WAGENINGEN UNIVERSITY, CARUS

Research topics:	The research facility of Wageningen University (WU) 'Carus' is a state of the art facility for applied and strategic research concerning farm and companion animals. Research at Carus is conducted in the fields of sustainable animal husbandry, behaviour and welfare, biology and aquaculture. For the present TNA in SmartCow, the description below focuses on cattle. Research with dairy cattle at Carus focuses on nutrition, physiology, health, and environmental impacts of milk production including aspects of milk composition and human health. The facility includes climate respiration chambers, characterized by a high level of accuracy and flexibility, for research on gas emissions (CO_2, $^{13}CO2$, NH_3, CH_4, H_2 etc.), digestion, and energy metabolism. Selected publications of studies performed at the WU 'Carus' facilities include: Van Gastelen, S., Antunes-Fernandes, E.C., Hettinga, K.A. &. Dijkstra, J. (2018). The relationship between milk metabolome and methane emission of Holstein Friesian dairy cows: Metabolic interpretation and prediction potential. Journal of Dairy Science 101, 2110-2126 Klop, G., Dijkstra, J., Dieho, K., Hendriks, W.H. & Bannink, A. (2017). Enteric methane production in lactating dairy cows with continuous feeding of essential oils or rotational feeding of essential oils and lauric acid. Journal of Dairy Science 100, 3563-3575 Macome, F., Pellikaan, W.F., Schonewille, J.T., Bannink, A., Laar, H. van, Hendriks, W.H., Warner, D. & Cone, J.W. (2017). In vitro rumen gas and methane production of grass silages differing in plant maturity and nitrogen fertilisation, compared to in vivo enteric methane production. Animal Feed Science and Technology 230, 96 - 102. Van Gastelen, S., Antunes-Fernandes, E.C., Hettinga, K.A. & Dijkstra, J. (2017). Relationships between methane emission of Holstein Friesian dairy cows and fatty acids, volatile metabolites and non-volatile metabolites in milk. Animal 11, 1539-1548. Van Gastelen, S., Visker, M.H.P.W., Edwards, J.E., Antunes-Fernandes, E.C., Hettinga, K.A., Alferink, S.J.J., Hendriks, W.H., Bovenhuis, H., Smidt, H. & Dijkstra, J. (2017). Linseed oil and DGAT1 K232A polymorphism: Effects on methane emission, energy and nitrogen metabolism, lactation performance, ruminal fermentation, and rumen microbial composition of Holstein-Friesian cows. Journal of Dairy Science 100, 8939-8957. Van Lingen, H.J., Edwards, J.E., Vaidya, J.D., van Gastelen, S., Saccenti, E., van den Bogert, B., Bannink, A., Smidt, H., Plugge, C.M. and Dijkstra, J. (2017). Diurnal dynamics of gaseous and dissolved metabolites and microbiota composition in the bovine rumen. Frontiers in Microbiology 8, 425. Warner, D., Bannink, A., Hatew, B., van Laar, H. & Dijkstra, J. (2017). Effects of grass silage quality and level of feed intake on enteric methane production in lactating dairy cows. Journal of Animal Science 95, 3687-3699. Antunes-Fernandes, E.C., van Gastelen, S., Dijkstra, J., Hettinga, K.A. & Vervoort, J. (2016). Milk metabolome relates enteric

	methane emission to milk synthesis and energy metabolism pathways. Journal of Dairy Science 99, 6251-6262. Ellis, J.L., Hindrichsen, I.K., Klop, G., Kinley, R.D., Milora, N., Bannink, A. & Dijkstra, J. (2016). Effects of lactic acid bacteria silage inoculation on methane emission and productivity of Holstein Friesian dairy cattle. Journal of Dairy Science 99, 7159-7174. Hatew, B., Bannink, A., van Laar, H., de Jonge, L.H. & Dijkstra, J. (2016). Increasing harvest maturity of whole-plant corn silage reduces methane emission of lactating dairy cows. Journal of Dairy Science 99, 354-368. Huyen, N.T., Desrues, O., Alferink, S.J.J., Zandstra, T., Verstegen, M.W.A., Hendriks, W.H., & Pellikaan, W.F. (2016). Inclusion of sainfoin (*Onobrychis viciifolia*) silage in dairy cow rations affects nutrient digestibility, nitrogen utilization, energy balance, and methane emissions. Journal of Dairy Science 99, 3566 - 3577. Klop, G., Bannink, A., Dieho, K., Gerrits, W.J.J. & Dijkstra, J. (2016). Using diurnal patterns of ^{13}C enrichment of CO_2 to evaluate the effects of nitrate and docosahexaenoic acid on fiber degradation in the rumen of lactating dairy cows. Journal of Dairy Science 99, 7216-7220. De Jonge, L.H., van Laar, H. & Dijkstra, J. (2015). Estimation of the in situ degradation of the washout fraction of starch by using a modified in situ protocol and in vitro measurements. Animal 9, 1465-1472. Van Gastelen, S., Antunes-Fernandes, E.C., Hettinga, K.A., Klop, G., Alferink, S.J.J., Hendriks, W.H. & Dijkstra, J. (2015). Enteric methane production, rumen volatile fatty acid concentrations, and milk fatty acid composition in lactating Holstein-Friesian cows fed grass silage- or corn silage-based diets. Journal of Dairy Science 98, 1915-1927. Warner, D., Podesta, S.C., Hatew, B., Klop, G., van Laar, H., Bannink, A. & Dijkstra, J. (2015). Effect of nitrogen fertilization rate and regrowth interval of grass herbage on methane emission of zero-grazing lactating dairy cows. Journal of Dairy Science 98, 3383-3393. Warner, D., Dijkstra, J., Tamminga, S. & Pellikaan, W.F. (2013). Passage kinetics of concentrates in dairy cows measured with carbon stable isotopes. Animal 7, 1935-1943. Warner, D., Ferreira, L.M.M., Breuer, M.J.H., Dijkstra, J. & Pellikaan, W.F. (2013). Stable isotope labeled n-alkanes to assess digesta passage kinetics through the digestive tract of ruminants. PLoS ONE 8 (10), e75496.
Activities and services currently offered by the infrastructure/installation:	The research facility Carus is a small-scaled, high-tech facility for a wide range of animals. For dairy cattle, research is performed in the free-stall barn, tie-stall, and in respiration chambers, on digestion and metabolism including resource efficiency, methane emissions and nitrogen (N) and phosphorus (P) efficiency. The research facility is in close collaboration with the Wageningen University & research facility 'Dairy Campus' (Leeuwarden, the Netherlands; 550 dairy cows), for example in expanding the pool of cows available for research. The ruminant research facility of Carus has a maximum of 32 lactating and 8 dry dairy cattle. Carus has supporting in-house surgical facilities. The farm has 4 ha of grassland for grazing and 20 ha for ensiling purposes. Maize

	silage is bought elsewhere. The facility includes climate respiration chambers, characterized by a high level of accuracy and flexibility. Eight individual cow climate respiration chambers are available to measure gaseous exchange and energy metabolism of cows, with adaptation to diets usually taking place in the tie-stall. This unique facility can be used for research on gas emissions (CO_2, $^{13}CO_2$, NH_3, CH_4, H_2 etc.), digestion, and energy metabolism. 12 cows can be individually housed in tie-stalls, mainly for digestion studies. Animal performance (milk production and composition), body weight and body condition score is recorded. On average 12 rumen-fistulated cows are available for research including abomasal infusion studies. The facilities allow measurements of rumen and total tract digestibility, N balance, and digesta fractional passage rates performed using a combination of total collection and external markers. The diurnal pattern of rumen pH, volatile fatty acids and ammonia concentrations and in situ (nylon bag) degradability of feeds are measured via rumen fistula, and animals can be abomasally infused if required. Nearby laboratory facilities are also available for measurements of in vitro degradability of feeds using gas production techniques. Technical and farm staff members are highly skilled to support experimental research work. At Carus, in addition to strictly scientific research, an average of 2-3 projects a year are carried out at the request of multinational private companies, mainly in the fields of animal feeding, genetics, and animal housing and husbandry. Previous cutting-edge research includes the measurement of rumen passage rates using ^{13}C labeled plant material; lactic acid bacteria silage inoculants in relation to energy and nitrogen balance of cows; and research into dietary CH_4 mitigation options including changes in gaseous exchange of H_2 and other gases.
Description of the access to be provided under SmartCow TNA calls:	Access includes animals (including rumen fistulated animals if available), animal housing (respiration chambers in particular), preparation, feeding and daily care. Support offered under this proposal also includes experienced staff providing full technical and logistic support to users. Adjacent laboratories of the Animal Nutrition Group can provide complementary expertise and additional technologies where necessary/requested. Users can be present and may participate in the experiment, according to their preferences and practical competencies, subject to legal restrictions as to animal experimentation. The unit of access for this installation is defined as one cow.week, with 3 wks per cow to complete methane and energy balance. One typical access for a project (84 units of access) covers preparatory work which will be required at least 4 months before the access to facilities, and help with data analysis, also training in use of equipment (graduate students). Approval of Animal Ethics committee requires at least 6 mths in advance. The data collected will respect the SmartCow data management plan to allow their integration into the cloud-based database. Offices and meeting rooms with internet connection are available.

	Assistance with finding short-term accommodation can be provided.
Animal types, diets, housing and experimental conditions that can be worked on in this infrastructure/installation:	A wide range of animals may be evaluated at Carus facilities, including farm animals and companion animals. In the SmartCow project, dry and lactating cattle are of interest. Housing of dairy cattle is in a free-barn, in tie-stall (digestion studies mainly), or in climate-controlled respiration chambers (for methane and energy metabolism studies mainly). Diets may vary and may include grass silage, maize silage, and concentrate; other feeds may be bought and used. In climate respiration chambers, environmental conditions (e.g., light/dark period, temperature and humidity) may be set at desired levels.
Travel and subsistence costs:	Travel/subsistence costs for applicants in WU experiments at Carus facilities is available. Reimbursement is limited to one person per application for travel and 10-day stay. Travel and subsistence costs of applicants can be reimbursed on production of original receipts.
Infrastructure/installation ethical rules:	WU has animal facilities that are licensed by the Dutch Government to perform studies with animals for experimental purposes. Animal experimental work is carried out at WU in accordance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes regulation, S.I. 543/2012. The EU Directive aims for better protection of animals involved in scientific research; fair competitive conditions for businesses and scientific research within the EU; encouraging the application of the 3R's (i.e., replacement, reduction and refinement). The Dutch legislation is available at http://wetten.overheid.nl/BWBR0003081/2014-12-18. In the Netherlands, the EU Guidelines are incorporated in the Experiments on Animals Act (WOD, 2014). The principle behind this Act is that no experiments should be conducted on animals unless there are good reasons for doing so, and no alternatives are available that would produce the result without using animals. At WU, the procedure for animal experiments is as follows. A research project proposal is submitted to the local Animal Welfare Body (AWB) of WU. After approval by AWB, WU sends the application to the Animal Experiment Committee (AEC) of WU. The AEC will review the scientific quality of the proposal and makes an ethical decision based on the potential discomfort in relation to the importance of the experiment. The AEC then submits a recommendation to the national Central Authority for Scientific Procedures on Animals (CCD) (see: https://www.centralecommissiedierproeven.nl/). If granted, the CCD issues the license for the project. Subsequently, every animal experiment in the authorized project has to be approved by the AWB of WU. Only after approval by AWB, the experiment can start. The application of the 3Rs is considered at WU: i) replace animal experiments by in vitro investigations or in silico simulations whenever possible; ii) reduce the number of animals involved to the necessary minimum for each experiment; and iii) refine

	experimental protocols in order to diminish to a minimum the amount of stress imposed on those animals that will be used. In compliance to the legislation, all researchers, assistant researchers and technicians employed at WU possess the necessary qualifications to perform experiments with (farm) animals, and hold a certificate of competence required by law, that was obtained after satisfactory completion of an official course on Laboratory and Farm Animal Science.