Algemene gegevens	
PPS-nummer	AF-16602
Titel	Partnership DSM-STW
Thema	
Uitvoerende kennisinstelling(en)	WUR, UU-UMCU, RUG-UMCG
Projectleider onderzoek (naam + emailadres)	Project 1: Walter Gerrits, walter.gerrits@wur.nl Project 2: Stephan Bakker, s.j.l.bakker@umcg.nl Project 3: Saskia van Mil, s.w.c.vanmil@umcutrecht.nl Project 4:Jaap Keijer, jaap.keijer@wur.nl Project 5:René Kwakkel, rene.kwakkel@wur.nl
Penvoerder (namens private partijen)	Arie Kies - DSM
Contactpersoon overheid (indien relevant)	P. Spierenburg – NWO-TTW
Adres projectwebsite	https://www.nwo.nl/onderzoek-en-resultaten/programmas/partnership/partnership-programmas/stw-dsm+partnership
Startdatum	1-12-2016
Einddatum	30-11-2021

Goedkeuring penvoerder/consortium De jaarrapportage dient te worden besproken met de penvoerder/het consortium. De TKI's nemen graag kennis van eventuele opmerkingen over de jaarrapportage.	
De penvoerder heeft namens het consortium de jaarrapportage	✓ goedgekeurd ☐ niet goedgekeurd
Eventuele opmerkingen over de jaarrapportage:	

Inhoudelijke samenvatting van het project	
Probleemomschrijving	Politics have put a target to the agri-world to reduce the use of antibiotics in animal production, to decrease possible induction of microbial resistance against antibiotics. However, this may increase the occurrence of health problems, and, consequently, significantly reduce the profitability of the industry. Improving the health status of animals, permitting a reduction of antibiotic use without decreasing productivity, is one of the large challenges for the animal production chain. Indigestible protein affects (gut) health negatively. Consequently, this protein fraction is causing increased need for antibiotic use for economic animal production. A better knowledge of the mode of action of this potential negative effect will help to find solutions to improve animal health with lower antibiotic use. It will also result in better utilisation of protein, allowing the industry to increase the use of co-products from human food industry, and increase effective and sustainable animal production. Improving health status of animals is also important from animal welfare perspective.

Doelen van het project	General aim: Understanding how animal (gut) health is affected by feeding and feed composition with emphasis on protein digestibility. The project consists of 5 sub-projects: Project 1: WUR, UMCU: The relation between protein fermentation and associated metabolites, microbiota and post-weaning diarrhoea in pigs (Walter Gerrits) The relation between protein fermentation, associated metabolites and microbiota in relation to gut health and pig performance. Project 2: UMCG, WUR: A Feed-Forward Approach of New Knowledge Arising from Human Nutritional Studies in Susceptible Groups to Application in Animal Nutrition (Stephan Bakker) The influence of protein intake, vitamins and minerals on gut microbiota, synthesis of uremic toxins and organic acids in post kidney transplantation patients. Project 3: UMCU: An unbiased metabolomics approach to understand how the undigested protein fraction in chickens and pigs impacts on gut health (Saskia van Mil) The influence of ileal undigested protein on metabolites in different parts of the intestinal lumen, blood, intestine and liver tissue in chicken and pigs. Focus on changes in microbiome. Project 4: WUR: Understanding the impact of protein fermentation end-products on pig intestinal metabolic health to optimize its barrier function (Jaap Keijer). The role of mitochondrial metabolism in the relationship to protein fermentation and the impaired gut barrier function. Development of novel feed components that improve gut health. Project 5: WUR, UMCU : Dynamics of proteolytic fermentation and the effects on intestinal health of broilers and piglets (René Kwakkel) Evaluate the impact of ileal undigested protein on microbial activity, proteolytic fermentation, intestinal health and animal performance.

Resultaten	
Beoogde resultaten 2019	<u>Project 1</u> - Finalize second organoid paper on transcriptome identification after long-term growth - Pathogen-specific analyses on-farm piglet experiment according to step-wise approach (May 2019) - Targeted metabolomics in fresh faeces (summer 2019) - Data analyses on-farm piglet experiment (summer 2019) - Organoid studies according to step-wise approach (summer 2019) - Design of next in vivo experiment <u>Project 2</u> - Data collection: TransplantLines Biobank and Cohort Study. Closing the first cohort January 2019. However data collection will off course continue. First cohort can be used for the first analyses. (first cohort will contain ±800 RTR). - Data entry stool frequency questionnaire in Utopia: start summer 2018. (Deadline: January 2019). Perform statistical analyses to investigate the prevalence of diarrhoea and gastro-intestinal symptoms among RTR and donors. First results are reported in 2-pager of February 2019. Plan: continue with statistical analyses and explore possibility of a scientific publication.

	- Microbiome analyses. (Deadline has yet to be determined). Start DNA extraction feces samples December 2018. Deadline DNA extraction: March 2018). Planning: sending samples to novogene in May or June 2019. - Analysis of organic acids in 24-h urine samples from the TransplantLines study. (Deadline had yet to be determined). Analyses of the 24-h urine samples from the pilot are expected in May 2019. - Analysis gut permeability markers: Start citrulline measurements in TransplantLines samples august 2018. Results statistical analyses expected in May 2019. - Laboratory analysis and data analysis phase 1 (WUR deadline end year 2) - Submission of publication written on the experiments performed in phase 1 (WUR deadline end year 2) - Finalize the experimental design for phase 2 and start with the preparations for the experiment (WUR deadline end of year 2018) - Finish preparations and start experiment 2 (WUR end year 2 and beginning year 3) <u>Project 3</u> - Publication on MetaboShiny, 5/6-2019 (current status: resubmission 7/2020). - Expanding DIMS database even more with serum metabolite profiles from broilers and piglets from around the world (12-2019). - Progress towards the definition of a m/z signature associated with poor/good performance or gut health (12-2019). - Implementation of more features, bug fixing and making user-friendly of METABOshiny application (continuous) - Metagenomic sequencing of fecal samples (spring 2019) - Metabolic modelling of fecal/cecal microbial protein fermentation (12-2019). - Expanding experiments using pig organoids to identify genes involved in development that could potentially be influenced by nutrition (8-2019). - Investigating the mechanism of action of the effect of certain nutrients on gut development (6-2019). <u>Project 4</u> - Submit document to get animal ethical approval for the second animal experiment (tentatively September 2019) - Perform in vitro studies with protein fermentation end-products, using both conventional, organoid cell cultures and/or primary enterocytes (continuous) - Analyze results from ongoing animal experiment that is performed in May/June (starting July 2019) <u>Project 5</u> - Perform an extra broiler growth trial (in-vivo 2) - Statistical analysis of data in-vivo 1 - Writing literature review - Targeted metabolomics in ceca contents collected in in-vivo1 and in droppings collected in the extra broiler trial - Writing first publication - Designing next broiler experiment 3 - First pig trial (May - June 2019) in collaboration with project 4 - Laboratory procedures, data analyses of the first pig trial

Behaalde resultaten 2019	<u>Project 1</u> - Analyses of ammonia and volatile fatty acids in faecal/digesta samples - Comparison of preparation methods on metabolomics data - Completing intestinal morphology analyses - Performing faecal microbiota composition analyses - Analysis of Rotavirus A presence in faecal material - Performing wound-healing assays (in presence of different metabolites) - Evaluating the relation between metabolites, colon morphology and post-weaning diarrhoea - Evaluating the relation between microbial parameters and post-weaning diarrhoea - Performing untargeted metabolomics to faecal and digesta samples - Performing microbial toxin analyses in faecal samples - Organoid scratch assay results (ammonia and hydrogen sulphide) - General design of next piglet experiment <u>Project 2</u> - Very good inclusion of renal transplant recipients in TransplantLines Biobank and Cohort Study. - Large amount of fecal samples, 24-h urine collections and samples, blood samples. Status 10-07-2018: 1379 independent biobank moments, including follow-up visits (3, 6, 12 and 24 months after renal transplantation). - Start Organic acids/ uremic toxin analysis under supervision of prof. Heiner-Fokkema. Samples from the pilot study in which the microbiome was already measured are analyzed. Biostatistician is involved on how to proceed with statistical analyses. - First 250 feces samples are analyzed using 16s rRNA method, which resulted in a publication. DNA is extracted from a 1000 fecal samples and results from metagenomic sequencing just arrived (November 2019) Cas Swarte is currently analyzing these data. - Pilot study investigating breath hydrogen and methane concentrations in RTR, which resulted in a publication by Tim Knobbe. Breath samples are still collected in the TransplantLines study for future analysis. - Questionnaire about stool frequency and diarrhea symptoms is incorporated in UTOPIA and questionnaires are imported in the Database. - Marker for gut wall permeability: citrulline, is measured in the previous TransplantLines-cohort (2008-2011). Citrulline is also measured in the TransplantLines cohort study. Results just arrived and Rianne presented some preliminary results at the ASN kidney week November 2019 in Washington D.C. - Rianne Douwes was enrolled in several courses and conferences including: Medical Statistics and Survival analysis course, PhD management course, winner best oral presentation at the NTV-BTS transplantation congress. She recently finished the course publishing in English. - Kim Lammers-Jannink enrolled in a training school entitled Gut Biology and Health (2017) at Aarhus University. - Project license next animal experiments - Combined in vivo and in vitro study in pigs (reported in several two-pagers of 2018, Kim Jannink; <u>Project 3</u> - Metaboshiny tool version 1.0 has been finished. - MetaboShiny paper is in the process of submission / final editing. - A clear understanding of the amount of samples needed for a predictive metabolite signature has been calculated and plans to increase sample size have been made. - Gathered genomes belonging to microbial genera identified in pig 16S rRNA studies from WUR. - Collected set of genes responsible for different protein fermentation pathways in bacteria. - Set up MAMBO simulation pipeline for simulating microbial community metabolomes.

	- Identified that galactose, a milk oligosaccharide, next to acetate, affects the Wnt signalling pathway. - Set up an organoid model to investigate gut maturation - Identified that acetate positively affects the expression of intestinal stem cells markers in the fetal organoids. <u>Project 4</u> - Feed-intervention trial in pigs was successfully preformed. In this trial the effects of protein fermentation and the influence of different fiber-types on intestinal mitochondrial function mitochondrial function was investigated. - Our technique to isolate intact pig intestinal cells has been optimized. - Inverted sac technique was implemented and applied (in collaboration with project 1). - The freshly isolated pig intestinal cells form the intervention trial have been used to measure in vivo mitochondrial function. - In vitro studies (cells,organoids) with protein fermentation products have been performed. - A review paper on protein fermentation products (spermine and spermindine) and the gut has been published. <u>Project 5</u> - Analysis of crude protein digestibility of in-vivo 1 broilers. - Analysis of leaky gut by FITC-dextran and litter scores of in-vivo 1 broilers. - Histology of ileum of in-vivo 1 broilers (statistical analyses not yet finished). - In-vivo 2 broiler experiment is performed and statistical analyses on growth and feed intake are finished. - Statistical analyses on crude protein and amino acids of in-vivo 1 broiler experiment are finished. - Finished pig in-vivo 1 (batch 1 in April/May 2019 and batch 2 in August/September 2019), in collaboration with project 4 - Analysis of ammonia concentrations in digesta of different intestinal segments in pigs. - Analysis of intestinal permeability by FITC-dextran and TRITC-dextran in jejunum and proximal colon in pigs.
Beoogde resultaten 2020	<u>Project 1</u> - Request/obtain license for the animal experiment - Finalize design of piglet experiment 2 (in collaboration with project 5) - Perform piglet experiment 2 (September – November 2020) - Analyses on-farm trial (microbiota metagenomics, intestinal cell expression, targeted metabolomics) - Multivariate data analyses on-farm trial - Organoids stimulations for transcriptomics (to finish a paper) - Writing of a review on (intestinal) metabolite-host interactions <u>Project 2</u> - Data collection: TransplantLines Biobank and Cohort Study. Closing the first cohort January 2020. However data collection will off course continue. First cohort can be used for the first analyses. (first cohort will contain ±800 RTR). - Microbiome analyses. (Deadline has yet to be determined). Metagenomic sequencing data just arrived. Focus will first be on diarrhea vs. no diarrhea and effect of medication use on the gut microbiota composition. - Analysis of organic acids in 24-h urine samples from the TransplantLines study. (Deadline had yet to be determined). Analyses of the 24-h urine samples from the pilot are available from November 2019. Currently, samples from Kim (pig study) are measured. - Analysis gut permeability markers: citrulline from TransplantLines study just arrived in November. Rianne will focus on statistical analyses and writing of a manuscript in the coming period. - Laboratory analysis and data analysis phase 1 (WUR deadline june year 2020) - Submission of publication written on the experiments performed in phase 1 (WUR deadline june year 2020)

	- Start experiment 2 (WUR) (deadline year 2020) Project 3 - Publishing MetaboShiny (submitted 02-2020 to Metabolomics, resubmission in 7-2020). - Progress towards the definition of a m/z signature associated with poor/good performance or gut health (10-2020). - Developing a neural network to enhance metabolite annotation (~ 06-2020) - Implementation of more features, bug fixing and making user-friendly of MetaboShiny application (all 2020) Project 4 - Continue analyzing data from the performed animal trial. - Perform in vitro studies with protein fermentation end-products, using both conventional, organoid cell cultures and/or primary enterocytes (continuous) - Present the results about the in vitro effects of butyrate exposure on metabolic function, using also data from the animal experiment, at an international conference. - Write a paper about the in vitro effects of butyrate exposure on metabolic function, using also data from the animal experiment. - Perform second in vivo trial (finalized by 12-2020, depending on Corona). Project 5 - Microbiome and histology analyses of in-vivo 1 are almost finished. - Targeted metabolomics in cecal contents (broiler experiment 1) and droppings (broiler experiment 2) - Plan was originally to start broiler experiment 3 on March 23th. This is rescheduled due to Corona to start on May 18th. - Starting gene expression analyses after broiler experiment 3. - Writing broiler manuscripts (in-vitro work, broiler experiment 1 and 2) - Laboratory analyses of first pig experiment - Statistical analysis of data first pig experiment - Second pig experiment, in collaboration with project 1 (September-November 2020) - Writing first pig publication

Opgeleverde producten in 2019 (geef de titels en/of omschrijvingen van de producten / deliverables of een link naar de producten op de projectwebsite of andere openbare websites)
Wetenschappelijke artikelen: Project 1 - B. van der Hee, L.M.P. Loonen, N. Taverne, J.J. Taverne-Thiele, H. Smidt, and J.M. Wells. Optimized procedures for generating an enhanced, near physiological 2D culture system from porcine intestinal organoids. Stem Cell Research 28 (2018) 165–171. - M. S. Gilbert, N. J. J. Ijssennagger, A. K. Kies, and S. W. C. van Mil. Protein fermentation in the gut; implications for intestinal dysfunction in humans, pigs and poultry. American Journal of Physiology-Gastrointestinal and Liver Physiology 315: G159-G170, 2018. - B. van der Hee, O. Madsen, H. Smidt, J.M. Wells. Congruence of location-specific transcriptional programs in intestinal organoids during long-term culture. BioRxiv, 600940, under review at journal. - Gilbert, B van der Hee, M Gulersonmez, E Stigter, A Kies* and W Gerrits. 2018. Post-weaning diarrhoea in piglets in practice is associated with protein fermentation, but specific protein fermentation metabolites contribute differently. Page S66 in 14th International Symposium on Digestive Physiology of Pigs, Brisbane, Australia. Vol. 9. E. Roura and F. Dunshea, ed. Cambridge University Press - Gilbert, M. S., B. van der Hee, and W. J. J. Gerrits. 2019. The role of protein fermentation metabolites in post-weaning diarrhoea in piglets. Pages 361 - 362 in 6th EAAP International Symposium on Energy and Protein Metabolism and Nutrition, Belo Horizonte, Brazil. M. L. Chizzotti, ed. Wageningen Academic Publishers. - S.K. Kar, B. Van der Hee, L.M.P. Loonen, N. Taverne, A. Jansman, D.J. Schokker, A. Taverne, M.A. Smits, J.M. Wells. 2018. Evaluation of functional properties of current and novel protein sources using enteroids. Chapter in PhD thesis; under review at journal.

- N. Benis, J.M. Wells, M.A. Smits, S.K. Kar, B. van der Hee, V.A.P. Martins dos Santos, M. Suarez-Diez, D.J. Schokker. 2019. High-level integration of murine intestinal transcriptomics data highlights the importance of the complement system in mucosal homeostasis. BMC Genomics, accepted for publication
- R.J.J. van Erp, S. de Vries, B. van der Hee, H. Smidt, T.A.T.G. van Kempen, W.J.J. Gerrits. 2019. Quantification of ileal and total tract starch fermentation in pigs fed resistant starch. Chapter in PhD thesis.
- N. Winaris*, B. Van der Hee*, E. Kranenbarg, J.M. Wells. 2020. Transcriptional responses of porcine intestinal organoids exposed to acetate and butyrate. Chapter in PhD thesis.

Project 2
- Publication (currently under revision at PLOS Medicine). The Assocation between Use of Proton-Pump Inhibitors and Excess Mortality after Kidney Transplantation: A Prospective Cohort Study. Rianne M. Douwes1.†, António W. Gomes-Neto1.†, Michele F. Eisenga, Joëlle C. Schutten1, Rijk O.B. Gans1, Maarten Naesens, Else van den Berg1, Ben Sprangers, Stefan P. Berger1, Gerjan Navis1, Hans Blokzijl2, Björn Meijers, Stephan J.L. Bakker1, Dirk Kuypers.
- Publication Amino Acids. Effect of Renal Function on Homeostasis of Asymmetric Dimethylarginine (ADMA): Studies in Donors and Recipients of Renal Transplants. M. Yusof Said1, Rianne M. Douwes1, Marco van Londen1, Isidor Minović2, Anne-Roos Frenay1, Martin H. de Borst1 , Else van den Berg1, M. Rebecca Heiner-Fokkema2, A. Arinc Kayacelebi3, Harry van Goor4 , Gerjan Navis1, Dimitrios Tsikas3, Stephan J.L. Bakker1.
- Publication Journal of Clinical Medicine. Chronic Use of Proton-Pump Inhibitors and Iron Status in Renal Transplant Recipients. Rianne M. Douwes, António W. Gomes-Neto, Michele F. Eisenga, Joanna Sophia J. Vinke, Martin de Borst, Else van den Berg, Stefan P. Berger, Daan J. Touw, Eelko Hak, Hans Blokzijl, Gerjan Navis, Stephan J.L. Bakker.
- Publication Journal of Clinical Medicine. Proton-Pump Inhibitors and Hypomagnesaemia in Kidney Transplant Recipients. Rianne M. Douwes, António W. Gomes-Neto, Joëlle C. Schutten, Else van den Berg, Martin de Borst, Stefan P. Berger, Daan J. Touw, Eelko Hak, Hans Blokzijl, Gerjan Navis, Stephan J.L. Bakker.
- Publication Journal of Clinical Medicine. Altered Gut Microbial Fermentation and Colonization with Methanobrevibacter smithii in Renal Transplant Recipients. Tim J. Knobbe, Rianne M. Douwes, Daan Kremer, J. Casper Swarte, Michele F. Eisenga, António W. Gomes-Neto, Marco van Londen, Frans T.M. Peters, Hans Blokzijl, Ilja M. Nolte, Wouter H. Hendriks, Hermie J.M. Harmsen and Stephan J.L. Bakker.
- Publication Journal of Clinical Medicine. Characteristics and Dysbiosis of the Gut Microbiome in Renal Transplant Recipints. J.Casper Swarte, Rianne M. Douwes, Shixian Hu, Arnau Vich Vila, Michele F. Eisenga, Marco van Londen, António W. Gomes-Neto, Rinse K. Weersma, Hermie J.M. Harmsen and Stephan J.L. Bakker.

Project 3
- Protein fermentation in the gut; implications for intestinal dysfunction in humans, pigs and poultry' by Gilbert, M; IJssennagger, N; Kies, A and van Mil SWC, is published in American Journal of Physiology-Gastrointestinal and Liver Physiology 315: G159-G170, 2018.
- MetaboShiny - interactive processing, analysis and identification of untargeted metabolomics data. by Joanna C. Wolthuis, Stefania Magnusdottir, Mia Pras-Raves, Judith J.M. Jans, Boudewijn Burgering, Saskia van Mil, Jeroen de Ridder. bioRxiv 734236; doi: https://doi.org/10.1101/734236

Project 4
- Literature review, Nutrients, 11th January 2020, Anna F Bekebrede, Jaap Keijer, Walter JJ Gerrits and Vincent CJ de Boer."The molecular and physiological effects of protein-derived polyamines in the intestine"

Project 5
- NA

Externe rapporten:

Artikelen in vakbladen:

Inleidingen/posters tijdens workshops, congressen en symposia:

Project 1

- B. van der Hee. 5/2/2018 "Using intestinal stem cells to generate a near-physiological multicellular in vitro model", WIAS Science Day, Wageningen, poster presentation
- M.S. Gilbert. 22-08-2018 "Post-weaning diarrhoea in piglets in practice is associated with protein fermentation, but specific protein fermentation metabolites contribute differently", 14th International Symposium on Digestive Physiology of Pigs, 22-08-2018, Brisbane, Australia, oral presentation
- B. van der Hee. 16/02/2019 "Development of equine intestinal organoid monolayers to study location-specific epithelial responses", 9th European Equine Health and Nutrition Congress, Utrecht, The Netherlands (oral presentation)
- M.S. Gilbert. 11-09-2019. "The role of protein fermentation metabolites in post-weaning diarrhoea in piglets", 6th EAAP International Symposium on Energy and Protein Metabolism and Nutrition, Belo Horizonte, Brazil, oral presentation
- M.S. Gilbert. 25-09-2019. "About piglets, protein fermentation and diarrhoea", DSM conference on Transition Nutrition, Belper, UK, oral presentation.
- B. van der Hee. 19-6-2019. "Organoids as models to study probiotics", IPC2019: International scientific conference on probiotics, prebiotics, gut microbiota, and health, oral presentation

Project 2

- Oral presentation Rianne Douwes. Joint NTV-BTS Transplantation Congress 16-03-2018. Price for best presentation. (abstract publicatie 1 zie boven)
- Oral presentation, Kim Lammers-Jannink. Ignite 2018 - DSM Animal Nutrition & Health Scientific Symposium 03-04-2018.

Project 3

- Poster presentation Joanna Wolthuis, BioSB, 5th Dutch Bioinformatics & Systems Biology Conference, Lunteren, The Netherlands
- Poster & e-poster presentation Joanna Wolthuis, Metabolomics2019 Conference, the Hague, 2019
- Visit: Dutch Society for Stem Cell Research Meeting, UMC Utrecht, May 2019
- Visit: Gut Day, Amsterdam UMC, November 2019
- Visits: Utrecht Bioinformatics Symposium 2019 Utrecht (3rd poster prize), Bioinformatics and Systems Biology (BioSB) conference 2019, Metabolomics2019 the Hague(e-poster opportunity)

Project 4

- WIAS science day, 5th February 2018, Wageningen, The Netherlands.The impact of food-derived components on intestinal mitochondrial function. Anna F Bekebrede; Walter J.J. Gerrits; Jaap Keijer; Vincent CJ de Boer (pitch presentation, posterwalk session with poster)
- WIAS science day, 18th March 2019, Lunteren, The Netherlands. Mitochondria: energy for the gut. AF Bekebrede, VCJ de Boer (poster presentation).
- WIAS science day, 13-14th February 2020, Lunteren, The Netherlands. Butyrate induces metabolism of other mitochondrial substrates in colonocytes. AF Bekebrede, WJJ Gerrits, T van Deuren, J keijer, VCJ de Boer (poster presentation).

Project 5

- Miranda Elling-Staats "Developing an in-vitro method to predict caecal protein fermentation in broilers". Poster presentation at WIAS Science Day (5/2/2018)
- Miranda Elling-Staats, September 2018. "Effects of different heat damaged protein ingredients on protein digestibility and caecal fermentation in broilers". European Poultry Conference, Dubrovnik 17-21 September 2018, poster.
- Miranda Elling-Staats, February 14th 2020. Oral presentation at the 'WIAS Annual Conference' about the digesta flows in in-vivo 1 of the Chicken part.

TV/ Radio / Social Media / Krant:

Overig (Technieken, apparaten, methodes etc.):

Project 3

- MetaboShiny tool (zie publicatielijst voor meer info)