(19) DANMARK

(10) DK/EP 2431044 T3

(12) Oversættelse af europæisk patentskrift

Patent- og Varemærkestyrelsen

(51) Int.Cl.: *A 61 K 35/74 (2015.01)* *A 61 P 3/04 (2006.01)* *C 12 N 1/20 (2006.01)*
*C 12 R 1/23 (2006.01)* *C 12 R 1/245 (2006.01)*

(45) Oversættelsen bekendtgjort den: **2016-04-11**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2016-01-13**

(86) Europæisk ansøgning nr.: **11191774.6**

(86) Europæisk indleveringsdag: **2006-10-02**

(87) Den europæiske ansøgnings publiceringsdag: **2012-03-21**

(30) Prioritet: **2005-10-07 SE 0502214**

(62) Stamansøgningsnr: **06799717.1**

(84) Designerede stater: **AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LI LT LU LV MC NL PL PT RO SE SI SK TR**

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(54) Benævnelse: **Probiotika til at påvirke fedtmetabolisme og fedme**

(56) Fremdragne publikationer:
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# DESCRIPTION

**Field of the Invention**

**[0001]** The invention relates to the use of probiotic bacteria for the manufacture of a food product, feed product, dietary supplement, nutritional product, and natural remedy, to be used for lowering weight gain, preventing obesity, increasing satiety, prolonging satiety, reducing food intake, reducing fat deposition, or lowering storage of abdominal fat with the proviso that the use of said bacteria is not for therapeutic treatment.

**Background of the Invention**

**[0002]** The interest in the use of food to influence health is rapidly increasing among researchers, health professionals and in the food industry. The metabolic syndrome including obesity abdominal fat storage and type II diabetes is one of the main threat to human health and wellbeing. Today more people are dying from eating too much than the number of people dying from eating too little. Thus the possibility to lower the risk of obesity and body fat storage is of great value in a worldwide health perspective and the possibility to develop food products in this area is of great interest to the food industry.

**[0003]** The food industry has already responded to this demand by developing products with low energy density e.g. low fat and low carbohydrate products. The possibilities to find bioactive components, natural occurring in food or possible to add, that influence the risk of obesity, fat metabolism, glucose metabolism and satiety opens new possibility for the industry to develop i.e. functional foods with documented health effects. Recently studies showing a correlation between consumption of high levels of calcium in milk and a low body mass index (BMI) have been published. Today the conclusion that calcium in milk, and milk products, lowers the risk for obesity, body fat storage and insulin resistance has been drawn in a number of review articles. Calcium by itself does not seem to have those properties and it has been speculated that additional to calcium there is a need for bioactive components present in the milk.

**[0004]** Probiotics are defined by ILSE (International life science institute) as added "microorganisms with a beneficial effect on health". Probiotics are commonly used in foods, feed products and dietary supplements for their influence on gut health, infection and the immune system. Some probiotic strains, but not all studied, have also been shown to influence blood cholesterol level and recently the possibility to lower blood pressure by the use of milk products fermented by proteolytic lactic acid bacteria has been found. Thus the interest in probiotics is wide and the central role of the gut flora for gut health and wellbeing and the immune system is strongly established.

**[0005]** During the last years techniques, often referred to as genomics and nutrigenomics, have been developed. Those techniques allow new unique possibilities to study the influence of foods, nutritional components and probiotics on health and wellbeing. The techniques allow the study on gene-expression of thousands of genes simultaneously. Thus new and unexpected areas for health effects can be found and the mechanism behind a health effect can be suggested.

**[0006]** The basis for this application is studies on man and mice interestingly showing that a probiotic product influences satiety, decreases fat storage and the risk of obesity. Possible mechanisms behind the observed effect were explained from gene-expression studies after consumption of the probiotic products.

**Description of background art**

**[0007]** Metabolic functions contributing to overweight, the metabolic syndrome and risk of diabetes type II have been described and also the link between general nutrition and those diseases is well known. However there is a need for diet based intervention studies to establish the role of individual foods and food group in diminishing the risk to develop those diseases. In studies in this area there has been a focus on energy density, fat and carbohydrates.

**[0008]** An important factor in the metabolic syndrome is the glucose metabolism including the insulin sensitivity. There is a link between body fat deposition, insulin insensitivity and overweight. There is also a link to satiety and satiation. The interactions and relationships between all those functions are complex and it is difficult to identify the exact sequence of events leading to health or disease.

**[0009]** A number of genes and their corresponding proteins that are involved in energy homeostasis have been described. Adipose tissue synthesizes a number of proteins with structural resemblance to cytokines and therefore these proteins are named adipokines. Some of those are also synthesised in the gut or the synthesis is regulated from the gut. The nuclear receptor PPAR-gamma is involved in the synthesis of e.g. adiponectine and the synthesis is associated with calorie intake and leptin signalling. Increased synthesis of adiponectine improves insulin sensitivity by increasing fatty-acid transport, oxidation and dissipation in skeletal muscle and by increasing the sensitivity of the hepatocyte to insulin. Another nutritionally regulated adipocyte-derived factor influencing insulin sensitivity is resistin. Circulating resistin levels are increased in obesity while treatment of mice with resistin resulted in increased glucose production and impaired insulin action. Thus resistin has a function in glucose homeostasis, and acts as an antagonist to insulin action, giving rise to insulin resistance. Resistin-like proteins are also expressed in the gastrointestinal tract (14). The protein apolipoprotein A-IV is secreted by the small intestine in humans and in rodents. The synthesis is stimulated by fat intake and the protein is probably involved in inhibiting food intake after ingestion of fat. Studies have shown that apolipoprotein A-IV probably is involved both in the short-time and long-time regulation of food intake.

**[0010]** Hooper et al (11) studied the gene expression in the gut of mice exposed to *Bacteroides thetraiotaomicron.* They concluded that the experiments show the essential role for the interaction between gut microflora and the host. No effects on energy metabolism or obesity were reported. The influence of probiotics on the gene expression in the small bowel mucosa has been studied for *Lactobacillus rhamnosus* GG (8). Their conclusion is that the administration of Lactobacillus GG is associated with a complex genetic response. The main responses seen are effects on the immune system, inflammation, apoptosis, cellgrowth and differentiation, cell adhesion and signal transcription and transduction. No effects on energy, fat, glucose or insulin metabolism are reported. In an oral presentation by W. de Vos, at the 4$^{th}$ NIZO Dairy conference in 2005, the influence of lactobacilli e.g. *L. plantarum* 299v on gene expression in the gut was presented and the conclusion was that the effect on gene expression from different strains varies considerably. No effect on energy or fat metabolism was reported. A few articles linking gut flora to weight gain and fat storage have been published. Bäckhed et al (7) studied the importance of the normal gut flora for weight gain and body fat storage. It was shown that gnotobiotic mice conventionalized with a normal microbiota increased their body fat with 60% and increased insulin resistance during a 14 day feeding period despite a reduced food intake. A suggestion for how the microbiota can influence triglyceride storage is presented. Thus it is known that the normal gut microbiota influences fat metabolism however until this investigation it was not known whether the normal flora could be modified concerning fat metabolism. Ali et al (2, 3) have studied the influence of soybean isoflavones with or without probiotics to influence the cholesterol metabolism and fat deposition and find that the probiotics used had no influence on cholesterol or fat deposition. Other investigations ref. has shown that some probiotic strains may influence the level of blood cholesterol (1, 13). This and other studies where probiotic strains are compared shows that different probiotic strains have different characters and it is important to choose the right strains for specific health effects.

**[0011]** In the PCT patent application WO 04/014403 (15), the use of probiotics to reduce the amount of monosaccharides and disaccharides in food, and thereby treat or prevent the risk of obesity are described. In the US patent no 6 696 057 Bojrab (4) has patented a composition of yoghurt bacteria e.g. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* for the treatment of gastrointestinal disorders, hyperlipidemia and autoimmune diseases, and in the US patent 6 641 808 Bojrab (5) describes the use of the same composition also for the treatment of obesity. This invention is also described in a European patent application 1 177 794 (6). The patents/applications have a long description on the influence of probiotics on gut health, infection and immunity but the only experiment presented that confirms the influence on obesity is a single feeding trial on one person. The use of green tea or green tea extracts in combination with a number of bioactive components and probiotics for weight loss has been patented by Gorsek in US patents 6 383 482 (9) and 6 565 847 (10). In both patents the main active components are the green tea and the bioactive components, while probiotics are added as part of the basic composition. In the US patent 6 808 703 and the Japanese patent application 2001-292728 (16) a way to prepare a food for obesity treatment containing yeasts, enterobacteria, lactic acid bacteria, oligosaccharides and vegetable fibre is described. No examples or claims concerning treatment of obesity are presented.

**Description of the invention**

**[0012]** An object of the invention is use of probiotic bacteria for the manufacture of a food product, feed product, dietary supplement, nutritional product ,and natural remedy, to be used for loweringweight gain, increasing satiety, prolonging satiety, reducing food intake, reducing fat deposition, or lowering storage of abdominal fat, with the proviso that the use of said bacteria is not for therapeutic treatment.

**[0013]** This object is achieved in that the probiotic bacteria being selected from the group consisting of *Lactobacillus casei* F19

(LMG P-17806), *Lactobacillus acidophilus* NCFB 1748, and *Bifidobacterium lactis* Bb12 (the bacteria are deponated at LMG culture collection in Gent, available from the NCFB culture collection and from Chr. Hansen Company DK, respectively). The strains will be referred to as LMG P17806, NCFB 1748 and Bb12 below.

**[0014]** *Lactobacillus casei* F19 (LMG P-17806), *Lactobacillus acidophilus* NCFB 1748, and *Bifidobacterium lactis* Bb12 are well characterised and the LMG P-17806 strain and its use is previously patented (WO 99/29833). The three strains survive well in food products and during the passage through the gut.

**[0015]** The impact of the invention is of considerable importance since the common lifestyle of today often means excess food intake leading to increased risk for the metabolic syndrome including obesity, abdominal fat deposition and type II diabetes. Type II diabetes is caused by a low sensitivity to insulin or insulin resistance. This threat to human health increases continuously in all age groups all over the world. Lactobacilli as probiotics are accepted as a food product and feed product, natural product, dietary supplements, and in natural remedies, pharmaceutical active formulations and medicinal products. They are easy to use in those delivery systems and can easily be consumed on a daily basis. The regular consumption of those bacteria will help to control weight gain, prevent obesity, increase satiety, prolong satiation, reduce food intake, reduce fat deposition, improve energy metabolism, enhance insulin sensitivity, treat obesity and treat insulin insensitivity and thereby the risk for diseases included in the metabolic syndrome.

**[0016]** Included in the invention are the following studies:

A study has promisingly shown that a probiotic product can lower the weight gain in humans. Further studies have shown that man given probiotics reported a higher satiety and had a longer satiation than individuals given the same acidified milk without probiotics or placebo. Mice given probiotics had a lower total energy intake, less fat deposition in adipose tissue, and a lower total weight gain than individuals not given probiotics. Gene expression studies confirm that the probable mechanism behind the observed effects is an influence on a cluster of genes involved in energy, fat, sugar and insulin metabolism and on satiation.

**Examples**

**1) Weight regulation in humans**

**[0017]** In a double blind placebo controlled study, humans were given either an acidified milk containing probiotics (LMG P-17806 and NCFB 1748) or the same acidified milk without probiotics or a calcium tablet. The groups consisted of 15, 14 and 10 persons respectively. The probiotic bacteria were added to the product in final concentration 5x10E7 CFU/ml and the number of bacteria was stable in the product during the experimental time. The participants were eating 2x2.5 dl of the product each day. The persons included hade a slight overweight (BMI 25-30). The aim of the trial was to study effects of probiotics on cholesterol metabolism and weight gain was measured as a control measure. No advises concerning weight control was given but all the participants were offered a weight reduction treatment after the trial. The bacteria survived the passage in the gut and increased the number of lactobacilli found in faeces from the participants by a factor 5. After 4 weeks there was a surprising but significant difference in weight gain between the three groups.
The mean figure for weight gain was 0,25 kg in the group receiving the probiotic product, 0,75 kg in the group receiving the acidified milk without probiotics and 1,4 kg in the group receiving the tablet.

**[0018]** See Figure 1.

**2) Satiety and satiation in humans**

**[0019]** In a double blind placebo controlled study, humans were given either an acidified milk containing probiotics in two different dosages or a placebo no 1 consisting of the same acidified milk without probiotics or a placebo no 2 consisting of acidified milk without any lactic acid bacteria at all. The probiotic microorganisms, LMG P 17806, NCFB 1748, and Bb12, were added to the product in final concentration 1x10E6 CFU/ml and 5x10E7 CFU/ml of each strain. After a controlled standard breakfast, including 3 dl of any of the four products, the participants were asked to indicate satiety and hunger on a VAS-scale (Visual Analogue Scale) directly after the meal and continuously every half hour for 4 hours. Totally 10 persons were included in the study and

every participant was given all four products but at different occasions and the relative differences were calculated. Directly after the meal there was a slight difference in the satiety scores between the products. The acidified milk with probiotics in concentration 5x10E7 CFU/ml and 1x10E6 giving the highest scores (relative VAS-score 6.6 and 6.4 respectively), and placebo no 1 had an intermediate score (relative VAS-score 6.2) and placebo no 2 the lowest (relative VAS-score 5.9). The satiety scores dropped after the 3.5 hours to 3.1, 3.0, 2.5 and 2.2 respectively. The difference was small but the difference between the products persisted and an influence on satiation was noted for the probiotic product with two different concentrations of bacteria. Concerning hunger the opposite situation could be seen, i.e. the probiotic products giving a lower hunger score than the two placebo products. Directly after the meal the two probiotic product resulted in hunger scores 0.4 for both products on the VAS scale, placebo no 1 was intermediate (VAS score 0.9) and placebo no 2 gave a VAS score on hunger at 1.1. After 4 hours the figures were 6.0, 6.2, 7.0 and 6.9 respectively.

**[0020]** After the standard breakfast above and the four hour when data on satiety and hunger were noticed the participants were offered to eat a standard lunch meal. The participants were asked to eat until they were pleasantly satisfied and to try to get the same level of fullness after each different breakfast. After eating the probiotic products the energy intake at lunch was 3690 KJ for the product with high level of probiotics, it was 3810 for the low level probiotic. For placebo no 1 it was 3850 and for placebo no 2 it was 3995. This shows that the probiotic products can reduce the energy intake in a postprandial meal after eating the probiotic products as part of the breakfast.

**Food intake of mice**

**[0021]** Normal flora mice of Swiss Webster strain were given acidified milk containing either LMG P-17806 or NCFB 1748 or a placebo product with the same composition but without probiotics for 10 days. The mice were between 6 and 8 weeks when the administration was started. The groups given probiotics contained 7 mice in each group and the placebo group consisted of 5 animals. The probiotic microrganisms were added to the products in final concentration of 1x10E8 CFU/ml and the number of bacteria was stable in the product during the feeding period. The mice were given two standard daily dosages of the products, one by oral gavage feeding of 1 ml and one by sublingual injection. Both probiotic bacteria survived the passage in the gut and were present in significant numbers in the small and large intestine of the mice. Besides the probiotic products and the placebo product the mice had *ad libitum* access to purified ingredient diet (D12450B from Research Diets Inc., New Jersey, USA). The daily food intake was compared between the two groups of mice receiving *Lactobacillus* strains versus the placebo group of mice. The products were well tolerated by the mice.

**[0022]** During the second part of the feeding period the food intake was significantly reduced in the groups of animals given probiotics compared to the group receiving the placebo product. See Figure 2.

**3) Abdominal fat storage in mice**

**[0023]** Normal flora mice were given either an acidified milk probiotic product containing LMG P-17806 or a placebo product with the same composition but without probiotics or kept as a control group given the same feed as the two other groups but without the acidified milk. The mice strain chosen was C57B16 (Charles River) which is sensitive to diet induced obesity. The number of animals included in the different groups was 15 in each of the groups given acidified milk and 3 in the control group. The mice were introduced into the study when they were 9 weeks old. The number of probiotic bacteria in the product was 1x10E8 CFU/ml and the mice were allowed to eat the product *ad libitum,* 5 days a week, for a total number of 12 weeks. For rapid weight gain the mice were fed with a high fat diet (D12309 from Research Diets Inc. New Jersey, USA containing 36% fat for the first 5 weeks and then D12492 (35% fat) from the same company for the remaining weeks of the trial). After 12 weeks the mice were sacrificed and the data on weight gain, food consumption and amount of abdominal fat was analysed. The mice given acidified milk product gained less weight than the control mice not receiving any acidified milk. The mice given acidified milk with probiotics also had a lower storage of abdominal fat compared to the group receiving acidified milk without probiotics and the control group not receiving any acidified milk at all.

**5) Gene-expression in mice**

**[0024]** Germ free and normal flora mice of Swiss Webster strain were given acidified milk containing either LMG P-17806 or NCFB 1748 or a placebo product with the same composition but without probiotics for 10 days. The mice were between 6 and 8 weeks of age when the administration was started. The groups given probiotics contained 6 mice in each group and the placebo

group consisted of 4 animals. The probiotic microrganisms were added to the products in final concentration of 1x10E8 CFU/ml and the number of bacteria was stable in the product during the feeding period. The mice were given the product daily by oral gavage feeding. During the study the mice had *ad libitum* access to purified ingredient diet (D12450B from Research Diets Inc., New Jersey, USA). Both probiotic bacteria survived the passage in the gut and were present in significant numbers in the small and large intestine of the mice. The mice were sacrificed and the gene-expression in the small intestine was analysed by oligonucleotid micro array technology. Besides expected effects on the immune system unexpected effects on the expression of a number of genes involved in energy metabolism and homeostasis was found to be different in the placebo group compared to the two probiotic groups. Among the genes showing a significant change in expression were Scd1, Acrp30, Adn, Thrsp, Car3, and Apoa-4, all of which were up regulated in the probiotic groups and Retnlb which was down regulated in the probiotic groups, compared to the placebo group. The pattern of differential gen-expression was very similar for the probiotic strains used. Differential expression of adipsin (Adn), adiponectin (Acrp 30), carbonic anhydrase 3 (Car3) and resistin like beta (Retnlb) genes in germ free mice was confirmed by quantitative Real-Time PCR technique, see Figure 3. Figure 4 shows the expression levels of apolipoprotein A-IV (Apoa4) in normal flora colonised mice as verified by quantitative Real-Time PCR approach.

**6) Dosage**

**[0025]** The total dosage of the individual strains of probiotic bacteria in the different experiments described above was 1x10E8-1x10E9 CFU (mice experiments) and 2,5x10E8-2,5x10E10 (human experiments). This dosage needs to be administrated in a portion or as a daily dose meaning that e.g. a drink for human use that is consumed in amounts 200-500 ml need to contain 0,5x10E6-1,25x10E8 CFU/ml of the individual strain for efficacy and a capsule need to contain the total amount of bacteria in approximately 1 g of the content in the capsule i.e 1x10E8-2,5x10E10. For the highest concentrations of bacteria the bacteria need to be concentrated by freeze drying or spray drying.

**7) Preparation of product**

**[0026]** The probiotic bacteria have to be added to products based on milk, cereals or fruits. The bacteria were added as a concentrate e.g. lyophilized and the survival has been analysed in all tree matrixes. The survival was very good in milk based and cereal based products. In fruit based product the survival is influenced by the fruit type. As a lyophilised powder care needs to be taken concerning the water activity and exposure to oxygen. The results from product production can be seen in table 1.

**[0027]** The probiotic bacteria can also be added to milk based products together with other lactic acid bacteria for the production of different kinds of cultured products. In this environment the probiotic bacteria can multiply and will be well adjusted to the acid environment giving a good survival also at the low final pH in those products. The results from product production can be seen in table 1.

Table 1. Survival of probiotic bacteria in different product matrixes measured as million CFU/g. The products were stored at 8°C.

| Bacteria | Milk | Smoothie Milk/fruit | Cereal drink | Lyophilized powder in cereal mix | Fruit juice Black curran t | Fruit juice Apple | Yoghurt drink | Yoghurt | Sour milk |
|---|---|---|---|---|---|---|---|---|---|
| LMG P-17806, day 1 | 53 | 56 | 50 | 45 | 46 | 53 | 67 | 70 | 74 |
| LMG P-17806, day 21 | 55 | 51 | 52 | 39 | 13 | 35 | 55 | 75 | 63 |
| NCFB 1748, day 1 | 50 | 53 | 56 | 43 | 50 | 55 | 57 | 56 | 60 |
| NCFB 1748, day 21 | 42 | 43 | 43 | 37 | 3 | 30 | 32 | 42 | 44 |
| Bb12, day 1 | 59 | 60 | 65 | 65 | 62 | 67 | 62 | 73 | 68 |
| Bb 12, day 21 | 49 | 47 | 60 | 31 | 14 | 49 | 51 | 68 | 60 |

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**[0028]**

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**Patentkrav**

1. Anvendelse af mindst en probiotisk bakterie til reduktion af vægtforøgelse, hvor den mindst ene probiotiske bakterie er valgt fra gruppen bestående af *Lactobacillus casei* F19 (LMG P-17806), *Lactobacillus acidophilus* (NCFB 1748) og *Bifidobacterium lactis* Bb12 (DSM 15954) med det forbehold at anvendelsen af denne bakterie ikke er til terapeutisk behandling.

2. Anvendelse af mindst en probiotisk bakterie til forøgelse af mæthed, hvor den mindst ene probiotiske bakterie er valgt fra gruppen bestående af *Lactobacillus casei* F19 (LMG P-17806), *Lactobacillus acidophilus* (NCFB 1748), *Bifidobacterium lactis* Bb12 (DSM 15954) med det forbehold at anvendelsen af denne bakterie ikke er til terapeutisk behandling.

3. Anvendelse af mindst en probiotisk bakterie til forlængelse af mæthed, hvor den mindst ene probiotiske bakterie er valgt fra gruppen bestående af *Lactobacillus casei* F19 (LMG P-17806), *Lactobacillus acidophilus* (NCFB 1748), *Bifidobacterium lactis* Bb12 (DSM 15954) med det forbehold at anvendelsen af denne bakterie ikke er til terapeutisk behandling.

4. Anvendelse af mindst en probiotisk bakterie til reduktion af fødeindtagelse, hvor den mindst ene probiotiske bakterie er valgt fra gruppen bestående af *Lactobacillus casei* F19 (LMG P-17806), *Lactobacillus acidophilus* (NCFB 1748), *Bifidobacterium lactis* Bb12 (DSM 15954) med det forbehold at anvendelsen af denne bakterie ikke er til terapeutisk behandling.

5. Anvendelse af mindst en probiotisk bakterie til reduktion af fedtdeponering, hvor den mindst ene probiotiske bakterie er valgt fra gruppen bestående af *Lactobacillus casei* F19 (LMG P-17806), *Lactobacillus acidophilus* (NCFB 1748) og *Bifidobacterium lactis* Bb12 (DSM 15954) med det forbehold at anvendelsen af denne bakterie ikke er til terapeutisk behandling.

6. Anvendelse af mindst en probiotisk bakterie til reduktion af deponering af abdominal fedt, hvor den mindst ene probiotiske bakterie er valgt fra gruppen bestående af *Lactobacillus casei* F19 (LMG P-17806), *Lactobacillus acidophilus*

(NCFB 1748) og *Bifidobacterium lactis* Bb12 (DSM 15954) med det forbehold at anvendelsen af denne bakterie ikke er til terapeutisk behandling.

7. Anvendelse ifølge et hvilket som helst af kravene 1-6, kendetegnet ved at den probiotiske bakterie anvendes til fremstilling af et produkt valgt fra gruppen bestående af et produkt baseret på mælk, et produkt baseret på ceralier og et produkt baseret på frugt eller sammensætninger afledt deraf.

8. Anvendelse ifølge et hvilket som helst af kravene 1-7, kendetegnet ved at den probiotiske bakterie anvendes som et koncentrat f.eks. lyofiliseret.

9. Anvendelse ifølge et hvilket som helst af kravene 1-8, kendetegnet ved at den probiotiske bakterie er indbefattet i en kapsel.

10. Anvendelse ifølge krav 9, kendetegnet ved at kapslen indeholder en dosis af probiotisk bakterie på mellem $1x10^8$ CFU til $2,5x10^8$ CFU.

11. Anvendelse ifølge et hvilket som helst af kravene 1-10, hvor det probiotiske produkt er valgt fra gruppen bestående af et fødevareprodukt, et foderprodukt, et kosttilskud, et naturlægemiddel og en farmaceutisk aktiv formulering.

# DRAWINGS

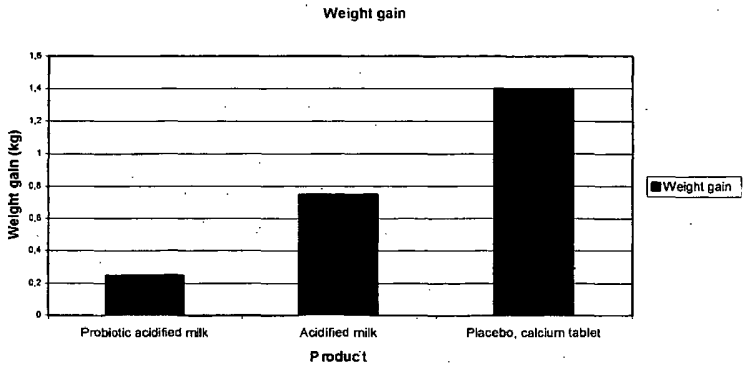


Figure 1. Effect of probiotics on weight gain in humans after 4 weeks of treatment.

| Product | Weight gain (kg) | Sd | Significance* |
|---|---|---|---|
| Probiotic acidified milk | 0,25 | 0,25 | a |
| Acidified milk | 0,75 | 0,3 | a, b |
| Placebo, calcium tablet | 1,4 | 0,35 | B |

* Different letters=significant difference $p<0.05$

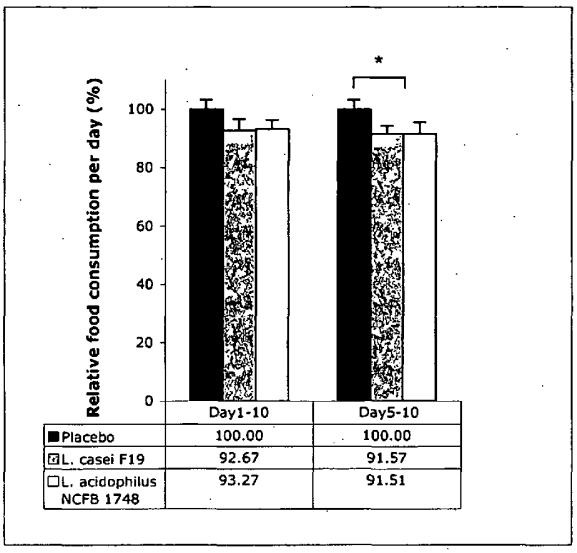


Figure 2. Daily food intake in two groups of mice receiving probiotic bacteria *Lactobacillus casei* F19, LMG P-17806 or *Lactobacillus acidophilus* NCFB 1748 in acidified milk compared to the placebo group of mice receiving acidified milk only. Food consumption is estimated for the whole period of product administration (day 1-10) and after the period of adjustment (day 5-10). The results are expressed as the percentage compared to the placebo group of mice. The statistical significance is determined by two-tailed unpaired Student´s t-tests. * represents $p \leq 0.05$.

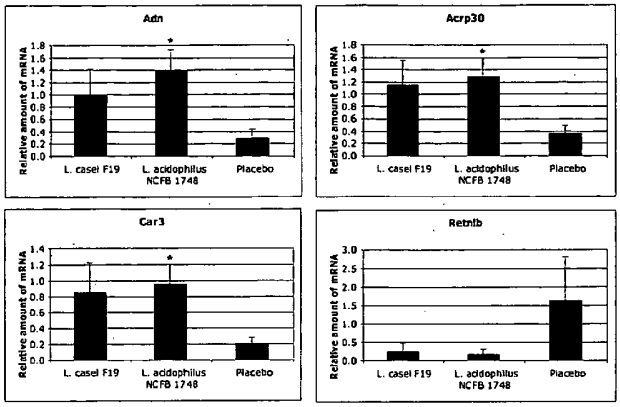


Figure 3. Expression of adipsin (Adn), adiponectin (Acrp30), carbonic anhydrase 3 (Car3), and resistin like beta (Retnlb) genes as quantified by RT-PCR in germ free mice. The two test groups of mice received probiotic bacteria *Lactobacillus casei* F19, LMG P-17806 or *Lactobacillus acidophilus* NCFB 1748 in acidified milk while the placebo group of mice received acidified milk only. Each bar represents the mean ± SEM. The statistical significance is determined by two-tailed unpaired Student´s t-tests. * represents $p \leq 0.05$. n = 6 for the two probiotic groups and n = 5 for the placebo group.

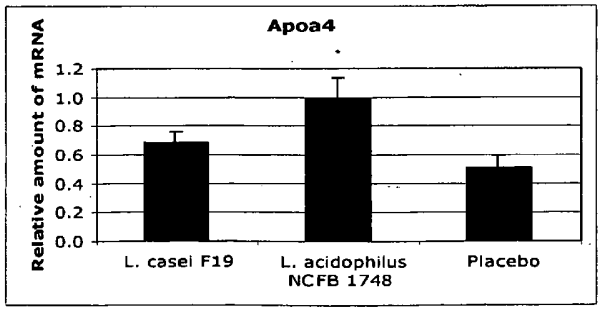


Figure 4. Expression of apolipoprotein A-IV (Apoa4) gene as quantified by RT-PCR in normal microflora colonized mice. The two test groups of mice received probiotic bacteria *Lactobacillus casei* F19, LMG P- 17806 or *Lactobacillus acidophilus* NCFB 1748 in acidified milk while the placebo group of mice received acidified milk only. Each bar represents the mean ± SEM. The statistical significance is determined by two-tailed unpaired Student´s t-tests. * represents $p \leq 0.05$. n = 6 for the two probiotic groups and n = 4 for the placebo group.