel®) could affect the incidence of acute diarrhoea when compared with traditional yogurt.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

928 children (aged 6-24 months) were recruited from 49 day-care centres.

Inclusion criteria

- Healthy children of both genders, aged 6-24 months attending day-care centres 5 full days per week and taking at least two meals there.
- Diet has included dairy products for more than one month before the study and they are well tolerated.

Exclusion criteria

- Long-term medication.
- Breastfeeding or special diet.
- Allergy or intolerance to cow's milk.
- Absorption disorders.

DESIGN

The trial was randomised, double-blind, controlled and multicentric.

Subjects were randomly assigned to one of two parallel groups and were supplemented daily with standard yogurt or Actimel®. The total duration of the study was 18 weeks divided into 2 periods. A supplementation period (12 weeks) during which the children consumed the products (2x100g/day) five days a week was followed by an observation period (6 weeks) where no special diet was required and children were under surveillance for diarrhoea outcome.

Other fermented dairy products were excluded during the whole investigation period.

OUTCOMES

- Incidence of acute diarrhoea during the supplementation period and the observation period. (Acute diarrhoea was defined as the proportion of subjects experiencing at least one episode. An episode was defined as loose or watery stools for 2 consecutive days or more).
- Duration of diarrhoea episodes (defined by cessation of watery stools) during the supplementation period and observation period.

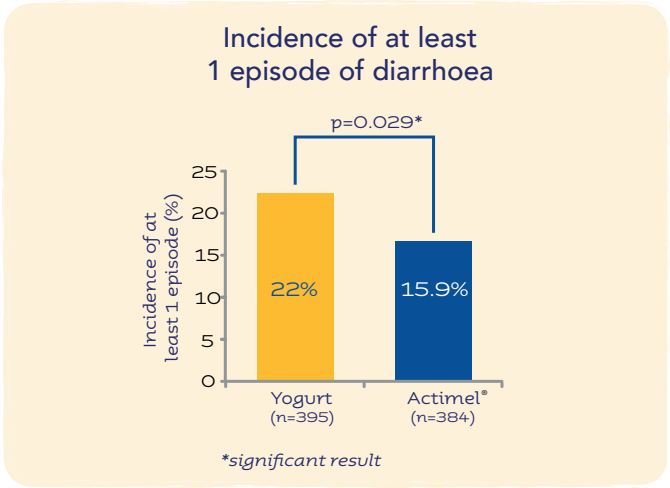
POPULATION ANALYSED

- Of the 928 children recruited (463 in the Actimel® group and 465 in the standard yogurt group), 149 were excluded from the analysis (because of loss of follow-up or poor compliance evenly distributed between groups).
- For the supplementation period, a per protocol analysis was performed on 779 children (384 in the Actimel® group and 395 in the standard yogurt group).
- For the observation period, an additional 55 subjects were not documented, so the analysis was performed on 724 children (360 in the Actimel® group and 364 in the standard yogurt group).
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

During the supplementation period, the incidence of acute diarrhoea was significantly reduced in the Actimel® group compared with the standard yogurt group (respectively 61 (15.9%) and 87 (22%), p=0.029). During the observation period, the difference between the groups was not statistically significant (21 children in total, 1.9% and 3.8 % in the Actimel® and standard yogurt groups).

Regarding the duration of diarrhoea, the Actimel® and the standard yogurt groups did not significantly differ.



CONCLUSION

This study shows that Actimel® is of more benefit than standard yogurt in preventing diarrhoea in healthy children attending day-care centres.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE
ESSENTIAL DAIRY
& PLANT-BASED

The effect of fermented milk containing *Lactobacillus casei* on the immune response to exercise

Pujol P, Huguet J, Drobnic F, Banquells M, Ruiz O, Galilea P, Segarra N, Aguilera S, Burnat A, Mateos JA, Postaire ER. Sports Med, Training and Rehab. 2000; 9(3): 209-223.

OBJECTIVE

The aim of the present study was to determine if fermented milk containing standard yogurt cultures and *Lactobacillus casei* DN-114 001 (Actimel®) would provide protection against an exercise induced immune system depression of natural killer (NK) cells.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

99 recreational athletes (aged 18-41 years, 19<BMI<27kg/m^2).

Inclusion criteria

- Practise of aerobic physical activity during a minimum of 3 times a week, for more than 30 min at minimal intensity of 60% VO_{2max}.
- Refrain of ergogenic aid for at least 2 months before starting the study.

Exclusion criteria

- Hypersensitivity or intolerance to milk products.
- Special diets (e.g. vegetarian or weight loss).
- Experience of exercise induced asthma.
- Antibiotics or prescribed drug treatment within 1 month prior to the beginning of the study.
- Pregnant women.

DESIGN

This study was a double-blind, randomised, controlled trial, made in 2 parallel groups and consisted of a 2-part cross-over design.

First part

99 subjects were included. The first part consisted of a 60-minute exercise stress test at 75% of $VO_{2\,max}$. to measure the decrease in the number of NK cells relative to the base cell concentration in plasma 2 hours after the test. 5 subjects out of the 99 dropped out.

Second part

25 athletes were selected out of 94 for the significant drop (over 3%) in their NK cell concentration compared with the normal base concentration in plasma 2 hours after the exercise stress test in the first phase of the study. The subjects consumed Actimel® or a standard milk product according to a cross-over design. First they ingested daily 500mL of Actimel® (containing 3.2 10^8 CFU/mL of *Lactobacillus casei* DN-114 001) for one month – the Actimel® phase. There then followed a washout period of one month. Then, for another month, they consumed 500mL of a standard milk diet – the milk phase. After each phase of dieting, subjects were investigated before, then 5 minutes and 2 hours after an exercise stress test, testing for various immune cell concentrations.

Other fermented dairy products were excluded during the whole investigation period.

OUTCOMES

Quantitative determination of NK cells, lymphocytes B receptors (IgA, IgM, IgG), lymphocytes T receptors (CD8, CD4, CD3) and cytokines (IL-1β, IL-6, IL-2, sIL-2 receptor and IFNγ).

POPULATION ANALYSED

- Over the 99 enrolled subjects, 5 dropped out. Out of the 94 subjects who completed the first part, 25 were selected for the second part and none of them dropped out.
- The analysis was performed on all the evaluable subjects at the end of the second part according to the intention-to-treat principle (25 in total).
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

No significant differences between Actimel® and milk phases in NK cell concentration at baseline, 5 minutes or 2 hours were observed. However, the NK cell concentration response between 5 minutes and 2 hours was significantly different in the Actimel® and milk phases: respectively from 16.19 ± 8.30 to 13.04 ± 6.34 and from 17.20 ± 7.05 to 11.96 ± 4.86, $p \leq 0.05$).

Analysis of IL-6 showed an increase following ingestion of milk and Actimel® between baseline and 5 minutes ($p \leq 0.05$); and a decrease was observed between 5 minutes and 2 hours in both phases ($p \leq 0.05$).

Most baseline and post-exercise IFN-γ values were below the detection limit (0.5 U/mL).

The other immune parameters were unaffected by exercise and Actimel® consumption.

CONCLUSION

This study shows that Actimel® may contribute to modulating the response of innate immune cells during intensive exercise.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE
ESSENTIAL DAIRY & PLANT-BASED

The effect of supplementation with milk fermented by *Lactobacillus casei* (strain DN-114 001) on acute diarrhoea in children attending day care centres

Pedone CA, Bernabeu AO, Postaire ER, Bouley CF, Reinert P. Int J Clin Pract. 1999 Apr-May; 53(3): 179-84.

OBJECTIVE

The objective of the present study was to determine if supplementation with milk fermented by yogurt cultures and *Lactobacillus casei* DN-114 001 at 10^8 CFU/mL (Actimel®) could lessen acute diarrhoea in healthy children.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

287 healthy children aged 7-32 months attending nursery day care centres.

Inclusion criteria

- Healthy children of both sexes, aged 6-36 months, attending day care centres 5 days a week, where they have lunch and an afternoon snack at 4pm.
- Dairy products introduced into the diet more than one month before the study, and well tolerated.

Exclusion criteria

- Long-term medication (except for some common treatments).
- Breast-fed children.
- Known history of allergy or intolerance to cow's milk.
- Absorption disorders.

DESIGN

The trial was multicentric, double-blind, randomised and controlled.

The study was conducted over six months comprising three periods of one month supplementation, alternating with one month without supplementation. Subjects were randomly assigned to one of three parallel groups and supplemented daily with either 125g (6-18 months old) or 250g (> 18 months old) of one of three tested dairy products: standard yogurt, Actimel®, or jellied milk (control).

Other fermented dairy products were excluded during the whole investigation period.

OUTCOMES

- Incidence of acute diarrhoea (diarrhoea was defined as 3 or more loose or watery stools per 24hours, acute diarrhoea as an episode that lasted less than 2 weeks).
- Duration of acute diarrhoea, diarrhoea attacks, diarrhoea episode, and the normalisation period.

An episode of diarrhoea was defined as the total period of stool perturbation, from the acute phase to the return to normal. The magnitude of diarrhoea was assessed by 2 duration parameters: 'one diarrhoea episode' was defined as the mean number of days from the beginning of the diarrhoea to the end of the normalisation period (the duration being taken as the longest episode in those experiencing more than one episode during the study); and 'diarrhoea attack' was defined as the mean number of days between the beginning of the diarrhoea and the end of the normalisation period (defined as the time between the last watery stools until a return to normal consistency over 2 consecutive days).

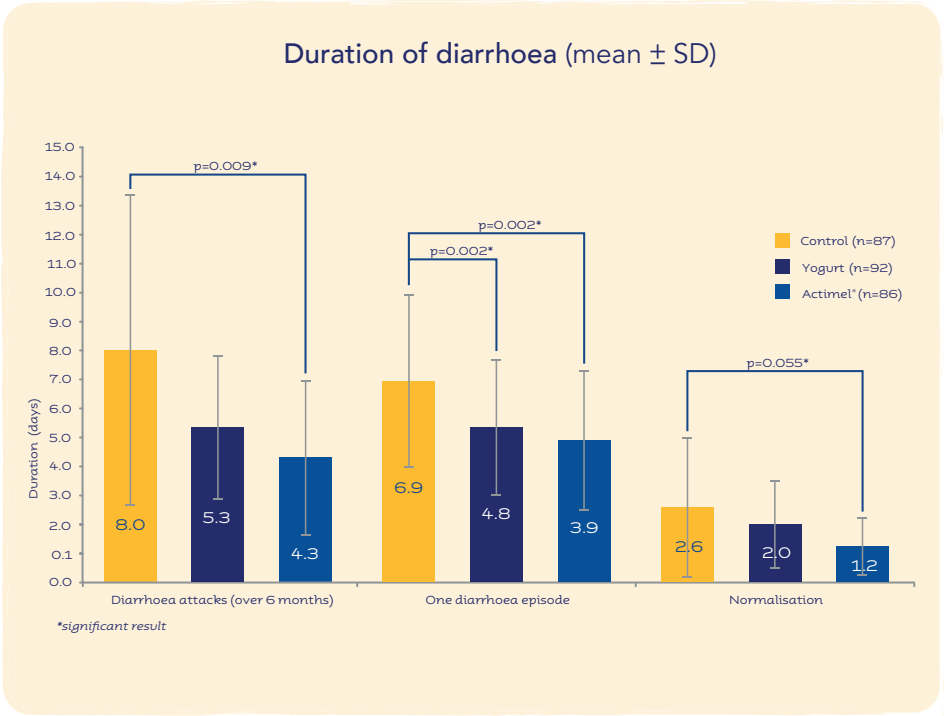
POPULATION ANALYSED

- Of the 287 children recruited, 29 prematurely withdraw (withdrawals were evenly distributed across the groups and were for reasons such as moving, holidays, severe diseases, surgical interventions and refusal to consume the product). Seven of the 29 had well documented case reports for at least 3 months follow-up and were included in the analysis.
- The analysis of incidence was performed on all the evaluable subjects in accordance with the intention-totreat principle (265 in total, 86 in the Actimel® group, 92 in the standard yogurt group, and 87 in the jellified milk group). Results are expressed as number of subjects and percentage.
- The analysis of diarrhoea duration was performed on 62 evaluable children with diarrhoea episodes. Results are expressed as mean time [standard deviation] in days.
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

The incidence of acute diarrhoea during the six-month observation period was not significantly different between the 3 groups (number of subjects and percentage respectively in the Actimel®, standard yogurt, and jellified milk groups, 17 (23.3%), 21 (28.3%), 20 (26.4%), p=0.745).

In children with diarrhoea, the severity of diarrhoea attacks was significantly different between the 3 groups (4.3 [2.7], 5.3 [2.5], 8.0 [5.3] days, p=0.009). Only the difference between Actimel® and jellified milk was significant. Both Actimel® and standard yogurt gave a significantly shorter duration of "one episode of diarrhoea" than with jellified milk (respectively 3.9 [2.4], 4.8 [2.3], 6.9 [3.0] days, p=0.002). Stool normalisation was not significantly different between the 3 groups also it seems to be quicker in the Actimel® group than in the two others groups (respectively 1.2 [1.0], 2.0 [1.6], 2.6[2.4] days p=0.055).



CONCLUSION

This study demonstrates the clinical benefit of fermented milks, with special reference to Actimel®, in decreasing severity of acute diarrhoea episodes in children in an everyday situation.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE ESSENTIAL DAIRY & PLANT-BASED

New insights in the validation of systemic biomarkers for the evaluation of the immunoregulatory properties of milk fermented with yogurt culture and *Lactobacillus casei* (Actimel®): A prospective trial

Yoon H, Dubarry M, Bouley C, Meredith C, Portier A, Tome D, Renevot O, Blachon JL, Dugas B, Drewitt P, Postaire E.
Int J Immunotherapy. 1999; 15(2): 79-89.

OBJECTIVE

The aim of the present study was to assess the potent immunoregulatory properties of fermented milk containing standard yogurt cultures and *Lactobacillus casei* DN-114 001 (Actimel®) in healthy adults and to evaluate the validity of immunological markers that could be affected by Actimel®.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

60 healthy adults (30 males and 30 females), aged 18-50 years, BMI between 19 and 30kg/m^2.

Inclusion criteria

- Clinically normal blood biochemistry, haematology and urinalysis values 21 days prior to the start of the study.
- Negative antigen screening hepatitis B and C, HIV 1 and 2, and *Salmonella*.

Exclusion criteria

- History of renal disorder, cardiovascular or gastrointestinal disease.
- Digestive tract surgery other than appendectomy.
- Hypersensitivity or intolerance to milk products.
- Special diets, unbalanced or irregular diet.
- History of diabetes mellitus, alcohol or drug abuse.
- Antibiotics or other prescribed drug treatment during 1 month prior the start of the study.
- Intake of any over-the-counter drugs or vitamins, or locally applied preparation during 1 week prior the start of the study.
- Participation clinical trials or donated 1-1.5 l of blood during 3 months prior the start of the study.
- History of asthma, allergic skin rash or other allergic reactions.
- Pregnancy or lactation.
- Participation in competitive sports.
- Start of antimalarial prophylaxis before the end of the study.

DESIGN

The trial was randomised, single-center, double-blind and controlled.

Subjects were randomly allocated to one of two parallel groups (Actimel® and control groups). All subjects eliminated commercially fermented milk products from their diet for 35 days before the start of the trial. This restriction applied until the study had been completed on day 28. Subjects were orally vaccinated against *Salmonella typhimurium* on days 0, 2 and 4. Actimel® or semi-skimmed milk were given 1 week before vaccination (starting on day -7) and for a further 2 weeks after vaccination. Blood was collected on days -21, -14, -7, 0, +7, +14, and +21, and saliva on days -7, 0, +7, +14, and +21.

	DAY -35	DAY -21	DAY -14	DAY -7	DAY 0	DAY 2	DAY 4	DAY 7	DAY 14	DAY 21	DAY 28
	Exclusion of commercially fermented milks from the diet										
PRODUCTS				Actimel® or milk intake							
VACCINATION					•	•	•				
BLOOD COLLECTION		•	•	•	•			•	•	•	
SALIVA COLLECTION				•	•			•	•	•	

OUTCOMES

Innate immunity was evaluated by measuring the phagocytic properties of polymorphonuclear (PMN) cells and monocytes. The specific adaptive immunity was evaluated, after vaccination, by the production of salivary and serum specific antibodies (IgA, IgG and IgM) against S. *typhimurium* type 21a and *L. casei*.

POPULATION ANALYSED

> The analysis was performed on all the evaluable subjects according to the intention-to-treat principle (60 in total, 30 in the Actimel® group, and 30 in the control group).
> All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

A significant variation of the phagocytic activities was observed for the two products, the level of significance being higher for the Actimel® group than for the control group (respectively $p<0.001$ and $p=0.001$). After vaccination, specific salivary and serum IgA were detected but these results were not statistically significant.

The other immune parameters seem not to be affected by Actimel®.

CONCLUSION

This study shows that Actimel® affects innate rather than acquired immunity. The variability of the data obtained with IgA leads to the conclusion that serological markers (IgA, at least) seem not to be valuable enough for the follow-up of the immunoregulatory properties of Actimel®.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE
ESSENTIAL DAIRY & PLANT-BASED

Milk fermented with yogurt cultures and *Lactobacillus casei* compared with yogurt and gelled milk : influence on intestinal microflora in healthy infants

Guerin-Danan C, Chabanet C, Pedone C, Popot F, Vaissade P, Bouley C, Szylit O, Andrieux C. Am J Clin Nutr. 1998 Jan; 67(1): 111-7.

OBJECTIVE

The aim of the present study was to compare the effects of consumption of standard yogurt and fermented milk Actimel® containing standard yogurt cultures and at least 10^8 CFU/g of *Lactobacillus casei* DN-114 001 on the faecal flora of healthy infants.

DANONE NUTRICIA RESEARCH

STUDY POPULATION

39 infants aged 10-18 months.

Inclusion criteria

- Attending day-care centres in an urban area.
- Infants were not receiving any medical treatment.

DESIGN

This study was a multricentric, randomised, controlled trial, divided into 3 periods: a 1-week baseline period, a 1-month supplementation period, and a 1-week follow-up period.

Infants were randomly allocated to one of three parallel groups, receiving daily for a month 125g/day of a standard yogurt, or of Actimel®, or of gelled non-fermented milk (control). The total study duration was 6 weeks for each subject.

OUTCOMES

Faecal bacterial counts (total anaerobic bacteria, bifidobacteria, bacteroides, enterobacteria, enterococci, and lactobacilli), pH and faecal moisture, amounts of bacterial metabolites (SCFAs, D- and L-lactate, ammonia), and glycolytic activity of β-glucuronidase, β-glucosidase, β-galactosidase, β-glucosidase, nitroreductase and nitrate reductase. All measures were performed before (days -8 and 0) during (days 15 and 30), and after (day 38) supplementation.

POPULATION ANALYSED

- Among the 39 infants enrolled, 26 had available data for the two observation periods (8/13 in the control group, 11/14 in the yogurt group, and 7/12 in the Actimel® group).
- For biochemical results and bacterial counts, the analysis was performed on 26 infants with available data for the two observation periods (7 in the Actimel® group, 11 in the standard yogurt group, 8 in the control group).
- For count of lactobacilli classified in two categories (<6 or >6 $\log_{10}$ CFU/g faces), the analysis was performed on all the evaluable subjects according to the intentionto-treat principle (39 in total, 12 in the Actimel® group, 14 in the standard yogurt group, 13 in the control group).
- All statistical tests were two-sided at a 5% significance level.

MAIN RESULTS

In the Actimel® group, the percentage of children with up to 6 $\log_{10}$ CFU lactobacilli/g faeces increased ($p<0.05$), whereas the potentially harmful enzyme activity of β-glucuronidase and β-glucosidase decreased after 2 weeks of supplementation ($p<0.05$). These decreases were particularly marked in those infants in the Actimel® group in whom activity of the enzymes was initially unusually high.

There was no specific correlation between bacterial and metabolic modifications in any group. Higher concentrations of lactobacilli in the faeces were not systematically associated with either lower β-glucuronidase or β-glucosidase activity or with a higher concentration of the fermentable metabolites that characterises the two lactobacilli strains used (i.e., D-lactate for *L. bulgaricus* and L-lactate and acetate for *L. casei*).

In the yogurt group the number of faecal enterococci increased ($p<0.05$) from day -15 and day -38 of supplementation, whereas the percentage of branchedchain and long-chain fatty acids, which are markers of proteolytic fermentation, decreased ($p<0.05$).

Other indexes were not modified during the supplementation period or for 1 week after the end of the supplementation period.

CONCLUSION

This study shows that consumption of yogurt and Actimel® might be advantageous for infants after weaning, Actimel® appearing especially beneficial for infants with high ß-glucuronidase or ß-glucosidase activity. This study also suggests that *Lactobacillus casei* DN-114 001 survives during transit through the digestive tract as supplementation with Actimel® led to an increase of faecal lactobacilli.

This study is part of the overall body of scientific evidence of Actimel

For healthcare professionals only

DANONE
ESSENTIAL DAIRY & PLANT-BASED