CARBOHYDRATE
COMPETENCE CENTER

CARBOHEALTH

CARBOHYDRATES FOR IMPROVING HEALTH

TABLE OF CONTENTS

PREFACE - CCC PRESENTS....

Carbohydrate Competence Center (CCC) proudly presents the final report with the results of about four years of hard work in the CarboHealth programme. Seven ambitious researchers and their supervisors, four knowledge institutes, six industrial partners, TKI Agri Food and representatives of CCC made sure the goals of CarboHealth were achieved. The result: an integral toolbox for the identification and functional characterization of healthy carbohydrates.

CarboHealth is a research programme of a consortium of companies and knowledge institutes, partly financed by TKI Agri Food, managed and coordinated by CCC. The full partners of this programme are: the University of Groningen, University Medical Center Groningen, Wageningen University & Research, Wageningen Food & Biobased Research (part of WUR), Royal FrieslandCampina N.V. (owned by Zuivelcoöperatie FrieslandCampina U.A.), Coöperatie AVEBE U.A., Nutricia Research B.V. (part of Danone Nutricia Research), Trouw Nutrition (a Nutreco company), Sensus B.V. (part of Royal COSUN) and Winclove B.V. At a later stage Radboud University Nijmegen and Maastricht University provided samples from two human cohorts.

PAUL DE VOS,

PROFESSOR IMMUNO-ENDOCRINOLOGY UMCG

'Thanks to this technology platform, we now know that 80% of the carbohydrates we selected have no influence on immunity at all. Of the 20% selected for further research, we know they impact health in one way or the other. For some of these fibres we have proven actual health benefits. But there is still so much to learn: we are by no means finished yet.'

STUDY OF CARBOHYDRATES THAT BENEFIT HEALTH

The programme is aimed at the study of carbohydrates that benefit health. The participants focused on an integral and coordinated analysis and identification of beneficial effects of oligosaccharides and polysaccharides on health. The industrial partners contributed to the programme by providing funds, carbohydrates and bacterial strains and also by generating ideas and insights, matching the demand-driven approach of CCC. The researchers used the same samples and substrates, so their results could be compared and validated.

BENEFICIAL EFFECTS ON HEALTH

Commercially, the Netherlands is one of the leading countries as far as development and marketing of functional oligosaccharides and polysaccharides are concerned. At the same time, Dutch universities are carrying out important, fundamental research in order to analyse and explain beneficial effects of these carbohydrates on health. The main goal of CarboHealth was to deliver a toolbox for the identification and functional characterization of healthy carbohydrates. It would have been great if the industrial partners could have used the results to support nutritional and health claims at the European Food Safety Authority (EFSA). Since EFSA requirements have become much stricter, this turned out to be not feasible yet. However, the Carbo-Health programme delivered highly valuable knowledge, improved and created novel methods and yielded large datasets on the relation between indigestible carbohydrates, bacteria in the gut and health.

JURRIAAN MES, EXPERT LEADER FOOD & HEALTH RESEARCH, WAGENINGEN FBR:

'This programme delivered strong results and promising leads for future research. The combination of long-chain inulin carbohydrates and the *Lactobacillus* W37 bacterial strain is very interesting for incorporation in future human trials. If we can prove that this combination leads to a positive effect on health, then it is possible to get EFSA support for a health claim for products containing these ingredients. Other compounds have also shown interesting leads which should be further tested in specific human trial designs.'

VALUABLE TOOLBOX

The goal of this programme was to develop a technology platform for the rapid identification and validation of the health promoting effects of indigestible oligo- and polysaccharides provided by Dutch industries. In addition, an interesting set of biological samples of human milk and infant faeces was included, focusing on complex carbohydrates, the human milk oligosaccharides, and their effect on the microbiome in early life. From a broad range of ingredients, the most promising carbohydrates and bacterial strains were selected. These were analysed in detail and tested in different ways. After four years, the participants of CarboHealth succeeded in delivering a valuable toolbox of scientific methods and specific data, available to all participants. The industrial partners will use the results to support their own research and development. This new knowledge and innovative methods generated in CarboHealth also provided input for two new CCC programmes: CarboKinetics and CarboBiotics. Both programmes are already underway, three of the industrial partners are also participating in one or both of them.

SCIENTIFIC PUBLICATIONS

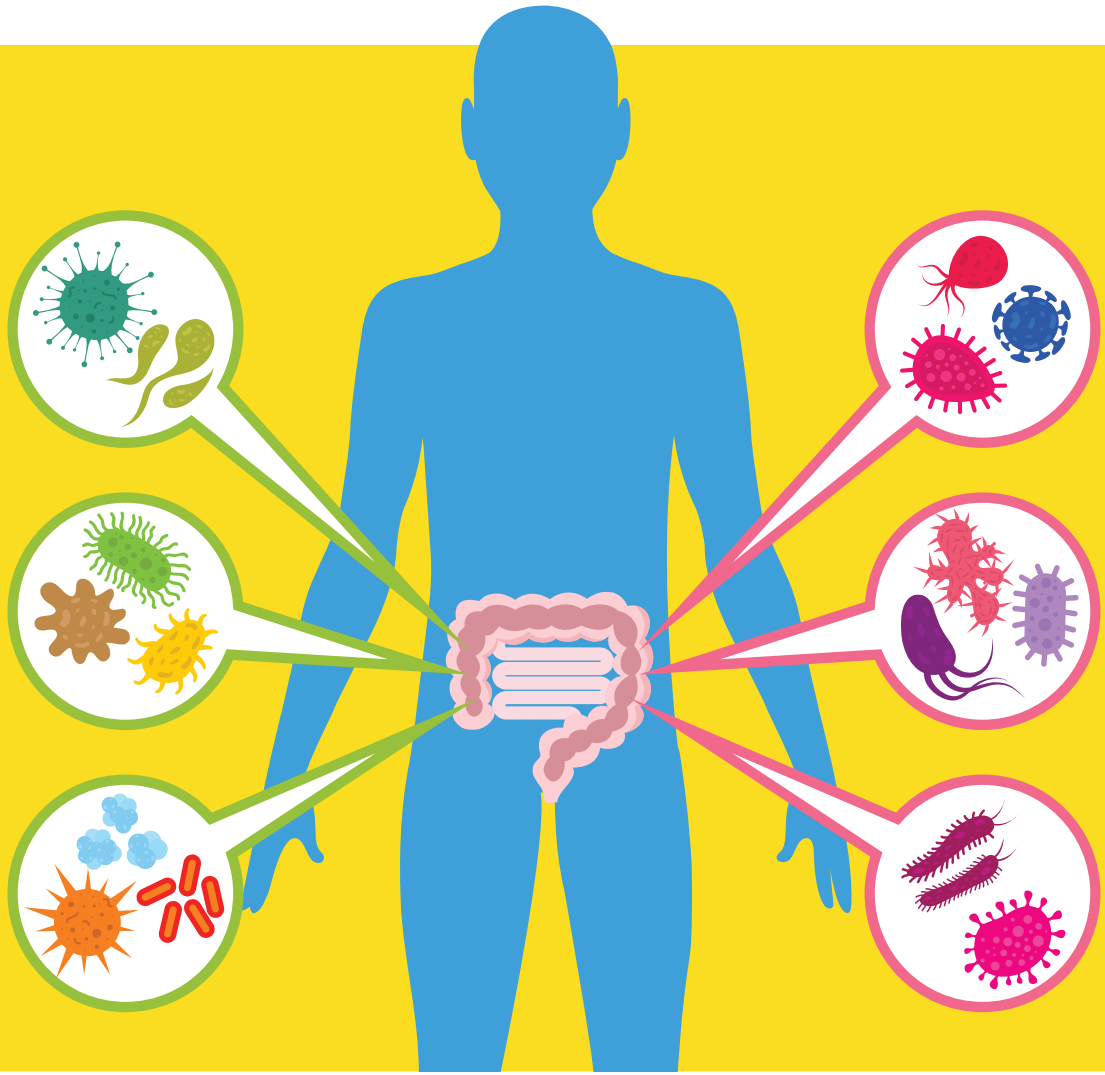
The programme is expected to deliver no less than 25 scientific publications, 5 of which are dissertations. All seven researchers contributed and used the toolbox extensively, adding to the knowledge base regarding carbohydrates themselves, their beneficial effects on human and animal health and the way they interact with beneficial bacteria in the intestinal tract.

LOOKING BACK

In this report the results of all five subprojects are summarised and illustrated by the researchers, their supervisors, the industrial partners and other CCC members. They provide information about their own contributions to the programme, explaining the various steps and phases they went through, their goals and results as well as the impact of their findings on society. At the closing symposium on 30th of November 2017, three (inter)national keynote speakers, specialists with strong contributions in the research field of carbohydrates and health, presented their views, which are also incorporated in this report.

PIET BUWALDA, MANAGER INNOVATION CENTRE AVEBE AND ASSOCIATE PROFESSOR WAGENINGEN UNIVERSITY AND RESEARCH:

‘Carbohydrates are indispensable for our health. Although they may have a somewhat negative connotation at the moment, we need carbohydrates for sustaining our brains and inducing a healthy colon population.
But only if we eat a variety of fibres, because each fibre influences our health in a different way. CarboHealth is very important in this respect, because these research projects unlock some of the secrets of the bacteria in our gut and the way carbohydrates influence their balance and our health.’



INTRODUCING CARBOHEALTH

Currently, carbohydrates, a generic name for a diverse group of simple and complex sugars, have rather a bad reputation. Self-styled food experts advise the general public emphatically to avoid sugar like the plague. In their opinion sugar equals poison. The same used to be said about fat in the sixties. However, both assertions are false. The human body needs both fat and carbohydrates to function properly. Now, with respect to fatty food ingredients, there is plenty of scientific evidence to suggest that some fatty acids are good for us, but others not so much. The same seems to be the case for carbohydrates.

The focus of CarboHealth was on finding out which carbohydrates are beneficial to the health of humans and animals. It was already known that indigestible carbohydrates, the main component of dietary fibre, improve and even protect human and animal health because they benefit the intestinal flora. The low-molecular weight indigestible carbohydrates, so-called prebiotics, are not digested until they reach the lower intestinal tract, where beneficial bacteria grow on them. The same goes for carbohydrates that are the third largest component of human milk: the human milk oligosaccharides (hMOS). Most manufacturers of infant formula now add a variety of prebiotic carbohydrates to improve bottled milk for babies. Manufacturers of animal feed also experiment with prebiotics in order to reinforce the immunity and metabolism of livestock. Generally speaking, it would be a very good thing if the use of prebiotics could also lead to a better protection and recovery of beneficial bacteria in the intestinal tract in case antibiotics must be used. In addition, social pressure also contributes to the efforts to reduce the use of antibiotics in general. Whether using prebiotics can help to reach this goal, cannot be predicted yet.

IDENTIFICATION AND ANALYSIS

In CarboHealth a great number of prebiotic carbohydrates were identified and analysed in detail. Initially, the pro-

gramme was provided with samples and substrates by the industrial partners. These included galacto-oligo-saccharides (GOS), provided by FrieslandCampina and fructose-oligosaccharides (FOS), contributed by Sensus. In addition, AVEBE provided exciting new compounds derived from starch, namely IsoMalto/Malto-Poly-saccharides (IMMP) and pectin-oligosaccharides. Winclove Probiotics contributed some 10 probiotic strains of bacteria. Additionally, human milk and baby faeces samples from the BINGO cohort study of the Radboud University Nijmegen and the KOALA cohort study of the Maastricht University were included in the programme. The other industrial partners, Nutricia Research and Trouw Nutrition, participated actively in the research projects and adopted the outcome of several subprojects.

OVERVIEW OF SUBPROJECTS

The first subproject (SP1) focused on the analysis and identification of samples and substrates, which resulted in the creation of a detailed toolbox. This result on its own is considered to be a valuable outcome by all partners. In the other subprojects the developed advanced analysis

PREBIOTICS AND PROBIOTICS

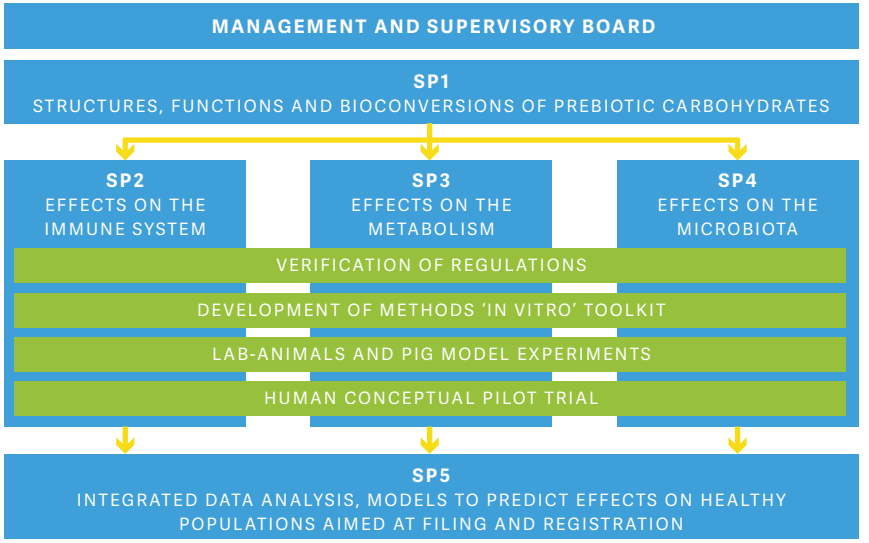
Prebiotics Prebiotics is the term used for carbohydrates built up of 10 or less sugar building units. These carbohydrates are not digested by human enzymes in the digestive track before they reach the colon. They are also called non-digestible carbo-hydrates. Prebiotics naturally occur in a number of vegetable sources, like soy and chicory, but also in human milk. Prebiotics form a good substrate for the probiotics in the colon.

Probiotics is used to indicate the 'good' bacteria living in the gut, in particular Bifidobacteria and Lactobacilli. These bacteria play an important role in the metabolism and reinforce the immune system. To do so, they need to feed on the non-digestible carbohydrates that pass the upper digestive tract unchanged: the prebiotics.

CCC: TOWARDS A HEALTHIER AND SUSTAINABLE SOCIETY

Carbohydrate Competence Center (CCC) generates and develops the interdisciplinary science of carbohydrates with the aim to stimulate innovation and contribute to a healthier and more sustainable society. At the heart of all of the projects are both our industry- driven research questions and excellent research capabilities. This way CCC offers the agro-food industry a unique meeting place where industry and high-quality research institutions are brought together. At CCC, fundamental research and market-oriented innovation lead to new products, new processes and new jobs. It forms an excellent starting point for a leading role in a bio-based economy.

methods and the characterized prebiotic substrates of this subproject were used extensively for further research and testing. These subprojects focused particularly in a coordinated approach on the effects of prebiotics on the mammalian immune system, metabolism and on gut microbiota. A final project was implemented to wrap up the entire programme, by integrating all data and offering researchers and industrial partners methods they can use for further research. At the start of the programme, this project was also meant to validate all techniques and methods in the laboratory, using human and animal trials in order to study correlations. During the programme, however, it turned out that this was not feasible.



SHARING DATA AND FINDINGS

The programme consisted of five subprojects, each with their own goals. However, all participants shared information, ideas and results freely. All CarboHealth participants have contributed in a positive way to joint meetings. The programme was concluded with a symposium, in Zwolle on 30th of November 2017. Three invited keynote speakers shed their light on the subject of carbohydrates and health, while the seven researchers showed the results of their work during their presentations and on posters in the hallway.

KOALA AND BINGO

The original CarboHealth research programme started out with a broad approach and by a funnel procedure. Promising assays and pre- and probiotics would be identified, leading to a human trial. During this process, however, it was concluded that the results obtained were not sufficient to allow such a trial. Besides, such a trial, especially with infants, turned out to be very time-consuming and very expensive and requires a dedicated approach. As an alternative approach, two cohort studies from Radboud University Nijmegen and Maastricht University were added to the CarboHealth programme.

KOALA is a cohort study of Maastricht University, consisting of almost 3,000 children born between 2001 and 2003. The aim of this cohort was to study allergy and asthma as well as growth and development. In total 146 samples of human milk and the faeces of corresponding babies were collected and stored when the infants were one month old. These samples were added to the CarboHealth programme. Results were shared with Maastricht University.

BINGO is a cohort study of the Radboud University Nijmegen, and of a later date. The first babies contributing to this study were born in 2015. While the KOALA samples were collected only once after four weeks of age, the BINGO study provided samples of human milk and faeces, after three, six and twelve weeks of age. The BINGO study focused on biological factors during pregnancy and after birth, that can predict a child's health. This cohort consists of 80 mothers and babies.

ELLEN VAN LEUSEN, PROGRAMME LEADER AND LIAISON FRIESLANDCAMPINA:

‘VALUABLE TOOLBOX’

‘This project led to the creation of a valuable toolbox, which we can all use for research purposes and actual applications. I really hope that further research will prove that prebiotic ingredients are beneficial to people of all ages: not only for infants, as this project nicely shows.’

‘The way we worked together was a good example of the benefits of synergy.’

ALIGNMENT IS KEY

‘In private public collaborations, it is important to realise that everybody has their own agenda. It is important to agree in advance on what the joined plan will be and what everybody will contribute and share. It is also important to take into account each other’s wishes and keep track of that. That is why we decided to evaluate halfway the programme how things were progressing.’

PROUD OF WHAT WE HAVE ACHIEVED

‘One of the great things about CarboHealth was that all scientists used the same methods, samples and substrates, which resulted in valuable, new knowledge, shared by all partners. The way we worked together was a good example of co-creation. In the end, I am very proud of what we achieved: CarboHealth rendered beautiful data as well as relevant leads for all participants. The final symposium in Zwolle combined relevant topics and high-quality research, but it also felt like a party, our CarboHealth party.’

‘CarboHealth rendered beautiful data as well as leads for all participants.’

PROGRAMME LEADER FONS VORAGEN:

‘THERE IS STILL MUCH TO LEARN’

‘I am very happy to say that CarboHealth has been a wonderful example of private public cooperation. In my career I have not often experienced a group of participants this committed and so much in control. They complemented each other very well.’

‘We now know more about what goes on in the colon.’

INTERESTING LEADS

‘We did a good job on studying the compatibility of prebiotics and probiotics and the programme added to our knowledge of what they do in the colon and rendered many interesting leads for further research on the effects on the immune system and metabolism. A new CCC-NWO research programme CarboKinetics follows up on these leads. It is also good to remember that this programme integrated a lot of data delivered by previous CCC programmes. I am pleased how CCC takes on scientific issues in an integral way. This is an important area of research and the coordinated way we are studying this field helps us to understand how intestinal tract, metabolism and immunity are connected.’

PROMISING RESULTS

‘The programme achieved its main goal. We now know so much more about the way carbohydrates and bacteria work together. Looking back, I can say that we obtained many promising results. But what this programme has taught me is that this subject matter is even more complicated than I ever imagined. Even though it is clear that prebiotics have a positive effect on the human body, we still cannot prove exactly how. Circumstantial evidence shows clearly that people with colonic disorders benefit from prebiotics. Our challenge is to find out exactly what goes on in the colon and how we can influence this process for health benefits.’

LUBBERT DIJKHUIZEN, PROFESSOR MICROBIOLOGY UNIVERSITY OF GRONINGEN AND SCIENTIFIC DIRECTOR CCC:

‘LEARNING ABOUT THE ROLE OF BACTERIA IN THE GUT’

‘Not all scientists are used to working with industrial partners. It is important to stress that scientists must always carry out research independently. This programme was all about testing the ingredients the partners provided, allowing them to use the results to improve their own products. This also included the organization of various valorisation meetings to ensure the optimal transfer of the methods and scientific techniques developed by the knowledge institutes to the industrial partners, for use in their own laboratories.’

BACTERIA IN THE LEAD

‘For me as a microbiologist, this is a wonderful time to do research. We are learning so much about the role that bacteria play in our intestinal tract. Over a 1,000 different species of bacteria coexist in a balanced ecosystem. Changes in that system can cause severe problems. We now also know that there is a connection between these bacteria and our brain, which may very well explain disorders like some forms of autism.’

‘We are learning so much about the role that bacteria play in our intestinal tract.’

THE GOLDEN STANDARD

‘An important part of the programme was dedicated to the research into the prebiotic carbohydrates in human milk and infant formula. We wanted to know what these mixtures are composed of and how they impact the bacteria in the gut. Human milk is the golden standard: it contains between 100 and 200 oligosaccharides, which feed the bacteria growing in the baby’s intestinal tract. After birth, this system is still vulnerable: the bacteria protecting the baby

are still developing and growing. They are fed best by the oligosaccharides from human milk. But we also see that the current mixtures of prebiotics in infant formula have a positive effect on the gut.'

'This knowledge will hopefully lead to an even greater decrease of antibiotics use in animal feed.'

REDUCING ANTIBIOTICS USE

'Another theme concerned animal feed. Over the years, the agricultural industry has gradually reduced the amount of antibiotics given to livestock. Antibiotics wipe out a large part of the bacteria in our gut, which is why it often takes so long to regain our vitality. This also goes for livestock. Even worse, due to overuse of antibiotics more and more pathogenic bacteria have become resistant. This Carbo-Health programme also has produced new knowledge about the use of prebiotics to fight pathogens, which will hopefully lead to an even greater decrease of antibiotics use in animal feed.'

SUBPROJECT 1:

STRUCTURES AND FUNCTIONS OF PREBIOTIC CARBOHYDRATES

It is generally known that breastfeeding babies is best for their health. Human milk is the golden standard, because it contains everything infants need to give them a healthy start in life. In the past years, it has become possible to study this biological reference in detail. This has resulted in valuable leads for future developments in infant nutrition.

One of the aims of CarboHealth was to study the effects of prebiotic carbohydrates on health. The goal of this subproject was to analyse a great number of prebiotic carbohydrates, mapping their structures and functions. To do so, various partners provided ingredients for analysis. Researchers analysed these samples and studied their effect on the beneficial bacteria in the gut. Their work resulted in a database with valuable results for the partners as well as for further research. The researchers used complementary analytical techniques to analyse the samples, combining the excellent research capacity of Groningen and Wageningen.

ANALYSIS OF PREBIOTIC CARBOHYDRATES FROM INDUSTRIAL SAMPLES

FrieslandCampina, Winclove Probiotics, Sensus and AVEBE provided carbohydrate samples for analysis in the laboratories of the University of Groningen (UoG) and

HUMAN MILK OLIGOSACCHARIDES (HMOS)

Human milk oligosaccharides (hMOS) are complex, prebiotic carbohydrates that are not digested by the baby itself. Instead they reach the gut intact, where beneficial bacteria consume them. At a molecular level, they consist of complex sugar chains. In total, human milk contains 100 to 200 different hMOS.

FRUCTO-OLIGOSACCHARIDES (FOS)

FOS are a class of prebiotic carbohydrates mainly manufactured by degradation of inulin extracted from chicory roots. Inulin is a naturally occurring carbohydrate, produced by several plants including fruits and vegetables, such as bananas and onions. FOS is widely used to enrich or improve nutritional products.

Wageningen University & Research (WUR). These samples consisted of different mixtures of galacto-oligosaccharides (GOS), fructose-oligosaccharides (FOS) and IsoMalto-/Malto-Polysaccharides (IMMP). These are all different kinds of prebiotic carbohydrates. The work done at this stage of the programme resulted in a novel analytical approach for the identification and quantification of individual prebiotic molecules, a valuable database containing a detailed over-view of the molecular structures and properties to be used to understand their effects on the gut and more specifically on the beneficial intestinal bacteria as investigated by subproject 4 Carbohydrates and the Microbiota.

IN VITRO AND *IN VIVO* EXPERIMENTS

Sander van Leeuwen (SP1), Fangjie Gu (SP1), Markus Böger (SP1&4) and Klaudyna Borewicz (SP4) collaborated in this project on the analysis of the samples provided by the different partners. They were responsible for the in-depth analysis of prebiotic carbohydrates in human milk and infant faecal samples in order to study the fate of these sugars during fermentation by the bacteria in the gut. They carried out their research using complementary techniques available in their respective labs in order to have a more

detailed depiction of individual oligosaccharides present. At a later stage, samples of human milk and baby's faeces from the KOALA and BINGO cohorts were added to the programme. Newly developed and validated NMR and LC-MS methods were not only able to establish the genetic status of the mother in relation to hMOS composition, they also made it possible to monitor the individual in the faeces during the lactation time. Huge differences were observed between individuals as well as between structurally rather similar oligosaccharide isomers. This type of novel and highly relevant information now enables us for the first time to link the consumption of individual hMOS structures as present in the human milk by babies' own microbiota as analysed in subproject 4.

IMPORTANT RESULTS

This subproject developed and improved various NMR (Groningen) and LC-MS-MS (Wageningen) methods, allowing the analysis of specific oligosaccharides which have been overlooked in most previous studies. As intended, this subproject delivered a huge amount of data about prebiotic carbohydrates. These data were shared with the partners that can apply this new knowledge in understanding the gut's microbiotal activity and for the development and improvement of new food products. The data also provided plenty of opportunities for further research within the scope of CarboHealth by the researchers of the various subprojects.

GALACTO-OLIGOSACCHARIDES (GOS)

These are non-digestible prebiotic carbohydrates that are the result of the conversion of lactose from cow's milk by certain enzymes. These prebiotics are widely used in nutritional applications, especially infant formula.

ISOMALTO-/MALTO-POLYSACCHARIDES

The GtfB enzyme is used to produce a new generation of starch derivatives called IsoMalto-/Malto-Polysaccharides (IMMP). These starch derivatives are not easily degraded by human enzymes. IMMP degrade only slowly in the small intestine. In contrast, IMMP are completely degraded by bacteria in the gut which is also expected to stimulate growth of beneficial bacteria (like Bifidobacteria) living in the colon.

SANDER VAN LEEUWEN, POSTDOC (SP 1):

‘GENETIC DIFFERENCES BETWEEN TYPES OF HUMAN MILK’

‘Human milk oligosaccharides (hMOS) are in fact complex chains of sugar molecules which are not significantly consumed by the baby, but reach the gut mostly unharmed to be fermented by the intestinal bacteria. From previous studies we know that hMOS contribute to the baby’s health in a number of ways. They benefit bacteria that keep the gut healthy and they stop pathogens from sticking to the colon wall, preventing infections. In addition, they also have a role in stimulating a balanced immune system.’

FOUR TYPES OF MOTHERS

‘I used samples from the KOALA cohort to identify four types of mothers. There are genetic differences between mothers determining the kind of hMOS they can produce. From the faeces samples we learned that there are three groups of babies. Some babies at four weeks of age have bacteria that consume all hMOS from the milk and others digest only a part of what they are offered. The third group yielded mixed results. From the BINGO cohort, which was used by other CarboHealth research projects, we know that as the babies get older and the bacteria develop further, more babies become like the first group: they consume all hMOS they are given. The next goal is investigating whether this genetic difference in hMOS complexity has long-term health implications for the babies.’

THE NEXT STEP

‘This research helps us to understand the influence of hMOS on the bacteria in our gut. We have seen this influence is not always as direct as we assumed. For the companies it is now possible to take the next step, using our data to develop hMOS to add to their infant formula. They can use our data to substantiate the need and the applications for such additions. What may help is the fact that the composition of faeces samples from formula-fed children from the more recent BINGO study resemble more closely the faeces of breastfed babies. This was not the case with formula-fed children from the older KOALA study where no prebiotics had been added to the infant formula yet.’

FANGJIE JU, PHD CANDIDATE (SP 1): ‘CREATING A MODEL THAT SHOWS PREBIOTIC POTENTIAL’

‘One of my projects concerned a type of starch, called IsoMalto-/Malto-Polysaccharides (IMMP). These starch molecules have been converted by enzymes in such a way that beneficial bacteria like Bifidobacteria are able to ferment them. We put IMMP in a laboratory gut model to simulate the gut fermentation process. We looked at the rate of fermentation and studied what happens during this fermentation. I also compared how the bacteria in the babies’ gut ferment human milk oligosaccharides (hMOS) from human milk.’

‘I found that the concentrations of hMOS in human milk decreased over time.’

TRACKING THE DIGESTION OF CARBOHYDRATES

‘I also studied the composition of oligosaccharides in human milk. For this purpose, I used samples from the KOALA and the BINGO cohorts. We mapped the exact structures of these hMOS and tried to find out their effects on the beneficial bacteria in the gut. Using both cohorts meant I could track how the fermentation of hMOS by infant gut microbiota developed. I found, for example, that the concentrations of hMOS in human milk decreased over time. A similar trend was found in faeces samples. The fermentation of hMOS by infants showed multiple stages and and we observed a huge variation in the utilisation of individual hMOS. Together with a detailed analysis of the gut bacteria of these infants, individual differences can be observed. Looking at the gut bacteria of these infants, we understand better how the hMOS influence the health of the baby’s gut. These data can be used by companies that wish to add specific hMOS to enrich infant formula.’

INFLUENCE OF DELIVERY MODE AND FEEDING MODE

'This research will help us to understand the relation between the ingredients of human milk and the way the baby's gut microbiota develops. In literature people found differences in the gut microbiota babies that were born naturally and babies born by C-section. The latter group starts out with bacteria from the mother's skin instead of her vagina. In our study, together with subproject 4, it was found that differences in the developing stages of hMOS fermentation patterns by the infant guts were influenced by a number of external factors observed in the first weeks of life. One of these factors was delivery mode: whether or not a baby was born naturally or born by C-section. The other factor was whether the baby was breastfed exclusively or was given a mixed diet of human milk as well as infant formula.'

'This research will help us to understand the relation between the ingredients of human milk and the way the baby's gut microbiota develops.'

HENK SCHOLS, PROFESSOR FOOD CHEMISTRY, WAGENINGEN UNIVERSITY & RESEARCH:

‘WE STILL HAVE MANY QUESTIONS’

‘What is the role of sugar molecules and what are they doing in the body? That has always been our take on CarboHealth. So, we have been focusing on individual molecules in the complex oligosaccharide mixtures. Here in Wageningen, we used samples from the BINGO cohort that our colleagues of Radboud University were so kind to provide. These were used to imitate what actually happens in the intestine of a child.’

KIND OF DELIVERY MAKES A DIFFERENCE

‘One of the things we proved was how the growth of the bacterial population in the intestinal tract is influenced by the manner of delivery of the child. All new-born babies need to develop their gut microbiota, starting with the bacteria they are contaminated with by their mother. These bacteria need to be fed and that is why human milk is so rich in hMOS, especially right after birth. Unfortunately, finding correlations between human milk and microbiota is still not easy. We need more evidence to prove these correlations really exist.’

‘The next step is to create a new generation of prebiotic carbohydrates to add to food components meant for infants and the elderly. I also expect breakthroughs with respect to the effects of nutrition on the immune system. It is up to the industry to take this new knowledge further and convert these finding into practical applications and improved or new products.’

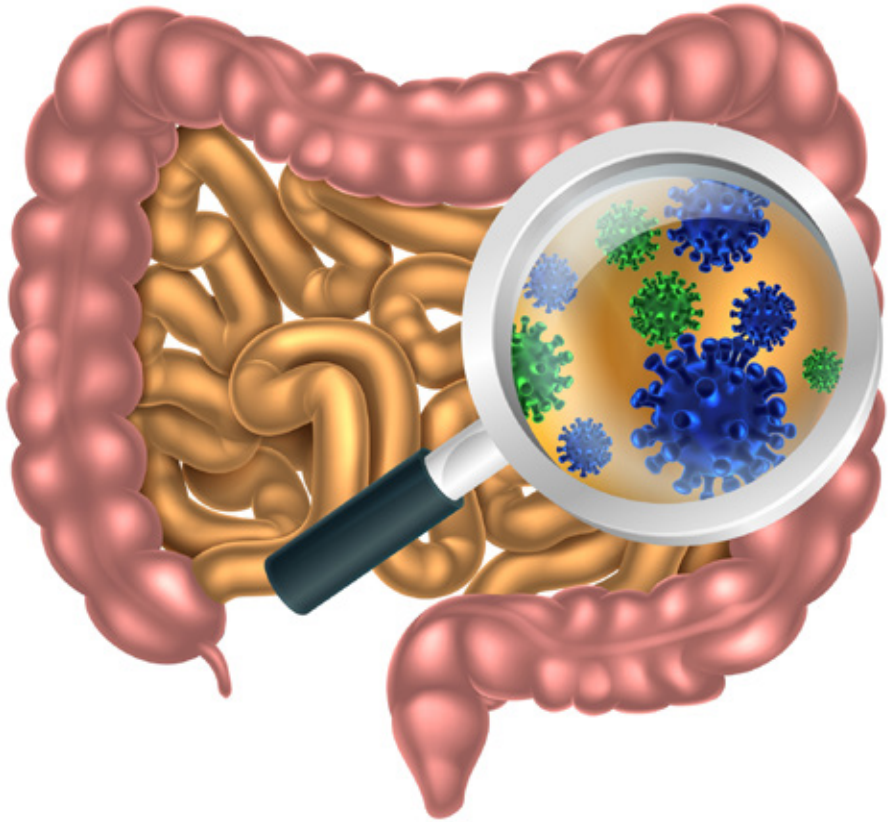
MANY QUESTIONS

‘We still have many questions. For instance, is it beneficial for the child if the mother adds galacto-oligosaccharides to her diet during the pregnancy? What are the effects of early life programming? How can we influence the health of the unborn child by means of healthy nutrition? We observe correlations between disorders of the bowels and premature babies and babies born by C-section, but we cannot prove them yet. We suspect there is a strong correlation between the brain and the bowels, but we cannot yet say with any certainty that disorders like dyslexia, adhd and autism are caused by a misbalance in the bowels early in life. I think there is a connection but proving this scientifically is not yet possible.’

SUBPROJECT 2: CARBOHYDRATES AND THE IMMUNE SYSTEM

To remain healthy and vital, people and animals alike depend on their immune system. In our gut millions of bacteria work together to keep sickness at bay. They prevent malignant pathogens from attaching themselves to the lining of the gut and then flush them out. To do this they need to be fed the right stuff: specific carbohydrates that reach the colon unharmed by our digestive system and beneficial bacterial strains to help the population in the gut along.

This subproject focuses on the health effects of prebiotics and probiotics on the immune system. Like vitamins, prebiotics and probiotics seem to have impact on specific parts of the body. The bacterial strains appear to have different functional roles. Some strains reinforce the colon wall, others help to build the immune system. This works well until pathogens win the battle and the immune system fails. One way to cure a sick body is to administer antibiotics, that wipe out most of the bacteria in the gut: the pathogens as well as the beneficial bacteria. Unfortunately, many pathogens have become resistant to antibiotics, making it increasingly hard to beat them. Perhaps prebiotics and probiotics can do something about



this. Therefore, a better understanding of how they work is required.

This subproject focused on the health effects of a combination of prebiotics and probiotics on the immunity of pigs. More specifically, the project was meant to find out whether such a combination would reinforce vaccination against *Salmonella*. The chosen composition turned out to show most promising results in various *in vitro* trials on the intestinal barrier and with immune cells. According to the toolbox this combination was most promising. Whether this is true was tested in this trial.

The piglet trial turned out to be successful. Not only were the piglets better equipped to deal with a *Salmonella* infection, their general health was better than that of the piglets that had not been given prebiotics and probiotics. The conclusion of this subproject is that a diet intervention with this particular combination of prebiotics and probiotics seems to be beneficial for the health of pigs.

NICOLE DE WIT, POSTDOC (SP 2):

'CREATING NEW KNOWLEDGE'

'Being part of a public private project means you are actively creating new knowledge that may benefit science, business and society. I really enjoyed that part of this project. Everybody was very committed, and the meetings were pleasant as well as challenging.'

'One probiotic strain showed a highly positive effect on intestine integrity.'

THE EFFECT OF PROBIOTIC STRAINS ON THE IMMUNE SYSTEM

'For me the showpiece of this programme was finding leads in the laboratory and validate in an animal model that probiotic strains really affect the immune system. We set up a number of tests to screen health effects on the intestinal barrier. The first part of this research used an *in vitro* model of a human intestinal system. Intestinal cells were grown to form a strong barrier between the food stuffs that reach the intestine and the components that will ultimately be absorbed by the body. Then we subjected this system to the prebiotic and probiotic compounds provided by several industrial partners in CarboHealth and we tested what their effect was on the integrity and permeability of the intestinal barrier.'

'The problem with prebiotics and probiotics is that it is very hard to prove how they work in the human system.'

LACTOBACILLUS W37 BACTERIUM

'Of the three probiotic strains we tested, one (W37) showed a highly positive effect on intestine integrity. This *Lactobacillus* strain also caused changes in gene expression of the intestinal cells, which may mean that

these bacteria stimulate the intestine to produce more M cells. This effect benefits the immune system and can support oral vaccination. That is why we used this strain in an experiment involving the vaccination of piglets against *Salmonella*.'

'The in vitro toolbox developed in this project is a valuable tool to preselect the most promising prebiotics and probiotics that can then be tested in a human study.'

HARD TO TEST ON HUMANS

'The problem with prebiotics and probiotics is that it is very hard to prove how they work in the human system. You cannot look into the intestine to see what is going on. People are also quite variable in their reactions to dietary interventions. When one person reacts in a positive way to a prebiotic fibre, his does not mean that everybody benefits in the same way. This depends on many things, including for instance, the diet that people normally eat and the bacteria that are already present in their gut. Because people can react so differently, you need to test the reactions of many people to get a good and general idea on the effects of prebiotics and probiotics in humans. The *in vitro* toolbox developed in this project is a valuable tool to preselect the most promising prebiotics and probiotics that can then be tested in a human study. Because in the end you want to make sure it works in people! We also have to keep in mind that there is a major difference between research into nutrition and pharmacy. To administer a drug is like hitting with a hammer, while a food intervention can only tickle the system. Food is almost never black or white: there are so many grey areas to consider.'

ALEXIA LEPINE, PHD CANDIDATE (SP2): ‘LOOKING FOR EFFECTS ON THE IMMUNE SYSTEM’

‘For me, with an engineering degree in food science, this was a very interesting programme. I liked working with partners like Sensus, Winclove Probiotics and Trouw Nutrition. I never felt limited in my scientific approach. Everybody was really helpful and whenever I asked for support I got it.’

‘The gut lining provides an important barrier in your bowels.’

THE LINING OF THE GUT

‘My part of the programme concerned the influence of prebiotics and probiotics on the lining of the gut and the associated mucosal immunity. This lining provides an important barrier in your bowels. If it is strong enough, harmful bacteria cannot pass into your bloodstream. If bacteria, or any compound, do get through, they can cause all kinds of problems, like infections and allergies. What we wanted to find out is how prebiotics and probiotic bacterial strains help this lining to remain strong.’

‘We built a complex model to mimic the gut lining in the laboratory, in vitro.’

COMPLEX MODEL

‘We built a complex model to mimic the gut lining in the laboratory, *in vitro*. The predictive accuracy of this model could later be tested in an experiment with newly-born piglets. We chose piglets because they react a lot like human babies: their immune system is rather similar. We divided the piglets into four groups. All groups were fed differently from birth. One group received a mixture containing the *Lactobacillus* strain called W37. We had

already found this to be the most promising strain with the addition of prebiotics, the so-called long-chain inulin carbohydrates. A second group was also fed with the inulin but without the W37. These two groups were vaccinated against *Salmonella* when they were three weeks old. Half of the piglets that were not fed any additional compounds were also vaccinated. 52 days after birth all four groups were infected with *Salmonella*.'

'The combination of the fibre and W37 strengthen the piglets' response towards the vaccination independently of microbiota.'

EFFECT OF THE DIET INTERVENTION

'The goal of this experiment was to prove that this diet intervention has an actual effect on the immune system. We wanted to see if it was possible to indirectly measure a better protection of vaccinated piglets with the dietary intervention as measured by blood parameters. We saw most effects in the quantity of antibodies that specifically target and protect against *Salmonella*. We also found some correlations between this intervention and the effects on the microbiota of the piglets, but we could not explain fully the benefits of the interventions. There is a direct immune effect: the combination of the fibre and W37 strengthen the piglets' response towards the vaccination independently of microbiota.'

PAUL DE VOS, PROFESSOR IMMUNO-ENDOCRINOLOGY UMCG: ‘A TOOLBOX OFFERING MANY OPPORTUNITIES FOR STUDY’

‘We used the funnel approach to select a particular combination of prebiotics and probiotics to test in a group of new-born piglets. This was not easy, as little is known about the immune system of pigs. The results were very promising: not only did this dietary intervention strengthen the effect of the vaccination against *Salmonella*, the piglets were visibly healthier than the piglets that were not fed prebiotics and probiotics. It is now up to the industry to use this knowledge to improve the feed they develop for livestock.’

MORE QUESTIONS

‘This particular dietary intervention was only one of many we could test. The toolbox this programme delivered, contains many opportunities to study other combinations and effects. As a scientist I am never done asking questions. For instance, we think the probiotic strain we used in this trial also has effects on the metabolism of pigs, but we could not test this within the scope of this programme. Doing so would require more time and more funds.’

INFORMATION TO THE GENERAL PUBLIC

‘One of the strengths of CCC is that as a scientist you get to work closely with industrial partners. This leads to great projects and knowledge that is shared widely beyond academia. In addition, it is absolutely necessary to improve the information about carbohydrates to the general public. Just telling people to eat more fibres is out of date. We need to tell people about how many different fibres there are, all with their own properties. People over seventy need different fibres to protect their microbiota than young people. When the mucus layer in their intestinal tract becomes thinner, it is easier for pathogens to take hold. It is important to determine which prebiotics can be used to repair this layer, reinforcing the immunity against illness. Currently, the information about such matters falls short.’

SUBPROJECT 3:

CARBOHYDRATES AND METABOLISM

How can indigestible carbohydrates influence the intestinal microbiota in such a way that our metabolic health benefits? Can these fibres reduce the risk of developing metabolic syndromes, like obesity, type II diabetes, insulin resistance or atherosclerosis? And if these effects are found, what are the best fibres to feed the bacteria in the gut? And which bacteria are most beneficial? This CarboHealth subproject focused on the effects of carbohydrates on our metabolism.

From the start, this subproject had three objectives: to determine the impact of carbohydrates the programming of disease, the effect on the modulation of an established disease risk and the dependency of a health effect of carbohydrates on the intestinal microbiota. To do this, the researchers used ingredients supplied by several industrial partners. At a later stage, they also used the results of the analysis of new carbohydrates, acquired in the first subproject.

FEEDING BACTERIA

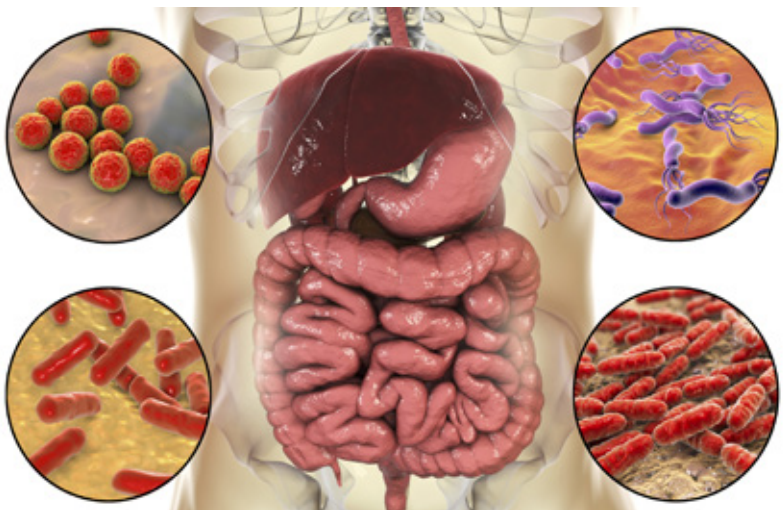
One of the major questions of this subproject was how carbohydrates interact with the bacteria in the intestinal tract. A lot of species of bacteria are active in the gut, and a few of them were selected for further study. These bacteria were cultured in the laboratory under conditions that resemble what goes on in the gut. Specific carbohydrates were added to find out how these bacteria reacted. It is generally known that these prebiotic carbohydrates stimulate digestion, keep the gut clean and prevent inflammation. In this subproject a number of prebiotics were tested on the bacterial strains in order to find out which fibres make the bacteria grow best. The main objective of course was to identify which carbohydrates have most impact on health. In this, the research succeeded: the CarboHealth toolbox now contains data about specific carbohydrates that have a positive effect on specific bacterial strains.

SIMULATING THE HUMAN GUT

In addition to the *in vitro* laboratory research, this sub-project also tested the effects of dietary fibres on intestinal bacteria of mice. The researchers fed them a diet that resembles the regular western diet many people are used to: high in fat. One group of mice was fed this diet, supplemented with indigestible carbohydrates. These mice didn't gain as much weight as the ones that were not fed extra fibres. They were also healthier in general than the other mice. Not all fibres had the same effect, but some of them certainly improved the health of the mice. Adding fibres to food thus appears to have a positive effect on the metabolism, reducing the risk of becoming obese or contracting metabolic syndromes.

UNDERSTANDING THE MECHANISM

Of course, it is important to understand the mechanism behind this process: how do these carbohydrates influence the bacteria? What is exactly going on in the gut? How do these positive effects come about? This part of the study is still underway. Being able to produce a chain of evidence that explains these effects, could very well be the foundation of a strong case for an EFSA health claim. This is still in the future: first this mechanism must be identified in the human body as well.



SYNBIOTICS

This programme proves that adding prebiotics or probiotics to food components have a certain effect on health. What happens when both are added? That is the field of synbiotics, a field that is still largely unknown territory. To apply synbiotics well, it is very important to know which prebiotics and probiotics work best together.

RIMA MISTRY, PHD CANDIDATE (SP 3):

‘THIS RESEARCH COULD SAVE LIVES’

‘It is my job to study the impact of dietary fibres on the bacteria in the gut, leading to a healthier metabolism. I also looked into how dietary interventions can influence diseases like obesity, diabetes and non-alcoholic liver disease. We used the results from other subprojects. In fact, we translated the *in vitro* work of other project members into *in vivo* tests. The results were positive. For instance, we now know which fibres can prevent fat accumulation or reduce the risk of inflammation.’

‘We now know which fibres can prevent fat accumulation or reduce the risk of inflammation.’

‘It would be wonderful if this programme helps prevent people from becoming sick.’

PINPOINTING THE MECHANISM

‘We are not finished yet: we still cannot pinpoint exactly how the basic mechanism of fibres, bacteria and metabolism works. Understanding this principle would be a huge breakthrough. It would mean that the industry can add fibres to food components that will improve the health of people at risk. It could even save lives. It is still early days, but if you could incorporate specific complementary carbohydrates in your diet to improve health, this would be very interesting for people who run a high risk of developing a metabolic syndrome. ’

MARKUS BÖGER, PHD CANDIDATE (SP1&4):

'PROMISING RESULTS FOR FUTURE RESEARCH'

'At present, there is a lot of attention for prebiotics as potential replacers for antibiotics. Yet the function of most prebiotics in the commercial market is yet unknown. That is why we analysed the complete structural composition of carbohydrates provided by the CarboHealth partners and studied stimulatory effects on growth of selected probiotic bacteria.'

'Only a limited number of components present in carbohydrates had been consumed by bacteria.'

STUDYING CARBOHYDRATES AND PROBIOTICS

'Analysis revealed that the carbohydrates tested, consisted of many individual components, differing in size and the way they are linked to each other. For example, in Vivinal-GOS (FrieslandCampina) we identified over forty structurally different components. After we had grown probiotic bacteria as single cultures on the commercial carbohydrates, we found that only a limited number of components present in carbohydrates had been consumed by bacteria. In addition, these bacteria differ in components used. To find out why certain carbohydrates stimulate growth more than others, we looked at the enzymes that bacteria use to degrade the carbohydrates.'

'Knowing the pathways means we can predict which prebiotics are best for which probiotics.'

HOW BACTERIA BEHAVE IN THE GUT

'We also put different bacteria together to find out wat is happening: we saw that some bacteria benefited from each other, but others were in fierce competition. This

gave us more insight into how the bacterial world of the microbiota works. Knowing the pathways means we can predict which prebiotics are best for which probiotics. This knowledge is valuable for further research in how the total microbiota functions. The industrial partners can apply our findings to improve the composition of their products. In all, this hopefully leads to clear health effects.'

'This knowledge is valuable for further research in how the total microbiota functions.'

PROMISING RESULTS FOR FUTURE RESEARCH

'These results overall show that commercial carbohydrates comprise components that can be utilized by multiple probiotic bacteria. These observations are of particular interest for the probiotic industry which is selling combinations of probiotics and therefore can now select single prebiotics that match the abilities of all probiotics added to the mixture. The results further highlight the individual role of gut bacteria in degrading carbohydrates. Future research will further explore the specific abilities of gut bacteria to utilize carbohydrates, in particular how combinations of prebiotics and probiotics lead to effective synbiotics.'

'These results overall show that commercial carbohydrates comprise components that can be utilized by multiple probiotic bacteria.'

UWE TIETGE, ASSOCIATE PROFESSOR PAEDIATRICS, UMCG:

‘CARBOHYDRATES SHOW HEALTH BENEFITS IN PRECLINICAL MODELS OF THE METABOLIC SYNDROME’

‘In my view, CarboHealth was a good example of a well-working consortium. The first thing we did in tour subproject, was confirming that indigestible carbohydrates are safe and do not create major harmful metabolic side effects *in vivo*. Even more interesting, in our mouse model for metabolic syndrome, they were beneficial for plasma lipid levels and, as Rima’s research shows, they help keeping body weight down and the metabolism in a healthy state. If we can prove that adding these fibres to the human diet improves insulin resistance, prevents us from gaining more weight and reduces the accumulation of fat in our liver, then that would be good news for our health.’

‘CarboHealth was a good example of a well working consortium.’

CONVERTING STARCH INTO PREBIOTICS

‘It is also important to know how we can change carbohydrates, like with the starch molecules provided by AVEBE, to achieve beneficial effects on the microbiota. Shaping the composition and function of our intestinal bacteria is a more and more recognized means of tuning the balance

between health and disease. However, before our industrial partners are allowed to claim that adding these fibres to their products is good for our health, we would still need to make the step to a human trial. We thus have to provide a chain of evidence that also incorporates showing exactly the mechanism behind such a claim. A proof of concept trial would therefore be desirable to show how carbohydrates influence human health by stimulating growth of bacteria perceived as beneficial in the human gut.'

'I think that novel indigestible carbohydrates have a good chance for being beneficial supplements without major side effects.'

CONVINCED OF THE BENEFICIAL EFFECT OF CARBOHYDRATES

'Personally, I think that novel indigestible carbohydrates have a good chance for being beneficial supplements without major side effects. To continue in the line of Carbo-Health, I would be interested in setting up a short-term trial with metabolic syndrome patients to test whether an intervention with dietary fibres improves insulin resistance and leads to weight loss, similar to our preclinical results. Ultimately, I think that the field of nutrition also needs well-designed long-term prospective randomized controlled trials to provide hard scientific evidence for health benefits.'

HENKJAN VERKADE, PROFESSOR OF PAEDIATRICS, UMCG

‘PREVENTION IS THE KEY’

‘As a paediatrician, it is my concern that children can become healthy adults. To achieve this, prevention of illness is very important. Very early in life, even before conception, our bodies are already subject to factors that can lead to health problems. This mechanism is called programming. Programming can be positive and negative.’

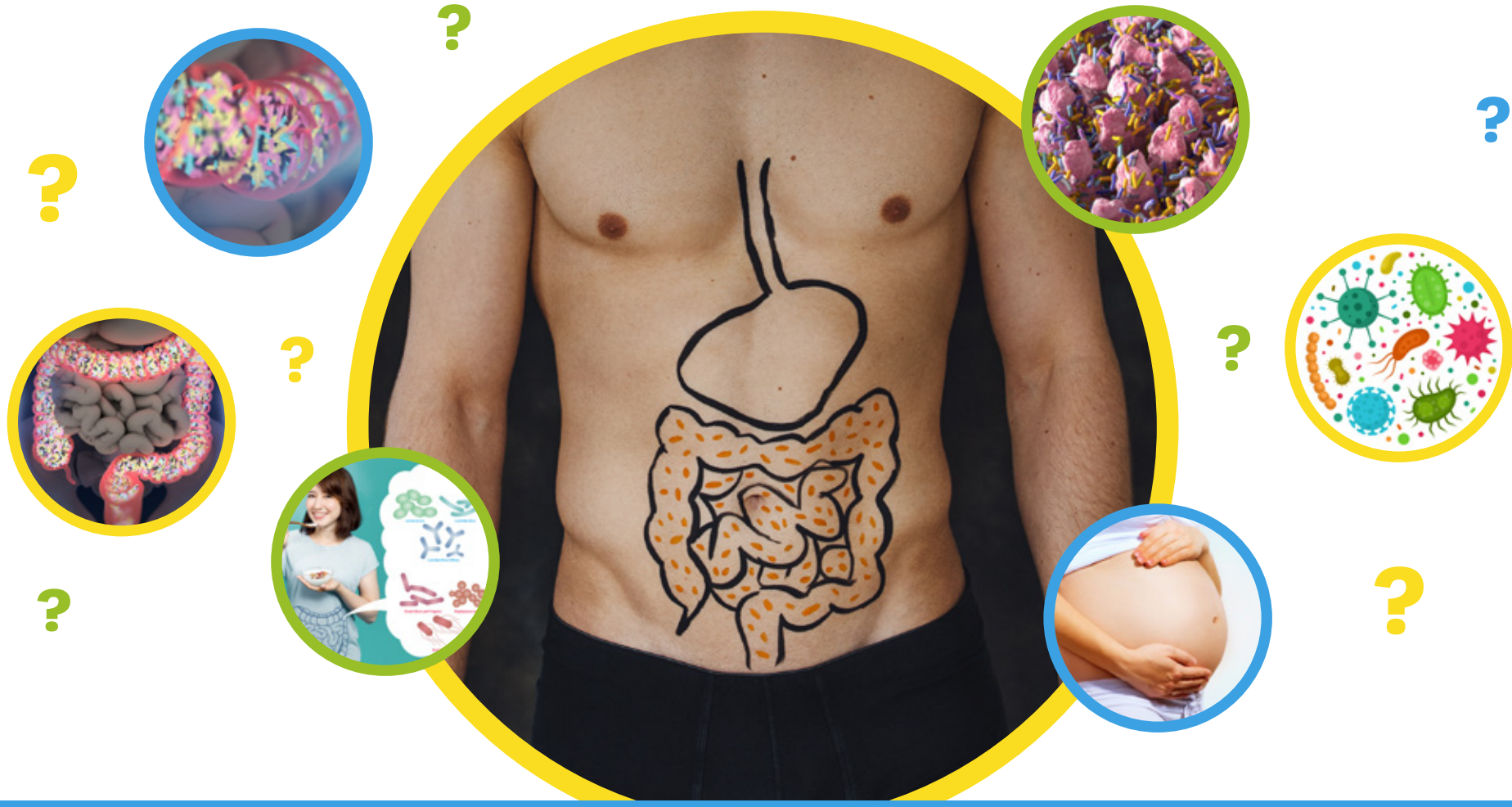
‘Very early in life, even before conception, our bodies are already subject to factors that can lead to health problems.’

EFFECTS OF BREASTFEEDING

‘One of the things we are looking at in CarboHealth is the effect of breastfeeding babies on health later in life. We know that the oligosaccharides in human milk affect the bacteria in the gut and, directly or indirectly, the immune system. In this project we want to discover the relation between these indigestible carbohydrates and our health. The research resulted in a toolbox which provides promising leads for further research into infant nutrition.’

DIET INTERVENTION

‘Since this kind of research takes years, we cannot test the effect of carbohydrates in the long run on humans. We cannot wait sixty years for the results. We chose to test how mice on a western high fat diet reacted to the addition of fibres to their food. We also tested a combination of prebiotics and probiotics. This way we can predict accurately the effects of this dietary intervention. If we can target specific factors or biomarkers with effects on health, we can set up short term human trials, to discover whether these effects can be found in humans as well.’



SUBPROJECT 4: CARBOHYDRATES AND THE MICROBIOTA

The human gut teems with microbial life: trillions of bacterial cells share our intestinal tract with other tiny life forms, including archaea, viruses and fungi. All of them are competing, but also collaborating intensely to stay alive and to multiply. Little is known about the function of individual strains. Some we call beneficial bacteria, because it is known - or at least suspected - that they can protect their host from pathogenic infections, improve the metabolism and strengthen the host's immunity. This subproject looked into what actually goes on in the gut. How do bacterial strains differ from each other? Which 'food' do they prefer? What are their functions? And how do they coexist?

No colon is the same when it comes to the composition of the microbiota. Each individual person and animal carries a unique community in his gut. This community largely determines our physical and mental health. In relation to the latter, there is growing evidence that a slight imbalance of bacteria in the intestinal tract can lead to disease, stress, anxiety and even depression. Our gut needs to remain in balance, while being bombarded by outside influences.

LEARNING ABOUT INFLUENCES ON THE MICROBIOTA

The goal of this subproject was to study the effects of prebiotics and probiotics on gut microbiota composition and function in humans and using animal and *in vitro* models.

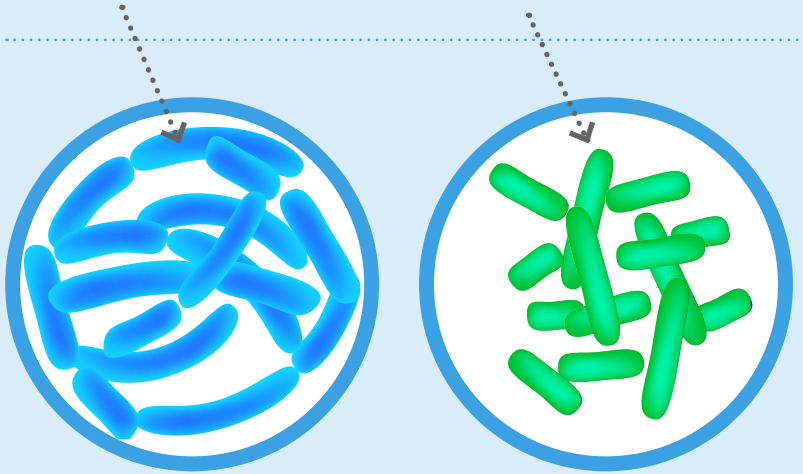
The human studies investigated the role of naturally occurring human milk oligosaccharides (hMOS) and commercially available infant formulas on faecal microbiota in healthy infants. *In vitro* the modulatory properties of IsoMalto/Malto-Polysaccharides (IMMP). The piglet trials tested the effects of a prebiotic (inulin) and a synbiotic (a mixture of inulin and a probiotic strain, namely *Lactobacillus* W37) on microbiota composition and vaccine efficiency.

BALANCE OF THE GUT AS WELL AS IMMUNITY AND METABOLISM OF THE HOST

In general, our studies aimed to investigate the influence of various prebiotics, probiotics or synbiotics on the balance

of our gut, as well as the host's immunity and metabolism. They also aimed to identify which carbohydrates stimulate which specific bacteria and which prebiotics could be used to stimulate beneficial bacteria like **Lactobacilli** and **Bifidobacteria**. The studies on IMMP confirmed the prebiotic potential of these novel starch derived compounds. The results could be used as foundation for further research leading to development of commercial products that could be used as functional ingredients by the food and feed industry. The modulatory effects varied, depending on the IMMP chemical structure, which may be interesting to investigate further. Together, the studies made it possible to gather more knowledge that in the future could help to develop personalised nutrition targeted interventions.

BENEFICIAL LACTOBACILLI AND BIFIDOBACTERIA



These strains of bacteria are called beneficial, because they help clean the gut and fight pathogens. Lactobacilli convert fibres into lactic acid, Bifidobacteria produce lactic acid as well as acetic acid. These acids prevent harmful bacteria to multiply quickly, or not at all.

INSIGHT INTO BABIES' MICROBIOTA

Both the KOALA and BINGO birth cohort studies were used. In the CarboHealth programme the studies aimed to gain more insight into the role that different hMOS structures play in the development of the intestinal microbial community in early life. The link between hMOS composition and changes in the composition of the microbiota in the faeces of the corresponding breastfed infants was studied. In addition, the relation between microbiota composition and the extent of intestinal hMOS metabolism was investigated, and it was found that various Bifidobacteria and Lactobacilli preferentially degraded certain hMOS.

KLAUDYNA BOREWICZ, PHD CANDIDATE (SP4): **'THE IMPACT OF DIET INTERVENTIONS ON THE MICROBIOTA'**

'I was involved in studies revolving around gut microbiota in humans and animal models. I was responsible for processing samples in the lab, analysing data, preparing written reports, making posters, writing manuscripts and distributing the findings during meetings and conferences. It was very interesting to see how different prebiotics and probiotics impacted the balance of the gut of humans and animals. For this purpose, we designed a number of experiments, including the animal and *in vitro* trials. In addition, we also integrated findings obtained in other subprojects to gain better understanding of the effect that intestinal microbiota plays in hosts' metabolism and immune function.'

'The addition of prebiotics to infant formula increases the abundance of beneficial bacteria.'

'It was very interesting to see how different prebiotics and probiotics impacted the balance of the gut of humans and animals.'

DIET INTERVENTIONS

'My favourite results came from the studies on infants, where we saw a large difference in microbiota between infants who received formulas that were fortified with prebiotics (GOS/FOS) and those who were fed formulas without prebiotics. The prebiotic group also showed faecal microbiota profiles that were very similar to faecal microbiota profiles of the breastfed infants. Thus, our findings showed that the addition of prebiotics to infant formula really does what it is supposed to do: it increases the abundance of beneficial bacteria in the intestines of the babies that are not breastfed.'

'I expect that the attention of science and industry will shift to producing compounds based on hMOS.'

THE HMOS OF THE FUTURE

'Looking to the future of infant nutrition, I expect that the attention of science and industry will shift to producing compounds based on hMOS. It is already possible to isolate compounds from cow's milk that could serve as a natural source for future products.'

'We still have so much to learn.'

VARIABILITY

'One of the most striking observations for me, which was common to all studies on microbial ecosystems in both humans as well as various animal species, was the large variation between individuals. Even in mice that were bred to resemble each other, we saw a lot of differences in the microbiota and how the animals responded to the different diets and dietary supplementations This teaches us how complicated this area is. We still have so much to learn.'

HAUKE SMIDT, PROFESSOR OF MICROBIOLOGY, WAGENINGEN UNIVERSITY AND RESEARCH:

‘PLENTY OF LEADS FOR FURTHER RESEARCH’

‘Working in a consortium with so many different partners enabled us to set up a varied toolbox programme containing data, methods and tools that can be used to show us how certain prebiotic interventions work and how they impact the microbiota and the immune system. From different areas, groups joined CCC to discuss these matters and to collaborate with the industrial partners that want to apply this new knowledge. This was more than a purely scientific programme, but also a project benefiting industrial partners who wish to gain more knowledge about the ingredients in their own products.’

COMPLETE PICTURE

‘This particular subproject concerns the complete picture of the community of microbiota in the gut. How do various substrates influence this community? This subproject was flooded with samples and it was quite a challenge to make the right choices. In the end, we succeeded in getting positive results and plenty of leads for further research.’

NEW KINDS OF CARBOHYDRATES

‘One of the things we looked at, were new kinds of carbohydrates, IMMP, of which the properties were still unknown. So, we gathered a lot of knowledge about how these new carbohydrates work. We studied how these carbohydrates are degraded in the gut and what happens to them afterwards. Gathering these data will help the industrial partners to improve their own products.’

DESIGNING NEW INTERVENTIONS

‘Of course, we want to know how we can contribute to health in general. But it is hard to prove the effects of prebiotics in healthy adults whose microbiota community is stable and resilient. In the end, these data will help us to design interventions to prevent vulnerable groups of people to improve their health.’

PARTNERS IN CARBOHEALTH

Within CarboHealth University of Groningen, UniversityMedical Center Groningen, Wageningen University & Research and Wageningen Food & Biobased Research collaborated with six industrial partners. These are: Royal FrieslandCampina N.V. (owned by Zuivelcoöperatie FrieslandCampina U.A.), coöperatie AVEBE U.A. , Nutricia Research B.V. (part of DanoneNutricia Research), TrouwNutrition (a Nutreco company), Sensus B.V. (part of Royal COSUN) and Winclove B.V. At a later stage Radboud University Nijmegen and Maastricht University joined the programme, each providing samples of two cohorts of mothers and babies. This research was financed by participating partners and allowances of the TKI Agri&Food programme, Ministry of Economic Affairs and coordinated by Carbohydrate Competence Center.

At the heart of the CarboHealth programme was the close cooperation of science and industry. The industry provided established or potentially new ingredients the scientists used in scientific research. Most participants were present at informative meetings, where results were presented, and new ideas were discussed. Together they achieved the main objective of CarboHealth: the creation of a valuable technology platform about prebiotics, probiotics, the role of microbiota and health. Data, results and methods were shared freely and have already led to promising leads for further research.

ROYAL FRIESLANDCAMPINA N.V.

Royal FrieslandCampina N.V. is fully owned by Zuivelcoöperatie FrieslandCampina U.A. With 18,645 members in the Netherlands, Germany and Belgium it is one of the world's largest dairy cooperatives. FrieslandCampina produces and sells consumer products such as dairy-based beverages, infant nutrition, cheese and desserts in many European countries, in Asia and in Africa via its own subsidiaries. Dairy products are also exported worldwide from the Netherlands. In addition, products are supplied to professional customers, including cream and butter products to bakeries and catering companies in West Europe.

FrieslandCampina sells ingredients and half-finished products to manufacturers of infant nutrition, the food industry and the pharmaceutical sector around the world.

FrieslandCampina has branch offices in 34 countries and their products find their way to over 100 countries. The company's central office is based in Amersfoort, the Netherlands. The company is divided into four market-oriented business groups, focussing on Consumer Dairy, Specialised Nutrition, Dairy Essentials and Ingredients.

ELLEN VAN LEUSEN, SENIOR RESEARCH MANAGER, FRIESLANDCAMPINA:

‘EVERY LEAD BRINGS US A STEP FURTHER TOWARD HEALTH’

For FrieslandCampina this programme, like the previous CCC programmes, is complementary to what we do in our own laboratories. Our main objective is to learn as much as possible about our own ingredients, especially about those we are still developing. We want to understand how these ingredients influence human health. This programme helps us to determine their nutritional value.’

‘This programme is complementary to what we do in our own laboratories.’

BINGO AND KOALA

The research into these samples has been very successful: we have gained knowledge about the value of the galacto-oligosaccharides that we produce and add to our infant formula. The data provide chemical insights as well as more knowledge about the influence of prebiotics on the microbiota of babies. We were pleasantly surprised to hear that this research proves that our galacto-oligosaccharides are of great value for their application in infant formula.’

PROPERTIES OF HUMAN MILK

'We are happy to learn more about the oligosaccharides in human milk and the way they affect the microbiota. These insights are very relevant for future developments regarding nutritional products. In the long run, as much more research is needed, I expect we can use these data not only within the context of infant nutrition but also for other target groups, who can benefit from the effects from prebiotics and/or probiotics.'

'The idea we can help the very young and the elderly to remain healthy by means of prebiotics and probiotics is very interesting.'

TRYING TO LOOK BEYOND THE HORIZON OF KNOWLEDGE

'We are not done yet: there is so much more to learn. We are at the border of what is known and cannot look beyond the horizon yet. But each lead takes us one step ahead, learning more about how we can improve the health of

the very young, whose immune system is still developing, as well as the vulnerable elderly, whose immune system is not as alert as it once was. The idea we can help both groups to remain healthy by means of prebiotics and probiotics is very interesting. That is why FrieslandCampina takes part in both follow-up CCC programmes: CarboBiotics as well as CarboKinetics.'

NUTRICIA RESEARCH B.V.

Nutricia Research B.V. (part of Danone Nutricia Research) pioneers in nutritional solutions for those with special nutritional needs. Over 500 employees with passion, drive and expertise work on life science, food science and technology. It is the belief of this company that all consumers and patients deserve optimal nutritional support. For over 120 years the company has been pioneering in nutritional solutions. Research and innovation continually evolve, building upon our insights into nutrients and their integral role in optimal health. These insights are used to tailor evidence-based nutritional solutions for infants, young children, pregnant women, patients and the elderly, supporting them throughout their lives.

JEROEN VAN BERGENHENEGOUWEN, SENIOR SCIENTIST, GLOBAL CENTRE OF EXCELLENCE IMMUNOLOGY:

'ESPECIALLY INTERESTED IN THE KOALA AND BINGO ANALYSIS'

'CarboHealth offered us the opportunity to gain more knowledge about the use of indigestible oligosaccharides in applications of our 'Early Life Nutrition' and 'Advanced Medical Nutrition' divisions. Thanks to this public private programme, it was possible to test a variety of fibres for their effect on the microbiota in general and on specific intestinal bacteria in particular. At a research centre like ours, we need these insights to make rational choices for prebiotic, probiotic and synbiotic concepts, of which the interaction between fibres, probiotic bacteria and host are important.'

INTERESTED IN KOALA AND BINGO RESULTS

'We did not provide our own ingredients to the programme, but we are very interested in the analysis of the human KOALA and BINGO cohorts. These results give us more insight into how human milk determines the development of the composition of the microbiota of infants. At a later date, these data may well be used to carry out research into the development of allergies and other non-communicable diseases. We think future research into these data should be correlated with clinical data concerning, for instance, the development of non-communicable and inflammations.'

FUTURE RESEARCH

'We shared these results widely with several stakeholders within our own divisions and are planning to present these data during an internal symposium. Possibly these insights will help us to develop more personalized fibre mixtures in the future.'

'These results give us more insight into how human milk determines the development of the composition of the microbiota of infants.'

SENSUS B.V.

Sensus B.V. is part of Royal COSUN, an agro-industrial cooperative with the ambition to optimally use vegetable raw materials and achieve excellence in sustainability. Royal Cosun manufactures ingredients for food, non-food applications and the chemical industry.
Sensus produces and supplies innovative ingredients based on soluble fibre from chicory roots, especially various types of inulin and oligofructose. The chicory seeds are sown in spring, the plant stores inulin in the roots, and this is harvested and extracted in the autumn to give powders and syrups with dietary fibre.

There is much scientific evidence for physiological benefits of chicory root fibres for improved digestive health, blood glucose management, bone health and weight management. They are prebiotic, giving a strong bifidogenic effect on the intestinal microbiota, and are also excellent substitutes for sugar and fat with sweetening and texturizing properties. Thanks to their qualities, these ingredients are extremely suitable as part of a healthy and responsible diet.
Sensus is active globally, with sales offices in Europe, North America and Asia and has two production locations in the Netherlands, in Roosendaal and Zwolle. Not only do they make standard products, but they also develop entirely new concepts and applications in cooperation with customers. The customers include professionals in the food industry, especially in the bread & baked goods, dairy, breakfast cereal and cereal bar sectors.

ELAINE VAUGHAN, SCIENTIFIC & REGULATORY AFFAIRS IN SENSUS B.V.:

'AN OPPORTUNITY TO LEARN MORE ABOUT OUR INGREDIENTS'

VALUABLE INSIGHTS IN THE MECHANICS OF OUR INGREDIENTS

'When I took over from my predecessor, who retired, the CarboHealth programme was already halfway. We became involved in the programme for several reasons, especially the opportunity to learn more about our ingredients role in human health such as impact on immunity, serum lipids and the human gut microbiome. There were several subprojects within the programme, some of which were more directly relevant to Sensus ingredients than others. Most subprojects were scientifically valuable for Sensus. For example, the subproject investigating the effect of human milk versus types of formula milk – to which inulin is often added as prebiotic - on the gut microbiota of infants. This subproject showed that modern formula milk gives a better bifidogenic effect - more like human milk - than older kinds of formula milk without prebiotics. While Sensus is focused on human health, we also valued the immunity study with piglets. Now at Sensus we do not encourage animal trials for human research, but in the case of the piglet study, the trial was to improve the piglets' health upon weaning, and so it supported research for both animals and humans.'

'We are also pleased to learn that specific Lactobacilli in the human intestine can grow on prebiotic inulin.'

INTERESTING COMBINATION OF PREBIOTICS AND PROBIOTICS

'Scientifically, we were very interested in hearing about the effect of both long chain inulin as well as the combination of the probiotic W37 of Winclove Probiotics and our inulin on piglets. We need to learn the mechanisms underlying

immune health effects, so we can apply this knowledge in our own research projects and support the health benefits of chicory root fibre. We were really pleased to learn that inulin helped the piglets to overcome *Salmonella* infection more easily and improved the feed efficiency, and there were synergistic effects with the probiotic too for enhanced immunity.'

'The programme gave us more insights in the mechanisms of our chicory root fibre ingredients on health.'

VALUABLE INSIGHTS ABOUT INULIN

'We are also pleased to learn that specific Lactobacilli in the human intestine can grow on prebiotic inulin, and also in such a manner that other gut bacteria may also grow. That is a valuable insight for us to explain the growth of Lactobacilli due to prebiotic inulin. The bifidogenic effect of inulin, which underlies its prebiotic effect, is well established. Still here also new insights were generated showing the preferential manner of digestion of inulin by intestinal Bifidobacteria. We also learned about the impact of inulin on the gene expression of the intestinal tract, which is really pioneering research using the latest molecular technology'.

JOINING CARBOKINETICS

'All in all, the programme delivered interesting results that gave us at Sensus more insights in the mechanisms of our chicory root fibre ingredients on health. Working on this project together with the academics was very enjoyable and we all learned from one another. That is why we decided to join CarboKinetics, one of CCC's new programmes.'

TROUW NUTRITION

Trouw Nutrition (part of Nutreco company) is the global leader in innovative feed specialities, premixes and nutritional services for the animal nutrition industry. The company provides species-specific nutritional solutions, consisting of feed concepts, products and nutritional know-how. The unique combination of products, models and services Trouw Nutrition offers, boosts productivity and supports animal health through all life stages, contributing to our customers' peace of mind. Trouw Nutrition solutions are designed to meet the needs of farmers, home-mixers, feed producers, integrators and distributors. Trouw Nutrition employs over 8000 people located in 28 companies.

HUBÈRT VAN HEES, SENIOR RESEARCHER TROUW NUTRITION RESEARCH AND DEVELOPMENT:

'PLEASED WITH THE RESULTS OF THE TRIAL'

'As a specialist in livestock feed, we are very interested in learning how we can improve animal health by means of nutritional innovations. We expected to learn more about the science of prebiotics and probiotics and their effect on health of livestock. We decided to join this project and offered the researchers the possibility to test their hypotheses about the influence of prebiotics and probiotics on the microbiota in the gut. For this purpose, we invited them to test a group of piglets in our testing facility.'

PROMISING FINDINGS

'We were pleased with the preliminary results that were presented during the symposium in November 2017 and we expect to hear more once the final data become available. For the agricultural sector these findings are very promising. Worldwide, we wish to reduce the use of antibiotics on animals and in the Netherlands, we are at the forefront in this field.'

MAKING VACCINATION MORE EFFECTIVE

'This project shows that vaccination is more effective if livestock is fed the right mixture of prebiotics and probiotics. Being able to manufacture feed that contributes to the reduction of antibiotics is a very promising idea and we will certainly take these results to the next level. What I also learned, is that it is important to start feeding these mixtures at a very early age. That way the immune system is trained to become more effective, which also benefits the health in the long run.'

'What I also learned is that it is important to start feeding the right mixture of prebiotics and probiotics at a very early age.'

WINCLOVE B.V.

In close collaboration with business partners, research institutes and academic hospitals, Winclove B.V. develops evidence-based, indication-specific probiotics for a variety of indications.
Research is the foundation of Winclove's probiotic formulations, stretching out over the complete probiotic development process from bacterial selection to clinical human trials. Winclove's product development is based on identifying key alterations in the microbiome that are associated with specific diseases.

This knowledge, combined with a proprietary library of over 100 well-studied microbial strains, is used to develop indication-specific probiotic formulations that restore the microbial balance.

The company continuously invests in research and technologies in order to develop the most effective probiotics and more importantly, to ensure that the end-user is provided with the best solution.

ISOLDE BESSELING, MANAGER RESEARCH PARTNERSHIPS:

‘IMPROVING THE FUNCTIONALITY OF OUR PROBIOTIC STRAINS’

‘At Winclove Probiotics we have an extensive collection of probiotic strains, which we use in our dietary supplements. Our supplements are usually combinations of several probiotic strains, more and more combined with other active ingredients like prebiotics. These are sold worldwide: 95% of our products are exported.’

USING PROBIOTICS TO IMPROVE HEALTH

‘All bacteria have their own characteristics and properties, so you cannot use the same bacterial strains in every situation. We do our best to determine exactly what each probiotic can do in order to make well-founded choices when to apply them and how to combine them. Our supplements are for instance used to prevent side-effects of antibiotics. Antibiotics kill or inhibit the growth of pathogens involved in infections, but they are not only targeting the pathogen, but also create a disbalance of the commensal microbiota. One of our probiotic formulations was developed to overcome this effect and to prevent diarrhoea. Other focus areas for research and development of our probiotic formulations are more beyond the gut, like in the area of allergies, gut-brain relations and women’s health, like vaginal and urinary tract infections.’

‘We do our best to determine exactly what each probiotic can do in order to make well-founded choices when to apply them and how to combine them.’

INTERACTION OF PREBIOTICS AND PROBIOTICS

'We joined CarboHealth because we are interested in the interaction of prebiotics and probiotics. Using the right prebiotics in the growth medium of our bacterial strains hopefully improves their functionality. We are also interested in what happens when you combine prebiotics and probiotics in a supplement, to have a synergistic effect in the body. Looking back, I must say we were very happy with the work that was done specifically in two subprojects focused on the growth of bacteria strains on several prebiotic substrates, as well as more on functional assays like tests on gut barrier function and immune modulation. We now know more precisely which probiotics and prebiotics match best. Most promising seems to be the combination of one of our strains, *Lactobacillus acidophilus* W37, and a long chain inulin of Sensus, which was tested in the piglet trial of subproject 2. This successful trial may lead to further research into the effectiveness of this combination on human vaccination efficiency.'

'This trial may lead to further research into the effectiveness of this combination on human vaccination efficiency.'

PROOF OF CONCEPT

'This programme offers us a proof of concept, which is very useful in our ambition to make our probiotic supplements more effective. The next step is to use these new insights in our own laboratories and to expand the screenings of prebiotics and probiotics to more of our bacterial strains. Hopefully, we will find more matches that are as effective as the one used in the piglet trial.'

COÖPERATIE AVEBE U.A.

Coöperatie AVEBE U.A. is a cooperative of starch-potato growers in Veendam. AVEBE's 1350 employees work every day on using everything the potato has to offer to make life nicer, healthier and easier. To AVEBE, the potato is one of nature's special gifts: a source of possibilities with even more valuable ingredients to which value can be added. AVEBE's breeding company Averis Seeds B.V. works on the natural breeding and development of potato varieties, which have added value. Clients choose these ingredients because they simplify the production process, improve the flavour of the product or extent its shelf life. In the years to come, AVEBE will continue to unlock the secrets of the potato and to add value to them. AVEBE wants to achieve optimum returns for their growers, not just today, but also tomorrow and in the distant future the cooperative strives to achieve harmony between making a profit, the environment and people: growers, employees and customers.

PIET BUWALDA, MANAGER INNOVATION CENTRE AVEBE
AND ASSOCIATE PROFESSOR WAGENINGEN UNIVERSITY AND RESEARCH :

'CONVERTING OUR STARCH INTO USEFUL FIBRES'

'For AVEBE it is important to find out how enzymes can convert our starch into useful fibres. We apply this knowledge for the development of new products and to discover new markets. For us it is not sufficient to prove how these fibres work, they also have to have added value for our products. We cannot put a fibre in a nutritional product just because it is healthy to do so. Our products also need to have the right texture.'

CONVERTING STARCH INTO FIBRES

'We like to cooperate with scientists to find out the possibilities our carbohydrates have to offer to improve intestinal health. That is why we provided CarboHealth with two kinds of ingredients: the GtfB enzyme and our IMMP compounds. The GtfB enzyme was characterized in a previous CCC programme with AVEBE and University of Groningen. This enzyme converts starch into an indigestible fibre. During CarboHealth the effects of these fibres was tested in several ways. We want to know what happens in the colon when these converted fibres get there. It is still not quite clear what these fibres do. However, we achieved nice results and could demonstrate prebiotic effects. We will be part of the CarboKinetics programme to find out more.'

'We learned that our carbohydrates are fermented in the colon at a slow rate and stimulate the growth of both Bifidobacteria and Lactobacilli.'

EVERYTHING IS CONNECTED

'This programme taught us a lot. We learned that our carbohydrates, the IMMP compounds, are fermented in the colon at a slow rate and stimulate the growth of both Bifidobacteria and Lactobacilli. This means that they may be beneficial for the very young and for elderly people who are convalescing. We also learned more about techniques to unravel a carbohydrate compound in order to find what exactly happens to its components during its journey through the colon. All these findings came from different scientists, working on different subprojects. This underlines the strength of CarboHealth: the way everything is connected.'

BINGO COHORT, RADBOUD UNIVERSITY NIJMEGEN

BINGO is the cohort study of the Radboud University Nijmegen, that was started in 2015. This cohort consists of 80 mothers, 50 fathers, and 80 babies. The objective of this research programme is to gain more insight into the biological factors that can predict a baby's health and development during pregnancy and after birth. For this purpose, human milk samples, babies' faecal samples and data about the babies' behaviour are collected. The parents also keep a diary about their baby's behaviour, especially about how much the baby cries. The samples used by CarboHealth were collected at two, six and twelve weeks after birth. The BINGO cohort was added to CarboHealth at a later stage.

CAROLINA DE WEERTH, PROFESSOR BEHAVIOURAL SCIENCE INSTITUTE AND PROFESSOR OF DEVELOPMENT PSYCHOLOGY, RADBOUD UNIVERSITY NIJMEGEN: **'FOLLOWING MOTHERS AND THEIR BABIES ACROSS TIME'**

'Our research covers biological as well as behavioural factors that influence a baby's health. For instance, before the babies were born, we studied how the new parents dealt with a baby that was crying all the time by using a simulator infant. This can predict how they will cope with their own children later on. As regards the biological factors, we study the influence of nutrition on health early in life. We assume that the composition of the milk babies drink influences their development to a certain extent. We already found that a mother's stress during the pregnancy results in a different pattern in the microbiota of the child after birth.'

INFLUENCES OF HUMAN MILK OVER TIME

'Like in CarboHealth, we study the composition of human milk of the mothers and the faecal samples of the corresponding children. Our idea is to find out whether factors like stress, depression, anxiety and other factors influence the composition of the milk. We also look at other health factors that are transferred from mother to child at an early stage of life. Since this is a longitudinal study, we can follow mothers and children over time. For instance, the babies will turn three years old this year, so we are going to test them again to find out if we can see any effects in their behaviour related to the human milk they were fed as babies. Does breastfeeding babies impact their health and behaviour in the long run? That is one of the questions we are going to investigate.'

'Does breastfeeding babies impact their health and behaviour in the long run?'

SUCCESSFUL COLLABORATION

'Our collaboration with CarboHealth was a success. We provided the samples for research and made clear which questions we needed to be answered. And that is how things went: we were sent the results we asked for and were invited to the symposium in November. I really hope we will continue to work together, for instance with Henk Schols and his group.'

KOALA COHORT, MAASTRICHT UNIVERSITY

KOALA is a cohort study of Maastricht University, consisting of almost 3000 children born between 2001 and 2003. The aim of this cohort was to study allergy and asthma as well as growth and development. Samples of human milk and the faeces of corresponding babies were collected and stored when the infants were one month old. This study was carried out at the Maastricht Centre for Systems Biology. This centre wishes to develop a set of models, applicable in science and clinic that will advance the understanding of biological systems, and predict progression and treatment of complex diseases over time. These KOALA samples were added to the CarboHealth programme. Results were shared with Maastricht University.

CAREL THIJS, ASSOCIATE PROFESSOR EPIDEMIOLOGY, SCHOOL FOR PUBLIC HEALTH AND PRIMARY CARE, MAASTRICHT UNIVERSITY:

'TESTING EFFECTS OF BREASTFEEDING IN THE LONG RUN'

'The KOALA Cohort was set up around the year 2000 as a means to study the effects of human milk. We wanted to know if the effects of breastfeeding were the same for all mothers and children and how human milk is affected by the mother's diet and her exposure to infections and microbes in her environment. The children in this cohort were born between 2001 and 2003.'

'In all, we tested around 3000 children and took different samples.'

PIECES OF THE PUZZLE

'In all, we tested around 3000 children and took different samples. The samples used in CarboHealth were 146 human milk samples and faecal samples collected when the children were one month old. One of the more exciting aspects of the collaboration with CarboHealth was that Groningen and Wageningen were using different techniques to measure the samples. The great thing is that the results matched, even taking into account genetic variation. The pieces of the puzzle fitted.'

HYGIENE HYPOTHESIS

'We use the faecal samples to carry out research into allergy and asthma, overweight and growth, and development of children. At the moment, I am working on a project to test the hygiene hypothesis, which assumes that more children contract allergies and asthma because the immune system has become bored. We are working on the assumption that not only infections, but also bacteria in the gut have a role in the development of the immune system.'

ILJA ARTS, PROFESSOR AND DIRECTOR MAASTRICHT CENTRE FOR SYSTEMS BIOLOGY

‘RICH SOURCE OF DATA’

‘I was interested in the KOALA Cohort because of the samples that were collected, and I wanted to study them to measure biomarkers in my research into obesity and diabetes. This cohort offers a rich source of data you can use for all kinds of research projects. So, when Carbo-Health asked us to cooperate in some subprojects, this was a perfect match. We had the right samples in our fridge. An in-depth analysis takes a lot of time and is very expensive.’

BETTER RESULTS THAN WE COULD HAVE HOPED FOR

‘For us it was a pleasant surprise to see how a number of hypotheses about the relation between the intake of hMOS and the composition of the microbiota were confirmed perfectly. The data that were found based on samples that had been lying around in our freezers for fifteen years were more promising than we could have hoped for. Sometimes people frown upon these kinds of large studies, but as you see, it is a wonderfully rich source of data. It can be used by many researchers to come, who can use the available samples without having to set up their own cohorts.’

FULL PARTNER OF CARBOBIOTICS

‘This cooperation led to us joining the next CCC programme, CarboBiotics, as a full partner. It is important to find out how the microbiota of babies develops when babies are very, very young. In that period, you can influence the composition of the bacteria in the gut by feeding the baby hMOS. We think this positively influences the rest of a baby’s life. In this new project, we will study if hMOS can compensate the negative effects of antibiotics young children often get at a very young age.’

‘It is important to find out how the microbiota of babies develops when babies are very, very young.’

WRAPPING UP CARBOHEALTH

After four years of hard work, CarboHealth is done. During the symposium in Zwolle on 30th of November 2017, all researchers presented their findings and conclusions and keynote speakers spoke about the significance of the CarboHealth theme. Looking back, everyone is pleased with the results CarboHealth achieved. Especially with the leads that have led to new lines of research, like the new CCC programmes CarboBiotics and CarboKinetics.

The only subproject not yet described in this document is subproject 5, which concerned an integrated data analysis and claim support. Initially, the idea was to validate all techniques and methods in the laboratory with both human and animal trials and to study correlations. The goal was to integrate all data that were collected. The second objective was to use the results of this data integration to support health claims to be submitted to EFSA. Subproject 5 served as means to link *in vitro* and *in vivo* data and to draw conclusions on the toolbox all participants of CarboHealth can use freely. In order to submit promising health claims to EFSA more research is required.

During the programme the aim began to shift. For instance, it was decided to dispense with the proposed human trial, because there was still so much to learn about the interaction between carbohydrates and bacteria. The ongoing subprojects delivered so many leads and new knowledge, that it was deemed too early to set up a human trial. Instead, human samples were provided by the universities of Nijmegen and Maastricht. This was a perfect example of the way the partners within CarboHealth cooperated and shared information.

Looking at the complete overview of conclusions, data and leads, it is fair to say that CarboHealth has taken our

knowledge about the relation between carbohydrates, gut bacteria and health a step ahead. As a result of the laboratory work, valuable new knowledge was gained. We now know more precisely which fibres are good for beneficial bacteria in the gut. We have found that certain combinations of prebiotics and probiotics reinforce the efficiency of certain vaccinations. It is much clearer now what human milk contains and the potential relation with microbiota composition of the babies. All this new knowledge will serve as the basis for new research.

For the industrial partners, CarboHealth has been a valuable programme. They learned more about the composition of their own ingredients and how these compounds interact with bacteria. At intermediate meetings the partners were invited to learn about the new findings and to offer their ideas and views. Some partners will continue their collaboration with CCC by joining new programmes like CarboKinetics and CarboBiotics.

JURRIAAN MES, EXPERT LEADER FOOD & HEALTH RESEARCH, WAGENINGEN

FOOD & BIOBASED RESEARCH: ‘BEST OF BOTH WORLDS’

‘I was involved with the CarboHealth programme from the very start, working with Paul de Vos to write the proposal. At the start of the programme we were very ambitious. During the programme, however, we had to back away slightly from our original plans. Not everything we wanted to achieve was feasible in the time and budget we had. So, we decided to dispense with a new human trial. Changing course slightly during the course of such a broad programme as CarboHealth is not strange at all. You always have to be flexible and adapt your plans to reality and new insights.’

CHARACTERIZING FIBRES IN DETAIL

‘Most ingredients we tested are already used in products our partners produce and sell. However, in order to put on the package that a product benefits health, you have to be able to characterize in detail what a fibre looks like, how it works and why you say it is beneficial for health. So, this is largely what we did during CarboHealth. Before testing ingredients on humans, it is important to have clues on the mechanism of action based on laboratory experiments. You want to develop products that have

‘There are more interesting things to find in human milk.’

a high potency of success and use the right human trial design. Only then it is possible to prepare a health claim for EFSA. This is still feasible in the future.'

'You want to develop products that have a high potency of success and use the right human trial design.'

HUMAN MILK

'Another important part of CarboHealth was finding out the properties of human milk. What is the difference in composition between human and cow's milk and why are these differences important for our health? What can we add to cow's milk in order to approach the quality of human milk? In this field we gained a lot of new insights, which will benefit both new research projects and the product development of partners like FrieslandCampina and Nutricia Research. It is interesting to continue this line of research, since there are more interesting things to find in human milk.'

EVERYTHING IS CONNECTED

'The cooperation between various scientific disciplines and industrial partners was a major part of CarboHealth and led to interesting new leads and insights. You get much more from a study if several specialists are involved. In our bodies everything is connected, so it makes sense to study our bodily processes from different perspectives. That is why it was a good thing microbiologists and immunologists were involved. Programmes like this deliver fundamental research that can actually be applied by the industry. The best of both worlds, to be sure.'

On 30th of November 2017, all CarboHealth participants met in Zwolle to attend the closing CarboHealth symposium. All researchers involved presented their findings in lectures and on posters. In addition, three internationally renowned keynote speakers were invited to shed their light on the theme of CarboHealth. These were: Ardythe L. Morrow, professor and Director at Cincinnati Childrens Hospital Medical Center, Geert-Jan Boons, professor Pharmaceutical Sciences and Molecular Pharmacy, Utrecht University, and Hauke Smidt, professor Microbiology at Wageningen University & Research. Some of their remarks during their speeches and afterwards are highlighted below.

ARDYTHE L. MORROW

'Everybody should understand the idea that mother's milk contains bio-active sugars that can feed beneficial bacteria with special properties like reducing inflammation and discouraging pathogens from making the baby sick.'

'We need to understand how these different structures in human milk work. Many of these sugars are doing similar things, that is good because all breastfed babies benefit. But specific structures do specific things. We are still trying to understand the specificity, in order to know more about the effect of mother's milk on the baby's health.'

'Mother's milk contains bio-active sugars that can feed beneficial bacteria.'

'Not all mothers are able or willing to breastfeed and then they have two options: donor milk or infant formula. We need to know more about these oligosaccharides, so we can make the right choices and enable the industry to add affordable and reliable oligosaccharides to their formula. It took us fifteen years to do the research. And still, you cannot make formula that is exactly like human milk, because mother's milk changes over time, while formula is standard and always the same. Mother's milk is awesome, it is worth working on, so we can learn more about how to improve infant formula.'

‘It is the common experience of women that it is hard to continue to breastfeeding once they go back to work. They should be given breaks and a room where they can extract milk. That is not always supported. Mothers should not have to fight to be able to do this. Our history and evolution prove that it is best for us to have mother’s milk in the first years of life, because it reduces the risks of allergies, infection, microbial disorders and other diseases. It is really worth the time and effort, but society should be supportive of women who breastfeed their babies, because we know this benefits health in the short and long run.’

GEERT-JAN BOONS

‘First we had a genome revolution. We thought that if we knew every gene, we could solve every health problem. That turned out to be not true. Then came the revolution of proteins: if we can measure all proteins in the body, we can diagnose more easily and treat patients in a personalised way. We are now learning that almost all proteins are modified by very complex carbohydrates. If you don’t include them in your analysis, you miss so much important information. So now, a new revolution is on its way: we need to understand the carbohydrates attached to the proteins, the so called glycans. It turns out that in almost all diseases these carbohydrates are involved. We know they are important but how or in what manner, that is a black box. It is very difficult to make these carbohydrates and study them individually.’

‘Almost all proteins are modified by very complex carbohydrates.’

‘We try to make artificially what is found in nature. We synthesise one molecule at the time to study its functions. Can we make all for these carbohydrates, study how they are involved in diseases and health and use this knowledge for interventions, better diagnostics and real personalised medicine? We think this knowledge can be used to find biomarkers for cancer, better ways to treat viral infections, diabetes and inflammatory diseases for instance.’

‘We can already make oligosaccharides in the laboratory that are exactly like those from mother’s milk. The only difference is that they are made in a laboratory and not in

a human breast. For this we use bacteria that make these human sugars and spit them out. This way we can produce these sugars more easily and on a large scale. How this works exactly we show in a video. These are stepping stones on our way to better knowledge about these carbohydrates, leading in the end to more personalised medicine.'

HAUKE SMIDT

'At a very young age, our microbiota is developing, and it is influenced by our surroundings. Right after birth, a baby is colonised by microbes from its mother's vagina and faeces, and from its immediate environment. This development is important for the rest of our lives. Our microbiota keeps developing during early childhood and only at old age, its diversity starts to diminish.'

'One of the first jolts to the microbiota occurs when a mother stops breastfeeding or when a child is given antibiotics. From a Finnish cohort study of 142 children from two to seven years old, we know how antibiotics influence the composition and function of a child's microbiota. This study also teaches us that antibiotics in early life can lead to an increased risk of asthma and weight gain. Earlier piglet studies have even shown us that when mothers are given antibiotics, this also affects intestinal microbiota and function of their offspring.'

'Our microbiota keeps developing during early childhood and only at old age, its diversity starts to diminish.'

'One of the most recent eye-openers in the area of gut microbiota research is that each individual's gut microbiota not only is different, almost with fingerprint-like forensic qualities, but that our gut microbiota also to a large extent determines how we respond and deal with specific dietary ingredients. This is maybe best exemplified by studies that showed large differences in each individual's glycaemic response to for instance white versus sourdough bread. This discovery leads the way towards personalized nutrition and microbiome management strategies.'

LUBBERT DIJKHUIZEN, PROFESSOR MICROBIOLOGY UNIVERSITY OF GRONINGEN AND SCIENTIFIC DIRECTOR CCC

CLOSING STATEMENT

CarboHealth was a perfect example of a programme focused on fundamental research in collaboration with industrial partners. We listened carefully to what these companies are looking for and need for their business. On the other hand, as fundamental scientists we also have our own research questions. As in all programmes like this one, we invited the companies to join us and talk about what we could achieve together. This way, we reached a consensus about the range of subjects we incorporated in the programme.

It was a pleasure to work with the six partners in this programme. They worked well together, with the scientists and with us as CCC. At the start of the programme we committed to find the best scientists for the different sub-projects. Mostly young, ambitious scientists who proved to us they were up for the job. In the end, this kind of fundamental research benefits both scientific progress and the industry. Our partners need innovative breakthroughs to keep ahead of national and international competition.

Fundamental research results in the best of the best. Encouraging young scientists to do fundamental, free research often leads to the most surprising outcomes. Results that benefit both science and industry. We need each other, and we can help each other. That is something we proved during CarboHealth and will continue to do so in the next CCC programmes.

Personally, I would very much like to help smaller companies to benefit from our research as well. Small companies are often unable to join a large consortium but can elaborate on what we found within the scope of the programme. Knowledge and data that are not used by any of our partners, could well serve to create interesting, local spinoffs.

CARBOHYDRATE COMPETENCE CENTER

COLOPHON

Carbohydrate Competence Center is a public-private partnership for research in nutrition and health as well as biobased economy.
Final report TKI-project AF12203 CarboHealth, Carbohydrates for improving Health, 2013-2017. Published by Carbohydrate Competence Center (CCC), Groningen, Netherlands.
CarboHealth has been financed by the participating partners and allowances of the TKI Agri&Food programme, Ministry of Economic Affairs.

April 2018

Topsector Agri& Food

Text
Marjan Brouwers, Bureau Brouwers, Groningen

Design
Mariët Visser, marvis concept+vorm, Groningen

CARBOHYDRATE COMPETENCE CENTER

PARTNERS CARBOHEALTH:

FrieslandCampina nourishing by nature

Avebe

DANONE NUTRICIA RESEARCH | NUTRICIA RESEARCH

trouw nutrition a Nutreco company

SENSUS

rijksuniversiteit groningen

WAGENINGEN UNIVERSITY & RESEARCH

WINCLOVE PROBIOTICS

umcg

RESEARCH PARTNERS:

Maastricht University

Radboud University

CARBOHYDRATE COMPETENCE CENTER

Visiting address:
Nijenborgh 7, 9747 AG Groningen
The Netherlands

Postal address:
P.O. Box 11103, 9700 CC Groningen
The Netherlands

Phone:
+31 (0)50 363 21 50 (secretariat)

Mail:
info@cccresearch.nl

Website:
www.cccresearch.nl