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Probiotics, Functional and Baby Foods
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Research and development new symbiotic product and its clinical effect

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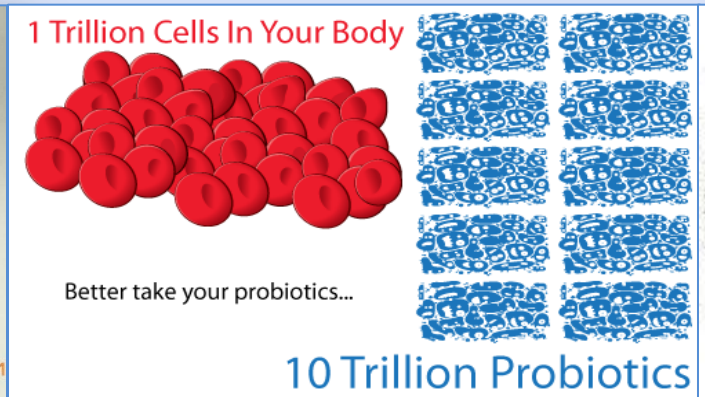


First stage: To study the gut microbiome

To determine gut microbiome types and to compare microbial composition of the gut microbiome in different aged Kazakhstani

Subjects were divided into 3 groups:

I group - to 44 years

II group – 50 – 70 years

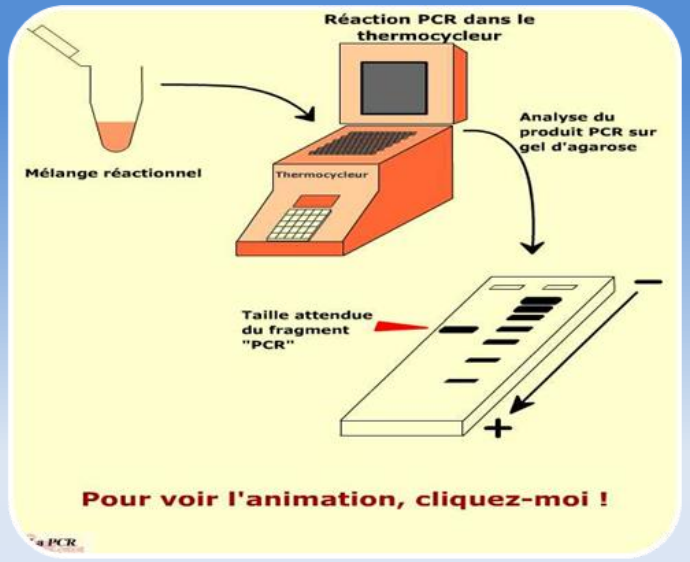
III group - 90 years and above

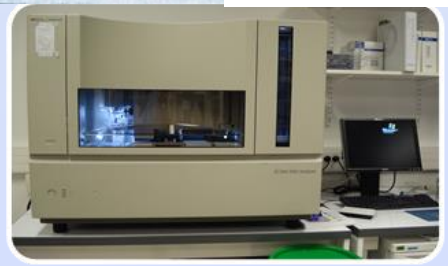
Patients signed informed consent on the procedure, screening and collection of samples

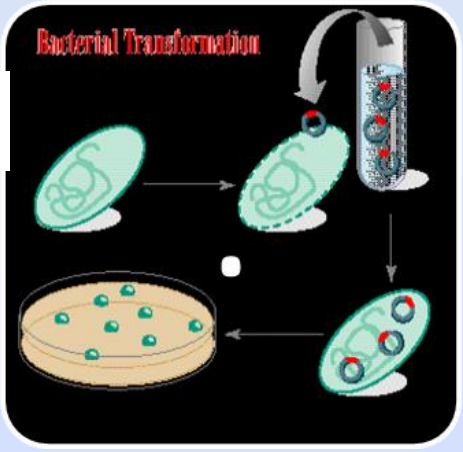
Questionnaire

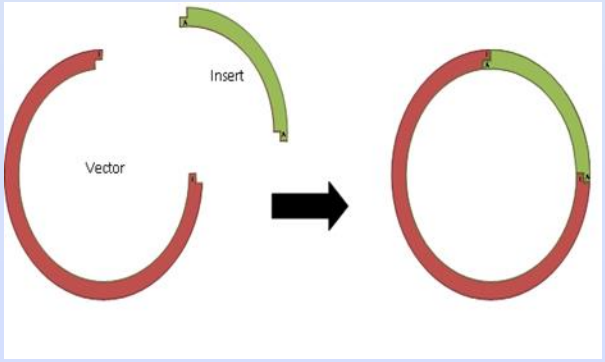
1. General information (date and place of birth, weight, growth, gender, smoking habits, diet, nationality)
2. Information about diseases of gastro-intestinal tract (IBD, *H. pylori*, dyspepsia, etc.)
3. General clinical information (blood group, antibiotics (last 2 months), birth, nutrition in infancy, stool frequency, regularity of the cycle, etc.)
4. Information about the diet (alcohol, probiotics, vegetables, dairy products)

16S rRNA approach



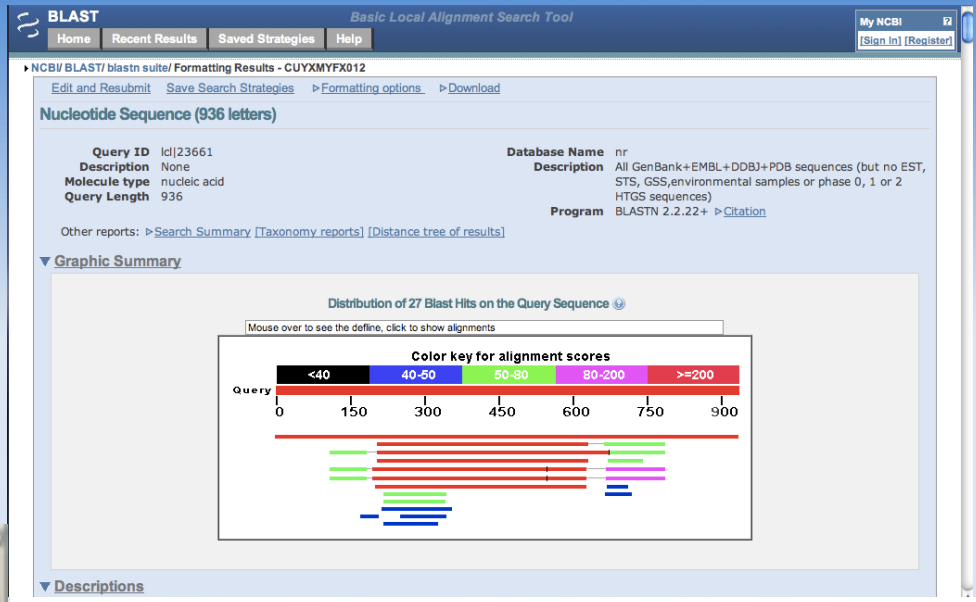


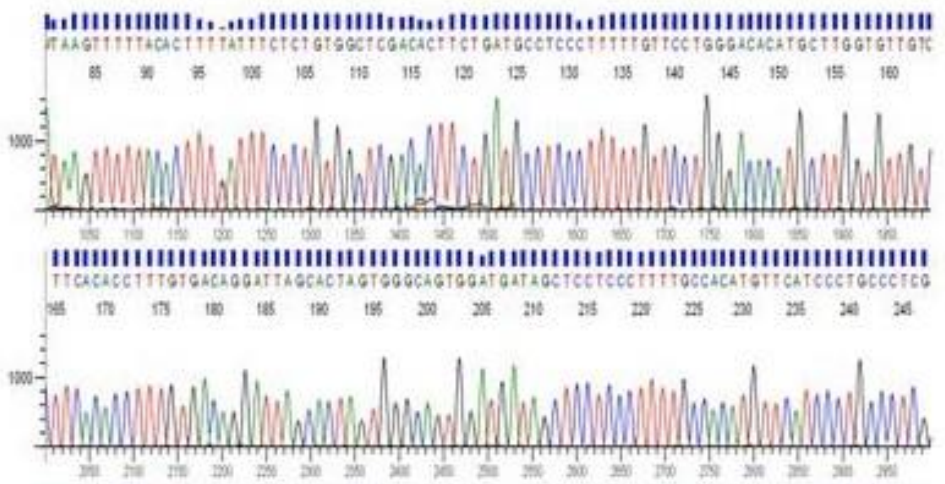




Data processing

Sequence Scanner v 1.0

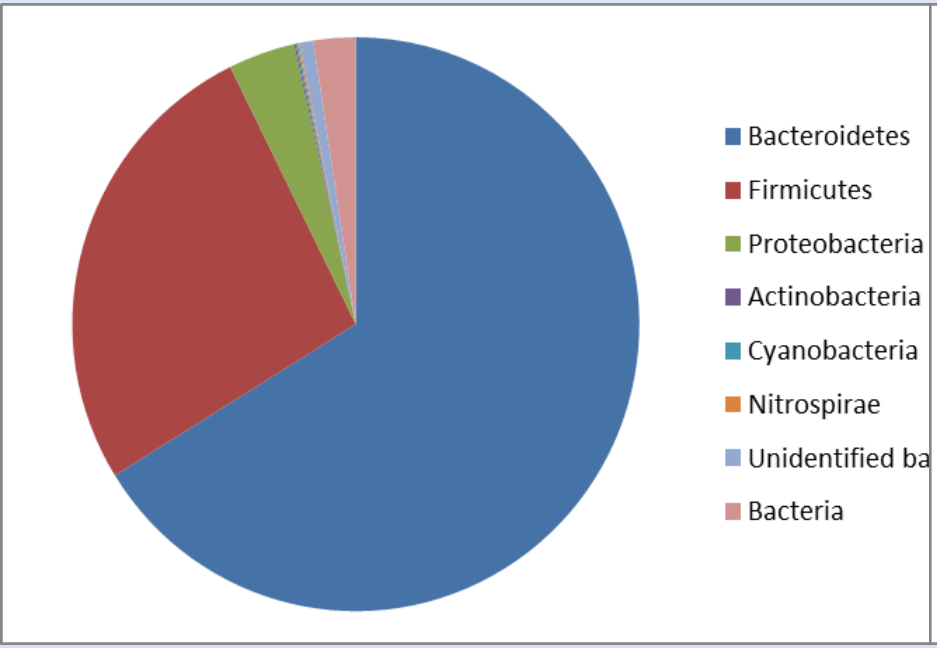


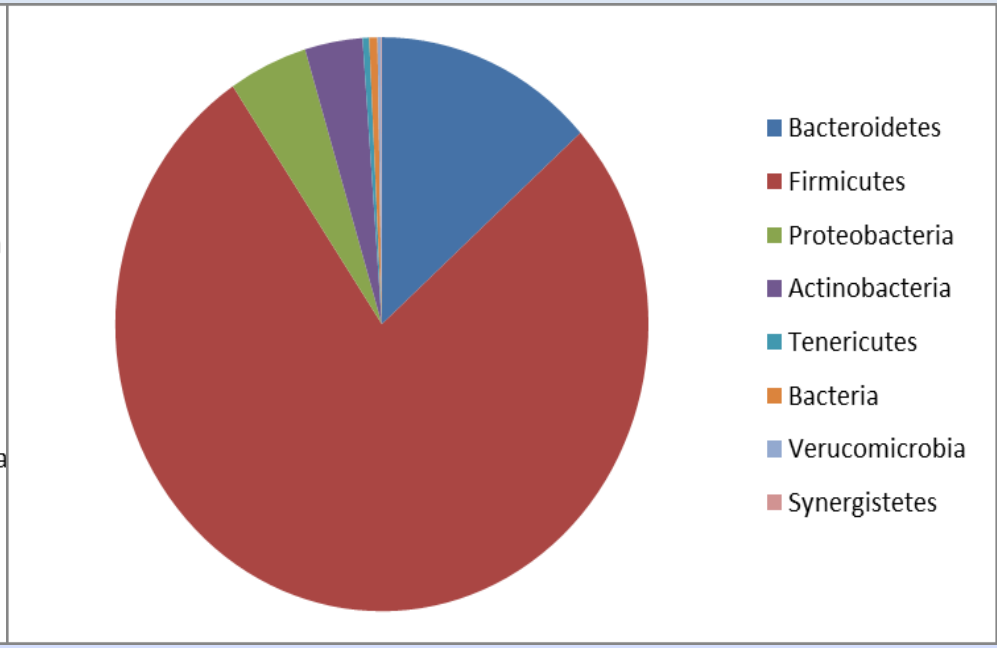


Results

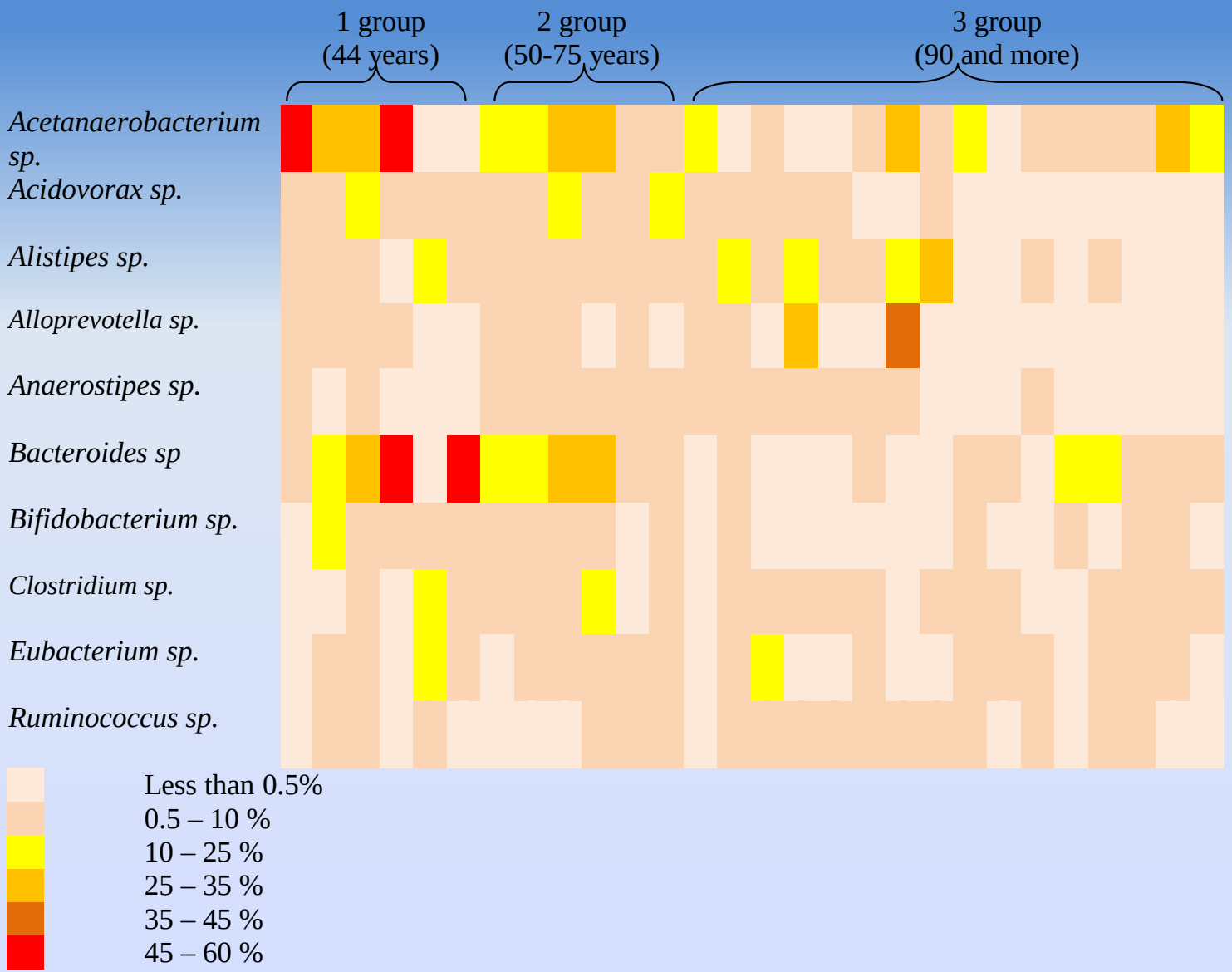
It was observed that in all groups independently on age prevail two enterotypes

Bacteroides enterotype ***Firmicutes enterotype***

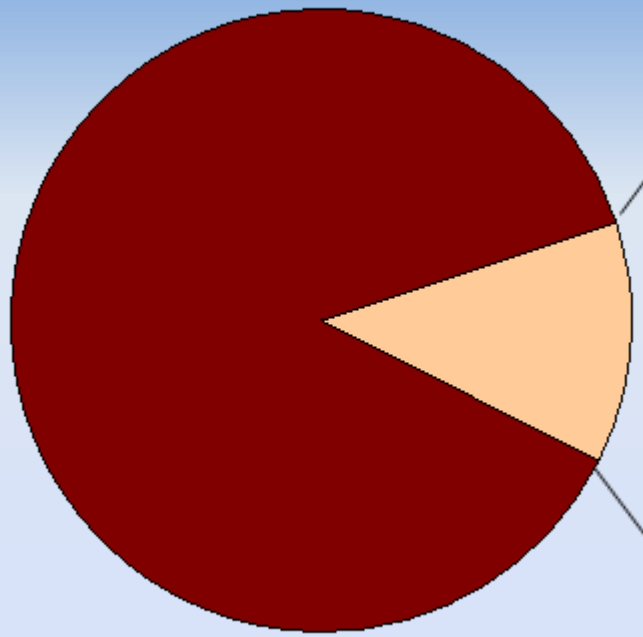




Most numerous members of the gut microbiome

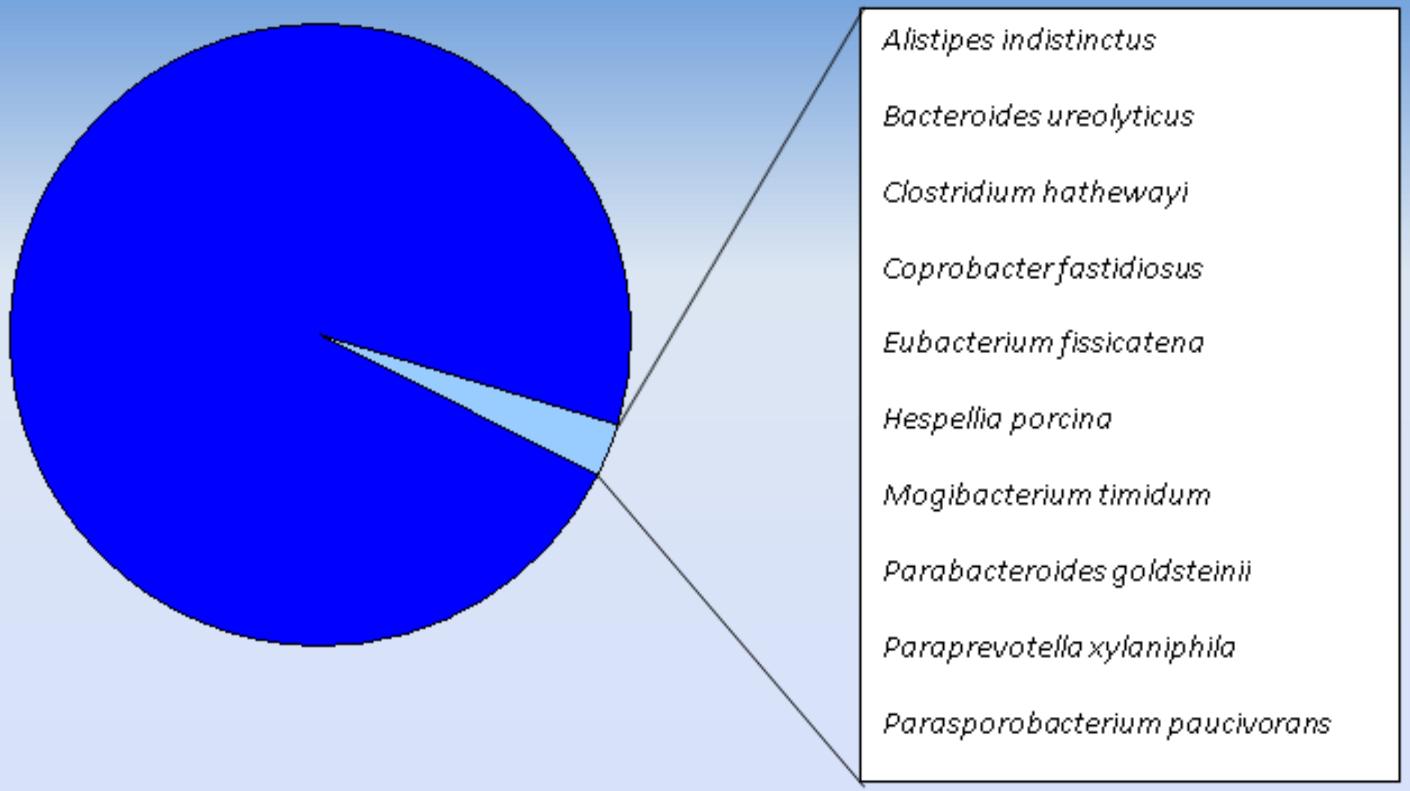


Species' variety in subjects of 30-70 years



- *Bacteroides xylanisolvens*
- *Lactonifactor longoviformis*
- *Akkermansia muciniphila*
- *Alloprevotella rava*
- *Catenibacterium mitsuokai*
- *Clostridium cellulolyticum*
- *Coprococcus catus*
- *Eubacterium biforme*
- *Lactobacillus delbrueckii*
- *Lactobacillus mucosae*
- *Lactobacillus plantarum*
- *Lactobacillus rogosae*
- *Leuconostoc mesenteroides*
- *Prevotella salivae*
- *Pyramidobacter piscolens*
- *Citrobacter diversus*
- *Clostridium indolis*
- *Coprococcus comes*
- *Salmonella typhi*
- *Sarcina ventriculi*
- *Prevotella melaninogenica*

Species' variety in subjects of 90 years and above



Functional characteristics of species

Functional characteristics	30-70 years	90 years and above
Plant substrates degrading bacteria (cellulose)	+	+
Mucin-degrading bacteria	+	-
Butyrate-producing bacteria	+	-
Carbohydrates converting bacteria (production of organic acids, acetate, ethanol,carbon dioxide, hydrogen)	+	+
Protein and starch degrading bacteria	+	-
Mucus-binding bacteria	+	-
Infections causing pathogens	+	+

1. Two enterotypes of the gut microbiome: *Bacteroidetes* and *Firmicutes* independently on age of subjects

2. Significant differences in bacterial species between all 3 groups according to age

3. The richest diversity occured in younger individuals and decreased in the elderly. It can be explained by the age factor

4. The percentage of bifidobacteria and lactobacilli is low in all research groups

5. More research is needed for better understanding of compositional structure of the gut microbiome of Kazakhstani population

Synbiotic "HƏP" NAR

A formula and a production technology were developed and a pilot batch of a symbiotic bio-product "HƏP" was produced (Dairy plant Astana – onim)

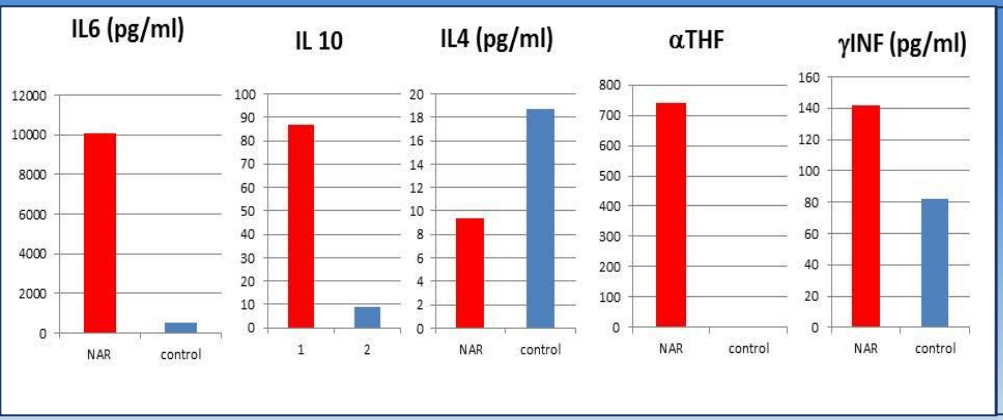
Probiotic component
Prebiotic - inulin
Plant fiber – pectin
Fish collagen
Milk

Consortium content:
Lactobacillus plantarum;
Lactobacillus fermentum;
Lactobacillus acidophilus;
Bifidobacterium longum;
Bifidobacterium bifidum

NAR has been tested in JSC "Kazakh Academy of Nutrition"

The probiotic component is deposited in the National Depository of industrial microorganisms.

Synbiotic “HƏP” NAR: properties



Synbiotic activates primary immune response.

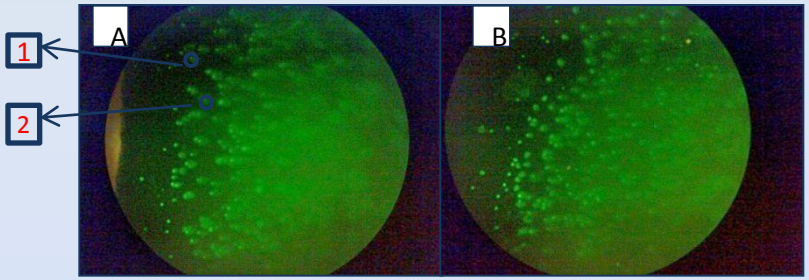
First of all, synbiotic coming into an organism interacts with colon epithelium cells. Reaction of macrophages and dendritic cells of lamina propria is induced, and as a result induction of IL-6 (10080,0±238,0 pg/ml)

IL-6 promotes clonal expansion of IgA-lymphocytes, increase in quantity of IgA-producing cells and passing them through plasmatic cells in lamina propria of intestinal mucous (64,7±0,7).

In the analysis of induced cytokines, we observed significant increase in TNF-α and IFN-γ and regulatory cytokine IL-10

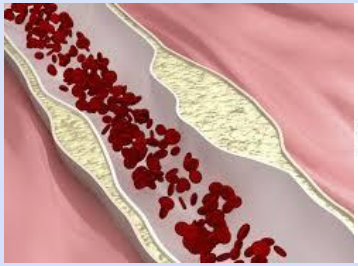
Induction of γ IFN along with increase in production of IL-12 (228,9±17,8) inhibits producing IL4 due to activation of signal pathway of NF-Kb and STAT

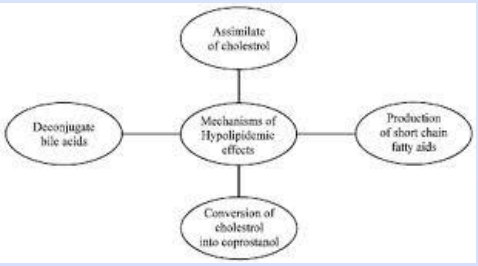
The listed changes bring Th1/Th2 balance towards Th1



Antioxidant activity

Total antioxidant activity of the synbiotic product 67.4 mmol/ml
SOD is 1.42 U/mg
Glutathione reductase 0.06 U/ml
Damage index–0.60

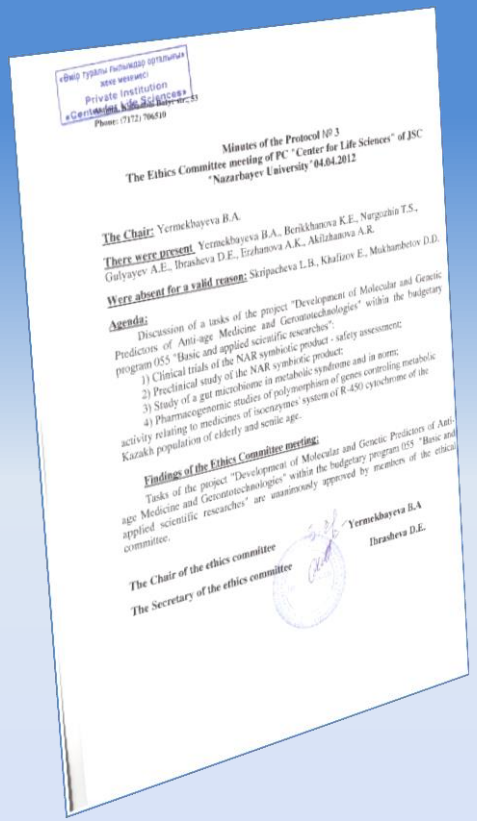




Cholesterol lowering properties

in MRS broth medium 22 %
in the presence of 0.2 % bovine bile 50 %.

Preclinical and clinical study



Minutes of the Protocol № 3
The Ethics Committee meeting of PC "Center for Life Sciences" of JSC "Nazarbayev University" 04.04.2012

The Chair: Yermekbayeva B.A.

There were present: Yermekbayeva B.A., Berikkhanova K.E., Nurgazhin T.S., Gulyayev A.E., Ibrasheva D.E., Erzhanova A.K., Akilzhanova A.R.

Were absent for a valid reason: Skripacheva L.B., Khafizov E., Mukhanbetov D.D.

Agenda:

Discussion of a tasks of the project "Development of Molecular and Genetic Predictors of Anti-age Medicine and Geroantotechnologies" within the budgetary program 055 "Basic and applied scientific researches":

1) Clinical trials of the NAR symbiotic product - safety assessment;
2) Preclinical study of the NAR symbiotic product;
3) Study of a gut microbiome in metabolic syndrome and in norm;
4) Pharmacogenomic studies of polymorphism of genes controling metabolic activity relating to medicines of isoenzymes system of R-450 cytochrome of the Kazakh population of elderly and senile age.

Findings of the Ethics Committee meeting:

Tasks of the project "Development of Molecular and Genetic Predictors of Anti-age Medicine and Geroantotechnologies" within the budgetary program 055 "Basic and applied scientific researches" are unanimously approved by members of the ethical committee.

The Chair of the ethics committee — Yermekbayeva B.A

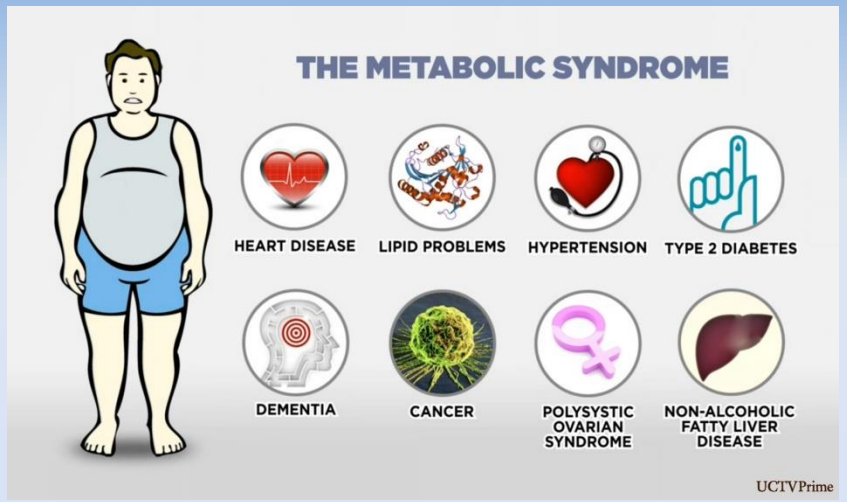
The Secretary of the ethics committee — Ibrasheva D.E.

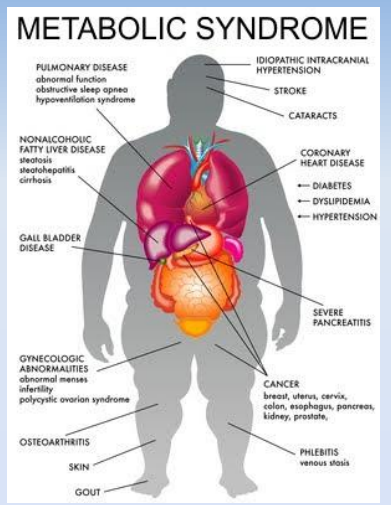
Group	Sample collection			total
	Isolation period (5 days)	+ 14 days od experiment	14 days after feeding post effect	
Control 1 (standart feeding)	5	5	5	15
Control 2 (standart+prebiotic)	5	5	5	15
Exp 1 1 dose NAR	5	5	5	15
Exp 2 probiotic component 1 dose	5	5	5	15
Exp 3 2 dose NAR	5	5	5	15
Exp 4 probiotic component 2 dose	5	5	5	15
Total	30	30	30	90

1 stage of clinical trials -70 patients: 35m and 35 f
Recruiting and Clinical - laboratory investigation were performed in two different Medical Center

Introduction

The aim of this study is to investigate the efficacy of a synbiotic in treatment of adult with metabolic syndrome





Metabolic syndrome is an umbrella name for a collection of health risk factors — *high blood pressure, high blood sugar, high triglycerides, low HDL cholesterol, high LDL cholesterol, and excess belly fat*.

Study design

recruiting volunteers

initial clinical and laboratory examination

sampling: blood and stool

survey

Placebo group

Synbiotic group

Duration of synbiotic/placebo taking is 3 months

Dairy plant Astana - onim

repeated clinical and laboratory examination

repeated collection of stool samples

Data analysis

Metagenome analysis of gut microbiome in normal and with metabolic syndrome, before and after receiving the symbiotic product NAR

SNP analysis on markers associated with metabolic syndrome

Randomisation and blinding

Random selection of patients from the database of Medical Center of President's Affairs Administration of Republic of Kazakhstan

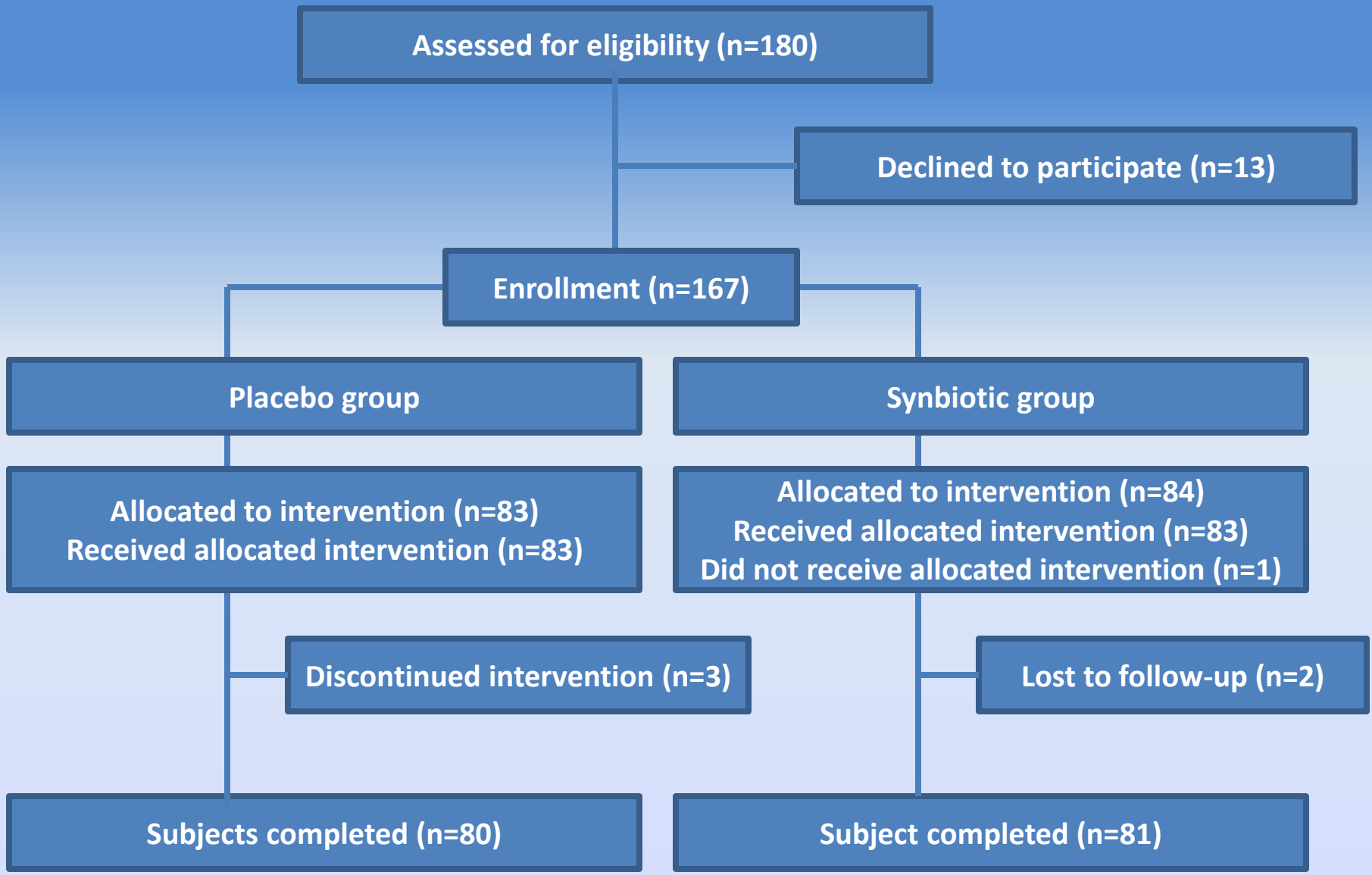
The physician-researchers responsible for enrolling patients

Study leader is responsible for the distribution of participants by placebo-symbiotic groups

Placebo and synbiotic provided in identical packages

The physician-researchers collecting the reporting forms and performing the patients investigations were blind to the patient's treatment assignment

Participants and subject eligibility



Participants and subject eligibility

We enrolled 161 adult

92 patients with metabolic syndrome

key criteria for inclusion:

1. No history of the use of probiotics or antibiotic for 3 months
2. Blood pressure: = 130/90 mmHg
3. Raised fasting plasma glucose (FPG): >100 mg/dL (5.6 mmol/L), or previously diagnosed type 2 diabetes
4. DyslipidemiaTG: = 1.695 mmol/L; HDL-C = 0.9 mmol/L (male), = 1.0 mmol/L (female)
5. Central obesity: waist:hip ratio > 0.90 (male); > 0.85 (female), or body mass index > 30 kg/m2

69 patients with no symptoms of metabolic syndrome

key criteria for inclusion:

1. No history of the use of probiotics or antibiotic for 3 months
2. healthy on items 2-5 above for the first group

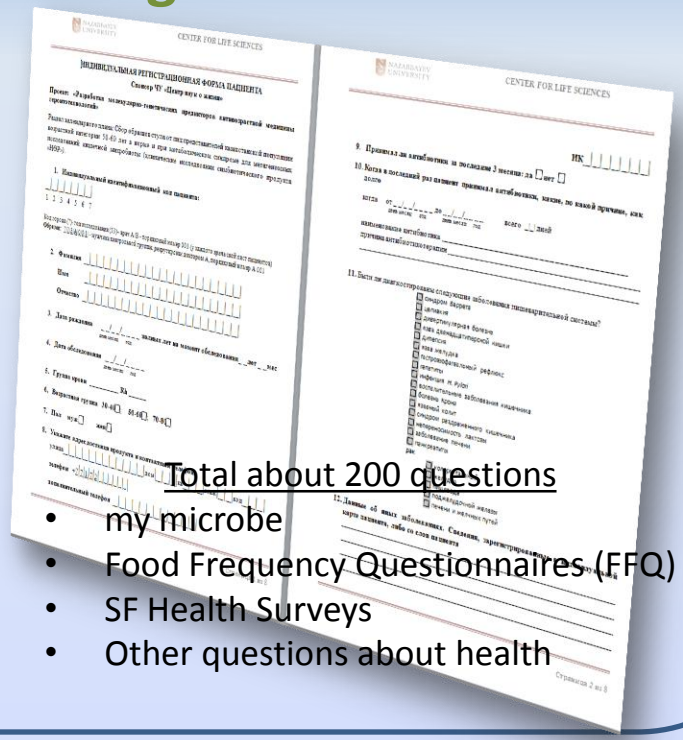
Participants and subject eligibility

Age categories

30-39; 40-49; 50-59; 60-69; 70-79

Before the start of the study, all patients were examined comprehensively, including the following list of clinical and laboratory examination

- family history
- information on the use of antibiotics
- anthropometry
- characterization of the cardiovascular system
- characterization of the stool and its frequency
- characterization of immune status
- data for the blood glucose, ApoE, C-reactive protein, total cholesterol, HDL, LDL

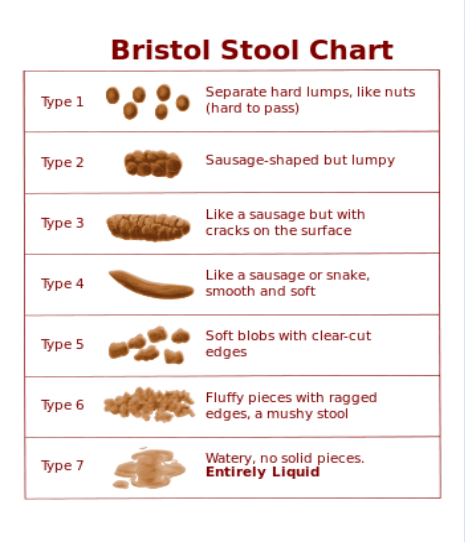


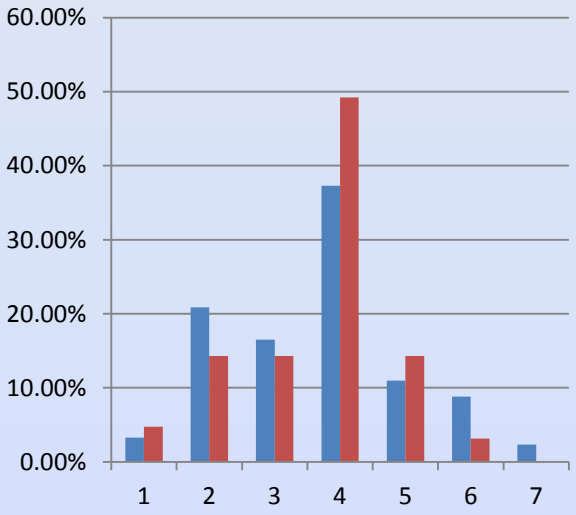
Total about 200 questions

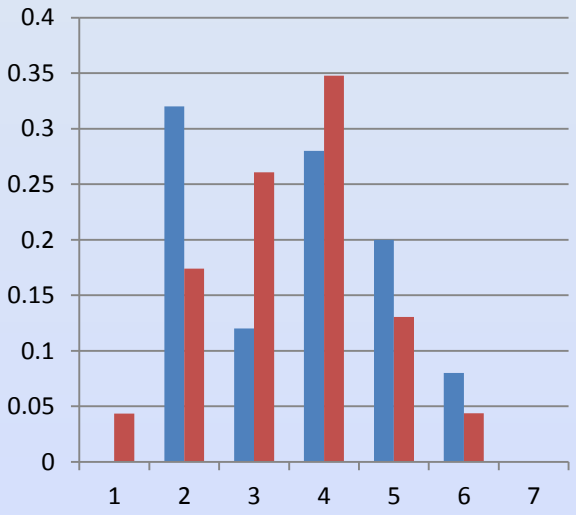
- my microbe
- Food Frequency Questionnaires (FFQ)
- SF Health Surveys
- Other questions about health

Effect of synbiotic on function of Digestive tract

average stool frequency	Synbiotic		Placebo	
	before	after	before	after
>twice daily	14.9%	21.9%	15.3%	7.1%
once daily	14.2%	65.6%	3.8%	10.7%
once every 2 days	70%	12.5%	76.9%	82%
<once every 2 days	0.9%	0	4%	0.2







Clinical outcome

BMI Categories:
Underweight = <18.5
Normal weight = 18.5–24.9
Overweight = 25–29.9
Obesity = BMI of 30 or greater

34.3% no change in BMI
66.7% BMI decreased by 0.3 - 3.8

713B053	158	62.5	53	25.03605	21.23057
713A038	173	77	73.5	25.72756	24.55812
713B065	164	88	86	32.71862	31.97501

Waistline

synbiotic
42.86% - a decrease by 1-3 cm
40% - no changes
17.14% - an increase by 1 - 2 cm

placebo
14,2% - a decrease by 0,5-1 cm
63% - no changes
22,8% - an increase by 0,5 – 4 cm

Effect of synbiotics on Lipid Profile

mmol/L

Data	Synbiotic		Placebo	
	before	after	before	after
Total Blood Cholesterol	4.92±0.25	4.58±0.23	5.02±0.06	4.97±0.07
LDL-Cholesterol	3.37±0.28	2.98±0.27	2.94±0.08	3.08±0.1
HDL-Cholesterol	1.09±0.08	1.20±0.07	1.13±0.08	1.27±0.12
TG	1.59±0.07	1.50±0.06	1.61±0.06	1.73±0.09

Effect of synbiotic on Inflammatory Markers

C-reactive protein (CRP)

Before 3,162±0,122 mg/L
After 1,987±0,124 mg/L

Low Risk: less than 1.0 mg/L
Average risk: 1.0 to 3.0 mg/L
High risk: above 3.0 mg/L

The erythrocyte sedimentation rate (ESR)

Before 11,19±1,00 mm/hour
After 9,88±0,85 mm/hour

Leukocytes (White Blood Cells)

Before 6,75±0,46 10^9/L
After 6,16±0,39 10^9/L

Effect of synbiotic on HB

Data	before	after
average	134±3	138±3
min	82	104
max	165	177

Bifidobacterium contributes to increasing permeability through the intestinal wall of the ions of calcium, iron, vitamin D.

Bioeng Bugs. May 1, 2012; 3(3): 157–167.

Conclusion

Synbiotic NAR

- ✓ Improves motor function of the intestine
- ✓ Improves metabolism of macroorganism
- ✓ Lowers Blood Cholesterol
- ✓ Possesses anti - Inflammatory effects

synbiotic is proposed as an addition to the basic treatment, not an alternative to main therapy

- In the study group the percentage of colds were significantly lower than in placebo group
- the majority of patients reported an improvement in abdominal discomfort

Eat Probiotics foods, live healthy life

Acknowledgment

Center for life sciences

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Baiskhanova Dinara

Prof. Francesco Marotta
Regenera Research Group

Medical Center of President's Affairs Administration of Republic of Kazakhstan

USM NU

Dairy plant Astana - onim
Usenov KZh - collaborator of dairy plant
Baimenova BA - collaborator of dairy plant
Kozhentaeva ZT - dairy technologist
Zhabagenova A - microbiologist at the milk plant

Thank you for your attention!

So…..

Eat the “good bugs” every day…….

Invite them in……..

You will find they make very friendly houseguests.