PPP Project Annual Report 2018
The PPP-projects that have been established under the direction of the top sectors must submit an annual report on their technical and financial progress. This format is to be used for reporting the technical progress. A separate format ('PPP final report') is available for PPP-projects that have been completed in 2018.
The annual reports will be published in full on the websites of the TKIs/top sector, excluding the blocks 'Approval coordinator/consortium' and 'Planning and progress' . Please ensure that no confidential matters are left in the remaining blocks.
The PPP Project Annual Reports must be submitted by 15 February 2019 to Hans van der Kolk

General information	
PPP number	**AF14322**
Title	**Algae Linkages**
Theme	**TKI Agri & Food CIRCULAIR**
Executive knowledge institution(s)	**WFBR, WLR**
Research project leader (name + e-mail address)	**Lolke Sijtsma; e-mail: lolke.sijtsma@wur.nl**
Coordinator (on behalf of private parties)	**Lolke Sijtsma**
Government contact person	**Patricia Wagenmakers/Jan van Esch**
Total project size (k€)	**1466**
Address project website	**https://topsectoragrifood.nl/project/algaelinkages/; https://www.wur.nl/nl/project/Algaelinkages-1.htm**
Start date	**01.03.2016**
End date	**01.03.2020**

Approval coordinator/consortium The annual report should be discussed with the coordinator/the consortium. The TKIs appreciate being informed of possible feedback on the annual report.	
The coordinator has assessed the annual report on behalf of the consortium:	X approved □ rejected
Possible feedback on the annual report:	

Short content description/aim PPS What is going on and how is this project involved? What will be delivered by the project and what is the effect of this?
Objective: In AlgaeLinkages a new, integrated and sustainable agrifood chain will be developed, using drain water from the Mexican greenhouse horticulture as a nutrient source for microalgae production. Next, his algal biomass will be studied as a healthy chicken feed to produce omega-3 enriched eggs. Future implementation of this agrifood chain will reduce the problems related to water quality in Mexico, and at the same time create a healthy and sustainable feed(ingredient) for enriched, healthy eggs, thereby using Dutch expertise and technology. Once proven successful and economically feasible the sustainable approach used in this project can be replicated in Mexico, the Netherlands and the rest of the world.

Planning and progress (if there are changes to the project plan, please explain)	
Is the PPP going according to plan?	In 2018 we had a delay in production of sufficient amounts of *Nannochloropsis limnetica* biomass containing relevant amounts of n-3 fatty acids. This delay affected the timing of feeding trials. Additional activities aiming to investigate the gut-health promoting properties of this micro algae in *in vitro* test with Intestinal Porcine Epithelial Cells (IPEC-J2) have been performed.
Have there been changes in the consortium/project partners?	No
Is there a delay and/or deferred delivery date?	Due to problems in producing sufficient amount of algal biomass containing sufficient amount of n-3 fatty acids there is a delay in the start of feed trials on chickens (WP3). We foresee, this work can start in Q2-3 2019. This delay might impact the estimated end date with 3-6 month.
Are there any substantive bottlenecks?	No
Are there any deviations from the projected budget?	Project partners most likely exceed their "in kind" contribution as agreed initially.

Results in 2018/ so far
Give a short description of the high-lights and (most important) project deliverable in 2018 / so far and their target group

Biomass production
Stable production of algal biomass in outdoor photobioreactors under temperature and light conditions that are highly variable, faced several challenges.

Drain water quality: The microfiltration system (installed by the end of 2017 and tested in 2018 with two membranes, one of 5 µm and the second one of 0.2 µm plus a Smart UV 2040 Sterilizer) shows to be enough to guarantee the microbiological quality of the greenhouse drain water. However, the green-house drain water, as a medium to grow microalgae, has a pH of about 5.5 whereas 8.4 is required to grow *Nannochloropsis limnetica*. After several tests, sodium bicarbonate was selected as the most suitable to increase the pH because of its low cost and because it produces less precipitating salts as compared to other reagents

Temperature. In Queretaro, Mexico temperatures can be as high as 33 °C in summer and as low as 1 °C in winter. To grow *Nannochloropsis limnetica* optimally, the temperature has to be controlled between 20 and 25°C. Sprinklers and a heating system using coils to cool and heat, respectively, are being used and tested with good results so far.

Biomass quality: The first biomass, produced at scale, and sent to the Netherlands to be analyzed for the chicken feed showed that the production process in the lab and in the outdoor photobioreactors needed to be improved because biomass was contaminated with another algae

(later identified via DNA sequencing as *Desmodesmus pleiomorphus* and looking similar to Nannochloropsis) and with some pathogens (maybe due to drying conditions at 40°C). The algae *Desmodesmus pleiomorphus* does not contain omega 3 fatty acids and is therefore not useful for our research. Consequently, a new inoculation "train", including improved quality control, was set up. Furthermore, a new disinfection and validation process was implemented to guarantee microalgae free of undesired bacteria.

Storage, processing and transport.
It is agreed that after harvesting the biomass paste needs to be dried (above 50°C). After drying, the biomass will be transported in vacuum sealed bags to the Netherlands for further processing and testing as chicken feed in Q2-Q3 2019

In vitro gut-health promoting activities
Nannochloropsis limnetica biomass, cultivated in Mexico (small scale), has been subjected to an *in vitro* test with Intestinal Porcine Epithelial Cells (IPEC-J2) aiming to investigate the gut-health promoting properties of this micro algae. The algae were used either in intact form or processed by beat-milling to degrade the cell-wall matrix. Both forms were tested in the *in vitro* system with and without the presence of an *E. coli* bacteria, which brought the IPEC-cells in a stress condition. Genes expression of the IPEC-cells were measured by using microarrays and bioinformatics was applied to understand the functionality of the different expressed genes.

The results show that both algae treatments substantially affected the expression of genes. Moreover, the genes were highly upregulated in the presence of *E. coli.* Beat-milling negatively affected the number of different expressed genes, indicating that intensively degrading of the cell-wall matrix resulted in a loss of functional properties of the micro algae. Expressed genes were involved in many super pathways. More super pathways were activated in the presence of E. coli. Super pathways demonstrate the positive impact of algae on immune responses, gut cell development and survival, and energy metabolism of cells.

Based on these results we can conclude that intact *Nannochloropsis limnetica* has high potential as functional ingredient to promote gut health of monogastric farm animals. *In vivo* studies are needed to demonstrate these potential effects in pigs and/or poultry.

Number of delivered products in 2018 / so far *(in an appendix, please provide the titles and/or description of the products or a link to the products on public websites)*			
Academic articles	Reports	Articles in journals	Introductions/workshops
1			

Appendix: Names of the products or a link to the products on a public website

***Scientific Presentations/ introductions*:**
Guiscafre, F., Vanthoor, M., Kleinegris, D., Fernado García, J., Salinas, M., Escoto, H., Veloo, R., **Sijtsma, L*** (2018). Outdoor cultivation of nannochloropsis in Mexico: challenges and solutions. Abstract book pp 312-313. 5th AlgaeEurope, 4-6 December, Amsterdam, The Netherlands

Sijtsma, L (2017) AlgaeLinkages: Production of microalgae on greenhouse drain water for the production of poultry feed. Wetsus Congress 2017, Synergy in Research and Innovation , October 9-10, Leeuwarden, The Netherlands (https://www.wetsus.nl/home/wetsus-news/wetsus-congress-2017)

Wijers, T. (2017) Lipid content in microalgae for treatment of wastewater in greenhouses. ECO innovations from biomass congress, 28029 June 2017, Papenburg, Germany

Agostino, L (2017) Key note speaker. Tecnologias para aproveitamento de águas salinas: experiência Internacional. (Technology development in Water Technology. The Dutch System and Experiences) I CIESA, 26 Nov – 1 Dec 2017; Belem, Brazil. http://www.funasa.gov.br/web/i-ciesa

Newsitems:
Onderzoeksprogramma Topsectoren Wageningen University & Research-WR 2018, P53
https://www.wur.nl/upload_mm/1/8/7/87679e63-8396-4887-8ae4-473d85be0d90_Onderzoeksprogramma%20Topsectoren%20Wageningen%20University%20%26%20Research-WR%202018.pdf

Presentatie voortgang Algae Linkages project (Landbouwraad, ambassade)
Nieuwsbericht | 06-06-2017 | 16:27
https://www.agroberichtenbuitenland.nl/actueel/nieuws/2017/06/06/presentatie-voortgang-algae-linkages-project

Universidad Autónoma de Querétaro (UAQ), Producción de microalgas para impulsar la sustentabilidad alimenticia (2017): https://ingenieria.uaq.mx/facultad-de-ingenieria-desarrolla-produccion-de-microalgas-para-impulsar-la-sustentabilidad-alimenticia/

Bezoek aan Mexico voor Algalinkages project VHL (2017):
https://www.hvhl.nl/nieuws/items/2017/bezoek-aan-mexico-voor-algalinkages-project.html

Website (2017): http://www.alfa-editores.com.mx/tecnologia-agricola-mexicana-para-enriquecer-huevos-con-omega-3/

Website: http://www.elfinanciero.com.mx/bajio/en-la-uaq-producen-microalgas-para-alimentar-gallinas.html

Link naar Kennisonline/TKI AF:
http://topsectoragrifood.nl/project/algaelinkages/