	Inventaris Wob-verzoek W23-03									
		wordt verstrekt				weigeringsgronden				
nr.	document NTS 202316684	reeds openbaar	niet	geheel	deels	5.1, lid 1c	5.1, lid 2e	5.1, lid 2f	5.1, lid 2h	5.2, lid 1
1	Aanvraag projectvergunning, d.d. 06-01-2023				x		x		x	
2	Projectvoorstel bij aanvraag				x				x	
3	Bijlage dierproeven_1 bij aanvraag				x				x	
4	Bijlage dierproeven_2 bij aanvraag				x				x	
5	Bijlage dierproeven_3 bij aanvraag				x				x	
6	Bijlage dierproeven_4 bij aanvraag				x				x	
7	NTS bij aanvraag			x						
8	E-mail aan DEC om advies projectvergunning, d.d. 15-02-2023				x		x		x	
9	DEC-advies, d.d. 15-2-2023				x		x		x	
10	Projectvoorstel na DEC advies				x				x	
11	Bijlage dierproeven_1 na DEC advies				x				x	
12	Bijlage dierproeven_2 na DEC advies				x				x	
13	Bijlage dierproeven_3 na DEC advies				x				x	
14	Bijlage dierproeven_4 na DEC advies				x				x	
15	NTS na DEC advies			x						
16	Adviesnota aan CCD, d.d. 28-02-2023_met opmerkingen				x		x		x	x
17	Adviesnota aan CCD, d.d. 03-03-2023				x		x		x	x
18	E-mail CCD aan vergunninghouder over projectvergunning, 03-03-2023				x		x		x	
19	Antwoorden vergunninghouder na vragen CCD				x				x	
20	Projectvoorstel na vragen CCD				x				x	
21	Bijlage dierproeven_1 na vragen CCD				x				x	
22	Bijlage dierproeven_2 na vragen CCD				x				x	
23	Bijlage dierproeven_3 na vragen CCD				x				x	
24	Bijlage dierproeven_4 na vragen CCD				x				x	
25	E-mail intern beraad, d.d. 14-03-2023				x		x			
26	E-mail intern beraad, d.d. 14-03-2023				x		x			x
27	E-mail intern beraad, d.d. 17-03-2023				x		x			x
28	Adviesnota aan CCD, d.d.17-03-2023				x		x		x	x
29	NTS na vragen CCD en definitieve versie			x						
30	Beschikking, d.d. 20-03-2023				x		x		x	
31	E-mail CCD aan DEC over projectvergunning, d.d. 10-05-2023				x		x		x	

Centrale Commissie Dierproeven

Aanvraag
Projectvergunning Dierproeven
Administratieve gegevens

- U bent van plan om één of meerdere dierproeven uit te voeren.
- Met dit formulier vraagt u een vergunning aan voor het project dat u wilt uitvoeren. Of u geeft aan wat u in het vergunde project wilt wijzigen.
- Meer informatie over de voorwaarden vindt u op de website www.centralecommissiedierproeven.nl. of in de toelichting op de website.
- Of bel met 0900-2800028 (10 ct/min).

1 Gegevens aanvrager

1.1 Heeft u een deelnemernummer van de NVWA?
Neem voor meer informatie over het verkrijgen van een deelnemernummer contact op met de NVWA.

☒ Ja > *Vul uw deelnemernummer in*	5.1 lid2h
☐ Nee > *U kunt geen aanvraag doen*	

1.2 Vul de gegevens in van de instellingsvergunninghouder die de projectvergunning aanvraagt.

Naam instelling of organisatie	5.1 lid2h
Naam van de portefeuillehouder of diens gemachtigde	5.1 lid2e
KvK-nummer	5.1 lid2h
Straat en huisnummer	5.1 lid2h
Postbus	5.1 lid2h
Postcode en plaats	5.1 lid2h 5.1 lid2h
IBAN	5.1 lid2h
Tenaamstelling van het rekeningnummer	5.1 lid2h

1.3 Vul de gegevens van het postadres in.
Alle correspondentie van de CCD gaat naar de portefeuillehouder of diens gemachtigde en de verantwoordelijke onderzoeker.

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	5.1 lid2e	☐ Dhr. ☒ Mw.
Functie	5.1 lid2e	
Afdeling	5.1 lid2h	
Telefoonnummer	5.1 lid2e	
E-mailadres	5.1 lid2e	

1.5 *(Optioneel)* Vul hier de gegevens in van de plaatsvervangende verantwoordelijke onderzoeker.

(Titel) Naam en voorletters		☐ Dhr. ☐ Mw.
Functie		
Afdeling		
Telefoonnummer		
E-mailadres		

1.6	*(Optioneel)* Vul hier de gegevens in van de persoon die er verantwoordelijk voor is dat de uitvoering van het project in overeenstemming is met de projectvergunning.	(Titel) Naam en voorletters	☐ Dhr. ☐ Mw.
		Functie	
		Afdeling	
		Telefoonnummer	
		E-mailadres	
1.7	Is er voor deze projectaanvraag een gemachtigde?	☐ Ja > *Stuur dan het ingevulde formulier Melding Machtiging mee met deze aanvraag*	
		☒ Nee	

2 Over uw aanvraag

2.1	Wat voor aanvraag doet u?	☒ Nieuwe aanvraag > Ga verder met vraag 3
		☐ Wijziging op (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn Vul uw vergunde projectnummer in en ga verder met vraag 2.2
		☐ Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn Vul uw vergunde projectnummer in en ga verder met vraag 2.3
2.2	Is dit een *wijziging* voor een project of dierproef waar al een vergunning voor verleend is?	☐ Ja > Beantwoord dan in het projectplan en de niet-technische samenvatting alleen de vragen waarop de wijziging betrekking heeft en onderteken het aanvraagformulier
		☐ Nee > Ga verder met vraag 3
2.3	Is dit een *melding* voor een project of dierproef waar al een vergunning voor is verleend?	☐ Nee > Ga verder met vraag 3
		☐ Ja > Geef hier onder een toelichting en ga verder met vraag 6

3 Over uw project

3.1	Wat is de geplande start- en einddatum van het project?	Startdatum	1 - 6 - 2023
		Einddatum	31 - 5 - 2028
3.2	Wat is de titel van het project?	Development of nutritional strategies to better meet piglet requirements after weaning, while increasing sustainability, performance, welfare, and health.	
3.3	Wat is de titel van de niet-technische samenvatting?	Ontwikkelen van voerstrategiëen die beter aansluiten bij de nutriëntbehoeftes van biggen na spenen en tevens duurzaamheid, groeiprestaties, welzijn en gezondheid bevorderen.	
3.4	Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?	Naam DEC	5.1 lid2h
		Postadres	
		E-mailadres	

4 Betaalgegevens

4.1	Om welk type aanvraag gaat het?	☒ Nieuwe aanvraag Projectvergunning € 2322,- Lege ☐ Wijziging € Lege
4.2	Op welke wijze wilt u dit bedrag aan de CCD voldoen. *Bij een eenmalige incasso geeft u toestemming aan de CCD om eenmalig het bij 4.1 genoemde bedrag af te schrijven van het bij 1.2 opgegeven rekeningnummer.*	☐ Via een eenmalige incasso ☒ Na ontvangst van de factuur

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

☒ Projectvoorstel

☒ Niet-technische samenvatting

Overige bijlagen, indien van toepassing

☐ Melding Machtiging

☐

6 Ondertekening

6.1 Print het formulier uit, onderteken het en stuur het inclusief bijlagen via de beveiligde e-mailverbinding naar de CCD of per post naar:

Centrale Commissie
Dierproeven
Postbus 20401
2500 EK Den Haag

Ondertekening door de instellingsvergunninghouder of gemachtigde (zie 1.7). De ondergetekende verklaart:

- dat het projectvoorstel is afgestemd met de Instantie voor Dierenwelzijn.
- dat de personen die verantwoordelijk zijn voor de opzet van het project en de dierproef, de personen die de dieren verzorgen en/of doden en de personen die de dierproeven verrichten voldoen aan de wettelijke eisen gesteld aan deskundigheid en bekwaamheid.
- dat de dieren worden gehuisvest en verzorgd op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van richtlijn 2010/63/EU, behalve in het voorkomende geval de in onderdeel F van de bijlage bij het bij de aanvraag gevoegde projectvoorstel gemotiveerde uitzonderingen.
- dat door het ondertekenen van dit formulier de verplichting wordt aangegaan de leges te betalen voor de behandeling van de aanvraag.
- dat het formulier volledig en naar waarheid is ingevuld.

Naam	5.1 lid2e
Functie	
Plaats	5.1 lid2h
Datum	2023 - 01 06 -
Handtekening	5.1 lid2e

Centrale Commissie Dierproeven

Form
Project proposal

- This form should be used to write the project proposal for animal procedures.
- The appendix 'description animal procedures' is an appendix to this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed.
- For more information on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 lid2h
1.2	Provide the name of the licenced establishment.	5.1 lid2h
1.3	Provide the title of the project.	Development of nutritional strategies to better meet piglet requirements after weaning, while increasing sustainability, performance, welfare, and health.

2 Categories

2.1 Please tick each of the following boxes that applies to your project.

- **X** Basic research
- **X** Translational or applied research
- ☐ Regulatory use or routine production
- ☐ Research into environmental protection in the interest of human or animal
- ☐ Research aimed at preserving the species subjected to procedures
- ☐ Higher education or training
- ☐ Forensic enquiries
- ☐ Maintenance of colonies of genetically altered animals not used in other animal procedures

3 General description of the project

3.1 Background

Describe the project (motivation, background and context) with respect to the categories selected in 2.1.

The weaning process, in general, is the most stressful period in the life of a pig. It is accompanied by removal from the sow, mixing of piglets, changing environment with accompanying microbiological changes, and change in feed from highly digestible sow milk to a solid feed diet containing typically less digestible raw materials. Altogether, this results in a drop in feed intake which often results in gut wall damage such as atrophy of the villi and increases in permeability (Pluske, et al., 1997). This renders the piglets vulnerable to digestive pathogens like pathogenic *E. coli*, responsible for post-weaning diarrhoea (PWD). Although abrupt and early weaning are known to exacerbate the above-mentioned processes (Buchet et al., 2017), piglets in all husbandry systems will experience some level of stress around weaning, especially considering the sub-optimal hygiene conditions often occurring on farms. This stress is the main factor for health problems in the nursery and this, in turn, is responsible for a large part of the antibiotic usage in swine production. In this proposal we aim to develop nutritional solutions that will support the piglet before, during and after the weaning process by providing nutrients that better match its requirements than the currently available diets.

The current requirements for weaned piglets are largely based on those determined in older pigs (>35 kg body weight) while requirements for suckling piglets are lacking or largely based on nutrient levels in sow milk. The proposed nutritional solutions spinning off from this project should increase piglet health and welfare making them more robust to face the challenges around weaning. Moreover, they should increase sustainability of pig production by reducing mortality and the use of antibiotics, by using sustainable raw material sources, or by including strategies to optimize the use of raw materials sources (i.e., using enzymes or feed additives). It is highly important to start with nutritional interventions before weaning, for example, by providing piglets with supplemental milk or (solid) creep feed in order to adapt them in early life to raw materials present in diets after weaning (Huting et al., 2021). The intake of creep feed before weaning has been found to stimulate feed intake early after weaning (Bruininx et al., 2002), alter microbial populations towards a profile typically seen after weaning, increase microbial fermentation products (short chain fatty acids) exerting beneficial effects in the gastrointestinal tract, modulate intestinal development (length and weight of the gastrointestinal tract, increased absorptive area as indicated by an increased villus height to crypt depth ratio) at the time of weaning with subsequently less PWD (Choudhury et al., 2021a; Choudhury et al., 2021b). These studies indicate that there is a window of opportunity to prepare the piglet for the weaning process by nutritional solutions provided prior to weaning. Moreover, in the wild, piglets start to eat non-milk items from the first week of life onwards (Van Hees et al., 2022) indicating that their natural instinct is to search for food items other than sow milk already in early life. Thus, starting with nutritional solutions before weaning fits with the piglet's natural behaviour. In this proposal, we aim to focus on the following dietary components present in diets before and after weaning: energy, protein, fibres, minerals, and vitamins. Our studies are performed under standard practical conditions but in a research facility.

Energy

Energy is typically provided to piglets in the form of starch and fat sources. Piglets before weaning mainly get their energy from milk fat, while after weaning the main energy source is starch from cereals. The fat content in sow's milk is around ~34% on dry matter basis, as compared to ~7% in a typical nursery diet (Jensen et al., 1997). Thus, during the weaning process, the piglet needs to change from fat digestion to starch digestion while at the same time the production of all pancreatic enzymes (i.e., lipase, amylase, trypsin and chymotrypsin) is hampered (Jensen et al., 1997). Thus, the digestive capacity of starch and fat is limited in the week(s) immediately after weaning. We have performed a study with different energy levels (2200 – 2700 kCal/kg) of the feed either coming from starch or from fat. The diets were fed for 2.5 weeks from day 14 after weaning (~9 kg body weight) onwards. We found that piglets were able to maintain similar energy intake by adapting their feed intake. A higher energy level from starch was beneficial in terms of fat and protein digestibility. Our findings were in contrast to Kim et al. (2021) who found that pigs with a body weight below 20 kg were not able to adjust feed intake to diets differing in energy level. Thus, there is a lack of understanding on the relation between feed intake and energy intake of young piglets (~24 to 38 days of age; ~5 kg body weight), but also on how piglets respond to different sources of energy (i.e., fat, starch or potentially protein or lactose).
Piglet diets are typically formulated to provide an optimum ratio between amino acids and energy. This optimum has been determined in older pigs (~40 kg body weight) and extrapolated to piglets around

weaning (~4-10 kg body weight). However, with potential changes in energy level, we also need to re-evaluate the ratio between amino acids and energy, particularly in young piglets (~7kg).

Protein level and amino acid ratio's

Studies to determine the optimal protein level and ratio between essential and non-essential amino acids with the main aim to reduce nitrogen excretion in faeces and urine are typically done using older pigs and results are extrapolated to piglets around weaning. However, the gastrointestinal physiology of the weaned piglet is known to be different and, therefore, estimations of optimal protein level and amino acid ratios might be incorrect. Moreover, the optimal amino acid ratios might depend on the level of stress and presence of pathogens. Piglets with a high weaning stress (no feed before weaning and restricted feeding for 24 h after weaning) had a higher protein breakdown and higher utilization of amino acids by the gastrointestinal tract as compared to piglets with low weaning stress (Resink et al., 2022). Consequently, arginine and glutamine were found to be most limiting in this phase early after weaning as opposed to lysine in older pigs. An increased threonine supplementation was found to improve gut health when piglets were challenged with *E. coli* on day 7 after weaning (Trevisi et al., 2015). The interaction between fibres and kinetics of digestion of proteins (i.e., level of fermentable protein) can also influence requirements of amino acids such as threonine because of its role in mucus formation and antioxidant capacity (Wellington et al., 2020).

One of the strategies to combat PWD is through reducing dietary crude protein level (Heo et al., 2009) while supplementing the diet with synthetic essential amino acids. In a non-health challenge situation, we have studied the effect of supplementing several amino acids (histidine, valine, threonine, isoleucine, leucine, tryptophan, and methionine) to a low protein diet on growth of piglets after weaning. We found that growth was lower on the low protein diet compared to a high protein control diet and that none of the studied amino acids was able to sustain growth. This suggested that none of these amino acids were limiting in the diet contradicting our hypothesis. In low protein diets supplemented with synthetic amino acids, the essential:non-essential amino acid (EAA:NEAA) ratio is skewed towards the EAA potentially resulting in a lack of NEAA. Consequently, we hypothesize that the ratio between EAA:NEAA is more important in this phase of a piglet's life, especially in low protein diets. The NEAA can be synthesized by the body from EAA, but this process is not as efficient as supplying the right amount of NEAA through the diet. Thus, there is a general lack of understanding on protein and amino acids requirements in the youngest pigs and how they are modulated by weaning stress. A better understanding is expected to improve the use of protein sources and reduce PWD and nitrogen losses in faeces and urine.

Gastrointestinal development: dietary acidification and fibres

Before weaning, piglets rely on lactic acid bacteria to control stomach pH as hydrochloric acid production (HCl) in the stomach is still low (Cranwell et al.,1976). A piglet's maximum capacity to produce HCl is around 10 weeks of age. Thus, at the time of weaning, the piglet has limited capability to reduce the pH in the stomach because HCl production is not in place and lactic acid produced from lactose is decreasing because of the removal of sow milk. The pH in the stomach remained above 4.5 during the first hours after a meal in piglets at 2 weeks after weaning (weaned at ~24 days of age; in-house study) while a pH of 3 is considered optimal for stomach enzymes (Heo et al., 2013). A low stomach pH is important (1) to reduce the survival of pathogens and, thereby, also the flow of pathogens into other parts of the gastrointestinal tract, (2) activation of protein digestive enzymes (i.e., pepsinogen to pepsin) resulting in better gastric protein predigestion with less protein flowing into the large intestine providing nutrients to pathogens, and (3) releasing pancreatic enzymes for the digestion of e.g., starch and fat (Heo et al., 2013). The latter occurs when digesta with a low pH enters the duodenum. Stomach retention time and acidification are known to be influenced by soluble fibres (in-house study) and particle size of raw materials (Kiarie and Mills, 2019). Recently, we have developed a laboratory method to determine the buffering capacity of raw materials. Certain feed ingredients (e.g., calcium salts) have high buffering capacity, i.e., need large amounts of HCl to reduce the pH, while others (e.g., organic acids) have low buffering capacity. We aim to steer towards a diet formulation that helps piglets regulate the stomach pH in order to enhance gastric barrier function and increase digestibility of nutrients. In an in-house study, we determined the effect of buffering capacity on growth performance in order to determine recommendations for feed formulation. We found that the buffering capacity in typical diets is able to support piglet growth. Although the diets were similar in amino acids to energy ratio, we did observe

differences in the feed efficiency (i.e., amount of feed needed for growth). Thus, we hypothesize that dietary buffering capacity can impact the piglet's physiology via changes in protein digestion in the stomach and an imbalance in the presence of amino acids and energy after absorption. This study was done under optimal environmental conditions (i.e., low disease pressure, low animal density), and it can be hypothesized that the optimal dietary buffering capacity differs in suboptimal (i.e., low sanitary conditions or high pathogenic load) conditions. Thus, there is a need to better understand the role of the stomach to optimize health of the gastrointestinal tract and reduce PWD.
The extent and rate (i.e., kinetics) of protein, starch and fat digestion can be affected by stomach functioning. There is, thus, a complicated interaction between stomach functioning and nutrient availability with effects on nutrient utilization and subsequent nutrient losses in faeces and urine. In this project, we aim to unravel this interaction, especially in the period after weaning.

Translation of results from experimental conditions to field farm conditions
Studying the effect of nutritional strategies on PWD on commercial farms is difficult because of variability in the incidence of PWD and uncontrolled pathogenic pressure. Moreover, different husbandry systems impose a variety of environmental challenges for piglets leading to a subclinical disease state. These conditions include level of ventilation, temperature control, humidity, presence of dust, faeces, etc. and with that the related pathogen load (airborne or soil). The variability in responses would increase the number of animals needed to show the efficacy of a nutritional strategy when experiments are conducted at field farms. Our research facility is well maintained, and the climate and sanitary conditions are controlled and do not represent the average commercial farm. For this reason, we recently designed and operate a separate unit at our research facility. This unit contains four fully isolated rooms having their own climate control system (state-of-the-art ventilation and temperature installations). In this way, we can perform studies where we can mimic commercial farm conditions. Its design allows challenge studies with specific pathogens in one room without running the risk of spreading the infection to other rooms and to the rest of our facility. An *E. coli* challenge model has recently been validated and used to test different nutritional strategies. It induces an increase in diarrhoea immediately after weaning. We have multiple years of experience with this model and the procedure is documented as standard operating procedure in our company. During this procedure, piglets are challenged with pathogenic *E. coli* (O149:F4ac) according to a method described in Roubos-van den Hil et al. (2017). Piglets are tested for susceptibility or resistance towards ETEC O149:F4ac by a DNA marker-based test on biological samples collected during the suckling phase. This is done to ensure that only susceptible piglets will be used, which means a reduction in variation in the outcomes and, thus, in the number of animals needed to show an effect.
We have conducted a validation experiment testing climate and sanitary parameters involved in the so-called management model. However, there is a need for a follow-up study to further validate this model (to be used up to 25 kg body weight) and develop a standard operating procedure for this unit. An older protocol for a management model included increased ventilation by shortening the P-band by 2°C (=P-band is the range in which the ventilation is steered from minimum to maximum), suboptimal temperature (2°C lower than standard settings), spreading of dust, and spreading of sow manure. However, these parameters showed to be insufficient to mimic the conditions on commercial farms. Recently, other research groups were able to reproduce a sanitary model (e.g., Van der Meer et al., 2016 and Le Floc'h et al., 2009) and parameters in those models (e.g., frequent spreading of manure from different batches of pigs) could be tested to improve our old protocol.
Both the *E. coli* challenge model and management model resemble conditions on commercial farms. The most promising nutritional strategies will first be tested under the controlled *E. coli* challenge model and/or management model and subsequently under commercial conditions on field farms around the world to further validate the results. This needs to be done to ensure that the nutritional strategies also work with e.g., different management, genetics, dietary (raw material) composition.

References
Bruininx, E.M.A.M., G.P. Binnendijk, C.M.C. van der Peet-Schwering, J.W. Schrama, L.A. den Hartog, H. Everts, and A.C. Beynen. 2002. Effect of creep feed consumption on individual feed intake characteristics and performance of group-housed weanling pigs. Journal of Animal Science 80:1413-1418.

Buchet, A. C. Belloc, M. Leblanc-Maridor, and E. Merlot. 2017. Effects of age and weaning conditions on blood indicators of oxidative status in pigs. PloS ONE 12(5):e0178487.

Choudhury, R., A. Middelkoop, J. Boekhorst, W.J.J. Gerrits, B. Kemp, J.E. Bolhuis, and M. Kleerebezem. 2021a. Early life feeding accelerates gut microbiome maturation and suppresses acute post-weaning stress in piglets. Environmental Microbiology, 23(11): 7201-7213.

Choudhury, R., A. Middelkoop, J.G. de Souza, L.A. van Veen, W.J.J. Gerrits, B. Kemp, J.E. Bolhuis, and M. Kleerebezem. 2021b. Impact of early-life feeding on local intestinal microbiota and digestive system development in piglets. Scientific reports 11:4213, doi.org/10.1038/s41598-021-83756-2.

Cranwell, P.D., D.E. Noakes, and K.J. Hill. 1976. Gastric secretion and fermentation in the suckling pig. British Journal of Nutrition 36:71-86.

Heo J.M., J.C. Kim, C.F. Hansen, B.P. Mullan, D.J. Hampson, and J.R. Pluske. 2009. Feeding a diet with decreased protein content reduces indices of protein fermentation and the incidence of postweaning diarrhea in weaned pigs challenged with an enterotoxigenic strain of Escherichia coli. Journal of Animal Science 87:2833-2843.

Heo, J.M., F.O. Opapeju, J.R. Pluske, J.C. Kim, D.J. Hampson, and C.M. Nyachoti. 2013. Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds. Journal of Animal Physiology and Animal Nutrition 97:207-237.

Huting, A.M.S., A. Middelkoop, X. Guan, and F. Molist. 2021. Using nutritional strategies to shape the gastro-intestinal tracts of suckling and weaned piglets. Animals 11, 402, doi.org/10.3390/ani11020402.

Jensen, M.S., S.K. Jensen, and K. Jakobsen. 1997. Development of digestive enzymes in pigs with emphasis on lipolytic activity in the stomach and pancreas. Journal of Animal Science 75:437-445.

Kiarie, E.G. and A. Mills. 2019. Role of feed processing on gut health and function in pigs and poultry: conundrum of optimal particle size and hydrothermal regimens. Frontiers in Veterinary Science 6:19 doi: 10.3389/fevts.2019.00019.

Kim, J.W., B. Koo, and C.M. Nyachoti. 2021. Pigs weighing less than 20 kg are unable to adjust feed intake in response to dietary net energy density regardless of diet composition. Canadian Journal of Animal Science 101:118-125.

Koopmans, S.J., A.C. Guzik, J. van der Meulen, R. Dekker, J. Kogut, B.J. Kerr, and L.L. Southern. 2006. Effects of supplemental L-tryptophan on serotonin, cortisol, intestinal integrity, and behavior in weanling pigs. Journal of Animal Science 84:963-971.

Le Floc'h, N., L. LeBellego, J.J. Matte, D. Melchior, and B. Sève. 2009. The effect of sanitary status degradation and dietary tryptophan content on growth rate and tryptophan metabolism in weaning pigs. Journal of Animal Science 87:1686-1694.

Pluske, J.R., D.J. Hampson, and I.H. Williams. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livestock Production Science 51(1-3): 215-236.

Resink, J.W., W.J.J. Gerrits, T.G. Hulshof, N. Deutz, and G. Ten Have. 2022. P215 Higher amino acid demand in portal drained viscera of post-wean piglets. Animal – Science Proceedings 13: 157-226.

Roubos-van den Hil, P.J., R. Litjens, A.-K. Oudshoorn, J.W. Resink, and C.H.M. Smits. 2017. New perspectives to the enterotoxigenic *E. coli* F4 porcine infection model: Susceptibility genotypes in relation to performance, diarrhoea and bacterial shedding. Veterinary Microbiology 202:58-63.

Trevisi, P., E. Corrent, M. Mazzoni, S. Messori, D. Priori, Y. Gherpelli, A. Simongiovanni, and P. Bosi. 2015. Effect of added dietary threonine on growth performance, health, immunity and gastrointestinal function of weaning pigs with different genetic susceptibility to *Escherichia coli* infection and challenged with *E. coli* K88ac. Journal of Animal Physiology and Animal Nutrition 99:511-520.

Van Hees, H.M.J., S.A. Ballari, L. Dieste-Pérez, B.N. Carpinetti, and G.P.J. Janssens. 2022. Diet and stomach characteristics of feral pigs (*Sus scrofa*): implications for farmed piglets. Journal of Animal Physiology and Animal Nutrition *in press*, https://doi.org/10.1111/jpn.13726.

Van Der Meer, Y., A. Lammers, A.J.M. Jansman, M.M.J.A Rijnen, W.H. Hendriks, and W.J.J. Gerrits. 2016. Performance of pigs kept under different sanitary conditions affected by protein intake and amino acid supplementation. Journal of Animal Science 94:4704-4719.

Wellington, M.O., J.K. Htoo, A.G. Van Kessel, and D.A. Columbus. 2019. Estimating the optimal threonine requirement for 25-50 kg pigs fed a mixture of soluble and insoluble dietary fibre. Canadian Journal of Animal Science 99:634-638.

Wellington, M.O., R.B. Thiessen, A.G. Van Kessel, and D.A. Columbus. 2020. Intestinal health and threonine requirement of growing pigs fed diets containing high dietary fibre and fermentable protein. Animals 10:2055.

3.2 Purpose

3.2.1 Describe the project's immediate and ultimate goals. Describe to which extent achieving the project's immediate goal will contribute to achieving the ultimate goal.

- If applicable, describe all subobjectives

The ultimate goal of this project is to develop nutritional strategies that will better meet the requirements of piglets before, around, and after weaning in optimal and sub-optimal (including pathogenic pressure) environments. With these nutritional strategies, we aim to increase the sustainability of pig production by helping to reduce mortality rate and the use of antibiotics, by using raw materials that meet animal requirements, by supporting feed intake and gastrointestinal tract development around weaning, and by improving animal welfare for example by reducing abnormal behaviour (tail, biting, belly nosing).

The immediate goals are:

1. To validate a management model that simulates commercial conditions but in a controlled environment (Appendix 2).
2. To determine the optimal lysine to energy ratio and energy level in piglets after weaning (Appendix 1).
3. To determine the effect of creep feed, fatty acid level and fatty acid composition on fat digestibility, bile acid production, enzyme secretion, gastrointestinal development, and blood lipid metabolite profile after weaning (Appendix 1). Socializing the piglets before weaning might be used as model to reduce weaning stress and, thus, to disentangle the effect of stress from feed transition (sow milk to solid feed) from social stress (mixing of piglets) on fat digestibility. The optimal fatty acid level and fatty acid composition will also be determined under suboptimal sanitary conditions (Appendix 2) in order to translate the knowledge into practical conditions.
4. To determine the optimal ratio between unsaturated and saturated (U:S ratio) fatty acids on fat digestibility under optimal (Appendix 1) and suboptimal sanitary conditions (Appendix 2).
5. To determine the interaction between dietary protein level and specific amino acids requirements under optimal and suboptimal sanitary conditions. Nitrogen balance experiments will be done to determine protein deposition under optimal and suboptimal sanitary conditions (Appendix 4). The optimal protein and amino acid levels will be verified in a growth performance trial under optimal (no animal experiment) and suboptimal sanitary conditions (Appendix 2).
6. To determine the interaction between feed particle size and the optimal inclusion level of coarse raw materials in the diet given before and after weaning on stomach and intestinal development and nutrient digestibility after weaning under optimal (Appendix 1), suboptimal sanitary conditions (Appendix 2) and a specific pathogen challenge (*E. coli* challenge model; Appendix 3).
7. To determine the effect of feed form (mash, pellet, crumble, extruded feed) and transition between feed forms at weaning on gastrointestinal development and health (Appendix 1).
8. To determine the optimal buffering capacity in diets for piglets after weaning under suboptimal sanitary conditions (Appendix 2) conditions. Optimal levels under optimal conditions were already established in an in-house study.
9. To determine the interactive effect between buffering capacity and feed particle size distribution on gastrointestinal development and health (Appendix 1).

Next to the response parameters specified in the appendices, we will also evaluate sustainability metrics when applicable. The metrics are divided in diet-related and animal-related metrices. Diet-related: resource (use of fossil fuel) and water use, environmental acidification, nitrogen and phosphorus excretion during pig production, CO_2 emission from transport of raw materials or from raw material production itself. Animal-related: behaviour scores, body condition scores, mortality, and morbidity.

3.2.2 Provide a justification for the project's feasibility.

We are an international animal nutrition company that develops nutritional additives and feeding strategies for most livestock species. The company has the ambition to be a global leader in this field. Our focus area is sustainable livestock farming which for us includes reducing antibiotic use, improving animal health and welfare, and reducing environmental burden of livestock production by e.g., reducing nutrient losses in urine and faeces and reducing emissions associated with production. The need to go for a more circular food production system could also lead to the use of feed with lower nutrient availability/digestibility, which increases the need for additives and more in-depth knowledge on nutrient delivery. This will allow for improved digestion and utilization of raw materials with lower nutrient availability/digestibility. Our company has a dedicated R&D department with research teams for all species in scope, including pigs. Each species team has around 6 research scientists holding a PhD related to animal or veterinary science and they maintain a broad international science network. Studies will be performed at our research centre in The Netherlands. We have recently invested in a state-of-the-art unit where we can perform trials that mimic and control specific environmental conditions (as described above in section 3.1 Background). High biosecurity and hygiene standards are maintained to protect our personnel and animals. Daily care of animals, measurements, and experimental techniques are performed by certified competent and experienced staff. Competence of personnel is controlled by a ISO9001 quality management system and our animal welfare body. The company has state-of-the-art and GLP certified laboratory facilities with a range of assays and technologies, including *in vitro* techniques for first screening.
Project results will be disseminated through scientific journals and conferences and ultimately translated into nutritional strategies applied via our global network of nutritionists.

3.2.3 Are, for conducting this project, other laws and regulations applicable that may affect the welfare of the animals and/or the feasibility of the project?

X No

☐ Yes > Describe which laws and regulations apply en describe the effects on the welfare of the animals and the feasibility of the project.

3.3 Relevance

3.3.1 What is the scientific and/or social relevance of the objectives described above?

The scientific relevance of the immediate goals is to better understand the piglet's requirements around weaning which is one of the most stressful events in a pig's life. The lack of understanding of the physical state and the variable response to weaning of the post-weaning piglet makes it challenging for nutritionists to establish an optimal diet. However, there are certain aspects that are similar between piglets: they all need energy to survive, nutrients to develop their gastrointestinal tract, and need adequate nutrition to combat pathogens and stay healthy. Moreover, changes in legislation, e.g., the expected ban on tail docking and use of in-feed antibiotics and pharmacological levels of zinc oxide, stress the need to understand the nutritional requirements of piglets in this phase, also to prevent damaging behaviours. Nutrient requirements for weaned piglets are mostly extrapolated from older pigs and we have just started to unravel the complex physiology of a piglet around weaning and its accompanying nutrient requirements. Results from the immediate project goals will be used to further optimize piglet feeding strategies internally but our results will also be published in scientific journals. Regarding the social relevance of the ultimate goal, main aspects are to further reduce reliance on antibiotics for pigs in order to maintain future availability of antibiotics for human medicine. Secondly,

this project will contribute to the use of non-human-edible feed ingredients (e.g., co-products from food production) for feed, leaving human-edible ingredients available as food.

3.3.2 Who are the project's stakeholders? Describe their specific interests.

Target animals (piglets): Go through the weaning process with less nutritional stress and having an improved (gastrointestinal) development after weaning. In this way, have a lower risk for diseases incidence, remain healthier after weaning, and have lower mortality.
Experimental animals (piglets): Contribute to research needed to study nutritional requirements around weaning more in-depth. Violation of piglet integrity by housing conditions (specifically without bedding), housing under suboptimal sanitary conditions, or challenge with a pathogen.
License holder: Increase the sustainability of pig production and improve animal health and welfare. Be able to provide optimized nutritional strategies for feeding piglets before and after weaning to farmers. The license holder also has an economic interest to market high-end piglet feeds and nutritional advice to farmers.
Researchers: Increase their understanding of the weaning process and the nutritional requirements of piglets around weaning. Moreover, to publish the results of this project in scientific journals.
Environment: Reduce nitrogen and phosphorus excretion into the environment. More efficient use of raw materials and to reduce the competition between food and feed and reduce fossil fuel and water resource use.
Pig industry: Increase its sustainability by increased piglet robustness, resilience to weaning and sanitary stressors, reduced damaging behaviours, PWD, antimicrobial use, and mortality around weaning. Increased economic sustainability by increasing the flexibility to use raw materials based on nutrient values and costs.
Farmer: Smoother weaning process of piglets and lower veterinary costs, resulting in a sustainable income from less morbidity and mortality. Improved growth performance of their herd as result from lower morbidity and mortality and improved nutrient delivery.
Society: Increase animal wellbeing and higher environmentally sustainable pig production. Reduce the reliance on antimicrobials in pig production and, thereby, reduce the risk of antibiotic resistance for humans.

3.4 Strategy

3.4.1 Provide an overview of the overall design of the project (strategy). If applicable, describe the different phases in the project, the coherence, the milestones, selection points and decision criteria.

To answer the immediate goals specified in 3.2.1, a total of 18 animal experiments are designed. The design of the project is schematically represented below, to be interpreted from top to bottom. Go/no-go decisions on continuation of specific studies within this project are made by a dedicated project team. This project team consists of researchers, nutritionists, and people working in the company's sustainability, regulatory, and marketing departments. Together, they decide on the continuation of a project based on pre-set key performance indicators set. Typical key performance indicators are:

1. Growth performance: does one of the tested nutritional strategies maintain or even increases feed intake to a level that is adequate to support or improve growth and health.
2. Sustainability metrics: does one of the tested nutritional strategies improve sustainability by e.g., reducing antibiotic use, reducing mortality, or increase the use of more sustainable raw materials and increasing feed efficiency (i.e., reducing nutrient losses into the environment).
3. Animal health and welfare metrics: does one of the tested nutritional strategies lead to lower occurrence of PWD or better body condition scores including reducing damaging behaviours.

For goals 2-4 (energy) and 6-9 (gastrointestinal development), a series of experiments (1-9) are designed that relate to the respective immediate goals using a Nutrient utilization model (Appendix 1). Results of exp. 1-9 will be discussed in the project team that decides on go/no-go with experiments under suboptimal sanitary conditions (Management model; Appendix 2) and/or subsequently using an *E. coli* challenge model (E. coli challenge model; Appendix 3) based on the predetermined key performance indicators. Validation of the management model is required before exp. 13-17 can start. This will be done

in exp. 12 (goal 1; Appendix 2). After exp. 12, a go/no go decision needs to be made on the validity of the management model.
For goal 5, first one experiment will be conducted under optimal conditions (exp. 10; Appendix 4). A go /no go decision has to be taken before moving to exp. 11 which has a similar experimental design as exp. 10 but then under health challenge conditions (suboptimal sanitary conditions). Experiment 11 (nitrogen balance under suboptimal sanitary conditions) can only be done if experiment 12 (development of management model) is successful. With this project governance structure, only the most promising nutritional strategies will be tested in challenge models (Management model and E. coli challenge model). The nutritional strategies will ultimately be validated in large-scale field trials (non-animal experiments).

Energy **Goals 2-4**	**Gastrointestinal development: acidification and fibres** **Goals 6-9**	**Protein level and amino acid ratio's** **Goal 5**

Hypotheses creation based on literature studies, in vitro testing (digestibility or buffering capacity), existing in-house in vivo studies

Appendix 1: Nutrient utilization model (n=888)		*Appendix 4: Nitrogen balance optimal and suboptimal conditions (n=160)*
Exp. 1 Energy level × lysine/energy ratio	Exp. 6 Fibre coarseness × start of feeding before weaning	Exp. 10 Protein level × amino acid level optimal conditions
Exp. 2 Feed level × lysine/energy ratio	Exp. 7 Inclusion level coarse raw materials before and after weaning	**GO/NO GO DECISION MOMENT**
Exp. 3 Creep feed effect on fat digestion	Exp. 8 Feed form	Exp. 11 Protein level × amino acid level suboptimal sanitary conditions
Exp. 4 SCFA+MCFA source × level	Exp. 9 Buffering capacity × coarseness	
Exp. 5 U:S ratio dose-response		

GO/NO GO DECISION MOMENT

Appendix 2: Management model (n=1254) *Exp. 12: development of management model* = **Goal 1**		
GO/NO GO DECISION MOMENT ON MANAGEMENT MODEL		
Exp. 13 SCFA+MCFA source × level	Exp. 15 Optimal strategy regarding coarseness before and after weaning with suboptimal conditions after weaning	Exp. 17 Protein +amino acids level × housing conditions (optimal vs suboptimal)
Exp. 14 U:S ratio dose-response	Exp. 16 Buffering capacity under suboptimal conditions	

GO/NO GO DECISION MOMENT

Appendix 3: E. coli challenge model (n= 207)
Exp. 18 Optimal strategy regarding coarseness before and after weaning with pathogen challenge conditions after weaning

Validation studies under field conditions

3.4.2 Provide a justification for the strategy described above.

Hypotheses of each experiment are based on available knowledge (literature or earlier in-house studies). When possible, *in vitro* studies will first be conducted to find the most promising combinations of, for example, unsaturated to saturated fatty acid ratios. Those will subsequently be used in *in vivo* experiments. Only the nutritional strategies that showed promising effects under optimal sanitary conditions will be tested in the management and/or E. coli challenge models. In this way, the number of animals and discomfort per animal will be minimized. The management model will be validated first prior to using this model to test nutritional strategies. The results obtained from the validation trial will be used to calculate the exact number of animals needed to find the relevant difference. This will also reduce the number of animals.

3.4.3 List the different types of animal procedures. Use a different appendix 'description animal procedures' for each type of animal procedure.

Serial number	Type of animal procedure
1	Nutrient utilization model
2	Management model
3	*E. coli* challenge model
4	Nitrogen balance optimal and suboptimal conditions
5	
6	
7	
8	
9	
10	

Centrale Commissie Dierproeven

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 lid2h
1.2	Provide the name of the licenced establishment.	5.1 lid2h
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 1 — Type of animal procedure: Nutrient utilization model

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

This model is used to study the nutrient utilization of energy, protein, fat, and fibres but also the effect of fat and fibres on gastrointestinal development. Sampling blood and/or different parts of the gut will provide more insight into the dynamics of digestion and the locations where different nutrients are released and absorbed by the gut. In order to study digestibility and retention time of feed in specific parts of the gastrointestinal tract, known amounts of markers will be fed to the piglets (e.g., chromium EDTA as soluble marker and insoluble ash as in-feed marker). By sampling digesta contents of different sections of the gastrointestinal tract, digestibility and retention time for each section can be determined. When for example ileal contents are sampled, ileal apparent digestibility data will be obtained, which can be used for formulating more optimal diets because the requirements are also expressed on an ileal digestibility basis. Sampling faecal material is needed to study the overall nutrient digestion and gives an indication of the absolute requirement. The primary outcome parameter depends on the goal of the experiment. Experiments 1-8 will be done under this Appendix. The treatments will be given during pre-weaning or at maximum 3 weeks after weaning since that is the most sensitive period in a piglet's life.

Experiment 1
Treatments after weaning: 2 energy levels × 5 levels of lysine/energy ratios = 10 experimental diets.

- The energy levels are at the borders of the current recommendations while some of the tested lysine/energy ratios might fall outside current recommendations, i.e., either lower or higher.

Sampling: Blood samples from a subset of animals to determine plasma urea nitrogen as measure for protein deposition. Faecal samples to determine protein and energy digestibility.

Main outcome parameter: growth; blood and faecal samples will help to explain observed growth responses.

Experiment 2
Treatments after weaning: 3 lysine/energy levels (based on Exp 1) × 3 feeding levels (80, 90 and 100% of ad libitum feed intake). Feed intake might be restricted in praxis and in this way, we validate our nutritional strategies under more commercial-like conditions.
Main outcome parameter: growth; no other samples will be taken.

Experiment 3
Treatments before weaning: socializing piglets by combining litters (i.e., open fences between farrowing crates) and with or without creep feed. Thus, 4 treatments before weaning: social or traditional and creep feed or no creep feed.
Treatments after weaning: 1 experimental diet.
Sampling: blood, gastrointestinal content, pancreatic + gastrointestinal tissue at 4 timepoints around weaning. Faecal sampling in the period after weaning.
Main outcome parameter: fat digestibility at the end of the ileum; other parameters, such as bile acid production, enzyme secretion, gastrointestinal development (weight, length, histology), blood lipid metabolite profile, will help to explain the observed effects on fat digestibility.

Experiment 4
Treatments before weaning: socializing piglets by combining litters (i.e., open fences between farrowing crates). Thus, 2 treatments before weaning: social or traditional.
Treatments after weaning: 3 SCFA+MCFA composition × 3 SCFA+MCFA levels = 9 experimental diets.
Sampling: blood, gastrointestinal content, pancreatic + gastrointestinal tissue. Faecal sampling in the period after weaning.
Main outcome parameter: fat digestibility at the end of the ileum; other parameters, such as bile acid production, enzyme secretion, gastrointestinal development (weight, length, histology), blood lipid metabolite profile, will help to explain the observed effects on fat digestibility.

Experiment 5
Treatments after weaning: 4 ratios of unsaturated:saturated fatty acids in dose-response.
Sampling: gastrointestinal content, pancreatic + gastrointestinal tissue. Faecal sampling in the period after weaning.
Main outcome parameter: fat digestibility at the end of the ileum; other parameters, such as bile acid production, enzyme secretion, gastrointestinal development (weight, length, histology), blood lipid metabolite profile, will help to explain the observed effects on fat digestibility.

Experiment 6
Treatments before weaning: 3 levels of coarseness of the diet × 2 starting times of feeding = 6 experimental treatments.
Treatments after weaning: same level of coarseness as before weaning = 3 experimental diets.
Sampling: gastrointestinal development, e.g., acidification, ulceration of the stomach and length, weight and histomorphology of different parts of the gastrointestinal tract, and gastrointestinal content at and after weaning.
Main outcome parameter: nutrient digestibility at the end of the ileum; other parameters determined from the gastrointestinal samples will help to explain observed effects on nutrient digestibility.

Experiment 7
Treatments after weaning: 3 inclusion levels coarse raw materials before weaning × 3 inclusion levels coarse raw materials after weaning = 9 experimental diets.
Sampling: gastrointestinal development, e.g., acidification, ulceration of the stomach and length, weight and histomorphology of different parts of the gastrointestinal tract, and gastrointestinal content at and after weaning.
Main outcome parameter: nutrient digestibility at the end of the ileum; other parameters determined from the gastrointestinal samples will help to explain observed effects on nutrient digestibility.

Experiment 8

Treatments after weaning: 3 feed forms (mash, pellet, extruded feed) before weaning × 3 feed forms (mash, pellet, extruded feed) after weaning = 9 experimental diets.
Sampling: gastrointestinal development and gastrointestinal content at and after weaning. Faecal sampling in the period after weaning.
Main outcome parameter: nutrient digestibility at the end of the ileum; other parameters determined from the gastrointestinal samples (e.g., retention time) will help to explain observed effects on nutrient digestibility.

Experiment 9
Treatments after weaning: 2 levels of buffering capacity × 2 levels of coarseness (standard vs optimized from Exp 5 and 6) = 4 experimental diets.
Sampling: gastrointestinal development, e.g., acidification, ulceration of the stomach and length, weight and histomorphology of different parts of the gastrointestinal tract, and gastrointestinal content after weaning.
Main outcome parameter: nutrient digestibility at the end of the ileum; other parameters determined from the gastrointestinal samples (e.g., retention time) will help to explain observed effects on nutrient digestibility.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Proposed animal procedures all experiments:

1. Before weaning, piglets will be individually ear-tagged and birth weights will be recorded. Creep feed will be given as free-choice. Piglets will be weaned at a minimum age of 21 days old into specialised housing which are cleaned before entering of a new group and which is separated from housings where the sows are kept (Council Directive 2008/120/EC).
2. Piglets will be housed in groups of 3-6 piglets depending on the barn/pen sizes to be used.
3. Housing without bedding for maximally 6 weeks after weaning. This is required to properly assess the effects of the nutritional strategies. Consumption of bedding materials (e.g., high in fibre) might interfere with the response leading to more variation and a higher number of animals required to show an effect.
4. Animal weighing every 7 days for a maximum of 10 weeks (before and after weaning periods).

Additional animal procedure experiment 1:

5. Blood sampling; maximal 6 times within 6 weeks after weaning with a maximum 10 mL and of 8ml/kg/14 days for young animals. Route: intravenous.

Additional animal procedure experiment 2:

6. Feed restriction at 80% of ad libitum intake.

Additional animal procedure experiment 3-4:

7. Socializing before weaning.

Additional animal procedure experiment 3, 4 and 6:

8. Faecal swab to determine if a piglet had eaten the creep feed. A colorant will be added to the feed for 3 days and a faecal swap will be taken after those days to determine the presence of the colorant in faeces.

Additional animal procedure experiments 3-9:

9. Euthanasia for the sampling of digesta from the different sections of the gut and tissue sampling in order to determine digestibility / retention time / enzyme production. Euthanasia can take place before (reference samples in Exp 3) and after weaning. A blood sample can be taken just before euthanasia when animals are sedated.
10. Imposed feeding pattern to get a continuous flow of digesta through the gastrointestinal tract after weaning. A feeding pattern means that the total feed typically eaten is provided in smaller meals throughout the day instead of free access to feed. The amount of feed to be given will be determined based on feed intake of individual piglets (average of previous studies) and will always be above the level needed for maintenance.

Additional animal procedure experiments 1, 3, 4, 5 and 8:

11. Faeces sampling via rectum stimulation; maximal 7 times in the experimental period of 10 weeks (before and after weaning periods) with max 2 samples taken before weaning and max 5 samples after weaning.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

Digestibility studies are done by measuring the loss of nutrients in the gut with the help of inert digestibility markers such as insoluble ash. Therefore, the individual pig is the experimental unit. Analysis in blood samples (experiment 1), are also on individual pig level. The relevant outcome parameter will be taken (e.g., protein digestibility) and error variances from previous studies will be used to estimate sample size at a power of 80% and a probability of 95%. This will be done for every experiment.

For example, ileal protein digestibility
500 studies are simulated in a statistical software package using relevant digestibility means ranging between 60 and 80%. We aim to detect an absolute difference of 7 percentage-points within this range of digestibility means. Previous studies with nutritional strategies have shown that we can get this difference in our facility. The 500 simulations are, thereafter, analysed using a linear mixed model (MIXED) which is also the model used to analyse the data after a study is completed. The proportion of simulations showing a significant treatment effect should be >80% in order to ensure a power of 80% and a probability of 95%.
Error variance = 19.96
Mean digestibility = 70 with absolute differences ranging from 0-12 (mean range = 58 – 82%)
Relevant difference = 6
Number of replicates per treatment = 10. However, piglets at a young age might not have sufficient digesta at the end of the small intestine for chemical analyses or a piglet might need to be removed from a study (e.g., feed intake below specified criteria or death). Therefore, sample size is estimated to be 10 + 2 = 12 in total per treatment in order to maintain sufficient power.

B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
1	Sus scrofa	Own facility	Pre-/weaned piglets	888	Males and females	No	Hypor Libra × Hypor Maxter

Provide justifications for these choices

Species	Sus scrofa is the target species
Origin	We have our own facility with a sow herd, where we breed our own piglets
Life stages	Piglets before and after weaning up to 6 weeks after weaning are the target group
Number	Total experimental treatments experiment 1-9 is 72 (Exp 1: 10, Exp 2: 5 (3 with 80% of ad libitum feeding and 2 with lysine/energy below current recommendations), Exp 3: 16 (4 timepoints × 4 diets), Exp 4: 9, Exp 5: 4, Exp 6: 6, Exp 7: 9, Exp 8: 9, and Exp 9: 4). In Exp 3, 12 socialized piglets and 12 traditionally housed piglets will form the reference sample group. 72 treatments × 12 piglets/treatment + 24 reference piglets (Exp 4) = 888 piglets in total
Gender	Males and females are representative for the commercial situation
Genetic alterations	Not applicable
Strain	This strain is present at our own facility and representative of the commercial situation

C. Accommodation and care

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

Vertrouwelijk

☐ Yes

X No > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices

During the pre-weaning phase of the experiment piglets will be housed in conventional farrowing crates together with the dam.
Piglets will be housed without bedding material. Non-edible pen enrichment will be provided to allow animals to play and exhibit normal behaviour. The pen enrichment meets the requirements set by the NVWA.
The pens have a tenderfoot slatted floor.

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

X Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

X No > Justify why pain relieving methods will not be used.

Blood sampling might induce pain, but no sedation will be used. A single insertion of a needle to sample blood is not expected to cause severe pain and the duration is short.

☐ Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Describe which other adverse effects on the animals' welfare may be expected?

Piglets will not get bedding material in the pens. However, we expect little adverse effects because other (non-edible) pen enrichment will be present.
The imposed feeding pattern is not expected to result in adverse effects on animal welfare since the animals will be fed their daily amount (always above their requirements for maintenance) but then divided across a day. The feeding level at 80% of ad libitum feed intake might result in adverse effects.

Explain why these effects may emerge.

Diets are designed to provide the recommended nutrients when given ad libitum.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

The lower feeding level will be given for a maximum of 3 weeks after weaning. Care will be given that all vitamin and mineral requirements will be met even at this lower feeding level.

E. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

X No > Continue with question F

☐ Yes > Describe the criteria that will be used to identify the humane endpoints.

Indicate the likely incidence.

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

1. Ear tagging: less than mild (standard commercial procedure)
2. Group housing: less than mild

3. Housing without bedding: less than mild
4. Animal weighing: less than mild
5. Blood sampling intravenously (~13% of piglets): mild
6. Feed restriction at 80% of ad libitum / nutrients below requirements (~6% of piglets): mild
7. Socializing before weaning: less than mild
8. Faecal swab: less than mild
9. Euthanasia after sedation (~79% of piglets): mild
10. Imposed feeding pattern: less than mild
11. Faeces sampling: less than mild

The expected level of discomfort is mild for all animals (n=888).

G. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement	We have an in-house in vitro digestion kinetics model in which we can screen raw materials and diets. Wherever possible, first a screening with this model will be done in order to select the raw materials and diets to be tested in in vivo studies. Interactions between raw materials and /or nutrients on e.g., digestibility and nutrient utilization, passage through the gastrointestinal tract, and acidification in the stomach cannot be studied outside animals. Especially not in a weaned piglet where digestive physiology changes rapidly due to weaning. Animal data is also needed to validate the in vitro digestion kinetics model. Faecal samples could be obtained non-invasively but are not representative for what is happening in different parts of the gastrointestinal tract. For this project, the stomach and small intestine are the most important parts of the gastrointestinal tract. Changes in digestive physiology and the stress around weaning occurs in all husbandry systems.
Reduction	Sample size estimations are done using data from our own facility. Per nutritional strategy, we use previous studies and literature data (see 3.1 Background in the project proposal) to decide on the treatments to be studied. Go/no go decisions per nutritional strategy ensures that only the most promising strategies will be tested in follow-up studies, allowing for the reduction in the number of animals potentially required.
Refinement	Piglets after weaning are the target animals and their physiology cannot be obtained in other species or in models. Animals will be sedated before euthanasia. In the absence of bedding, extra care will be taken that piglets have access to enrichment material at all times. The enrichment material should be manipulated, is chewable, interesting for a longer time and available for all animals in a pen (e.g., chains reaching the floor, rope, plastic toys, etc.). Additional health checks will be done for experiment 2 when for the treatment where feed intake is restricted. Standard operating procedures will be used for faeces collection to reduce variation between studies.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

X No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

X No > Continue with question I.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

X No

☐ Yes > Provide specific justifications for the re-use of these animals during the procedures.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

Not applicable.

J. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question K.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

☐ No > Provide information on the destination of the animals.

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

Tissue and/or digesta from the gastrointestinal tract can only be sampled after euthanasia. Only a part of the animals will be euthanised. The other animals will be kept at our own research facility or sold to a commercial fattening farm.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

X Yes > Will a method of killing be used for which specific requirements apply?

X No > Describe the method of killing.

Overdose of barbiturate after sedation

☐ Yes > Describe the method of killing that will be used and provide justifications for this choice.

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

Centrale Commissie Dierproeven

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 iid2h
1.2	Provide the name of the licenced establishment.	5.1 iid2h
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 2 — Type of animal procedure: Management model

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

Experiment 12 development of management model
In our old stable, we were able to find differences in growth, feed intake, and feed efficiency (growth obtained with a certain amount of feed) between piglets housed under standard conditions and piglets housed under suboptimal / management model conditions. Feed intake and growth are measures of animal wellbeing (i.e., if an animal feels sick it will not eat and grow) and feed efficiency is a measure for sustainability (i.e., how well nutrients are used by the animal and thus not lost in urine and faeces). Last year we performed a small-scale study looking at several factors (body weight at weaning, spreading of dirt, partially closed vs open floor, lowering temperature) to start validating the old model in our new barn. We did not use different types of diets and also the number of replicates per factor was too low (n=10) to draw firm conclusions regarding the model. A second study is, therefore, needed to further develop and verify the outcomes of the first study. In this study we will use different dietary treatments. Since this model is aimed at finding differences between nutritional strategies, we also need to validate this first before we continue to test new nutritional strategies.
Treatments after weaning: 2 commercial-like diets × 3 housing conditions = 6 treatments.

- The housing conditions will be the optimal conditions (as control treatment) and the 2 most promising combinations of factors from the previous study (2 test treatments).

Sampling: blood for inflammatory markers and faeces for protein digestibility (as reduced protein digestibility is a main driver for post-weaning diarrhoea).
Main outcome parameter: inflammatory response in blood (e.g., haptoglobin). Faecal sampling will be done to verify the model. Growth response and diarrhoea incidence will be also be determined.

This study will be used to determine the residual variance which is required to do power and sample size calculations for subsequent studies. After the model is validated, we will use it for experiments 12-16 in which we want to examine the effect of nutritional strategies under sub-optimal conditions where the pathogenic pressure is long-lasting (up to 2 weeks after weaning). Moreover, poor management conditions immediately after weaning are expected to influence feed intake during the first 24-48 hours after weaning which might result in a higher occurrence of post-weaning diarrhoea.
The measurements and sampling in this experiment (no. 12) are needed to verify the outcomes of the first study (executed last year) and necessary to develop a standard operating procedure. This standard operating procedure will subsequently be used for experiments 13-14 and 11 which is in Appendix 4.

Experiment 13
Treatments before weaning: socializing piglets by combining litters (i.e., open fences between farrowing crates).
Treatments after weaning: 2 SCFA+MCFA composition × 2 SCFA+MCFA levels = 4 experimental diets.

- Composition and level based on Exp 3.

Sampling: blood, gastrointestinal content, pancreatic + gastrointestinal tissue. Faecal sampling in the period after weaning.
Main outcome parameter: fat digestibility at the end of the ileum; other parameters, such as bile acid production, enzyme secretion, gastrointestinal development (weight, length, histology), blood lipid metabolite profile, will help to explain the observed effects on fat digestibility.

Experiment 14
Treatments after weaning: 2 ratios of unsaturated:saturated fatty acids.

- Ratios based on Exp 4.

Sampling: gastrointestinal content, pancreatic + gastrointestinal tissue. Faecal sampling in the period after weaning.
Main outcome parameter: fat digestibility at the end of the ileum; other parameters, such as bile acid production, enzyme secretion, gastrointestinal development (weight, length, histology), blood lipid metabolite profile, will help to explain the observed effects on fat digestibility.

Experiment 15
Treatments before and/or after weaning: the 4 most promising nutritional strategies from Exp 5 and 6 will be used. This is regarding the level of coarseness, starting time of coarser feed before weaning, and inclusion level of coarse raw materials.
Sampling: gastrointestinal development, e.g., acidification, ulceration of the stomach and length, weight and histomorphology of different parts of the gastrointestinal tract, and gastrointestinal content at and after weaning.
Main outcome parameter: nutrient digestibility at the end of the ileum; other parameters determined from the gastrointestinal samples will help to explain observed effects on nutrient digestibility.

Experiment 16
Treatments after weaning: 4 diets with differing buffering capacity in a dose-response.
Sampling: gastrointestinal development, e.g., acidification, ulceration of the stomach and length, weight and histomorphology of different parts of the gastrointestinal tract, and gastrointestinal content at and after weaning.
Main outcome parameter: nutrient digestibility at the end of the ileum; other parameters determined from the gastrointestinal samples will help to explain observed effects on nutrient digestibility.

Experiment 17
Treatments after weaning: 2 combinations of amino acids and protein levels × 2 housing conditions (optimal vs management model) = 4 treatments of which 2 are regarded as animal experiment. The combinations of amino acids and protein levels are obtained in Exp 9 and 10.
Main outcome parameter: growth.

All experiments
Non-challenge control piglets receiving the same experimental diets will be included in experiments 12-16, but only non-invasive measurements will be done on these animals, i.e., growth, faecal sampling, fecal scoring for diarrhoea incidence.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Proposed animal procedures all experiments:

1. Before weaning, piglets will be individually ear-tagged and birth weights will be recorded. Creep feed will be given as free-choice. Piglets will be weaned at a minimum age of 21 days old into specialised housing which is separated from housings where the sows are kept (Council Directive 2008/120/EC).
2. Piglets will be housed in groups of 3 after weaning.
3. Housing without bedding for maximally 6 weeks after weaning. This is required to properly assess the effects of the nutritional strategies. Consumption of bedding might interfere with the response leading to more variation and a higher number of animals required to show an effect.
4. Animal weighing every 7 days for a maximum of 10 weeks (before and after weaning periods).
5. Management model with an average weaning age of 24 days (minimum 21 days): Factors in the management model that will be validated are e.g., reduced temperature (e.g., 2°C), increased ventilation (reduced P-band), spreading of manure, no disinfection of the unit before animals enter. The parameters will be incorporated in a standard operating procedure to ensure repeatability of the model.

Additional animal procedure experiment 12:

6. Blood sampling; maximal 6 times within 6 weeks after weaning with a maximum of 10 mL or 8ml/kg/14 days for young animals. Route: intravenous.

Additional animal procedure experiment 13:

7. Socializing before weaning.

Additional animal procedure experiment 13-16:

8. Euthanasia for the sampling of digesta samples from the gut and tissue sampling in order to determine digestibility / retention time / enzyme production. Euthanasia can take place before (reference samples in Exp 12) and after weaning. A blood sample can be taken just before euthanasia when animals are sedated.
9. Imposed feeding pattern to get a continuous flow of digesta through the gastrointestinal tract after weaning. A feeding pattern means that the total feed typically eaten is provided in smaller meals throughout the day instead of free access to feed. The amount of feed to be given will be determined based on feed intake of individual piglets (average of previous studies) and will always be above the level needed for maintenance.

Additional animal procedure experiment 12-14:

10. Faeces sampling via rectum stimulation; maximal 7 times in the experimental period of 10 weeks (before and after weaning periods) with max 2 samples taken before weaning and max 5 samples after weaning.

Example of a timeline for experiment 12:

Day in experiment	**Procedure**
Birth	Ear tag birth weight
~24 of age = day 0	Weaning into groups of 3: 4 groups of animals (2 per diet) housed under management model conditions and 2 groups of animals (1 per diet) housed in optimal conditions
Day 6	Blood sample + body weight
Day 10	Faecal sample
Day 13	Blood sample + body weight
Day 17	Faecal sample
Day 20	Blood sample + body weight
Day 27	Blood sample + body weight
Day 41 (=6 weeks after weaning)	Blood sample + body weight

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

Calculating sample size (power analysis) in order to minimise the number of animals needed per experiment will be determined based on the main outcome parameters. For experiment 12, it is based on haptoglobin concentrations in blood. For experiments 13-17, the power calculation is based on the amount of piglets needed to find an effect of the management model. For now, this will be based on growth but this might be adjusted based on outcomes of Exp 12.

Experiment 12
500 studies are simulated in a statistical software package using relevant differences in blood haptoglobin concentration found in a previous trial. The 500 simulations are, thereafter, analysed using a linear mixed model (MIXED) which is also the model used to analyse the data after a study is completed. The proportion of simulations showing a significant treatment effect should be >80% in order to ensure a power of 80% and a probability of 95%.
Error variance = 0.31
Mean haptoglobin concentration optimal conditions vs management model conditions (~day 14 after weaning) = 0.90 deviating between 0.70 vs 1.20 (based on in-house trial and Le Floc'h et al., 2009, J. Anim. Sci. 87:1686-1694; Van der Meer et al., 2016, J. Anim. Sci. 94:4704-4719; Van der Peet-Schwering et al., 2021, Wageningen Livestock Research, Public Report 1319)
Relevant difference = 0.60
Number of replicates per treatment = 15.

Experiment 13-17
Data from previous studies were used to estimate the expected mean, and error and block variances. 500 studies are simulated in a statistical software package using a mean growth of 460 g/d (day 0-42 after weaning) with differences ranging from 0-45 g/d. We aim to detect an absolute difference of 35. The 500 simulations are, thereafter, analysed using a linear mixed model (MIXED) which is also the model used to analyse the data after a study is completed. The proportion of simulations showing a significant treatment effect should be >80% in order to ensure a power of 80% and a probability of 95%.

Error variance = 1553
Block variance = 983
Mean = 460 g/d with absolute differences ranging from 0-45 (mean range = 415 – 505 g/d)
Relevant difference = 35 g/d
Number of replicates per treatment = 20.

B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
1	Sus scrofa	Own facility	Pre-/weaned piglets	1254	Males and females	No	Hypor Libra × Hypor Maxter

Provide justifications for these choices

Species	Sus scrofa is the target species
Origin	We have our own facility with a sow herd, where we breed our own piglets
Life stages	Piglets before and after weaning up to 6 weeks after weaning are the target group

Number	Experiment 12: 2 diets × 15 piglets = 30 piglets for control/optimal conditions (blood sample only). For the management model (procedure 5), experiments 12-17: 20 treatments (Exp 12: 4, Exp 13: 4, Exp 14: 2, Exp 15: 4, Exp 16: 4, and Exp 17: 2) in total. In Exp 13, 12 socialized piglets and 12 traditionally housed piglets will form the reference sample group. 20 treatments × 20 pens × 3 piglets + 24 reference piglets = 1224 piglets Total number of piglets 30 + 1224 = 1254
Gender	Males and females are representative for the commercial situation.
Genetic alterations	Not applicable
Strain	This strain is present at our own facility and representative of the commercial situation

C. Accommodation and care

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

☐ Yes

X No > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices

During the pre-weaning phase of the experiment piglets will be housed in conventional farrowing crates together with the dam.
Piglets will be housed without bedding material. Non-edible pen enrichment will be provided to allow animals to play and exhibit normal behaviour. The pen enrichment meets the requirements set by the NVWA.
The pens have a tenderfoot slatted floor.

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

X Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

X No > Justify why pain relieving methods will not be used.

Blood sampling might induce pain, but no sedation will be used. A single insertion of a needle to sample blood is not expected to cause severe pain and the duration is short.

☐ Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Describe which other adverse effects on the animals' welfare may be expected?

Piglets might experience lung problems due to increased ventilation and reduced environmental temperature. The incidence of health issues (not specific for lung problems) in earlier trials with the management model was 4% of which lung problems were 0.3%. The 4% incidence of health issues is similar to other trials where no challenge was given to the animals. In literature, animals kept under low sanitary conditions for the entire fattening period (+/- 15 weeks) had higher pleuritis scores (0.3) and greater percentage of lung surface with pleuritis (1%) at slaughter (Van der Meer et al., 2016, J. Anim. Sci. 94:4704-4719). In a study with weaned piglets (4 weeks of age until 9 weeks of age), there was no difference in the incidence of veterinary treated piglets between the low and high sanitary condition (both ~4%; Van der Peet-Schwering et al., 2021, Wageningen Livestock Research, Public Report 1319). In praxis, almost 10% of the pigs at slaughter show signs of pleuritis (https://duurzaamvarkensvlees.nl/themas/smart-farming/varkenshouder-2030/gebruik-van-data/).

Piglets will not get bedding material in the pens. However, we expect little adverse effects because other (non-edible) pen enrichment will be present.

Explain why these effects may emerge.

The effects on health are inherent to the management model and not different from praxis.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

In case of symptoms of respiratory distress, animals will be treated with antibiotics.

E. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question F

X Yes > Describe the criteria that will be used to identify the humane endpoints.

No recovery from respiratory distress. In that case, the animal will be removed from the study and transferred to standard housing if possible or humanely euthanized. An animal will also be removed from the study when it ends up alone in a pen (penmates removed from the study because of health reasons or death).

Indicate the likely incidence.

Unlikely. Literature and previous in-house trials, indicate that the management model gives similar subclinical health issues as seen in other trials.

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

1. Ear tagging: less than mild (standard commercial procedure)
2. Group housing: less than mild
3. Housing without bedding: less than mild
4. Animal weighing: less than mild
5. Management model: mild for ~95% of the piglets in this appendix (1200 out of 1254 piglets)
6. Blood sampling: mild for ~7% of the piglets in this appendix (90 out of 1254 piglets (Exp 11 only)
7. Socializing before weaning: less than mild
8. Euthanasia after sedation (~13% of piglets): mild for ~13% of the piglets in this appendix (piglets in Exp 12-15 with a total of 14 treatments and with n=12 per treatment (as in Appendix 1) results in 168 out of 1254 piglets)
9. Imposed feeding pattern: less than mild
10. Faeces sampling: less than mild

The overall expected level of discomfort is mild for piglets under management model conditions (n=1200), for non-challenged piglets (n=30 for blood sampling in Exp 11) and piglets euthanised pre-weaning (n=24 in Exp 12 piglets).

G. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement	A response in growth of an animal to a nutritional strategy cannot be determined without using animals. The physiology of a piglet, especially after weaning, and its response to suboptimal environmental conditions cannot be modelled in in vitro or in silico systems. Suboptimal conditions are common in commercial husbandry, but the parameters are not controlled. In order to study the response to nutritional strategies in a reliable and repeatable way, we need controlled conditions such as those obtained with the management model.
Reduction	Sample size estimations are done using data from our own facility. Per nutritional strategy, we use previous studies and literature (see 3.1 Background in the project

	proposal) to decide on the treatments to be studied. The model will be validated first and a go/no go decision will follow before continuing with the other experiments (12-16). Go/no go decisions per nutritional strategy ensures that only the most promising strategies will be tested in challenge studies, allowing for the reduction in the number of animals potentially required.
Refinement	Piglets after weaning are the target animals and their physiology cannot be obtained in other species or in models. Animals will be group housed. Animals will be sedated before euthanasia. In the absence of bedding, extra care will be taken that piglets have access to enrichment material at all times. The enrichment material should be manipulated, is chewable, interesting for a longer time and available for all animals in a pen (e.g., chains reaching the floor, rope, plastic toys, etc.). Animals will be checked daily by trained staff. Water and feed intake will be monitored daily at least for the first 14 days after weaning to get an indication of wellbeing. The goal of the management model is to get a subclinical immune response and it is, therefore, not a disease challenge model. In case of signs of disease, animals will be treated and if needed removed from the study (Humane Endpoint).

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

X No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

X No > Continue with question I.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

X No

☐ Yes > Provide specific justifications for the re-use of these animals during the procedures.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

Not applicable

J. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question K.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

☐ No > Provide information on the destination of the animals.

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

Tissue and/or digesta from the gastrointestinal tract can only be sampled after euthanasia. Only a part of the animals will be euthanised. The other animals will be kept at our own research facility or sold to a commercial fattening farm.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

X Yes > Will a method of killing be used for which specific requirements apply?

X No > Describe the method of killing.

Overdose of barbiturate after sedation

☐ Yes > Describe the method of killing that will be used and provide justifications for this choice.

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

Centrale Commissie Dierproeven

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 lid2h
1.2	Provide the name of the licenced establishment.	5.1 lid2h
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 3 — Type of animal procedure: *E. coli* challenge model

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

The *E. coli* challenge model will be applied to validate the optimized nutritional strategy regarding the use of coarse raw materials in feed before and after weaning (Exp. 17). The 2 most promising nutritional strategies will be tested against a standard/control diet, resulting in a total of 3 treatments. The 2 nutritional strategies come from Exp. 5, 6 and 14 (see 3.4.1 in Project proposal) and the control will be the strategy that is currently used in praxis. The primary outcome parameter is the diarrhoea incidence after the *E. coli* challenge. Other outcome parameters are feed intake and health (to be measured as body condition, behaviour and skin condition). The *E. coli* challenge will result in an immediate drop in feed intake that typically lasts for max 7 days and an increase in diarrhoea that typically lasts max 10 days.

Piglets will be housed in groups of 3 and receive the *E. coli* inoculum using a revolver syringe to ensure each piglet receives it. Piglets will be selected based on their susceptibility towards ETEC O149:F4ac by a DNA-marker test as described in 3.1 Background of the Project proposal. In the past, we used tails for this purpose since tail docking was standard practise. However, we are already validating alternatives for the tails such as hair, oral swab (like for human DNA tests) or a piece of ear that is removed during the standard procedure of inserting the ear label needed for identification purposes. In this way, we do not need to inflict additional discomfort to the piglets for the purpose of the DNA-marker test.
Only susceptible heterozygote (RS) and susceptible homozygote (SS) animals are used in the challenge since these will show a greater response to the challenge as opposed to non-susceptible homozygote (RR) animals. This will reduce variation and, therefore, the number of animals needed. The genotype of the mother sow could also be tested but the sow is not always inseminated with semen from the same boar. Thus, the parent genotype is not suitable to select the susceptible piglets. SS piglets show the greatest response but are at

greatest risk of getting dehydrated. Therefore, RS and SS piglets will be mixed in a pen which will result in a lower response on pen level but less risk of losing piglets, and thus replicates and experimental power, during the trial.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Proposed animal procedures:

1. Before weaning, piglets will be individually ear-tagged and birth weights will be recorded. Creep feed will be given as free-choice. Piglets will be weaned at a minimum age of 21 days old into specialised housing which are cleaned before entering of a new group and which is separated from housings where the sows are kept (Council Directive 2008/120/EC).
2. Piglets will be housed in groups of 3 with a mix of RS and SS piglets.
3. Housing without bedding for maximally 6 weeks after weaning. This is required to properly assess the effects of the nutritional strategies. Consumption of bedding (typically coarse and fibrous materials) might interfere with the response leading to more variation and a higher number of animals required to show an effect.
4. Animal weighing every 7 days for a maximum of 10 weeks (before and after weaning periods).
5. Blood sampling. Maximal 8 times within 10 weeks before and after weaning with a maximum of 10 mL and 8ml/kg/14 days for young animals. Route: intravenous. Out of the 8 samples, a maximum of 2 blood samples will be taken before weaning (max 4 weeks time-period) in case samples before administration of creep feed and on the day before weaning (without weaning stress) are required. Before administration of creep feed, it is not always known which animals will be included in the experiment after weaning. Thus, a blood sample might be taken from an animal that is not used after weaning. Out of the 8 samples, a maximum of 6 samples will be taken after weaning (max 6 weeks time-period). Analysis: markers for gut permeability or inflammation.
6. *E. coli* challenge: oral route 1 ml pathogenic *E. coli* (O149K91K88ac) for maximal 3 consecutive days within the first two weeks post-weaning. Piglets will be minimally 28 days of age when receiving the *E. coli* inoculum. The *E. coli* inoculum will be provided using a revolver syringe.

Example of a timeline of the experiment:

Day in experiment	**Procedure**
Birth	Ear tag + remove piece of ear for DNA-marker test + birth weight
~10 of age	Start experimental feed + blood sample + body weight
~23 of age	Blood sample + body weight
~24 of age = day 0	Weaning into groups of 3
Day 6	Blood sample + body weight
Day 6-8	*E. coli* challenge
Day 13	Blood sample + body weight
Day 20	Blood sample + body weight
Day 27	Blood sample + body weight
Day 34	Blood sample + body weight
Day 41 (=6 weeks after weaning)	Blood sample + body weight

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

Data from studies using 3 piglets per pen were used to estimate the error variance in this specific animal facility for the period day 0-14 after weaning. This is the period where we expect most effects of the nutritional strategies and *E. coli* challenge. 500 studies are simulated in a statistical software package using relevant means ranging between 0.15 and 0.45 (i.e., 15-45% diarrhoea incidence). We aim to detect an absolute difference of 0.15 within this range of means. Previous studies with nutritional strategies have shown that we can detect this difference in our facility using this *E. coli* challenge model. The 500 simulations are, thereafter, analysed using a generalized linear mixed model (GLIMMIX) which is also the model used to analyse the data after a study is completed. The proportion of simulations showing a significant treatment effect should be >80% in order to ensure a power of 80% and a probability of 95%.
Error variance = 7.37

Means = 0.15 – 0.30 – 0.45 (relevant difference of 0.15 absolute)
Number of replicates (=pens) per treatment = 18

B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
1	Sus scrofa	Own facility	Pre-/weaned piglets	207	Males and females	No	Hypor Libra × Hypor Maxter

Provide justifications for these choices

Species	Sus scrofa is the target species
Origin	We have our own facility with a sow herd, where we breed our own piglets
Life stages	Piglets before and after weaning up to 6 weeks after weaning are the target group
Number	Post-weaning: 3 treatments (see A) × 18 pens × 3 animals = 162 piglets undergoing the *E. coli* challenge. Pre-weaning: 3 treatments × maximally 15 animals = maximally 45 animals undergoing blood sampling but not selected for the post-wean phase. At the start of the experiment, it is not always known which piglets will be included in the post-wean phase; for example, piglets of poor health or treated with antibiotics before weaning will be excluded but might have been used for blood sampling. 15 animals per treatment are required to find a relevant difference in e.g., plasma haptoglobin concentration (see Appendix 2).
Gender	Males and females are representative for the commercial situation
Genetic alterations	Not applicable
Strain	This strain is present at our own facility and representative of the commercial situation

C. Accommodation and care

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

☐ Yes

X No > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices

During the pre-weaning phase of the experiment piglets will be housed in conventional farrowing crates together with the dam.
Piglets will be housed without bedding material. Non-edible pen enrichment will be provided to allow animals to play and exhibit normal behaviour. The pen enrichment meets the requirements set by the NVWA.
The pens have a tenderfoot slatted floor.

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

X Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

X No > Justify why pain relieving methods will not be used.

Blood sampling might induce pain, but no sedation will be used. A single insertion of a needle to sample blood is not expected to cause severe pain and the duration is short.

☐ Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Describe which other adverse effects on the animals' welfare may be expected?

Due to the *E. coli* infection, all piglets will experience a period of mild diarrhoea (typically lasts max 10 days). Piglets will not get bedding material in the pens. However, we expect little adverse effects because other (non-edible) pen enrichment will be present.

Explain why these effects may emerge.

This specific *E. coli* strain causes diarrhoea especially in susceptible (RS and SS) piglets. This effect is essential for answering the research question: can our nutritional interventions reduce diarrhoea incidence?

Indicate which measures will be adopted to prevent occurrence or minimise severity.

Before the *E. coli* inoculum is provided, piglets will be weighed and their body condition judged visually by experienced personnel. If piglets have lost more than 9% of their body weighed since weaning, they will be removed from the study, moved to another department, and put on commercial feed. This is done to ensure all piglets are healthy at the start of the *E. coli* challenge.
All animals will be checked on health status twice daily in the week immediately following the *E. coli* challenge (period with highest risk). When an animal or a pen is suspected of dehydration, additional measures can be taken such as body temperature, skin pinch test, and water intake. In case animals respond with severe dehydration symptoms, electrolytes and antibiotics will be provided.

E. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question F

X Yes > Describe the criteria that will be used to identify the humane endpoints.

Body weight loss before the *E. coli* challenge.
Dehydration after the *E. coli* challenge by visual examination, body temperature, skin pinch test, water intake. Monitoring of the recovery from dehydration after the use of electrolytes and antibiotics.

Indicate the likely incidence.

Dehydration is unlikely. In the past studies (2021-2022) involving a total of 1200 animals, we did not have to euthanize an animal because of severe dehydration and lack of recovery.
In the week following the *E. coli* challenge it is possible that an animal dies. In that case the cause of death, as determined by the animal health service (Royal GD, Deventer, The Netherlands), will come back as *E. coli*. Although we increase the frequency of monitoring of the piglets after the *E. coli* challenge, it is possible that a piglet dies in that week. We expect that this is at maximum 3% of the animals. These animals will be classified as having severe discomfort.
In past studies, 6 out of 1200 piglets (0.5%) were removed before the *E. coli* challenge started because of too high weight loss. For these animals, severe discomfort was avoided.
An animal will be removed from the study when it ends up alone in a pen (penmates removed from the study because of health reasons or death).

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

1. Ear tagging: less than mild (standard commercial procedure)
2. Group housing: less than mild
3. Housing without bedding: less than mild
4. Animal weighing: less than mild
5. Blood sampling: mild
6. *E. coli* challenge: moderate

The expected level of discomfort is moderate for *E. coli* challenged animals (n=157) and maximally mild for animals only used for blood sampling pre-weaning (n=45). Severe discomfort due to the *E. coli* infection will

be prevented by monitoring the animals closely especially in the period immediately following the *E. coli* challenge. However, an animal can die in that period following the *E. coli* challenge. These animals will be classified as severe discomfort and we expect that this will be maximum 3% of the animals undergoing the *E. coli* challenge (n=5; 3% of 162).

G. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement	A response in diarrhoea incidence of an animal to a nutritional strategy cannot be determined without using animals. The physiology of a piglet, especially after weaning, cannot be modelled in in vitro or in silico systems. Organoids could be considered but organoids resemble the physiology of the state of the animal that it was made from. For example, organoids from slaughter material will resemble the physiology of the slaughtered animal and not from a weaned piglet. In order to obtain organoids from weaned piglets, piglets in this life phase need to be sacrificed. An organoid, however, is not suitable to look at the whole gastrointestinal tract, including stomach, small and large intestine, and the residing microbiota. The interaction within the gastrointestinal tract can only be studied in an animal of the appropriate age. Post-weaning diarrhoea and the stress around weaning occurs in all husbandry systems. Pathogens are present in all systems and stress will give room for health issues caused by these pathogens.
Reduction	Sample size estimations are done using data from our own facility. Per nutritional strategy, we use previous studies (outside this project proposal), literature (see 3.1 Background in the project proposal), and Exp 5, 6, and 14 to decide on the treatments to be studied. Only the most promising nutritional strategies, i.e., decided based on go/no go decisions, will be tested in this Appendix. This results in a reduction in the number of animals required for this Appendix. RS and SS animals will be used to ensure that we obtain a difference in diarrhoea incidence (=main outcome parameter). This will lower the variation and, thus, increase the power and reduce the number of animals needed to show an effect.
Refinement	Piglets will be housed in groups of 3. In case 1 piglet needs to be removed (Humane End Point) or dies, piglets are still with 2 in a pen. If a second piglets needs to be removed or dies, the remaining piglet will be removed from the study and housed with other piglets in a separate barn. In the absence of bedding, extra care will be taken that piglets have access to enrichment material at all times. The enrichment material should be manipulated, is chewable, interesting for a longer time and available for all animals in a pen (e.g., chains reaching the floor, rope, plastic toys, etc.). Animals will be checked twice daily by trained staff after the *E. coli* challenge at least until the trained staff member indicates that there are no further health issues observed. The animals will then be checked once daily again. Water and feed intake will be monitored daily at least for the first 14 days after weaning to get an indication of wellbeing. Piglets will be weighed before the *E. coli* inoculum is given. When a piglet has lost too much body weight or is in poor health before the *E. coli* challenge, the piglet is removed from the study and transferred to a standard housing pen at the same facility.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

X No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

X No > Continue with question I.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

X No

☐ Yes > Provide specific justifications for the re-use of these animals during the procedures.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

Not applicable.

J. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question K.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

X No > Provide information on the destination of the animals.

Animals will be kept at our own research facility or sold to a commercial fattening farm. Before transferring animals to another farm, an antibiotic treatment will be given at the end of the experiment to ensure that there is no *E. coli* from the challenge remaining.

☐ Yes > Explain why it is necessary to kill the animals during or after the procedures.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☐ Yes > Will a method of killing be used for which specific requirements apply?

☐ No > Describe the method of killing.

☐ Yes > Describe the method of killing that will be used and provide justifications for this choice.

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

Centrale Commissie Dierproeven

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 lid2h
1.2	Provide the name of the licenced establishment.	5.1 lid2h
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 4 — Type of animal procedure: Nitrogen balance optimal and suboptimal conditions

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

The studies described here aim to determine the effect of dietary protein level on the requirement of amino acids in pigs from 2-weeks after weaning until the end of the nursery (~25 kg body weight). This will be determined under optimal and suboptimal sanitary conditions. With the results, we will be able to optimize diet formulations for growth, health, and reducing nitrogen emissions in urine and faeces under different practically relevant conditions.
Two protein levels in combination with 5 levels of a test amino acid will be used. An indigestible marker will be included in the diets to calculate nitrogen digestibility in faeces. A total of 10 dietary treatments will be provided to piglets from 2 weeks to 6 weeks after weaning (end of the nursery). We have chosen for this timeframe since we first want to establish requirements in a period where the piglet is not facing stressors due to weaning. Subsequently, we will investigate the requirements of younger (weaning – 2 weeks after weaning) piglets. The requirements of amino acids in this period (week 2-6 after weaning) are currently extrapolated from older pigs. We hypothesize that in this period, the requirement of different amino acids changes rapidly and, therefore, we propose to determine the amino acid requirements on a weekly basis.

With the proposed diets, we can estimate the amino acid requirements in the context of a low and high protein diet by using a breakpoint plateau model for protein deposition and plasma urea nitrogen levels. Protein deposition will be determined from nitrogen (=protein) intake and nitrogen excretion through faeces and urine. For this, feed intake needs to be determined per individual piglet and faeces and urine needs to be quantitatively and accurately collected over a 3-day period of an individual piglet. Plasma urea nitrogen levels will be determined at the end of the (3-day) nitrogen balance period by collecting a blood sample.

Plasma urea nitrogen is a measure for body protein breakdown and, therefore, is an indicator for protein utilization efficiency. Thus, the main outcome parameters are protein deposition and plasma urea nitrogen.

Experiment 1. Optimal housing conditions
Piglets will be housed in groups of 3 after weaning until 2 weeks after weaning. Thereafter, 1 piglet per pen will be randomly allocated to one of the ten dietary treatments for the remaining 4 weeks of the experiment. The other 2 pigs of a pen (non-experimental animals) will be returned to the commercial herd (group housing) and sold to a commercial fattener after the nursery phase (~25 kg body weight). Pigs will be adapted to the experimental diet for 4 days which is then followed by a 3-day collection period of feed intake, faeces and urine. Feed intake will be restricted to 2.2× energy required for maintenance, which is based on body weight, (close to ad libitum feed intake at this age) to ensure a uniform intake of energy between piglets. This will reduce variation between piglets.
For the separate collection of faeces and urine, stoma bags will be attached to the rear end of the piglet for the collection of faeces. Urine will be collected in urine pans situated underneath the pens.
Piglets will receive the same diet throughout the experimental period. There will be 4 collection periods: ~d19-21, d26-28, d33-35, d40-42 after weaning which are all preceded by a 4-day adaptation period to adapt the piglets to the new feeding level. A blood sample will be taken on the last day of the collection period (4 blood samples per piglet).

Example of a timeline of the experiment:

Day in experiment	**Procedure**
~24 of age = day 0	Body weight and weaning into groups of 3
Day 7	Body weight
Day 15	Body weight and selection of animals and individual housing (removal of 2 piglets per pen: return to commercial herd)
Day 15-18	Adaptation experimental diet + feeding level
Day 19	Body weight + attachment of stoma bags for faecal collection
Day 19-21	N balance (replacing stoma bags regularly)
Day 21	Blood sample and body weight
Day 22-25	Adaptation feeding level
Day 26	Body weight + attachment of stoma bags for faecal collection
Day 26-28	N balance (replacing stoma bags regularly)
Day 28	Blood sample and body weight
Day 29-32	Adaptation feeding level
Day 33	Body weight + attachment of stoma bags for faecal collection
Day 33-35	N balance (replacing stoma bags regularly)
Day 35	Blood sample and body weight
Day 36-39	Adaptation feeding level
Day 40	Body weight + attachment of stoma bags for faecal collection
Day 40-42	N balance (replacing stoma bags regularly)
Day 42	Blood sample and body weight + end experiment return of piglets to commercial herd

Experiment 2. Suboptimal sanitary conditions
The same procedure is followed as for experiment 1 except piglets will be housed under suboptimal sanitary conditions from weaning onwards as specified in Appendix 2. The N balance period will be minimized to 2 weeks (week 3-4 after weaning) to reduce the time that piglets are housed individually and are exposed to suboptimal sanitary conditions.

Example of a timeline of the experiment:

Day in experiment	**Procedure**
~24 of age = day 0	Body weight and weaning into groups of 3 under suboptimal sanitary conditions
Day 7	Body weight
Day 15	Body weight and selection of animals and individual housing (removal of 2 piglets per pen)
Day 15-18	Adaptation experimental diet + feeding level

Day 19	Body weight + attachment of stoma bags for faecal collection
Day 19-21	N balance (replacing stoma bags regularly)
Day 21	Blood sample and body weight
Day 22-25	Adaptation feeding level
Day 26	Body weight + attachment of stoma bags for faecal collection
Day 26-28	N balance (replacing stoma bags regularly)
Day 28	Blood sample and body weight + end experiment return of piglets to commercial herd

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Proposed animal procedures:

1. Housing without bedding for the entire experiment (~6 weeks). This is required to properly assess nitrogen efficiency, since consumption of bedding material interferes with these responses.
2. Individual housing for 4 weeks (experiment 1) or 2 weeks (experiment 2) starting from 2 weeks after weaning.
3. Attachment of stoma bags for faecal collection for 4 times (experiment 1) or 2 times (experiment 2) 3 days.
4. Animal weighing at weaning and around day 7 and 14 after weaning and before and after balance period (maximally 11 times in total in 6 weeks).
5. Blood sampling: maximally 4 times within 4 weeks after weaning with a maximum 10 mL and of 8ml/kg/14 days for young animals. Route: intravenous.
6. Amino acid levels of the experimental diets may fall outside current recommendations (lower) in order to determine the optimum levels in a breakpoint analysis.
7. Management model with an average weaning age of 24 days (minimum 21 days): Factors in the management model that will be validated are e.g., reduced temperature (e.g., 2°C), increased ventilation (reduced P-band), spreading of manure, no disinfection of the unit before animals enter.
8. Feed restriction at 2.2× energy required for maintenance.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

For nitrogen efficiency the piglet is the experimental unit. Sample size estimation for nitrogen efficiency are made for every single experiment with a power of 80% and a probability of 95%. Using a two-tailed t-test based on variation in nitrogen efficiency the sample size estimation was performed. Effect size: 5% (μ=53% nitrogen efficiency; SD 5.7%); number of piglets per treatment=10. Breakpoint analyses (regression) lowers the required samples size to n=8 per treatment.

B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
1	Sus Scrofa	Own facility	Piglets	160	Males and Females	No	Hypor Libra X Hypor Maxter

Provide justifications for these choices

Species	Sus Scrofa target species of interest
Origin	Own facility/produce own piglets from onside sow herd
Life stages	Grower-Finisher target life span with largest feed consumption
Number	160 (based on power analyses and number of studies and sample points) are considered experimental animals.
Gender	Males (the choice for one gender will not lead to a surplus in breeding stock of experimental animals because animals will be obtained from the own herd)
Genetic alterations	Not Applicable

Strain	Own facility genetics

C. Accommodation and care

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

☐ Yes

X No > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices

Piglets will be individually housed for 2 (experiment 2) to 4 weeks (experiment 1). Piglets will have visual, tactile and olfactory contact with neighbouring pens via a hole in the wall. Piglets will be housed without bedding material. Non-edible pen enrichment will be provided to allow animals to play and exhibit normal behaviour. The pen enrichment meets the requirements set by the NVWA.
The pens have a tenderfoot slatted floor.

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

X Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

X No > Justify why pain relieving methods will not be used.

Blood sampling might induce pain, but no sedation will be used. A single insertion of a needle to sample blood is not expected to cause severe pain and the duration is short.

☐ Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Describe which other adverse effects on the animals' welfare may be expected?

The pigs will not get bedding material in the pens. However, limited adverse effects are expected because pens will contain (non-edible) enrichment materials.
Pigs will be housed individually for 2 or 4 weeks, which may affect the pig behaviour.
Piglets might experience lung problems due to increased ventilation and reduced environmental temperature. The incidence of health issues (not specific for lung problems) in earlier trials with the management model was 4% of which lung problems were 0.3%. The 4% incidence of health issues is similar to other trials where no challenge was given to the animals. In literature, animals kept under low sanitary conditions for the entire fattening period (+/- 15 weeks) had higher pleuritis scores (0.3) and greater percentage of lung surface with pleuritis (1%) at slaughter (Van der Meer et al., 2016, J. Anim. Sci. 94:4704-4719). In a study with weaned piglets (4 weeks of age until 9 weeks of age), there was no difference in the incidence of veterinary treated piglets between the low and high sanitary condition (both ~4%; Van der Peet-Schwering et al., 2021, Wageningen Livestock Research, Public Report 1319). In praxis, almost 10% of the pigs at slaughter show signs of pleuritis (https://duurzaamvarkensvlees.nl/themas/smart-farming/varkenshouder-2030/gebruik-van-data/).

Explain why these effects may emerge.

Pigs are social animals and like to eat, huddle, sleep and play together with pen mates.
The effects on health from suboptimal sanitary conditions are inherent to the management model and not different from praxis.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

The time that animals will be housed individually is minimized to 2 (experiment 2) or 4 weeks (experiment 1). Furthermore, pigs are housed in pens specially designed for individual housing having an opening in the wall allowing pigs to have nose-to-nose contact with a neighbouring piglet. Floor heating below the pens, ensures the temperature in the pen is within the thermoneutral zone for piglets of a certain age.
In case of symptoms of respiratory distress, animals will be treated with antibiotics.

E. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question F

X Yes > Describe the criteria that will be used to identify the humane endpoints.

No recovery from respiratory distress. In that case, the animal will be removed from the study and transferred to standard housing if possible or humanely euthanized. An animal will also be removed from the study when it ends up alone in a pen (penmates removed from the study because of health reasons or death).

Indicate the likely incidence.

Unlikely. Literature and previous in-house trials, indicate that the management model gives similar subclinical health issues as seen in other trials.

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

Proposed animal procedures:

1. Housing without bedding material: less than mild
2. Individual housing for 2 weeks (experiment 2) or 4 weeks (experiment 1): moderate
3. Attachment of stoma bags: mild
4. Weighing: less than mild
5. Blood sampling: mild
6. Amino acid levels below recommendations: mild
7. Suboptimal sanitary conditions: mild
8. Feed restriction: mild

The expected level of discomfort is moderate for all experimental animals (n=160).

G. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement	Responses of animals towards different protein and amino acid levels under optimal and specific pathogen conditions needs to be determined in the animal itself. The used dietary protein and amino acids levels will be based on literature. In silico models will be used to assure that the desired effect will be seen. The in silico model, however, is not capable of estimating the effect of a specific pathogen on requirements. The results of the in vivo experiment will be used to improve the in silico model.
Reduction	Sample size estimations are done using data from our own facility. RS and SS animals will be used to ensure that we obtain a difference in diarrhoea incidence (=main outcome parameter). This will lower the variation and, thus, increase the power and reduce the number of animals needed to show an effect.
Refinement	Piglets are the target animals. Using other animals (mice, rats) as a model will not result in less discomfort to an individual. In addition, protein deposition is species specific. Therefore, the experimental treatments should be tested in the target animal directly. Pigs will receive non-edible enrichment material to reduce stress and expression of abnormal behaviour. Pigs are closely followed to check health and welfare issues during the experiments, with both being documented daily and medical treatments will be applied if necessary. Nitrogen efficiency will be measured indirectly using total collection of faeces and urine to reduce discomfort. Pigs are social animals and will be housed in

	groups for the first two weeks. Period and number of pigs that need to be housed individually are limited as much as possible. When housed individually nose to nose contact is still possible. Pigs are housed in pens specially designed for individual housing with floor heating ensuring the temperature is within the thermoneutral zone. Blood sampling will be performed by trained personnel. We will do whatever is possible to adapt piglets to human handling prior to the start of the experiment in order to refine the procedure of attaching the stoma bags.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

X No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

X No > Continue with question I.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

X No

☐ Yes > Provide specific justifications for the re-use of these animals during the procedures.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

Not applicable.

J. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☒ No > Continue with question K.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

X No > Provide information on the destination of the animals.

Animals will be kept at our own research facility or sold to a commercial fattening farm. Before transferring animals to another farm, an antibiotic treatment will be given at the end of the experiment to ensure that there is no *E. coli* from the challenge remaining.

☐ Yes > Explain why it is necessary to kill the animals during or after the procedures.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☐ Yes > Will a method of killing be used for which specific requirements apply?

☐ No > Describe the method of killing.

☐ Yes > Describe the method of killing that will be used and provide justifications for this choice.

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

Format Niet technische samenvatting
Let op: bij gebruik van dit word-format dient uiteindelijk alsnog het Excel-format te worden ingevuld voordat uw aanvraag vergund kan worden (zie Procesbeschrijving word-document NTS)

Tab NTS

Country	NL
Language	NL
EU submission	☒ yes ☐ no
Title of the project	Ontwikkelen van voerstrategieën die beter aansluiten bij de nutriëntbehoeftes van biggen na spenen en tevens duurzaamheid, groeiprestaties, welzijn en gezondheid bevorderen
NTS identifier	Deze wordt door de CCD ingevuld
NTS national identifier	Deze wordt door EC ingevuld
Duration of the project	60 (in months)
Keywords	
Keyword 1	Biggen
Keyword 2	Speenproblematiek
Keyword 3	Darmgezondheid
Keyword 4	Nutriëntbehoefte
Keyword 5	

Purpose(s) of the project
Objectives of the project
Describe the objectives of the project (for example, addressing certain scientific unknowns, of scientific or clinical needs). Compulsory! Maximum length is 2500 characters
Het speenproces is een stressvolle periode in het leven van biggen. Het gaat gepaard met het weghalen van biggen bij de zeug, mixen van biggen uit verschillende tomen, veranderende omgeving met de daarbij horende veranderingen in omgevingsbacteriën, en een verandering van zeugenmelk naar minder goed verteerbaar vast voer. Dit gaat vaak gepaard met een vermindering in voeropname net na spenen wat zorgt voor een verminderde functie van de darmwand. Hierdoor hebben ziekteverwekkende bacteriën kans om zich te vestigen in de darm wat vervolgens speendiarree veroorzaakt. Daarnaast is een gezonde start de basis voor een gezonder leven. Speendiarree kan worden behandeld met antibiotica, maar door het streven naar het voorkomen van antibioticaresistentie bij bacteriën moet er worden gezocht naar alternatieven zoals bijvoorbeeld via optimalisatie van voeding. Een andere managementstrategie namelijk het bijvoeren van biggen voor het spenen heeft verschillende voordelen. Voorbeelden zijn: (1) het verteringstelsel en de microbiota in de darm kunnen alvast aangepast worden aan vast voer, (2) voeropname na het spenen wordt bevorderd en (3) het risico op speendiarree wordt verminderd. Er is echter nog veel onduidelijk over wat de beste samenstelling van het voer voor spenen is wat betreft energie-, eiwit-, en vezelgehaltes. Daarnaast is het van belang dat deze bijvoeding voor spenen goed aansluit op de voeding ná het spenen om verteringsstoornissen te voorkomen. De huidige formulering van speenvoer is voornamelijk gebaseerd op gegevens verkregen bij oudere varkens. Maar de fysiologische uitdagingen van het speenproces zijn anders dan de fysiologische omstandigheden van oudere varkens. In de laatste jaren is er veel onderzoek gedaan naar de nutriëntbehoeftes van biggen na spenen. Er blijven echter nog steeds vragen over de meest optimale voeding vooral in relatie tot het gebruik van duurzamere/circulaire grondstoffen en het reduceren van de uitscheiding van bijvoorbeeld stikstof. Daarom zijn de directe doelen van dit project gericht op het ontwikkelen van voerstrategieën die beter aansluiten bij de nutriëntbehoeftes van biggen voor, tijdens en na het spenen onder zowel optimale als

suboptimale varkenshouderij omstandigheden. Binnen dit project wordt gekeken naar energiegehalte en bronnen, eiwitgehalte en aminozuursamenstelling, alsmede het bevorderen van de maagfunctie om daarmee gezondheid van de biggen te verhogen en speendiarree en sterfte rond spenen te verminderen.

Potential benefits likely to derive from this project
What are the potential benefits likely to derive from this project? Explain how science could be advanced, or humans, animals or environment may ultimately benefit from the projects. Where applicable, differentiate between short-term benefits (within the duration of the project) and long-term benefits (which may accrue after the project is finished). Compulsory! Maximum length is 2500 characters
De belangrijkste potentiële voordelen van het project is dat wij voeders kunnen ontwikkelen die beter voldoen aan de nutriëntenbehoeftes van biggen voor, tijdens en na spenen door middel van het beter begrijpen wat de behoeftes onder optimale en suboptimale varkenshouderij omstandigheden zijn. Met deze kennis kan vervolgens de vertaalslag naar praktijkomstandigheden gemaakt worden. Het ultieme doel van het project is het verhogen van de duurzaamheid van de varkenshouderij door het verlagen van de sterfte bij biggen, vermindering van het antibiotica gebruik, en door grondstoffen in het voer te verwerken zodat aan de behoeftes van de big wordt voldaan en de ontwikkeling van het maagdarmstelsel geholpen wordt. Uiteindelijk zal hierdoor ook het welzijn van de biggen verhoogd worden. In dit project zal ook inzicht verkregen worden in de werking en ontwikkeling van de maag welke tot nu toe weinig aandacht in de wetenschap heeft gekregen, maar wel een belangrijke rol speelt bij het bevorderen van de gezondheid van de big, meer specifiek het maagdarmstelsel. Alleen de voerstrategieën die geen negatieve effecten laten zien onder gecontroleerde proefomstandigheden op groeiprestatie, duurzaamheid, dierwelzijn en darmgezondheid zullen verder getoetst worden onder suboptimale varkenshouderij omstandigheden. Hiermee wordt bedoeld het nabootsen van praktijkomstandigheden op het proefbedrijf door middel van bijvoorbeeld het aanpassen van de hygiënestatus tijdens de proeven. Door te kijken naar de nutriëntensamenstelling van het voer in plaats van de grondstoffen zelf, kan ook gewerkt worden naar alternatieve voersamenstellingen waarin duurzamere/circulaire en eventueel goedkopere grondstoffen verwerkt kunnen worden zonder negatieve effecten voor de gezondheid en groeiprestaties van het dier.

Predicted harms

In what procedures will the animals typically be used
In what procedures will the animals typically be used (for example, injections, surgical procedures)? Indicate the number and duration of these procedures. Compulsory! Maximum length is 2500 characters
1. Voor spenen worden biggen gewogen en krijgen ze een oornummer. Vast voer wordt verstrekt gedurende de dag. In bepaalde studies worden biggen voor spenen gesocialiseerd door biggen van verschillende tomen samen te voegen als model voor verminderde stress na spenen. 2. Biggen worden in groepen of individueel gehuisvest afhankelijk van de onderzoeksvraag. Individuele huisvesting zal maximaal 4 weken achtereen plaatsvinden en een big kan in de periode nog steeds andere biggen zien, besnuffelen, horen en ruiken. 3. Biggen worden gehuisvest zonder bodemmateriaal voor maximaal 6 weken na spenen. Consumptie van dit materiaal kan de meting van vertering en nutriëntbenutting

beïnvloeden. Biggen krijgen wel ander verrijkingsmateriaal aangereikt om aan hun exploratie- en spelgedrag tegemoet te komen.

4. Biggen worden meerdere malen voor en na spenen gewogen.
5. Een managementmodel waarbij een suboptimale hygiënestatus wordt nagebootst. Factoren in het managementmodel zijn bijvoorbeeld de verlaging van de staltemperatuur, verhoging van de ventilatieluchtsnelheid, verspreiden van mest, en het niet desinfecteren van de ruimte.
6. Bloedafname uit de halsader voor maximaal 6 keer in 6 weken.
7. Verzamelen van mestmonsters via het stimuleren van het rectum voor maximaal 7 keer in de totale experimentele periode van 10 weken waarvan maximaal 2 keer voor spenen en maximaal 5 keer na spenen.
8. Verzamelen van urine door het opvangen van urine in bakken onder de hokken. Om besmetting van urine met mest te voorkomen zullen er stomazakjes bevestigd worden rond de anus zodat mest apart van de urine verzameld kan worden. De stomazakjes zullen maximaal 4 keer in 4 weken worden aangebracht en zullen maximaal 3 keer 24 uur blijven zitten.
9. Biggen worden gedood na verdoving om monsters uit het maagdarmkanaal te verzamelen of om weefsels te verzamelen. Biggen kunnen een voerpatroon opgelegd krijgen zodat er een continue stroom van nutriënten door de darm komt en er voldoende darminhoud aanwezig is. Biggen kunnen ook beperkt, echter wel boven onderhoudsbehoefte, gevoerd worden om de variatie in voeropname en daardoor variatie in de resultaten te verminderen.
10. Bepaalde nutriëntgehaltes in het voer kunnen afwijken van de huidige aanbevelingen zodat preciezer de behoefte ervan kan worden vastgesteld.
11. Orale toediening van de E. colibacterie voor maximaal 3 dagen op rij in 5 weken door middel van een spuit in de mondholte.

Expected impacts/adverse effects on the animals

What are the expected impacts/adverse effects on the animals for example pain, weight loss, inactivity/reduced mobility, stress, abnormal behaviour, and the duration of those effects? Compulsory! Maximum length is 2500 characters

Biggen worden gehuisvest zonder bodemmateriaal, zoals stro of zaagsel, omdat consumptie van dit materiaal de meetresultaten kan beïnvloeden en daarmee de variatie verhogen. De hokken worden uitgerust met niet eetbare hokverrijking die voldoet aan de eisen voor wat betreft manipuleerbaarheid, kauwbaarheid, interessant voor een langere periode en beschikbaar voor alle dieren in een hok. Hiermee wordt het vertonen van spel- en exploratiegedrag mogelijk en eventuele negatieve effecten van de afwezigheid van bodemmateriaal voorkomen.
In bepaalde experimenten worden biggen individueel gehuisvest in hokken die speciaal zijn ontworpen voor individuele huisvesting, maar hebben dan wel de mogelijkheid tot het zien, aanraken/besnuffelen, ruiken en horen van naburige biggen. De hokken staan op hoogte en er is vloerverwarming aanwezig onder de hokken zodat de temperatuur in het hok binnen de thermo-neutrale zone voor biggen ligt.
Het managementmodel levert mogelijk respiratieproblemen op maar de incidentie is naar verwachting laag (0.3% van de biggen in voorgaande proeven).
Het toedienen van de E. colibacterie zal diarree veroorzaken die na een aantal dagen afneemt. De gezondheid van de biggen zal scherp gemonitord worden en waar nodig zullen biggen behandeld worden (bijvoorbeeld door het verstrekken van elektrolyten) om verdere negatieve effecten op diergezondheid te voorkomen.

Reasons for the planned fate of the animals after the procedure
Please provide reasons for the planned fate of the animals after the procedure. Compulsory! Maximum length is 2500 characters
Dieren worden gedood als het voor het beantwoorden van de onderzoeksvraag nodig is om monsters van de darminhoud te nemen. Wanneer het doden niet nodig is, zullen biggen grootgebracht worden op een commerciële varkenshouderij of op de eigen proeffaciliteit.

Application of the Three Rs

1. Replacement
State which non-animal alternatives are available in this field and why they cannot be used for the purposes of the project. Compulsory! Maximum length is 2500 characters
Verteringsprocessen in biggen kunnen deels in vitro nagebootst worden. Het in vitro verteringsmodel zal worden toegepast waar mogelijk om grondstoffen en voeders te bestuderen voordat deze worden toegepast in dierexperimenten. Echter, interacties tussen voeders en het maagdarmkanaal, bv vertering, absorptie, nutriëntenpassage door de darm en aanzuring in de maag, kunnen niet bestudeerd worden zonder gebruikt te maken van doeldieren. Met name de fysiologie van de gespeende big is complex en moeilijk na te bootsen buiten het dier. Verteringsgegevens van biggen zijn ook nodig om het in vitro verteringsmodel te valideren zodat deze verbeterd wordt voor toekomstig gebruik. Mest- en urinemonsters kunnen zonder ongerief voor het dier genomen worden. Echter deze zijn niet representatief voor wat er in de verschillende segmenten van het maagdarmkanaal, zoals de maag en de dunne darm, plaatsvindt. De toedienen van E. coli en het managementmodel zouden op commerciële bedrijven getest kunnen worden. Echter daar zijn de houderij omstandigheden variabel en tamelijk onvoorspelbaarder waardoor een hoger dieraantal nodig is vanwege een grotere variatie in de meetresultaten. Lichaamsgroei als gevolg van een voerstrategie is moeilijk middels computermodellen te simuleren omdat deze afhankelijk is van verschillende factoren vooral bij gespeende biggen gehouden onder verschillende huisvestingsomstandigheden.

2. Reduction
Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce the number of animals to be used, and principles used throughout the project to minimise the number of animals used consistent with scientific objectives. Those practices may include e.g. pilot studies, computer modelling, sharing of tissue and reuse. Compulsory! Maximum length is 2500 characters
Het aantal dieren wat gebruikt wordt per experiment wordt geminimaliseerd zoals is bepaald door middel van statistische powerberekeningen. De variatie gebruikt voor deze berekeningen is gebaseerd op eerdere soortgelijke proeven in dezelfde proeffaciliteit. Voor elk experiment zal een nieuwe power berekening gedaan worden op basis van de meest recente resultaten. Voor elke voerstrategie zal voorafgaand aan een experiment eerst de al gedane studies en literatuur bestudeerd worden en eventueel in vitro onderzoek gedaan worden om zo de meest veelbelovende strategieën te kiezen. Go/no go beslissingen worden op basis van vastgestelde criteria (dat wil zeggen groeiprestatie, dierwelzijn en gezondheid) gemaakt voordat vervolgstudies worden opgezet. Het managementmodel zal eerst gevalideerd worden voordat vervolgstudies ingezet worden.

Biggen in het E. coli model zullen geselecteerd worden op genotype en alleen de genotypen die hoog gevoelig zijn voor de specifieke E. coli zullen gebruikt worden. Dit vermindert de variatie in de resultaten waardoor uiteindelijk minder biggen nodig zijn.

3. Refinement
Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms to take up emerging refinement techniques during the lifetime of the project. Compulsory! Maximum length is 2500 characters
Biggen zijn de doeldieren en hun fysiologie kan niet vergeleken worden met die van andere diersoorten of bestudeerd worden met behulp van computermodellen. Dieren worden waar mogelijk in groepen gehuisvest. Als individuele huisvesting noodzakelijk is, zal de periode zo kort mogelijk worden gehouden. Neus-neus contact met naburige biggen is altijd mogelijk. Biggen worden altijd verdoofd voor het doden. Biggen krijgen geen bodemmateriaal in hun hok, maar krijgen wel te allen tijde andere verrijkingsmaterialen. Dit verrijkingsmateriaal moet, om aan de ethologische behoeften tegemoet te komen, manipuleerbaar en kauwbaar zijn en interessant voor een langere periode. Voorbeelden zijn kettingen die over de grond gaan, touw, plastic speeltjes, enz. De gezondheid van de biggen zal dagelijks worden gecontroleerd door gecertificeerd en getraind deskundig personeel. Na het toedienen van de E. colibacterie en in het geval van het managementmodel zullen de biggen vaker gecontroleerd worden zodat kan worden ingegrepen voordat de gezondheid van de biggen het humaan eindpunt bereikt. Hiervoor zal ook de water- en voeropname dagelijks gecontroleerd worden tenminste voor de eerste 2 weken na spenen aangezien dit ook een maat is voor welzijn. Biggen die gewicht zijn verloren voordat de E. colibacterie toegediend wordt, worden uit de proef gehaald en overgebracht naar een standaardhok op dezelfde proeffaciliteit om te herstellen. Deze biggen krijgen dus geen E. colibacterie toegediend. Biggen in het E. coli model zullen geselecteerd worden op genotype en alleen de genotypen die hoog gevoelig zijn voor de specifieke E. coli zullen gebruikt worden. Dit zorgt voor verfijning aangezien de minder gevoelige dieren niet onnodig blootgesteld worden aan de bacterie. Standaardprocedures voor het verzamelen van mest en urine zullen gebruikt worden om variatie tussen proeven te verminderen. Voor het aanbrengen van stomazakjes rond de anus om mest te verzamelen moeten de biggen gehanteerd worden. Hiervoor zullen biggen vooraf worden gewend aan het hanteren door mensen.

Explain the choice of species and the related life stages
Compulsory! Maximum length is 2500 characters
Het speenproces bij het doeldier (varken) is uniek en kan moeilijk gesimuleerd of bestudeerd worden bij andere diersoorten. Voor de experimenten in dit project wordt de doelgroep van biggen tot 6 weken na spenen gebruikt omdat oudere varkens niet representatief zijn.

Tab Purpose of the project
Basic research: Gastrointestinal System including Liver [PB5]
Translational and applied research: Animal Nutrition [PT38]
Translational and applied research: Animal Welfare [PT34]
Choose a purpose
Choose a purpose

Tab Expected harms

What species and numbers of animals are expected to be used? What are the expected severities and the numbers of animals in each severity category (per species)?

Estimated numbers per severity				
Please fill in all categories for selected species with whole numbers (0 or higher)!				
Species	Non-recovery	Mild	Moderate	Severe
Pigs (Sus scrofa domesticus) [A14]	0	2187	317	5
Choose a species				
Choose a species				
Choose a species				
Choose a species				
Choose a species				
Choose a species				
Choose a species				
Choose a species				
Choose a species				
Choose a species				
Choose a species				
Choose a species				
Choose a species				

Tab Fate of animals kept alive

What will happen to the animals kept alive at the end of the procedure?

Estimated numbers per severity			
Please fill in all categories for selected species with whole numbers (0 or higher)!			
Species	Reused	Returned	Rehomed
Pigs (Sus scrofa domesticus) [A14]		1801	
Choose a species			
Choose a species			
Choose a species			
Choose a species			
Choose a species			
Choose a species			
Choose a species			
Choose a species			
Choose a species			
Choose a species			
Choose a species			
Choose a species			
Choose a species			

8

Van: 5.1 lid2h
Verzonden: woensdag 15 februari 2023 15:53
Aan: info@zbo-ccd.nl
Onderwerp: RE: Verzoek om advies over projectvergunningsaanvraag AVD5.1 lid2h202316684

Categorieën: DEC adviezen

Geachte CCD,

Het wachtwoord is 5.1 lid2h

Van: info@zbo-ccd.nl <info@zbo-ccd.nl>
Verzonden: vrijdag 6 januari 2023 14:11
Aan: 5.1 lid2h
Onderwerp: Verzoek om advies over projectvergunningsaanvraag AVD5.1 lid2h**202316684**

Geachte leden van 5.1 lid2h

De Centrale Commissie Dierproeven (hierna: CCD) verzoekt u in het kader van vergunningverlening advies te geven over het project met als titel: "Development of nutritional strategies to better meet piglet requirements after weaning, while increasing sustainability, performance, welfare, and health." en aanvraagnummer: AVD5.1 lid2h**202316684.**

Uw commissie wordt verzocht op grond van artikel 10.a.2 van de Wet op de dierproeven de aanvraag te beoordelen en een ethische toetsing uit te voeren waarbij wordt afgewogen of de doelstelling van het project, de verwachte voordelen voor mens, dier of milieu en de haalbaarheid van de doelstellingen, het gebruik van dieren en de schade die zal worden toegebracht aan de dieren in de vorm van lijden, pijn en angst kan rechtvaardigen.

Graag ontvangen wij van u bericht dat deze e-mail goed is ontvangen en wanneer u dit advies in de vergadering gaat bespreken.

Voor het in te dienen advies dient de DEC gebruik te maken van de meest actuele versie van het op de website van de CCD gepubliceerde Format DEC-advies en de toelichting daarbij. U dient deze aanvraag vertrouwelijk te behandelen. Voor de communicatie met de CCD dient u gebruik te maken van FileSecure.

De CCD verzoekt u uiterlijk binnen 20 werkdagen, na 06-01-2023, uw advies bij de CCD in te dienen. Indien de aanvraag door uw commissie niet in behandeling kan worden genomen, dient u dit per ommegaande per e-mail aan de CCD te melden.

Ingeval uw commissie tussentijds aanvullende informatie wil inwinnen bij de aanvrager wordt de termijn opgeschort en geeft u in uw advies aan wanneer dit is geweest. Opschorting van de adviestermijn vindt niet plaats ingeval u ten behoeve van uw advies een onafhankelijk extern expert raadpleegt. Mocht u verwachten door een andere reden dan opschorting uw advies later dan 20 werkdagen na 06-01-2023 bij de CCD in te dienen, dan verzoeken wij u dit direct aan de CCD te melden.

Mocht u vragen hebben, dan kunt u uiteraard contact met ons opnemen.

Met vriendelijke groet,
Centrale Commissie Dierproeven

www.centralecommissiedierproeven.nl

.....
Postbus 93118 | 2509 AC | Den Haag
.....
T: 0800 789 0789
E: info@zbo-ccd.nlDit bericht kan informatie bevatten die niet voor u is bestemd. Indien u niet de geadresseerde bent of dit bericht abusievelijk aan u is gezonden, wordt u verzocht dat aan de afzender te melden en het bericht te verwijderen.
De Staat aanvaardt geen aansprakelijkheid voor schade, van welke aard ook, die verband houdt met risico's verbonden aan het elektronisch verzenden van berichten.

This message may contain information that is not intended for you. If you are not the addressee or if this message was sent to you by mistake, you are requested to inform the sender and delete the message.
The State accepts no liability for damage of any kind resulting from the risks inherent in the electronic transmission of messages.

ADVIES PROJECTVOORSTEL DIERPROEVEN

5.1 lid2h 9

A. Algemene gegevens over de procedure

1. Aanvraagnummer : AVD[redacted]202316684
2. Titel van het project : Development of nutritional strategies to better meet piglet requirements after weaning, while increasing sustainability, performance, welfare, and health.
3. Titel van de NTS : Ontwikkelen van voerstrategieën die beter aansluiten bij de nutriëntbehoeftes van biggen na spenen en tevens duurzaamheid, groeiprestaties, welzijn en gezondheid bevorderen

4. Type aanvraag:
   - ☒ nieuwe aanvraag projectvergunning
   - ☐ wijziging van vergunning met nummer :

5. Contactgegevens DEC

Naam DEC	: [redacted]
Telefoonnummer contactpersoon	: [redacted]
Emailadres contactpersoon	: [redacted]

6. Adviestraject (data dd-mm-jjjj):
   - ☒ ontvangen door DEC: 6-1-2023
   - ☐ aanvraag compleet:
   - ☒ in vergadering besproken: 18-1-2023
   - ☐ anderszins behandeld:
   - ☒ termijnonderbreking(en) van / tot : 23-1-2023/ 24-1-2023
   - ☐ besluit van CCD tot verlenging van de totale adviestermijn met max. 15 werkdagen:
   - ☐ aanpassing aanvraag:
   - ☒ advies aan CCD: 15-2-2023

7. De aanvraag is afgestemd met de IvD en deze is hiermee akkoord.

8. Eventueel horen van aanvrager
   - Datum: 18-1-2023
   - Plaats: via Teams
   - Aantal aanwezige DEC-leden: 8
   - Aanwezige (namens) aanvrager: verantwoordelijk onderzoeker en IvD lid
   - Gestelde vragen en verstrekte antwoorden: De DEC heeft de onderzoekers o.a. gehoord over of het klopt dat in bijlage 2 de metingen (in de nieuwe stal) eenmalig gevalideerd worden om de resultaten van de 1e studie te verifiëren en om de vervolgstudies te kunnen uitvoeren. Leest de DEC het goed dat, als het 1e experiment laat zien dat als de resultaten van dat experiment overeenkomen met de data van de oude stal, de validatie daarna niet meer hoeft? En dat de vraag voor experiment 2 te rechtvaardigen is binnen de context van deze

onderzoeksvraag? Zou je die vraag onafhankelijk van welke stal sowieso willen stellen? Is het experiment bedoeld om voor de eerste keer deze stal te valideren, zodat het later niet meer hoeft? De onderzoeker bevestigt deze vragen.
Er is dus wel eenmalig extra diergebruik voor het valideren van de stal? Ja, ook dat klopt.
In bijlage 3 F staat, dat 3% van de dieren sterft als gevolg van E. Coli infectie, terwijl bij E was uitgelegd, dat geen HEP toegepast hoeft te worden. De onderzoeker zal dit met elkaar in overeenstemming brengen.

- Hieruit zijn onderstaande vragen, zoals vermeld bij punt A9, voortgekomen, die schriftelijk aan de onderzoekers werden voorgelegd.
- Het horen van de aanvrager heeft geleid tot aanpassing van de aanvraag.

9. Correspondentie met de aanvrager
- Datum vragen: 23-1-2023
- Datum antwoord: 25-2-2023
- Gestelde vragen en antwoorden:

Projectvoorstel

Algemeen: wilt u de tekst nog een keer goed doorkijken en nummering 1-9 i.p.v. 1-8 gebruiken en 11 en 12 niet door elkaar halen?
De tekst in het projectvoorstel bij 3.4.1 is aangepast. Er is een zin toegevoegd aan 3.4.1 om duidelijk te maken dat experiment 11 (nitrogen balance under suboptimal sanitary conditions) alleen plaats kan vinden als experiment 12 (development of management model) een goed werkend model oplevert. In bijlage 2 stond experiment 11 vermeld maar dit is veranderd in experiment 12 om gelijk te zijn aan de nummering in het projectvoorstel.

Bijlage 2
- A. Experimentele aanpak en primaire uitkomstparameters:
- Klopt het dat de metingen in de nieuwe stal eenmalig gevalideerd worden om de resultaten van de 1e studie te verifiëren en om de vervolgstudies te kunnen uitvoeren?
  Dat klopt en een zin is toegevoegd om dit te verduidelijken in bijlage 2.
- Lezen wij het goed dat, als het 1e experiment laat zien dat als de resultaten van dat experiment overeenkomen met de data van de oude stal, de validatie daarna niet meer hoeft?
- *In het eerste experiment, welke vorig jaar is uitgevoerd en geen onderdeel is van de huidige aanvraag, hebben wij op kleine schaal (10 hokken per parameter) en zonder verschillende voeders een aantal parameters getest. We hebben nu aanwijzingen dat het verlagen van de staltemperatuur (2 graden) en het aanbrengen van biggenmest (van de eigenfaciliteit) mogelijk een goed werkend model zullen opleveren. Dit zal echter nog wel bij een groter aantal hokken (20 hokken per experimentele behandeling) en met verschillend voer (2 voeder zoals ook aangegeven in bijlage 2) gevalideerd moeten worden. Dit is experiment 12 en deze zal daarom sowieso gedaan moeten worden voordat er verder gegaan kan worden met experimenten 13-17 en 11 (in bijlage 4). De tekst in bijlage 2 is aangepast.*

5.1 lid2h [redacted]

- Is de vraag voor experiment 2 te rechtvaardigen binnen de context van deze onderzoeksvraag? Zou je die vraag onafhankelijk van welke stal sowieso willen stellen?
  Deze vraag stellen we alleen specifiek voor deze stal en is nodig om de andere vragen in deze projectaanvraag te beantwoorden.
- Is het experiment bedoeld om voor de eerste keer deze stal te valideren, zodat het later niet meer hoeft?
  Dat klopt. Dit experiment is bedoeld om uiteindelijk de procedure van het management model op te schrijven in een standard operating procedure en deze vervolgens in vervolgexperimenten te gebruiken.
- Is er dus wel eenmalig extra diergebruik voor het valideren van de stal?
  Dat klopt.
- F. Mist u bij 'cumulatief ongerief' soms het woordje 'overall' expected?
  Het woord 'overall' is toegevoegd om te benadrukken dat het om cumulatief ongerief gaat.

Bijlage 3

- E. Humane eindpunten &
- F. Classificatie van ongerief: Hier staat dat 3% van de dieren sterft als gevolg van E. Coli infectie, terwijl bij E was uitgelegd, dat geen HEP toegepast hoeft te worden. Kunt u dat met elkaar in overeenstemming brengen?
  De Humane eindpunten en Classificatie van ongerief zijn aangepast zodat deze in overeenstemming met elkaar zijn. Daarnaast is ook de NTS op het punt Verwacht ongerief aangepast.

- De antwoorden hebben geleid tot aanpassing van de aanvraag.

10. Eventuele adviezen door experts (niet lid van de DEC); n.v.t.

B. Beoordeling (adviesvraag en behandeling)

1. Het project is vergunningplichtig (dierproeven in de zin der wet).
2. De aanvraag betreft een nieuwe aanvraag.
3. De DEC is competent om hierover te adviseren.
4. Er zijn geen DEC-leden betrokken bij het betreffende project.

C. Beoordeling (inhoud):

1. De aanvraag is toetsbaar en heeft voldoende samenhang. De aanvraag beschrijft het onderzoek naar verbeterde voedingsstrategieën rond de speenperiode voor biggen. Het speenproces is een stressvolle periode in het leven van biggen. De daarbij horende veranderingen in omgevingsbacteriën, de verandering van zeugenmelk naar minder goed verteerbaar vast voer en de andere samenstelling van tomen spelen een grote rol en kunnen gepaard gaan met een verminderde voeropname net na spenen en verminderde functie van de darmwand. Hierdoor hebben ziekteverwekkende bacteriën kans om zich te vestigen in de darm wat vervolgens speendiarree veroorzaakt. Onderzoekers beschrijven een stapsgewijs onderzoek naar de werking

[redacted]

en ontwikkeling van de maag. Dit heeft tot nu toe weinig aandacht gekregen in de wetenschap, maar speelt wel een belangrijke rol bij het bevorderen van de gezondheid van de big, meer specifiek het maagdarmstelsel. Alleen de voerstrategieën die geen negatieve effecten laten zien onder gecontroleerde proefomstandigheden op groeiprestatie, duurzaamheid, dierwelzijn en darmgezondheid zullen verder getoetst worden onder suboptimale varkenshouderij omstandigheden. Hiermee wordt bedoeld, dat praktijkomstandigheden worden nagebootst op het proefbedrijf door middel van bijvoorbeeld het aanpassen van de hygiënestatus tijdens de proeven. Door te kijken naar de nutriëntensamenstelling van het voer in plaats van de grondstoffen zelf, kan ook gewerkt worden naar alternatieve voersamenstellingen waarin duurzamere/circulaire en eventueel goedkopere grondstoffen verwerkt kunnen worden zonder negatieve effecten voor de gezondheid en groeiprestaties van het dier. De DEC heeft nog aanvullende vragen gesteld, die naar tevredenheid zijn beantwoord.
De aanvraag komt het meest overeen met voorbeeld 1 uit de nieuwe Handreiking "Invulling Definitie Project".

2. Voor zover de DEC bekend, is er geen mogelijk tegenstrijdige wetgeving die het uitvoeren van de dierexperimenten in de weg zou kunnen staan.

3. De in de aanvraag aangekruiste doelcategorie(ën), te weten fundamenteel onderzoek en translationeel onderzoek, sluiten aan bij de hoofddoelstellingen.

Belangen en waarden

4. Het directe doel van het project is om voedingsstrategieën te ontwikkelen, die beter aan de eisen voldoen van biggen voor, rond en na het spenen in optimaal en suboptimale (inclusief pathogene druk) omstandigheden. Het uiteindelijke doel van het project is een bijdrage leveren aan de verduurzaming van de varkenshouderij door antibioticagebruik en sterfte van jonge biggen te verminderen. Daarnaast worden alternatieve voersamenstellingen onderzocht waarin duurzamere/ circulaire en eventueel goedkopere grondstoffen verwerkt kunnen worden zonder negatieve effecten voor de gezondheid en groeiprestaties van het dier. Ook kan bij een aangepaste voederstrategie dierenwelzijn worden verbeterd door het verminderen van afwijkend gedrag (staart, bijten, buikknikken).
De DEC is van mening dat er een duidelijke relatie is tussen het directe en het uiteindelijke doel, en dat het doel gerechtvaardigd is in de context van onderzoekers, instelling/ voederfabrikanten en de behoeften vanuit varkenshouderij.

5. De belangrijkste belanghebbenden in dit onderzoeksproject zijn weergegeven in onderstaande tabel:

Belanghebbende	**Morele waarde die wordt bevorderd**
Individuele veehouders	Verbetering problemen rond spenen, minder antibiotica, minder sterfte

5.1 lid2h

Doeldieren / biggen rond speenleeftijd	Verbeterd voer geeft betere vertering, minder darmproblemen, minder gevoelig voor E. coli infecties, minder agressief gedrag
Wetenschappers	Levert nieuwe inzichten in hoe het maagdarmstelsel rond de speenleeftijd van biggen ontwikkelt en hoe voeder daar een positieve impact op kan hebben, publicaties
Maatschappij	Minder antibiotica, minder kans op resistenties.
Voederproducent	Kan beter aangepaste voeders produceren met meer omzet / winst
	Morele waarde die in het geding is
Proefdieren	Worden blootgesteld aan voor hen niet noodzakelijke handelingen die ongerief kunnen veroorzaken

6. De aanvrager geeft niet aan nadelige effecten op het milieu te verwachten. De DEC ziet geen aanleiding om aan te nemen dat zich toch nadelige effecten zullen voordoen.

Proefopzet en haalbaarheid

7. De kennis en kunde van de onderzoeksgroep en andere betrokkenen bij de dierproeven zijn voldoende gewaarborgd en dragen eraan bij dat de doelstellingen behaald kunnen worden, dat aan de 3V-beginselen voldaan kan worden en dat voorkomen kan worden dat mens, dier en milieu negatieve effecten ondervinden als gevolg van de dierproeven. De instelling is een internationaal diervoedingsbedrijf dat voedingsadditieven en voedingsstrategieën ontwikkelt voor de meeste diersoorten. Het bedrijf heeft de ambitie om een wereldleider te zijn op dit gebied en focussed duurzame veehouderij, zoals het verminderen van antibioticagebruik, het verbeteren van de gezondheid en het welzijn van dieren en het verminderen van de milieubelasting van de veehouderij door bijvoorbeeld het verminderen van nutriëntenverliezen in urine en uitwerpselen en het verminderen van emissies in verband met de productie. Het bedrijf wil kennis vergaren over de afgifte van voedingsstoffen in de darm en een betere vertering en gebruik van grondstoffen met een lagere beschikbaarheid/ verteerbaarheid van voedingsstoffen. Het bedrijf heeft een speciale R&D-afdeling met onderzoeksteams voor alle soorten in omvang, inclusief varkens. Elk soortenteam heeft ongeveer 6 onderzoekswetenschappers die een doctoraat hebben met betrekking tot dier- of diergeneeskunde en ze onderhouden een breed internationaal wetenschappelijk netwerk. De studies zullen worden uitgevoerd in het eigen onderzoekscentrum in Nederland, waar is geïnvesteerd in een state-of-the-art unit en waar proeven kunnen worden uitgevoerd die specifieke omgevingscondities nabootsen en beheersen. Hoge bioveiligheids- en hygiënenormen worden gehandhaafd om personeel en dieren te beschermen. Dagelijkse verzorging van dieren, metingen en experimentele technieken worden uitgevoerd door gecertificeerd competent en ervaren personeel. Projectresultaten zullen worden verspreid via

wetenschappelijke tijdschriften en conferenties en uiteindelijk worden vertaald in voedingsstrategieën, die worden toegepast via het wereldwijde netwerk van voedingsdeskundigen.

8. Het project is goed opgezet, de voorgestelde experimentele opzet en uitkomstparameters sluiten logisch en helder aan bij de aangegeven doelstellingen. De gekozen strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project. De verschillende onderzoeken worden na elkaar uitgevoerd op basis van beslismomenten en zijn gedetailleerd beschreven in de verschillende bijlagen. Omdat gekozen is de dieren voortaan op eigen locatie te huisvesten in plaats van gebruik te maken van bestaande varkenshouderijen, heeft men een validatiestudie opgenomen die goed is toegelicht.

Welzijn dieren

9. Er is geen sprake van de volgende bijzonderheden op het gebied van categorieën van dieren, omstandigheden of behandeling van de dieren:
   - ☐ Bedreigde diersoort(en) (10e lid 4)
   - ☐ Niet-menselijke primaten (10e)
   - ☐ Dieren in/uit het wild (10f)
   - ☐ Niet gefokt voor dierproeven (11, bijlage I EU richtlijn)
   - ☐ Zwerfdieren (10h)
   - ☐ Hergebruik (1e lid 2)
   - ☐ Locatie: buiten instelling vergunninghouder (10g)
   - ☐ Geen toepassing verdoving/pijnbestrijding (13)
   - ☐ Dodingsmethode niet volgens bijlage IV EU richtlijn (13c lid 3)

10. De dieren worden niet gehuisvest en verzorgd op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van de EU richtlijn. Tijdens de fase voorafgaand aan het spenen zijn de biggen samen met de zeug ondergebracht in conventionele kraamhokken. De biggen worden gehuisvest zonder strooiselmateriaal. Er zal wel niet-eetbare kooiverrijking zijn om de dieren in staat te stellen te spelen en normaal gedrag te vertonen. De kooiverrijking voldoet aan de eisen die de NVWA stelt. De kooien hebben een zachte tenderfoot bodem.

11. Het cumulatieve ongerief als gevolg van de dierproeven is realistisch ingeschat en geclassificeerd. Per experiment zijn de handelingen beschreven die uitgevoerd gaan worden met het daarbij behorende ongerief. Het cumulatieve ongerief is weergegeven als het totaal van alle uitgevoerde handelingen en de DEC heeft geen reden dit cumulatieve ongerief per experiment anders in te schatten.

12. De integriteit van de dieren wordt fysiek aangetast door o.a. wegen, het afnemen van swabs en bloedmonsters en blootstellen aan E. coli met een infectie en diarree tot gevolg.

5.1 lid2h [redacted]

13. De humane eindpunten zijn in de bijlage dierproeven goed gedefinieerd en het percentage dieren dat naar verwachting een humaan eindpunt bereikt is goed ingeschat. Per bijlage is beschreven of voor die bijlage een HEP verwacht kan worden of niet en hoe die HEP per experiment wordt vastgesteld. Het percentage is gebaseerd op ervaringen uit eerdere experimenten.

3V's

14. De aanvrager heeft voldoende aannemelijk gemaakt dat er geen geschikte vervangingsalternatieven zijn. Verteringsprocessen in biggen kunnen deels *in vitro* nagebootst worden. Het *in vitro* verteringsmodel zal worden toegepast waar mogelijk om grondstoffen en voeders te bestuderen voordat deze worden toegepast in dierexperimenten. Echter, interacties tussen voeders en het maagdarmkanaal, bv vertering, absorptie, nutriëntenpassage door de darm en aanzuring in de maag, kunnen niet bestudeerd worden zonder gebruikt te maken van doeldieren. Met name de fysiologie van de gespeende big is complex en moeilijk na te bootsen buiten het dier. Verteringsgegevens van biggen zijn ook nodig om het *in vitro* verteringsmodel te valideren, zodat dit verbeterd wordt voor toekomstig gebruik. Mest- en urinemonsters kunnen genomen worden zonder ongerief voor het dier. Echter deze zijn niet representatief voor wat er in de verschillende segmenten van het maagdarmkanaal, zoals de maag en de dunne darm, plaatsvindt. Het toedienen van E. coli en het managementmodel zouden op commerciële bedrijven getest kunnen worden, maar daar zijn de houderij-omstandigheden variabel en tamelijk onvoorspelbaarder waardoor een hoger dieraantal nodig is vanwege een grotere variatie in de meetresultaten. Lichaamsgroei als gevolg van een voerstrategie is moeilijk middels computermodellen te simuleren, omdat deze afhankelijk is van verschillende factoren vooral bij gespeende biggen gehouden onder verschillende huisvestingsomstandigheden.

15. Het aantal te gebruiken dieren is realistisch ingeschat en er is een heldere strategie om ervoor te zorgen dat tijdens het project met het kleinst mogelijke aantal dieren wordt gewerkt waarmee nog een betrouwbaar resultaat kan worden verkregen. Het aantal dieren, dat gebruikt wordt per experiment, wordt geminimaliseerd zoals is bepaald door middel van statistische powerberekeningen. De variatie gebruikt voor deze berekeningen is gebaseerd op eerdere soortgelijke proeven in dezelfde proeffaciliteit. Voor elk experiment zal een nieuwe power berekening gedaan worden op basis van de meest recente resultaten. Voor elke voerstrategie zal voorafgaand aan een experiment eerst de al gedane studies en literatuur bestudeerd worden en eventueel *in vitro* onderzoek gedaan worden om zo de meest veelbelovende strategieën te kiezen. Go/no go beslissingen worden op basis van vastgestelde criteria (dat wil zeggen groeiprestatie, dierwelzijn en gezondheid) gemaakt voordat vervolgstudies worden opgezet. Het management model zal eerst gevalideerd worden voordat vervolgstudies ingezet worden. Biggen in het E. coli model zullen geselecteerd worden op genotype en alleen de genotypen die hoog gevoelig zijn voor de specifieke E. coli zullen gebruikt worden. Dit vermindert de variatie in de resultaten waardoor uiteindelijk minder biggen nodig zijn.

5.1 lid2h

16. Het project is in overeenstemming met de vereiste van verfijning van dierproeven en het project is zodanig opgezet dat de dierproeven zo humaan mogelijk kunnen worden uitgevoerd. Biggen zijn de doeldieren en hun fysiologie kan niet vergeleken worden met die van andere diersoorten of bestudeerd worden met behulp van computermodellen. Dieren worden waar mogelijk in groepen gehuisvest. Als individuele huisvesting noodzakelijk is, zal de periode zo kort mogelijk worden gehouden. Neus-neus contact met naburige biggen is altijd mogelijk. Biggen worden altijd verdoofd voor het doden. Biggen krijgen geen bodemmateriaal in hun hok, maar krijgen wel te allen tijde andere verrijkingsmaterialen. Dit verrijkingsmateriaal moet, om aan de ethologische behoeften tegemoet te komen, manipuleerbaar en kauwbaar zijn en interessant voor een langere periode. De gezondheid van de biggen zal dagelijks worden gecontroleerd door gecertificeerd en getraind deskundig personeel. Na het toedienen van de E. colibacterie en in het geval van het managementmodel zullen de biggen vaker gecontroleerd worden, zodat kan worden ingegrepen voordat de gezondheid van de biggen het humaan eindpunt bereikt. Hiervoor zal ook de water- en voeropname dagelijks gecontroleerd worden tenminste voor de eerste 2 weken na spenen, aangezien dit ook een maat is voor welzijn. Biggen die gewicht zijn verloren voordat de E. colibacterie toegediend wordt, worden uit de proef gehaald en overgebracht naar een standaardhok op dezelfde proeffaciliteit om te herstellen. Deze biggen krijgen dus geen E. colibacterie toegediend. Biggen in het E. coli model zullen geselecteerd worden op genotype en alleen de genotypen die hoog gevoelig zijn voor de specifieke E. coli zullen gebruikt worden. Dit zorgt voor verfijning aangezien de minder gevoelige dieren niet onnodig blootgesteld worden aan de bacterie. Standaardprocedures voor het verzamelen van mest en urine zullen gebruikt worden om variatie tussen proeven te verminderen. Voor het aanbrengen van stomazakjes rond de anus om mest te verzamelen moeten de biggen gehanteerd worden. Hiervoor zullen biggen vooraf worden gewogen.

17. Er is geen sprake van wettelijk vereist onderzoek.

Dieren in voorraad gedood en bestemming dieren na afloop proef

18. Dieren (biggen) van beide geslachten zullen in gelijke mate worden ingezet.

19. De dieren worden niet gedood in het kader van het project.

20. Hergebruik voor andere experimenten is niet overwogen omdat de biggen na afloop naar varkenshouderijen gaan: herplaatsing.

NTS

21. De niet-technische samenvatting is een evenwichtige weergave van het project en begrijpelijk geformuleerd.

5.1 lid2h [redacted]

D. Ethische afweging

1. De morele vraag die de DEC dient te beantwoorden is of het belang van dit onderzoek, namelijk om voedingsstrategieën te ontwikkelen die beter aan de eisen voldoen van biggen voor, rond en na het spenen in optimaal en suboptimale (inclusief pathogene druk) omstandigheden met als uiteindelijke doel een bijdrage te leveren aan de verduurzaming van de varkenshouderij door antibioticagebruik en sterfte van jonge biggen te verminderen, de onvermijdelijke aantasting van het welzijn en de integriteit van de gebruikte proefdieren kan rechtvaardigen.

2. Er vindt een beperkte aantasting van welzijn en integriteit van de 2187 proefdieren plaats, met mild ongerief, voor 322 dieren kan maximaal matig ongerief optreden.
   Indien de hierboven genoemde doelstellingen behaald worden, dan zal dit project er toe bijdragen, dat meer kennis wordt verkregen over de ontwikkeling van maag/darmstelsel van jonge biggen en de invloed van voeder/nutriënten rondom de speenleeftijd. Ook zal met verbeterde voedingsstrategieën een duurzamer houderijsysteem kunnen worden ontwikkeld. Het is aannemelijk dat de fundamentele en translationele doelstelling behaald zal worden. Daarvoor is de inzet van proefdieren noodzakelijk, maar de onderzoekers doen al het mogelijke om het ongerief voor de dieren en het aantal dieren tot een minimum te beperken. Dat het voor de instelling van belang kan zijn om daarmee commerciële voeders te ontwikkelen is juist, maar in de uiteindelijke afweging kent de DEC daar geen gewicht aan toe. Omdat de aanvrager heeft besloten eigen stallen te ontwikkelen om het onderzoek 'in huis' te kunnen uitvoeren, is een eenmalige validatie studie noodzakelijk. De DEC heeft dit meegenomen in haar afweging.

3. Op grond van het bovenstaande is de DEC van oordeel dat het doel om voedingsstrategieën te ontwikkelen die beter aan de eisen voldoen van biggen voor, rond en na het spenen in optimaal en suboptimale (inclusief pathogene druk) omstandigheden met als uiteindelijke doel een bijdrage te leveren aan de verduurzaming van de varkenshouderij door antibioticagebruik en sterfte van jonge biggen te verminderen een reëel belang vertegenwoordigt en dat dit reële belang opweegt tegen de beperkte aantasting van het welzijn en de integriteit van de proefdieren. De relatie tussen het directe en het uiteindelijk doel is voldoende helder. Het is aannemelijk dat de directe doelstelling behaald zal worden. De commissie is overtuigd van de kwaliteit van het werk van de aanvrager. De aanvrager heeft voldoende aannemelijk gemaakt, dat er geen geschikte vervangingsalternatieven zijn, dat het doel niet met minder dieren behaald kan worden, dat de gebruikte aanpak de meest verfijnde is en dat er geen sprake zal zijn van onbedoelde negatieve effecten voor mens, dier en milieu als gevolg van de dierproeven. Het gebruik van de proefdieren zoals beschreven in de aanvraag is daarmee gerechtvaardigd.

E. Advies

1. Advies aan de CCD

☒ De DEC adviseert de vergunning te verlenen.

☐ De DEC adviseert de vergunning te verlenen onder de volgende voorwaarden.

5.1 lid2h [redacted]

☐ Op grond van het wettelijk vereiste dient de projectleider bij beëindiging van het project een beoordeling achteraf aan te leveren die is afgestemd met de IvD.
☐ Voor de uitvoering van dit project is tevens ministeriële ontheffing vereist
☐ Overige door de DEC aan de uitvoering verbonden voorwaarden, te weten...

☐ De DEC adviseert de vergunning niet te verlenen vanwege:
☐ De vaststelling dat het project niet vergunningplichtig is om de volgende redenen:...
☐ De volgende doorslaggevende ethische bezwaren:...
☐ De volgende tekortkomingen in de aanvraag:...

2. Het uitgebrachte advies is gebaseerd op consensus.

3. Er zijn geen knelpunten/dilemma's naar voren gekomen tijdens het beoordelen van de aanvraag en het opstellen van het advies.

5.1 lid2h [redacted]

Centrale Commissie Dierproeven

Form
Project proposal

- This form should be used to write the project proposal for animal procedures.
- The appendix 'description animal procedures' is an appendix to this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed.
- For more information on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 lid2h
1.2	Provide the name of the licenced establishment.	5.1 lid2h
1.3	Provide the title of the project.	Development of nutritional strategies to better meet piglet requirements after weaning, while increasing sustainability, performance, welfare, and health.

2 Categories

2.1	Please tick each of the following boxes that applies to your project.	**X** Basic research
		X Translational or applied research
		☐ Regulatory use or routine production
		☐ Research into environmental protection in the interest of human or animal
		☐ Research aimed at preserving the species subjected to procedures
		☐ Higher education or training
		☐ Forensic enquiries
		☐ Maintenance of colonies of genetically altered animals not used in other animal procedures

3 General description of the project

3.1 Background

Describe the project (motivation, background and context) with respect to the categories selected in 2.1.

The weaning process, in general, is the most stressful period in the life of a pig. It is accompanied by removal from the sow, mixing of piglets, changing environment with accompanying microbiological changes, and change in feed from highly digestible sow milk to a solid feed diet containing typically less digestible raw materials. Altogether, this results in a drop in feed intake which often results in gut wall damage such as atrophy of the villi and increases in permeability (Pluske, et al., 1997). This renders the piglets vulnerable to digestive pathogens like pathogenic *E. coli*, responsible for post-weaning diarrhoea (PWD). Although abrupt and early weaning are known to exacerbate the above-mentioned processes (Buchet et al., 2017), piglets in all husbandry systems will experience some level of stress around weaning, especially considering the sub-optimal hygiene conditions often occurring on farms. This stress is the main factor for health problems in the nursery and this, in turn, is responsible for a large part of the antibiotic usage in swine production. In this proposal we aim to develop nutritional solutions that will support the piglet before, during and after the weaning process by providing nutrients that better match its requirements than the currently available diets.

The current requirements for weaned piglets are largely based on those determined in older pigs (>35 kg body weight) while requirements for suckling piglets are lacking or largely based on nutrient levels in sow milk. The proposed nutritional solutions spinning off from this project should increase piglet health and welfare making them more robust to face the challenges around weaning. Moreover, they should increase sustainability of pig production by reducing mortality and the use of antibiotics, by using sustainable raw material sources, or by including strategies to optimize the use of raw materials sources (i.e., using enzymes or feed additives). It is highly important to start with nutritional interventions before weaning, for example, by providing piglets with supplemental milk or (solid) creep feed in order to adapt them in early life to raw materials present in diets after weaning (Huting et al., 2021). The intake of creep feed before weaning has been found to stimulate feed intake early after weaning (Bruininx et al., 2002), alter microbial populations towards a profile typically seen after weaning, increase microbial fermentation products (short chain fatty acids) exerting beneficial effects in the gastrointestinal tract, modulate intestinal development (length and weight of the gastrointestinal tract, increased absorptive area as indicated by an increased villus height to crypt depth ratio) at the time of weaning with subsequently less PWD (Choudhury et al., 2021a; Choudhury et al., 2021b). These studies indicate that there is a window of opportunity to prepare the piglet for the weaning process by nutritional solutions provided prior to weaning. Moreover, in the wild, piglets start to eat non-milk items from the first week of life onwards (Van Hees et al., 2022) indicating that their natural instinct is to search for food items other than sow milk already in early life. Thus, starting with nutritional solutions before weaning fits with the piglet's natural behaviour. In this proposal, we aim to focus on the following dietary components present in diets before and after weaning: energy, protein, fibres, minerals, and vitamins. Our studies are performed under standard practical conditions but in a research facility.

Energy

Energy is typically provided to piglets in the form of starch and fat sources. Piglets before weaning mainly get their energy from milk fat, while after weaning the main energy source is starch from cereals. The fat content in sow's milk is around ~34% on dry matter basis, as compared to ~7% in a typical nursery diet (Jensen et al., 1997). Thus, during the weaning process, the piglet needs to change from fat digestion to starch digestion while at the same time the production of all pancreatic enzymes (i.e., lipase, amylase, trypsin and chymotrypsin) is hampered (Jensen et al., 1997). Thus, the digestive capacity of starch and fat is limited in the week(s) immediately after weaning. We have performed a study with different energy levels (2200 – 2700 kCal/kg) of the feed either coming from starch or from fat. The diets were fed for 2.5 weeks from day 14 after weaning (~9 kg body weight) onwards. We found that piglets were able to maintain similar energy intake by adapting their feed intake. A higher energy level from starch was beneficial in terms of fat and protein digestibility. Our findings were in contrast to Kim et al. (2021) who found that pigs with a body weight below 20 kg were not able to adjust feed intake to diets differing in energy level. Thus, there is a lack of understanding on the relation between feed intake and energy intake of young piglets (~24 to 38 days of age; ~5 kg body weight), but also on how piglets respond to different sources of energy (i.e., fat, starch or potentially protein or lactose).

Piglet diets are typically formulated to provide an optimum ratio between amino acids and energy. This optimum has been determined in older pigs (~40 kg body weight) and extrapolated to piglets around

weaning (~4-10 kg body weight). However, with potential changes in energy level, we also need to re-evaluate the ratio between amino acids and energy, particularly in young piglets (~7kg).

Protein level and amino acid ratio's

Studies to determine the optimal protein level and ratio between essential and non-essential amino acids with the main aim to reduce nitrogen excretion in faeces and urine are typically done using older pigs and results are extrapolated to piglets around weaning. However, the gastrointestinal physiology of the weaned piglet is known to be different and, therefore, estimations of optimal protein level and amino acid ratios might be incorrect. Moreover, the optimal amino acid ratios might depend on the level of stress and presence of pathogens. Piglets with a high weaning stress (no feed before weaning and restricted feeding for 24 h after weaning) had a higher protein breakdown and higher utilization of amino acids by the gastrointestinal tract as compared to piglets with low weaning stress (Resink et al., 2022). Consequently, arginine and glutamine were found to be most limiting in this phase early after weaning as opposed to lysine in older pigs. An increased threonine supplementation was found to improve gut health when piglets were challenged with *E. coli* on day 7 after weaning (Trevisi et al., 2015). The interaction between fibres and kinetics of digestion of proteins (i.e., level of fermentable protein) can also influence requirements of amino acids such as threonine because of its role in mucus formation and antioxidant capacity (Wellington et al., 2020).

One of the strategies to combat PWD is through reducing dietary crude protein level (Heo et al., 2009) while supplementing the diet with synthetic essential amino acids. In a non-health challenge situation, we have studied the effect of supplementing several amino acids (histidine, valine, threonine, isoleucine, leucine, tryptophan, and methionine) to a low protein diet on growth of piglets after weaning. We found that growth was lower on the low protein diet compared to a high protein control diet and that none of the studied amino acids was able to sustain growth. This suggested that none of these amino acids were limiting in the diet contradicting our hypothesis. In low protein diets supplemented with synthetic amino acids, the essential:non-essential amino acid (EAA:NEAA) ratio is skewed towards the EAA potentially resulting in a lack of NEAA. Consequently, we hypothesize that the ratio between EAA:NEAA is more important in this phase of a piglet's life, especially in low protein diets. The NEAA can be synthesized by the body from EAA, but this process is not as efficient as supplying the right amount of NEAA through the diet. Thus, there is a general lack of understanding on protein and amino acids requirements in the youngest pigs and how they are modulated by weaning stress. A better understanding is expected to improve the use of protein sources and reduce PWD and nitrogen losses in faeces and urine.

Gastrointestinal development: dietary acidification and fibres

Before weaning, piglets rely on lactic acid bacteria to control stomach pH as hydrochloric acid production (HCl) in the stomach is still low (Cranwell et al.,1976). A piglet's maximum capacity to produce HCl is around 10 weeks of age. Thus, at the time of weaning, the piglet has limited capability to reduce the pH in the stomach because HCl production is not in place and lactic acid produced from lactose is decreasing because of the removal of sow milk. The pH in the stomach remained above 4.5 during the first hours after a meal in piglets at 2 weeks after weaning (weaned at ~24 days of age; in-house study) while a pH of 3 is considered optimal for stomach enzymes (Heo et al., 2013). A low stomach pH is important (1) to reduce the survival of pathogens and, thereby, also the flow of pathogens into other parts of the gastrointestinal tract, (2) activation of protein digestive enzymes (i.e., pepsinogen to pepsin) resulting in better gastric protein predigestion with less protein flowing into the large intestine providing nutrients to pathogens, and (3) releasing pancreatic enzymes for the digestion of e.g., starch and fat (Heo et al., 2013). The latter occurs when digesta with a low pH enters the duodenum. Stomach retention time and acidification are known to be influenced by soluble fibres (in-house study) and particle size of raw materials (Kiarie and Mills, 2019). Recently, we have developed a laboratory method to determine the buffering capacity of raw materials. Certain feed ingredients (e.g., calcium salts) have high buffering capacity, i.e., need large amounts of HCl to reduce the pH, while others (e.g., organic acids) have low buffering capacity. We aim to steer towards a diet formulation that helps piglets regulate the stomach pH in order to enhance gastric barrier function and increase digestibility of nutrients. In an in-house study, we determined the effect of buffering capacity on growth performance in order to determine recommendations for feed formulation. We found that the buffering capacity in typical diets is able to support piglet growth. Although the diets were similar in amino acids to energy ratio, we did observe

differences in the feed efficiency (i.e., amount of feed needed for growth). Thus, we hypothesize that dietary buffering capacity can impact the piglet's physiology via changes in protein digestion in the stomach and an imbalance in the presence of amino acids and energy after absorption. This study was done under optimal environmental conditions (i.e., low disease pressure, low animal density), and it can be hypothesized that the optimal dietary buffering capacity differs in suboptimal (i.e., low sanitary conditions or high pathogenic load) conditions. Thus, there is a need to better understand the role of the stomach to optimize health of the gastrointestinal tract and reduce PWD.
The extent and rate (i.e., kinetics) of protein, starch and fat digestion can be affected by stomach functioning. There is, thus, a complicated interaction between stomach functioning and nutrient availability with effects on nutrient utilization and subsequent nutrient losses in faeces and urine. In this project, we aim to unravel this interaction, especially in the period after weaning.

Translation of results from experimental conditions to field farm conditions
Studying the effect of nutritional strategies on PWD on commercial farms is difficult because of variability in the incidence of PWD and uncontrolled pathogenic pressure. Moreover, different husbandry systems impose a variety of environmental challenges for piglets leading to a subclinical disease state. These conditions include level of ventilation, temperature control, humidity, presence of dust, faeces, etc. and with that the related pathogen load (airborne or soil). The variability in responses would increase the number of animals needed to show the efficacy of a nutritional strategy when experiments are conducted at field farms. Our research facility is well maintained, and the climate and sanitary conditions are controlled and do not represent the average commercial farm. For this reason, we recently designed and operate a separate unit at our research facility. This unit contains four fully isolated rooms having their own climate control system (state-of-the-art ventilation and temperature installations). In this way, we can perform studies where we can mimic commercial farm conditions. Its design allows challenge studies with specific pathogens in one room without running the risk of spreading the infection to other rooms and to the rest of our facility. An *E. coli* challenge model has recently been validated and used to test different nutritional strategies. It induces an increase in diarrhoea immediately after weaning. We have multiple years of experience with this model and the procedure is documented as standard operating procedure in our company. During this procedure, piglets are challenged with pathogenic *E. coli* (O149:F4ac) according to a method described in Roubos-van den Hil et al. (2017). Piglets are tested for susceptibility or resistance towards ETEC O149:F4ac by a DNA marker-based test on biological samples collected during the suckling phase. This is done to ensure that only susceptible piglets will be used, which means a reduction in variation in the outcomes and, thus, in the number of animals needed to show an effect.
We have conducted a validation experiment testing climate and sanitary parameters involved in the so-called management model. However, there is a need for a follow-up study to further validate this model (to be used up to 25 kg body weight) and develop a standard operating procedure for this unit. An older protocol for a management model included increased ventilation by shortening the P-band by 2°C (=P-band is the range in which the ventilation is steered from minimum to maximum), suboptimal temperature (2°C lower than standard settings), spreading of dust, and spreading of sow manure. However, these parameters showed to be insufficient to mimic the conditions on commercial farms. Recently, other research groups were able to reproduce a sanitary model (e.g., Van der Meer et al., 2016 and Le Floc'h et al., 2009) and parameters in those models (e.g., frequent spreading of manure from different batches of pigs) could be tested to improve our old protocol.
Both the *E. coli* challenge model and management model resemble conditions on commercial farms. The most promising nutritional strategies will first be tested under the controlled *E. coli* challenge model and/or management model and subsequently under commercial conditions on field farms around the world to further validate the results. This needs to be done to ensure that the nutritional strategies also work with e.g., different management, genetics, dietary (raw material) composition.

References
Bruininx, E.M.A.M., G.P. Binnendijk, C.M.C. van der Peet-Schwering, J.W. Schrama, L.A. den Hartog, H. Everts, and A.C. Beynen. 2002. Effect of creep feed consumption on individual feed intake characteristics and performance of group-housed weanling pigs. Journal of Animal Science 80:1413-1418.

Buchet, A. C. Belloc, M. Leblanc-Maridor, and E. Merlot. 2017. Effects of age and weaning conditions on blood indicators of oxidative status in pigs. PloS ONE 12(5):e0178487.

Choudhury, R., A. Middelkoop, J. Boekhorst, W.J.J. Gerrits, B. Kemp, J.E. Bolhuis, and M. Kleerebezem. 2021a. Early life feeding accelerates gut microbiome maturation and suppresses acute post-weaning stress in piglets. Environmental Microbiology, 23(11): 7201-7213.

Choudhury, R., A. Middelkoop, J.G. de Souza, L.A. van Veen, W.J.J. Gerrits, B. Kemp, J.E. Bolhuis, and M. Kleerebezem. 2021b. Impact of early-life feeding on local intestinal microbiota and digestive system development in piglets. Scientific reports 11:4213, doi.org/10.1038/s41598-021-83756-2.

Cranwell, P.D., D.E. Noakes, and K.J. Hill. 1976. Gastric secretion and fermentation in the suckling pig. British Journal of Nutrition 36:71-86.

Heo J.M., J.C. Kim, C.F. Hansen, B.P. Mullan, D.J. Hampson, and J.R. Pluske. 2009. Feeding a diet with decreased protein content reduces indices of protein fermentation and the incidence of postweaning diarrhea in weaned pigs challenged with an enterotoxigenic strain of Escherichia coli. Journal of Animal Science 87:2833-2843.

Heo, J.M., F.O. Opapeju, J.R. Pluske, J.C. Kim, D.J. Hampson, and C.M. Nyachoti. 2013. Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds. Journal of Animal Physiology and Animal Nutrition 97:207-237.

Huting, A.M.S., A. Middelkoop, X. Guan, and F. Molist. 2021. Using nutritional strategies to shape the gastro-intestinal tracts of suckling and weaned piglets. Animals 11, 402, doi.org/10.3390/ani11020402.

Jensen, M.S., S.K. Jensen, and K. Jakobsen. 1997. Development of digestive enzymes in pigs with emphasis on lipolytic activity in the stomach and pancreas. Journal of Animal Science 75:437-445.

Kiarie, E.G. and A. Mills. 2019. Role of feed processing on gut health and function in pigs and poultry: conundrum of optimal particle size and hydrothermal regimens. Frontiers in Veterinary Science 6:19 doi: 10.3389/fevts.2019.00019.

Kim, J.W., B. Koo, and C.M. Nyachoti. 2021. Pigs weighing less than 20 kg are unable to adjust feed intake in response to dietary net energy density regardless of diet composition. Canadian Journal of Animal Science 101:118-125.

Koopmans, S.J., A.C. Guzik, J. van der Meulen, R. Dekker, J. Kogut, B.J. Kerr, and L.L. Southern. 2006. Effects of supplemental L-tryptophan on serotonin, cortisol, intestinal integrity, and behavior in weanling pigs. Journal of Animal Science 84:963-971.

Le Floc'h, N., L. LeBellego, J.J. Matte, D. Melchior, and B. Sève. 2009. The effect of sanitary status degradation and dietary tryptophan content on growth rate and tryptophan metabolism in weaning pigs. Journal of Animal Science 87:1686-1694.

Pluske, J.R., D.J. Hampson, and I.H. Williams. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livestock Production Science 51(1-3): 215-236.

Resink, J.W., W.J.J. Gerrits, T.G. Hulshof, N. Deutz, and G. Ten Have. 2022. P215 Higher amino acid demand in portal drained viscera of post-wean piglets. Animal – Science Proceedings 13: 157-226.

Roubos-van den Hil, P.J., R. Litjens, A.-K. Oudshoorn, J.W. Resink, and C.H.M. Smits. 2017. New perspectives to the enterotoxigenic *E. coli* F4 porcine infection model: Susceptibility genotypes in relation to performance, diarrhoea and bacterial shedding. Veterinary Microbiology 202:58-63.

Trevisi, P., E. Corrent, M. Mazzoni, S. Messori, D. Priori, Y. Gherpelli, A. Simongiovanni, and P. Bosi. 2015. Effect of added dietary threonine on growth performance, health, immunity and gastrointestinal function of weaning pigs with different genetic susceptibility to *Escherichia coli* infection and challenged with *E. coli* K88ac. Journal of Animal Physiology and Animal Nutrition 99:511-520.

Van Hees, H.M.J., S.A. Ballari, L. Dieste-Pérez, B.N. Carpinetti, and G.P.J. Janssens. 2022. Diet and stomach characteristics of feral pigs (*Sus scrofa*): implications for farmed piglets. Journal of Animal Physiology and Animal Nutrition *in press*, https://doi.org/10.1111/jpn.13726.

Van Der Meer, Y., A. Lammers, A.J.M. Jansman, M.M.J.A Rijnen, W.H. Hendriks, and W.J.J. Gerrits. 2016. Performance of pigs kept under different sanitary conditions affected by protein intake and amino acid supplementation. Journal of Animal Science 94:4704-4719.

Wellington, M.O., J.K. Htoo, A.G. Van Kessel, and D.A. Columbus. 2019. Estimating the optimal threonine requirement for 25-50 kg pigs fed a mixture of soluble and insoluble dietary fibre. Canadian Journal of Animal Science 99:634-638.

Wellington, M.O., R.B. Thiessen, A.G. Van Kessel, and D.A. Columbus. 2020. Intestinal health and threonine requirement of growing pigs fed diets containing high dietary fibre and fermentable protein. Animals 10:2055.

3.2 Purpose

3.2.1 Describe the project's immediate and ultimate goals. Describe to which extent achieving the project's immediate goal will contribute to achieving the ultimate goal.

- If applicable, describe all subobjectives

The ultimate goal of this project is to develop nutritional strategies that will better meet the requirements of piglets before, around, and after weaning in optimal and sub-optimal (including pathogenic pressure) environments. With these nutritional strategies, we aim to increase the sustainability of pig production by helping to reduce mortality rate and the use of antibiotics, by using raw materials that meet animal requirements, by supporting feed intake and gastrointestinal tract development around weaning, and by improving animal welfare for example by reducing abnormal behaviour (tail, biting, belly nosing).

The immediate goals are:

1. To validate a management model that simulates commercial conditions but in a controlled environment (Appendix 2).
2. To determine the optimal lysine to energy ratio and energy level in piglets after weaning (Appendix 1).
3. To determine the effect of creep feed, fatty acid level and fatty acid composition on fat digestibility, bile acid production, enzyme secretion, gastrointestinal development, and blood lipid metabolite profile after weaning (Appendix 1). Socializing the piglets before weaning might be used as model to reduce weaning stress and, thus, to disentangle the effect of stress from feed transition (sow milk to solid feed) from social stress (mixing of piglets) on fat digestibility. The optimal fatty acid level and fatty acid composition will also be determined under suboptimal sanitary conditions (Appendix 2) in order to translate the knowledge into practical conditions.
4. To determine the optimal ratio between unsaturated and saturated (U:S ratio) fatty acids on fat digestibility under optimal (Appendix 1) and suboptimal sanitary conditions (Appendix 2).
5. To determine the interaction between dietary protein level and specific amino acids requirements under optimal and suboptimal sanitary conditions. Nitrogen balance experiments will be done to determine protein deposition under optimal and suboptimal sanitary conditions (Appendix 4). The optimal protein and amino acid levels will be verified in a growth performance trial under optimal (no animal experiment) and suboptimal sanitary conditions (Appendix 2).
6. To determine the interaction between feed particle size and the optimal inclusion level of coarse raw materials in the diet given before and after weaning on stomach and intestinal development and nutrient digestibility after weaning under optimal (Appendix 1), suboptimal sanitary conditions (Appendix 2) and a specific pathogen challenge (*E. coli* challenge model; Appendix 3).
7. To determine the effect of feed form (mash, pellet, crumble, extruded feed) and transition between feed forms at weaning on gastrointestinal development and health (Appendix 1).
8. To determine the optimal buffering capacity in diets for piglets after weaning under suboptimal sanitary conditions (Appendix 2) conditions. Optimal levels under optimal conditions were already established in an in-house study.
9. To determine the interactive effect between buffering capacity and feed particle size distribution on gastrointestinal development and health (Appendix 1).

Next to the response parameters specified in the appendices, we will also evaluate sustainability metrics when applicable. The metrics are divided in diet-related and animal-related metrices. Diet-related: resource (use of fossil fuel) and water use, environmental acidification, nitrogen and phosphorus excretion during pig production, CO_2 emission from transport of raw materials or from raw material production itself. Animal-related: behaviour scores, body condition scores, mortality, and morbidity.

3.2.2 Provide a justification for the project's feasibility.

We are an international animal nutrition company that develops nutritional additives and feeding strategies for most livestock species. The company has the ambition to be a global leader in this field. Our focus area is sustainable livestock farming which for us includes reducing antibiotic use, improving animal health and welfare, and reducing environmental burden of livestock production by e.g., reducing nutrient losses in urine and faeces and reducing emissions associated with production. The need to go for a more circular food production system could also lead to the use of feed with lower nutrient availability/digestibility, which increases the need for additives and more in-depth knowledge on nutrient delivery. This will allow for improved digestion and utilization of raw materials with lower nutrient availability/digestibility. Our company has a dedicated R&D department with research teams for all species in scope, including pigs. Each species team has around 6 research scientists holding a PhD related to animal or veterinary science and they maintain a broad international science network. Studies will be performed at our research centre in The Netherlands. We have recently invested in a state-of-the-art unit where we can perform trials that mimic and control specific environmental conditions (as described above in section 3.1 Background). High biosecurity and hygiene standards are maintained to protect our personnel and animals. Daily care of animals, measurements, and experimental techniques are performed by certified competent and experienced staff. Competence of personnel is controlled by a ISO9001 quality management system and our animal welfare body. The company has state-of-the-art and GLP certified laboratory facilities with a range of assays and technologies, including *in vitro* techniques for first screening.
Project results will be disseminated through scientific journals and conferences and ultimately translated into nutritional strategies applied via our global network of nutritionists.

3.2.3 Are, for conducting this project, other laws and regulations applicable that may affect the welfare of the animals and/or the feasibility of the project?

X No

☐ Yes > Describe which laws and regulations apply en describe the effects on the welfare of the animals and the feasibility of the project.

3.3 Relevance

3.3.1 What is the scientific and/or social relevance of the objectives described above?

The scientific relevance of the immediate goals is to better understand the piglet's requirements around weaning which is one of the most stressful events in a pig's life. The lack of understanding of the physical state and the variable response to weaning of the post-weaning piglet makes it challenging for nutritionists to establish an optimal diet. However, there are certain aspects that are similar between piglets: they all need energy to survive, nutrients to develop their gastrointestinal tract, and need adequate nutrition to combat pathogens and stay healthy. Moreover, changes in legislation, e.g., the expected ban on tail docking and use of in-feed antibiotics and pharmacological levels of zinc oxide, stress the need to understand the nutritional requirements of piglets in this phase, also to prevent damaging behaviours. Nutrient requirements for weaned piglets are mostly extrapolated from older pigs and we have just started to unravel the complex physiology of a piglet around weaning and its accompanying nutrient requirements. Results from the immediate project goals will be used to further optimize piglet feeding strategies internally but our results will also be published in scientific journals.
Regarding the social relevance of the ultimate goal, main aspects are to further reduce reliance on antibiotics for pigs in order to maintain future availability of antibiotics for human medicine. Secondly,

this project will contribute to the use of non-human-edible feed ingredients (e.g., co-products from food production) for feed, leaving human-edible ingredients available as food.

3.3.2 Who are the project's stakeholders? Describe their specific interests.

Target animals (piglets): Go through the weaning process with less nutritional stress and having an improved (gastrointestinal) development after weaning. In this way, have a lower risk for diseases incidence, remain healthier after weaning, and have lower mortality.
Experimental animals (piglets): Contribute to research needed to study nutritional requirements around weaning more in-depth. Violation of piglet integrity by housing conditions (specifically without bedding), housing under suboptimal sanitary conditions, or challenge with a pathogen.
License holder: Increase the sustainability of pig production and improve animal health and welfare. Be able to provide optimized nutritional strategies for feeding piglets before and after weaning to farmers. The license holder also has an economic interest to market high-end piglet feeds and nutritional advice to farmers.
Researchers: Increase their understanding of the weaning process and the nutritional requirements of piglets around weaning. Moreover, to publish the results of this project in scientific journals.
Environment: Reduce nitrogen and phosphorus excretion into the environment. More efficient use of raw materials and to reduce the competition between food and feed and reduce fossil fuel and water resource use.
Pig industry: Increase its sustainability by increased piglet robustness, resilience to weaning and sanitary stressors, reduced damaging behaviours, PWD, antimicrobial use, and mortality around weaning. Increased economic sustainability by increasing the flexibility to use raw materials based on nutrient values and costs.
Farmer: Smoother weaning process of piglets and lower veterinary costs, resulting in a sustainable income from less morbidity and mortality. Improved growth performance of their herd as result from lower morbidity and mortality and improved nutrient delivery.
Society: Increase animal wellbeing and higher environmentally sustainable pig production. Reduce the reliance on antimicrobials in pig production and, thereby, reduce the risk of antibiotic resistance for humans.

3.4 Strategy

3.4.1 Provide an overview of the overall design of the project (strategy). If applicable, describe the different phases in the project, the coherence, the milestones, selection points and decision criteria.

To answer the immediate goals specified in 3.2.1, a total of 18 animal experiments are designed. The design of the project is schematically represented below, to be interpreted from top to bottom. Go/no-go decisions on continuation of specific studies within this project are made by a dedicated project team. This project team consists of researchers, nutritionists, and people working in the company's sustainability, regulatory, and marketing departments. Together, they decide on the continuation of a project based on pre-set key performance indicators set. Typical key performance indicators are:

1. Growth performance: does one of the tested nutritional strategies maintain or even increases feed intake to a level that is adequate to support or improve growth and health.
2. Sustainability metrics: does one of the tested nutritional strategies improve sustainability by e.g., reducing antibiotic use, reducing mortality, or increase the use of more sustainable raw materials and increasing feed efficiency (i.e., reducing nutrient losses into the environment).
3. Animal health and welfare metrics: does one of the tested nutritional strategies lead to lower occurrence of PWD or better body condition scores including reducing damaging behaviours.

For goals 2-4 (energy) and 6-9 (gastrointestinal development), a series of experiments (1-9) are designed that relate to the respective immediate goals using a Nutrient utilization model (Appendix 1). Results of exp. 1-9 will be discussed in the project team that decides on go/no-go with experiments under suboptimal sanitary conditions (Management model; Appendix 2) and/or subsequently using an *E. coli* challenge model (E. coli challenge model; Appendix 3) based on the predetermined key performance indicators. Validation of the management model is required before exp. 13-17 can start. This will be done

in exp. 12 (goal 1; Appendix 2). After exp. 12, a go/no go decision needs to be made on the validity of the management model.
For goal 5, first one experiment will be conducted under optimal conditions (exp. 10; Appendix 4). A go /no go decision has to be taken before moving to exp. 11 which has a similar experimental design as exp. 10 but then under health challenge conditions (suboptimal sanitary conditions). Experiment 11 (nitrogen balance under suboptimal sanitary conditions) can only be done if experiment 12 (development of management model) is successful. With this project governance structure, only the most promising nutritional strategies will be tested in challenge models (Management model and E. coli challenge model). The nutritional strategies will ultimately be validated in large-scale field trials (non-animal experiments).

Energy **Goals 2-4**	**Gastrointestinal development: acidification and fibres** **Goals 6-9**	**Protein level and amino acid ratio's** **Goal 5**

Hypotheses creation based on literature studies, in vitro testing (digestibility or buffering capacity), existing in-house in vivo studies

Appendix 1: Nutrient utilization model (n=888)		*Appendix 4: Nitrogen balance optimal and suboptimal conditions (n=160)*
Exp. 1 Energy level × lysine/energy ratio	Exp. 6 Fibre coarseness × start of feeding before weaning	Exp. 10 Protein level × amino acid level optimal conditions
Exp. 2 Feed level × lysine/energy ratio	Exp. 7 Inclusion level coarse raw materials before and after weaning	**GO/NO GO DECISION MOMENT**
Exp. 3 Creep feed effect on fat digestion	Exp. 8 Feed form	Exp. 11 Protein level × amino acid level suboptimal sanitary conditions
Exp. 4 SCFA+MCFA source × level	Exp. 9 Buffering capacity × coarseness	
Exp. 5 U:S ratio dose-response		

GO/NO GO DECISION MOMENT

Appendix 2: Management model (n=1254) *Exp. 12: development of management model* = **Goal 1**		
GO/NO GO DECISION MOMENT ON MANAGEMENT MODEL		
Exp. 13 SCFA+MCFA source × level	Exp. 15 Optimal strategy regarding coarseness before and after weaning with suboptimal conditions after weaning	Exp. 17 Protein +amino acids level × housing conditions (optimal vs suboptimal)
Exp. 14 U:S ratio dose-response	Exp. 16 Buffering capacity under suboptimal conditions	

GO/NO GO DECISION MOMENT

Appendix 3: E. coli challenge model (n= 207)
Exp. 18 Optimal strategy regarding coarseness before and after weaning with pathogen challenge conditions after weaning

Validation studies under field conditions

3.4.2 Provide a justification for the strategy described above.

Hypotheses of each experiment are based on available knowledge (literature or earlier in-house studies). When possible, *in vitro* studies will first be conducted to find the most promising combinations of, for example, unsaturated to saturated fatty acid ratios. Those will subsequently be used in *in vivo* experiments. Only the nutritional strategies that showed promising effects under optimal sanitary conditions will be tested in the management and/or E. coli challenge models. In this way, the number of animals and discomfort per animal will be minimized. The management model will be validated first prior to using this model to test nutritional strategies. The results obtained from the validation trial will be used to calculate the exact number of animals needed to find the relevant difference. This will also reduce the number of animals.

3.4.3 List the different types of animal procedures. Use a different appendix 'description animal procedures' for each type of animal procedure.

Serial number	Type of animal procedure
1	Nutrient utilization model
2	Management model
3	*E. coli* challenge model
4	Nitrogen balance optimal and suboptimal conditions
5	
6	
7	
8	
9	
10	

Centrale Commissie Dierproeven

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 lid2h
1.2	Provide the name of the licenced establishment.	5.1 lid2h
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 1 — Type of animal procedure: Nutrient utilization model

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

This model is used to study the nutrient utilization of energy, protein, fat, and fibres but also the effect of fat and fibres on gastrointestinal development. Sampling blood and/or different parts of the gut will provide more insight into the dynamics of digestion and the locations where different nutrients are released and absorbed by the gut. In order to study digestibility and retention time of feed in specific parts of the gastrointestinal tract, known amounts of markers will be fed to the piglets (e.g., chromium EDTA as soluble marker and insoluble ash as in-feed marker). By sampling digesta contents of different sections of the gastrointestinal tract, digestibility and retention time for each section can be determined. When for example ileal contents are sampled, ileal apparent digestibility data will be obtained, which can be used for formulating more optimal diets because the requirements are also expressed on an ileal digestibility basis. Sampling faecal material is needed to study the overall nutrient digestion and gives an indication of the absolute requirement. The primary outcome parameter depends on the goal of the experiment. Experiments 1-8 will be done under this Appendix. The treatments will be given during pre-weaning or at maximum 3 weeks after weaning since that is the most sensitive period in a piglet's life.

Experiment 1
Treatments after weaning: 2 energy levels × 5 levels of lysine/energy ratios = 10 experimental diets.

- The energy levels are at the borders of the current recommendations while some of the tested lysine/energy ratios might fall outside current recommendations, i.e., either lower or higher.

Sampling: Blood samples from a subset of animals to determine plasma urea nitrogen as measure for protein deposition. Faecal samples to determine protein and energy digestibility.

Main outcome parameter: growth; blood and faecal samples will help to explain observed growth responses.

Experiment 2
Treatments after weaning: 3 lysine/energy levels (based on Exp 1) × 3 feeding levels (80, 90 and 100% of ad libitum feed intake). Feed intake might be restricted in praxis and in this way, we validate our nutritional strategies under more commercial-like conditions.
Main outcome parameter: growth; no other samples will be taken.

Experiment 3
Treatments before weaning: socializing piglets by combining litters (i.e., open fences between farrowing crates) and with or without creep feed. Thus, 4 treatments before weaning: social or traditional and creep feed or no creep feed.
Treatments after weaning: 1 experimental diet.
Sampling: blood, gastrointestinal content, pancreatic + gastrointestinal tissue at 4 timepoints around weaning. Faecal sampling in the period after weaning.
Main outcome parameter: fat digestibility at the end of the ileum; other parameters, such as bile acid production, enzyme secretion, gastrointestinal development (weight, length, histology), blood lipid metabolite profile, will help to explain the observed effects on fat digestibility.

Experiment 4
Treatments before weaning: socializing piglets by combining litters (i.e., open fences between farrowing crates). Thus, 2 treatments before weaning: social or traditional.
Treatments after weaning: 3 SCFA+MCFA composition × 3 SCFA+MCFA levels = 9 experimental diets.
Sampling: blood, gastrointestinal content, pancreatic + gastrointestinal tissue. Faecal sampling in the period after weaning.
Main outcome parameter: fat digestibility at the end of the ileum; other parameters, such as bile acid production, enzyme secretion, gastrointestinal development (weight, length, histology), blood lipid metabolite profile, will help to explain the observed effects on fat digestibility.

Experiment 5
Treatments after weaning: 4 ratios of unsaturated:saturated fatty acids in dose-response.
Sampling: gastrointestinal content, pancreatic + gastrointestinal tissue. Faecal sampling in the period after weaning.
Main outcome parameter: fat digestibility at the end of the ileum; other parameters, such as bile acid production, enzyme secretion, gastrointestinal development (weight, length, histology), blood lipid metabolite profile, will help to explain the observed effects on fat digestibility.

Experiment 6
Treatments before weaning: 3 levels of coarseness of the diet × 2 starting times of feeding = 6 experimental treatments.
Treatments after weaning: same level of coarseness as before weaning = 3 experimental diets.
Sampling: gastrointestinal development, e.g., acidification, ulceration of the stomach and length, weight and histomorphology of different parts of the gastrointestinal tract, and gastrointestinal content at and after weaning.
Main outcome parameter: nutrient digestibility at the end of the ileum; other parameters determined from the gastrointestinal samples will help to explain observed effects on nutrient digestibility.

Experiment 7
Treatments after weaning: 3 inclusion levels coarse raw materials before weaning × 3 inclusion levels coarse raw materials after weaning = 9 experimental diets.
Sampling: gastrointestinal development, e.g., acidification, ulceration of the stomach and length, weight and histomorphology of different parts of the gastrointestinal tract, and gastrointestinal content at and after weaning.
Main outcome parameter: nutrient digestibility at the end of the ileum; other parameters determined from the gastrointestinal samples will help to explain observed effects on nutrient digestibility.

Experiment 8

Treatments after weaning: 3 feed forms (mash, pellet, extruded feed) before weaning × 3 feed forms (mash, pellet, extruded feed) after weaning = 9 experimental diets.
Sampling: gastrointestinal development and gastrointestinal content at and after weaning. Faecal sampling in the period after weaning.
Main outcome parameter: nutrient digestibility at the end of the ileum; other parameters determined from the gastrointestinal samples (e.g., retention time) will help to explain observed effects on nutrient digestibility.

Experiment 9
Treatments after weaning: 2 levels of buffering capacity × 2 levels of coarseness (standard vs optimized from Exp 5 and 6) = 4 experimental diets.
Sampling: gastrointestinal development, e.g., acidification, ulceration of the stomach and length, weight and histomorphology of different parts of the gastrointestinal tract, and gastrointestinal content after weaning.
Main outcome parameter: nutrient digestibility at the end of the ileum; other parameters determined from the gastrointestinal samples (e.g., retention time) will help to explain observed effects on nutrient digestibility.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Proposed animal procedures all experiments:

1. Before weaning, piglets will be individually ear-tagged and birth weights will be recorded. Creep feed will be given as free-choice. Piglets will be weaned at a minimum age of 21 days old into specialised housing which are cleaned before entering of a new group and which is separated from housings where the sows are kept (Council Directive 2008/120/EC).
2. Piglets will be housed in groups of 3-6 piglets depending on the barn/pen sizes to be used.
3. Housing without bedding for maximally 6 weeks after weaning. This is required to properly assess the effects of the nutritional strategies. Consumption of bedding materials (e.g., high in fibre) might interfere with the response leading to more variation and a higher number of animals required to show an effect.
4. Animal weighing every 7 days for a maximum of 10 weeks (before and after weaning periods).

Additional animal procedure experiment 1:

5. Blood sampling; maximal 6 times within 6 weeks after weaning with a maximum 10 mL and of 8ml/kg/14 days for young animals. Route: intravenous.

Additional animal procedure experiment 2:

6. Feed restriction at 80% of ad libitum intake.

Additional animal procedure experiment 3-4:

7. Socializing before weaning.

Additional animal procedure experiment 3, 4 and 6:

8. Faecal swab to determine if a piglet had eaten the creep feed. A colorant will be added to the feed for 3 days and a faecal swap will be taken after those days to determine the presence of the colorant in faeces.

Additional animal procedure experiments 3-9:

9. Euthanasia for the sampling of digesta from the different sections of the gut and tissue sampling in order to determine digestibility / retention time / enzyme production. Euthanasia can take place before (reference samples in Exp 3) and after weaning. A blood sample can be taken just before euthanasia when animals are sedated.
10. Imposed feeding pattern to get a continuous flow of digesta through the gastrointestinal tract after weaning. A feeding pattern means that the total feed typically eaten is provided in smaller meals throughout the day instead of free access to feed. The amount of feed to be given will be determined based on feed intake of individual piglets (average of previous studies) and will always be above the level needed for maintenance.

Additional animal procedure experiments 1, 3, 4, 5 and 8:

11. Faeces sampling via rectum stimulation; maximal 7 times in the experimental period of 10 weeks (before and after weaning periods) with max 2 samples taken before weaning and max 5 samples after weaning.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

Digestibility studies are done by measuring the loss of nutrients in the gut with the help of inert digestibility markers such as insoluble ash. Therefore, the individual pig is the experimental unit. Analysis in blood samples (experiment 1), are also on individual pig level. The relevant outcome parameter will be taken (e.g., protein digestibility) and error variances from previous studies will be used to estimate sample size at a power of 80% and a probability of 95%. This will be done for every experiment.

For example, ileal protein digestibility
500 studies are simulated in a statistical software package using relevant digestibility means ranging between 60 and 80%. We aim to detect an absolute difference of 7 percentage-points within this range of digestibility means. Previous studies with nutritional strategies have shown that we can get this difference in our facility. The 500 simulations are, thereafter, analysed using a linear mixed model (MIXED) which is also the model used to analyse the data after a study is completed. The proportion of simulations showing a significant treatment effect should be >80% in order to ensure a power of 80% and a probability of 95%.
Error variance = 19.96
Mean digestibility = 70 with absolute differences ranging from 0-12 (mean range = 58 – 82%)
Relevant difference = 6
Number of replicates per treatment = 10. However, piglets at a young age might not have sufficient digesta at the end of the small intestine for chemical analyses or a piglet might need to be removed from a study (e.g., feed intake below specified criteria or death). Therefore, sample size is estimated to be 10 + 2 = 12 in total per treatment in order to maintain sufficient power.

B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
1	Sus scrofa	Own facility	Pre-/weaned piglets	888	Males and females	No	Hypor Libra × Hypor Maxter

Provide justifications for these choices

Species	Sus scrofa is the target species
Origin	We have our own facility with a sow herd, where we breed our own piglets
Life stages	Piglets before and after weaning up to 6 weeks after weaning are the target group
Number	Total experimental treatments experiment 1-9 is 72 (Exp 1: 10, Exp 2: 5 (3 with 80% of ad libitum feeding and 2 with lysine/energy below current recommendations), Exp 3: 16 (4 timepoints × 4 diets), Exp 4: 9, Exp 5: 4, Exp 6: 6, Exp 7: 9, Exp 8: 9, and Exp 9: 4). In Exp 3, 12 socialized piglets and 12 traditionally housed piglets will form the reference sample group. 72 treatments × 12 piglets/treatment + 24 reference piglets (Exp 4) = 888 piglets in total
Gender	Males and females are representative for the commercial situation
Genetic alterations	Not applicable
Strain	This strain is present at our own facility and representative of the commercial situation

C. Accommodation and care

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

☐ Yes

X No > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices

During the pre-weaning phase of the experiment piglets will be housed in conventional farrowing crates together with the dam.
Piglets will be housed without bedding material. Non-edible pen enrichment will be provided to allow animals to play and exhibit normal behaviour. The pen enrichment meets the requirements set by the NVWA.
The pens have a tenderfoot slatted floor.

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

X Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

X No > Justify why pain relieving methods will not be used.

Blood sampling might induce pain, but no sedation will be used. A single insertion of a needle to sample blood is not expected to cause severe pain and the duration is short.

☐ Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Describe which other adverse effects on the animals' welfare may be expected?

Piglets will not get bedding material in the pens. However, we expect little adverse effects because other (non-edible) pen enrichment will be present.
The imposed feeding pattern is not expected to result in adverse effects on animal welfare since the animals will be fed their daily amount (always above their requirements for maintenance) but then divided across a day. The feeding level at 80% of ad libitum feed intake might result in adverse effects.

Explain why these effects may emerge.

Diets are designed to provide the recommended nutrients when given ad libitum.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

The lower feeding level will be given for a maximum of 3 weeks after weaning. Care will be given that all vitamin and mineral requirements will be met even at this lower feeding level.

E. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

X No > Continue with question F

☐ Yes > Describe the criteria that will be used to identify the humane endpoints.

Indicate the likely incidence.

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

1. Ear tagging: less than mild (standard commercial procedure)
2. Group housing: less than mild

3. Housing without bedding: less than mild
4. Animal weighing: less than mild
5. Blood sampling intravenously (~13% of piglets): mild
6. Feed restriction at 80% of ad libitum / nutrients below requirements (~6% of piglets): mild
7. Socializing before weaning: less than mild
8. Faecal swab: less than mild
9. Euthanasia after sedation (~79% of piglets): mild
10. Imposed feeding pattern: less than mild
11. Faeces sampling: less than mild

The expected level of discomfort is mild for all animals (n=888).

G. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement	We have an in-house in vitro digestion kinetics model in which we can screen raw materials and diets. Wherever possible, first a screening with this model will be done in order to select the raw materials and diets to be tested in in vivo studies. Interactions between raw materials and /or nutrients on e.g., digestibility and nutrient utilization, passage through the gastrointestinal tract, and acidification in the stomach cannot be studied outside animals. Especially not in a weaned piglet where digestive physiology changes rapidly due to weaning. Animal data is also needed to validate the in vitro digestion kinetics model. Faecal samples could be obtained non-invasively but are not representative for what is happening in different parts of the gastrointestinal tract. For this project, the stomach and small intestine are the most important parts of the gastrointestinal tract. Changes in digestive physiology and the stress around weaning occurs in all husbandry systems.
Reduction	Sample size estimations are done using data from our own facility. Per nutritional strategy, we use previous studies and literature data (see 3.1 Background in the project proposal) to decide on the treatments to be studied. Go/no go decisions per nutritional strategy ensures that only the most promising strategies will be tested in follow-up studies, allowing for the reduction in the number of animals potentially required.
Refinement	Piglets after weaning are the target animals and their physiology cannot be obtained in other species or in models. Animals will be sedated before euthanasia. In the absence of bedding, extra care will be taken that piglets have access to enrichment material at all times. The enrichment material should be manipulated, is chewable, interesting for a longer time and available for all animals in a pen (e.g., chains reaching the floor, rope, plastic toys, etc.). Additional health checks will be done for experiment 2 when for the treatment where feed intake is restricted. Standard operating procedures will be used for faeces collection to reduce variation between studies.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

X No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

X No > Continue with question I.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

X No

☐ Yes > Provide specific justifications for the re-use of these animals during the procedures.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

Not applicable.

J. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question K.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

☐ No > Provide information on the destination of the animals.

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

Tissue and/or digesta from the gastrointestinal tract can only be sampled after euthanasia. Only a part of the animals will be euthanised. The other animals will be kept at our own research facility or sold to a commercial fattening farm.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

X Yes > Will a method of killing be used for which specific requirements apply?

X No > Describe the method of killing.

Overdose of barbiturate after sedation

☐ Yes > Describe the method of killing that will be used and provide justifications for this choice.

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

Centrale Commissie Dierproeven

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 lid2h
1.2	Provide the name of the licenced establishment.	5.1 lid2h
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 2 — Type of animal procedure: Management model

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

Experiment 12 development of management model

In our old stable, we were able to find differences in growth, feed intake, and feed efficiency (growth obtained with a certain amount of feed) between piglets housed under standard conditions and piglets housed under suboptimal / management model conditions. Feed intake and growth are measures of animal wellbeing (i.e., if an animal feels sick it will not eat and grow) and feed efficiency is a measure for sustainability (i.e., how well nutrients are used by the animal and thus not lost in urine and faeces). Last year we performed a small-scale study looking at several factors (body weight at weaning, spreading of dirt, partially closed vs open floor, lowering temperature) to start validating the old model in our new barn. We did not use different types of diets and also the number of replicates per factor was too low (n=10) to draw firm conclusions regarding the model. A second study is, therefore, needed to further develop and verify the outcomes of the first study. In this study we will use different dietary treatments. Since this model is aimed at finding differences between nutritional strategies, we also need to validate this first before we continue to test new nutritional strategies.

Treatments after weaning: 2 commercial-like diets × 3 housing conditions = 6 treatments.

- The housing conditions will be the optimal conditions (as control treatment) and the 2 most promising combinations of factors from the previous study (2 test treatments).

Sampling: blood for inflammatory markers and faeces for protein digestibility (as reduced protein digestibility is a main driver for post-weaning diarrhoea).

Main outcome parameter: inflammatory response in blood (e.g., haptoglobin). Faecal sampling will be done to verify the model. Growth response and diarrhoea incidence will be also be determined.

This study will be used to determine the residual variance which is required to do power and sample size calculations for subsequent studies. After the model is validated, we will use it for experiments 12-16 in which we want to examine the effect of nutritional strategies under sub-optimal conditions where the pathogenic pressure is long-lasting (up to 2 weeks after weaning). Moreover, poor management conditions immediately after weaning are expected to influence feed intake during the first 24-48 hours after weaning which might result in a higher occurrence of post-weaning diarrhoea.
The measurements and sampling in this experiment (no. 12) are needed to verify the outcomes of the first study (executed last year) and necessary to develop a standard operating procedure. This standard operating procedure will subsequently be used for experiments 13-14 and 11 which is in Appendix 4.

Experiment 13
Treatments before weaning: socializing piglets by combining litters (i.e., open fences between farrowing crates).
Treatments after weaning: 2 SCFA+MCFA composition × 2 SCFA+MCFA levels = 4 experimental diets.

- Composition and level based on Exp 3.

Sampling: blood, gastrointestinal content, pancreatic + gastrointestinal tissue. Faecal sampling in the period after weaning.
Main outcome parameter: fat digestibility at the end of the ileum; other parameters, such as bile acid production, enzyme secretion, gastrointestinal development (weight, length, histology), blood lipid metabolite profile, will help to explain the observed effects on fat digestibility.

Experiment 14
Treatments after weaning: 2 ratios of unsaturated:saturated fatty acids.

- Ratios based on Exp 4.

Sampling: gastrointestinal content, pancreatic + gastrointestinal tissue. Faecal sampling in the period after weaning.
Main outcome parameter: fat digestibility at the end of the ileum; other parameters, such as bile acid production, enzyme secretion, gastrointestinal development (weight, length, histology), blood lipid metabolite profile, will help to explain the observed effects on fat digestibility.

Experiment 15
Treatments before and/or after weaning: the 4 most promising nutritional strategies from Exp 5 and 6 will be used. This is regarding the level of coarseness, starting time of coarser feed before weaning, and inclusion level of coarse raw materials.
Sampling: gastrointestinal development, e.g., acidification, ulceration of the stomach and length, weight and histomorphology of different parts of the gastrointestinal tract, and gastrointestinal content at and after weaning.
Main outcome parameter: nutrient digestibility at the end of the ileum; other parameters determined from the gastrointestinal samples will help to explain observed effects on nutrient digestibility.

Experiment 16
Treatments after weaning: 4 diets with differing buffering capacity in a dose-response.
Sampling: gastrointestinal development, e.g., acidification, ulceration of the stomach and length, weight and histomorphology of different parts of the gastrointestinal tract, and gastrointestinal content at and after weaning.
Main outcome parameter: nutrient digestibility at the end of the ileum; other parameters determined from the gastrointestinal samples will help to explain observed effects on nutrient digestibility.

Experiment 17
Treatments after weaning: 2 combinations of amino acids and protein levels × 2 housing conditions (optimal vs management model) = 4 treatments of which 2 are regarded as animal experiment. The combinations of amino acids and protein levels are obtained in Exp 9 and 10.
Main outcome parameter: growth.

All experiments
Non-challenge control piglets receiving the same experimental diets will be included in experiments 12-16, but only non-invasive measurements will be done on these animals, i.e., growth, faecal sampling, fecal scoring for diarrhoea incidence.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Proposed animal procedures all experiments:

1. Before weaning, piglets will be individually ear-tagged and birth weights will be recorded. Creep feed will be given as free-choice. Piglets will be weaned at a minimum age of 21 days old into specialised housing which is separated from housings where the sows are kept (Council Directive 2008/120/EC).
2. Piglets will be housed in groups of 3 after weaning.
3. Housing without bedding for maximally 6 weeks after weaning. This is required to properly assess the effects of the nutritional strategies. Consumption of bedding might interfere with the response leading to more variation and a higher number of animals required to show an effect.
4. Animal weighing every 7 days for a maximum of 10 weeks (before and after weaning periods).
5. Management model with an average weaning age of 24 days (minimum 21 days): Factors in the management model that will be validated are e.g., reduced temperature (e.g., 2°C), increased ventilation (reduced P-band), spreading of manure, no disinfection of the unit before animals enter. The parameters will be incorporated in a standard operating procedure to ensure repeatability of the model.

Additional animal procedure experiment 12:

6. Blood sampling; maximal 6 times within 6 weeks after weaning with a maximum of 10 mL or 8ml/kg/14 days for young animals. Route: intravenous.

Additional animal procedure experiment 13:

7. Socializing before weaning.

Additional animal procedure experiment 13-16:

8. Euthanasia for the sampling of digesta samples from the gut and tissue sampling in order to determine digestibility / retention time / enzyme production. Euthanasia can take place before (reference samples in Exp 12) and after weaning. A blood sample can be taken just before euthanasia when animals are sedated.
9. Imposed feeding pattern to get a continuous flow of digesta through the gastrointestinal tract after weaning. A feeding pattern means that the total feed typically eaten is provided in smaller meals throughout the day instead of free access to feed. The amount of feed to be given will be determined based on feed intake of individual piglets (average of previous studies) and will always be above the level needed for maintenance.

Additional animal procedure experiment 12-14:

10. Faeces sampling via rectum stimulation; maximal 7 times in the experimental period of 10 weeks (before and after weaning periods) with max 2 samples taken before weaning and max 5 samples after weaning.

Example of a timeline for experiment 12:

Day in experiment	**Procedure**
Birth	Ear tag birth weight
~24 of age = day 0	Weaning into groups of 3: 4 groups of animals (2 per diet) housed under management model conditions and 2 groups of animals (1 per diet) housed in optimal conditions
Day 6	Blood sample + body weight
Day 10	Faecal sample
Day 13	Blood sample + body weight
Day 17	Faecal sample
Day 20	Blood sample + body weight
Day 27	Blood sample + body weight
Day 41 (=6 weeks after weaning)	Blood sample + body weight

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

Calculating sample size (power analysis) in order to minimise the number of animals needed per experiment will be determined based on the main outcome parameters. For experiment 12, it is based on haptoglobin concentrations in blood. For experiments 13-17, the power calculation is based on the amount of piglets needed to find an effect of the management model. For now, this will be based on growth but this might be adjusted based on outcomes of Exp 12.

Experiment 12
500 studies are simulated in a statistical software package using relevant differences in blood haptoglobin concentration found in a previous trial. The 500 simulations are, thereafter, analysed using a linear mixed model (MIXED) which is also the model used to analyse the data after a study is completed. The proportion of simulations showing a significant treatment effect should be >80% in order to ensure a power of 80% and a probability of 95%.
Error variance = 0.31
Mean haptoglobin concentration optimal conditions vs management model conditions (~day 14 after weaning) = 0.90 deviating between 0.70 vs 1.20 (based on in-house trial and Le Floc'h et al., 2009, J. Anim. Sci. 87:1686-1694; Van der Meer et al., 2016, J. Anim. Sci. 94:4704-4719; Van der Peet-Schwering et al., 2021, Wageningen Livestock Research, Public Report 1319)
Relevant difference = 0.60
Number of replicates per treatment = 15.

Experiment 13-17
Data from previous studies were used to estimate the expected mean, and error and block variances. 500 studies are simulated in a statistical software package using a mean growth of 460 g/d (day 0-42 after weaning) with differences ranging from 0-45 g/d. We aim to detect an absolute difference of 35. The 500 simulations are, thereafter, analysed using a linear mixed model (MIXED) which is also the model used to analyse the data after a study is completed. The proportion of simulations showing a significant treatment effect should be >80% in order to ensure a power of 80% and a probability of 95%.

Error variance = 1553
Block variance = 983
Mean = 460 g/d with absolute differences ranging from 0-45 (mean range = 415 – 505 g/d)
Relevant difference = 35 g/d
Number of replicates per treatment = 20.

B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
1	Sus scrofa	Own facility	Pre-/weaned piglets	1254	Males and females	No	Hypor Libra × Hypor Maxter

Provide justifications for these choices

Species	Sus scrofa is the target species
Origin	We have our own facility with a sow herd, where we breed our own piglets
Life stages	Piglets before and after weaning up to 6 weeks after weaning are the target group

Number	Experiment 12: 2 diets × 15 piglets = 30 piglets for control/optimal conditions (blood sample only). For the management model (procedure 5), experiments 12-17: 20 treatments (Exp 12: 4, Exp 13: 4, Exp 14: 2, Exp 15: 4, Exp 16: 4, and Exp 17: 2) in total. In Exp 13, 12 socialized piglets and 12 traditionally housed piglets will form the reference sample group. 20 treatments × 20 pens × 3 piglets + 24 reference piglets = 1224 piglets Total number of piglets 30 + 1224 = 1254
Gender	Males and females are representative for the commercial situation.
Genetic alterations	Not applicable
Strain	This strain is present at our own facility and representative of the commercial situation

C. Accommodation and care

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

☐ Yes

X No > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices

During the pre-weaning phase of the experiment piglets will be housed in conventional farrowing crates together with the dam.
Piglets will be housed without bedding material. Non-edible pen enrichment will be provided to allow animals to play and exhibit normal behaviour. The pen enrichment meets the requirements set by the NVWA.
The pens have a tenderfoot slatted floor.

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

X Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

X No > Justify why pain relieving methods will not be used.

Blood sampling might induce pain, but no sedation will be used. A single insertion of a needle to sample blood is not expected to cause severe pain and the duration is short.

☐ Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Describe which other adverse effects on the animals' welfare may be expected?

Piglets might experience lung problems due to increased ventilation and reduced environmental temperature. The incidence of health issues (not specific for lung problems) in earlier trials with the management model was 4% of which lung problems were 0.3%. The 4% incidence of health issues is similar to other trials where no challenge was given to the animals. In literature, animals kept under low sanitary conditions for the entire fattening period (+/- 15 weeks) had higher pleuritis scores (0.3) and greater percentage of lung surface with pleuritis (1%) at slaughter (Van der Meer et al., 2016, J. Anim. Sci. 94:4704-4719). In a study with weaned piglets (4 weeks of age until 9 weeks of age), there was no difference in the incidence of veterinary treated piglets between the low and high sanitary condition (both ~4%; Van der Peet-Schwering et al., 2021, Wageningen Livestock Research, Public Report 1319). In praxis, almost 10% of the pigs at slaughter show signs of pleuritis (https://duurzaamvarkensvlees.nl/themas/smart-farming/varkenshouder-2030/gebruik-van-data/).

Piglets will not get bedding material in the pens. However, we expect little adverse effects because other (non-edible) pen enrichment will be present.

Explain why these effects may emerge.

The effects on health are inherent to the management model and not different from praxis.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

In case of symptoms of respiratory distress, animals will be treated with antibiotics.

E. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question F

X Yes > Describe the criteria that will be used to identify the humane endpoints.

No recovery from respiratory distress. In that case, the animal will be removed from the study and transferred to standard housing if possible or humanely euthanized. An animal will also be removed from the study when it ends up alone in a pen (penmates removed from the study because of health reasons or death).

Indicate the likely incidence.

Unlikely. Literature and previous in-house trials, indicate that the management model gives similar subclinical health issues as seen in other trials.

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

1. Ear tagging: less than mild (standard commercial procedure)
2. Group housing: less than mild
3. Housing without bedding: less than mild
4. Animal weighing: less than mild
5. Management model: mild for ~95% of the piglets in this appendix (1200 out of 1254 piglets)
6. Blood sampling: mild for ~7% of the piglets in this appendix (90 out of 1254 piglets (Exp 11 only)
7. Socializing before weaning: less than mild
8. Euthanasia after sedation (~13% of piglets): mild for ~13% of the piglets in this appendix (piglets in Exp 12-15 with a total of 14 treatments and with n=12 per treatment (as in Appendix 1) results in 168 out of 1254 piglets)
9. Imposed feeding pattern: less than mild
10. Faeces sampling: less than mild

The overall expected level of discomfort is mild for piglets under management model conditions (n=1200), for non-challenged piglets (n=30 for blood sampling in Exp 11) and piglets euthanised pre-weaning (n=24 in Exp 12 piglets).

G. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement	A response in growth of an animal to a nutritional strategy cannot be determined without using animals. The physiology of a piglet, especially after weaning, and its response to suboptimal environmental conditions cannot be modelled in in vitro or in silico systems. Suboptimal conditions are common in commercial husbandry, but the parameters are not controlled. In order to study the response to nutritional strategies in a reliable and repeatable way, we need controlled conditions such as those obtained with the management model.
Reduction	Sample size estimations are done using data from our own facility. Per nutritional strategy, we use previous studies and literature (see 3.1 Background in the project

	proposal) to decide on the treatments to be studied. The model will be validated first and a go/no go decision will follow before continuing with the other experiments (12-16). Go/no go decisions per nutritional strategy ensures that only the most promising strategies will be tested in challenge studies, allowing for the reduction in the number of animals potentially required.
Refinement	Piglets after weaning are the target animals and their physiology cannot be obtained in other species or in models. Animals will be group housed. Animals will be sedated before euthanasia. In the absence of bedding, extra care will be taken that piglets have access to enrichment material at all times. The enrichment material should be manipulated, is chewable, interesting for a longer time and available for all animals in a pen (e.g., chains reaching the floor, rope, plastic toys, etc.). Animals will be checked daily by trained staff. Water and feed intake will be monitored daily at least for the first 14 days after weaning to get an indication of wellbeing. The goal of the management model is to get a subclinical immune response and it is, therefore, not a disease challenge model. In case of signs of disease, animals will be treated and if needed removed from the study (Humane Endpoint).

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

X No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

X No > Continue with question I.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

X No

☐ Yes > Provide specific justifications for the re-use of these animals during the procedures.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

Not applicable

J. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question K.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

☐ No > Provide information on the destination of the animals.

X Yes > Explain why it is necessary to kill the animals during or after the procedures.

Tissue and/or digesta from the gastrointestinal tract can only be sampled after euthanasia. Only a part of the animals will be euthanised. The other animals will be kept at our own research facility or sold to a commercial fattening farm.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

X Yes > Will a method of killing be used for which specific requirements apply?

X No > Describe the method of killing.

Overdose of barbiturate after sedation

☐ Yes > Describe the method of killing that will be used and provide justifications for this choice.

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

Centrale Commissie Dierproeven

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 lid2h
1.2	Provide the name of the licenced establishment.	5.1 lid2h
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 3 — Type of animal procedure: *E. coli* challenge model

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

The *E. coli* challenge model will be applied to validate the optimized nutritional strategy regarding the use of coarse raw materials in feed before and after weaning (Exp. 17). The 2 most promising nutritional strategies will be tested against a standard/control diet, resulting in a total of 3 treatments. The 2 nutritional strategies come from Exp. 5, 6 and 14 (see 3.4.1 in Project proposal) and the control will be the strategy that is currently used in praxis. The primary outcome parameter is the diarrhoea incidence after the *E. coli* challenge. Other outcome parameters are feed intake and health (to be measured as body condition, behaviour and skin condition). The *E. coli* challenge will result in an immediate drop in feed intake that typically lasts for max 7 days and an increase in diarrhoea that typically lasts max 10 days.

Piglets will be housed in groups of 3 and receive the *E. coli* inoculum using a revolver syringe to ensure each piglet receives it. Piglets will be selected based on their susceptibility towards ETEC O149:F4ac by a DNA-marker test as described in 3.1 Background of the Project proposal. In the past, we used tails for this purpose since tail docking was standard practise. However, we are already validating alternatives for the tails such as hair, oral swab (like for human DNA tests) or a piece of ear that is removed during the standard procedure of inserting the ear label needed for identification purposes. In this way, we do not need to inflict additional discomfort to the piglets for the purpose of the DNA-marker test.
Only susceptible heterozygote (RS) and susceptible homozygote (SS) animals are used in the challenge since these will show a greater response to the challenge as opposed to non-susceptible homozygote (RR) animals. This will reduce variation and, therefore, the number of animals needed. The genotype of the mother sow could also be tested but the sow is not always inseminated with semen from the same boar. Thus, the parent genotype is not suitable to select the susceptible piglets. SS piglets show the greatest response but are at

greatest risk of getting dehydrated. Therefore, RS and SS piglets will be mixed in a pen which will result in a lower response on pen level but less risk of losing piglets, and thus replicates and experimental power, during the trial.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Proposed animal procedures:

1. Before weaning, piglets will be individually ear-tagged and birth weights will be recorded. Creep feed will be given as free-choice. Piglets will be weaned at a minimum age of 21 days old into specialised housing which are cleaned before entering of a new group and which is separated from housings where the sows are kept (Council Directive 2008/120/EC).
2. Piglets will be housed in groups of 3 with a mix of RS and SS piglets.
3. Housing without bedding for maximally 6 weeks after weaning. This is required to properly assess the effects of the nutritional strategies. Consumption of bedding (typically coarse and fibrous materials) might interfere with the response leading to more variation and a higher number of animals required to show an effect.
4. Animal weighing every 7 days for a maximum of 10 weeks (before and after weaning periods).
5. Blood sampling. Maximal 8 times within 10 weeks before and after weaning with a maximum of 10 mL and 8ml/kg/14 days for young animals. Route: intravenous. Out of the 8 samples, a maximum of 2 blood samples will be taken before weaning (max 4 weeks time-period) in case samples before administration of creep feed and on the day before weaning (without weaning stress) are required. Before administration of creep feed, it is not always known which animals will be included in the experiment after weaning. Thus, a blood sample might be taken from an animal that is not used after weaning. Out of the 8 samples, a maximum of 6 samples will be taken after weaning (max 6 weeks time-period). Analysis: markers for gut permeability or inflammation.
6. *E. coli* challenge: oral route 1 ml pathogenic *E. coli* (O149K91K88ac) for maximal 3 consecutive days within the first two weeks post-weaning. Piglets will be minimally 28 days of age when receiving the *E. coli* inoculum. The *E. coli* inoculum will be provided using a revolver syringe.

Example of a timeline of the experiment:

Day in experiment	**Procedure**
Birth	Ear tag + remove piece of ear for DNA-marker test + birth weight
~10 of age	Start experimental feed + blood sample + body weight
~23 of age	Blood sample + body weight
~24 of age = day 0	Weaning into groups of 3
Day 6	Blood sample + body weight
Day 6-8	*E. coli* challenge
Day 13	Blood sample + body weight
Day 20	Blood sample + body weight
Day 27	Blood sample + body weight
Day 34	Blood sample + body weight
Day 41 (=6 weeks after weaning)	Blood sample + body weight

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

Data from studies using 3 piglets per pen were used to estimate the error variance in this specific animal facility for the period day 0-14 after weaning. This is the period where we expect most effects of the nutritional strategies and *E. coli* challenge. 500 studies are simulated in a statistical software package using relevant means ranging between 0.15 and 0.45 (i.e., 15-45% diarrhoea incidence). We aim to detect an absolute difference of 0.15 within this range of means. Previous studies with nutritional strategies have shown that we can detect this difference in our facility using this *E. coli* challenge model. The 500 simulations are, thereafter, analysed using a generalized linear mixed model (GLIMMIX) which is also the model used to analyse the data after a study is completed. The proportion of simulations showing a significant treatment effect should be >80% in order to ensure a power of 80% and a probability of 95%.
Error variance = 7.37

Means = 0.15 – 0.30 – 0.45 (relevant difference of 0.15 absolute)
Number of replicates (=pens) per treatment = 18

B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
1	Sus scrofa	Own facility	Pre-/weaned piglets	207	Males and females	No	Hypor Libra × Hypor Maxter

Provide justifications for these choices

Species	Sus scrofa is the target species
Origin	We have our own facility with a sow herd, where we breed our own piglets
Life stages	Piglets before and after weaning up to 6 weeks after weaning are the target group
Number	Post-weaning: 3 treatments (see A) × 18 pens × 3 animals = 162 piglets undergoing the *E. coli* challenge. Pre-weaning: 3 treatments × maximally 15 animals = maximally 45 animals undergoing blood sampling but not selected for the post-wean phase. At the start of the experiment, it is not always known which piglets will be included in the post-wean phase; for example, piglets of poor health or treated with antibiotics before weaning will be excluded but might have been used for blood sampling. 15 animals per treatment are required to find a relevant difference in e.g., plasma haptoglobin concentration (see Appendix 2).
Gender	Males and females are representative for the commercial situation
Genetic alterations	Not applicable
Strain	This strain is present at our own facility and representative of the commercial situation

C. Accommodation and care

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

☐ Yes

X No > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices

During the pre-weaning phase of the experiment piglets will be housed in conventional farrowing crates together with the dam.
Piglets will be housed without bedding material. Non-edible pen enrichment will be provided to allow animals to play and exhibit normal behaviour. The pen enrichment meets the requirements set by the NVWA.
The pens have a tenderfoot slatted floor.

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

X Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

X No > Justify why pain relieving methods will not be used.

Blood sampling might induce pain, but no sedation will be used. A single insertion of a needle to sample blood is not expected to cause severe pain and the duration is short.

☐ Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Describe which other adverse effects on the animals' welfare may be expected?

Due to the *E. coli* infection, all piglets will experience a period of mild diarrhoea (typically lasts max 10 days). Piglets will not get bedding material in the pens. However, we expect little adverse effects because other (non-edible) pen enrichment will be present.

Explain why these effects may emerge.

This specific *E. coli* strain causes diarrhoea especially in susceptible (RS and SS) piglets. This effect is essential for answering the research question: can our nutritional interventions reduce diarrhoea incidence?

Indicate which measures will be adopted to prevent occurrence or minimise severity.

Before the *E. coli* inoculum is provided, piglets will be weighed and their body condition judged visually by experienced personnel. If piglets have lost more than 9% of their body weighed since weaning, they will be removed from the study, moved to another department, and put on commercial feed. This is done to ensure all piglets are healthy at the start of the *E. coli* challenge.
All animals will be checked on health status twice daily in the week immediately following the *E. coli* challenge (period with highest risk). When an animal or a pen is suspected of dehydration, additional measures can be taken such as body temperature, skin pinch test, and water intake. In case animals respond with severe dehydration symptoms, electrolytes and antibiotics will be provided.

E. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question F

X Yes > Describe the criteria that will be used to identify the humane endpoints.

Body weight loss before the *E. coli* challenge.
Dehydration after the *E. coli* challenge by visual examination, body temperature, skin pinch test, water intake. Monitoring of the recovery from dehydration after the use of electrolytes and antibiotics.

Indicate the likely incidence.

Dehydration is unlikely. In the past studies (2021-2022) involving a total of 1200 animals, we did not have to euthanize an animal because of severe dehydration and lack of recovery.
In the week following the *E. coli* challenge it is possible that an animal dies. In that case the cause of death, as determined by the animal health service (Royal GD, Deventer, The Netherlands), will come back as *E. coli*. Although we increase the frequency of monitoring of the piglets after the *E. coli* challenge, it is possible that a piglet dies in that week. We expect that this is at maximum 3% of the animals. These animals will be classified as having severe discomfort.
In past studies, 6 out of 1200 piglets (0.5%) were removed before the *E. coli* challenge started because of too high weight loss. For these animals, severe discomfort was avoided.
An animal will be removed from the study when it ends up alone in a pen (penmates removed from the study because of health reasons or death).

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

1. Ear tagging: less than mild (standard commercial procedure)
2. Group housing: less than mild
3. Housing without bedding: less than mild
4. Animal weighing: less than mild
5. Blood sampling: mild
6. *E. coli* challenge: moderate

The expected level of discomfort is moderate for *E. coli* challenged animals (n=157) and maximally mild for animals only used for blood sampling pre-weaning (n=45). Severe discomfort due to the *E. coli* infection will

be prevented by monitoring the animals closely especially in the period immediately following the *E. coli* challenge. However, an animal can die in that period following the *E. coli* challenge. These animals will be classified as severe discomfort and we expect that this will be maximum 3% of the animals undergoing the *E. coli* challenge (n=5; 3% of 162).

G. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement	A response in diarrhoea incidence of an animal to a nutritional strategy cannot be determined without using animals. The physiology of a piglet, especially after weaning, cannot be modelled in in vitro or in silico systems. Organoids could be considered but organoids resemble the physiology of the state of the animal that it was made from. For example, organoids from slaughter material will resemble the physiology of the slaughtered animal and not from a weaned piglet. In order to obtain organoids from weaned piglets, piglets in this life phase need to be sacrificed. An organoid, however, is not suitable to look at the whole gastrointestinal tract, including stomach, small and large intestine, and the residing microbiota. The interaction within the gastrointestinal tract can only be studied in an animal of the appropriate age. Post-weaning diarrhoea and the stress around weaning occurs in all husbandry systems. Pathogens are present in all systems and stress will give room for health issues caused by these pathogens.
Reduction	Sample size estimations are done using data from our own facility. Per nutritional strategy, we use previous studies (outside this project proposal), literature (see 3.1 Background in the project proposal), and Exp 5, 6, and 14 to decide on the treatments to be studied. Only the most promising nutritional strategies, i.e., decided based on go/no go decisions, will be tested in this Appendix. This results in a reduction in the number of animals required for this Appendix. RS and SS animals will be used to ensure that we obtain a difference in diarrhoea incidence (=main outcome parameter). This will lower the variation and, thus, increase the power and reduce the number of animals needed to show an effect.
Refinement	Piglets will be housed in groups of 3. In case 1 piglet needs to be removed (Humane End Point) or dies, piglets are still with 2 in a pen. If a second piglets needs to be removed or dies, the remaining piglet will be removed from the study and housed with other piglets in a separate barn. In the absence of bedding, extra care will be taken that piglets have access to enrichment material at all times. The enrichment material should be manipulated, is chewable, interesting for a longer time and available for all animals in a pen (e.g., chains reaching the floor, rope, plastic toys, etc.). Animals will be checked twice daily by trained staff after the *E. coli* challenge at least until the trained staff member indicates that there are no further health issues observed. The animals will then be checked once daily again. Water and feed intake will be monitored daily at least for the first 14 days after weaning to get an indication of wellbeing. Piglets will be weighed before the *E. coli* inoculum is given. When a piglet has lost too much body weight or is in poor health before the *E. coli* challenge, the piglet is removed from the study and transferred to a standard housing pen at the same facility.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

X No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

X No > Continue with question I.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

X No

☐ Yes > Provide specific justifications for the re-use of these animals during the procedures.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

Not applicable.

J. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

X No > Continue with question K.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

X No > Provide information on the destination of the animals.

Animals will be kept at our own research facility or sold to a commercial fattening farm. Before transferring animals to another farm, an antibiotic treatment will be given at the end of the experiment to ensure that there is no *E. coli* from the challenge remaining.

☐ Yes > Explain why it is necessary to kill the animals during or after the procedures.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☐ Yes > Will a method of killing be used for which specific requirements apply?

☐ No > Describe the method of killing.

☐ Yes > Describe the method of killing that will be used and provide justifications for this choice.

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

Centrale Commissie Dierproeven

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	5.1 lid2h
1.2	Provide the name of the licenced establishment.	5.1 lid2h
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 4 — Type of animal procedure: Nitrogen balance optimal and suboptimal conditions

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

The studies described here aim to determine the effect of dietary protein level on the requirement of amino acids in pigs from 2-weeks after weaning until the end of the nursery (~25 kg body weight). This will be determined under optimal and suboptimal sanitary conditions. With the results, we will be able to optimize diet formulations for growth, health, and reducing nitrogen emissions in urine and faeces under different practically relevant conditions.
Two protein levels in combination with 5 levels of a test amino acid will be used. An indigestible marker will be included in the diets to calculate nitrogen digestibility in faeces. A total of 10 dietary treatments will be provided to piglets from 2 weeks to 6 weeks after weaning (end of the nursery). We have chosen for this timeframe since we first want to establish requirements in a period where the piglet is not facing stressors due to weaning. Subsequently, we will investigate the requirements of younger (weaning – 2 weeks after weaning) piglets. The requirements of amino acids in this period (week 2-6 after weaning) are currently extrapolated from older pigs. We hypothesize that in this period, the requirement of different amino acids changes rapidly and, therefore, we propose to determine the amino acid requirements on a weekly basis.

With the proposed diets, we can estimate the amino acid requirements in the context of a low and high protein diet by using a breakpoint plateau model for protein deposition and plasma urea nitrogen levels. Protein deposition will be determined from nitrogen (=protein) intake and nitrogen excretion through faeces and urine. For this, feed intake needs to be determined per individual piglet and faeces and urine needs to be quantitatively and accurately collected over a 3-day period of an individual piglet. Plasma urea nitrogen levels will be determined at the end of the (3-day) nitrogen balance period by collecting a blood sample.

Plasma urea nitrogen is a measure for body protein breakdown and, therefore, is an indicator for protein utilization efficiency. Thus, the main outcome parameters are protein deposition and plasma urea nitrogen.

Experiment 1. Optimal housing conditions

Piglets will be housed in groups of 3 after weaning until 2 weeks after weaning. Thereafter, 1 piglet per pen will be randomly allocated to one of the ten dietary treatments for the remaining 4 weeks of the experiment. The other 2 pigs of a pen (non-experimental animals) will be returned to the commercial herd (group housing) and sold to a commercial fattener after the nursery phase (~25 kg body weight). Pigs will be adapted to the experimental diet for 4 days which is then followed by a 3-day collection period of feed intake, faeces and urine. Feed intake will be restricted to 2.2× energy required for maintenance, which is based on body weight, (close to ad libitum feed intake at this age) to ensure a uniform intake of energy between piglets. This will reduce variation between piglets.

For the separate collection of faeces and urine, stoma bags will be attached to the rear end of the piglet for the collection of faeces. Urine will be collected in urine pans situated underneath the pens.

Piglets will receive the same diet throughout the experimental period. There will be 4 collection periods: ~d19-21, d26-28, d33-35, d40-42 after weaning which are all preceded by a 4-day adaptation period to adapt the piglets to the new feeding level. A blood sample will be taken on the last day of the collection period (4 blood samples per piglet).

Example of a timeline of the experiment:

Day in experiment	**Procedure**
~24 of age = day 0	Body weight and weaning into groups of 3
Day 7	Body weight
Day 15	Body weight and selection of animals and individual housing (removal of 2 piglets per pen: return to commercial herd)
Day 15-18	Adaptation experimental diet + feeding level
Day 19	Body weight + attachment of stoma bags for faecal collection
Day 19-21	N balance (replacing stoma bags regularly)
Day 21	Blood sample and body weight
Day 22-25	Adaptation feeding level
Day 26	Body weight + attachment of stoma bags for faecal collection
Day 26-28	N balance (replacing stoma bags regularly)
Day 28	Blood sample and body weight
Day 29-32	Adaptation feeding level
Day 33	Body weight + attachment of stoma bags for faecal collection
Day 33-35	N balance (replacing stoma bags regularly)
Day 35	Blood sample and body weight
Day 36-39	Adaptation feeding level
Day 40	Body weight + attachment of stoma bags for faecal collection
Day 40-42	N balance (replacing stoma bags regularly)
Day 42	Blood sample and body weight + end experiment return of piglets to commercial herd

Experiment 2. Suboptimal sanitary conditions

The same procedure is followed as for experiment 1 except piglets will be housed under suboptimal sanitary conditions from weaning onwards as specified in Appendix 2. The N balance period will be minimized to 2 weeks (week 3-4 after weaning) to reduce the time that piglets are housed individually and are exposed to suboptimal sanitary conditions.

Example of a timeline of the experiment:

Day in experiment	**Procedure**
~24 of age = day 0	Body weight and weaning into groups of 3 under suboptimal sanitary conditions
Day 7	Body weight
Day 15	Body weight and selection of animals and individual housing (removal of 2 piglets per pen)
Day 15-18	Adaptation experimental diet + feeding level

Day 19	Body weight + attachment of stoma bags for faecal collection
Day 19-21	N balance (replacing stoma bags regularly)
Day 21	Blood sample and body weight
Day 22-25	Adaptation feeding level
Day 26	Body weight + attachment of stoma bags for faecal collection
Day 26-28	N balance (replacing stoma bags regularly)
Day 28	Blood sample and body weight + end experiment return of piglets to commercial herd

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Proposed animal procedures:

1. Housing without bedding for the entire experiment (~6 weeks). This is required to properly assess nitrogen efficiency, since consumption of bedding material interferes with these responses.
2. Individual housing for 4 weeks (experiment 1) or 2 weeks (experiment 2) starting from 2 weeks after weaning.
3. Attachment of stoma bags for faecal collection for 4 times (experiment 1) or 2 times (experiment 2) 3 days.
4. Animal weighing at weaning and around day 7 and 14 after weaning and before and after balance period (maximally 11 times in total in 6 weeks).
5. Blood sampling: maximally 4 times within 4 weeks after weaning with a maximum 10 mL and of 8ml/kg/14 days for young animals. Route: intravenous.
6. Amino acid levels of the experimental diets may fall outside current recommendations (lower) in order to determine the optimum levels in a breakpoint analysis.
7. Management model with an average weaning age of 24 days (minimum 21 days): Factors in the management model that will be validated are e.g., reduced temperature (e.g., 2°C), increased ventilation (reduced P-band), spreading of manure, no disinfection of the unit before animals enter.
8. Feed restriction at 2.2× energy required for maintenance.

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

For nitrogen efficiency the piglet is the experimental unit. Sample size estimation for nitrogen efficiency are made for every single experiment with a power of 80% and a probability of 95%. Using a two-tailed t-test based on variation in nitrogen efficiency the sample size estimation was performed. Effect size: 5% (μ=53% nitrogen efficiency; SD 5.7%); number of piglets per treatment=10. Breakpoint analyses (regression) lowers the required samples size to n=8 per treatment.

B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
1	Sus Scrofa	Own facility	Piglets	160	Males and Females	No	Hypor Libra X Hypor Maxter

Provide justifications for these choices

Species	Sus Scrofa target species of interest
Origin	Own facility/produce own piglets from onside sow herd
Life stages	Grower-Finisher target life span with largest feed consumption
Number	160 (based on power analyses and number of studies and sample points) are considered experimental animals.
Gender	Males (the choice for one gender will not lead to a surplus in breeding stock of experimental animals because animals will be obtained from the own herd)
Genetic alterations	Not Applicable

Strain	Own facility genetics

C. Accommodation and care

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

☐ Yes

X No > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices

Piglets will be individually housed for 2 (experiment 2) to 4 weeks (experiment 1). Piglets will have visual, tactile and olfactory contact with neighbouring pens via a hole in the wall. Piglets will be housed without bedding material. Non-edible pen enrichment will be provided to allow animals to play and exhibit normal behaviour. The pen enrichment meets the requirements set by the NVWA.
The pens have a tenderfoot slatted floor.

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

X Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

X No > Justify why pain relieving methods will not be used.

Blood sampling might induce pain, but no sedation will be used. A single insertion of a needle to sample blood is not expected to cause severe pain and the duration is short.

☐ Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Describe which other adverse effects on the animals' welfare may be expected?

The pigs will not get bedding material in the pens. However, limited adverse effects are expected because pens will contain (non-edible) enrichment materials.
Pigs will be housed individually for 2 or 4 weeks, which may affect the pig behaviour.
Piglets might experience lung problems due to increased ventilation and reduced environmental temperature. The incidence of health issues (not specific for lung problems) in earlier trials with the management model was 4% of which lung problems were 0.3%. The 4% incidence of health issues is similar to other trials where no challenge was given to the animals. In literature, animals kept under low sanitary conditions for the entire fattening period (+/- 15 weeks) had higher pleuritis scores (0.3) and greater percentage of lung surface with pleuritis (1%) at slaughter (Van der Meer et al., 2016, J. Anim. Sci. 94:4704-4719). In a study with weaned piglets (4 weeks of age until 9 weeks of age), there was no difference in the incidence of veterinary treated piglets between the low and high sanitary condition (both ~4%; Van der Peet-Schwering et al., 2021, Wageningen Livestock Research, Public Report 1319). In praxis, almost 10% of the pigs at slaughter show signs of pleuritis (https://duurzaamvarkensvlees.nl/themas/smart-farming/varkenshouder-2030/gebruik-van-data/).

Explain why these effects may emerge.

Pigs are social animals and like to eat, huddle, sleep and play together with pen mates.
The effects on health from suboptimal sanitary conditions are inherent to the management model and not different from praxis.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

The time that animals will be housed individually is minimized to 2 (experiment 2) or 4 weeks (experiment 1). Furthermore, pigs are housed in pens specially designed for individual housing having an opening in the wall allowing pigs to have nose-to-nose contact with a neighbouring piglet. Floor heating below the pens, ensures the temperature in the pen is within the thermoneutral zone for piglets of a certain age.
In case of symptoms of respiratory distress, animals will be treated with antibiotics.

E. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question F

X Yes > Describe the criteria that will be used to identify the humane endpoints.

No recovery from respiratory distress. In that case, the animal will be removed from the study and transferred to standard housing if possible or humanely euthanized. An animal will also be removed from the study when it ends up alone in a pen (penmates removed from the study because of health reasons or death).

Indicate the likely incidence.

Unlikely. Literature and previous in-house trials, indicate that the management model gives similar subclinical health issues as seen in other trials.

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

Proposed animal procedures:

1. Housing without bedding material: less than mild
2. Individual housing for 2 weeks (experiment 2) or 4 weeks (experiment 1): moderate
3. Attachment of stoma bags: mild
4. Weighing: less than mild
5. Blood sampling: mild
6. Amino acid levels below recommendations: mild
7. Suboptimal sanitary conditions: mild
8. Feed restriction: mild

The expected level of discomfort is moderate for all experimental animals (n=160).

G. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement	Responses of animals towards different protein and amino acid levels under optimal and specific pathogen conditions needs to be determined in the animal itself. The used dietary protein and amino acids levels will be based on literature. In silico models will be used to assure that the desired effect will be seen. The in silico model, however, is not capable of estimating the effect of a specific pathogen on requirements. The results of the in vivo experiment will be used to improve the in silico model.
Reduction	Sample size estimations are done using data from our own facility. RS and SS animals will be used to ensure that we obtain a difference in diarrhoea incidence (=main outcome parameter). This will lower the variation and, thus, increase the power and reduce the number of animals needed to show an effect.
Refinement	Piglets are the target animals. Using other animals (mice, rats) as a model will not result in less discomfort to an individual. In addition, protein deposition is species specific. Therefore, the experimental treatments should be tested in the target animal directly. Pigs will receive non-edible enrichment material to reduce stress and expression of abnormal behaviour. Pigs are closely followed to check health and welfare issues during the experiments, with both being documented daily and medical treatments will be applied if necessary. Nitrogen efficiency will be measured indirectly using total collection of faeces and urine to reduce discomfort. Pigs are social animals and will be housed in

	groups for the first two weeks. Period and number of pigs that need to be housed individually are limited as much as possible. When housed individually nose to nose contact is still possible. Pigs are housed in pens specially designed for individual housing with floor heating ensuring the temperature is within the thermoneutral zone. Blood sampling will be performed by trained personnel. We will do whatever is possible to adapt piglets to human handling prior to the start of the experiment in order to refine the procedure of attaching the stoma bags.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

X No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

X No > Continue with question I.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

X No

☐ Yes > Provide specific justifications for the re-use of these animals during the procedures.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

Not applicable.

J. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☒ No > Continue with question K.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

X No > Provide information on the destination of the animals.

Animals will be kept at our own research facility or sold to a commercial fattening farm. Before transferring animals to another farm, an antibiotic treatment will be given at the end of the experiment to ensure that there is no *E. coli* from the challenge remaining.

☐ Yes > Explain why it is necessary to kill the animals during or after the procedures.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☐ Yes > Will a method of killing be used for which specific requirements apply?

☐ No > Describe the method of killing.

☐ Yes > Describe the method of killing that will be used and provide justifications for this choice.

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

Format Niet technische samenvatting
Let op: bij gebruik van dit word-format dient uiteindelijk alsnog het Excel-format te worden ingevuld voordat uw aanvraag vergund kan worden (zie Procesbeschrijving word-document NTS)

Tab NTS

Country	NL
Language	NL
EU submission	☒ yes ☐ no
Title of the project	Ontwikkelen van voerstrategieën die beter aansluiten bij de nutriëntbehoeftes van biggen na spenen en tevens duurzaamheid, groeiprestaties, welzijn en gezondheid bevorderen
NTS identifier	Deze wordt door de CCD ingevuld
NTS national identifier	Deze wordt door EC ingevuld
Duration of the project	60 (in months)
Keywords	
Keyword 1	Biggen
Keyword 2	Speenproblematiek
Keyword 3	Darmgezondheid
Keyword 4	Nutriëntbehoefte
Keyword 5	

Purpose(s) of the project
Objectives of the project
Describe the objectives of the project (for example, addressing certain scientific unknowns, of scientific or clinical needs). Compulsory! Maximum length is 2500 characters
Het speenproces is een stressvolle periode in het leven van biggen. Het gaat gepaard met het weghalen van biggen bij de zeug, mixen van biggen uit verschillende tomen, veranderende omgeving met de daarbij horende veranderingen in omgevingsbacteriën, en een verandering van zeugenmelk naar minder goed verteerbaar vast voer. Dit gaat vaak gepaard met een vermindering in voeropname net na spenen wat zorgt voor een verminderde functie van de darmwand. Hierdoor hebben ziekteverwekkende bacteriën kans om zich te vestigen in de darm wat vervolgens speendiarree veroorzaakt. Daarnaast is een gezonde start de basis voor een gezonder leven. Speendiarree kan worden behandeld met antibiotica, maar door het streven naar het voorkomen van antibioticaresistentie bij bacteriën moet er worden gezocht naar alternatieven zoals bijvoorbeeld via optimalisatie van voeding. Een andere managementstrategie namelijk het bijvoeren van biggen voor het spenen heeft verschillende voordelen. Voorbeelden zijn: (1) het verteringstelsel en de microbiota in de darm kunnen alvast aangepast worden aan vast voer, (2) voeropname na het spenen wordt bevorderd en (3) het risico op speendiarree wordt verminderd. Er is echter nog veel onduidelijk over wat de beste samenstelling van het voer voor spenen is wat betreft energie-, eiwit-, en vezelgehaltes. Daarnaast is het van belang dat deze bijvoeding voor spenen goed aansluit op de voeding ná het spenen om verteringsstoornissen te voorkomen. De huidige formulering van speenvoer is voornamelijk gebaseerd op gegevens verkregen bij oudere varkens. Maar de fysiologische uitdagingen van het speenproces zijn anders dan de fysiologische omstandigheden van oudere varkens. In de laatste jaren is er veel onderzoek gedaan naar de nutriëntbehoeftes van biggen na spenen. Er blijven echter nog steeds vragen over de meest optimale voeding vooral in relatie tot het gebruik van duurzamere/circulaire grondstoffen en het reduceren van de uitscheiding van bijvoorbeeld stikstof. Daarom zijn de directe doelen van dit project gericht op het ontwikkelen van voerstrategieën die beter aansluiten bij de nutriëntbehoeftes van biggen voor, tijdens en na het spenen onder zowel optimale als

suboptimale varkenshouderij omstandigheden. Binnen dit project wordt gekeken naar energiegehalte en bronnen, eiwitgehalte en aminozuursamenstelling, alsmede het bevorderen van de maagfunctie om daarmee gezondheid van de biggen te verhogen en speendiarree en sterfte rond spenen te verminderen.

Potential benefits likely to derive from this project
What are the potential benefits likely to derive from this project? Explain how science could be advanced, or humans, animals or environment may ultimately benefit from the projects. Where applicable, differentiate between short-term benefits (within the duration of the project) and long-term benefits (which may accrue after the project is finished). Compulsory! Maximum length is 2500 characters
De belangrijkste potentiële voordelen van het project is dat wij voeders kunnen ontwikkelen die beter voldoen aan de nutriëntenbehoeftes van biggen voor, tijdens en na spenen door middel van het beter begrijpen wat de behoeftes onder optimale en suboptimale varkenshouderij omstandigheden zijn. Met deze kennis kan vervolgens de vertaalslag naar praktijkomstandigheden gemaakt worden. Het ultieme doel van het project is het verhogen van de duurzaamheid van de varkenshouderij door het verlagen van de sterfte bij biggen, vermindering van het antibiotica gebruik, en door grondstoffen in het voer te verwerken zodat aan de behoeftes van de big wordt voldaan en de ontwikkeling van het maagdarmstelsel geholpen wordt. Uiteindelijk zal hierdoor ook het welzijn van de biggen verhoogd worden. In dit project zal ook inzicht verkregen worden in de werking en ontwikkeling van de maag welke tot nu toe weinig aandacht in de wetenschap heeft gekregen, maar wel een belangrijke rol speelt bij het bevorderen van de gezondheid van de big, meer specifiek het maagdarmstelsel. Alleen de voerstrategieën die geen negatieve effecten laten zien onder gecontroleerde proefomstandigheden op groeiprestatie, duurzaamheid, dierwelzijn en darmgezondheid zullen verder getoetst worden onder suboptimale varkenshouderij omstandigheden. Hiermee wordt bedoeld het nabootsen van praktijkomstandigheden op het proefbedrijf door middel van bijvoorbeeld het aanpassen van de hygiënestatus tijdens de proeven. Door te kijken naar de nutriëntensamenstelling van het voer in plaats van de grondstoffen zelf, kan ook gewerkt worden naar alternatieve voersamenstellingen waarin duurzamere/circulaire en eventueel goedkopere grondstoffen verwerkt kunnen worden zonder negatieve effecten voor de gezondheid en groeiprestaties van het dier.

Predicted harms

In what procedures will the animals typically be used
In what procedures will the animals typically be used (for example, injections, surgical procedures)? Indicate the number and duration of these procedures. Compulsory! Maximum length is 2500 characters
1. Voor spenen worden biggen gewogen en krijgen ze een oornummer. Vast voer wordt verstrekt gedurende de dag. In bepaalde studies worden biggen voor spenen gesocialiseerd door biggen van verschillende tomen samen te voegen als model voor verminderde stress na spenen. 2. Biggen worden in groepen of individueel gehuisvest afhankelijk van de onderzoeksvraag. Individuele huisvesting zal maximaal 4 weken achtereen plaatsvinden en een big kan in de periode nog steeds andere biggen zien, besnuffelen, horen en ruiken. 3. Biggen worden gehuisvest zonder bodemmateriaal voor maximaal 6 weken na spenen. Consumptie van dit materiaal kan de meting van vertering en nutriëntbenutting

beïnvloeden. Biggen krijgen wel ander verrijkingsmateriaal aangereikt om aan hun exploratie- en spelgedrag tegemoet te komen.

4. Biggen worden meerdere malen voor en na spenen gewogen.
5. Een managementmodel waarbij een suboptimale hygiënestatus wordt nagebootst. Factoren in het managementmodel zijn bijvoorbeeld de verlaging van de staltemperatuur, verhoging van de ventilatieluchtsnelheid, verspreiden van mest, en het niet desinfecteren van de ruimte.
6. Bloedafname uit de halsader voor maximaal 6 keer in 6 weken.
7. Verzamelen van mestmonsters via het stimuleren van het rectum voor maximaal 7 keer in de totale experimentele periode van 10 weken waarvan maximaal 2 keer voor spenen en maximaal 5 keer na spenen.
8. Verzamelen van urine door het opvangen van urine in bakken onder de hokken. Om besmetting van urine met mest te voorkomen zullen er stomazakjes bevestigd worden rond de anus zodat mest apart van de urine verzameld kan worden. De stomazakjes zullen maximaal 4 keer in 4 weken worden aangebracht en zullen maximaal 3 keer 24 uur blijven zitten.
9. Biggen worden gedood na verdoving om monsters uit het maagdarmkanaal te verzamelen of om weefsels te verzamelen. Biggen kunnen een voerpatroon opgelegd krijgen zodat er een continue stroom van nutriënten door de darm komt en er voldoende darminhoud aanwezig is. Biggen kunnen ook beperkt, echter wel boven onderhoudsbehoefte, gevoerd worden om de variatie in voeropname en daardoor variatie in de resultaten te verminderen.
10. Bepaalde nutriëntgehaltes in het voer kunnen afwijken van de huidige aanbevelingen zodat preciezer de behoefte ervan kan worden vastgesteld.
11. Orale toediening van de E. colibacterie voor maximaal 3 dagen op rij in 5 weken door middel van een spuit in de mondholte.

Expected impacts/adverse effects on the animals
What are the expected impacts/adverse effects on the animals for example pain, weight loss, inactivity/reduced mobility, stress, abnormal behaviour, and the duration of those effects? Compulsory! Maximum length is 2500 characters
Biggen worden gehuisvest zonder bodemmateriaal, zoals stro of zaagsel, omdat consumptie van dit materiaal de meetresultaten kan beïnvloeden en daarmee de variatie verhogen. De hokken worden uitgerust met niet eetbare hokverrijking die voldoet aan de eisen voor wat betreft manipuleerbaarheid, kauwbaarheid, interessant voor een langere periode en beschikbaar voor alle dieren in een hok. Hiermee wordt het vertonen van spel- en exploratiegedrag mogelijk en eventuele negatieve effecten van de afwezigheid van bodemmateriaal voorkomen. In bepaalde experimenten worden biggen individueel gehuisvest in hokken die speciaal zijn ontworpen voor individuele huisvesting, maar hebben dan wel de mogelijkheid tot het zien, aanraken/besnuffelen, ruiken en horen van naburige biggen. De hokken staan op hoogte en er is vloerverwarming aanwezig onder de hokken zodat de temperatuur in het hok binnen de thermo-neutrale zone voor biggen ligt. Het managementmodel levert mogelijk respiratieproblemen op maar de incidentie is naar verwachting laag (0.3% van de biggen in voorgaande proeven). Het toedienen van de E. colibacterie zal diarree veroorzaken die na een aantal dagen afneemt. De gezondheid van de biggen zal scherp gemonitord worden en waar nodig zullen biggen behandeld worden (bijvoorbeeld door het verstrekken van elektrolyten) om verdere negatieve effecten op diergezondheid te voorkomen.

Reasons for the planned fate of the animals after the procedure
Please provide reasons for the planned fate of the animals after the procedure. Compulsory! Maximum length is 2500 characters
Dieren worden gedood als het voor het beantwoorden van de onderzoeksvraag nodig is om monsters van de darminhoud te nemen. Wanneer het doden niet nodig is, zullen biggen grootgebracht worden op een commerciële varkenshouderij of op de eigen proeffaciliteit.

Application of the Three Rs

1. Replacement
State which non-animal alternatives are available in this field and why they cannot be used for the purposes of the project. Compulsory! Maximum length is 2500 characters
Verteringsprocessen in biggen kunnen deels in vitro nagebootst worden. Het in vitro verteringsmodel zal worden toegepast waar mogelijk om grondstoffen en voeders te bestuderen voordat deze worden toegepast in dierexperimenten. Echter, interacties tussen voeders en het maagdarmkanaal, bv vertering, absorptie, nutriëntenpassage door de darm en aanzuring in de maag, kunnen niet bestudeerd worden zonder gebruikt te maken van doeldieren. Met name de fysiologie van de gespeende big is complex en moeilijk na te bootsen buiten het dier. Verteringsgegevens van biggen zijn ook nodig om het in vitro verteringsmodel te valideren zodat deze verbeterd wordt voor toekomstig gebruik. Mest- en urinemonsters kunnen zonder ongerief voor het dier genomen worden. Echter deze zijn niet representatief voor wat er in de verschillende segmenten van het maagdarmkanaal, zoals de maag en de dunne darm, plaatsvindt. De toedienen van E. coli en het managementmodel zouden op commerciële bedrijven getest kunnen worden. Echter daar zijn de houderij omstandigheden variabel en tamelijk onvoorspelbaarder waardoor een hoger dieraantal nodig is vanwege een grotere variatie in de meetresultaten. Lichaamsgroei als gevolg van een voerstrategie is moeilijk middels computermodellen te simuleren omdat deze afhankelijk is van verschillende factoren vooral bij gespeende biggen gehouden onder verschillende huisvestingsomstandigheden.

2. Reduction
Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce the number of animals to be used, and principles used throughout the project to minimise the number of animals used consistent with scientific objectives. Those practices may include e.g. pilot studies, computer modelling, sharing of tissue and reuse. Compulsory! Maximum length is 2500 characters
Het aantal dieren wat gebruikt wordt per experiment wordt geminimaliseerd zoals is bepaald door middel van statistische powerberekeningen. De variatie gebruikt voor deze berekeningen is gebaseerd op eerdere soortgelijke proeven in dezelfde proeffaciliteit. Voor elk experiment zal een nieuwe power berekening gedaan worden op basis van de meest recente resultaten. Voor elke voerstrategie zal voorafgaand aan een experiment eerst de al gedane studies en literatuur bestudeerd worden en eventueel in vitro onderzoek gedaan worden om zo de meest veelbelovende strategieën te kiezen. Go/no go beslissingen worden op basis van vastgestelde criteria (dat wil zeggen groeiprestatie, dierwelzijn en gezondheid) gemaakt voordat vervolgstudies worden opgezet. Het managementmodel zal eerst gevalideerd worden voordat vervolgstudies ingezet worden.

Biggen in het E. coli model zullen geselecteerd worden op genotype en alleen de genotypen die hoog gevoelig zijn voor de specifieke E. coli zullen gebruikt worden. Dit vermindert de variatie in de resultaten waardoor uiteindelijk minder biggen nodig zijn.

3. Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms to take up emerging refinement techniques during the lifetime of the project. Compulsory! Maximum length is 2500 characters

Biggen zijn de doeldieren en hun fysiologie kan niet vergeleken worden met die van andere diersoorten of bestudeerd worden met behulp van computermodellen.
Dieren worden waar mogelijk in groepen gehuisvest. Als individuele huisvesting noodzakelijk is, zal de periode zo kort mogelijk worden gehouden. Neus-neus contact met naburige biggen is altijd mogelijk.
Biggen worden altijd verdoofd voor het doden.
Biggen krijgen geen bodemmateriaal in hun hok, maar krijgen wel te allen tijde andere verrijkingsmaterialen. Dit verrijkingsmateriaal moet, om aan de ethologische behoeften tegemoet te komen, manipuleerbaar en kauwbaar zijn en interessant voor een langere periode. Voorbeelden zijn kettingen die over de grond gaan, touw, plastic speeltjes, enz.
De gezondheid van de biggen zal dagelijks worden gecontroleerd door gecertificeerd en getraind deskundig personeel. Na het toedienen van de E. colibacterie en in het geval van het managementmodel zullen de biggen vaker gecontroleerd worden zodat kan worden ingegrepen voordat de gezondheid van de biggen het humaan eindpunt bereikt. Hiervoor zal ook de water- en voeropname dagelijks gecontroleerd worden tenminste voor de eerste 2 weken na spenen aangezien dit ook een maat is voor welzijn. Biggen die gewicht zijn verloren voordat de E. colibacterie toegediend wordt, worden uit de proef gehaald en overgebracht naar een standaardhok op dezelfde proeffaciliteit om te herstellen. Deze biggen krijgen dus geen E. colibacterie toegediend.
Biggen in het E. coli model zullen geselecteerd worden op genotype en alleen de genotypen die hoog gevoelig zijn voor de specifieke E. coli zullen gebruikt worden. Dit zorgt voor verfijning aangezien de minder gevoelige dieren niet onnodig blootgesteld worden aan de bacterie.
Standaardprocedures voor het verzamelen van mest en urine zullen gebruikt worden om variatie tussen proeven te verminderen. Voor het aanbrengen van stomazakjes rond de anus om mest te verzamelen moeten de biggen gehanteerd worden. Hiervoor zullen biggen vooraf worden gewend aan het hanteren door mensen.

Explain the choice of species and the related life stages

Compulsory! Maximum length is 2500 characters

Het speenproces bij het doeldier (varken) is uniek en kan moeilijk gesimuleerd of bestudeerd worden bij andere diersoorten. Voor de experimenten in dit project wordt de doelgroep van biggen tot 6 weken na spenen gebruikt omdat oudere varkens niet representatief zijn.

Tab Purpose of the project
Basic research: Gastrointestinal System including Liver [PB5]
Translational and applied research: Animal Nutrition [PT38]
Translational and applied research: Animal Welfare [PT34]
Choose a purpose
Choose a purpose

Tab Expected harms

What species and numbers of animals are expected to be used? What are the expected severities and the numbers of animals in each severity category (per species)?

Estimated numbers per severity				
Please fill in all categories for selected species with whole numbers (0 or higher)!				
Species	Non-recovery	Mild	Moderate	Severe
Pigs (Sus scrofa domesticus) [A14]	0	2187	317	5
Choose a species				
Choose a species				
Choose a species				
Choose a species				
Choose a species				
Choose a species				
Choose a species				
Choose a species				
Choose a species				
Choose a species				
Choose a species				
Choose a species				
Choose a species				

Tab Fate of animals kept alive

What will happen to the animals kept alive at the end of the procedure?

Estimated numbers per severity			
Please fill in all categories for selected species with whole numbers (0 or higher)!			
Species	Reused	Returned	Rehomed
Pigs (Sus scrofa domesticus) [A14]		1801	
Choose a species			
Choose a species			
Choose a species			
Choose a species			
Choose a species			
Choose a species			
Choose a species			
Choose a species			
Choose a species			
Choose a species			
Choose a species			
Choose a species			
Choose a species			

Centrale Commissie Dierproeven

Advies aan CCD

Datum 28 februari 2023
Betreft Advies Secretariaat over Aanvraag projectvergunning Dierproeven AVD202316684

Instelling:	5.1 lid2h
Onderzoeker:	5.1 lid2e
Project:	Development of nutritional strategies to better meet piglet requirements after weaning, while increasing sustainability, performance, welfare, and health.
Aanvraagnummer:	AVD202316684
Betreft:	Nieuwe aanvraag
Categorieën:	Fundamenteel onderzoek Translationeel of toegepast onderzoek

1 Inzicht in aanvraag en de eventuele knelpunten en risico's

Proces	Vragen project. - Kunt u toelichten op welke bestaande inzichten de hypothese: "the optimal dietary buffering capacity diffe[1]s in suboptimal (i.e., low sanitary conditions or high pathogenic loa[2] conditions" is gebaseerd? Het is voor de beoordeling van uw aanvraag van belang dat het nut en de noodzaak van de [3]uboptimal sanitary conditions proeven helder is onderbouwd. - De beslisstrategie omtrent go/no-go momenten is onvoldoende helder. In het projectvoorstel geeft u aan dat de go/no-go beslissingen gebaseerd zijn op een vooraf bepaald 'key performance indicat[4]s set', maar noemt enkel 3 voorbeelden van performance indicat[5]n. Kunt u de gehele set benoemen? Kunt u ook aangeven hoe deze indicatoren gewogen zullen worden? - Kunt u toelichten waarom 'Growth performance' onder 3.4.1 als typical key performance indicator wordt benoemd voor het maken van go/no-go beslissingen en op welke manier deze indicator aansluit bij de onder 3.2.1 benoemde projectdoelen? - Wij verzoeken u om in de bijlagen, onder C, te onderbouwen waarom bij de huisvesting van de dieren zal worden afgeweken van Richtlijn 2010/63/EU. - Kunt u in bijlage 3.4.3.1 ook onder C. (huisvesting en verzorging) benoemen dat een deel van de dieren voedselrestrictie zal ondergaan? [6] In het projectvoorstel staat dat zowel het E. coli challenge model als het

Overzicht van opmerkingen bij 17. AdviesNotaCCD_[redacted], d.d. 280223.pdf

Pagina: 1

Nummer: 1 Auteur: [redacted] Onderwerp: Notitie Datum: 1-3-2023 12:38:06 +01'00'
[redacted]

Status
[redacted] Geaccepteerd 3-3-2023 16:12:24 +01'00'
Auteur: [redacted] Onderwerp: Notitie Datum: 1-3-2023 14:20:50 +01'00'
Dit typ je zelf over de suboptimale condities in de samenvatting: modellen worden gebruikt om voorspellingen te doen of een voedingsstrategie in de praktijk effectief zal zijn.

Auteur: [redacted] Onderwerp: Sticky Note Datum: 3-3-2023 16:12:24 +01'00'
[redacted]

Nummer: 2 Auteur: [redacted] Onderwerp: Notitie Datum: 1-3-2023 12:08:42 +01'00'
Ik heb de vragen over de NTS er even bijgeplakt:
- Kunt u voor jargonwoorden, zoals spenen, tomen en management model toelichten wat deze betekenen?

[redacted]

- 3% van de dieren die een e-coli challenge krijgen worden zo ziek dat ze komen te overlijden. Deze oorzaak van het ernstige ongerief wordt niet benoemd in 'adverse effects' sectie. Kunt u deze sectie aanpassen zodat het voor de lezer duidelijk is dat dit challenge model ernstige gezondheidsproblemen kan veroorzaken?

Status
[redacted] Geannuleerd 3-3-2023 16:13:56 +01'00'
Auteur: [redacted] Onderwerp: Notitie Datum: 1-3-2023 12:13:59 +01'00'
[redacted] Alleen de voerstrategieën die geen negatieve effecten laten zien onder gecontroleerde proefomstandigheden op groeiprestatie, duurzaamheid, dierwelzijn en darmgezondheid zullen verder getoetst worden onder suboptimale varkenshouderij omstandigheden. Hiermee wordt bedoeld het nabootsen van praktijkomstandigheden op het proefbedrijf door middel van bijvoorbeeld het aanpassen van de hygiënestatus tijdens de proeven.

Auteur: [redacted] Onderwerp: Sticky Note Datum: 3-3-2023 16:15:21 +01'00'
[redacted]

Nummer: 3 Auteur: [redacted] Onderwerp: Markering Datum: 1-3-2023 12:39:49 +01'00'
[redacted]

Nummer: 4 Auteur: [redacted] Onderwerp: Sticky Note Datum: 3-3-2023 16:10:35 +01'00'

Nummer: 5 Auteur: [redacted] Onderwerp: Markering Datum: 1-3-2023 12:44:39 +01'00'
[redacted]

Status
[redacted] Geannuleerd 3-3-2023 16:15:48 +01'00'
Nummer: 6 Auteur: [redacted] Onderwerp: Notitie Datum: 1-3-2023 14:16:24 +01'00'
[redacted]

Auteur: [redacted] Onderwerp: Sticky Note Datum: 3-3-2023 16:20:41 +01'00'
[redacted]

	management model (suboptimale condities) de omstandigheden op commerciële bedrijven nabootsen om voorspellingen te kunnen doen over de effectiviteit van voedingsstrategieën in de praktijk. Uit uw projectaanvraag blijkt echter niet waarom het noodzakelijk is om beide modellen hiervoor te gebruiken. Kunt u toelichten welke inzichten het challenge model toevoegt aan de inzichten die verworven door middel van het management model verworven zullen worden en waarom deze relevant zijn voor het (beter) kunnen voorspellen van de effectiviteit van een nieuwe voedingsstrategie in de praktijk? - Kunt u toelichten met welke frequentie de dieren zullen worden gemonitord na het toedienen van E. coli in bijlage 3.4.3.3? Kunt u ook uitleggen waarom het niet mogelijk is om humane eindpunten te formuleren die in combinatie met scherpe monitoring kunnen voorkomen dat dieren komen te overlijden aan een E. coli infectie?

Naam proef	Diersoort	Stam	Aantal dieren	Herkomst
3.4.3.1 Nutrient utilization model				
	Varkens (Sus scrofa domesticus)	Biggen tot 6 weken na spenen	888	Dieren die voor onderzoek gefokt zijn
3.4.3.2 Management model				
	Varkens (Sus scrofa domesticus)	Biggen tot 6 weken na spenen	1.254	Dieren die voor onderzoek gefokt zijn
3.4.3.3 E. coli challenge model				
	Varkens (Sus scrofa domesticus)	Biggen tot 6 weken na spenen	207	Dieren die voor onderzoek gefokt zijn
3.4.3.4 Nitrogen balance optimal and suboptimal conditions				
	Varkens (Sus scrofa domesticus)		160	Dieren die voor onderzoek gefokt zijn

Pagina: 2

Nummer: 1 Auteur: 5.1 lid2e Onderwerp: Markering Datum: 1-3-2023 12:50:28 +01'00'

Uit

Status

5.1 lid2e Geaccepteerd 3-3-2023 16:21:43 +01'00'

Nummer: 2 Auteur: 5.1 lid2e Onderwerp: Notitie Datum: 1-3-2023 14:13:11 +01'00'

Bij de strategie staat dat er afhankelijk van de uitkomsten of voor beide modellen wordt gekozen of voor het ene model of voor het andere wordt gekozen

Status

5.1 lid2e Geaccepteerd 3-3-2023 16:26:33 +01'00'

Auteur: 5.1 lid2e Onderwerp: Sticky Note Datum: 3-3-2023 16:26:26 +01'00'

Ja, het zijn beide challenge modellen, 5.2 lid1 .

Nummer: 3 Auteur: 5.1 lid2e Onderwerp: Markering Datum: 1-3-2023 14:02:56 +01'00'

weg

Status

5.1 lid2e Geaccepteerd 3-3-2023 16:26:37 +01'00'

Nummer: 4 Auteur: 5.1 lid2e Onderwerp: Markering Datum: 1-3-2023 13:11:11 +01'00'

5.2 lid1 , staat bij D: All animals will be checked on health status twice daily in the week immediately following the *E. coli* challenge (period with highest risk).
En ook onder G bij refinement

Status

5.1 lid2e Geaccepteerd 3-3-2023 16:27:39 +01'00'

Auteur: 5.1 lid2e Onderwerp: Sticky Note Datum: 3-3-2023 16:27:43 +01'00'

5.2 lid1

Nummer: 5 Auteur: 5.1 lid2e Onderwerp: Notitie Datum: 1-3-2023 13:06:15 +01'00'

5.2 lid1

Status

5.1 lid2e Geaccepteerd 3-3-2023 16:29:57 +01'00'

Auteur: 5.1 lid2e Onderwerp: Sticky Note Datum: 3-3-2023 16:29:57 +01'00'

5.2 lid1

Huisvesting en verzorging anders dan Bijlage III Richtlijn

3.4.3.1 Nutrient utilization model

Citaat.
During the pre-weaning phase of the experiment piglets will be housed in conventional farrowing crates together with the dam.
Piglets will be housed without bedding material. Non-edible pen enrichment will be provided to allow animals to play and exhibit normal behaviour. The pen enrichment meets the requirements set by the NVWA.
The pens have a tenderfoot slatted floor.

3.4.3.2 Management model

Zie 3.4.3.1.

3.4.3.3 E. coli challenge model

Zie 3.4.3.1.

3.4.3.4 Nitrogen balance optimal and suboptimal conditions

Citaat.
Piglets will be individually housed for 2 (experiment 2) to 4 weeks (experiment 1). Piglets will have visual, tactile and olfactory contact with neighbouring pens via a hole in the wall. Piglets will be housed without bedding material. Non-edible pen enrichment will be provided to allow animals to play and exhibit normal behaviour. The pen enrichment meets the requirements set by the NVWA.
The pens have a tenderfoot slatted floor.

Gebruik van mannelijke en vrouwelijke dieren

3.4.3.1 Nutrient utilization model

Varkens (Sus scrofa domesticus)	Er worden zowel mannelijke als vrouwelijke dieren gebruikt.

3.4.3.2 Management model

Varkens (Sus scrofa domesticus)	Er worden zowel mannelijke als vrouwelijke dieren gebruikt.

3.4.3.3 E. coli challenge model

Varkens (Sus scrofa domesticus)	Er worden zowel mannelijke als vrouwelijke dieren gebruikt.

3.4.3.4 Nitrogen balance optimal and suboptimal conditions

Varkens (Sus scrofa domesticus)	Er worden zowel mannelijke als vrouwelijke dieren gebruikt.

Locatie uitvoering experimenten	- Alle proeven vinden plaats in een instelling van een vergunninghouder. - Er zijn geen problemen bekend met de vergunninghouder.

2 DEC advies

DEC-advies	Citaat C8. [...] Omdat gekozen is de dieren voortaan op eigen locatie te huisvesten in plaats van gebruik te maken van bestaande varkenshouderijen, heeft men een validatiestudie opgenomen die goed is toegelicht. Citaat C10. De dieren worden niet gehuisvest en verzorgd op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van de EU richtlijn. Tijdens de fase voorafgaand aan het spenen zijn de biggen samen met de zeug ondergebracht in conventionele kraamhokken. De biggen worden gehuisvest zonder strooiselmateriaal. Er zal wel niet-eetbare kooiverrijking zijn om de dieren in staat te stellen te spelen en normaal gedrag te vertonen. De kooiverrijking voldoet aan de eisen die de NVWA stelt. De kooien hebben een zachte tenderfoot bodem. Citaat C20. Hergebruik voor andere experimenten is niet overwogen omdat de biggen na afloop naar varkenshouderijen gaan: herplaatsing. Ethische afweging van de DEC: Citaat. 1. De morele vraag die de DEC dient te beantwoorden is of het belang van dit onderzoek, namelijk om voedingsstrategieën te ontwikkelen die beter aan de eisen voldoen van biggen voor, rond en na het spenen in optimaal en suboptimale (inclusief pathogene druk) omstandigheden met als uiteindelijke doel een bijdrage te leveren aan de verduurzaming van de varkenshouderij door antibioticagebruik en sterfte van jonge biggen te verminderen, de onvermijdelijke aantasting van het welzijn en de integriteit van de gebruikte proefdieren kan rechtvaardigen. 2. Er vindt een beperkte aantasting van welzijn en integriteit van de 2187 proefdieren plaats, met mild ongerief, voor 322 dieren kan maximaal matig ongerief optreden. Indien de hierboven genoemde doelstellingen behaald worden, dan zal dit project er toe bijdragen, dat meer kennis wordt verkregen over de ontwikkeling van maag/darmstelsel van jonge biggen en de invloed van voeder/nutriënten rondom de speenleeftijd. Ook zal met verbeterde voedingsstrategieën een duurzamer houderijsysteem kunnen worden ontwikkeld. Het is aannemelijk dat de fundamentele en translationele doelstelling behaald zal worden. Daarvoor is de inzet van proefdieren noodzakelijk, maar de onderzoekers doen al het mogelijke om het ongerief voor de dieren en het aantal dieren tot een minimum te beperken. Dat het voor de instelling van belang kan zijn om daarmee

	commerciële voeders te ontwikkelen is juist, maar in de uiteindelijke afweging kent de DEC daar geen gewicht aan toe. Omdat de aanvrager heeft besloten eigen stallen te ontwikkelen om het onderzoek 'in huis' te kunnen uitvoeren, is een eenmalige validatie studie noodzakelijk. De DEC heeft dit meegenomen in haar afweging. 3. Op grond van het bovenstaande is de DEC van oordeel dat het doel om voedingsstrategieën te ontwikkelen die beter aan de eisen voldoen van biggen voor, rond en na het spenen in optimaal en suboptimale (inclusief pathogene druk) omstandigheden met als uiteindelijke doel een bijdrage te leveren aan de verduurzaming van de varkenshouderij door antibioticagebruik en sterfte van jonge biggen te verminderen een reëel belang vertegenwoordigt en dat dit reële belang opweegt tegen de beperkte aantasting van het welzijn en de integriteit van de proefdieren. De relatie tussen het directe en het uiteindelijk doel is voldoende helder. Het is aannemelijk dat de directe doelstelling behaald zal worden. De commissie is overtuigd van de kwaliteit van het werk van de aanvrager. De aanvrager heeft voldoende aannemelijk gemaakt, dat er geen geschikte vervangingsalternatieven zijn, dat het doel niet met minder dieren behaald kan worden, dat de gebruikte aanpak de meest verfijnde is en dat er geen sprake zal zijn van onbedoelde negatieve effecten voor mens, dier en milieu als gevolg van de dierproeven. Het gebruik van de proefdieren zoals beschreven in de aanvraag is daarmee gerechtvaardigd. De DEC heeft extern advies ingewonnen bij - de aanvrager is om aanvullingen gevraagd 1 Het DEC advies is Positief Het uitgebrachte advies is gebaseerd op consensus.

Pagina: 5

Nummer: 1 Auteur: 5.1 lid2e Onderwerp: Notitie Datum: 1-3-2023 14:25:24 +01'00'

Wat is de strekking van deze vragen?

Status

5.1 lid2e Geaccepteerd 3-3-2023 16:31:09 +01'00'

Auteur: 5.1 lid2e Onderwerp: Sticky Note Datum: 3-3-2023 16:31:05 +01'00'

5.2 lid1

3 Kwaliteit DEC advies

Kwaliteit DEC-advies	
Het DEC advies is niet geheel helder en navolgbaar. In het advies is op heldere wijze inzicht gegeven in de vragen die aan de aanvrager zijn gesteld en bij de beantwoording van de beoordelingsvragen verstrekt u een heldere uiteenzetting. Bij C10 hadden wij echter graag ook een oordeel teruggezien over de onderbouwing voor afwijkende huisvesting van de dieren. De ethische afweging volgt op logische wijze uit de beantwoording van de C vragen, maar gaat voorbij aan het ernstige ongerief dat de dieren in bijlage 3 zullen ondergaan. In verband met dit ernstige ongerief is de beoordeling achteraf een vereiste voorwaarde. Het project richt zich op voeding voor biggen in de veehouderij. Daarmee speelt ethische dilemma rondom de veehouderij ook bij dit project. 5.2 lid1 5.2 lid1	

4 Inhoudelijke beoordeling

Doelstelling	Citaat.
Doelstelling	The ultimate goal of this project is to develop nutritional strategies that will better meet the requirements of piglets before, around, and after weaning in optimal and sub-optimal (including pathogenic pressure) environments. With these nutritional strategies, we aim to increase the sustainability of pig production by helping to reduce mortality rate and the use of antibiotics, by using raw materials that meet animal requirements, by supporting feed intake and gastrointestinal tract development around weaning, and by improving animal welfare for example by reducing abnormal behaviour (tail, biting, belly nosing). The immediate goals are: 1. To validate a management model that simulates commercial conditions but in a controlled environment (Appendix 2). 2. To determine the optimal lysine to energy ratio and energy level in piglets after weaning (Appendix 1). 3. To determine the effect of creep feed, fatty acid level and fatty acid composition on fat digestibility, bile acid production, enzyme secretion, gastrointestinal development, and blood lipid metabolite profile after weaning (Appendix 1). Socializing the piglets before weaning might be used as model to reduce weaning stress and, thus, to disentangle the effect of stress from feed transition (sow milk to solid feed) from social stress (mixing of piglets) on fat digestibility. The optimal fatty acid level and fatty acid composition will also be determined under suboptimal sanitary conditions (Appendix 2) in order to translate the knowledge into

Pagina: 6

Nummer: 1 Auteur: 5.1 lid2e Onderwerp: Notitie Datum: 1-3-2023 14:28:48 +01'00'

5.2 lid1

Status

5.1 lid2e Geaccepteerd 3-3-2023 16:35:16 +01'00'

Nummer: 2 Auteur: 5.1 lid2e Onderwerp: Notitie Datum: 3-3-2023 16:36:01 +01'00'

het

Status

5.1 lid2e Geaccepteerd 3-3-2023 16:36:08 +01'00'

Nummer: 3 Auteur: 5.1 lid2e Onderwerp: Markering Datum: 1-3-2023 14:31:12 +01'00'

5.2 lid1

Status

5.1 lid2e Geaccepteerd 3-3-2023 16:36:12 +01'00'

Nummer: 4 Auteur: 5.1 lid2e Onderwerp: Markering Datum: 1-3-2023 14:30:20 +01'00'

5.2 lid1

Status

5.1 lid2e Geaccepteerd 3-3-2023 16:36:24 +01'00'

	practical conditions. 4. To determine the optimal ratio between unsaturated and saturated (U:S ratio) fatty acids on fat digestibility under optimal (Appendix 1) and suboptimal sanitary conditions (Appendix 2). 5. To determine the interaction between dietary protein level and specific amino acids requirements under optimal and suboptimal sanitary conditions. Nitrogen balance experiments will be done to determine protein deposition under optimal and suboptimal sanitary conditions (Appendix 4). The optimal protein and amino acid levels will be verified in a growth performance trial under optimal (no animal experiment) and suboptimal sanitary conditions (Appendix 2). 6. To determine the interaction between feed particle size and the optimal inclusion level of coarse raw materials in the diet given before and after weaning on stomach and intestinal development and nutrient digestibility after weaning under optimal (Appendix 1), suboptimal sanitary conditions (Appendix 2) and a specific pathogen challenge (E. coli challenge model; Appendix 3). 7. To determine the effect of feed form (mash, pellet, crumble, extruded feed) and transition between feed forms at weaning on gastrointestinal development and health (Appendix 1). 8. To determine the optimal buffering capacity in diets for piglets after weaning under suboptimal sanitary conditions (Appendix 2) conditions. Optimal levels under optimal conditions were already established in an in-house study. 9. To determine the interactive effect between buffering capacity and feed particle size distribution on gastrointestinal development and health (Appendix 1). Next to the response parameters specified in the appendices, we will also evaluate sustainability metrics when applicable. The metrics are divided in diet-related and animal-related metrices. Diet-related: resource (use of fossil fuel) and water use, environmental acidification, nitrogen and phosphorus excretion during pig production, CO2 emission from transport of raw materials or from raw material production itself. Animal-related: behaviour scores, body condition scores, mortality, and morbidity.

Wetenschappelijk en maatschappelijk belang	Citaat. The scientific relevance of the immediate goals is to better understand the piglet's requirements around weaning which is one of the most stressful events in a pig's life. The lack of understanding of the physical state and the variable response to weaning of the post-weaning piglet makes it challenging for nutritionists to establish an optimal diet. However, there are certain aspects that are similar between piglets: they all need energy to survive, nutrients to develop their gastrointestinal tract, and need adequate nutrition to combat pathogens and stay healthy. Moreover, changes in legislation, e.g., the expected ban on tail docking and use of in-feed antibiotics and pharmacological levels of zinc oxide, stress the need to understand the nutritional requirements of piglets in this phase, also to prevent damaging behaviours. Nutrient requirements for weaned piglets are mostly extrapolated from older pigs and we have just started to unravel the complex physiology of a piglet around weaning and its accompanying nutrient requirements. Results from the immediate project goals will be used to further optimize piglet feeding strategies internally but our results will also be published in scientific journals. Regarding the social relevance of the ultimate goal, main aspects are to further reduce reliance on antibiotics for pigs in order to maintain future availability of antibiotics for human medicine. Secondly, this project will contribute to the use of non-human-edible feed ingredients (e.g., co-products from food production) for feed, leaving human-edible ingredients available as food.
Onderbouwing wetenschappelijk en maatschappelijk belang	Het belang is voldoende uitgewerkt.

Wetenschappelijke kwaliteit Kwaliteit aanvrager/ onderzoeksgroep en onderzoek	Citaat DEC advies C7. De kennis en kunde van de onderzoeksgroep en andere betrokkenen bij de dierproeven zijn voldoende gewaarborgd en dragen eraan bij dat de doelstellingen behaald kunnen worden, dat aan de 3V-beginselen voldaan kan worden en dat voorkomen kan worden dat mens, dier en milieu negatieve effecten ondervinden als gevolg van de dierproeven. De instelling is een internationaal diervoedingsbedrijf dat voedingsadditieven en voedingsstrategieën ontwikkelt voor de meeste diersoorten. Het bedrijf heeft de ambitie om een wereldleider te zijn op dit gebied en focussed duurzame veehouderij, zoals het verminderen van antibioticagebruik, het verbeteren van de gezondheid en het welzijn van dieren en het verminderen van de milieubelasting van de veehouderij door bijvoorbeeld het verminderen van nutriëntenverliezen in urine en uitwerpselen en het verminderen van emissies in verband met de productie. Het bedrijf wil kennis vergaren over de afgifte van voedingsstoffen in de darm en een betere vertering en gebruik van grondstoffen met een lagere beschikbaarheid/ verteerbaarheid van voedingsstoffen. Het bedrijf heeft een speciale R&D-afdeling met onderzoeksteams voor alle soorten in omvang, inclusief varkens. Elk soortenteam heeft ongeveer 6 onderzoekswetenschappers die een doctoraat hebben met betrekking tot dier- of diergeneeskunde en ze onderhouden een breed internationaal wetenschappelijk netwerk. De studies zullen worden uitgevoerd in het eigen onderzoekscentrum in Nederland, waar is geïnvesteerd in een state-of-the-art unit en waar proeven kunnen worden uitgevoerd die specifieke omgevingscondities nabootsen en beheersen. Hoge bioveiligheids- en hygiënenormen worden gehandhaafd om personeel en dieren te beschermen. Dagelijkse verzorging van dieren, metingen en experimentele technieken worden uitgevoerd door gecertificeerd competent en ervaren personeel. Projectresultaten zullen worden verspreid via wetenschappelijke tijdschriften en conferenties en uiteindelijk worden vertaald in voedingsstrategieën, die worden toegepast via het wereldwijde netwerk van voedingsdeskundigen. [1]

3V's

Vervanging

Nummer: 1 Auteur: 5.1 lid2e Onderwerp: Notitie Datum: 1-3-2023 11:41:35 +01'00'

5.2 lid1

Status

5.1 lid2e Geaccepteerd 3-3-2023 16:37:02 +01'00'

	3.4.3.1 Nutrient utilization model: Citaat. We have an in-house in vitro digestion kinetics model in which we can screen raw materials and diets. Wherever possible, first a screening with this model will be done in order to select the raw materials and diets to be tested in in vivo studies. Interactions between raw materials and /or nutrients on e.g., digestibility and nutrient utilization, passage through the gastrointestinal tract, and acidification in the stomach cannot be studied outside animals. Especially not in a weaned piglet where digestive physiology changes rapidly due to weaning. Animal data is also needed to validate the in vitro digestion kinetics model. Faecal samples could be obtained non-invasively but are not representative for what is happening in different parts of the gastrointestinal tract. For this project, the stomach and small intestine are the most important parts of the gastrointestinal tract. Changes in digestive physiology and the stress around weaning occurs in all husbandry systems.
	3.4.3.2 Management model: Citaat. A response in growth of an animal to a nutritional strategy cannot be determined without using animals. The physiology of a piglet, especially after weaning, and its response to suboptimal environmental conditions cannot be modelled in in vitro or in silico systems. Suboptimal conditions are common in commercial husbandry, but the parameters are not controlled. In order to study the response to nutritional strategies in a reliable and repeatable way, we need controlled conditions such as those obtained with the management model.
	3.4.3.3 E. coli challenge model: Citaat. A response in diarrhoea incidence of an animal to a nutritional strategy cannot be determined without using animals. The physiology of a piglet, especially after weaning, cannot be modelled in in vitro or in silico systems. Organoids could be considered but organoids resemble the physiology of the state of the animal that it was made from. For example, organoids from slaughter material will resemble the physiology of the slaughtered animal and not from a weaned piglet. In order to obtain organoids from weaned piglets, piglets in this life phase need to be sacrificed. An organoid, however, is not suitable to look at the whole gastrointestinal tract, including stomach, small and large intestine, and the residing microbiota. The interaction within the gastrointestinal tract can only be studied in an animal of the appropriate age. Post-weaning diarrhoea and the stress around weaning occurs in all husbandry systems. Pathogens are present in all systems and stress will give room for health issues caused by these pathogens

	3.4.3.4 Nitrogen balance optimal and suboptimal conditions: Citaat. Responses of animals towards different protein and amino acid levels under optimal and specific pathogen conditions needs to be determined in the animal itself. The used dietary protein and amino acids levels will be based on literature. In silico models will be used to assure that the desired effect will be seen. The in silico model, however, is not capable of estimating the effect of a specific pathogen on requirements. The results of the in vivo experiment will be used to improve the in silico model.

Verminderen	
	3.4.3.1 Nutrient utilization model: Citaat. Sample size estimations are done using data from our own facility. Per nutritional strategy, we use previous studies and literature data (see 3.1 Background in the project proposal) to decide on the treatments to be studied. Go/no go decisions per nutritional strategy ensures that only the most promising strategies will be tested in follow-up studies, allowing for the reduction in the number of animals potentially required.
	3.4.3.2 Management model: Citaat. Sample size estimations are done using data from our own facility. Per nutritional strategy, we use previous studies and literature (see 3.1 Background in the project proposal) to decide on the treatments to be studied. The model will be validated first and a go/no go decision will follow before continuing with the other experiments (12-16). Go/no go decisions per nutritional strategy ensures that only the most promising strategies will be tested in challenge studies, allowing for the reduction in the number of animals potentially required.
	3.4.3.3 E. coli challenge model: Citaat. Sample size estimations are done using data from our own facility. Per nutritional strategy, we use previous studies (outside this project proposal), literature (see 3.1 Background in the project proposal), and Exp 5, 6, and 14 to decide on the treatments to be studied. Only the most promising nutritional strategies, i.e., decided based on go/no go decisions, will be tested in this Appendix. This results in a reduction in the number of animals required for this Appendix. RS and SS animals will be used to ensure that we obtain a difference in diarrhoea incidence (=main outcome parameter). This will lower the variation and, thus, increase the power and reduce the number of animals needed to show an effect.
	3.4.3.4 Nitrogen balance optimal and suboptimal conditions: Citaat. Sample size estimations are done using data from our own facility. RS and SS animals will be used to ensure that we obtain a difference in diarrhoea incidence (=main outcome parameter). This will lower the variation and, thus, increase the power and reduce the number of animals needed to show an effect.
Verfijnen	

	3.4.3.1 Nutrient utilization model: Citaat. Piglets after weaning are the target animals and their physiology cannot be obtained in other species or in models. Animals will be sedated before euthanasia. In the absence of bedding, extra care will be taken that piglets have access to enrichment material at all times. The enrichment material should be manipulated, is chewable, interesting for a longer time and available for all animals in a pen (e.g., chains reaching the floor, rope, plastic toys, etc.). Additional health checks will be done for experiment 2 when for the treatment where feed intake is restricted. Standard operating procedures will be used for faeces collection to reduce variation between studies.
	3.4.3.2 Management model: Citaat. Piglets after weaning are the target animals and their physiology cannot be obtained in other species or in models. Animals will be group housed. Animals will be sedated before euthanasia. In the absence of bedding, extra care will be taken that piglets have access to enrichment material at all times. The enrichment material should be manipulated, is chewable, interesting for a longer time and available for all animals in a pen (e.g., chains reaching the floor, rope, plastic toys, etc.). Animals will be checked daily by trained staff. Water and feed intake will be monitored daily at least for the first 14 days after weaning to get an indication of wellbeing. The goal of the management model is to get a subclinical immune response and it is, therefore, not a disease challenge model. In case of signs of disease, animals will be treated and if needed removed from the study (Humane Endpoint).