Locally produced probiotic yoghurt enhances health and wealth in East Africa

The results of a randomized double blind nutritional trial among 200 Ugandan school children

Wilbert Sybesma and Remco Kort

Probiota - Barcelona February 6-8 2023

Yoba for Life

Locally produced probiotic yoghurt enhances health and wealth in East Africa.

The results of a randomized double blind nutritional trial among 200 Ugandan school children

- **Introduction Yoba for Life**
  - What, Why, How
  - Probiotic starter culture
  - School feeding program
  - Previous trials conducted with Yoba probiotic yoghurt
- **Set up and results of nutritional trial**
  - Study design in a real life setting
  - RT and Skin infection incidence, survival curves and odds ratios
  - Bacterial load in stool samples
  - Urine metabolites
  - Saliva imune biomarkers
  - Antropometric data
- **Conclusion and Recommendations**
- **How to make your own Yoba yoghurt**
- **Acknowledgements**

Yoba for Life Foundation is a non-profit organization recognized by Dutch state as a Public Benevolent Institution

Disclaimer: Sybesma and Kort founded the Yoba for Life Foundation in 2009. Their work for the foundation is unpaid.

The reason why: Improving health and wealth by locally produced probiotic fermented food for people living in resource poor communities

Background:

- Africa - ancient history with fermented foods
- Sub-Saharan Africa - world's region with the highest % of chronically malnourished people and child mortality
- Mainly in the informal sector, locally produced probiotic fermented foods could contribute to increasing quality of life by providing business opportunities and a healthy product
- Western probiotic foods are not affordable for people living in resource poor communities

Lacticaseibacillus rhamnosus yoba 2012: World's first generic probiotic bacterium

Forum: Science & Society

Probiotics for every body

Remco Kort[1,2,3] and Wilbert Sybesma[1]

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[2] TNO Microbiology and Systems Biology, Utrechtseweg 48, 3704 HE, Zeist, The Netherlands
[3] VU University Amsterdam, Molecular Cell Physiology, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands

In this perspective we report for the first time the concept of 'generic probiotics', as a practical solution to create access to probiotics for people in the developing world. Analogous to generic drugs, we reason that patent-expired probiotics are free to be used by others. In this context we discuss the importance of probiotic genome stability for linking health claims of the mother strain to the generic strain.

To date, hundreds of studies on probiotic functionality and their impact on prevention and treatment of gastrointestinal diseases have been reported. One of the most widely described probiotic strains is *Lactobacillus rhamnosus* GG, for which the first publication on the efficacy against *Clostridium difficile* colitis appeared 25 years ago [1]. A recently conducted meta-analysis has concluded that probiotics, including *L. rhamnosus* GG, are generally beneficial in treatment and prevention of gastrointestinal disease, considering that different probiotic strains show different efficacy across these diseases [2].

At present, probiotic products are mainly available in the western world (including Japan and Oceania), where intestinal health is relatively good. However, in resource-disadvantaged countries, poor hygienic conditions, malnutrition, and acute and chronic enteric infections fre-

the introduction of generic probiotics in Africa through existing local production facilities (http://www.yoba4life.com). Accordingly, the local product portfolio of fermented dairy is extended with addition of a probiotic product, whereas related production costs are kept to a minimum. The net effect is that both the health of local communities is improved and some economic benefits are accrued based upon a social business model.

Generic drugs

A generic drug is a pharmaceutical product that is manufactured without a license and marketed after the expiry date of the related patents or other exclusive rights, according to a definition adapted from the WHO [http://www.who.int/trade/glossary/story034/en/index.html]. Generic drugs are frequently as effective as, but much cheaper than, brand-name drugs. Well-known examples are fluoxetine and acetaminophen of which patents expired in 2001 and 1963, respectively, and which are sold globally under many different brand names as an antidepressant and pain and fever reliever, respectively.

Unlike pharmaceuticals, bacteria cannot be synthesized *de novo*, even though recently the construction has been

DOI: 10.1016/j.tibtech.2012.09.002

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FOOD MICROBIOLOGY
Volume 79 Issue 7
https://doi.org/10.1128/AEM.03566-12

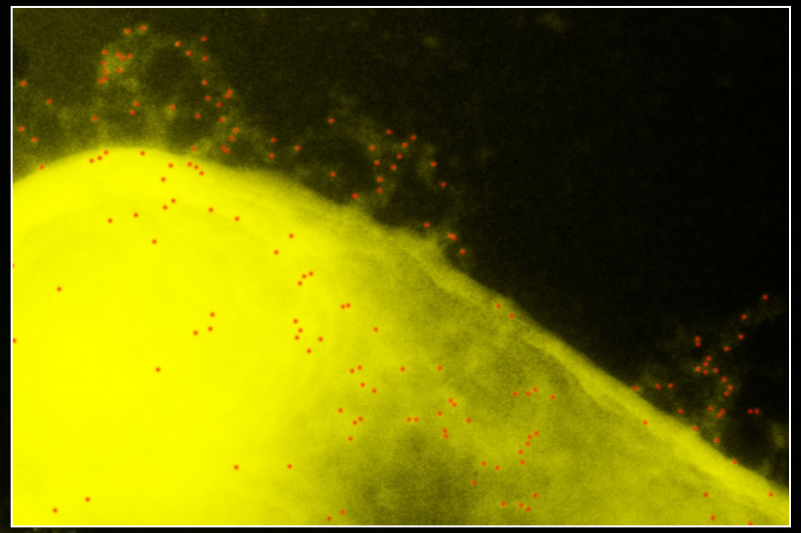
Genome Instability in *Lactobacillus rhamnosus* GG

Wilbert Sybesma[a], Douwe Molenaar[b], Wilfred van IJcken[c], Koen Venema[d], Remco Kort[a,b,e]

[a]Yoba for Life foundation, Amsterdam, The Netherlands
[b]VU University Amsterdam, Molecular Cell Physiology, Amsterdam, The Netherlands
[c]Erasmus MC, Center for Biomics, Rotterdam, The Netherlands
[d]TNO Pharmacokinetics and Human Studies, Zeist, The Netherlands
[e]TNO Microbiology and Systems Biology, Zeist, The Netherlands

ABSTRACT We describe here a comparative genome analysis of three dairy product isolates of *Lactobacillus rhamnosus* GG (LGG) and the ATCC 53103 reference strain to the published genome sequence of *L. rhamnosus* GG. The analysis showed that in two of three isolates, major DNA segments were missing from the genomic islands LGGISL1,2. The deleted DNA segments consist of 34 genes in one isolate and 84 genes in the other and are flanked by identical insertion elements. Among the missing genes are the *spaCBA* genes, which encode pilin subunits involved in adhesion to mucus and persistence of the strains in the human intestinal tract. Subsequent quantitative PCR analyses of six commercial probiotic products confirmed that two more products contain a heterogeneous population of *L. rhamnosus* GG variants, including genotypes with or without *spaC*. These results underline the relevance for quality assurance and control measures targeting genome stability in probiotic strains and justify research assessing the effect of genetic rearrangements in probiotics on the outcome of *in vitro* and *in vivo* efficacy studies.

DOI: 10.1128/AEM.03566-12



Lacticaseibacillus rhamnosus yoba 2012 showing pili with mucin binding protein illustrated by immuno-gold labeling

Yoba for Life INNOVATION

A tailor-made probiotic starter culture for small-scale producers in resource-poor settings

- 1 gram of starter culture costs 0.90 USD.
- To produce 100 liters of probiotic yoghurt

Kort *et al. Microb Cell Fact* (2015) 14:195
DOI 10.1186/s12934-015-0370-x

MICROBIAL CELL FACTORIES

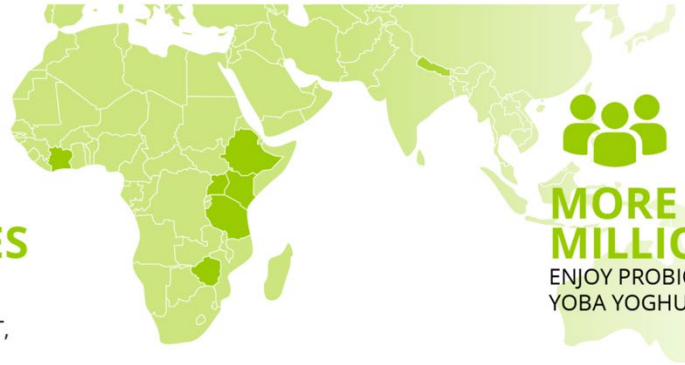
RESEARCH Open Access

A novel consortium of *Lactobacillus rhamnosus* and *Streptococcus thermophilus* for increased access to functional fermented foods

Remco Kort[1,2,3,4*], Nieke Westerik[1,3], L. Mariela Serrano[5], François P. Douillard[6], Willi Gottstein[3], Ivan M. Mukisa[7], Coosje J. Tuijn[1], Lisa Basten[5], Bert Hafkamp[5], Wilco C. Meijer[5], Bas Teusink[3], Willem M. de Vos[6,8,9], Gregor Reid[10,11] and Wilbert Sybesma[1]

Yoba for Life today

THE YOBA FOR LIFE CONCEPT IS DESIGNED TO ADDRESS COMMON HEALTH PROBLEMS IN RESOURCE-POOR COUNTRIES



7 COUNTRIES
UGANDA, TANZANIA, ETHIOPIA, KENYA, ZIMBABWE, IVORY COAST, NEPAL

MORE THAN MILLION
ENJOY PROBIOTIC YOBA YOGHURT

500 THOUSAND LITERS
PROBOTIC YOBA YOGHURT PRODUCED PER MONTH

300 PROBIOTIC
PRODUCING PROBIOTIC YOBA YOGHURT

1 QUADRILLION BACTERIA
OF LACTOBACILLUS RHAMNOSUS YOBA ARE CONSUMED PER DAY

Local branding

https://youtu.be/sj0OFtkUzec

https://youtu.be/iYtzi4pkdsM

Pre-primary Yoba Probiotic School Feeding Program

>30,000 children in the Mbarara region in Western Uganda

Prior to the present study, three nutritional trials have been conducted with Yoba probiotic yoghurt in Uganda and Ivory Coast

1. Pilot study Uganda, skin rashes, open label, 245 children

challenges MDPI

Article

Improving Health and Wealth by Introduction of an Affordable Bacterial Starter Culture for Probiotic Yoghurt Production in Uganda

Nieke Westerik [1,2], Alex Paul Wacoo [1,2], Esther Anyimo [3], William Matovu [3], Gregor Reid [4,5], Remco Kort [1,2,6,*] and Wilbert Sybesma [1,*]

2. Semi-randomized controlled trial, 3 arms, 251 children

Psychoneuroendocrinology 129 (2021) 105255

Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/psyneuen

ELSEVIER

Salivary biomarkers of stress and inflammation in first graders in Côte d'Ivoire: Effects of a probiotic food intervention

Bonnie E. Brett [a,*], Bruno K. Koko [b], Habib O.Y. Doumbia [b], Frédéric Kouadio Koffi [b], Savorgnan E. Assa [b], Kollet Y.A.S. Zahé [b], Hortense Faye-Ketté [b,c], Séraphin Kati-Coulibaly [b,c], Remco Kort [d,e], Wilbert Sybesma [d], Gregor Reid [f], Carolina de Weerth [a]

3. Open label Uganda, 2 arms, 1116 children

frontiers in Nutrition

ORIGINAL RESEARCH
published: 09 December 2020
doi: 10.3389/fnut.2020.574792

A Comparative Interrupted Times Series on the Health Impact of Probiotic Yogurt Consumption Among School Children From Three to Six Years Old in Southwest Uganda

Nieke Westerik [1,2], Arinda Nelson [1], Alex Paul Wacoo [1,2,3], Wilbert Sybesma [1] and Remco Kort [1,2]*

[1] Yoba for Life Foundation, Amsterdam, Netherlands, [2] Department of Molecular Cell Biology, Vrije Universiteit Amsterdam, Amsterdam, Netherlands, [3] Department of Food Technology and Nutrition, School of Food Technology Nutrition and Bioengineering, College of Agricultural and Environmental Sciences, Makerere University, Kampala, Uganda

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scientific reports

Check for updates

OPEN **Normative cognition and the effects of a probiotic food intervention in first grade children in Côte d'Ivoire**

Bonnie E. Brett[1,2], Habib O. Y. Doumbia[2], Bruno K. Koko[2], Frédéric Kouadio Koffi[2], Savorgnan E. Assa[2], Kollet Y. A. S. Zahé[2], Remco Kort[3,4], Wilbert Sybesma[3], Gregor Reid[5] & Carolina de Weerth[1]

Westerik, N., et al. Improving Health and Wealth by Introduction of an Affordable Bacterial Starter Culture for Probiotic Yoghurt Production in Uganda. *Challenges* **2019**, *10*, 2.

Westerik N., et al. Comparative Interrupted Times Series on the Health Impact of Probiotic Yogurt Consumption Among School Children From Three to Six Years Old in Southwest Uganda. Front Nutr. **2020** Dec 9;7:574792.

Brett B., et al. Salivary biomarkers of stress and inflammation in first graders in Côte d'Ivoire: Effects of a probiotic food intervention. Psychoneuroendocrinology. **2021** Jul;129:105255.

Brett B., et al. Normative cognition and the effects of a probiotic food intervention in first grade children in Côte d'Ivoire. Sci Rep. **2022** Nov 14;12(1):19491. doi: 10.1038/s41598-022-23797-3.

In a previous controlled nutritional trial in Western Uganda with 1,116 school children, Yoba probiotic yoghurt decreased the incidence of common cold and skin rashes

Set Up:

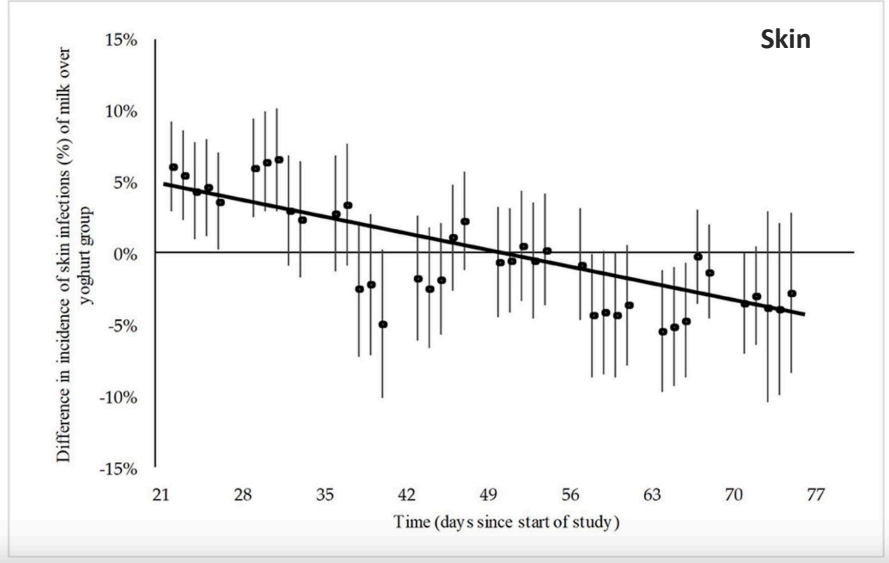
Comparative interrupted times series trial

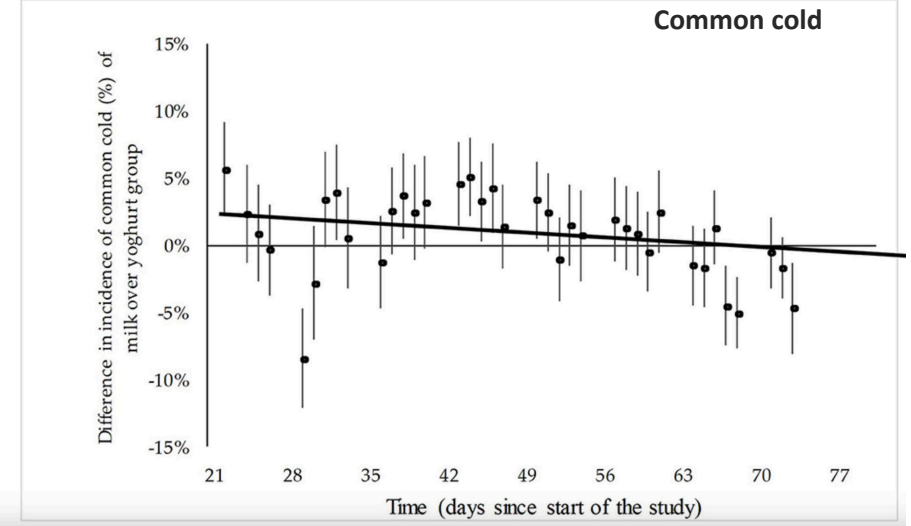
- 3 wks baseline + 9 wks 100 ml of probiotic yogurt or milk consumption, 5 days per week.
- 584 children in probiotic yogurt group (5 schools),
- 532 children in milk group (5 schools)

Results:

- Skin infections: Incidence rate decreased faster in the yogurt group compared to the milk group ($p < 0.0001$) resulting in a relative risk factor (RR) of 0.6 (CI: 0.4–0.9)

- Common cold symptoms: Incidence rate decreased faster in the yogurt group than in the milk group ($p = 0.09$) resulting in a final RR of 0.85 (95% CI: 0.5–1.4)





Conclusion:

- Positive trends, however differences at base line and fluctuations over the course of the intervention period

Recommendation:

- Repeat this study in a randomized, double blind trial, with a more uniform setting

→ One school, custard instead of milk as placebo

Westerik N., et al. Comparative Interrupted Times Series on the Health Impact of Probiotic Yogurt Consumption Among School Children From Three to Six Years Old in Southwest Uganda. Front Nutr. **2020** Dec 9;7:574792.

ClinicalTrials.gov BETA

Resources About

Home > Search Results > Study Record

COMPLETED

ClinicalTrials.gov Identifier: NCT04144491

Effect of *L. rhamnosus* Yoba on RTI and Other Health Outcomes Among Children (3-6 Years) in Uganda

Information provided by Remco Kort, VU University of Amsterdam (Responsible Party)

Last Update Posted: 2021-04-28

Daily monitoring by qualified school nurses

Primary outcome:

- Incidence of respiratory tract infections (cough, rhinitis)

Secondary outcomes:

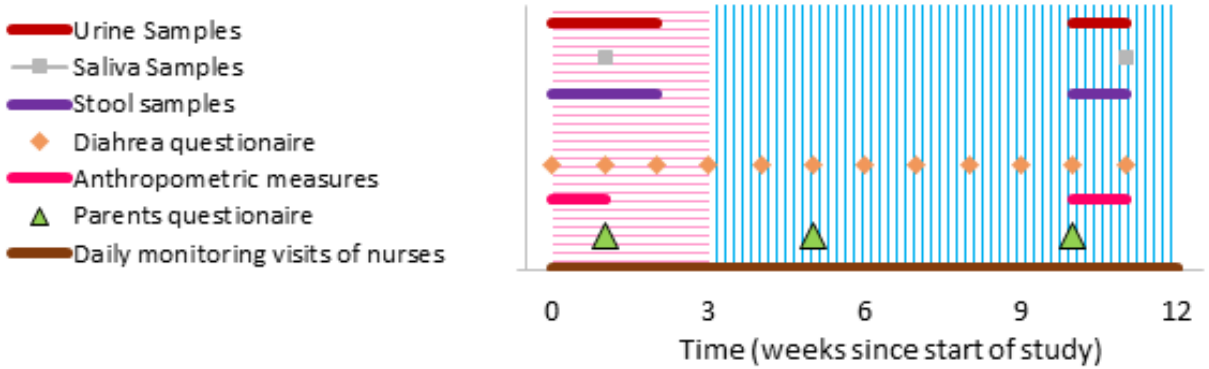
- Incidence of skin disease (incidence of skin rashes)
- Weight-for-age
- Length-for-age
- Metabolic profile of children's urine
- Microbial load of children's stool
- Immune markers of children's saliva

Reference: NCT04144491 (clinicaltrials.gov)

September 12

November 22 (2019)

N=196

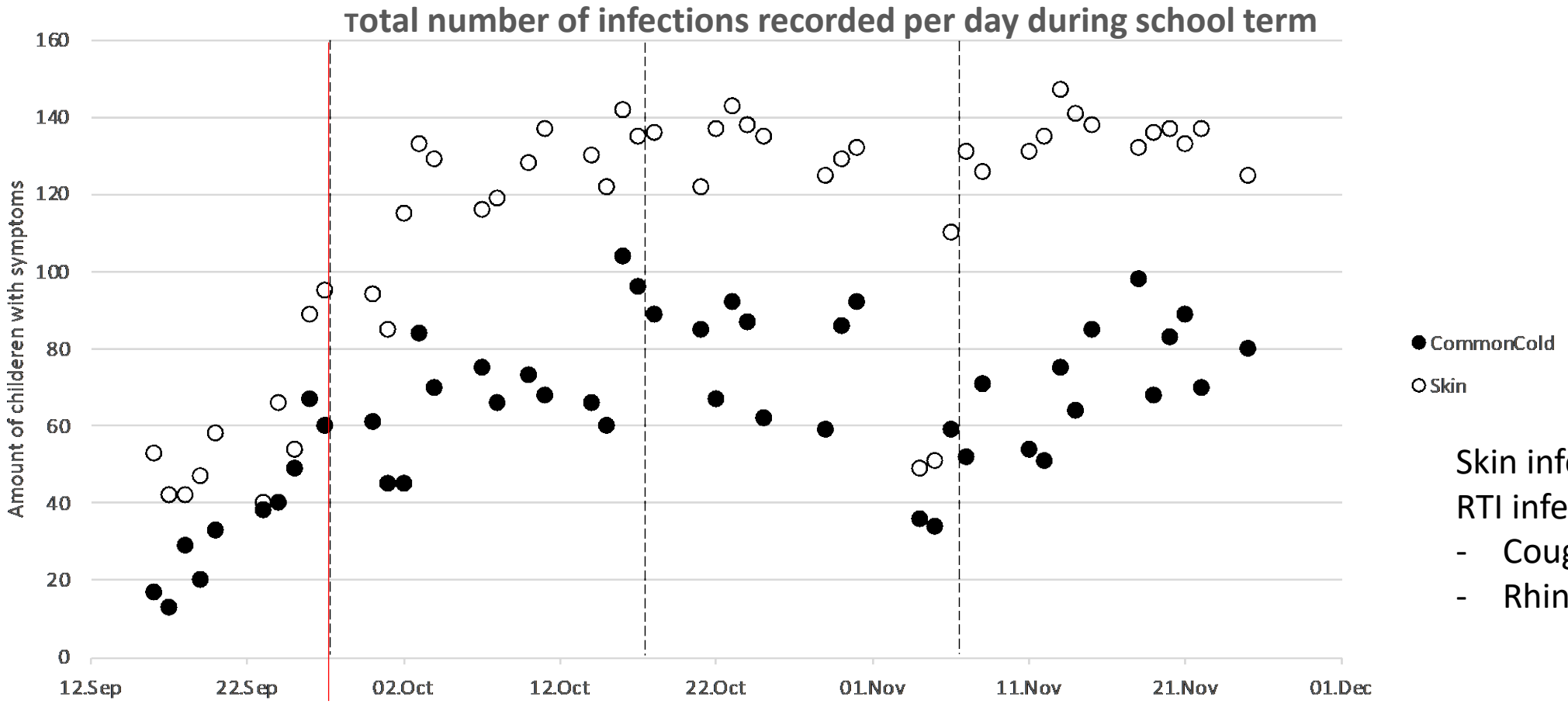


Daily dose of 125 ml, approx.: 1-3 x 10^9 cfu *L. rhamnosus* yoba 2012, 1-2 x 10^{11} cfu *S. thermophilus* /day

Overall infection incidence shows:

→ 1. Increase during the first weeks of school attendance, including baseline and two weeks after the start of the intervention

→ 2. More skin infections than common cold



Skin infections
RTI infections
- Cough
- Rhinitis

N=196 children

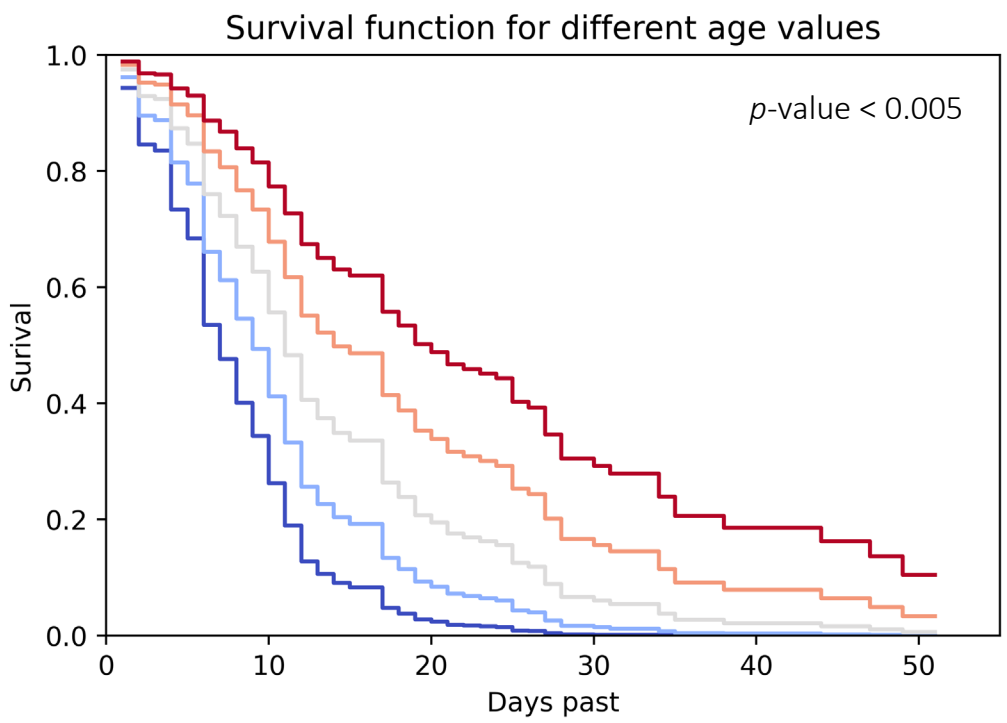
Intervention start

Observations related to all children - Clear correlation between age and incidence of skin infections and RTIs:

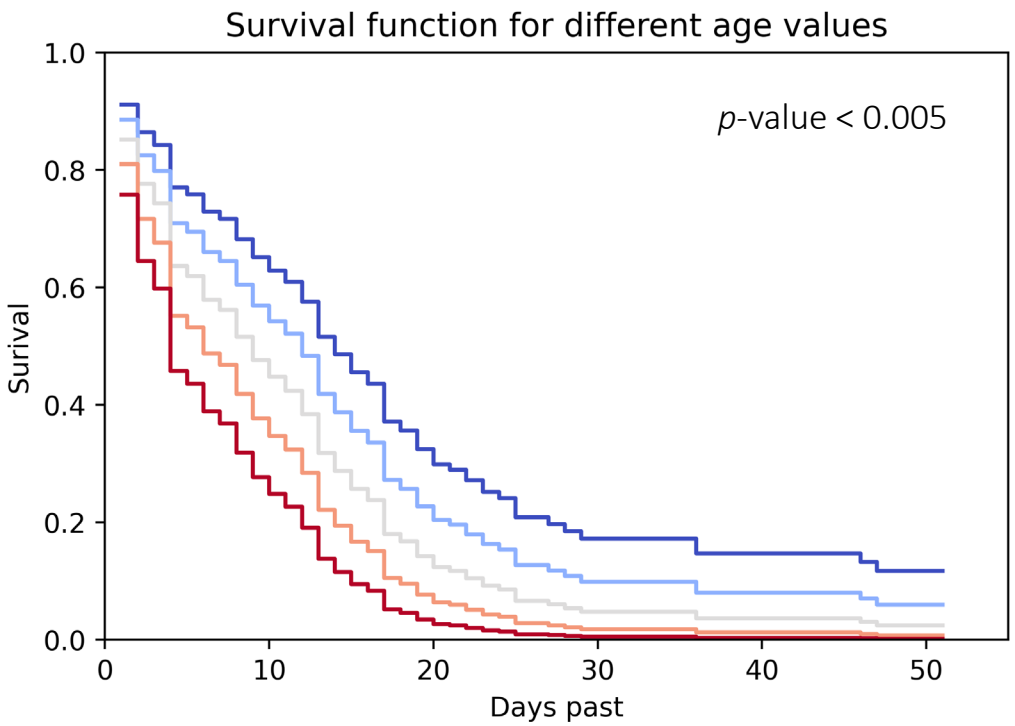
→ Skin infection is more prevalent in older children.

→ RTI is more prevalent in the younger children

A



B



800 days | 1300 days | 1800 days | 2300 days | 2800 days

During the course of the study, BMI for Age decreased in both groups

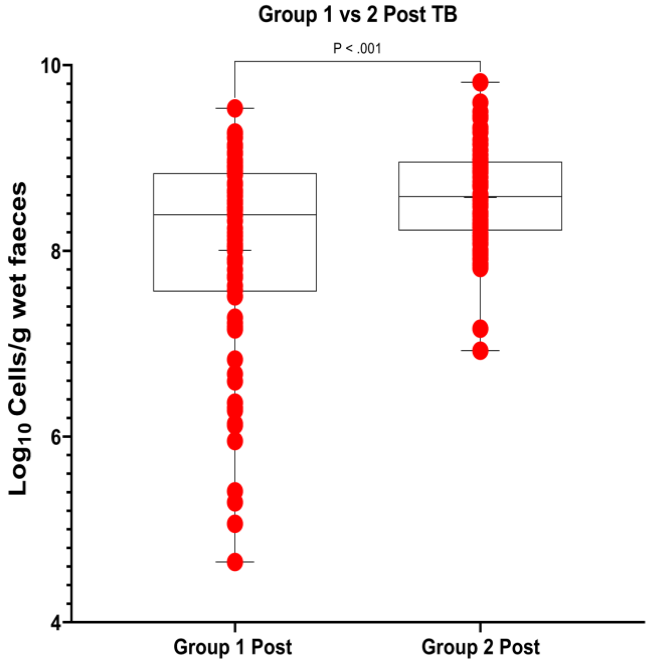
- **This observation was also made in the open label study with 1000 children**
- **These observations suggest that for children when coming back from holidays, their energy-intake-energy-expenditure-ratio got worse**

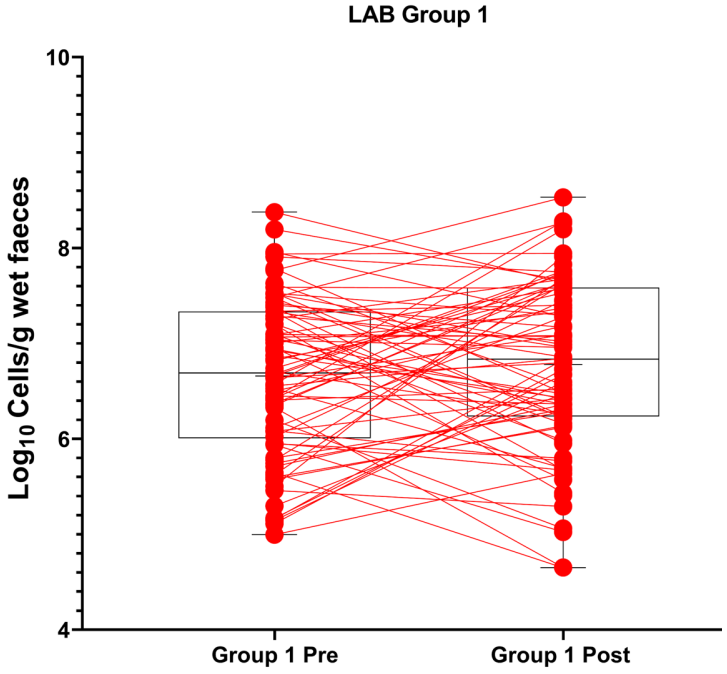
	Average weight (kg)	Average height (cm)	WAZ	HAZ	BAZ
Yoghurt					
Baseline	18.0 (± 2.6)	106.9 (± 7.9)	-0.05	-0.36	0.25
End line	18.0 (± 2.6)	108.5 (± 8.0)	-0.19	-0.26	-0.06
Difference	0.0	1.6	-0.14	0.10	-0.31
Control					
Baseline	17.9 (± 3.0)	106.7 (± 9.1)	-0.04	-0.24	0.18
End line	17.8 (± 3.0)	108.2 (± 9.0)	-0.22	-0.17	-0.17
Difference	0.0	1.5	-0.18	0.08	-0.34

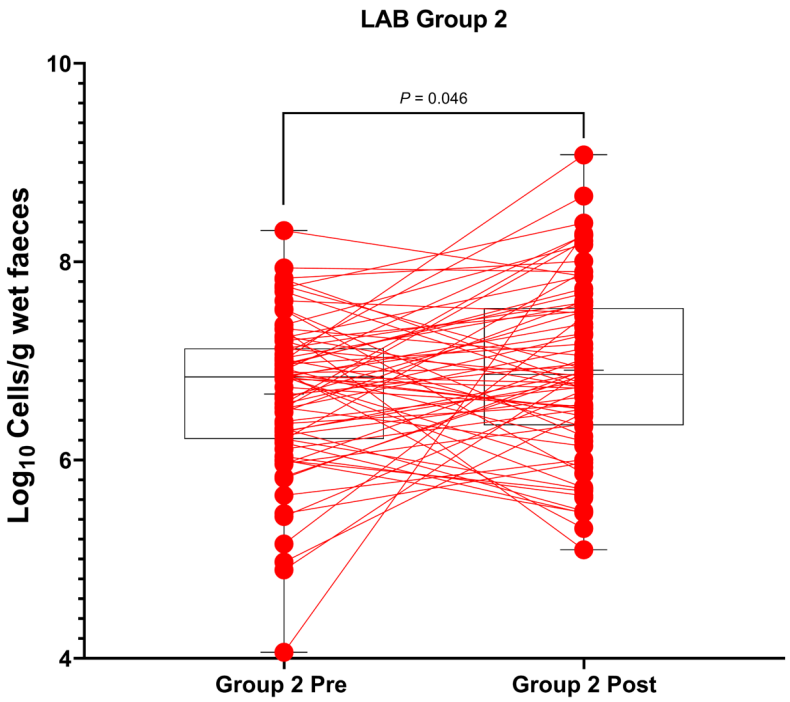
Table: *P*-values < 0.00001

Children in both groups start and end with similar lengths and weights
Average HAZ and WAZ values are slightly below and BAZ values are slightly above the WHO standards

The probiotic yoghurt group shows increased levels of LAB and total bacteria in stool







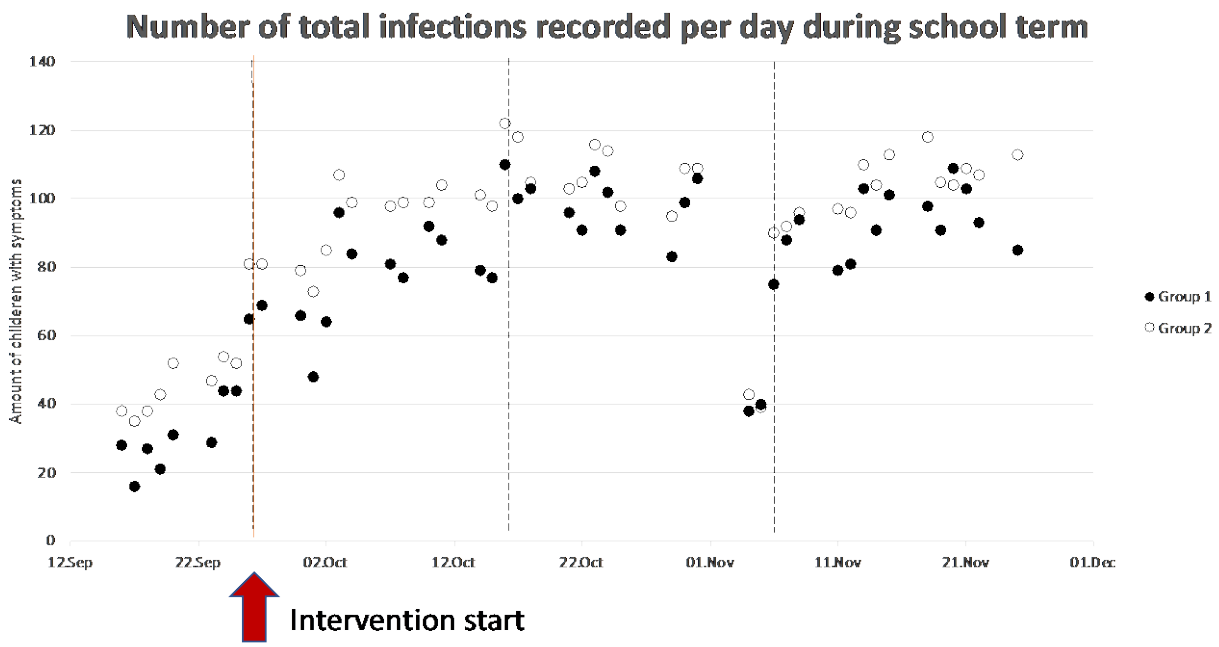
Total bacteria (*p*-value < 0.01)

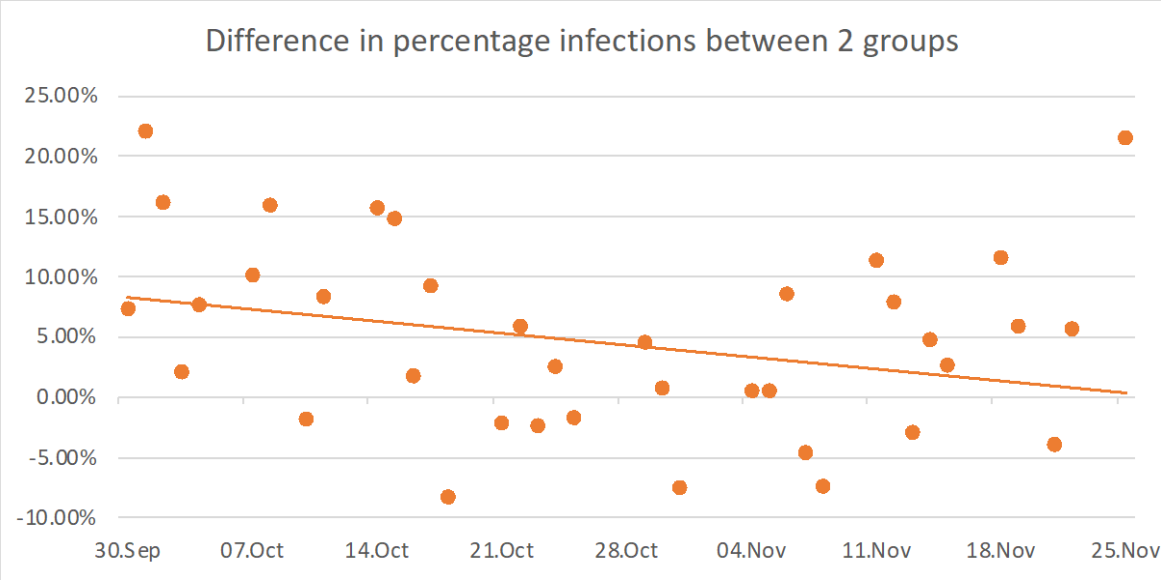
***Lactobacillus* (*p*-value < 0.05)**

Quantitative flow-cytometric data on stool bacteria

The difference in infection incidence between the two groups decreases over time, in favour of the Yoghurt group

A similar observation was seen as in the previous study



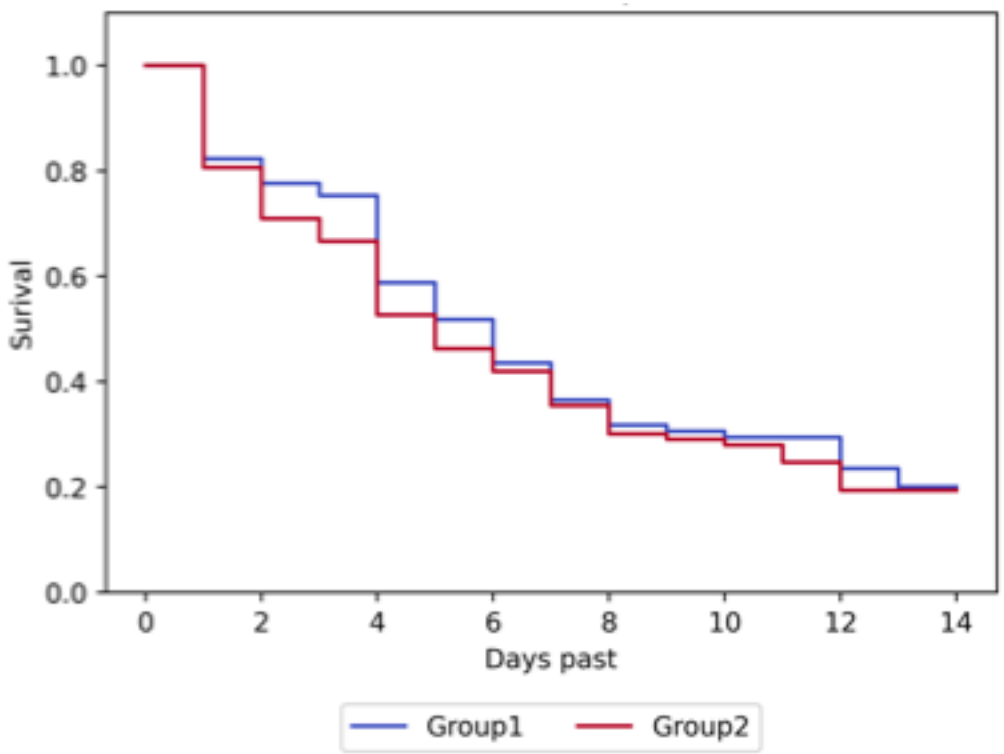


The number of infections in yoghurt group starts higher and ends at same level of the placebo /custard group

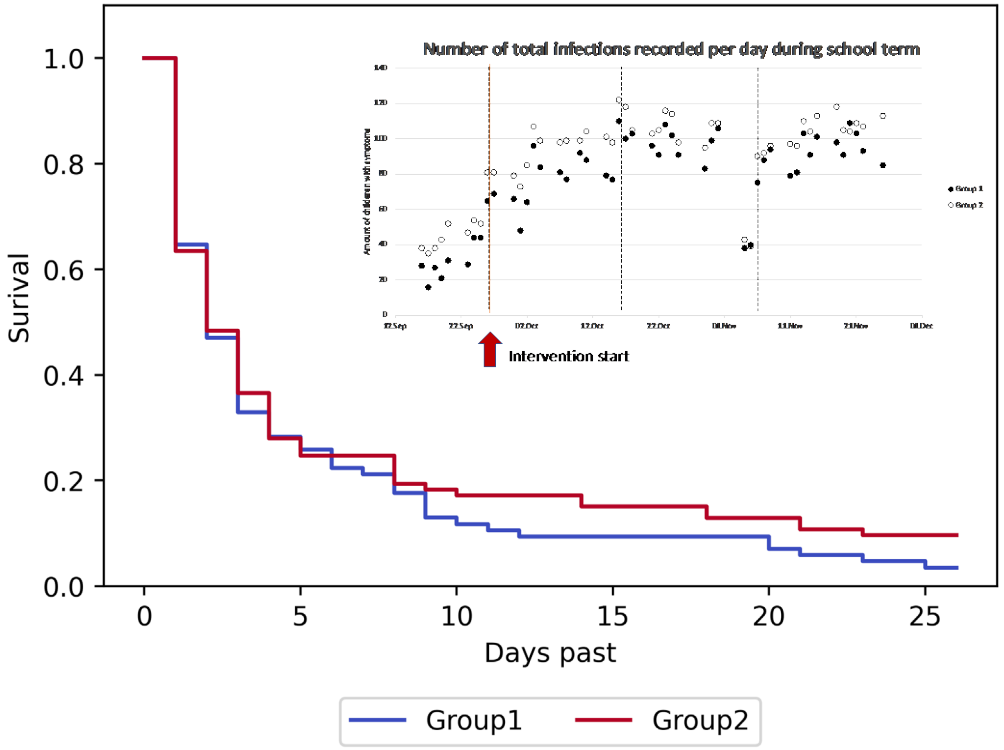
Group 1–Placebo: N= 85, Group 2-Yoghurt : N= 93, – Corrected for > 2 days of absence in any period

Total number of infections:
→ From two weeks after the start of the intervention, the survival curves tend to be in favour of the Yoghurt group

Survival Yoghurt vs Placebo group – Period 1



Survival Yoghurt vs Placebo group – Period 2.3

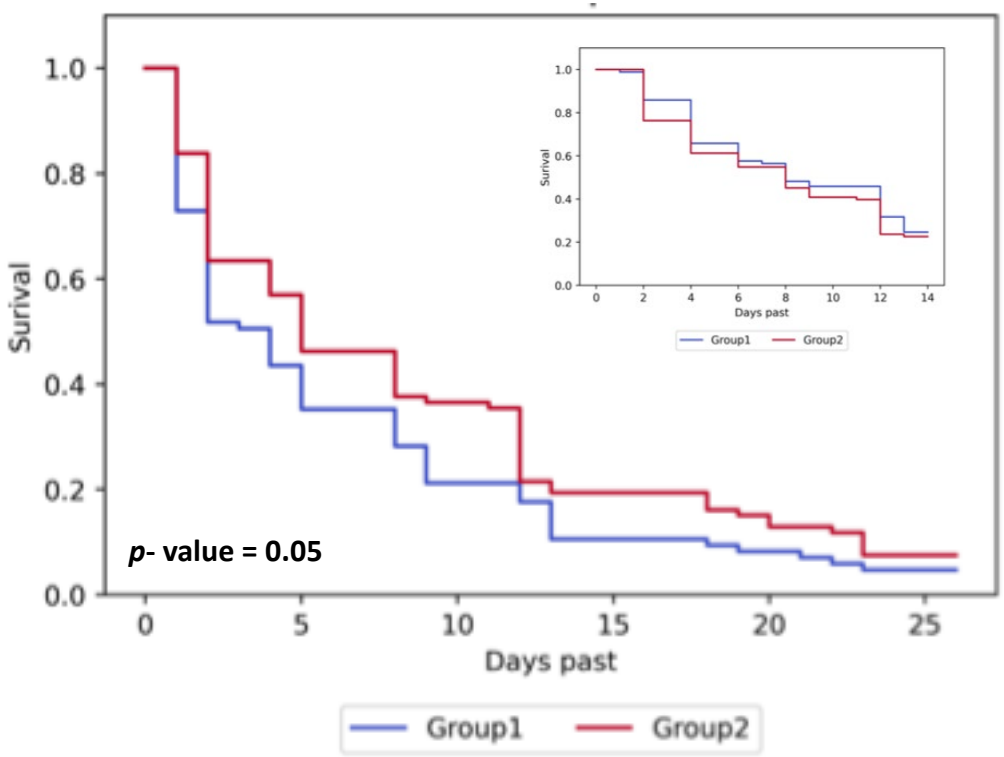


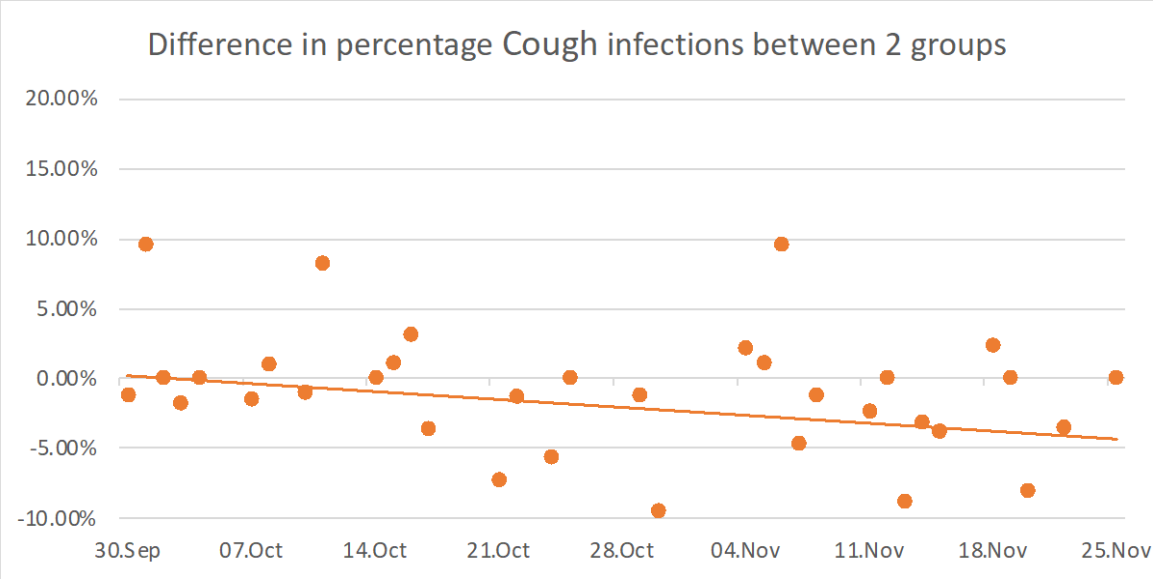
Group 1–Placebo: N= 85, Group 2-Yoghurt : N= 93, – Corrected for > 2 days of absence in any period

RT Infections, cough only

→ From two weeks of the start of the intervention, the survival curves tend to be in favour of the Yoghurt group
→ The difference in infection incidence between the two groups decreases over time, in favour of Yoghurt group

Survival Yoghurt vs Placebo group – Couch, Period 2,3
(Period 1 inserted)

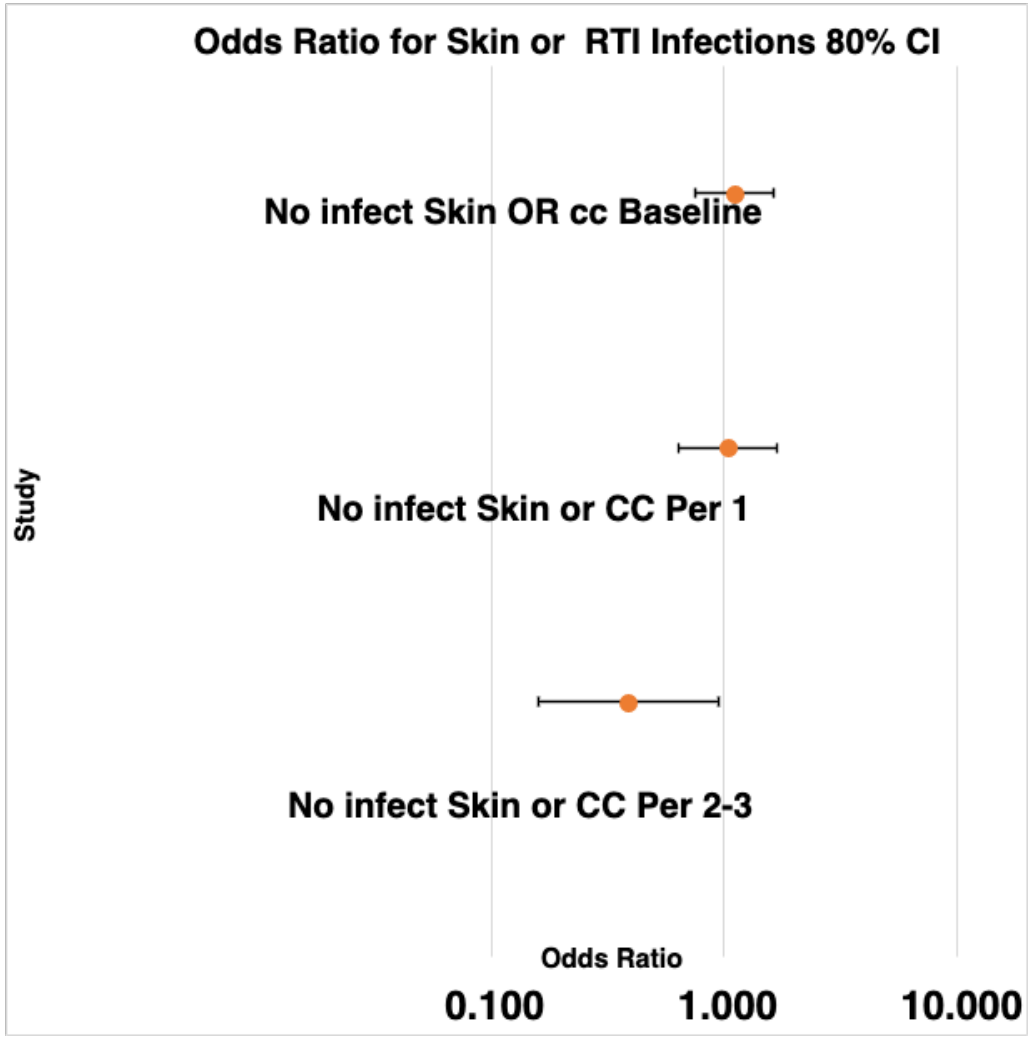




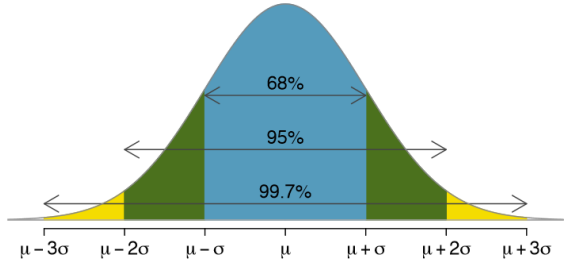
The number of infections in yoghurt group starts at the same level, and ends lower than the placebo /custard group

Group 1–Placebo: N= 85, Group 2-Yoghurt : N= 93, – Corrected for > 2 days of absence in any period

Also the odds ratio of 0.39 (approx. factor 2.5) is in favour of the probiotic yoghurt group, as displayed for the total number of infections.



P=0.17



Increased hippurate levels in the urine of the yogurt group

Gut microbiota

OPEN ACCESS

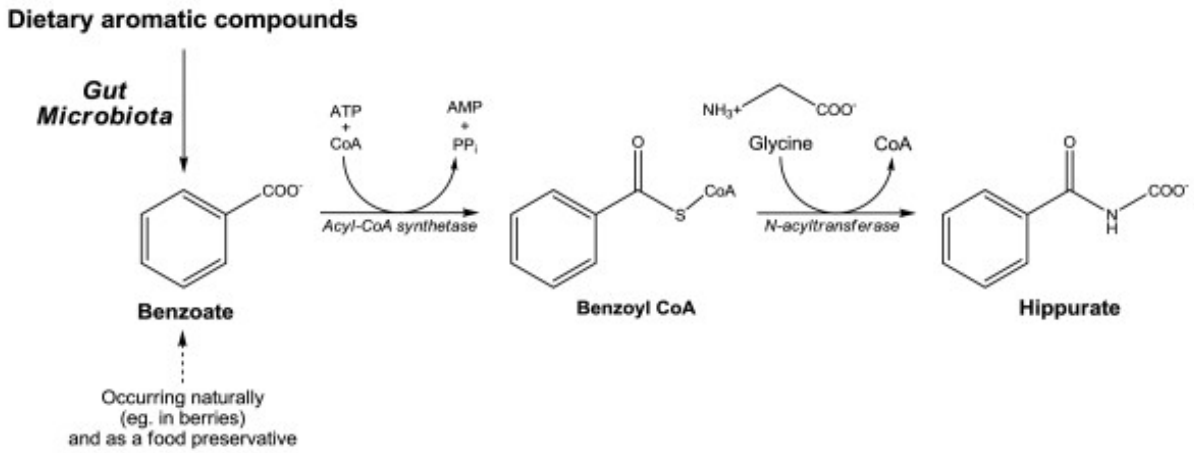
Original research

Human and preclinical studies of the host–gut microbiome co-metabolite hippurate as a marker and mediator of metabolic health

François Brial,[1] Julien Chilloux,[2] Trine Nielsen,[3] Sara Vieira-Silva,[4] Gwen Falony,[4] Petros Andrikopoulos,[2,5] Michael Olanipekun,[2,5] Lesley Hoyles,[6] Fatima Djouadi,[7,8] Ana L Neves,[2] Andrea Rodriguez-Martinez,[2] Ghiwa Ishac Mouawad,[1] Nicolas Pons,[9] Sofia Forslund,[10] Emmanuelle Le-chatelier,[9] Aurélie Le Lay,[1] Jeremy Nicholson,[2] Torben Hansen,[3] Tuulia Hyötyläinen,[11] Karine Clément,[12,13] Matej Oresic,[14] Peer Bork,[15] Stanislav Dusko Ehrlich,[9,16] Jeroen Raes,[4,17] Oluf Borbye Pedersen,[3] Dominique Gauguier,[1,18] Marc-Emmanuel Dumas [2,5,18,19]

Hippurate is a marker for improved gut metabolic function as dietary (aromatic) compounds from fruits and vegetables are converted to benzoic acid by the gut microbiota (including lactic acid bacteria) which is taken up and converted to hippuric acid in the liver

Conclusion Our human and experimental studies show that a high urine hippurate concentration is a general marker of metabolic health, and in the context of obesity induced by high-fat diets, hippurate contributes to metabolic improvements, highlighting its potential as a mediator of metabolic health.



Brial F, *et al. Gut* 2021;70:2105–2114

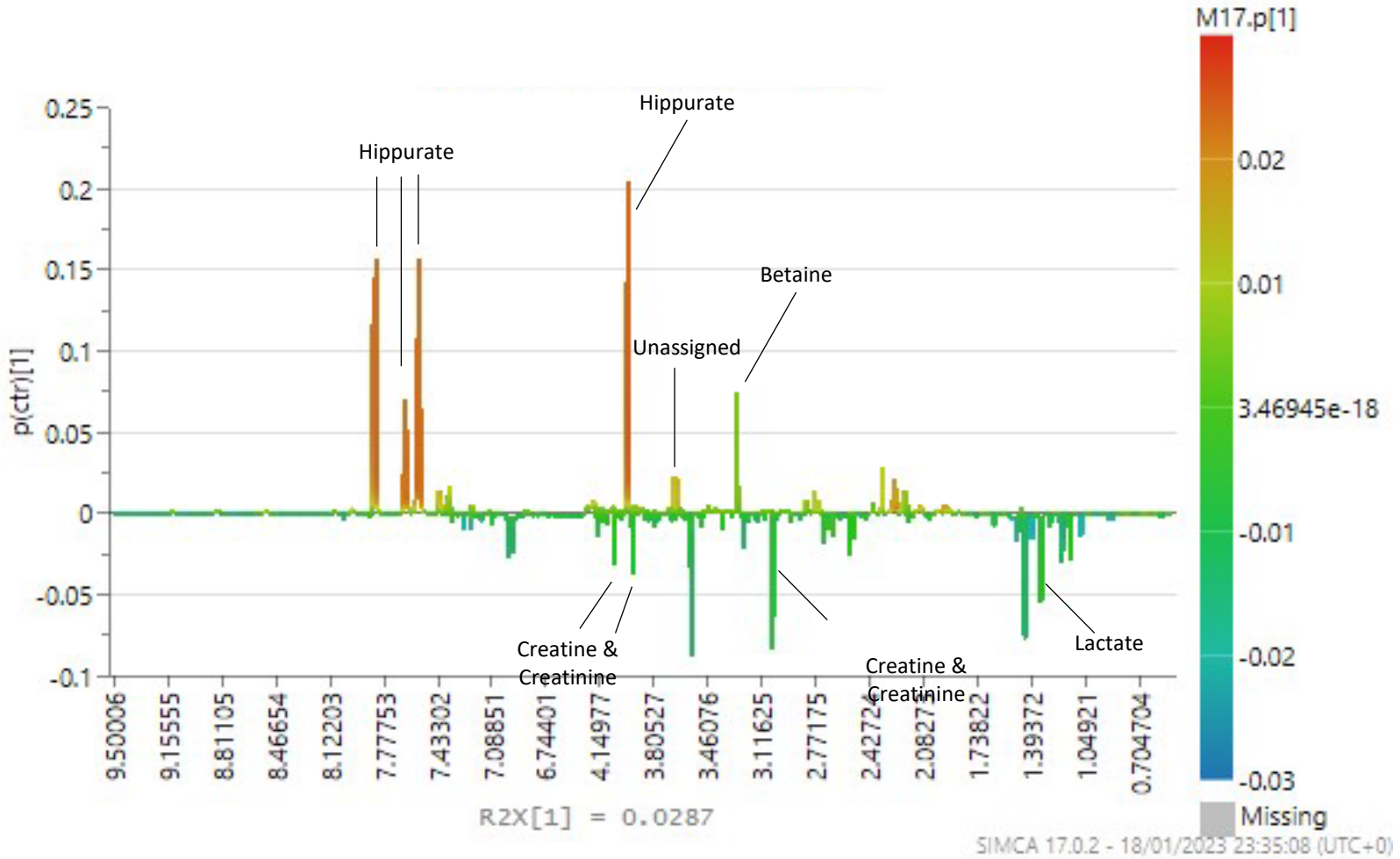
Williams et al. BMC Gastroenterol. 2010; 10:108.

Increased hippurate levels in the urine of the yogurt group

NMR-analysis* of urine metabolites

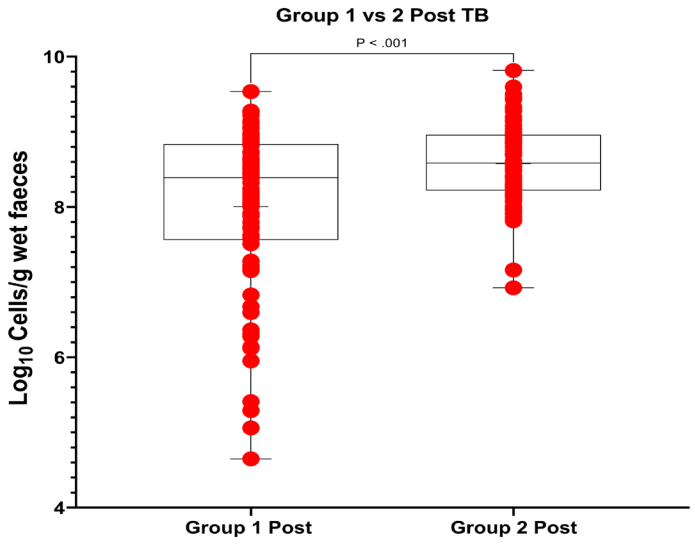
Urinary metabolites increased in Group 2

Urinary metabolites increased in Group 1



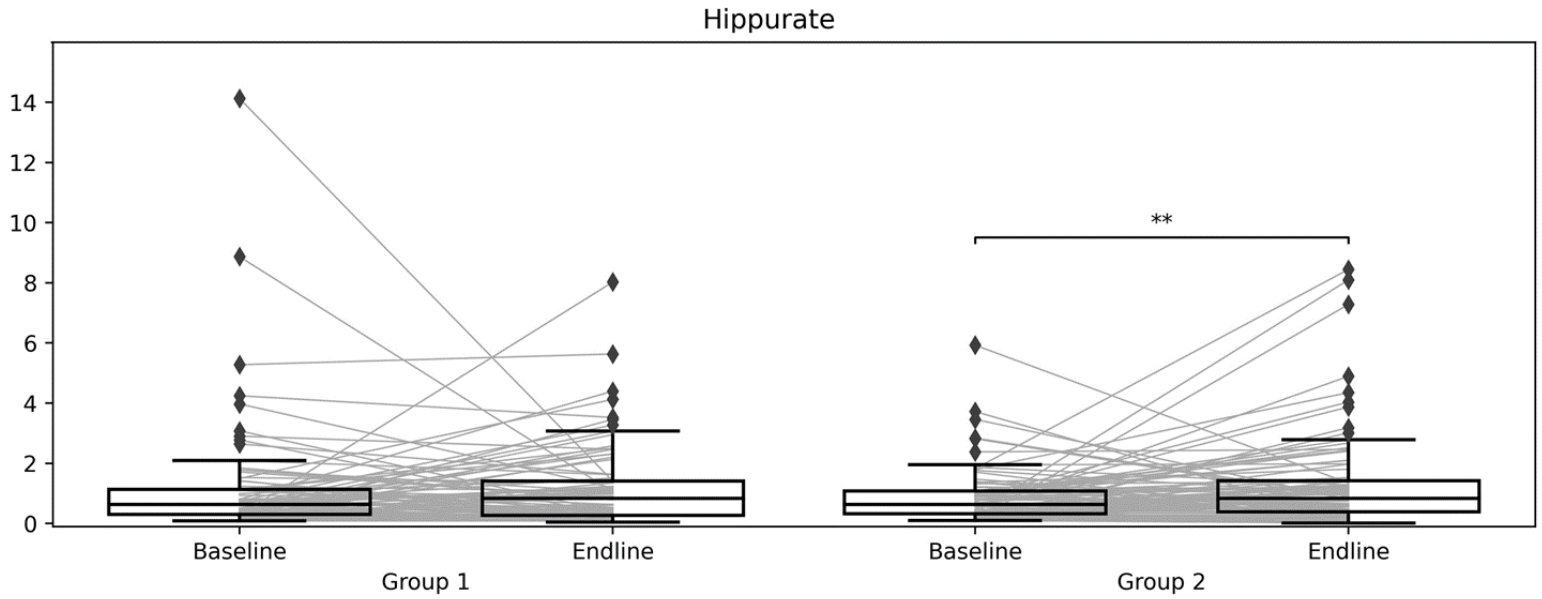
* Orthogonal Projection on Latent Structure Model Discriminant Analysis

The probiotic yoghurt group shows increased levels of hippurate in urine and increased levels of bacteria in stool



Quantitative flow-cytometric data on stool bacteria

Total bacteria (*p*-value < 0.01)

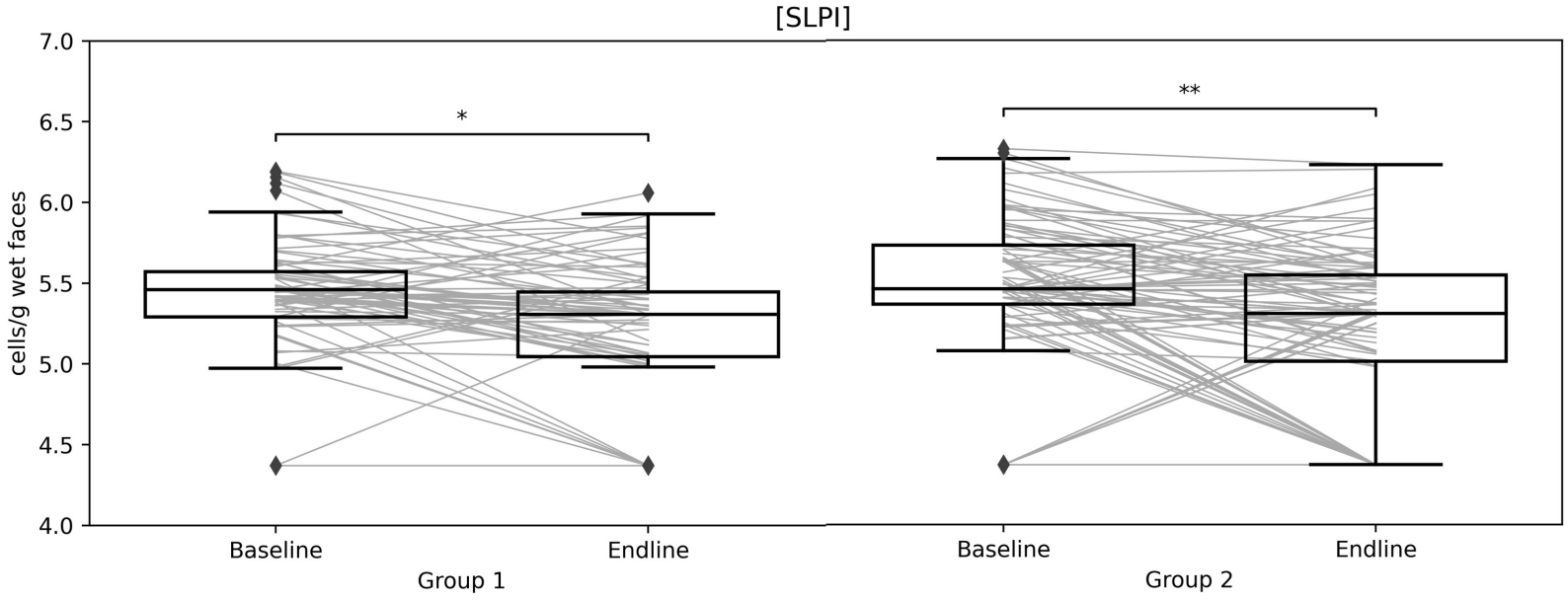


NMR-data on urine metabolites

***P*-value < 0.01**

The salivary inflammation marker secretory leukocyte peptidase inhibitor (SLPI) is drastically decreased in the probiotic yoghurt group

- Secretory leukocyte protease inhibitor (SLPI) is an immune factor relevant to defence response to external biotic stimuli including bacteria
- Previous transcriptome data in a pig model system show that probiotic supplementation down-regulates the SLPI immune associated genes (Shin *et al* (2019) Plos ONE)
- Probiotics could modify the crosstalk between commensal gut microbiota and the mouth via attenuating inflammation



***P*-value = 0.02**

***P*-value = 0.002**

Conclusions and recommendations

-Effect of probiotic yoghurt in real life setting-

Outcomes for both groups

- Strong level of cross infections week 1-4 start of school term
- Decrease of BMI for Age in both groups

Outcome for intervention

- Hippurate levels increased
- LAB and total bacteria increased
- Higher decrease of SLP1
- Locally produced probiotic yoghurt tend to provide protection against skin infections and respiratory tract infection

Recommendations - Study

To enhance the level of certainty of the impact of probiotic yoghurt, conduct a similar RCT, with

- A longer intervention period, and/or
- More children, and/or
- More uniform study environment (e.g. boarding school, or orphanage)?
- A third control group, in addition to yogurt and custard

What we would like to do next

- Aim for national school feeding programs with locally produced probiotics food
- Expand Yoba for Life concept in scale and scope
- Go beyond dairy and with locally produced probiotic Kwete, Zomkom, Obushera, Soy, Munkoyo, and more
- Investigate opportunity to use locally sourced probiotics

Science & Society

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Locally sourced probiotics, the next opportunity for developing countries?

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Yoba for Life – Partnership Workshop 20-22 February 2023

Igongo Cultural Center, Mbarara Uganda

Yoba for Life Symposium and workshop

20 – 22 February 2023, Mbarara, Uganda

Preliminary symposium program

Sunday 19th February		
until 14:00	Arrival in Entebbe	
14:00	Transport from Entebbe to Mbarara	
20:00	Arrival in Mbarara. Check-in hotel Mbarara. Dinner buffet with introduction round of people and organizations.	
Monday 20th February		
9:00	Welcome remarks by the Yoba team Uganda	Uganda team
9:15	An introduction in the work of Yoba for Life (production, certification, business development support, school yoghurt).	Dr. Nieke Westerik + country coordinators
9:45	Health benefits of probiotic fermented foods.	Dr. Wilbert Sybesma / Prof. Remco Kort
10:00	Visiting a (pre) primary school consuming probiotic yoghurt. Discussion with school management, teachers and parents.	Uganda team
13:00	Lunch	
14:00	Visiting a dairy cooperative Yoba producer and an individual Yoba producer.	Uganda team
16:00	Interactive work session on exploring synergistic opportunities to promote health and wealth.	Geert Westenbrink /Marco Streng
18:00	Closure of the day	
Tuesday 21st February		
9:00	Short discussion / reflection about previous day visit and work session	Dr. Nieke Westerik / Prof. Remco Kort
9:30	Presentation on collaboration between Yoba for Life and SNV/TIDE project	SNV
9:45	Presentation on collaboration between Yoba for Life and relevant government institutions	Dairy Development Authority
10:00	Testimonies of two school directors about the school yoghurt program	School directors
10:30	Visit women group Yoba producer and individual Yoba producer or second school	Uganda team
14:00	Lunch	
15:00	Yoba beyond dairy: to possibility of soy yoghurt and other fermented cereals	Dr. Alex Wacoo
15:00	Interactive work session on moving from challenges to opportunities	Geert Westenbrink /Marco Streng
17:00	Workshop closure, drinks and networking	Dr. Nieke Westerik + country coordinators
Wednesday 22nd February		
Selected/interested partners		**Returning participants**
9:00	Dedicated proposal writing session	8:00 Departure to Entebbe
18:00	Closure of the day	14:00 Lunch in Entebbe and departure
23rd February		
8:00	Departure to Entebbe	

Objectives of the symposium

- Create understanding about the Yoba for Life concept and its potential to success among potential partners across East Africa.
  - Generating supply and generating demand -
- Gain new ideas and opinions from a diverse audience about the possible future paths to explore as Yoba for Life.
  - From dairy to plant based fermented foods -
- Selected/interested partners: Dedicated session on day 3 to work out a project proposal.

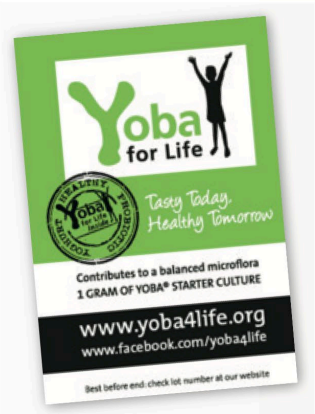
For info:
Contact Wilbert Sybesma / Remco Kort

How to make your own Yoba Yoghurt

- All kinds of milk are suitable for preparation of Yoba

- In case you prefer a more dink yoghurt format, use a kitchen blender for 30 seconds.

- Depending on personal taste preferences, you can add flavours, colorants, sugar, fruits, muesli, chocolate,



The Yoba for Life Team and Partners

Insights to share:

- Give away control (Inspire and Shape Aspirations)

- Look for partnerships with organizations to whom you can add value

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Uganda Team

Tanzania Team

Ethiopia Team

The Netherlands Team

Yoba for Life Foundation is a non-profit organization recognized by Dutch state as a Public Benevolent Institution

>25 publications and 2 PhD thesis