CARBOHYDRATE
COMPETENCE CENTER

CARBOKINETICS:

INTESTINAL CARBOHYDRATE FERMENTATION DYNAMICS AND ITS IMMUNOLOGICAL MODULATION

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DISCLAIMER

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PREFACE - CCC PRESENTS....

Carbohydrate Competence Center proudly presents the final report of CCC NWO Carbo-Kinetics. This program ran from 2016 to 2021 and involved the work of twelve ambitious researchers and their supervisors, four knowledge centers, seven industrial partners and the representatives of CCC. The result: an in-depth body of new knowledge and insights into the kinetic process of the intestinal degradation of dietary fibers and the effect of these fibers on human and animal health, immune system and metabolism.

CARBOHYDRATE COMPETENCE CENTER (CCC)

Carbohydrate Competence Center (CCC) is the leading carbohydrate knowledge center in the Netherlands, bringing together excellence in carbohydrate research and knowledge. It is our mission to generate, develop and share high-value knowledge in the field of carbohydrates, to promote innovation worldwide and to contribute to a healthy and sustainable society.

CCC was founded in 2008 and our first programs focused on the themes Health and Nutrition and Biobased Economy. These programs yielded many promising results, leading to follow-up research and a number of patented applications. In ten years' time we have gained valuable knowledge and experience in a wide range of research topics concerning carbohydrates. CCC is an initiative of the University of Groningen and Wageningen University & Research.

CCC NWO PARTNERSHIP PROGRAM CARBOKINETICS

To stimulate effective collaboration between academia and industry on the role of carbohydrates in human and animal health, CCC and NWO (Dutch Research Council) initiated the NWO CCC Partnership Program. This program offers

universities and industries the opportunity to participate in demand-driven, multidisciplinary, fundamental research in the precompetitive phase. The NWO CCC programs are funded by the industrial partners and NWO, with the industries paying up to 50% of all costs.
CCC NWO CarboKinetics is the first program to be realized by this partnership.

CCC TEAM

The CCC team consists of staff members from the University of Groningen and Wageningen University & Research. The secretariat is based in Groningen. The CCC team provides day-to-day management of all ongoing programs, develops new programs and maintains external contacts with companies, funders and governments. In addition to the management team, CCC has an advisory committee and a supervisory board. Members of the current CCC management team are Gert-Jan Euverink (director), Janneke Krooneman (business manager), Henk Schols (director), Éva Jermendi (project manager) and Yvonne Numan (secretariat).

During this program Lubbert Dijkhuizen, one of the original founders and director of the CCC, retired. He officially took his leave during the CCC Symposium in November 2020, but is glad to remain an active and involved member of the CCC community.

IN MEMORIAM **PIET BUWALDA**

On the 11th of July 2020 CCC received the sad news that Piet Buwalda had passed away. As a representative of Avebe he was involved with CCC from the start. Over the years he contributed a great deal to the CCC programs, always prepared to give his honest advice and profound insights. He helped to set up several programs, sharing his ideas and knowledge and contributing to the cooperation between universities and industrial partners. His enthusiastic attitude and heartfelt commitment will be sorely missed. We have lost a wonderful, honest partner in CCC.

INTRODUCING CARBOKINETICS

WHY CARBOHYDRATES MATTER

Carbohydrates are essential building blocks of nature: plants, animals, and humans cannot thrive without carbohydrates in the form of fibers and sugars. They provide sturdiness, energy and food reserves. Unfortunately, our Western diet usually doesn't contain the advised 30 to 40 grams of dietary fibers a day. This leads to the increase of the probability to develop chronic health problems like colon cancer, cardiovascular disorders and diabetes.

Various CCC programs aimed at finding out how carbohydrates can contribute to better health, prevention of diseases and reinforcement of the immune system. One of these programs was CCC NWO CarboKinetics. This program focused on the kinetics of the processes involved in the degradation of dietary fibers in the digestion tract of both humans and animals.

THE KINETICS OF FIBER DIGESTION: MANY QUESTIONS

Little is known about what exactly happens to dietary fibers at what stage during their journey through the digestive tract. At what stage during the intestinal passage do bacteria start to ferment fibers? Where exactly does this happen? And which fibers does a healthy microbiota prefer and why? Which enzymes are involved, and which short-chain fatty acids are produced by the bacteria? How does the kinetic process of fermentation change over time? Does a two-week old baby process fibers in the same way as an adult? How does the kinetic process change when people grow older?

FINDING ANSWERS

To find the answers to these questions, CCC and the Dutch Organization for Scientific Research (NWO) put out a call in 2015 for research proposals, focusing on the impact of the process kinetics on the intestinal degradation of

carbohydrates, more specifically dietary fibers, in human food and animal feed. One of the requirements was that each research project had to consist of at least two researchers from two different knowledge institutes. In this way, academic collaboration was encouraged, leading to new insights and innovative ideas. All participants had to be willing to collaborate closely, sharing their findings and presenting their results together.

SIX RESEARCH PROJECTS

The call led to many applications, which were all peer reviewed by an international group of scientists selected by NWO. These peers were informed beforehand that the program would involve universities as well as industrial partners. In all, six projects were selected, based on the quality of their research plan, fit to the call and societal impact. Twelve young researchers – eight PhD students and four post-docs - carried out the research. The program officially ran from 2016 to 2020 with a budget of € 3 million, 50% of which was contributed by the industrial partners. Due to unforeseen circumstances, in particular the COVID-19 pandemic, not all projects were finished in time. Some of them continue well into 2021, because of the lockdown of laboratories and hospital premises in 2020 and 2021. The final results of these delayed projects could not be included in this report.

PARTICIPATING PARTIES

Participating parties in this consortium were the companies FrieslandCampina, AVEBE, Sensus, DSM, Agrifirm, Nuscience, VanDrie Group and the knowledge institutes Wageningen University & Research, University Medical Center Groningen, Utrecht University and Maastricht University.

TAKING LEAVE OF **HARRY GRUPPEN**

Harry Gruppen of Wageningen University & Research has been a valued member of the CCC community for years and started working as supervisor of the pectin project after the start of CCC NWO CarboKinetics in 2016. Sadly, he fell ill and could no longer fulfill his tasks. He stepped back from the project, after which Henk Schols took over his role. The CCC team wishes to express their gratitude for Harry's work and wish him all the best for the future.

The nature of the research program was precompetitive. This ensured that all participants felt free to share knowledge and results during group meetings and discussions. Beforehand, the industrial partners were offered the opportunity to indicate which aspects of the program they were especially interested in and what kind of research they preferred. Some partners contributed ingredients for analysis and testing facilities. In addition, all partners actively participated in project meetings, offering ideas and insights.

SCIENTIFIC PUBLICATIONS, COLLABORATION AND PATENTS

The CCC NWO CarboKinetics Program already led to around fifteen scientific publications, and all eight PhD students should have finished their dissertation by the end of 2021. Together with the partners five patent applications were filed. During the program the researchers not only collaborated closely with their own counterpart, but also across the borders of their project with the other scientists within the program, sharing tools, insights and extensive analysis results. In fact, the exciting results and new promising ideas were the result of the open and collaborative spirit of CCC NWO CarboKinetics.

LOOKING BACK

In this report researchers and supervisors present an overview of their project and their results. The industrial partners also share their experiences with the program. CCC NWO CarboKinetics was officially concluded during the closing symposium on the 24th of November 2020.

HENK SCHOLS, DIRECTOR CCC AND PROFESSOR OF THE DEPARTMENT OF AGROTECHNOLOGY AND FOOD SCIENCES AT WAGENINGEN UNIVERSITY & RESEARCH

‘UNRAVELLING THE MECHANISM OF THE KINETIC PROCESS IN OUR BODIES’

CCC NWO CarboKinetics was a research program focused on the process kinetics of dietary fibers in our body. Most fibers are long sugar chain molecules that pass through the small intestine unharmed by the digestive system. Once they arrive in the colon, they feed the tens of thousands of bacteria that live in our gut: the microbiota. While fermenting the fibers, these bacteria produce all kinds of products which are utilized by our body. Some of these products are so-called short-chain fatty acids that have a beneficial effect on our health. They are used to strengthen the barrier between our colon and the rest of our body, for instance, and have a beneficial effect on our immune system and health.

‘Generally speaking, a healthy diet consists of plenty of fruit and vegetables, containing all kinds of dietary fibers. Nutritionists keep on telling us that we need to consume at least 35 grams of fibers a day. This seems a sound advice, but our research showed that not all fibers have the same effect. To make things more complicated: bacteria are very particular, preferring some fibers over others. There are also bacteria that only feed on organic acids that are produced by other bacteria after fermenting specific fibers. We know for sure that this process of feeding and fermenting is beneficial for our health and immune system. But how does it all work?’

UNRAVELLING THE MECHANICS

‘This program focused on unravelling the mechanism of the kinetic process that takes place in our bodies all the time. Hopefully new knowledge leads to a better understanding of how dietary fibers contribute to our health and immune system. In the end, this knowledge will contribute to the development of specific supplements aimed at certain groups of vulnerable people, like infants and frail elderly

who suffer from problems of the gut. In addition, one of our projects was focused on the effect of prebiotics on calves, aiming for ways to reduce the use of antibiotics.'

PROTECTING THE INTESTINAL WALL

'An example of the research dealt with the barrier function of the intestinal wall. This wall is reinforced by the production of short-chain fatty acids by the bacteria. Now, what you want is to optimize the integrity of this wall, so it is able to stop certain substances from entering the body, like undesired proteins, antigens or poisons. However, our western diet and our stress levels weaken this barrier function resulting in a leaky gut. Fortunately, our intestinal wall is quite sturdy. Skipping healthy food for one day will not harm it in any way. A slightly porous barrier is no problem, but when it leaks like a sieve you are in trouble. What you want to do for patients who suffer from serious bowel problems is to restore the balance in the gut by giving them fibers that will stimulate the growth of beneficial bifidobacteria. The fermentation process results in reinforcement of the barrier function and subsequently the immune system.'

BENEFICIAL LACTOBACILLI AND BIFIDOBACTERIA

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

Bifidobacteria

Lactobacilli

PROJECT 1:

HOW PECTINS FUEL IMMUNITY

Pectin is a dietary fiber which will possibly prove to be essential for the prevention of typically Western chronic diseases. The scientists of this project found evidence that beneficial effects of pectins highly depend on their chemical structure. The aim of this project was to characterize pectins and to measure how they can influence our immune system directly or indirectly.

Pectin is a dietary fiber that occurs in most fruits and vegetables. It is a polysaccharide, consisting of a complex chain of molecules. In comparison to other fibers, pectins are quite long, complex and consist of 500 sugar molecules or more. At face value, it is hard to spot the differences between various pectins. All of them consist of a long chain of similar building blocks, but some pectins are much longer and more complex than others and often branched in a complex fashion. Another difference concerns the level and distribution of methyl esters that were found to determine their health effects.

It is well-known that pectin can be used as a gelling agent to make jam, jelly and marmalade. About pectin's immune effects little was known before the start of this project, although in a previous CCC program, it had already become clear that the chemical structure of different pectins played an important role.

To characterize pectins, the scientists of project 1 used enzymatic fingerprinting techniques and to study the biological effects, they used complex culture systems as well as animal studies to validate findings. The data demonstrated that some pectins can prevent a leaky gut induced by chemotherapeutic agents for instance, while others prevent inflammation in the large intestine induced by pathogenic bacteria.

ÉVA JERMENDI, PHD STUDENT AT WAGENINGEN UNIVERSITY & RESEARCH:

‘PECTINS HAVE BEAUTIFULLY COMPLEX STRUCTURES’

Our Western diet contains too few fibers, which has a negative influence on our immune system. Fibers can influence our immune system in the large intestine directly through immune receptors and indirectly through the microbiota in the colon. In this project Éva worked with Martin Beukema in Groningen.

‘It was my job to analyze, characterize and tailor the pectins, using different methods. Martin tested my characterized and tailored pectins in immune assays and in mice. I also analyzed the short-chain fatty acids and microbiota occurring in the gut of mice that were supplemented with pectin. We wanted to find out which pectins could be used to improve human and animal health and enhance the immune system. I analyzed on a molecular level how pectins are structured and how they differ from each other.’

‘Pectins have beautifully complex structures. I could talk about them for hours, if you let me. When you characterize pectins, you find that although they have similar chemical compositions, their methyl-ester distribution patterns can differ. If you extract pectin from two oranges that have been harvested and stored differently, you will see that the pectins are different on a molecular level, because of pectin modifying enzymes present in the fruit.’

‘We wanted to find out which pectins could be used to improve human and animal health and enhance the immune system.’

‘Pectin has been researched for at least fifty years, but there is still space for more research and understanding. We could do so many more experiments, and each time we would learn something new. Really amazing, actually. Most interesting is that pectin has a beneficial influence on the immune system. That was a wonderful result of our project. It will take a lot more research to get to the bottom of what pectins can mean for health and the immune system.’

‘Pectins have beautifully complex structures. I could talk about them for hours, if you let me.’

‘During this project I also improved my presentation skills, and I learned a lot about teamwork. We had some very interesting meetings with the industrial partners, getting to know each other and having great discussions about our project. All in all, CCC offers a great community, where I feel right at home.’

PREBIOTICS AND PROBIOTICS

Prebiotics is the term used for carbohydrate chains containing 2-10 sugar units that are not digested by human enzymes in the digestive tract before they reach 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.

MARTIN BEUKEMA, PHD STUDENT AT UNIVERSITY MEDICAL CENTRE GRONINGEN (UMCG):

‘LOOKING AT THE INFLUENCE OF THE PECTIN STRUCTURE’

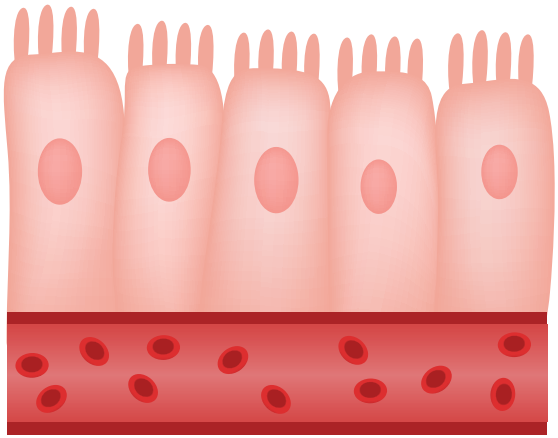
Martin’s contribution to project 1 was aimed at the biomedical aspects of pectin research. Working closely with Éva, he looked at how the specific structures of pectins influence the immune system, especially in the gut.

‘We wanted to detect how the structure of the different pectin fibers could influence the immune response.’

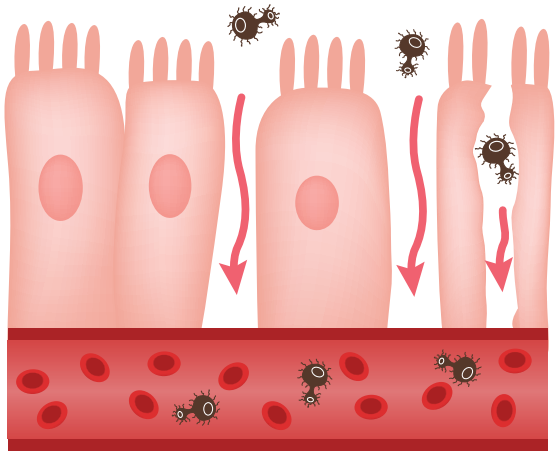
‘In one aspect of the study, I specifically looked at the so-called Toll-like receptors that are expressed on the immune and epithelial cells in the gut. These receptors are part of the immune system that form the first line of defense against the content of the gut. When these receptors recognize certain structures, like bacteria and pathogens, they can cause the immune system to respond with an immune reaction. Excessive activation of these receptors can lead to inflammation. When fibers are bound to these receptors, this can help to prevent the inflammatory reaction.’

‘We wanted to detect how different pectin structures could influence this Toll-like receptor mediated immune response. We found that the effects of pectins on the immune system differed. The effectivity of the pectins on the Toll-like receptors seems to depend largely on how the pectin molecule is structured.’

‘Our project showed that it is likely that pectin fibers with a certain structure can be used to reduce inflammation in both the small intestine and the colon. This finding could be important for various treatments, for instance, for cancer



Normal tight junction



Leaky and inflamed tight junction

patients who receive chemotherapy. Possibly, pectin fibers can reduce the inflammatory effects of this treatment. We also looked into how pectin fibers can reduce bowel infection caused by pathogens. Of course, more research is needed before these findings can be applied in human trials, but my results are certainly promising.'

'I enjoyed working with the industrial partners and we had interesting discussions. The regular meetings with the companies also taught me to get an overview of the results and present the results coherently. It was also a great opportunity to visit the companies and get to know how research is performed outside academia.'

'Possibly, pectin fibers can reduce the inflammatory effects of certain treatments, for instance for cancer patients who receive chemotherapy.'

PAUL DE VOS, IMMUNOLOGIST AT THE DEPARTMENT OF PATHOLOGY & MEDICAL BIOLOGY AT UNIVERSITY MEDICAL CENTER GRONINGEN (UMCG):

'LEARNING ABOUT PECTINS AND FIBER-SPECIFIC EFFECTS IN GENERAL'

Project 1 delivered new knowledge about the health effects of pectins. Some pectins, for instance, have a health effect in the small intestine. Others have an effect when they reach the colon, where they are fermented by microbiota. Sometimes it was necessary to combine various fibers to achieve any effects at all. We learned that not all, but only specific dietary fibers have health benefits. Especially pectins need specific chemistries to support health.

'This knowledge can be used in future to choose certain dietary fibers in order to achieve positive health effects.'

'This knowledge can be used in future to choose certain dietary fibers in order to achieve positive health effects. For instance, if you want to reduce inflammation in the small intestine, you need to take a specific pectin, consisting of a backbone with a low amount of methyl esters. This pectin will have a direct effect on immune receptors in the small intestine, reducing the immune response causing the inflammation.'

'When a pectin is fermented too soon, it will never reach the location in the gut where the correct beneficial bacteria are waiting.'

'If you want to treat a patient who suffers from an infection in the large intestine, you need a different pectin: one that will not be degraded or absorbed too soon. In the large intestine it is fermented by bacteria that produce the right kind of beneficial fatty acids. These acids stimulate

the immune system to produce new regulatory cells, reducing the infection.'

'When a pectin is fermented too soon, it will never reach the location in the gut where the correct beneficial bacteria are waiting. But if you combine such a fiber with another one, it will pass through unscathed to reach the right location, while its companion is devoured first. These are just a few examples of the results this project has yielded. It turns out we hardly knew anything about pectins at all and now Éva and Martin succeed in lifting the veil a little. The project showed that current views that just eating more fibers will improve health does not make any sense. These are specific fibers that support health while many have no effects at all.'

'It turns out we hardly knew anything about pectins at all and now Éva and Martin succeed in lifting the veil a little.'

HENK SCHOLS, DIRECTOR CCC AND PROFESSOR OF THE DEPARTMENT OF AGROTECHNOLOGY AND FOOD SCIENCES AT WAGENINGEN UNIVERSITY & RESEARCH :

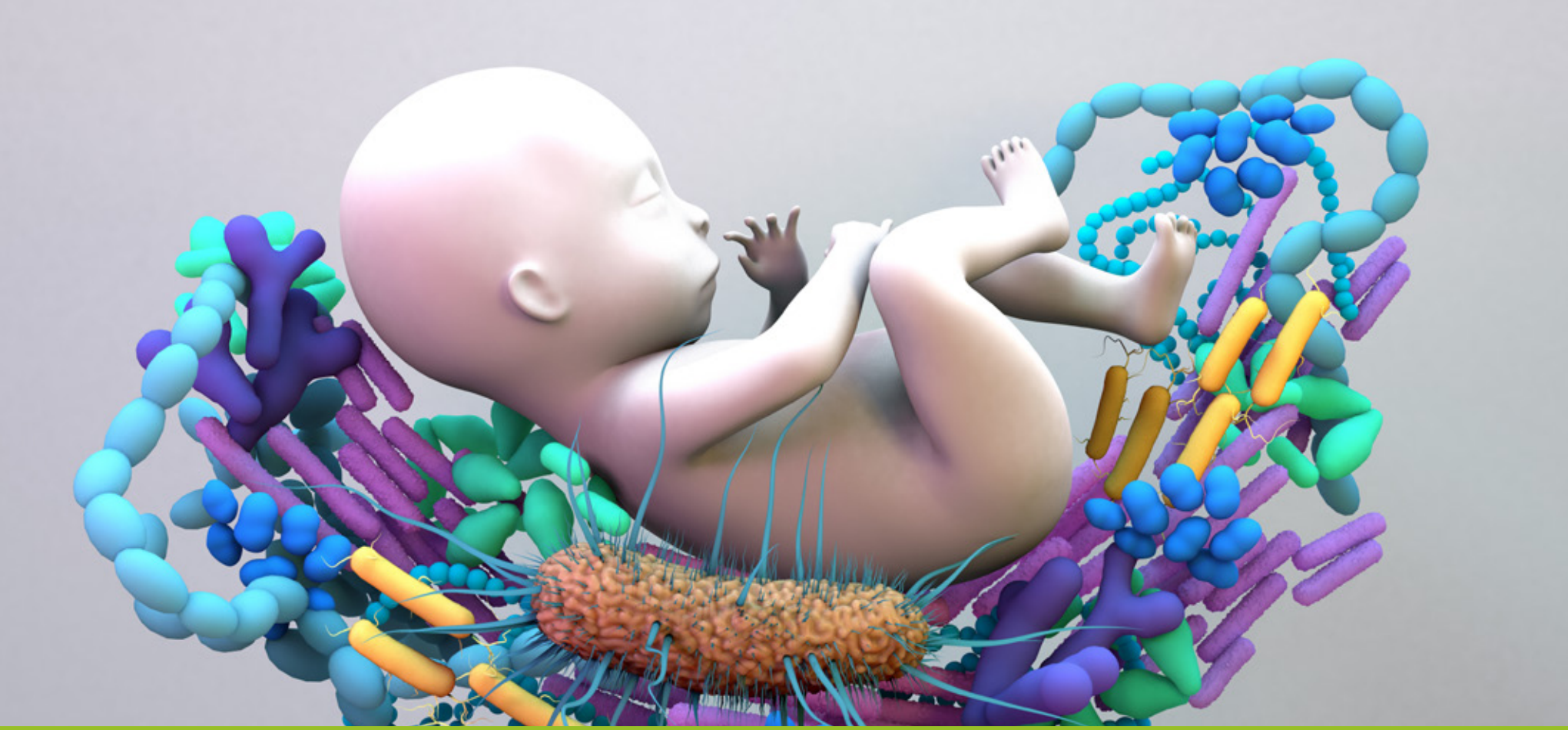
‘FUEL FOR NEW PROJECTS’

In this project we used pectins provided by our industrial partners. This was necessary to simplify the research process: extracting pectin from an apple would take much longer, and yield mixed results which would not be easy to translate to existing products. The industrial partners were highly interested in the molecular analyses of their ingredients.

‘Knowing more about the kinetic process of these fibres and their health effects is an important step.’

‘Project 1 yielded valuable information about the beneficial effects of different pectins. Knowing more about the kinetics of fermentation of these fibers and their health effects is an important step, although many more questions remain. It will be especially interesting to find out how we can combine pectins with other fibers. What will change when we add inulin in the mix? Will the pectins yield the same results or not? And how will pectins interact with poteins in the gut?’

‘One of the intriguing aspects of this project was that Éva Jermendi was able to show us the molecular structure of pectins. We now know exactly how these long, complex molecule chains are formed and what they look like. And even better: Martin found out how the way these molecules are structured influence the way they react. In the future we will know exactly which pectin to use for different disorders. That is why we certainly will continue research into this fiber in our next program.’



PROJECT 2: HOW CARBOHYDRATES CONTRIBUTE TO INFANTS' HEALTH AND IMMUNE SYSTEM

Right after birth, millions of bacteria find their way into the intestinal tract of a new-born infant, making sure that its immune system and the gut barrier protecting the infant are developed sufficiently. These bacteria need dietary carbohydrates that naturally occur in mother's milk, which is considered to be the gold standard for babies. However, infants that are not breastfed also need these complex carbohydrates.

Over the years, manufacturers of infant formula have supplemented their products with complex non-digestible carbohydrates. The purpose of these supplements is to stimulate the composition of the microbiota in the gut and to influence the baby's immune response to prevent and reduce inflammation.

Project 2 concentrated on finding out precisely how this process works: how, when and where during fermentation dietary carbohydrates influence the immune system and the composition of the microbiota of infants. The project also focused on the question if infants with special needs, such as children susceptible to allergies, need different dietary carbohydrates. The researchers carried out *in vitro* experiments in the lab and developed models to predict which combinations of carbohydrates are expected to yield the best results. During the lab experiments they copied the fermentation process in the infants' gut, using infant feces and carbohydrates provided by the industrial partners. The data this project delivered were widely shared among the other projects.

MADELON LOGTENBERG, PHD STUDENT AT WAGENINGEN UNIVERSITY & RESEARCH:

‘FINDING WAYS TO RESEMBLE MOTHER’S MILK’

The positive health effect of mothers’ milk is caused by the fermentation of so-called human milk oligosaccharides by bacteria in the intestinal tract. These bacteria produce short-chain fatty acids which are beneficial for the development of the infant’s immune system. Research showed that babies who were fed infant formula without these fibers are more likely to develop allergies when they get older than babies fed infant formula supplemented with specific non-digestible carbohydrates.

‘One of our goals was to unravel the chemical structure of a number of non-digestible carbohydrates and to learn how these are fermented in the gut of two-week and eight-week-old babies. We worked with mixtures of carbohydrates supplied by the industrial partners, such as inulin, FOS, GOS (fructo and galacto oligosaccharides) and isomalto-oligosaccharides. While my research concentrated on the fermentation and the chemical characterization of the carbohydrates, Renate Akkerman, my partner in Groningen for this project, concentrated on the immune effects.’

‘I hope our results will contribute to the improvement of infant formula and even to the development of tailor-made infant formula for different age groups of children with a different immune background.’

‘We collected feces from babies around Wageningen and Groningen. The parents scraped the feces from the nappies right after defecation. The microbiota of babies develop rapidly after birth and the differences turned out to be considerable. So, we decided to pool feces of different babies to create a two-week-old and an eight-week-old average baby microbiota for the purpose of our screening and analyses.’

'We took samples at certain points in time to measure the degradation of the carbohydrates and the production of so-called short-chain fatty acids. We also studied how bacteria reacted to single fibers and combinations of fibers. Next, we selected eight substrates to analyze the degradation process in more detail. We found that some of these fibers were fermented rapidly, while others left the body without being touched.'

'The microbiota of babies develop rapidly after birth and the differences turned out to be considerable.'

'Our research provided new, valuable insights into the structure and benefits of non-digestible carbohydrates. I hope our results will contribute to the improvement of infant formula and even to the development of tailor-made infant formula for different age groups of children with a different immune background.'

RENATE AKKERMAN, PHD STUDENT AT UNIVERSITY MEDICAL CENTER GRONINGEN (UMCG):

‘FOCUS ON THE EFFECTS OF FERMENTED FIBERS’

One of the benefits of mother’s milk is that it strengthens the infant’s immune system and develops the bacteria in the baby’s gut. This research hopefully contributed to the quality of cow-milk based infant formula for those babies that are not breastfed. Renate Akkerman focused on the effects of these fermented fibers on the immune system.

‘While working with fecal samples of two-week-old and eight-week-old babies, we found how much the composition of the bacteria in the gut changes in six weeks. At eight weeks babies not only ferment more kinds of fibers, the bacteria in their gut also start to produce more beneficial short-chain fatty acids. Our analyses of the eight selected fibers showed how important their structure is: the shorter the chains, the easier the fibers were fermented.’

‘In our study investigating the effects of the fibers on the immune system, we observed that fermentation of

‘Our analyses of the eight selected fibers showed how important their structure is: the shorter the chains, the easier the fibers were fermented.’

'We saw clearly that a high degree of fermentation leads to a significant anti-inflammatory response.'

the fibers and the production of short-chain fatty acids and other substances had a big impact on the immune cells that are present in the lining of the gut and are important for the immune response. We saw clearly that a high degree of fermentation leads to a significant anti-inflammatory response by these cells. This proved that adding specific mixtures of dietary fibers to infant formula can influence the immune system directly, causing a positive anti-inflammatory effect.'

'Due to COVID-19 our research progressed less smoothly than we had hoped: I had experiments planned in March and April of 2020, which had to be put on hold. I stayed on in the summer months to finish the work. It was also interesting to take part in discussions with the industrial partners, who tend to ask different questions. They helped me reflect on my own work and its relevance for society.'

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.

PAUL DE VOS, IMMUNOLOGIST AT THE DEPARTMENT OF PATHOLOGY & MEDICAL BIOLOGY AT UNIVERSITY MEDICAL CENTER GRONINGEN (UMCG):

'INFLUENCING THE BACTERIA IN THE GUT'

This project showed that it is possible to influence the way bacteria ferment dietary fibers in the gut. Fibers stay intact during their journey through the small intestine and have an effect in the large intestine. However, some fibers are totally degraded before reaching the end of the colon and only have an influence at the beginning of the intestine. Finding out exactly at what stage fibers are fermented and their effect on the immune system and health in general, is very valuable.

'The project also provided new information about the differences between infants and adults. Apparently, babies are unable to ferment certain fibers, because their enzyme makeup is not ready yet to do so. What this project taught us, is that the way we react to non-digestible carbohydrates differs from person to person. You cannot tell people to eat fibers without knowing their own distinct effect. How fibers influence our health and immune system is much more complicated than we ever knew. Not only does the microbiota composition change over time, the same goes for the type of enzymes produced by the microbiota. This project contributed to our knowledge about the extent in which we can steer microbiota and immunity in infants by means of non-digestible carbohydrates and their degradation products. We also found indications that we might need different supplements in infant formula for allergy or inflammation prone infants than for healthy babies. Obviously, we need more clinical trials to determine in detail how this kinetic process works and whether susceptible children will profit from our findings.'

'What this project taught us, is that the way we react to non-digestible carbohydrates differs from person to person.'

PROJECT 3: DEGRADATION OF CARBOHYDRATES IN HEALTHY ADULTS AND PRE-FRAIL ELDERLY

All through our lives the composition of the complex community of microbes in our intestinal tract is changing. Our microbiota starts to develop right after birth, changing and adapting over time, due to various health and lifestyle changes. The changes in the composition of our microbiota as well as its metabolic activity are often associated with the onset of age-related diseases. Little is known about the relation between microbiota, diet and health of elderly people.

This project aimed to study the composition of the microbiota of pre-frail elderly compared to that of healthy adults. Pre-frail elderly still lead active lives, usually live at home and do not yet experience serious disorders. Later in life, however, they often start to experience a gradual decline of their gut function and are more at risk to contract age-related disorders.

In addition to extensive *in vitro* analyses, two human trials were carried out, the first of which focused on the composition of the microbiota, metabolic activity and immune response. The second intervention trial studied the fermentation processes in the upper part of the gut. Because of the invasive nature of this second experiment, it was only carried out with young adult volunteers on hospital premises. Due to COVID-19, this intervention trial had to be put on hold for months. The final results of this study are expected sometime in 2021.

RAN AN, PHD STUDENT AT WAGENINGEN UNIVERSITY & RESEARCH:

‘COMPARING THE INFLUENCE OF FIBERS ON YOUNG ADULTS AND PRE-FRAIL ELDERLY’

Ran An focused on the way the composition of the microbiota of elderly people is changing. She compared this group to healthy adults. This way she wanted to observe the effect of adding dietary carbohydrates to their diet on the composition of the microbiota, their metabolic capacity and the immune system.

‘We set up a four-week trial with a group of young adults, aged between 25 and 50, and a group of relatively healthy elderly test subjects, aged 70 to 79. These groups were

‘It appeared to be quite tricky to pinpoint actual health changes as a direct result of the supplementation of fibers, because all test subjects were already quite healthy.’

'We found a significant difference between the microbiota of adults and pre-frail elderly, especially in the relative abundance of bifidobacteria.'

given GOS or a placebo. At various points in time, we collected samples of feces, blood and exhaled air (breaths). Moreover, we recorded the test subjects' food intake over three days. A subgroup of subjects from the *in vivo* intervention study was included to compare the metabolic capacity of the microbiota from different age groups, and this was studied via *in vitro* fermentation.'

'We found a significant difference between the microbiota of adults and pre-frail elderly, especially in the relative abundance of bifidobacteria. We also saw that supplementing GOS increased the number of bifidobacteria, but this intervention had no effect on metabolite production in feces or breath samples or on the immune function as measured in blood. It appeared to be quite tricky to pinpoint actual health changes as a direct result

'The main conclusion was that changes in microbiota composition during ageing is not directly linked to chronological ageing.'

of the supplementation of fibers, because all test subjects were already quite healthy. Still, it is important to find out what we can do to keep the microbiota healthy, slowing down the decline into frailty.'

'Finally, we were able to deliver an overall comparison of all results. Clearly, we saw strong individual differences. *In vitro*, we found that the structure of the carbohydrates had a significant effect on the microbiota composition and the metabolite production. The main conclusion was that changes in microbiota composition during ageing is not directly linked to chronological ageing but more to biological ageing, for instance changes in health status and medication use during ageing. In addition, the metabolic capacity of microbiota in adults and elderly differ in carbohydrate degradation and metabolites production.'

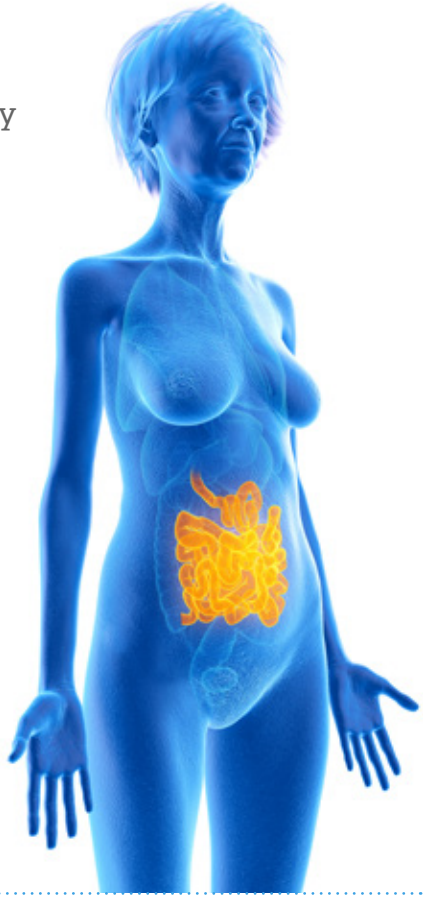
Ellen Wilms, PhD student at Maastricht University, took part in the research of this project until her maternity leave.

DAISY JONKERS, PROFESSOR AT MAASTRICHT UNIVERSITY, SCIENTIFIC DIRECTOR OF NUTRIM:

‘KEEPING PRE-FRAIL ELDERLY HEALTHY’

Elderly people are more prone to suffer from digestive problems and gut disorders and as they grow older their immune system becomes less effective. It has been known for years that the composition of the microbiota in the gut changes over time. Probably this contributes to the immune system becoming less effective, with the result that people run the risk of becoming ill more easily. To prevent comorbidity and to help the elderly to remain healthy, it is important to study what happens in the gut when people are ageing and when certain indigestible carbohydrates are added to their diet.

‘For this project we specifically looked at the effect of the intake of GOS for four weeks on the composition of the microbiota and the condition of the immune system. This project involved taking samples of blood and feces. Unfortunately, we found hardly any differences at all between the young and older populations. Possibly, pre-frail elderly, who were fit enough to take part in a study like this, were not the right target group and in future we should concentrate our efforts on elderly who are more vulnerable, perhaps in cooperation with general practitioners.’



‘What is left of the microbiota in feces does not tell the full story.’

‘A second, more invasive trial involved taking samples of the lumen content from the upper part of the colon. Again, we carried out a four-week trial, during which the subjects were instructed to add doses of GOS to their diet. In order to reach the upper part of the colon, our volunteers had to undergo an intestinal endoscopy. Now, this is not a pleasant treatment under normal circumstances. In this case, the endoscopy had to be performed without first flushing the intestines, which is even more invasive. That is why we selected extra volunteers in case some of them would withdraw during the proceedings. The endoscopy was performed on hospital premises. Due to COVID-19, however, we had to put this trial on hold. Hopefully, we will be able to complete our research in 2021.’

‘The reason why we wanted to take samples this way, is that what is left of the microbiota in feces does not tell the full story. The majority of soluble fermentable fibers, like GOS, are broken down by bacteria in the first part of the gut. Therefore, we want to know what happens before the lumen content leaves the body.’

‘I am proud of the way we worked together with other research groups within CCC NWO CarboKinetics.’

‘Looking back, I am proud of the way we worked together with other research groups within CCC NWO CarboKinetics. Combining our own expertise in human trials with the microbiological knowledge from Erwin Zoetendal and Ran An in Wageningen led to very fruitful discussions and promising methods.’

GALACTO-OLIGOSACCHARIDES (GOS)

GOS 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.

ERWIN ZOETENDAL, ASSOCIATE PROFESSOR AT WAGENINGEN UNIVERSITY & RESEARCH:

‘STEERING THE MICROBIOTA IN ORDER TO HALT OR EVEN PREVENT DISEASES’

In Wageningen the fecal microbiota composition during the intervention was studied. In addition, the researchers determined *in vitro* how the microbiota degraded different fibers supplied by the industrial partners.

‘It is our challenge to find out how we can use the microbiota to stay healthy for a longer part of our life.’

‘The *in vitro* analysis of the samples provided during project 3 yielded some surprising results. Surprising in the sense that we had not expected to see so few intestinal health differences between young and older subjects, as Daisy already indicated. We could not pinpoint any clear health effects, but we saw differences in microbiota composition, especially regarding the bifidobacteria. More experiments are necessary to determine the consequences of lower bifidobacteria in pre-frail elderly that are otherwise healthy. We also wanted to know what the effect of the ingredients were in the proximal gut, but as Daisy explained, this work was cancelled for now, due to COVID-19.’

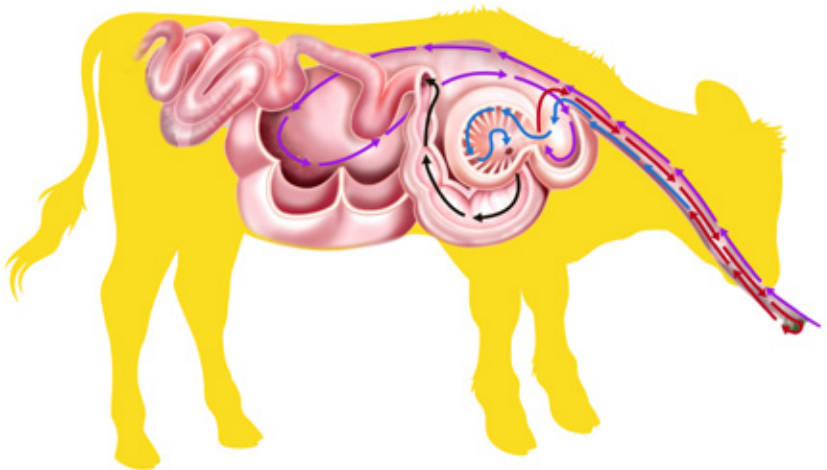
‘It is relevant to learn more about the ecosystem of the human gut. When you understand the causal relations between microbiota and diseases you can intervene and possibly prevent or cure certain gut disorders. We can now only treat by suppressing the symptoms. I am interested in learning how we can steer the microbiota in the gut, so people don’t fall ill and stay healthier for a longer time. It is our challenge to find out how we can use the microbiota to stay healthy for a longer part of our life.’

PROJECT 4:

IMPROVING CALVES' LUNG HEALTH

This project aimed at animal health and concerned the lung health of young calves. Every year dairy cows give birth to a calf. Dairy farms keep some of these, but some of the females and all male calves are sold for veal. Two weeks after birth, these calves are transported, first to a large collection center and then on to specialized calf veal farms. As the calves on a veal farm originate from different dairy farms, infection pressure and consequently the incidence of bovine respiratory disease is high, resulting in the need to use antibiotics.

Based on previous research into the beneficial effect of mixtures of oligosaccharides on the respiratory tract of mice, this project focused on calves in a controlled farm of the VanDrie Group, one of the industrial partners in this program. These calves were fed different dosages and mixtures of oligosaccharides, with or without additional fecal transplantation. The fecal transplantation has already proven its worth in human medicine. The aim of the project was to find out whether these different strategies aimed to affect the intestinal bacteria could improve lung health. If calves remain healthy there is no need to use antibiotics. This would contribute to the goal of reducing the use of antibiotics and with that the development of antibiotic resistance.



The calves that were studied in this program were part of the regular chain from farm to consumer. The scientists also used lung and intestinal tissues obtained from the slaughterhouse in their experiments.

MYRTHE GILBERT, ASSISTANT PROFESSOR ANIMAL NUTRITION AT WAGENINGEN UNIVERSITY & RESEARCH:

‘WE CONCENTRATED ON THE CONNECTION BETWEEN THE INTESTINE AND THE LUNGS’

Myrthe Gilbert joined this program at a later stage. As she did her own PhD on a previous CCC project, she was happy to step in. Due to COVID-19 this project was delayed, so it will be finished later this year.

‘This project focused on lung health of calves. Each calf carries its own bacteria and viruses. When put together, they tend to infect each other, often leading to diarrhea or lung problems. This is why the use of antibiotics in this livestock branch is still relatively high. In order to improve the calves’ health and reduce the use of antibiotics, this project looked into the beneficial effects of carbohydrates in their diet.’

‘We concentrated on the connection between the intestine and the lungs, using several mixtures of oligosaccharides to influence the microbiota in order to impact the immune

‘I was amazed to see the effect of this intervention in the lungs of the calves. Beforehand, I was skeptical about this being really possible.’

system in a beneficial way. By changing the feed and researching the immune molecules in the bloodstream and the lungs, we wanted to learn more about the connection between the gut and the lungs.'

'In order to improve the calves' health and reduce the use of antibiotics, this project looked into the beneficial effects of carbohydrates in their diet.'

'First a longlist of oligosaccharides provided by the industrial partners was analyzed, using a model of the lung at Utrecht University. This resulted in a much shorter list of oligosaccharides, giving us insights into their properties. We then performed a study, feeding groups of calves different oligosaccharides, dosages and mixtures. We also performed a test, giving calves one type of oligosaccharides by inhalation through the nose. We then monitored their growth and health. We took blood samples and collected cells from the lungs. This way we could determine inflammation parameters.'

'We saw that calves that were given oligosaccharides fared better as far as the inflammation and lung parameters were concerned. The most promising oligosaccharides turned out to be GOS. I was amazed to see the effect of this intervention in the lungs of the calves. Beforehand, I was skeptical about this being really possible.'

'The next calf study, which is still ongoing, concerned giving the calves a fecal and rumen transfer of bacteria, using the feces and rumen fluid of healthy calves. We are comparing the results of this group with calves that were fed GOS and a control group. We will also evaluate the combination of the bacteria transfer and GOS. These results are not in yet, but we are very curious whether this will positively influence lung health in calves.'

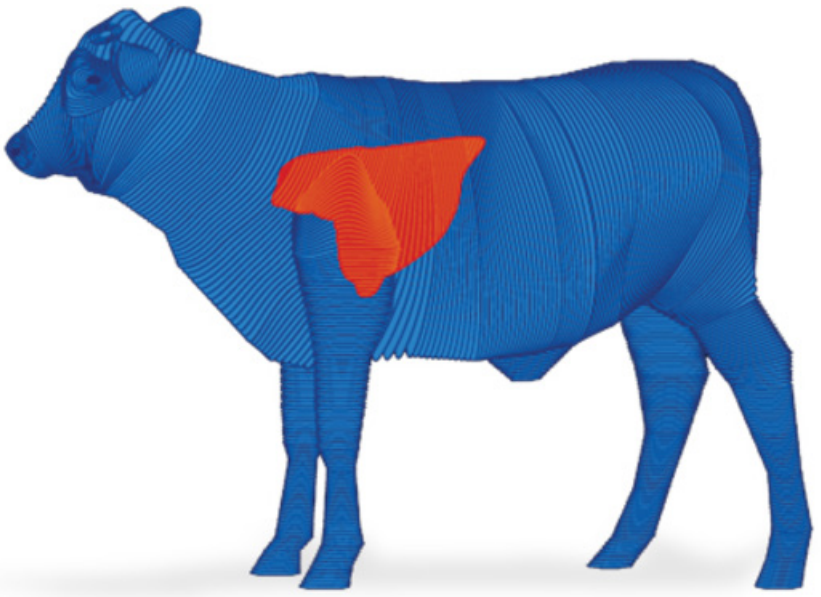
SASKIA BRABER, RESEARCHER AT UNIVERSITY UTRECHT:

‘FIBERS DO MUCH MORE THAN STIMULATE THE GROWTH OF SPECIFIC BACTERIA IN THE GUT’

Together with Walter Gerrits and Gert Folkerts, Saskia Braber wrote the proposal for this project. This way they could combine their knowledge of human and animal health care with the aim to contribute to the reduction of lung disorders in calves.

‘When we added oligosaccharides, we found that both the infection and inflammation were reduced.’

‘This project showed that fibers do much more than stimulate the growth of specific bacteria in the gut. They also reduce infections and have effects on other organs, like the lungs. Some of these oligosaccharides can even reach the lungs themselves. We went to the slaughterhouse of de VanDrie Group to collect lung tissue of calves and isolated the cells of these airways in the lab. We used these cells in an experiment mimicking the infection. When we added oligosaccharides, we found that both the infection and inflammation were reduced.’



'We also performed an experiment adding pathogens that frequently cause lung disorders in calves. Interestingly, when we added oligosaccharides, we observed again that the spread of pathogens was slowed down or halted entirely.'

'There is still so much we want to learn about the exact mechanism, the anti-inflammatory effects of fibers in the gut and the lungs and the effect on specific immune responses.'

'We also performed an experiment administering a mixture of fibers by inhalation through the nose, so the fibers would reach the lungs directly. This method yielded mixed results: a clear effect on the infection reactions, but less clear on the clinical symptoms of bovine respiratory disease. It is important to repeat this experiment in the future, using different mixtures and doses. There is still so much we want to learn about the exact mechanism, the anti-inflammatory effects of fibers in the gut and the lungs and the effect on specific immune responses. We need to perform additional mechanistical experiments to learn more about how it all works.'

WALTER GERRITS, PROFESSOR ANIMAL NUTRITION AT WAGENINGEN UNIVERSITY & RESEARCH

‘I WAS CONTENT WITH THE WAY WE COOPERATED WITHIN CCC, WE ALL HAVE A COMMON GOAL’

The aim of his project was to find out whether stabilizing the intestinal microbiota would lead to an improved immune response. The main objective was to reduce the use of antibiotics. On average, calves receive an antibiotics treatment three times during their lives. Antibiotics are known to damage the balance of the microbiota. What can be done to prevent this from happening?

‘The experiments that were carried out yielded interesting results. We now know which cell cultures we can use to screen the effects of the fibers. Working with groups of calves in a stable gave us the opportunity to test the effects of these fiber mixtures in an environment with a relevant infection pressure. We observed some promising results in this trial, but we need to repeat these experiments a number of times before these findings can be applied in practice. One of the current efforts tests the effectiveness of GOS in combination with a fecal transplantation.’

‘During this project we worked with the industrial partners, who provided the ingredients we could use in our experiments. VanDrie Group supplied the facilities to work with the calves. They also assisted our scientists during the experiments. A veterinarian taught us how to sample a broncho-alveolar lavage fluid of the lungs in calves. I was content with the way we cooperated within CCC. We all have a common goal.’

‘One of the current efforts tests the effectiveness of GOS in combination with a fecal transplantation.’

GERT FOLKERTS, PROFESSOR OF PHARMACOLOGY AT UTRECHT UNIVERSITY:

‘IF WE SUCCEED IN FINDING WAYS TO REDUCE THE USE OF ANTIBIOTICS IN CALVES, THIS WILL BENEFIT BOTH HUMAN AND ANIMAL HEALTH’

The relevance of this project mainly lies in the way we translated medicine from the lab to the clinic. We succeeded in getting strong results in the lab, which we applied *in vivo* with the calves.

‘The project is also relevant for human health. If we succeed in finding ways to reduce the use of antibiotics in calves, this will benefit both human and animal health. When calves are treated with antibiotics, the antimicrobial resistance may increase. We need to do something about the growing antibiotics resistance of bacteria and this project may well contribute to this goal.’

‘Furthermore, this project was a great example of collaboration. In Utrecht we know a lot about the *in vitro* effects of oligosaccharides on the epithelial cells of both the intestinal and respiratory tract, while in Wageningen they have *in vivo* experience with clinical trials involving calves. Together we set up a number of interesting experiments. This also shows the strength of the CCC programs: together we achieve more than on our own. That is why we will join a spinoff project, so we can take the results another step forward.’

‘In Utrecht we know a lot about the in vitro *effects of oligosaccharides on the epithelial cells of both the intestinal and respiratory tract, while in Wageningen they have experience with clinical trials involving calves.’*

PROJECT 5: **PREVENTING OVERWEIGHT-RELATED DISEASES**

Previously, research groups of Wageningen University & Research and Maastricht University collaborated on projects researching the influence of the gut on metabolism and overweight-related disorders. These projects led to an interesting result: acetates produced at the end of the colon have positive effects on the human metabolism. To stimulate this process, it is important to stimulate bacteria to ferment fibers at the right location: at the end of the colon.

The first step of project 5 was to look at *in vitro* fermentation models. These models were used to determine which fibers produce most of the short-chain fatty acids in the final part of the colon. Next, the composition of the microbiota producing most acetates was analyzed in Wageningen, while in Maastricht a human trial was set up. Two groups of volunteers were subjected to an acute, one-day trial: people with a healthy metabolic profile and people who were overweight and at risk of contracting type 2 diabetes.

This trial showed that fibers that were fermented at the end of the colon had a clear influence on the metabolism. The day after the fiber intake, participants experienced a higher energy output and an improved insulin sensitivity. This result correlated with the hypotheses. However, this result was only found in the group of healthy volunteers. The pre-diabetes group did not show the same effects.

GERBEN HERMES, POST-DOC AT WAGENINGEN UNIVERSITY & RESEARCH:

‘SCREENING FIBERS IN THE LAB’

Gerben Hermes studied the composition and potential of microbiota to produce acetate at the end of the colon, using an *in vitro* model that mimics the human intestinal tract. Such a model is not as complex as the human body but is sufficient for the purpose of screening the fibers provided by the industrial partners, for their acetate producing potential.

‘Interestingly, we only observed metabolic effects in the healthy group, but not in the overweight group.

‘Screening these fibers meant that we used the model to mimic the digestion of these fibers by the microbiota in the colon. This way, we were able to screen a substantial list of fibers and combinations of fibers. Based on the acetate production at the end of the model, which represented the colon, we chose the two most promising fiber combinations for the human trials.’

‘For the human trial we added these two fibers to the diet of two groups of people: healthy volunteers and over-weight adults. Interestingly, we only observed metabolic effects in the healthy group, but not in the overweight group. These results might be due to different processing of the fibers by the microbiota or differences in the metabolic processing of the subjects. Or thirdly: that it takes a longer time for the body to adapt or react to the intervention. I would have really liked to do a long-term trial, so we could have seen how the bacteria reacted over a longer period of time.’

‘During this project I saw once more how complex the interaction of the microbiota with human metabolism is. I think that in future we will move towards a more individual approach of overweight subjects. What works for one person, may not work for someone else.’

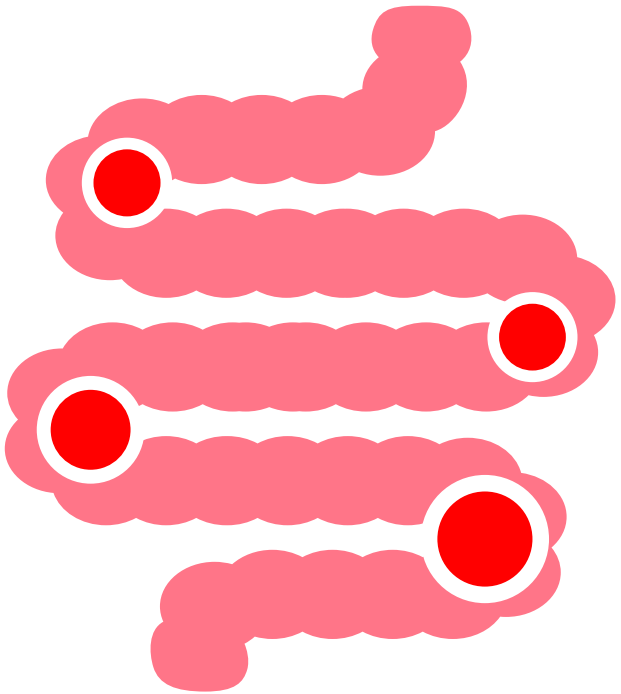
‘Screening these fibers meant that we used the model to mimic the digestion of these fibers by the microbiota in the colon.’

‘I think that in future we will move towards a more individual approach of overweight subjects. What works for one person, may not work for someone else.’

EMANUEL CANFORA, ASSISTANT PROFESSOR AT MAASTRICHT UNIVERSITY:

‘INCREASING THE SHORT-CHAIN FATTY ACID PRODUCTION IN THE GUT’

Now that it is clear how much intestinal health depends on including fibers in our diet, it seems quite easy to remedy chronic disorders. Add dietary fibers to your food and the bacteria in your gut will get the job done. Unfortunately, the process is more complicated than this.



‘When you want to increase the short-chain fatty acid production in order to fight overweight-related disorders, it is important that the fibers are fermented as late as possible. If fibers are fermented too early in the kinetic process, they will not reach the part of the colon we think is essential to reach to improve metabolic health. We tried to determine which combinations of fibers would yield the most promising results.’

‘To screen these fibers, we used a model that imitates the human intestinal tract. In this model we could analyze several combinations of fibers in order to find out whether they would lead to a higher production of short-chain fatty acids. Based on this analysis we selected three combinations for the human trials we had planned. Two of these trials were completed, a third one had to be postponed due to COVID-19.’

‘The most interesting result was that it seems that the fermentation process of fibers in the gut of overweight people with a high risk to develop type 2 diabetes differs from that of healthy people. Apparently, the bacteria react differently to the same fibers. These insights could lead to more a personalized diet. First, we will have to test which fiber combination works best for which person. The individual differences turned out to be considerable.’

‘First, we will have to test which fiber combination works best for which person. The individual differences turned out to be considerable.’

‘I enjoyed the collaboration within CCC very much. The discussions we had during meetings were fierce in a positive way. Nobody tried to steal the show. I always went home with a smile on my face.’

ELLEN BLAAK, PROFESSOR OF HUMAN BIOLOGY AT MAASTRICHT UNIVERSITY:

‘UNDERSTANDING THE IMPACT OF BACTERIA’

Ellen Blaak is involved in research focusing on how obese and overweight people contract complications like diabetes type 2. What can be done to prevent or reduce such complications? This project aimed at understanding the influence of the bacteria in the intestinal tract on the metabolism on overweight-related disorders.

‘The next step is to carry out more long-term interventions, because it takes the bacteria of prediabetic people longer to adapt to a new kind of fiber.’

‘The results of this project were promising. We learned more about the relevance of fiber combinations for overweight people and people with obesity and at high risk to develop type 2 diabetes. The next step is to carry out more long-term interventions, because it takes the bacteria of prediabetic people longer to adapt to a new kind of fiber. The short-term trial with healthy people confirmed our hypothesis that fermentation of fibers in the final part of the colon leads to significant metabolic health effects. Especially when you combine several fibers.

It is important to take this research to the next level. That is why we are happy to take part in the next CCC program CarboSupport. One of the things we want to know is if the fermentation of fibers in the final part of the colon, thereby reducing the harmful fermentation of proteins, will create a better balance in the gut with more pronounced health effects in the long run.'

'In general, it is increasingly clear that these strategies can no longer be based on a one size fits all attitude.'

'This program was a positive example of precompetitive research. The results led to patent applications, involving several industrial partners. The project also increased our understanding of the positive impact of dietary fibers on metabolism and health. I expect these insights will have implications for nutritional treatment and prevention strategies. In general, it is increasingly clear that these strategies can no longer be based on a one size fits all attitude. We need more research focused on personalized nutrition, especially for people who are overweight or suffer from metabolic disorders like diabetes type 2.'

ERWIN ZOETENDAL, ASSOCIATE PROFESSOR AT WAGENINGEN UNIVERSITY & RESEARCH:

‘PROMISING RESULTS FOR THE FUTURE’

Erwin Zoetendal was involved in two projects and both of them yielded promising results. The highlight of project 5 for him were the effects at the metabolic level during the acute human trial. How this connects to the microbiota is still to be determined, which will be quite complex given the individuality of the microbiota.

‘When you carry out an acute intervention, you give the body a shock effect. The advantage of such an acute study is that you can track the metabolic response of this shock effect. What happens to the body while these fibers are fermented? If nothing happens at all, it is unlikely you will see any effects over a longer period of time. But naturally, we need to perform long-term interventions to know this for sure. Determining the effect on the microbiota within 24 hours is complex, given the individuality of microbiota composition as well as transit time. We cannot exclude that for some individuals the effect of the acute study is not represented in the fecal sample due to slow transit.’

‘It was very interesting to work so closely with other research groups and the industrial partners. The interaction between the groups was great. I also saw how PhD students without a microbiology background came to our lab to do experiments, broadening their perspective.’

PROJECT 6:

LOOKING INSIDE THE GUT

When consuming a healthy diet consisting of plenty of vegetables, fruits and whole grain products it is not necessary to add any supplements to our food. However, for people suffering from certain disorders, such as diabetes type 2, stimulating the microbiota by adding extra fibers to their diet could be relevant. Making it easier to consume more fibers could certainly benefit these patients.

This project focused on measuring exactly how dietary fibers are fermented inside the gut of humans. Once lumen content is excreted as feces, the fermentation process has nearly been finished. Detailed knowledge of what really goes on during fermentation was lacking. To learn more, this project aimed to develop novel methods to observe intestinal bacteria fermenting fibers in the gut itself, leading to new insights into what exactly goes on in the gut and its impact on human health.

Two PhD-students, Mara van Trijp and Melany Ríos Morales, worked hard to prepare the use of a novel gastrointestinal sampling capsule that a test subject could swallow, after which it was possible to sample luminal content, including microbes. Halfway the project, the company that developed the capsule was not able to provide them.

Of course, this was a disappointment, but the project members did not give up: they designed and manufactured a three-meter-long custom-made catheter, which was used by the project members to perform the same tests. To make sure the precious intestinal sample was preserved, the project researchers developed a special reagent that inactivated the bacteria after sampling. This way the results of the analyses would correctly show the status of the bacteria at the moment of sampling. They successfully applied this method in two clinical trials and obtained the results they were after.

MELANY RÍOS MORALES, PHD STUDENT AT UNIVERSITY MEDICAL CENTER GRONINGEN (UMCG):

‘PROUD OF OUR CUSTOM-MADE CATHETER’

Since it is hard to reach the intestinal tract, Melany Ríos Morales and Mara van Trijp wanted to analyze exactly when and how bacteria start to ferment fibers. They focused on a new method to gain more insight into which fermentation products, such as short-chain fatty acids, were produced by these bacteria at what moment.

‘In humans most research on this topic is carried out using fecal samples, but we know that most of the short-chain fatty acids are absorbed by the body beforehand. So, analyzing the remainder of these fermentation products in the feces does not give you a representative picture on what is happening inside the gut. For example, only five percent of the acids that are produced in the gut are excreted in the feces.’

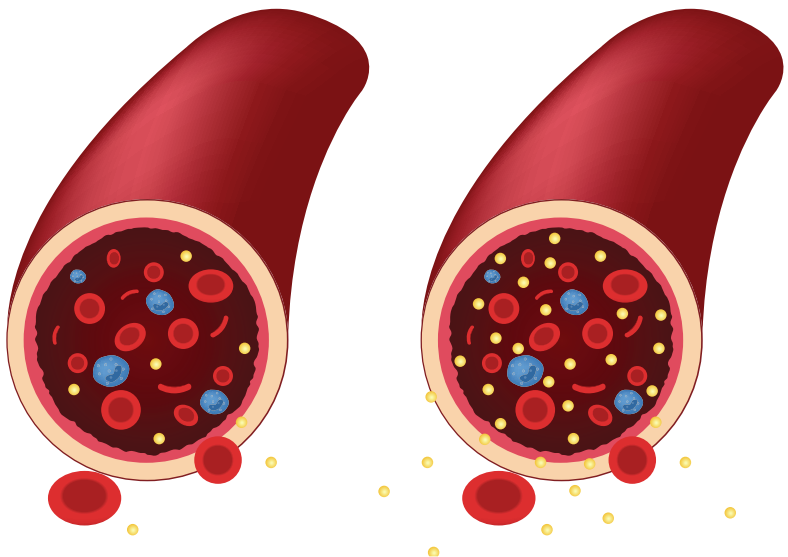
‘We used a special catheter that we could guide through the nose all the way down to the distal part of the small

intestine and the beginning of the colon. It was equipped with two little tubes: one to deliver labelled fermentation products with an isotopic marker to track their fate in the human body, and one to take a sample of luminal content. Once inside the beginning of the small intestine, we inflated a little balloon, so it moved gradually forward by the natural intestinal contractions, until it reached the position where we wanted to infuse the isotopes and take the luminal samples.'

'In humans most research on this topic is carried out using fecal samples, but we know that most of the short-chain fatty acids are absorbed by the body beforehand.'

'By tracking labelled short-chain fatty acids, we could measure the production of these acids by the bacteria over a period of time. Moreover, we saw what happened to these molecules when their label appeared in the blood. While I was responsible for the analysis of these molecules, my co-worker Mara van Trijp in Wageningen analyzed the fermented fibers and the composition of the microbiota.'

'One of our conclusions was that fermentation of the supplements GOS and FOS does not take place in the small intestine, but rather in the colon, which is good: otherwise, people may feel bloated. Another conclusion is that the fermentation process improves the glucose metabolism in the bloodstream.'



Normal blood glucose *High blood glucose*

MARA VAN TRIJP, PHD STUDENT AT THE DIVISION OF HUMAN NUTRITION AND HEALTH AT WAGENINGEN UNIVERSITY & RESEARCH:

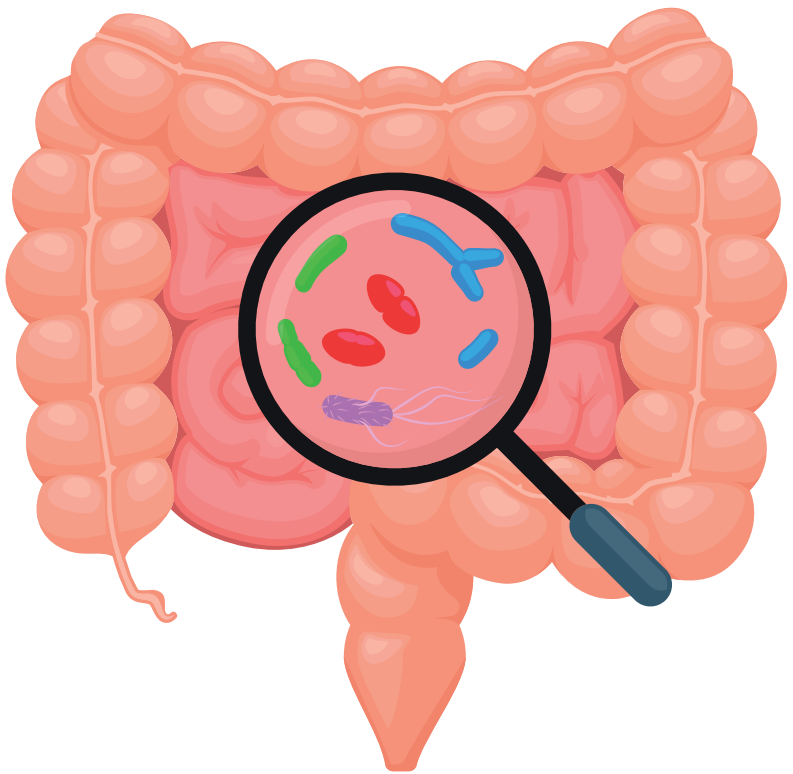
'USING FLUID FROM ILEOSTOMY BAGS'

This project aimed at unravelling parts of the fermentation process in the gut, which cannot be analyzed using fecal samples only. Mara van Trijp wanted to learn what exactly happens in the gut during fermentation and to study the effects on the health of the test subjects.

'It seems that the microbiota resembles our fingerprints in the sense that it is unique for each person.'

'We used a novel approach to analyze the breakdown of the prebiotic supplements galacto- and fructo-oligo-saccharides, also known as GOS and FOS, the production of short-chain fatty acids and the uptake and metabolism of the labelled short-chain fatty acids in the host. We first carried out a feasibility trial, which took two days for the study subjects. Using the intestinal catheter, we repeatedly collected luminal samples after consumption of a drink containing GOS and FOS, showing us exactly how quickly the fibers had been fermented and what happened in the body of the host. In a second trial, we aimed to examine the effects of a seven-day GOS and FOS intervention study on fiber fermentation and the microbiota inside the distal small intestine or the proximal colon. We are now analyzing the data.'

‘Before the start of the human trials, I also carried out *in vitro* work with lumen fluid that was sampled from ileostomy bags of patients who had their colon removed. Previous studies showed that bacteria in lumen fluid from ileostomy subjects are found in the intestinal lumen of healthy subjects. *In vitro* fermentation experiments taught us more about how quickly bacteria present in



‘In vitro fermentation experiments taught us more about how quickly bacteria present in the small intestine reacted when we added GOS, FOS or inulin into the mix.

the small intestine reacted when we added GOS, FOS or inulin into the mix. It turned out that the bacteria from the small intestine were capable of fermenting fibers, unless the molecular structures of these fibers were too complex. It took the bacteria longer to degrade inulin or pectin, for instance. Another conclusion was that the same bacteria reacted differently in each person. It seems that the microbiota resembles our fingerprints in the sense that it is unique for each person. This project showed that drawing conclusions for the general population is hard. Moving forward it seems important to look into personalized fiber-based nutritional strategies and also to study the effect of diet on bacteria in the intestinal lumen in more detail.’

GUIDO HOOIVELD, ASSISTANT PROFESSOR OF THE DIVISION OF HUMAN NUTRITION AND HEALTH AT WAGENINGEN UNIVERSITY & RESEARCH:

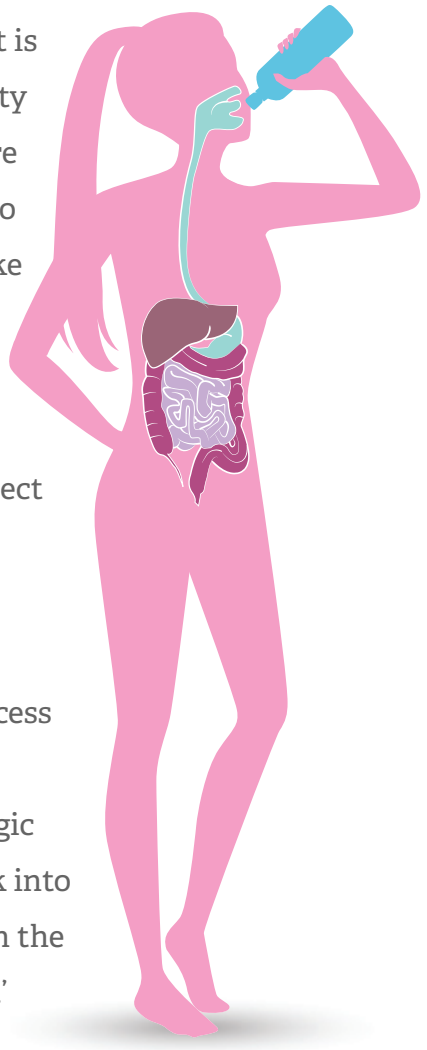
'PLENTY OF PROMISING DATA'

After receiving the call for this program, Guido Hooiveld contacted Barbara Bakker and together they wrote a proposal to research the kinetics of fiber degradation in the intestine. This project combined the expertise Guido had with running human dietary intervention trials with the advanced analytical methods Barbara and her colleague Dirk-Jan Reijngoud had at their disposal.

'It was wonderful to find out what goes on in the intestinal tract of a healthy person after consuming a drink containing fibers.'

'We are very happy with the results we achieved in project 6. Because of the innovative methods we used, most of these results are observed for the first time. It was wonderful to find out what goes on in the intestinal tract of a healthy person after consuming a drink containing fibers. We are still processing the data, but it is clear that the composition and activity of the microbiota changed much more rapidly than we had expected. We also found that fermentation does not take place in the small intestine, likely because the fibers pass through too quickly. Of course, we still have a hundred new questions, but this project helped us to take a leap forward.'

'We need to continue our research, looking at how the fermentation process influences the liver, as well as other processes in the body, including allergic reactions. It is also interesting to look into the details of the connection between the bacteria in our gut and brain activity.'



BARBARA BAKKER, PROFESSOR MEDICAL SYSTEMS BIOLOGY AT UNIVERSITY MEDICAL CENTER GRONINGEN (UMCG):

‘CONCENTRATING ON THE BIG SCIENTIFIC QUESTIONS’

Nutritional fibers are abundantly present in a healthy diet. Barbara Bakker is interested in how fiber fermentation in the gut affects whole-body glucose and fat metabolism. The collaboration within CCC provided a unique opportunity to investigate this in human volunteers.

‘I have been involved in computer models of metabolism for a long time, collaborating with Dirk-Jan Reijngoud, who retired as professor, but stayed on to work with us on analytical chemistry and stable isotopes. Together, we participated in a previous consortium working on the interaction between fibers, microbiota and metabolism. There we developed innovative methods using stable isotopes, which we used in this CCC project.’

‘In a previous research project, we applied an infusion directly in the gut of mice, containing butyric acids labelled with a stable carbon-13 marker to track how fast the bacteria would ferment the fibers and convert them into different molecules. We learned that concentrated fiber mixtures increased the insulin sensitivity and stimulated the production of more short-chain fatty acids. These insights we used to set up project 6, but now in a more challenging setting with human volunteers.’

Following the isotopes, we could trace in time how fermentation products formed in the intestine are converted by the body to glucose and fatty acids present in the blood.’

'The results are very exciting and there are more to come. Following the isotopes, we could trace in time how fermentation products formed in the intestine are converted by the body to glucose and fatty acids present in the blood. In addition to some exciting new methods, we now know more about the time it takes to ferment fibers. This will also help us to design future studies.'

'Working with industrial partners can be quite a challenge for scientists. In the context of this CCC program the companies engaged constructively within the projects. They shared their vast knowledge of dietary carbohydrates, so we could give the most relevant nutritional fibers to the subjects in the study. In addition, it was very valuable for the PhD students to work with the industrial partners. This gave them the opportunity to learn how companies operate. In this sense, CCC offers them a peek into the real world.'

'In addition, it was very valuable for the PhD students to work with the industrial partners.'

PARTNERS IN CCC NWO CARBOKINETICS

CCC NWO CarboKinetics was a partnership of knowledge centers and industrial partners. In addition to four universities, seven industrial partners took part in this program. These partners were: FrieslandCampina, DSM, Avebe, Nuscience, Agrifirm, VanDrie Group and Sensus. The knowledge centers involved were: Wageningen University & Research, University Medical Center Groningen (UMCG), Utrecht University and Maastricht University. The industrial partners provided 50% of the funds for this program. The other 50% was financed by NWO.

All CCC programs are the result of close cooperation between all partners in the consortium. The universities carried out the research and coached the young scientists involved, while the industrial partners provided ingredients, facilities and important industrial insights. Over time, the industrial partners were present during regular meetings with the scientists and their supervisors, providing ideas and advice. All partners played an important part in achieving the objectives of the program. In particular, some of the partners showed the participants in the program around on their own premises and facilitated discussions between the guests and their own staff and use of experimental facilities.

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 fiber from chicory roots, especially various types of inulin and oligofructose. The chicory seeds are sown in spring, the plant stores inulin in the root, and this is harvested and extracted in the autumn to give powders and syrups with dietary fiber.

Chicory root fibers are known for their benefits 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. That is why 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 two production locations in the Netherlands, in Roosendaal and Zwolle. 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 AT SENSUS B.V.:

‘UNDERSTANDING THE UNDERLYING MECHANISM OF PREBIOTIC FIBER HEALTH EFFECTS’

‘At Sensus we work with inulin and oligofructose harvested from chicory. These natural fibers are valued by food manufacturers for fiber enhancement and sugar replacement. In addition, our prebiotics are known to improve the composition of the gut microbiota. To prove that these ingredients have health effects it is important to understand the underlying mechanisms.’

‘We joined this program because we wanted to study the effects of our prebiotic inulin on the metabolic health of humans and in relation to weight and the immune system. We have in-depth knowledge of our own ingredients, which we could share with the researchers. We contributed essential data on structures, dose and application of our ingredients and information from relevant literature we already reviewed.’

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.

VALUABLE LESSONS LEARNED

'This program consisted of six projects, each concentrating on aspects of the kinetic process of non-digestible carbohydrates and all of them were valuable giving learnings besides improved scientific methods. To understand the breakdown of fibers and influence on health, the scientists needed to apply improved methodology, some of which they developed themselves. For example, in project 2, they developed a novel *in vitro* gut and immune model. We were pleased that the superior methods in this project showed complementary effects of prebiotic inulin and a human milk oligosaccharide on the infant microbiome and immune system.'

'We were pleased that the superior methods in this project showed complementary effects of prebiotic inulin and a human milk oligosaccharide on the infant microbiome and immune system.'

WEIGHT MANAGEMENT AND FIBER COMBINATIONS

'We also really appreciated project 5, which gave new insights for prebiotics and fibers on metabolic effects and weight management. This project carried out an intervention involving the combination of prebiotic inulin and resistant starch fibers. A one-day trial involving lean and obese men showed remarkable results in the lean men. They experienced a higher level of energy and an improved metabolism, and their gut microbiota could rapidly ferment this mix. Obese men did not show these effects; however, we suppose a longer-term trial is necessary for the inulin-resistant starch mix to change the microbiota composition to give such effects. The project also showed that only this particular mix of fibers resulted in this effect and we want to learn more about these mechanisms.'

'Such interactive collaboration between academia and industry on research themes relevant to society such as the gut microbiome with immunity or mental health motivated Sensus to participate in the next CCC program called CarboSupport.'

ROYAL FRIESLANDCAMPINA N.V.

Royal FrieslandCampina N.V. is fully owned by Zuivelcoöperatie Friesland Campina U.A. with 16,995 member dairy farmers in the Netherlands, Belgium and Germany. FrieslandCampina supplies consumer products, such as milk, yogurt, cheese, infant nutrition and desserts, products for the professional market, such as cream and butter products, ingredients and semi-finished products for producers of infant nutrition, the food industry and the pharmaceutical sector.

The company has branches in 38 countries, exports to over one hundred countries worldwide and employed at year-end 2020 some 23,783 people. The company's central office is based in Amersfoort, the Netherlands. The company is divided into four market-oriented business groups, focusing on Consumer Dairy, Specialised Nutrition, Dairy Essentials and Ingredients.

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ARJEN NAUTA, SENIOR SCIENTIST AT FRIESLANDCAMPINA:

‘LEARNING MORE ABOUT OUR OWN INGREDIENTS’

‘I have been part of the CCC from the very beginning. Carbohydrate research is very important for us, especially for our ingredients business. We develop ingredients for companies that use them in their own products. Our galacto-oligosaccharides, for instance, can be applied in infant formula but also in products for other age groups. Being part of this consortium helps us to get a better understanding of the mechanisms underlying the benefits of these ingredients and to find out more about possible health effects. Already some research projects we were involved in have led to promising new insights, publications and patent applications. This goes for other partners as well.’

‘The gut is like a black box: it is hard to find out what exactly goes on when carbohydrates are moving down the digestive tract.’

UNDERSTANDING THE BENEFITS OF OUR INGREDIENTS

‘In various CCC programs we focused our attention on ways to improve our understanding of the ingredients that are applied in infant formula. Quite a recent development is the market introduction of 2’-FL, an oligosaccharide that is present in mother’s milk. The various carbohydrates in human milk stimulate the growth and activity of specific bacteria in the infant’s gut which have been linked with a healthy development and implications later in live. Several new insights on the benefits of 2’-FL have been obtained, interestingly also for other age groups.

LOOKING INTO THE BLACK BOX

'The gut is like a black box: it is hard to find out what exactly goes on when carbohydrates are moving down the digestive tract. The CarboKinetics program helps us to find out more about this process, which can be very helpful for the development and improvement of our ingredients. More specifically, we were involved in an intervention study looking, literally, inside the human body, into the effects of GOS on the microbiota of adults and elderly. Another project delivered a detailed analytical analysis of the different oligosaccharides that are present in GOS. We knew this ingredient is complex, but even we were amazed by the number and variety of different beneficial compounds our own well-known ingredient contains. This could explain the differentiating performance as prebiotic in combination with 2'-FL when tested *in vitro* on infant microbiota and immune effects.'

MULTIPLYING EACH OTHER'S CONTRIBUTION

'Being part of the CCC community is also beneficial for the outside-in knowledge exchange within our own organization. Once a year I invite all CCC's highly motivated PhDs and their supervisors to participate in a workshop on our premises. This offers them the chance to present their work to my colleagues, leading to fruitful and exciting discussions. Taking part in programs like CarboKinetics means we can interact with twelve young and bright scientists carrying out valuable research, sharing results and ideas, and providing new insights. In fact, this joint pre-competitive program enables us to multiply our own contribution, to align with a state-of-the art multidisciplinary program which would not been possible on our own. I really enjoy the way we are working together, leading to new insights and ideas we can really use to understand and improve our products.'

COÖPERATIE AVEBE U.A.

Royal AVEBE is a cooperative of starch-potato growers in Veendam. Avebe's 1,350 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. Customers buy these ingredients because they simplify the production process, improve the flavor 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.

HANS LEEMHUIS, PRINCIPAL SCIENTIST AT ROYAL AVEBE:

‘BEING PART OF CCC DRIVES COLLABORATION AND INNOVATION’

‘Avebe has been involved in research programs of CCC for years, because we want to know more about the fibers we sometimes sell as a by-product of our starch and protein production. Our core business is functional starches, and we want to learn how we can change their properties in order to contribute to healthy food. Starch usually digests rapidly, releasing glucose in the bloodstream. We wish to find ways to slow down this digestion process. Fibers themselves are not our core business, but we are interested in their properties. Perhaps in the future fibers will become more important for us, which is why we gladly take part in CCC programs like this one.’

‘We excel in chemistry: isolating ingredients from natural products. By joining CCC programs we want to improve our understanding of how the degradation of fibers influences human and animal health. We can carry out *in vitro* experiments with fibers ourselves, but for highly

sophisticated *in vitro* and *in vivo* human trials we like to work with partners like CCC.’

WE NEED PROOF

‘During the CCC NWO CarboKinetics program a number of interesting models were developed, mimicking human and animal digestive tracts. Such models are still at an early stage, but they can teach us a lot about what happens to our own ingredients during fermentation. Of course, we have certain expectations about the fermentation process of our own fibers, but we need proof if we want to make any health claims.’

POOLING RESOURCES AND EXPERTISE

'Being part of the CCC community means you can choose to take part in interesting programs, like this one, pooling resources and expertise. The interaction with the scientists who all have different backgrounds and knowledge helps us to gain a better understanding of our own ingredients. Trying to analyze these data ourselves would take us more time and effort to reach the same conclusions. I enjoy the CCC meetings and like to listen to the presentations of all those young, motivated scientists. I like their drive and the way they concentrate on their research. At these meetings we also meet the other industrial partners. Since the program is all about precompetitive research, there is no friction because of commercial interests. To the contrary, we share the same interests which leads to interesting discussions.'

'Of course, we have certain expectations about the fermentation process of our own fibers, but we need proof if we want to make any health claims.'

'Fibers themselves are not our core business, but we are interested in their properties.'

DRIVING COLLABORATION AND INNOVATION

'Royal Avebe has been part of CCC for a long time and we are already participating in new programs, like CarboBiotics, CarboBased and CarboSupport. CCC offers a wonderful platform and network which drives collaboration and innovation.'

ROYAL DSM

Royal DSM is a global, purpose-led, science-based company active in Nutrition, Health and Sustainable Living. DSM's purpose is to create brighter lives for all. Our products and solutions address some of the world's biggest challenges while simultaneously creating economic, environmental and societal value for all its stakeholders - customers, employees, shareholders, and society at large.

DSM concentrates on Nutrition & Health, Climate & Energy and Resources & Circularity solutions.

'We were interested in all six projects of this program, because we wanted to learn more about carbohydrates in the broadest sense.'

MARCO VAN DEN BERG, PRINCIPAL SCIENTIST FOOD & APPLICATION AT DSM:

'FINDING OUT MORE ABOUT CARBOHYDRATES IN GENERAL'

'We were interested in all six projects of this program, because we wanted to learn more about carbohydrates in the broadest sense. Not only as a nutritional ingredient, but also to find out more about the function of carbohydrates in general. Taking part in the CCC consortium means we gained valuable knowledge, specifically about the Non-Digestible Carbohydrates (NDCs) we supplied ourselves: amongst others, various glucans and pectins.'

'Thanks to the efforts of the CCC management team, in particular of former director Lubbert Dijkhuizen, there were hardly any troubles during this program, even when things went less smoothly.'

'This program taught us much about all ingredients provided by the seven partners. Knowledge which we need to describe health benefits of new products. Looking back, I think the results of projects 1 and 5 were most interesting for our business. In Asia we produce pectins, mainly for applications in food. Learning about the complicated chemical structure of pectins in relation to health, stimulated our interest in the possibilities of developing these fibers for new applications.'

EYEOPENERS

'In general, it was interesting to hear about some of the surprising effects of a number of fibers on pathogenic microorganisms. Personally, it was an eyeopener for me to learn that some oligosaccharides can slow down the growth of pathogens. Moreover, it would be wonderful if the results of project 4 would lead to a strong reduction

of the use of antibiotics in calves. Our clients certainly benefit from our participation in CCC. Insights from previous programs have stimulated the launch of new DSM products. Recently, we also acquired a company that focuses its activities on research and development of oligosaccharides.'

'Personally, it was an eyeopener for me to learn that some oligosaccharides can slow down the growth of pathogens.'

SHARING IDEAS AND FINDINGS

'It is important that CCC programs remain precompetitive. Thanks to the efforts of the CCC management team, in particular of former director Lubbert Dijkhuizen, there were hardly any troubles during this program, even when things went less smoothly. The delays caused by COVID-19 and the sourcing problems with the capsule in project 6 were discussed openly with us. In a consortium of several industrial partners and universities like this one it is essential to act as transparently as possible. You don't want participants making deals behind closed doors. What you need is an open atmosphere where everyone feels free to share ideas and findings. This is what CCC offers.'

VANDRIE GROUP

Family business VanDrie Group is the global market leader in veal and the largest producer of calf milk. All companies operating as part of the VanDrie Group carry 100 percent responsibility to the optimum quality of the product to be supplied, fully supported by their own quality system Saftey Guard. Veal integration is VanDrie's major aim: every step, from the purchase of a new-born calf to the sale of veal is part of a chain, which is carried out inhouse.

VanDrie Group joined CCC in order to take part in CCC NWO CarboKinetics, supplying facilities to carry out research into the influence of fibers on calf health.

'Taking part in the CCC NWO CarboKinetics program will hopefully help us to improve the health and immune system of our calves.'

EELKE VAN DER WAL, R&D DEPARTMENT AT VANDRIE GROUP:

‘I WAS AMAZED TO SEE HOW MUCH WORK WAS DONE TO DEFINE THE MOLECULAR STRUCTURE OF THESE FIBERS’

‘In the first months of their lives, calves are still developing their immune system. Right after birth, their passive immunity is supported by the colostrum they are fed. But as the effect of the colostrum diminishes over time, they have to develop their immune system themselves. That is often the moment when calves are more likely to get ill, especially when they are put on transport and mixed with calves from other dairy farms and the first weeks after arrival at the veal farm. Taking part in the CCC NWO CarboKinetics program will hopefully help us to improve the health and immune system of our calves.’

‘We expect that these projects will contribute to the reduction of the use of antibiotics in the future.’

‘We supplied our premises and knowledge directly to project 4, but we also participated in the meetings with the other projects. In all, 300 calves were included in the project. These were not test animals reared for research, but part of our usual production chain. Our employees at the farm assisted the researchers when they needed to handle the calves. You have to know how to hold a calf correctly in order to prevent it from stressing out.’

NEW INSIGHTS INTO VARIOUS FIBERS

‘We learned a lot about various carbohydrates and their influence on our calves. I was amazed to see how much work was done to define the molecular structure of these fibers. These scientific insights are useful in my work, for instance when I am offered new products that claim to improve animal health. I now know better how to assess the scientific evidence.’

PROMISING QUESTIONS AND INTERESTING RESULTS

'The way CCC works also appealed to us, in particular because several knowledge institutes and industrial suppliers of fibers were involved. This led to promising research questions and interesting results, which can benefit all participants. We were interested in supplements that will help to improve calf health. The other partners wished to test their products *in vivo* and the scientists were interested in finding answers to their research questions. Within CCC all these interests were combined beautifully in one program.'

REDUCTION OF ANTIBIOTICS USE

'We also expect that these projects will contribute to the reduction of the use of antibiotics in the future. We have already successfully taken a number of steps to reduce our own use of antibiotics. It is a lot harder to take the next step but working with CCC may well lead to new insights in the way we can reduce the use of antibiotics even further.'

ROYAL AGRIFIRM GROUP

With almost 3,000 dedicated employees driven to excel every day, Royal Agrifirm Group contributes to a responsible food chain for future generations. The company delivers measurable, relevant and sustainable value at farm, field and industry. Founded over 120 years ago in the Netherlands, it is now a leading agricultural cooperative with an international network of subsidiaries within Europe, South America, United States of America, Asia and a worldwide distribution network.

The Royal Agrifirm Group is the parent company of the Belgian organization Nuscience. At the start of the CCC NWO CarboKinetics program, researchers from both Agrifirm and Nuscience were closely involved in the CCC programs. During the program, the different R&D departments of Royal Agrifirm Group were merged into one global R&D center. This led to a more centralized R&D approach and, subsequently, the decision that in the future only one Agrifirm colleague will represent Royal Agrifirm Group. Bianca Martens, who was the spokesperson for Agrifirm in CCC NWO CarboKinetics, has taken a step back in favor of Geert Bruggeman, who in future will represent Agrifirm via the Nuscience label in CCC projects.

BIANCA MARTENS, MANAGER R&D TEAM NEW TECHNOLOGIES AND MATERIALS AT AGRIFIRM:

‘JOINING CCC NWO CARBOKINETICS FELT LIKE COMING HOME’

‘I have known CCC for years, as I did my own PhD research within a CCC project on starch digestion kinetics in pigs. Joining CCC NWO CarboKinetics felt like coming home. The program was already running when I joined. Since Geert Bruggeman and I belong to the same team now, we have been working together closely. While he focused on the health aspects, I was involved in the chemistry of the carbohydrates.’

‘Taking part in this CCC program meant we were in the front row, watching how topnotch scientists carried out important research in the field of gut health.’

‘At Agrifirm we are especially interested in the value of fibers for a better gut health and a stronger immune system. This follows closely our global goal to reduce the use of antibiotics. That is why it is so important to gain more knowledge about how fibers can improve gut health. Pectins, for instance, seem to be quite interesting. Taking part in this CCC program meant we were in the front row, watching how topnotch scientists carried out important research in the field of gut health. This was very valuable for our global R&D group. This way we keep informed about new, relevant methods and scientific developments.’

TAKING THE LEAD

'As a major player in the field of animal feed it is essential for Agrifirm to take the lead. Clearly there is a lot of pressure on our sector to make a shift to a more circular system. In line with our vision to contribute to a responsible food chain for future generations, we strive to maximize the usage of raw materials in animal diets that are unsuitable for direct consumption by humans. As these streams are usually high in fibers and carbohydrates, it is of the utmost importance to understand their value. For this reason, we followed the research in the CCC NWO CarboKinetics program with great interest and will continue to do so via our colleague Geert Bruggeman in the future!'

'In line with our vision to contribute to a responsible food chain for future generations, we strive to maximize the usage of raw materials in animal diets that are unsuitable for direct consumption by humans.'

NUSCIENCE

Nuscience offers nutritional solutions and functional feed ingredients to the animal feed industry. Their nutritional solutions include a broad range of innovative young animal nutrition concepts and customized premixes and concentrates. Due to many years of scientific research and innovation, the key to further growth and development, Nuscience strives to be the preferred knowledge partner, offering economic quality solutions for the efficient production of feed and feed material for the animal feed industry.

Geert Bruggeman will represent Agrifirm via the Nuscience label in all CCC projects as a result of the merger of the two R&D departments into one global R&D center. All scientists involved in supplements and additions to feed now work together in the same team.

GEERT BRUGGEMAN, SENIOR SCOUT AT NUSCIENCE:

‘GLAD TO REMAIN PART OF THE CCC COMMUNITY’

‘We joined the CCC NWO CarboKinetics Program because we were very interested in the degradation of carbohydrates and in particular how these fibers can steer certain functionalities. Take GOS, for instance: a class of sugar molecules with a prebiotic function. But to learn which GOS variant we need for an optimal effect on the health of animals we need to know more about the kinetic process. What can we do about the formula of our feed in order to achieve health effects?’

‘We don’t expect CCC programs to provide us with ready-to-use solutions. That is not possible when you carry out precompetitive research, like it is done at CCC. But each program provides a piece of the puzzle. Hopefully in the future we can fit those pieces together, creating a new, valuable concept. Of course, we always give our due to the universities involved. We are very strict in observing intellectual property of new concepts. Previously, our company succeeded in applying a number of patents in conjunction with one of the partners. When we take concepts to the market, we always inform CCC what we have done, giving back what we have learned for future use. For the young researchers it is also great to know that their hard work does not end up in a closet somewhere but can be applied in practice.’

‘We don’t expect CCC programs to provide us with ready-to-use solutions. That is not possible when you carry out precompetitive research, like it is done at CCC.’

'One of the important aspects of CCC is that it forms a close community of committed people who want to enrich the world with new ideas. The atmosphere is safe, and we all show respect for each other's expertise and opinions.'

A CLOSE COMMUNITY

'One of the important aspects of CCC is that it forms a close community of committed people who want to enrich the world with new ideas. It is not some artificial platform paid by the taxpayers. Everything Lubbert Dijkhuizen set up and Gert-Jan Euverink and Henk Schols now continue, is done wisely with the aim of creating valuable knowledge to benefit society. The atmosphere is safe, and we all show respect for each other's expertise and opinions. The young scientists feel free to share their ideas and the supervisors act as coaches.'

ETHICAL PRINCIPLES

'The research that CCC carries out is quite complicated and also involves ethical principles. Babies are involved, and frail or overweight elderly people. Personal data need to be protected and CCC does this very well. Animal trials, quite a sensitive subject for most of the industrial partners, are reduced to a minimum. In future, we are happy to join new CCC programs, like CarboSupport that will kick off this year. We are already involved in CarboBiotics, aiming at the reduction of the use of antibiotics. Personally, I am very glad to remain part of the CCC community.'

WRAPPING UP
CCC NWO CARBOKINETICS

Officially CCC NWO CarboKinetics ran until 2020. Circumstances and the COVID-19 pandemic were the cause of some delays. Not all projects could be finished in time. Some of them will continue during 2021. Nevertheless, this report will be presented in April 2021, wrapping up CCC NWO CarboKinetics.

SOME CLOSING REMARKS BY HENK SCHOLS

CCC NWO CarboKinetics gave us a better grasp of what each individual fiber does and how we can influence its effect. But when we eat, we consume more than one fiber at the time. How does our body react to this mix of fibers in our diet? To find out the mechanism behind the way our body deals with all the signals it gets from what goes on in our gut we need more research. We need to refine our methods even further. That will be the focus of the CCC CarboSupport program, that will kick off sometime this year. The research in CarboSupport will also involve the effects of proteins on carbohydrate metabolism and vice versa in our gut.

LUBBERT DIJKHUIZEN, EMERITUS PROFESSOR MICROBIOLOGY AT UNIVERSITY OF GRONINGEN AND RETIRED DIRECTOR CCC

Lubbert Dijkhuizen was one of the founding fathers of CCC. CCC NWO CarboKinetics was the last program on his watch. He retired as professor in 2018 and stepped down as scientific director in September of 2019. During the symposium of CCC in November of that year the CCC community honored his contributions.

'This program was the last one I supervised as director, as I retired last year. It is a shame not all projects could be finished in time due to the pandemic. I am especially sorry for those brilliant young scientists whose progress was interrupted and who did not get the ceremony they deserved. We had to stop meeting in person. CCC is a kind of family and we like to create a warm, open atmosphere. That is a lot harder to do now. Still, I am very proud of what we achieved, in particular the patents we were able to file in cooperation with some industrial partners and the quality of the work that was delivered. I am especially pleased with the way we were able to involve NWO. Working with NWO means that our research is up to their quality standards, which increases the scientific reputation of our projects.'

WORKING WITH INDUSTRIAL PARTNERS

'I am glad we were able to involve all these industrial partners. Some universities are not keen to work with the industry out of fear that they will not be able to carry out uninhibited, fundamental research. In my experience carrying out precompetitive research is beneficial for all concerned, if you firmly agree

beforehand on what the research will focus on. Changing course during the program to meet commercial wishes of an industrial partner is out of the question for CCC and all participants know this in advance. Precompetitive research also means staying close to what the industrial partners are interested in, so they can apply the results sometime in the future. At the same time, our results are not going to lead to applications right away. Usually, more research is needed before they can be used commercially. This is also why these companies feel free to participate without having to worry about the competition.'

BIG SCIENTIFIC STEPS AHEAD

'Looking back on CCC NWO CarboKinetics I am proud to say this program has taken some bold steps, advancing science by the structural analysis of well-defined carbohydrates. The scientists observed closely how these fibers are degraded during their journey through the digestive tract and analyzed the effects of this process on the health and immune system. In addition, by working together and sharing findings, they were able to make comparisons between effects of dietary fibers in human food and animal feed. In addition, they all felt they belonged to a single program and were more than happy to assist each other by sharing data, findings, methods and insights.'

THE RESEARCH WILL CONTINUE

'This program has officially come to an end, but the research into carbohydrates continues. CCC NWO CarboBiotics, a program about the effects of antibiotics, is already halfway and CCC is on the verge of kicking off a new program called CarboSupport. They will do so with my blessing, but without my presence as their director. Now that I have retired, I have founded my own company exploring and analyzing carbohydrates. But naturally, I will remain interested in the CCC activities.'

CARBOHYDRATE COMPETENCE CENTER

COLOPHON

Carbohydrate Competence Center (CCC) is a public private partnership for research in nutrition and health as well as biobased economy.

Final report CCC NWO CarboKinetics, 2016 - 2021
Published by Carbohydrate Competence Center (CCC), Groningen, Netherlands.

CCC NWO CarboKinetics was financed by the participating partners and NWO. Research focused on the improvement of health based on a better knowledge of the degradation kinetics of carbohydrates present in food. This program offers unique opportunities unlocking available resources, to understand and to integrate as part of a healthy diet for humans and animals.

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CARBOHYDRATE COMPETENCE CENTER

PARTNERS CARBOKINETICS:

FrieslandCampina nourishing by nature

Sensus part of Royal Cosun

nuscience safe & innovative nutrition

DSM

agrifirm

Avebe

VanDrie Group CONTROLLED QUALITY VEAL

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