	Inventaris Wob-verzoek W17-09								
		wordt verstrekt				**weigeringsgronden**			
nr.	**document**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
	NTS2017874								
1	Aanvraagformulier				x		x	x	
2	Projectvoorstel oud			x					
3	Niet-technische samenvatting	x							
4	Bijlage beschrijving dierproeven 1 oud			x					
5	Bijlage beschrijving dierproeven 2 oud			x					
6	Bijlage beschrijving dierproeven 3 oud			x					
7	Bijlage beschrijving dierproeven 4 oud			x					
8	Ontvangstbevestiging				x		x	x	
9	Aanvulling aanvraag				x		x	x	
10	Projectvoorstel nieuw			x					
11	Bijlage beschrijving dierproeven 1 nieuw			x					
12	Bijlage beschrijving dierproeven 2 nieuw			x					
13	Bijlage beschrijving dierproeven 3 nieuw			x					
14	Bijlage beschrijving dierproeven 4 nieuw			x					
15	DEC-advies				x		x	x	
16	Advies CCD		x						x
17	Beschikking en vergunning				x		x	x	

1.

20 FEB 2017

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.zbo-ccd.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* 10400 874

☐ Nee > *U kunt geen aanvraag doen*

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

Naam instelling of organisatie	Wageningen University	
Naam van de portefeuillehouder of diens gemachtigde	[redacted]	
KvK-nummer	9215846	
Straat en huisnummer	Akkermaalsbos	12
Postbus	59	
Postcode en plaats	6700 AB	Wageningen
IBAN	NL10 RABO 0397066465	
Tenaamstelling van het rekeningnummer	Wageningen UR	

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	[redacted]	☐ Dhr. ☒ Mw.
Functie	Onderzoeker	
Afdeling	[redacted]	
Telefoonnummer	[redacted]	
E-mailadres	[redacted]	

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

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

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 - 5 - 2017
	Einddatum: 30 - 11 - 2017
3.2 Wat is de titel van het project?	Influence of solid feed intake on digesta passage rate and phosphorus requirements of veal calves
3.3 Wat is de titel van de niet-technische samenvatting?	Invloed van ruw- en krachtvoer op fosforbehoefte vleeskalveren

3.4 Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?

Naam DEC	DEC-WUR
Postadres	Postbus 9101, 6700 HB Wageningen
E-mailadres	dec@wur.nl

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

☒ Nieuwe aanvraag Projectvergunning € 1684 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

☒ Bestel order wordt nagestuurd.

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	[redacted]
Functie	[redacted]
Plaats	Wageningen
Datum	21 - 2 - 2017
Handtekening	[redacted]

2.

Form
Project proposal

- This form should be used to write the project propsal of 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 our website(www.zbo-ccd.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10400
1.2	Provide the name of the licenced establishment.	Wageningen University
1.3	Provide the title of the project.	Influence of solid feed intake on digesta passage rate and phosphorus requirements of veal calves

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 of routine production
[] Research into environmental protection in the interest of human or animal health or welfare dier
[] 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.

- For legally required animal procedures, indicate which statutory or regulatory requirements apply (with respect to the intended use and market authorisation).
- For routine production, describe what will be produced and for which uses.
- For higher education or training, explain why this project is part of the educational program and describe the learning targets.

Veal calves are traditionally fattened on a diet consisting only of milk replacer (MR). From a welfare and economic perspective, there is a strong incentive to replace a considerable portion of the MR by solid feeds (SF) in the diet (see e.g. Webb et al., 2015). Therefore, SF comprising roughages and concentrates, represent an increasingly important source of nutrients for veal calves. However, veal calves will still be fed a proportion of MR in their diet for approximately 9 months of age to achieve the paleness of white veal meat.
Interactions between MR and SF, mostly occurring in the gastro-intestinal tract, complicate the prediction of the nutritional value of these ration components. Quantitative information about passage rate kinetics of SF through the rumen and other gastro-intestinal compartments currently hampers progress in this field. Limited information available (Berends et al., 2015a) indicates that the level of SF feeding affects ruminal passage rates, more so for concentrates compared with straw, with estimates of mean retention time being considerable higher than in calves exclusively fed on SF. This impacts the nutritional value of SF and also the potential of nutriënt recycling via the rumen. Studies for measuring passage rate kinetics traditionally involve recovery of indigestible tracer inside various compartments of the gastro-intestinal tract (e.g. Berends et al. 2015a), or faecal excretion curves of indigestible markers. These techniques involve sacrificing experimental animals, thus preventing repeated measures on a subject, and/or individual housing on balance cages. Novel technologies to measure passage rate kinetics include the measurement of recovery of 13C tracers in breath (e.g. meCue and Welch, 2016) but these techniques have not been tested and validated in calves.
Recycling of nutrients from the MR back into the rumen has been demonstrated for nitrogen in many types of ruminant animals, including veal calves (Berends et al., 2014, 2015b). Also for phosphorus (P), recycling from blood, via saliva, back into the rumen has been demonstrated to occur in ruminants. Like with nitrogen, exploiting this potential can contribute to the P economy of the calf. Typically, MR ingredients are rather rich in P. Upon consumption, MR flows directly to the abomasum, i.e. bypassing the rumen, and from there to the small intestine. P absorbed from the MR thus enters the systemic circulation. Through saliva, P can recycle back into the rumen, thus providing P originating from MR with a second chance to be utilized. Currently, P contents of de SF portion of the veal calf diet are not optimized to take this type of recycling into account. Hence there is an opportunity to reduce the P content of SF, thereby contributing to steering or reducing in P excretion into the environment. For the Netherlands, a reduction of P excretion is vital to arrive below the maximum set by EU regulations.

Literature

Berends, H., van den Borne, J. J. G. C., Røjen, B. A., van Baal, J. & Gerrits, W. J. J. 2014. Urea Recycling Contributes to Nitrogen Retention in Calves Fed Milk Replacer and Low-Protein Solid Feed. The Journal of Nutrition. 144, 7, p. 1043-1049

Berends, H., van den Borne, J. J. G. C., Stockhofe-Zurwieden, N., Gilbert, M. S., Zandstra, T., Pellikaan, W. F., van Reenen, C. G., Bokkers, E. A. M. & Gerrits, W. J. J. 2015a. Effects of solid feed level and roughage-to-concentrate ratio on ruminal drinking and passage kinetics of milk replacer, concentrates, and roughage in veal calves. Journal of Dairy Science. 98, 8, p. 5621-5629

Berends, H., van den Borne, J. J. G. C., Røjen, B. A., Hendriks, W. H. & Gerrits, W. J. J. 2015b. Effect of protein provision via milk replacer or solid feed on protein metabolism in veal calves. Journal of Dairy Science. 98, 2, p. 1119-1126.
McCue, MD and Welch KC jr. 2016. 13C breath testing in animals: theory, applications and future directions. J. Comp. Physiol. B (2016) 186: 265-285.

Webb, L. E., van Reenen, C. G., Berends, H., Engel, B., de Boer, I. J. M., Gerrits, W. J. J. & Bokkers, E. A. M. 2015. The role of solid feed amount and composition and of milk replacer supply in veal calf welfare. Journal of Dairy Science. 98, 8, p. 5467-5481

3.2 Purpose

Describe the project's main objective and explain why this objective is achievable.

- If the project is focussed on one or more research objectives, which research questions should be addressed during this project?
- If the main objective is not a research objective, which specific need(s) does this project respond to?

In this project, we will address three important research questions:
1) Can digesta passage rate kinetics in calves (with emphasis on the rumen) be measured using non-invasive 13C tracer breath test approach?
2) What is the effect of SF intake and roughage to concentrate ratio on passage rate kinetics of MR and concentrates
3) What are the P requirements of calves fed rations with different MR to SF ratios and different SF compositions (i.e., different concentrate to roughage ratios).
The underlying issue of the latter 2 questions is to what extent can we exploit P recycling as a mechanism to steer/reduce P excretion in veal calves fed combinations of SF and MR.

3.3 Relevance

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

Inside the various compartments of the gastro-intestinal tract, there is always competition between passage and degradation of macronutrients. With increasing passage rate within a compartment, time for degradation decreases, hence reducing the efficiency of nutrient digestion in that compartment. Depending on the rate of feed intake, this reduced efficiency may lead to a decrease in nutriënt absorption or not. For predicting the feeding value of MR, concentrates and roughages (together comprising the SF component of the diet) it is pivotal to know passage rate kinetics. Such information typically is integrated into mathematical models predicting nutriënt absorption following the provision of various dietary regimes. Such models are already available for pigs, dairy cows and for calves fed exclusively on milk replacers. Currently, a calf model is being developed

integrating rumen development, rumen function and post-absorptive nutriënt metabolism. This model will be used for designing and evaluation of feeding strategies in veal calves, fed various combinations of MR and SF. Reliable information about passage rate kinetics of the different ration components has been identified as limiting the quality of such models. Current approaches to the measurement of passage rate kinetics involve the use of indigestible marker techniques, either requiring prolonged individual housing, or sacrificing of experimental animals, the latter preventing repeated measures on a subject. Novel, minimally invasive, 13CO2 breath test approaches could, in part, replace such approaches, but need to be developed and tested.

Reducing P excretion in the environment has been identified as an important target by the Dutch government, as P excretion has exceeded the limits set by EU in 2015. For extending the derogation for 2017 and beyond, prevention of P excretion exceeding these limits is of utmost importance. Furthermore, reducing P inputs (via dietary manipulation) will also contribute to a sustainable use of resources. This research will contribute positively to control both P inputs and P excretion. First, as mentioned previously, MR ingredients are rather rich in P. This, combined with the recycling of P in the rumen, provides the opportunity to reduce the P content of SF, thus reducing P inputs. Second, by determining the P requirements of calves, we are able to control the P excretion. Third, integrating knowledge into mathematical models will allow more accurate predictions of nutriënt use and environmental excretion under widely varying nutritional regimes.
Moreover, this research will contribute to less invasive measurements (increased refinement) of passage rate kinetics in future experimental animals.

3.4 Research Strategy

3.4.1 Provide an overview of the overall design of the project (strategy).

Within the present project we aim to quantify passage rate kinetics of MR, concentrates and roughages under 4 different dietary regimes. We will also determine P requirements in calves under the same 4 dietary regimes. As adaptation of calves to these dietary regimes will take time (about 4 weeks), we will minimize the number of calves required by performing a series of experiments, using the same calves. First, a pilot experiment will be performed (see section 3.4.2 and annex 1) to determine whether P requirements can be determined for individual calves with a step-wise within animal dose response approach, similar to Kampman van de Hoek et al. (2012) for studying limiting amino acids in pigs. The principle of the technique is that after providing an excess of P via MR, the P intake will be reduced via MR, leading to a reduction of P excreted via urine. The inflection point of the P-excretion curve against P intake will be assessed via nonlineair regression and assumed to represent the requirement of that calf. The plateau value, obtained at the lowest P intake will represent the maximum efficiency of P utilization, likely influenced by SF intake.
The advantage of such a technique is that it potentially provides estimates of P requirements of individual calves. For the pilot study, only the rate of adaptation of urinary P excretion following a substantial reduction in P intake via the MR will be monitored in a limited number of calves. If the rate of urinary P exretion stabilizes within 72h after the reduction in dietary P supply, the technique can be considered applicable for individual calves. If

this is the case, the different dietary regimes will be imposed on calves, subsequently followed by two studies to investigate passage rate kinetics of MR, concentrates and roughages (see section 3.4.2 and both annex 2 and 3), followed by a study to quantify P requirements under these nutritional conditions in each calf (see section 3.4.2 and annex 4). If the within-calf dose response technique does not work, a more traditional, between animal, dose response approach will be used (see e.g. Erickson et al., 2002), comparing P excretion at different levels of intake between animals. Hence, this project comprises 4 studies, with studies 2, 3, and 4 (annex 2, 3, and 4, respectively) being performed with the same calves.
The calves in the pilot study (annex 1) will be about 6 to 8 weeks of age. The age of the calves at each of the 3 consecutive studies (annex 2, 3, and 4) will differ, as these studies are performed sequentially, (re-)using the same 48 calves. These studies will be performed in six batches. At start of the first passage rate kinetics study (annex 2), calves of approximately 6 weeks of age will be purchased from a veal producer. The first batch of calves (n=8) will adapt to the diet for 5 weeks, subsequently followed by 7 days of measurements in the respiration chambers, and subsequently followed by the second passage rate kinetics study (annex 3) and the P requirement study (annex 4). For batch 2 to 6, the same procedure will be used, with a delay of about 1 week for each batch. Hence, the results will be obtained over an age-range of about 6 weeks. The common interest in these three studies is the combination of the nutritional treatments, of which it takes time for calves to adapt (rumen development). With discomfort for the calves estimated as mild for the studies in annex 2 and 3, we consider it the best option. to re-use calves from the study in annex 2 for annex 3 and 4. As argued in annex 2 and 4, the number of calves required is approximately the same. For the studies in annex 2 and 3, comparison of observations on the same animals is important.

Literature

Erickson GE, Klopfenstein TJ, Milton CT, Brink D, Orth MW and Whitted KM. 2002. Phosphorus requirements of finishing feedlot calves.. J. Anim,. Sci. 80(6) 1690-1695.
Kampman-van de Hoek, E, Gerrits, WJJ, van der Peet-Schwering, CMC Jansman, AJM and van den Borne, JJGC 2013. A simple amino acid dose-response technique to quantify amino acid requirements of individual meal-fed pigs Journal of Animal Science. 91, 10, p. 4788-4796

3.4.2 Provide a basic outline of the different components of the project and the type(s) of animal procedures that will be performed.

As described above, the project will consist of 4 studies:

1. P requirements in veal calves - a pilot study. The objective of this study is to test whether a within-animal approach can be used to study P requirements. The outcome will determine the design of study 4. This study will be conducted with calves, individually housed on balance cages and provided with faecal collection bags to allow separate, quantitative collection of urine and faeces. The study duration will be 4 days (adaptation) and 6 days (experiment)
2. Passage rate kinetics in veal calves - 13C tracer technique: Minimally invasive technique with stable isotope methods. This technique is based on a breath-test approach described by Van den Borne et al. (2015), and techniques widely used in humans and animals (MCue and Welch, 2016). Briefly, following a pulse dosis of a 13C tracer, 13C labelled CO2 is collected in exhaled air. If the tracer is well chosen, the exhalating pattern of the tracer will reflect the pattern of nutriënt absorption and metabolism of the tracee. As the primary interest is in the timing of nutrient absorption, metabolism patterns as well as delays in exhalation caused by dilution in body pools need to be corrected for. For this study, calves will be purchased at an age of 6 weeks and adapted to the nutritional treatments for a period of 5 weeks in group-housing. The measurements will be conducted in pair-housed calves in climatized respiration chambers, frequently fed a SF mix (i.e., 6 times a day to create a steady state), and fed a MR twice daily. Different isotope tracers will be administered through the diet, spread over the 7d experimental period.
3. Passage rate kinetics in veal calves - faecal excretion curves. Faecal excretion patterns of indigestible markers in faeces following a pulse dose of markers representative for MR, concentrates and roughages. Following the approach described by Dhanoa et al., (1985). Briefly, this approach is based on the assumption that excretion kinetics of an indigestible marker will reflect retention time of digesta in the slowest compartment. This study will be conducted in a setting similar to study 1, with frequent faecal collections following the pulse-dosed markers. The length of the adaptation and experiumental periods will be 4 and 5 days, respectively.
4. P requirements in veal calves fed different rations. The approach taken will depend on the outcome of the pilot. In the case the conventional approach will be used, the number of dietary regimes to be tested will be reduced from 4 to 2. This study will be conducted in a setting similar to study 1 and will last 21 days. In the case of a conventional approach, calves will be subjected to low P diets for 4 days, after which P excretion will be performed for a period of 7 d.

Literature

Dhanoa NS Siddons, RC, France J and Gale DL. 1985. A multicompartment model to describe marker excretion patterns in ruminant faeces. Br J Nutr 53: 663-671.
McCue, MD and Welch KC jr. 2016. 13C breath testing in animals: theory, applications and future directions. J. Comp. Physiol. B (2016) 186: 265-285.
Van den Borne JJGC, Heetkamp MJW, Buyse J, Niewold TA 2015 Fat coating of Ca butyrate results in extended butyrate release in the gastrointestinal tract of broilers . Livestock Science 2015 (175): 96-100.

3.4.3 Describe the coherence between the different components and the different steps of the project. If applicable, describe the milestones and selection points

For a description of the studies to be conducted as part of this project, please see the project outline above. Three milestones will be identified within this project, and a 4th one after completion of the animal experiments conducted within this project:
1) After study 1 (pilot), a decision will be made on how the fourth study will be conducted, i.e. a within-calf dose response approach or a between calf dose response approach.
2) An estimation of passage rate kinetics of MR and concentrates using the isotope method (study 2) and on MR, concentrates and roughages in the feacal collection method (study 3), both focusing on the first compartment of the GI tract (i.e. the rumen for the concentrates and roughages, the abomasum for the MR).
3) A validated approach of digesta passage kinetics using a combination of 13CO2 breath test approaches.
3) An estimate of P requirements as affected by the nutritional regimes applied in this project.
4) Upon completion of the 4 studies, the passage rate data will be used for improvement of the model simulating calf growth from nutritional inputs. P digestion and metabolism will be incorporated into this model, allowing the prediction of total P and N flows from ingestion to growth or excretion via urine or faeces.

3.4.4 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	P requirements in veal calves - a pilot study
2	Passage rate kinetics in veal calves - 13C tracer technique
3	Passage rate kinetics in veal calves - faecal excretion curves
4	P requirements in veal calves fed different rations

4.

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, see our website www.zbo-ccd.nl.
- Or contact us by phone. (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10400	
1.2	Provide the name of the licenced establishment.	Wageningen University	
1.3	List the different types of animal procedures. Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.	Serial number 1	Type of animal procedure P requirements in veal calves - a pilot study

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 direct goal of this pilot study is to determine whether we can measure the P requirements of individual veal calves using a step-wise within-calf dose respons approach. When succesfull, it provides a more refined technique to determine P requirements under varying nutritional regimes in the future.
As described in section 3.4.1 of the project proposal, the principle of this approach is that after providing an excess of P via MR, P intake will be step-wise reduced via MR (in 7 steps with every step lasting 72h), leading to a reduction of P excreted via urine. The inflection point of the P-excretion curve against P intake will be assessed via nonlineair regression and assumed to represent the requirement of the individual calfs. The plateau value, obtained at the lowest P intake will represent the maximum efficiency of P utilization, likely influenced by SF intake.
During this pilot study, we will only monitor the rate of adaptation of urinary P excretion following a substantial reduction in P intake via the MR in a limited number of calves (n=6). If the rate of urinary P exretion stabilizes within 72h after the reduction in dietary P supply, the technique can be considered applicable for individual calves.

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

Six calves of approximately 8 weeks of age will be individually housed in metabolic cages, and fitted with harnasses to which plastic bags are attached for the quantitative collection of faeces. Clean urine can be collected in buckets underneath a funnel, mounted underneath the cage. Calves will be fed a normal ration with a high P milk replacer for the 4-d adaptation period and for the first 3 days of the experimental period, after which they will be switched to a MR lowering the P content of the MR by 20%, which will be fed the remaining 4 days. Typically, if urinary P excretion sensitively responds to a reduction of P intake via MR by 20%, it can be assumed that calves will respond to each of the steps in the within-calf dose response study (i.e. the contrast chosen should not be much larger than the intended change in P intake between two steps).
During the entire experimental period, quantitative, 12h urinary collections, and 24h faecal collections will be performed. This is done to determine the rate of adaptation of urinary P excretion after a reduction in P intake via the MR. In addition, salivary P concentrations will be determined 4 times daily during the experimental period.

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

The number of calves used in this experiment is based on the average P excretion in urine and faeces in several experiments with calves housed under similar conditions. It is based on the analysis of P flows in a study published by Berends et al., (2015), of which the data on P flows are

reported in an internal report by Plomp et al (2015). That study involved 8 calves and used a between-calf dose response approach. A reduction in P input via MR by 15% resulted in a tendency for a reduced P output via urine.
In the present study however, we will use a reduction in P intake of 20% (as described in section 2A - animal procedures). Moreover, we will use a within-calf dose response approach, which will reduce variation. Performing a power analysis (one sided), revealed that at least 6 calves are needed to demonstrate a significant reduction of P excretion via urine when reducing P input by 20%.
We do want to emphasize that with this pilot study we want to determine whether this within-calf dose response approach is applicable to determine the P requirements of individual calves. This will be mainly based on the assessment of the P-excretion curves against P intake via nonlinear regression rather than strict statistical analysis. If we simply cannot find a stabilized urinary P excretion within 72h after the reduction in dietary P supply, we conclude that this within-calf dose response approach does not work.

Literature

Berends, H., van den Borne, J. J. G. C., Røjen, B. A., Hendriks, W. H. & Gerrits, W. J. J. 2015. Effect of protein provision via milk replacer or solid feed on protein metabolism in veal calves. Journal of Dairy Science. 98, 2, p. 1119-1126
Plomp M, Gerrits WJJ, Schop TA, Heeres-vd Tol, JJ 2015. Fosforstromen in vleeskalveren. P en Ca balans in kalveren gevoerd met melkvervanger en ruw- en krachtvoer. Rapport Wageningen Livestock Research, 46pp [in Dutch], available on request

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

In this pilot experiment, 6 male Holstein Friesian calves will be used. The age of these calves will be approximately 6 weeks, and we will purchased them from a veal producer or use the calves available at the experimental facilities. Male calves will be used to ease separate collection of faeces and urine. Calves will be about 80 kg of BW at the onset of the trial, and close to 90 kg BW when the trial is finished.

C. Re-use

Will the animals be re-used?

C. Re-use

[X] No, continue with question D.
[] Yes > Explain why re-use is considered acceptable for this animal procedure.

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

[] No
[] Yes > Provide specific justifications for the re-use of these animals during the procedures.

D. 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: The male Holstein Friesian calf is the target animal of this project. The rate of adaptation of urinary P excretion to changes in an excess or shortage of phosphorus cannot be estimated in another species. Rumination is essential. Reduction: this pilot study will be performed to investigate the possibility to study P requirements in individual calves rather than the conventional approach in which different levels of P are imposed on different calves, hence necessitating multiple groups of calves, estimating the average requirement of the calf population by regression based on observations obtained from different calves. When possible, less experimental animals are needed to obtain estimates of P requirements under various nutritional regimes. Refinement: As indivual housing on metabolism cages causes discomfort, we'll take precautions to minimize the impact. We place pairs of metabolic cages close to each other to facilitate physical contact between two calves. Additionally, we enrich the metabolic cages with brushes, a dry teat for suckling needs, and provide a ball inside the cage. Additionally, the use of chains is minimized (to avoid unneccesary noise). Furthermore, experienced animal care takers will monitor the calves to be sure a potential case of illness or reduced welfare will be diagnosed in an early stage. Handling of the calves will be performed by experienced personnel carefully and quietly, preferably in rest to prevent unnecessary distress. Analgesia and medication are applied where needed. If (in rare cases) analgesia or medication may influence trial results, calfs are treated and excluded from the trial. This study also contributes to the refinement of future research determining passage rate kinetics of animals. For example, the duration of the study 'P requirement in veal calves fed different rations' (annex 4) depends on the rate of adaptation of urinary P excretion to changes in P intake via MR. It is important for this study to provide an accurate estimate of this rate of adaptation in order to minimize the duration of the study 'P requirement in veal calves fed different rations' (annex 4), and thus the duration of the individual housing of these calves.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Housing on bedding material is not possible as clean urine needs to be collected from underneath the cages. The following measures will be taken to reduce the impact of housing in metabolism cages on welfare of the calves: - use of chains will be minimized as the noise can disturb the calves - availability of brushes, mounted on the cage and a dry teat to satisfy sucking needs - a ball inside the cage - cages will be organized in pairs close to each other to facilitate some physical contact between calves

Repetition and Duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Based on a literature search within the Pubmed and Scopus database using 'calf, phosphorus requirement, milk replacer, roughage, and concentrate' as searching criteria, we conclude that no study has been published before regarding the P requirements in calves fed a ration based on MR, concentrates and roughages. P requirement studies in beef calves are available, but these calves are fed a ration based on concentrates and roughages without MR, and therefore do not take the potential to recycle P from the MR into account.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

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

Calves will be housed individually in metabolism cages. Fecal collection bags will be harnassed to the calves, to facilitate the quantitative collection of faeces and thereby the quantitative collection of clean urine in buckets underneath funnels, mounted underneath the cage. The dimensions of the cage will be 0.8 x 2m, and calves will be fixed to the front of the cage, allowing them to stand or lie freely. Housing on bedding material is not possible as clean urine needs to be collected from underneath the cages. As individual housing on metabolism cages causes discomfort, we'll take precautions to minimize the impact (i.e., minimized use of chains, cage enrichment including brushes, dry teat, and ball, and cages will be close to each other to facilitate physical contact between calves).

G. 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 H.
[] 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.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

[X] No > Continue with question I.
[] Yes > Will anaesthesia, analgesiaor other pain relieving methods be used?

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

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

I. Other aspects compromising the welfare of the animals

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

Individual housing for 7 days, restricted movements, no bedding material on the floor, harnasses to fix faecal collection bags.

Explain why these effects may emerge.

Increased occurence of abnormal oral behaviours (e.g., repetitive self-licking, flank or object sucking, tongue rolling), as calves are deprived from exploring their environment. Some discomfort from wearing harnesses cannot be prevented.

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

The following measures will be taken to reduce the impact of housing in metabolism cages on welfare of the calves: - use of chains will be minimized as the noise can disturb the calves - availability of brushes, mounted on the cage and a dry teat to satisfy sucking needs - a ball inside the cage - cages will be organized in pairs close to each other to facilitate some physical contact between calves

J. 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 K.
[X] Yes > Describe the criteria that will be used to identify the humane endpoints.

• a calf has feed refusals exceeding 20% of the amount of MR offered for a period exceeding 3 days. • a calf has a fever for 3 successive days, not responding to medical treatments proposed by a veterinarian, and signs of infection and inflammation. • a calf suffers from problems related to the harnass, showing wounds at places the harness is connected. • in the expert judgement of the veterinarian, future observations on a calf will not provide reliable results. • in the expert judgement of the veterinarian, continuation of the experiment with a calf will cause discomfort higher than foreseen for this trial.

Indicate the likely incidence.

The likely incidence of calves to be removed from the experiment is estimated to be less than 10% during the duration of the trial, based on previous experience in trials of similar duration. The major portion of this 10% is expected to origin from feed refusals due to health problems unrelated to experimental procedures. In addition, problems arising from the harnesses will have a very short duration, as calves will then be removed from the trial.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned (non-recovery, mild, moderate, severe).

The level of discomfort is expected to be as listed below: • Individual housing on balance cages, in absence of bedding material during 10 days: moderate • Wearing of harnasses for the collection of faecal collection bags: mild Hence the cumulative discomfort in this trial is estimated at moderate. Based on previous experience, collecting saliva samples are considered to cause no discomfort. We will insert two cotton swabs (150 x 4 mm) into the mouth of a calf, who will chew on them voluntarily, subsequently we will place these into a salivette.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

[X] No > Continue with Section 3: 'Signatures'.
[] 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 > Descripe the method of killing that will be used and provide justifications for this choice.

[] Yes

5.

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, see our website www.zbo-ccd.nl.
- Or contact us by phone. (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10400	
1.2	Provide the name of the licenced establishment.	Wageningen University	
1.3	List the different types of animal procedures. Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.	Serial number 2	Type of animal procedure Passage rate kinetics in veal calves - 13C tracer technique

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.

For this study, 48 male, Holstein-Friesian calves will be purchased at about 6 weeks of age. They will be housed in groups and assigned to one of 4 dietary treatments, varying in the intake of solid feed (SF) and MR (factor 1) and the concentrate:roughage ratio within the SF (factor 2) in 2x2 factorial arrangement. The calves will be adapted to their diets for a period of 5 weeks to allow rumen development and rumination behaviour to completely adjust to the levels and composition of SF intake. After that, calves will be housed for 7 days in pairs in one of 4 respiration chambers, and frequently fed (i.e. 6x daily) their SF portion while receiving their MR supply twice daily.
This setup (pair or calves in one of 4 respiration chambers), results in a staggered planning with 6 batches of 8 calves (4 pairs) undergoing the same experimental procedure, each batch starting one week after the previous one. In this way, creating a range in age (as also described in section 3.4.1 - research strategy of the project proposal). The first batch of 8 calves will adapt to their diet for 5 weeks, subsequently followed by 7 days of measurements in the respiration chambers. The second batch of calves (also n=8) will adapt to the diet for 6 weeks (i.e., basis of 5 weeks + the 7 days that the first group of calves in housed in respiration chambers), subsequently followed by 7 days of measurements in the respiration chambers. This pattern continues for all 6 batches. In this manner, observations are achieved over an age range of 6 weeks.
When the calves are housed in the respiration chambers, every two days, one of the MR or SF meals will be spiked with a 13C stable isotope tracer, measuring the kinetics of recovery in 13CO2 in exhaled air as a measure of the rate of ruminal fermentation and/or digesta passage kinetics. 13C tracers will be selected after a thorough search of literature, but likely include naturally labelled yeast proteins (to be included in the concentrate), 13C-glycine (to be included in the MR), 13C octanoate and 13C-NaHCO3, to be injected intravenously. The latter is intended to correct for a delay between nutriënt oxidation and 13C exhaled in breath. 13CO2 excretion will be measured on-line in the facilities for indirect calorimetry of Wageningen University.

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

During the 5-week adaptation period, calves will be housed in groups (divided over several departments within the experimental facilities) and fed diets according to the experimental schedule. MR will be provided twice daily and SF will be provided in two portions upon completion of MR intake. During the 7d experimental period, calves will be housed in pairs inside a climatized respiration chamber. During this week, continuous measurement of CO2, 13CO2 and CH4 production and the consumption of O2 will be performed. During this period, SF will be provided in 6 equal portions spread over the 24h period. Following oral administration of 13C tracers, patterns of 13CO2 excretion will be measured. To account for a delay in 13CO2 exhalation following arrival in the bicarbonate pool, 13CO2 excretion patterns will be analyzed following intravenous injection of a bolus 13C-NaHCO3.
After the 7 days in the climate respiration chambers, the calves will directly in the study described in Annex 3.

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

This experiment is designed to quantify differences between the dietary treatments on digesta passage rate kinetics. As calves are housed in pairs and the gaseous exchanged is measured in a respiration chamber, a pair is considered as the experimental unit for this study. Treatment differences on dependent variables (mostly time of peak recovery of 13CO2 after a bolus of 13C tracer) will be analyzed by ANOVA using the dietary contrasts as fixed effects. In a previous study (Berends et al., 2015) it was demonstrated that provisional estimates of ruminal fractional passage rates could be evaluated using 8 calves per treatment, applying an approach using indigestible markers. A significant difference could be detected for a difference in feeding level of SF, similar to the one intended for this study. Considering a reduction in variation with two calves per experimental unit, we consider 6 experimental units for each treatment combination to be sufficient to estimate the effect of our dietary treatments on passage rate kinetics. For the methodology applied in this study, estimates of variation between experimental units are not available, although the 13CO2 breath test has been applied previously in calves in our facilities (see e.g. Gilbert et al., 2016), also with 2 calves in a respiration chamber. A 3-h difference in the time of peak metabolism of fructose and glucose could be quantitied using 5 pairs of calves per treatment ($P < 0.01$). Six pairs of calves per treatment combination would enable us to significantly detect a 2h difference in the time of peak of 13CO2 recovery.

Literature

Berends, H., van den Borne, J. J. G. C., Røjen, B. A., Hendriks, W. H. & Gerrits, W. J. J. 2015b. Effect of protein provision via milk replacer or solid feed on protein metabolism in veal calves. Journal of Dairy Science. 98, 2, p. 1119-1126.

Gilbert, M. S., Pantophlet, A. J., van den Borne, J. J. G. C., Hendriks, W. H., Schols, H. A. & Gerrits, W. J. J. 2016. Effects of replacing lactose from milk replacer by glucose, fructose, or glycerol on energy partitioning in veal calves. Journal of Dairy Science. 99, 2, p. 1121-1132

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

Male, Holstein Friesian calves are the target animals for the objective of this study.

C. Re-use

Will the animals be re-used?

C. Re-use

[X] No, continue with question D.
[] Yes > Explain why re-use is considered acceptable for this animal procedure.

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

[] No
[] Yes > Provide specific justifications for the re-use of these animals during the procedures.

D. 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: The male Holstein Friesian calf is the target animal of this project. Passage rate kinetics measured in other ruminants animals are not applicable for calves, because based on the results obtained by Berends et al. (2015) it can be concluded that the passage rate of digesta in calves is likely substantially lower compared with other ruminants. Reduction: the objective of this study is to evaluate the possibiltiy to use minimally invasive 13C breath test approach for the measurement of passage rate kinetics in calves. Outcomes will be validated with more conventional measures of passage rate kinetics (annex 3 of this project). When succesful, this approach can be used for repeated measurement of digesta passage rate in calves, or potentially also in other ruminant species. Refinement: Calves will be socially housed during the adaptation period and will be housed in pairs in the respiration chambers to avoid social isolation. We intent to use free housing, but if needed we restrain the calves during feeding. Measurements will be non-invasive, with the only exception of an intravenous injection of 13C labelled $NaHCO_3$. Furthermore, experienced animal care takers will monitor the calves to be sure a potential case of illness or reduced welfare will be diagnosed in an early stage. Handling of the calves will be performed by experienced personnel carefully and quietly, preferably in rest to prevent unnecessary distress. Analgesia and medication are applied where needed. If (in rare cases) analgesia or medication may influence trial results, calfs are treated and excluded from the trial.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

No adverse effects are foreseen

Repetition and Duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Based on a literature search within the Pubmed and Scopus database using 'calf, passage rate kinetics, milk replacer, roughage, and concentrate' as searching criteria, we conclude that no study has been published that uses the 13C tracer technique to measure digesta passage rate kinetics in calves fed combination of MR and solid feed are available. Hence this is not a repeat of research of others.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

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

Calves will be socially housed during the adaptation period and will be housed in pairs in the respiration chambers to avoid social isolation. We intent to use free housing, but if needed we will restrain the calves during feeding. In the respiration chambers, two calves will be housed in a pen of about 1.80x 3.5m. This is related to the maximum inner measures of the respiration chambers and needed for accurate measurement of short-term changes in 13CO2 exhalation.

G. 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 H.
[] 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.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

[] No > Continue with question I.
[X] Yes > Will anaesthesia, analgesiaor other pain relieving methods be used?

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

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

I. Other aspects compromising the welfare of the animals

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

We do not expect other adverse effects, including of the different dietary treatments. The four dietary treatments, varying in the intake of SF and MR (factor 1) and the concentrate:roughage ratio within the SF (factor 2), will be within the range which is fed in practice.

Explain why these effects may emerge.

n.a.

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

n.a.

J. 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 K.
[X] Yes > Describe the criteria that will be used to identify the humane endpoints.

Not as a result of the animal procedures, but health problems may arise necessitating animals to be removed from the trial. Humane endpoints are: - feed refusals exceeding 20% of the daily allowance for a period exceeding 3 days - a calf has a fever for 3 successive days, not responding to medical treatments proposed by a veterinarian. - in the expert judgement of the veterinarian, future observations on a calf will not provide reliable results. - in the expert judgement of the veterinarian, continuation of the experiment with a calf will cause discomfort higher than foreseen for this trial.

Indicate the likely incidence.

Likely 5% or less, because the calves are housed socially during the adaptation period and in pairs in the respiration chambers. Moreover, we intent to house the calves freely (not restrained, with the possible exception during feeding).

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned (non-recovery, mild, moderate, severe).

The level of discomfort is expected to be as listed below: • Group housing in adaptation phase and in respiration chambers, in absence of bedding material during 7 days: mild • if needed calves will be restrained during feeding: mild • iv injection of a pulse dose of NaHCO3: mild • weighing of the calves (weekly): mild Hence the cumulative discomfort in this trial is estimated at mild.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

L. Method of killing

[X] No > Continue with Section 3: 'Signatures'.

[] 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 > Descripe the method of killing that will be used and provide justifications for this choice.

[] Yes

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, see our website www.zbo-ccd.nl.
- Or contact us by phone. (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10400	
1.2	Provide the name of the licenced establishment.	Wageningen University	
1.3	List the different types of animal procedures. Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.	Serial number 3	Type of animal procedure Passage rate kinetics in veal calves - faecal excretion curves

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.

For this study, 48 male, Holstein-Friesian calves will be re-used from the study described in annex 2. The experimental procedures are staggered, with measurements on every batch of 8 calves starting a week after the previous one. The results from this study will thus be obtained over an age range of 6 weeks.

Upon completion of the study described in annex 2, the calves remain housed in pairs. They will be fed the same experimental dietary treatments, varying in the intake of solid feed (SF) and MR (factor 1) and the concentrate:roughage ratio within the SF (factor 2) in 2x2 factorial arrangement. The calves then have already been adapted to these diets for at least 6 weeks. Calves will be fitted with harnasses to which plastic bags are attached for the quantitative collection of faeces. Three indigestible markers will be used to trace the concentrates (likely TiO2 or alkanes), roughage (likely cr-mordant long straw) and the MR (likely Co-EDTA). The markers will be administered in a pulse dose 48h (roughage), 24h (concentrate) or 4h (MR) prior to the start of the faecal collection period. Faecal bags will be checked bi-hourly for a period of 4 days, and marker excretion will be quantified in time. Following methods published by Dhanoa et al. (1985), faecal excretion curves of markers will be analyzed to estimate the overall mean retention time of digesta and the passage rate of the slowest compartment (rumen for the markers of the concentrate and roughage; abomasum for the markers of the MR).

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

Calves will be housed in pairs, and fitted with a harness to which faecal collection bags will be attached. If the combination of housing in pairs and the harnasses is not succesfull, we will place a fence in between the calves. We will restrain calves during replacement of the faecal collection bags and, if needed, also during feeding.

Calves will be fed according to normal procedures and faeces will be collected 2-hourly during 4 days after the start of the trial.

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

As described in annex 2 and 4, we need 48 calves to achieve the objectives of these trials. Results from this study will be compared with the results

from the study described in annex 2 (passage rate kinetics - 13C tracer technique) and therefore needs to be conducted with the same calves continued on the same treatments.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

Male, Holstein Friesian calves are the target animals for the objective of this study.

C. Re-use

Will the animals be re-used?

[] No, continue with question D.
[X] Yes > Explain why re-use is considered acceptable for this animal procedure.

Discomfort during previous experiment (described in annex 2 of this project) is classified as mild. The comparison of the results of annex 2 with the results of this study is important, having similar conditions and working with the same calves is preferred. Additionally, if we would be working with a new batch of calves, we hape to adapt these calves to the diets for several weeks. This is not necessary if we re-use the calves from Annex 2.

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.

D. 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: The male Holstein Friesian calf is the target animal of this project. Passage rate kinetics measured in other species are not applicable for calves, because based on the results obtained by Berends et al. (2015) it can be concluded that the passage rate of digesta in calves is lower compared with other species. Reduction: the objective of this study is to estimate mean retention time of digesta and passage rate in the rumen, and secondly to provide a reference for the minimally invasive 13C breath test approach for the measurement of passage rate kinetics in calves, described in the study of annex 2. Using the same calves of the study described in annex 2, we reduce the total number of calves needed. Refinement: After careful consideration, it is decided to avoid the use of metabolism cages in this study. Therefore, the calves will be housed in pairs. We intent to house the calves in pairs while they are fitted with harnasses. If is questionable whether this possible, because it might be that the calves will start interacting with the harnass of the other calf. If all goes well, we continue housing them in pairs. If the housing in pairs in combination with the harnasses does not work, we will place a fence within their pen. Calves will be harnessed but will only be fixed when the faecal collection bags need to be replaced. We intent to use free housing, but if needed we restrain the calves during feeding. Furthermore, experienced animal care takers will monitor the calves to be sure a potential case of illness or reduced welfare will be diagnosed in an early stage. Handling of the calves will be performed by experienced personnel carefully and quietly, preferably in rest to prevent unnecessary distress. Analgesia and medication are applied where needed. If (in rare cases) analgesia or medication may influence trial results, calfs are treated and excluded from the trial.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Repetition and Duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Based on a literature search within the Pubmed and Scopus database using 'calf, passage rate kinetics, milk replacer, roughage, and concentrate' as searching criteria, we conclude that no study has been published in which digesta passage rate kinetics has been measured in calves fed combination of MR and solid feed are available. Hence this is not a repeat of research of others.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

F. Accommodation and care

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

Calves will be housed in pairs in a pen size meeting the EU directive. Calves will be harnessed, but will only be fixed when the faecal collection bags need to be replaced. If housing in pairs with calves that are harnessed is not possible, a fence will be placed in between the calves. We intent to use free housing, but if needed we restrain the calves during feeding. Bedding material cannot be used as the possible consumption can alter digesta passage rates.

G. 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 H.
[] 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.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

[X] No > Continue with question I.
[] Yes > Will anaesthesia, analgesiaor other pain relieving methods be used?

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

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

I. Other aspects compromising the welfare of the animals

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

Harnasses to fix faecal collection bags, fixing the calves when switching the faecal collection bags, the possible restrainment during feeding, and the possible individual housing if calves interfere with eachothers harnass.

Explain why these effects may emerge.

Some discomfort from wearing harnesses cannot be prevented. In addition, fixing the calves at times of changing the faecal collection bags as well as restraining the calves during feeding will cause some discomfort. Moreover, the possible restrainment during feeding (when eating from eachothers food) and the possible individual housing (when interfering with eachothers harnass), will cause some discomfort.

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

Harnesses will be checked daily and adjusted if needed. Fixing calves at the time of changing faecal collection bags is needed, but as opposed to housing on metabolism cages, it allows calves to move around freely when not fixed.

J. 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 K.
[X] Yes > Describe the criteria that will be used to identify the humane endpoints.

• a calf has feed refusals exceeding 20% of the amount of MR offered for a period exceeding 3 days. • a calf has a fever for 3 successive days, not responding to medical treatments proposed by a veterinarian. • a calf suffers from problems related to the harnass, showing wounds at places the harness is connected. • in the expert judgement of the veterinarian, future observations on a calf will not provide reliable results. • in the expert judgement of the veterinarian, a calf is not fit/happy/healthy (reduced welfare) without affecting experimental results.

Indicate the likely incidence.

The likely incidence of calves to be removed from the experiment is estimated at less than 10% during the duration of the trial. The major portion of this 10% is expected to origin from feed refusals due to health problems unrelated to experimental procedures. In addition, problems arising from the harnesses will have a very short duration, as calves will then be removed from the trial

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned (non-recovery, mild, moderate, severe).

The level of discomfort is expected to be as listed below: • Housing in pairs, or individually, fixing at the time of changing faecal collection bags during 7 days: mild • Wearing of harnasses for the collection of faecal collection bags: mild Hence the cumulative discomfort in this trial is estimated as mild.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

[X] No > Continue with Section 3: 'Signatures'.
[] 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 > Descripe the method of killing that will be used and provide justifications for this choice.

[] Yes

7.

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, see our website www.zbo-ccd.nl.
- Or contact us by phone. (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10400	
1.2	Provide the name of the licenced establishment.	Wageningen University	
1.3	List the different types of animal procedures. Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.	Serial number 4	Type of animal procedure P requirements in veal calves fed different rations

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 objective of this trial is to estimate minimal P requirements for growth of calves fed different mixtures of solid feed and milk replacer. There are two options for this trial, depending on the outcome of the pilot (annex 1). When the rate of adaptation of urinary P excretion following a substantial reduction in P intake via the MR stabilizes within 72 h after the reduction in dietary P supply, the technique can be considered applicable for individual calves. If this is the case, a within-calf response dose technique can be used to estimate P requirement for individual calves, in analogy to the dose response approach to estimate amino acid requirements, described by Kampman-van de Hoek (2013). If the results of the pilot demonstrate that more time is needed for urinary P excretion to stabilize, a more traditional, between animal, dose-response approach will be used (see e.g. Erickson et al., 2002), comparing P excretion at different levels of intake between animals.
The age of the calves at the start of the measurements in this experiment will range from 13 weeks of age to 18 weeks of age, depending on the batch of calves. This is needed because of the simultaneous availability of 4 climate-respiration chambers for the study described in annex 2 (calves are re-used). In each batch, 8 calves will be used. During the 6 batches, measurements on 48 calves are obtained. Hence differences between batches represent a composite effect of time, age and batch of calves.

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

Option 1: within-calf dose response approach
Upon completion of the study described in annex 3 of this project, calves will remain on their 4 nutritional treatments, but transferred to a MR with a high P level. All calves will be housed individually on metabolism cages. Calves will be fitted with harnesses to allow quantitative collection of faeces and collection of clean urine from funnels mounted underneath the cage. P intake via the MR will be reduced in a step-wise manner in 7 steps of 3d each (21d in total). Faecal P excretion will be measured daily, and urinary excretion in 12h periods. The response of total and urinary P excretion to a reduction in P intake will be analyzed using nonlinear regression techniques to estimate the inflection point (see Kampman-van de Hoek et al., 2013), which can be considered the minimum P requirement for each calf. Effects of the nutritional treatments on the estimated P requirements will be analyzed.
With this option, the calves will be followed for 21d in total. It could be that the minimum P requirement of a calf is reached before that (e.g., step 5 at 15 days or with step 6 at 18 days). However, the faecal and urine samples will be analyzed in the laboratory after the experiment. Therefore, we are unable to determine the minimum P requirement while the experiment is running, thus each calve has to be followed for 21d.

Option 2: between calf dose response approach
Upon completion of the study described in annex 3 of this project, calves will remain housed in pairs for two weeks and transferred to two dietary treatments: i.e. two levels of SF intake at a fixed roughage: concentrate ratio. Hence there will be 24 calves on each SF treatment. Within each SF treatment, calves will be assinged to a MR with one of 6 P concentrations, varying from low (at 50% of the estimated requirements) to high (at 150% of the estimated requirements). Thus, there will be 4 calves assigned to each P level within the SF treatment. Calves will then be housed individually on metabolism cages. Calves will be fitted with harnesses to allow quantitative collection of faeces and collection of clean urine from funnels mounted underneath the cage. Faecal and urinary P excretion will be quantified of the complete 7d collection period. The response of total and urinary P excretion to a increasing P intake will be analyzed using nonlinear regression techniques to estimate the inflection point. In this way, one estimate will be obtained for each SF treatment,

Literature

Erickson GE, Klopfenstein TJ, Milton CT, Brink D, Orth MW and Whitted KM. 2002. Phosphorus requirements of finishing feedlot calves. J. Anim,. Sci. 80(6) 1690-1695.
Kampman-van de Hoek, E, Gerrits, WJJ, van der Peet-Schwering, CMC Jansman, AJM and van den Borne, JJGC 2013. A simple amino acid dose-response technique to quantify amino acid requirements of individual meal-fed pigs Journal of Animal Science. 91, 10, p. 4788-4796

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

Depending on the option chosen, there will be one estimate of P requirements per calf, or one per SF treatment. Between-calf variation of such estimates are unknown, but previous experience using a similar dose response approach for amino acid requirements in pigs (Kampman van de Hoek et al, 2013; Bruininx et al., 2015) and for responses to graded levels of starch or starch products in calves (Gilbert et al., 2015) demonstrate sensitive responses to step-wise changes in the driving variable using 10 animals/treatment.
For estimating P requirements using a between-calf approach, 24 calves per treatment assigned to graded levels of P intake is an approach

previously shown to provide accurate P requirements, and can be expected to be adequate to develop reliable requirement estimates for our two nutritional conditions.

Literature

Bruininx, E. M. A. M., van den Borne, J. J. G. C., Eising, I., Vervenne, P., Sakkas, P. & Gerrits, W. J. J. 2015. Optimal lysine:DE ratio in growing pigs is independent of starch or fat as main energy source at two energy intake levels Journal of Animal Science. 93, 10, p. 4774-4780

Gilbert, M. S., van den Borne, J. J. G. C., Berends, H., Pantophlet, A. J., Schols, H. A. & Gerrits, W. J. J. 2015. A titration approach to identify the capacity for starch digeston in milk-fed calves. Animal. 9, 2, p. 249-257

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

In this experiment, 48 male Holstein Friesian calves will be re-used from the study described in annex 2 and 3 of this project. Male calves will be used to ease separate collection of faeces and urine.

C. Re-use

Will the animals be re-used?

C. Re-use

[] No, continue with question D.

[X] Yes > Explain why re-use is considered acceptable for this animal procedure.

Discomfort during the previous experiment was considered mild. The interest in this project is in the same treatments imposed on calves of annex 2, 3, and 4, facilitating repeated measurements.

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.

D. 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: The male Holstein Friesian calf is the target animal of this project. Effects of SF intake and composition on P requirements cannot be estimated in vitro or in model animal species. Reduction: Depending on the results of the pilot study (i.e. when a within-calf titration approach appears feasible), this study contributes to a reduction in the number of calves needed to obtain estimates of P requirements under various nutritional conditions. Refinement: The duration of the dose response study depends on the rate of adaptation of urinary P excretion to changes in P intake via MR. If changes in urinary P excretion following a reduction in P intake via the MR adapt within 48 hours, the duration of the dose response study can be reduced from 21 to 14 d, and thus reducing the duration of the individual housing of these calves. As indivual housing on metabolism cages causes discomfort, we'll take precautions to minimize the impact. We place pairs of metabolic cages close to each other to facilitate physical contact between two calves. Additionally, we enrich the metabolic cages with brushes, a dry teat for suckling needs, and provide a ball inside the cage. Additionally, the use of chains is minimized (to avoid unneccesary noice). Furthermore, experienced animal care takers will monitor the calves to be sure a potential case of illness or reduced welfare will be diagnosed in an early stage. Handling of the calves will be performed by experienced personnel carefully and quietly, preferably in rest to prevent unnecessary distress. Analgesia and medication are applied where needed. If (in rare cases) analgesia or medication may influence trial results, calfs are treated and excluded from the trial.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Housing on bedding material is not possible as clean urine needs to be collected from underneath the cages. The following measures will be taken to reduce the impact of housing in metabolism cages on welfare of the calves: - use of chains will be minimized as the noise can disturb the calves -

availability of brushes, mounted on the cage and a dry teat to satisfy sucking needs - a ball inside the cage. - cages will be organized in pairs close to each other to facilitate some physical contact between calves

Repetition and Duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Based on a literature search within the Pubmed and Scopus database using 'calf, phosphorous requirement, milk replacer, roughage, and concentrate' as searching criteria, we conclude that no study has been published that measured P requirements in calves fed combination of MR and solid feed are available. Hence this is not a repeat of research of others. P requirement studies in beef calves are available, but these calves are fed a ration based on concentrates and roughages without MR, and therefore do not take the potential to recycle P from the MR into account.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

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

Calves will be housed individually in metabolism cages. Fecal collection bags will be harnassed to the calves, to facilitate the quantitative collection of faeces and thereby the quantitative collection of clean urine in buckets underneath funnels, mounted underneath the cage. The dimensions of the cage will be 0.8 x 2m, and calves will be fixed to the front of the cage, allowing them to stand or lie freely.

G. 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 H.

G. Location where the animals procedures are performed

[] 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.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

[X] No > Continue with question I.

[] Yes > Will anaesthesia, analgesiaor other pain relieving methods be used?

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

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

I. Other aspects compromising the welfare of the animals

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

Prolonged individual housing, Restricted movements, no bedding material on the floor, harnasses to fix faecal collection bags. Please note: we are aware that in some occasions the calves receive a diet well below the P requirement. In comparison with studies performed with pigs, as well as the duration of the experiment, we do not consider this as a cause of discomfort. We do however acknowledge the risk, and will assess the calves daily to see the development of P deficiency symptoms in an early stage. After this experiment had ended, all calves will be fed the high P MR.

Explain why these effects may emerge.

Increased occurence of abnormal oral behaviours (e.g., repetitive self-licking, flank or object sucking, tongue rolling), as calves are deprived from exploring their environment due to a relative long period of individual housing in metabolic cages. Some discomfort from wearing harnesses cannot be prevented.

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

Housing on bedding material is not possible as clean urine needs to be collected from underneath the cages. The following measures will be taken to reduce the impact of housing in metabolism cages on welfare of the calves: - use of chains will be minimized as the noise can disturb the calves - availability of brushes, mounted on the cage and a dry teat to satisfy sucking needs - a ball inside the cage. - cages will be organized in pairs close to each other to facilitate some physical contact between calves After this experiment had ended, all calves will be fed a diet which had a P content equal or above P requirements.

J. 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 K.
[X] Yes > Describe the criteria that will be used to identify the humane endpoints.

• a calf has feed refusals exceeding 20% of the amount of MR offered for a period exceeding 3 days. • a calf has a fever for 3 successive days, not responding to medical treatments proposed by a veterinarian, and signs of infection and inflammation. • a calf suffers from problems related to the harnass, showing wounds at places the harness is connected. • a calf which clearly suffers from P deficiency (occurance of symptoms like bone fractures). • in the expert judgement of the veterinarian, future observations on a calf will not provide reliable results. • in the expert judgement of the veterinarian, continuation the experiment for a calf will cause more discomfort than foreseen.

Indicate the likely incidence.

The likely incidence of calves to be removed from the experiment is estimated at about 15% during the duration of the trial. The major portion of this 15% is expected to origin from feed refusals due to health problems unrelated to experimental procedures. In addition, problems arising from the harnesses will have a very short duration, as calves will then be removed from the trial. The higher incidence compared with the study described in annex 1 is related to the longer duration of this trial.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned (non-recovery, mild, moderate, severe).

The level of discomfort is expected to be as listed below: • Individual housing on balance cages, in absence of bedding material during 21 days: moderate • Wearing of harnasses for the collection of faecal collection bags: mild • Exposure to a P deficient diet for a period of about 10 days: moderate Hence the cumulative discomfort in this trial is estimated at moderate.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

[X] No > Continue with Section 3: 'Signatures'.
[] 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 > Descripe the method of killing that will be used and provide justifications for this choice.

[] Yes

8.

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Wageningen University
[redacted]
Postbus 59
6700 AB WAGENINGEN

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD104002017874

Bijlagen
2

Datum 20 februari 2017
Betreft Ontvangstbevestiging aanvraag projectvergunning Dierproeven

Geachte [redacted]

Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 17 februari 2017. Het gaat om uw project "Influence of solid feed intake on digesta passage rate and phosphorus requirements of veal calves". Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD104002017874. Gebruik dit nummer wanneer u contact met de CCD opneemt.

Wacht met de uitvoering van uw project
Als wij nog informatie van u nodig hebben dan ontvangt u daarover bericht. Uw aanvraag is in ieder geval niet compleet als de leges niet zijn bijgeschreven op de rekening van de CCD. U ontvangt binnen veertig werkdagen een beslissing op uw aanvraag. Als wij nog informatie van u nodig hebben, wordt deze termijn opgeschort. In geval van een complexe aanvraag kan deze termijn met maximaal vijftien werkdagen verlengd worden. U krijgt bericht als de beslisperiode van uw aanvraag vanwege complexiteit wordt verlengd. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.

Factuur
Bijgaand treft u de factuur aan voor de betaling van de leges. Wij verzoeken u de leges zo spoedig mogelijk te voldoen, zodat we uw aanvraag in behandeling kunnen nemen. Is uw betaling niet binnen dertig dagen ontvangen, dan kan uw aanvraag buiten behandeling worden gesteld. Dit betekent dat uw aanvraag niet beoordeeld wordt en u uw project niet mag starten.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Datum:
20 februari 2017
Aanvraagnummer:
AVD104002017874

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Datum:
20 februari 2017
Aanvraagnummer:
AVD104002017874

Gegevens aanvrager

Uw gegevens

Deelnemersnummer NVWA:	10400
Naam instelling of organisatie:	Wageningen University
Naam portefeuillehouder of diens gemachtigde:	[redacted]
KvK-nummer:	9215846
Straat en huisnummer:	Akkermaalsbos 12
Postbus:	59
Postcode en plaats:	6700 AB WAGENINGEN
IBAN:	NL10RABO0397066465
Tenaamstelling van het rekeningnummer:	Wageningen UR

Gegevens verantwoordelijke onderzoeker

Naam:	[redacted]
Functie:	Onderzoeker
Afdeling:	[redacted]
Telefoonnummer:	[redacted]6
E-mailadres:	[redacted]

Datum:
20 februari 2017
Aanvraagnummer:
AVD104002017874

Gegevens plaatsvervangende verantwoordelijke onderzoeker

Naam:	[redacted]
Functie:	[redacted]
Afdeling:	[redacted]
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Over uw aanvraag

Wat voor aanvraag doet u?	[x] Nieuwe aanvraag [ ] Wijziging op een (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn [ ] Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn

Over uw project

Geplande startdatum:	1 mei 2017
Geplande einddatum:	30 november 2017
Titel project:	Influence of solid feed intake on digesta passage rate and phosphorus requirements of veal calves
Titel niet-technische samenvatting:	invloed van ruw- en krachtvoer op fosforbehoefte vleeskalveren
Naam DEC:	DEC WUR
Postadres DEC:	Postbus 9101, 6700 HB Wageningen
E-mailadres DEC:	dec@wur.nl

Betaalgegevens

De leges bedragen:	€ 1.684,-
De leges voldoet u:	na ontvangst van de factuur

Checklist bijlagen

Verplichte bijlagen:	[x] Projectvoorstel [x] Beschrijving Dierproeven [x] Niet-technische samenvatting

Ondertekening

Naam:	[redacted]
Functie:	[redacted]
Plaats:	Wageningen
Datum:	17 februari 2017

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Wageningen University and Research Concernstaf+
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6708 PB WAGENINGEN

Centrale Commissie Dierproeven
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2500 EK Den Haag
centralecommissiedierproeven.nl
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Onze referentie
Aanvraagnummer
AVD104002017874

Bijlagen
2

Datum 20 februari 2017
Betreft Factuur aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 20 februari 2017
Vervaldatum: 22 maart 2017
Factuurnummer: 170874
Ordernummer: WUR1037014

Omschrijving	**Bedrag**
Betaling leges projectvergunning dierproeven Betreft aanvraag AVD104002017874	€ 1.684,00

Wij verzoeken u het totaalbedrag vóór de gestelde vervaldatum over te maken op rekening NL29INGB 070.500.1512 onder vermelding van het factuurnummer en aanvraagnummer, ten name van Centrale Commissie Dierproeven, Postbus 93144, 2509 AC te 's Gravenhage.

9.

[redacted]

Van: DEC WUR <dec@wur.nl>
Verzonden: maandag 27 maart 2017 11:42
Aan: 'info@zbo-ccd.nl'
Onderwerp: RE: Verzoek aanvullende informatie projectvergunningsaanvraag AVD104002017874

Categorieën: Dossier: [redacted]

Geachte [redacted]

In het advies, dat u van de DEC heeft ontvangen is helaas een passage weggevallen. Excuus hiervoor. T.a.v. onderstaand punt heeft de DEC geoordeeld, dat zij dit acceptabel acht, zeker gezien het feit dat de metingen noodzakelijk zijn in het kader van dit project. De dieren worden in principe in groepen gehuisvest, als ze individueel worden gezet is dit zo kort mogelijk. De onderzoekers ondervangen een toename in ongerief door het gezamenlijk (in paren) huisvesten en het aanbieden van extra verrijking.

Ik hoop hiermee uw vraag te hebben beantwoord.

Met vriendelijke groet,

[redacted]
Secretaris Dierexperimentencommissie
Wageningen University & Research

tel. [redacted]

http://www.wageningenur.nl/

Bezoekadres:
Droevendaalsesteeg 4
[redacted]

Postadres:
[redacted]
Postbus 9101
6700 HB Wageningen

Intern postadres:
Bode 75

Kantooruren: maandag, dinsdag, donderdag, 9.00-17.00 u

From: info@zbo-ccd.nl [mailto:info@zbo-ccd.nl]
Sent: Friday, March 24, 2017 12:26 PM
To: DEC WUR
Subject: Verzoek aanvullende informatie projectvergunningsaanvraag AVD104002017874

Geachte DEC WUR,

Op 17-02-2017 hebben wij een aanvraag voor een projectvergunning dierproeven ontvangen waarover uw DEC advies heeft uitgebracht. Het gaat om het project 'Influence of solid feed intake on digesta passage rate and phosphorus requirements of veal calves' met aanvraagnummer AVD104002017874.

In uw advies geeft u aan dat de dieren niet gehuisvest en verzorgd worden volgens bijlage III van de richtlijn. Kunt u ook aangeven of dit voldoende is onderbouwd en uw antwoord toelichten?

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

Met vriendelijke groet,

[redacted]
Centrale Commissie Dierproeven
www.centralecommissiedierproeven.nl

.....
Postbus 20401 | 2500 EK | Den Haag
.....
T: 0900 2800028
E: info@zbo-ccd.nl

10.

Form
Project proposal

- This form should be used to write the project propsal of 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 our website(www.zbo-ccd.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10400
1.2	Provide the name of the licenced establishment.	Wageningen University
1.3	Provide the title of the project.	Influence of solid feed intake on digesta passage rate and phosphorus requirements of veal calves

2 Categories

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

[] Basic Research
[X] Translational or applied research
[] Regulatory use of routine production
[] Research into environmental protection in the interest of human or animal health or welfare dier
[] 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.

- For legally required animal procedures, indicate which statutory or regulatory requirements apply (with respect to the intended use and market authorisation).
- For routine production, describe what will be produced and for which uses.
- For higher education or training, explain why this project is part of the educational program and describe the learning targets.

Veal calves are traditionally fattened on a diet consisting only of milk replacer (MR). From a welfare and economic perspective, there is a strong incentive to replace a considerable portion of the MR by solid feeds (SF) in the diet (see e.g. Webb et al., 2015). Therefore, SF comprising roughages and concentrates, represent an increasingly important source of nutrients for veal calves. However, veal calves will still be fed a proportion of MR in their diet for approximately 9 months of age to achieve the paleness of white veal meat.
Interactions between MR and SF, mostly occurring in the gastro-intestinal tract, complicate the prediction of the nutritional value of these ration components. Quantitative information about passage rate kinetics of SF through the rumen and other gastro-intestinal compartments currently hampers progress in this field. Limited information available (Berends et al., 2015a) indicates that the level of SF feeding affects ruminal passage rates, more so for concentrates compared with straw, with estimates of mean retention time being considerable higher than in calves exclusively fed on SF. This impacts the nutritional value of SF and also the potential of nutriënt recycling via the rumen. Studies for measuring passage rate kinetics traditionally involve recovery of indigestible tracer inside various compartments of the gastro-intestinal tract (e.g. Berends et al. 2015a), or faecal excretion curves of indigestible markers. These techniques involve sacrificing experimental animals, thus preventing repeated measures on a subject, and/or individual housing on balance cages. Novel technologies to measure passage rate kinetics include the measurement of recovery of 13C tracers in breath (e.g. meCue and Welch, 2016) but these techniques have not been tested and validated in calves.
Recycling of nutrients from the MR back into the rumen has been demonstrated for nitrogen in many types of ruminant animals, including veal calves (Berends et al., 2014, 2015b). Also for phosphorus (P), recycling from blood, via saliva, back into the rumen has been demonstrated to occur in ruminants. Like with nitrogen, exploiting this potential can contribute to the P economy of the calf. Typically, MR ingredients are rather rich in P. Upon consumption, MR flows directly to the abomasum, i.e. bypassing the rumen, and from there to the small intestine. P absorbed from the MR thus enters the systemic circulation. Through saliva, P can recycle back into the rumen, thus providing P originating from MR with a second chance to be utilized. Currently, P contents of de SF portion of the veal calf diet are not optimized to take this type of recycling into account. Hence there is an opportunity to reduce the P content of SF, thereby contributing to steering or reducing in P excretion into the environment. For the Netherlands, a reduction of P excretion is vital to arrive below the maximum set by EU regulations.

Literature

Berends, H., van den Borne, J. J. G. C., Røjen, B. A., van Baal, J. & Gerrits, W. J. J. 2014. Urea Recycling Contributes to Nitrogen Retention in Calves Fed Milk Replacer and Low-Protein Solid Feed. The Journal of Nutrition. 144, 7, p. 1043-1049

Berends, H., van den Borne, J. J. G. C., Stockhofe-Zurwieden, N., Gilbert, M. S., Zandstra, T., Pellikaan, W. F., van Reenen, C. G., Bokkers, E. A. M. & Gerrits, W. J. J. 2015a. Effects of solid feed level and roughage-to-concentrate ratio on ruminal drinking and passage kinetics of milk replacer, concentrates, and roughage in veal calves. Journal of Dairy Science. 98, 8, p. 5621-5629

Berends, H., van den Borne, J. J. G. C., Røjen, B. A., Hendriks, W. H. & Gerrits, W. J. J. 2015b. Effect of protein provision via milk replacer or solid feed on protein metabolism in veal calves. Journal of Dairy Science. 98, 2, p. 1119-1126.
McCue, MD and Welch KC jr. 2016. 13C breath testing in animals: theory, applications and future directions. J. Comp. Physiol. B (2016) 186: 265-285.

Webb, L. E., van Reenen, C. G., Berends, H., Engel, B., de Boer, I. J. M., Gerrits, W. J. J. & Bokkers, E. A. M. 2015. The role of solid feed amount and composition and of milk replacer supply in veal calf welfare. Journal of Dairy Science. 98, 8, p. 5467-5481

3.2 Purpose

Describe the project's main objective and explain why this objective is achievable.

- If the project is focussed on one or more research objectives, which research questions should be addressed during this project?
- If the main objective is not a research objective, which specific need(s) does this project respond to?

In this project, we will address three important research questions:
1) Can digesta passage rate kinetics in calves (with emphasis on the rumen) be measured using non-invasive 13C tracer breath test approach?
2) What is the effect of SF intake and roughage to concentrate ratio on passage rate kinetics of MR and concentrates
3) What are the P requirements of calves fed rations with different MR to SF ratios and different SF compositions (i.e., different concentrate to roughage ratios).
The underlying issue of the latter 2 questions is to what extent can we exploit P recycling as a mechanism to steer/reduce P excretion in veal calves fed combinations of SF and MR.

This project is achievable, both in terms of the technical aspect and the scientific aspect. This is due to the fact that this project involves project members with expertise and knowledge on this topic as well as on the practicability of the experiments. This enables us to design the experiments, which are described in more detail in the appendices, in such a way that we can answer the above mentioned questions while minimizing animal discomfort as much as possible.

3.3 Relevance

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

Inside the various compartments of the gastro-intestinal tract, there is always competition between passage and degradation of macronutrients. With increasing passage rate within a compartment, time for degradation decreases, hence reducing the efficiency of nutrient digestion in that compartment. Depending on the rate of feed intake, this reduced efficiency may lead to a decrease in nutriënt absorption or not. For predicting the feeding value of MR, concentrates and roughages (together comprising the SF component of the diet) it is pivotal to know passage rate kinetics. Such information typically is integrated into mathematical models predicting nutriënt absorption following the provision of various dietary regimes. Such models are already available for pigs, dairy cows and for calves fed exclusively on milk replacers. Currently, a calf model is being developed integrating rumen development, rumen function and post-absorptive nutriënt metabolism. This model will be used for designing and evaluation of feeding strategies in veal calves, fed various combinations of MR and SF. Reliable information about passage rate kinetics of the different ration components has been identified as limiting the quality of such models. Current approaches to the measurement of passage rate kinetics involve the use of indigestible marker techniques, either requiring prolonged individual housing, or sacrificing of experimental animals, the latter preventing repeated measures on a subject. Novel, minimally invasive, 13CO2 breath test approaches could, in part, replace such approaches, but need to be developed and tested.

Reducing P excretion in the environment has been identified as an important target by the Dutch government, as P excretion has exceeded the limits set by EU in 2015. For extending the derogation for 2017 and beyond, prevention of P excretion exceeding these limits is of utmost importance. Furthermore, reducing P inputs (via dietary manipulation) will also contribute to a sustainable use of resources. This research will contribute positively to control both P inputs and P excretion. First, as mentioned previously, MR ingredients are rather rich in P. This, combined with the recycling of P in the rumen, provides the opportunity to reduce the P content of SF, thus reducing P inputs. Second, by determining the P requirements of calves, we are able to control the P excretion. Third, integrating knowledge into mathematical models will allow more accurate predictions of nutriënt use and environmental excretion under widely varying nutritional regimes.
Moreover, this research will contribute to less invasive measurements (increased refinement) of passage rate kinetics in future experimental animals.

3.4 Research Strategy

3.4.1 Provide an overview of the overall design of the project (strategy).

Within the present project we aim to quantify passage rate kinetics of MR, concentrates and roughages under 4 different dietary regimes. We will also determine P requirements in calves under the same 4 dietary regimes. As adaptation of calves to these dietary regimes will take time (about 4 weeks), we will minimize the number of calves required by performing a series of experiments, using the same calves. First, a pilot experiment will be performed (see section 3.4.2 and annex 1) to determine whether P requirements can be determined for individual calves with a step-wise within animal dose response approach, similar to Kampman van de Hoek et al. (2012) for studying limiting amino acids in pigs. The principle of the

technique is that after providing an excess of P via MR, the P intake will be reduced via MR, leading to a reduction of P excreted via urine. The inflection point of the P-excretion curve against P intake will be assessed via nonlineair regression and assumed to represent the requirement of that calf. The plateau value, obtained at the lowest P intake will represent the maximum efficiency of P utilization, likely influenced by SF intake.
The advantage of such a technique is that it potentially provides estimates of P requirements of individual calves. For the pilot study, only the rate of adaptation of urinary P excretion following a substantial reduction in P intake via the MR will be monitored in a limited number of calves. If the rate of urinary P exretion stabilizes within 72h after the reduction in dietary P supply, the technique can be considered applicable for individual calves. If this is the case, the different dietary regimes will be imposed on calves, subsequently followed by two studies to investigate passage rate kinetics of MR, concentrates and roughages (see section 3.4.2 and both annex 2 and 3), followed by a study to quantify P requirements under these nutritional conditions in each calf (see section 3.4.2 and annex 4). If the within-calf dose response technique does not work, a more traditional, between animal, dose response approach will be used (see e.g. Erickson et al., 2002), comparing P excretion at different levels of intake between animals. Hence, this project comprises 4 studies, with studies 2, 3, and 4 (annex 2, 3, and 4, respectively) being performed with the same calves.
The calves in the pilot study (annex 1) will be about 6 to 8 weeks of age. The age of the calves at each of the 3 consecutive studies (annex 2, 3, and 4) will differ, as these studies are performed sequentially, (re-)using the same 48 calves. These studies will be performed in six batches. At start of the first passage rate kinetics study (annex 2), calves of approximately 6 weeks of age will be purchased from a veal producer. The first batch of calves (n=8) will adapt to the diet for 5 weeks, subsequently followed by 7 days of measurements in the respiration chambers, and subsequently followed by the second passage rate kinetics study (annex 3) and the P requirement study (annex 4). For batch 2 to 6, the same procedure will be used, with a delay of about 1 week for each batch. Hence, the results will be obtained over an age-range of about 6 weeks. The common interest in these three studies is the combination of the nutritional treatments, of which it takes time for calves to adapt (rumen development). With discomfort for the calves estimated as mild for the studies in annex 2 and 3, we consider it the best option. to re-use calves from the study in annex 2 for annex 3 and 4. As argued in annex 2 and 4, the number of calves required is approximately the same. For the studies in annex 2 and 3, comparison of observations on the same animals is important.

Literature

Erickson GE, Klopfenstein TJ, Milton CT, Brink D, Orth MW and Whitted KM. 2002. Phosphorus requirements of finishing feedlot calves.. J. Anim,. Sci. 80(6) 1690-1695.
Kampman-van de Hoek, E, Gerrits, WJJ, van der Peet-Schwering, CMC Jansman, AJM and van den Borne, JJGC 2013. A simple amino acid dose-response technique to quantify amino acid requirements of individual meal-fed pigs Journal of Animal Science. 91, 10, p. 4788-4796

3.4.2 Provide a basic outline of the different components of the project and the type(s) of animal procedures that will be performed.

As described above, the project will consist of 4 studies:

1. P requirements in veal calves - a pilot study. The objective of this study is to test whether a within-animal approach can be used to study P requirements. The outcome will determine the design of study 4. This study will be conducted with calves, individually housed on balance cages and provided with faecal collection bags to allow separate, quantitative collection of urine and faeces. The study duration will be 4 days (adaptation) and 6 days (experiment)
2. Passage rate kinetics in veal calves - 13C tracer technique: Minimally invasive technique with stable isotope methods. This technique is based on a breath-test approach described by Van den Borne et al. (2015), and techniques widely used in humans and animals (MCue and Welch, 2016). Briefly, following a pulse dosis of a 13C tracer, 13C labelled CO2 is collected in exhaled air. If the tracer is well chosen, the exhalating pattern of the tracer will reflect the pattern of nutriënt absorption and metabolism of the tracee. As the primary interest is in the timing of nutrient absorption, metabolism patterns as well as delays in exhalation caused by dilution in body pools need to be corrected for. For this study, calves will be purchased at an age of 6 weeks and adapted to the nutritional treatments for a period of 5 weeks in group-housing. The measurements will be conducted in pair-housed calves in climatized respiration chambers, frequently fed a SF mix (i.e., 6 times a day to create a steady state), and fed a MR twice daily. Different isotope tracers will be administered through the diet, spread over the 7d experimental period.
3. Passage rate kinetics in veal calves - faecal excretion curves. Faecal excretion patterns of indigestible markers in faeces following a pulse dose of markers representative for MR, concentrates and roughages. Following the approach described by Dhanoa et al., (1985). Briefly, this approach is based on the assumption that excretion kinetics of an indigestible marker will reflect retention time of digesta in the slowest compartment. This study will be conducted in a setting similar to study 1, with frequent faecal collections following the pulse-dosed markers. The length of the adaptation and experiumental periods will be 4 and 5 days, respectively.
4. P requirements in veal calves fed different rations. The approach taken will depend on the outcome of the pilot. In the case the conventional approach will be used, the number of dietary regimes to be tested will be reduced from 4 to 2. This study will be conducted in a setting similar to study 1 and will last 21 days. In the case of a conventional approach, calves will be subjected to low P diets for 4 days, after which P excretion will be performed for a period of 7 d.

Literature

Dhanoa NS Siddons, RC, France J and Gale DL. 1985. A multicompartment model to describe marker excretion patterns in ruminant faeces. Br J Nutr 53: 663-671.

McCue, MD and Welch KC jr. 2016. 13C breath testing in animals: theory, applications and future directions. J. Comp. Physiol. B (2016) 186: 265-285.
Van den Borne JJGC, Heetkamp MJW, Buyse J, Niewold TA 2015 Fat coating of Ca butyrate results in extended butyrate release in the gastrointestinal tract of broilers . Livestock Science 2015 (175): 96-100.

3.4.3 Describe the coherence between the different components and the different steps of the project. If applicable, describe the milestones and selection points

For a description of the studies to be conducted as part of this project, please see the project outline above. Three milestones will be identified within this project, and a 4th one after completion of the animal experiments conducted within this project:
1) After study 1 (pilot), a decision will be made on how the fourth study will be conducted, i.e. a within-calf dose response approach or a between calf dose response approach.
2) An estimation of passage rate kinetics of MR and concentrates using the isotope method (study 2) and on MR, concentrates and roughages in the feacal collection method (study 3), both focusing on the first compartment of the GI tract (i.e. the rumen for the concentrates and roughages, the abomasum for the MR).
3) A validated approach of digesta passage kinetics using a combination of 13CO2 breath test approaches.
3) An estimate of P requirements as affected by the nutritional regimes applied in this project.
4) Upon completion of the 4 studies, the passage rate data will be used for improvement of the model simulating calf growth from nutritional inputs. P digestion and metabolism will be incorporated into this model, allowing the prediction of total P and N flows from ingestion to growth or excretion via urine or faeces.

3.4.4 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	P requirements in veal calves - a pilot study
2	Passage rate kinetics in veal calves - 13C tracer technique
3	Passage rate kinetics in veal calves - faecal excretion curves
4	P requirements in veal calves fed different rations

11.

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, see our website www.zbo-ccd.nl.
- Or contact us by phone. (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10400	
1.2	Provide the name of the licenced establishment.	Wageningen University	
1.3	List the different types of animal procedures. Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.	Serial number 1	Type of animal procedure P requirements in veal calves - a pilot study

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 direct goal of this pilot study is to determine whether we can measure the P requirements of individual veal calves using a within-calf dose respons approach. When succesfull, it provides a more refined technique to determine P requirements under varying nutritional regimes in the future.
As described in section 3.4.1 of the project proposal, the principle of this approach is that after providing an excess of P via MR, P intake will reduced by 20% via MR (in 1 step), leading to a reduction of P excreted via urine. The inflection point of the P-excretion curve against P intake will be assessed via nonlineair regression and assumed to represent the requirement of the individual calfs. The plateau value, obtained at the lowest P intake will represent the maximum efficiency of P utilization, likely influenced by SF intake.
During this pilot study, we will only monitor the rate of adaptation of urinary P excretion following a substantial reduction in P intake via the MR in a limited number of calves (n=6). If the rate of urinary P exretion stabilizes within 72h after the reduction in dietary P supply, the technique can be considered applicable for individual calves.

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

Six calves of approximately 8 weeks of age will be individually housed in metabolic cages, and fitted with harnasses to which plastic bags are attached for the quantitative collection of faeces. Clean urine can be collected in buckets underneath a funnel, mounted underneath the cage. Calves will be fed a normal ration with a high P milk replacer for the 4-d adaptation period and for the first 3 days of the experimental period, after which they will be switched to a MR lowering the P content of the MR by 20%, which will be fed the remaining 4 days. Typically, if urinary P excretion sensitively responds to a reduction of P intake via MR by 20%, it can be assumed that calves will respond to the within-calf dose response study (i.e. the contrast chosen should not be much larger than the intended change in P intake).
During the entire experimental period, quantitative, 12h urinary collections, and 24h faecal collections will be performed. This is done to determine the rate of adaptation of urinary P excretion after a reduction in P intake via the MR. In addition, salivary P concentrations will be determined 4 times daily during the experimental period.

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

The number of calves used in this experiment is based on the average P excretion in urine and faeces in several experiments with calves housed under similar conditions. It is based on the analysis of P flows in a study published by Berends et al., (2015), of which the data on P flows are

reported in an internal report by Plomp et al (2015). That study involved 8 calves and used a between-calf dose response approach. A reduction in P input via MR by 15% resulted in a tendency for a reduced P output via urine.
In the present study however, we will use a reduction in P intake of 20% (as described in section 2A - animal procedures). Moreover, we will use a within-calf dose response approach, which will reduce variation. Performing a power analysis (one sided), revealed that at least 6 calves are needed to demonstrate a significant reduction of P excretion via urine when reducing P input by 20%.
We do want to emphasize that with this pilot study we want to determine whether this within-calf dose response approach is applicable to determine the P requirements of individual calves. This will be mainly based on the assessment of the P-excretion curves against P intake via nonlinear regression rather than strict statistical analysis. If we simply cannot find a stabilized urinary P excretion within 72h after the reduction in dietary P supply, we conclude that this within-calf dose response approach does not work.

Literature

Berends, H., van den Borne, J. J. G. C., Røjen, B. A., Hendriks, W. H. & Gerrits, W. J. J. 2015. Effect of protein provision via milk replacer or solid feed on protein metabolism in veal calves. Journal of Dairy Science. 98, 2, p. 1119-1126
Plomp M, Gerrits WJJ, Schop TA, Heeres-vd Tol, JJ 2015. Fosforstromen in vleeskalveren. P en Ca balans in kalveren gevoerd met melkvervanger en ruw- en krachtvoer. Rapport Wageningen Livestock Research, 46pp [in Dutch], available on request

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

In this pilot experiment, 6 male Holstein Friesian calves will be used. The age of these calves will be approximately 6 weeks, and we will purchased them from a veal producer or use the calves available at the experimental facilities. Male calves will be used to ease separate collection of faeces and urine. Calves will be about 80 kg of BW at the onset of the trial, and close to 90 kg BW when the trial is finished.

Species	Origin	Maximum number of animals	Life stage
calves	moderate	6	

C. Re-use

Will the animals be re-used?

[X] No, continue with question D.
[] Yes > Explain why re-use is considered acceptable for this animal procedure.

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

[] No
[] Yes > Provide specific justifications for the re-use of these animals during the procedures.

D. 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: The male Holstein Friesian calf is the target animal of this project. The rate of adaptation of urinary P excretion to changes in an excess or shortage of phosphorus cannot be estimated in another species. Additionally, passage rate kinetics are typically integrated into mathematical models predicting nutriënt absorption following the provision of various dietary regimes. Such models are already available for pigs, dairy cows, and for calves fed exclusively on milk replacers. However, no such model is available yet for calves fed a combination of milk replacer and solid feeds. Therefore, the rate of adaptation of urinary P excretion to changes in an excess or shortage of phosphorus cannot be estimated by the use of a mathematical model. Reduction: this pilot study will be performed to investigate the possibility to study P requirements in individual calves rather than the conventional approach in which different levels of P are imposed on different calves, hence necessitating multiple groups of calves, estimating the average requirement of the calf population by regression based on observations obtained from different calves. When possible, less experimental animals are needed to obtain estimates of P requirements under various nutritional regimes. Refinement: As indivual housing on metabolism cages causes discomfort, we'll take precautions to minimize the impact. We place pairs of metabolic cages close to each other to facilitate physical contact between two calves. Additionally, we enrich the metabolic cages with brushes, a dry teat for suckling needs, and provide a ball inside the cage. Additionally, the use of chains is minimized (to avoid unneccesary noise). Furthermore, experienced animal care takers will monitor the calves to be sure a potential case of illness or reduced welfare will be diagnosed in an early stage. Handling of the calves will be performed by experienced personnel carefully and quietly, preferably in rest to prevent unnecessary distress. Analgesia and medication are applied where needed. If (in rare cases) analgesia or medication may influence trial results, calfs are treated and excluded from the trial. This study also

contributes to the refinement of future research determining passage rate kinetics of animals. For example, the duration of the study 'P requirement in veal calves fed different rations' (annex 4) depends on the rate of adaptation of urinary P excretion to changes in P intake via MR. It is important for this study to provide an accurate estimate of this rate of adaptation in order to minimize the duration of the study 'P requirement in veal calves fed different rations' (annex 4), and thus the duration of the individual housing of these calves.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

The following measures will be taken to reduce the impact of housing in metabolism cages on welfare of the calves: - use of chains will be minimized as the noise can disturb the calves - availability of brushes, mounted on the cage and a dry teat to satisfy sucking needs - a ball inside the cage - cages will be organized in pairs close to each other to facilitate some physical contact between calves

Repetition and Duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Based on a literature search within the Pubmed and Scopus database using 'calf, phosphorus requirement, milk replacer, roughage, and concentrate' as searching criteria, we conclude that no study has been published before regarding the P requirements in calves fed a ration based on MR, concentrates and roughages. P requirement studies in beef calves are available, but these calves are fed a ration based on concentrates and roughages without MR, and therefore do not take the potential to recycle P from the MR into account.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

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

Calves will be housed individually in metabolism cages. Fecal collection bags will be harnassed to the calves, to facilitate the quantitative collection of faeces and thereby the quantitative collection of clean urine in buckets underneath funnels, mounted underneath the cage. The dimensions of the cage will be 0.8 x 2m, and calves will be fixed to the front of the cage, allowing them to stand or lie freely. Housing on bedding material is not possible as clean urine needs to be collected from underneath the cages. As individual housing on metabolism cages causes discomfort, we'll take precautions to minimize the impact (i.e., minimized use of chains, cage enrichment including brushes, dry teat, and ball, and cages will be close to each other to facilitate physical contact between calves).

G. 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 H.
[] 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.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

[X] No > Continue with question I.
[] Yes > Will anaesthesia, analgesiaor other pain relieving methods be used?

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

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

I. Other aspects compromising the welfare of the animals

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

Individual housing for 7 days, restricted movements, no bedding material on the floor (because clean urine needs to be collected from underneath the cages), harnasses to fix faecal collection bags. This could result in increased occurence of abnormal oral behaviours (e.g., repetitive self-licking, flank or object sucking, tongue rolling).

Explain why these effects may emerge.

The calves are deprived from exploring their environment and some discomfort from wearing harnesses cannot be prevented.

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

The following measures will be taken to reduce the impact of housing in metabolism cages on welfare of the calves: - use of chains will be minimized as the noise can disturb the calves - availability of brushes, mounted on the cage and a dry teat to satisfy sucking needs - a ball inside the cage - cages will be organized in pairs close to each other to facilitate some physical contact between calves

J. 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 K.
[X] Yes > Describe the criteria that will be used to identify the humane endpoints.

• a calf has feed refusals exceeding 20% of the amount of MR offered for a period exceeding 3 days. • a calf has a fever for 3 successive days, not responding to medical treatments proposed by a veterinarian, and signs of infection and inflammation. • a calf suffers from problems related to the harnass, showing wounds at places the harness is connected. • in the expert judgement of the veterinarian, future observations on a calf will not provide reliable results. • in the expert judgement of the veterinarian, continuation of the experiment with a calf will cause discomfort higher than foreseen for this trial.

Indicate the likely incidence.

The likely incidence of calves to be removed from the experiment is estimated to be less than 10% during the duration of the trial, based on previous experience in trials of similar duration. The major portion of this 10% is expected to origin from feed refusals due to health problems unrelated to

experimental procedures. In addition, problems arising from the harnesses will have a very short duration, as calves will then be removed from the trial.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned (non-recovery, mild, moderate, severe).

The level of discomfort is expected to be as listed below: • Individual housing on balance cages, in absence of bedding material during 10 days: moderate • Wearing of harnasses for the collection of faecal collection bags: mild Hence the cumulative discomfort in this trial is estimated at moderate. Based on previous experience, collecting saliva samples are considered to cause no discomfort. We will insert two cotton swabs (150 x 4 mm) into the mouth of a calf, who will chew on them voluntarily, subsequently we will place these into a salivette.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

[X] No > Continue with Section 3: 'Signatures'.
[] 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 > Descripe the method of killing that will be used and provide justifications for this choice.

[] Yes

12.

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, see our website www.zbo-ccd.nl.
- Or contact us by phone. (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10400	
1.2	Provide the name of the licenced establishment.	Wageningen University	
1.3	List the different types of animal procedures. Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.	Serial number 2	Type of animal procedure Passage rate kinetics in veal calves - 13C tracer technique

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.

For this study, 48 male, Holstein-Friesian calves will be purchased at about 6 weeks of age. They will be housed in groups and assigned to one of 4 dietary treatments, varying in the intake of solid feed (SF) and milk replacer (MR) (factor 1) and the concentrate:roughage ratio within the SF (factor 2) in 2x2 factorial arrangement. The calves will be adapted to their diets for a period of 5 weeks to allow rumen development and rumination behaviour to completely adjust to the levels and composition of SF intake. After that, calves will be housed for 7 days in pairs in one of 4 respiration chambers, and frequently fed (i.e. 6x daily) their SF portion while receiving their MR supply twice daily.
This setup (pair or calves in one of 4 respiration chambers), results in a staggered planning with 6 batches of 8 calves (4 pairs) undergoing the same experimental procedure, each batch starting one week after the previous one. In this way, creating a range in age (as also described in section 3.4.1 - research strategy of the project proposal). The first batch of 8 calves will adapt to their diet for 5 weeks, subsequently followed by 7 days of measurements in the respiration chambers. The second batch of calves (also n=8) will adapt to the diet for 6 weeks (i.e., basis of 5 weeks + the 7 days that the first group of calves in housed in respiration chambers), subsequently followed by 7 days of measurements in the respiration chambers. This pattern continues for all 6 batches. In this manner, observations are achieved over an age range of 6 weeks.
When the calves are housed in the respiration chambers, every two days, one of the MR or SF meals will be spiked with a 13C stable isotope tracer, measuring the kinetics of recovery in 13CO2 in exhaled air as a measure of the rate of ruminal fermentation and/or digesta passage kinetics. 13C tracers will be selected after a thorough search of literature, but likely include naturally labelled yeast proteins (to be included in the concentrate), 13C-glycine (to be included in the MR), 13C octanoate and 13C-NaHCO3, to be injected intravenously. The latter is intended to correct for a delay between nutriënt oxidation and 13C exhaled in breath.

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

During the 5-week adaptation period, calves will be housed in groups (divided over several departments within the experimental facilities) and fed diets according to the experimental schedule. MR will be provided twice daily and SF will be provided in two portions upon completion of MR intake.
During the 7d experimental period, calves will be housed in pairs inside a climatized respiration chamber. During this week, continuous measurement of CO2, 13CO2 and CH4 production and the consumption of O2 will be performed. During this period, SF will be provided in 6 equal portions spread over the 24h period to ensure a steady state. Following oral administration of 13C tracers, patterns of 13CO2 excretion will be measured. To account for a delay in 13CO2 exhalation following arrival in the bicarbonate pool, 13CO2 excretion patterns will be analyzed following intravenous injection of a bolus 13C-NaHCO3.
After the calves have been in the climate respiration chambers for 7 days, the calves subsequently enter the study described in Annex 3.

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

This experiment is designed to quantify differences between the dietary treatments on digesta passage rate kinetics. As calves are housed in pairs and the gaseous exchanged is measured in a respiration chamber, a pair is considered as the experimental unit for this study. Treatment differences on dependent variables (mostly time of peak recovery of 13CO2 after a bolus of 13C tracer) will be analyzed by ANOVA using the dietary contrasts as fixed effects. In a previous study (Berends et al., 2015) it was demonstrated that provisional estimates of ruminal fractional passage rates could be evaluated using 8 calves per treatment, applying an approach using indigestible markers. A significant difference could be detected for a difference in feeding level of SF, similar to the one intended for this study. Considering a reduction in variation with two calves per experimental unit, we consider 6 experimental units for each treatment combination to be sufficient to estimate the effect of our dietary treatments on passage rate kinetics. For the methodology applied in this study, estimates of variation between experimental units are not available, although the 13CO2 breath test has been applied previously in calves in our facilities (see e.g. Gilbert et al., 2016), also with 2 calves in a respiration chamber. A 3-h difference in the time of peak metabolism of fructose and glucose could be quantitied using 5 pairs of calves per treatment ($P < 0.01$). Six pairs of calves per treatment combination would enable us to significantly detect a 2h difference in the time of peak of 13CO2 recovery.

Literature

Berends, H., van den Borne, J. J. G. C., Røjen, B. A., Hendriks, W. H. & Gerrits, W. J. J. 2015b. Effect of protein provision via milk replacer or solid feed on protein metabolism in veal calves. Journal of Dairy Science. 98, 2, p. 1119-1126.

Gilbert, M. S., Pantophlet, A. J., van den Borne, J. J. G. C., Hendriks, W. H., Schols, H. A. & Gerrits, W. J. J. 2016. Effects of replacing lactose from milk replacer by glucose, fructose, or glycerol on energy partitioning in veal calves. Journal of Dairy Science. 99, 2, p. 1121-1132

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

For this study, 48 male Holstein Friesian calves will be used. The age of these calves will be approximately 6 weeks, and we will purchased them from a veal producer. Male calves will be used to ease separate collection of faeces and urine. The latter is not specifically needed for the study described in this Annex, but essential for the studies described in Annex 3 and Annex 4. Because we will use the same calves for all studies, we also

need male calves for this study. At the beginning of this experiment, all calves will be 6 weeks of age. At the end of the experiment, the age of the calves will range from 12 weeks to 19 weeks.

Species	Origin	Maximum number of animals	Life stage
calves	minor	48	

C. Re-use

Will the animals be re-used?

[X] No, continue with question D.
[] Yes > Explain why re-use is considered acceptable for this animal procedure.

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

[] No
[] Yes > Provide specific justifications for the re-use of these animals during the procedures.

D. 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: The male Holstein Friesian calf is the target animal of this project. Passage rate kinetics measured in other ruminants animals are not applicable for calves, because based on the results obtained by Berends et al. (2015) it can be concluded that the passage rate of digesta in calves is likely substantially lower compared with other ruminants. Additionally, passage rate kinetics are typically integrated into mathematical models predicting nutriënt absorption following the provision of various dietary regimes. Such models are already available for pigs, dairy cows, and for calves fed exclusively on milk replacers. However, no such model is available yet for calves fed a combination of milk replacer and solid feeds. Therefore, the rate of adaptation of urinary P excretion to changes in an excess or shortage of phosphorus cannot be estimated by the use of a mathematical model. Reduction: the objective of this study is to evaluate the possibiltiy to use minimally invasive 13C breath test approach for the measurement of passage rate kinetics in calves. Outcomes will be validated with more conventional measures of passage rate kinetics (annex 3 of this project). When succesful, this approach can be used for repeated measurement of digesta passage rate in calves, or potentially also in other ruminant species. Refinement: Calves will be socially housed during the adaptation period and will be housed in pairs in the respiration chambers to avoid social isolation. We intend to use free housing, but if needed we restrain the calves during feeding. Measurements will be non-invasive, with

the only exception of an intravenous injection of 13C labelled NaHCO3. Furthermore, experienced animal care takers will monitor the calves to be sure a potential case of illness or reduced welfare will be diagnosed in an early stage. Handling of the calves will be performed by experienced personnel carefully and quietly, preferably in rest to prevent unnecessary distress. Analgesia and medication are applied where needed. If (in rare cases) analgesia or medication may influence trial results, calfs are treated and excluded from the trial.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

No adverse effects are foreseen

Repetition and Duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Based on a literature search within the Pubmed and Scopus database using 'calf, passage rate kinetics, milk replacer, roughage, and concentrate' as searching criteria, we conclude that no study has been published that uses the 13C tracer technique to measure digesta passage rate kinetics in calves fed combination of MR and solid feed are available. Hence this is not a repeat of research of others.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

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

Calves will be socially housed during the adaptation period and will be housed in pairs in the respiration chambers to avoid social isolation. We intend to use free housing, but if needed we will restrain the calves during feeding. In the respiration chambers, two calves will be housed in a pen of about

1.80x 3.5m. This is related to the maximum inner measures of the respiration chambers and needed for accurate measurement of short-term changes in 13CO2 exhalation.

G. 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 H.
[] 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.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

[] No > Continue with question I.
[X] Yes > Will anaesthesia, analgesiaor other pain relieving methods be used?

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

No pain relieving methods will be used, as the intravenous injection will only cause momentary pain.

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

I. Other aspects compromising the welfare of the animals

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

Absence of bedding material during 7 days, restrainement during feeding if needed, iv injection of a pulse dose of NaHCO3, and weighing of the calves. We do not expect other adverse effects, including the different dietary treatments. The four dietary treatments, varying in the intake of SF and MR (factor 1) and the concentrate:roughage ratio within the SF (factor 2), will be within the range which is fed in practice.

Explain why these effects may emerge.

There will be some discomfort from the absence of bedding material. Moreover, the possible restrainment during feeding (when eating from eachothers food) will cause some discomfort. The iv injection will cause some discomfort, but only represents momentary pain.

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

We will house the calves in pairs to ensure social interaction.

J. 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 K.
[X] Yes > Describe the criteria that will be used to identify the humane endpoints.

Not as a result of the animal procedures, but health problems may arise necessitating animals to be removed from the trial. Humane endpoints are: - feed refusals exceeding 20% of the daily allowance for a period exceeding 3 days - a calf has a fever for 3 successive days, not responding to medical treatments proposed by a veterinarian. - in the expert judgement of the veterinarian, future observations on a calf will not provide reliable results. - in the expert judgement of the veterinarian, continuation of the experiment with a calf will cause discomfort higher than foreseen for this trial.

Indicate the likely incidence.

Likely 5% or less, because the calves are housed socially during the adaptation period and in pairs in the respiration chambers. Moreover, we intend to house the calves freely (not restrained, with the possible exception during feeding).

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned (non-recovery, mild, moderate, severe).

The level of discomfort is expected to be as listed below: • Group housing in adaptation phase and in respiration chambers, in absence of bedding material during 7 days: mild • if needed calves will be restrained during feeding: mild • iv injection of a pulse dose of NaHCO3: mild • weighing of the calves (weekly): mild Hence the cumulative discomfort in this trial is estimated at mild.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

[X] No > Continue with Section 3: 'Signatures'.
[] 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 > Descripe the method of killing that will be used and provide justifications for this choice.

[] Yes

13.

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, see our website www.zbo-ccd.nl.
- Or contact us by phone. (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10400	
1.2	Provide the name of the licenced establishment.	Wageningen University	
1.3	List the different types of animal procedures. Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.	Serial number 3	Type of animal procedure Passage rate kinetics in veal calves - faecal excretion curves

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.

For this study, 48 male, Holstein-Friesian calves will be re-used from the study described in annex 2. The experimental procedures are staggered, with measurements on every batch of 8 calves starting a week after the previous one. The results from this study will thus be obtained over an age range of 6 weeks.

Upon completion of the study described in annex 2, the calves remain housed in pairs. They will be fed the same experimental dietary treatments, varying in the intake of solid feed (SF) and milk replacer (MR) (factor 1) and the concentrate:roughage ratio within the SF (factor 2) in 2x2 factorial arrangement. The calves then have already been adapted to these diets for at least 6 weeks. Calves will be fitted with harnasses to which plastic bags are attached for the quantitative collection of faeces. Three indigestible markers will be used to trace the concentrates (likely TiO2 or alkanes), roughage (likely cr-mordant long straw) and the MR (likely Co-EDTA). The markers will be administered in a pulse dose 48h (roughage), 24h (concentrate) or 4h (MR) prior to the start of the faecal collection period. Faecal bags will be checked bi-hourly for a period of 4 days, and marker excretion will be quantified in time. Following methods published by Dhanoa et al. (1985), faecal excretion curves of markers will be analyzed to estimate the overall mean retention time of digesta and the passage rate of the slowest compartment (rumen for the markers of the concentrate and roughage; abomasum for the markers of the MR).

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

Calves will be housed in pairs, and fitted with a harness to which faecal collection bags will be attached. If the combination of housing in pairs and the harnasses is not succesfull, we will place a fence in between the calves. We will restrain calves during replacement of the faecal collection bags and, if needed, also during feeding.

Calves will be fed according to normal procedures and faeces will be collected 2-hourly during 4 days after the start of the trial.

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

The calves from this experiment, will be re-used from the study described in Annex 2 and will subsequently be re-used for the study described in

Annex 4. Therefore, the number of calves used in this study depends largly on the minimum number of calves needed for those two studies. To summarise:
The study described in Annex 2 needs 48 calves to achieve the objective of that trial. As calves are housed in pairs and the gaseous exchanged is measured in a respiration chamber, a pair is considered as the experimental unit for the study in Annex 2. In a previous study (Berends et al., 2015) it was demonstrated that provisional estimates of ruminal fractional passage rates could be evaluated using 8 calves per treatment, applying an approach using indigestible markers. A significant difference could be detected for a difference in feeding level of SF, similar to the one intended for the study in Annex 2. Considering a reduction in variation with two calves per experimental unit, we consider 6 experimental units for each treatment combination to be sufficient to estimate the effect of our dietary treatments on passage rate kinetics.
Additionally, results from this study will be compared with the results from the study described in annex 2 (passage rate kinetics - 13C tracer technique) and therefore needs to be conducted with the same calves continued on the same treatments.
Furthermore, the study described in Annex 3 needs 48 calves as well to achieve the objective of that trial. Depending on the option chosen, there will be one estimate of P requirements per calf, or one per SF treatment. Between-calf variation of such estimates are unknown, but previous experience using a similar dose response approach for amino acid requirements in pigs (e.g. Bruininx et al., 2015) and for responses to graded levels of starch or starch products in calves (Gilbert et al., 2015) demonstrate sensitive responses to step-wise changes in the driving variable using 10 animals/treatment. For estimating P requirements using a between-calf approach, 24 calves per treatment assigned to graded levels of P intake is an approach previously shown to provide accurate P requirements, and can be expected to be adequate to develop reliable requirement estimates for our two nutritional conditions.

Literature

Berends, H., van den Borne, J. J. G. C., Røjen, B. A., Hendriks, W. H. & Gerrits, W. J. J. 2015b. Effect of protein provision via milk replacer or solid feed on protein metabolism in veal calves. Journal of Dairy Science. 98, 2, p. 1119-1126.

Bruininx, E. M. A. M., van den Borne, J. J. G. C., Eising, I., Vervenne, P., Sakkas, P.& Gerrits, W. J. J. 2015. Optimal lysine:DE ratio in growing pigs is independent of starch or fat as main energy source at two energy intake levels Journal of Animal Science. 93, 10, p. 4774-4780

Gilbert, M. S., van den Borne, J. J. G. C., Berends, H., Pantophlet, A. J., Schols, H. A. & Gerrits, W. J. J. 2015. A titration approach to identify the capacity for starch digeston in milk-fed calves. Animal. 9, 2, p. 249-257

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

In this experiment, 48 male Holstein Friesian calves will be re-used from the study described in annex 2 of this project. These calves will be the same calves as decribed in Annex 2. Male calves will be used to ease separate collection of faeces and urine. At the beginning of this experiment, the age of the calves will range from 12 weeks to 19 weeks. At the end of the experiment, the age of the calves will range from 13 weeks to 20 weeks.

Species	Origin	Maximum number of animals	Life stage
Calves	mild	48	

C. Re-use

Will the animals be re-used?

[] No, continue with question D.

C. Re-use

[X] Yes > Explain why re-use is considered acceptable for this animal procedure.

Discomfort during previous experiment (described in annex 2 of this project) is classified as mild. The comparison of the results of annex 2 with the results of this study is important, having similar conditions and working with the same calves is preferred. Additionally, if we would be working with a new batch of calves, we have to adapt these calves to the diets for several weeks. This is not necessary if we re-use the calves from Annex 2.

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.

D. 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: The male Holstein Friesian calf is the target animal of this project. Passage rate kinetics measured in other species are not applicable for calves, because based on the results obtained by Berends et al. (2015) it can be concluded that the passage rate of digesta in calves is lower compared with other species. Additionally, passage rate kinetics are typically integrated into mathematical models predicting nutriënt absorption following the provision of various dietary regimes. Such models are already available for pigs, dairy cows, and for calves fed exclusively on milk replacers. However, no such model is available yet for calves fed a combination of milk replacer and solid feeds. Therefore, the rate of adaptation of urinary P excretion to changes in an excess or shortage of phosphorus cannot be estimated by the use of a mathematical model. Reduction: the objective of this study is to estimate mean retention time of digesta and passage rate in the rumen, and secondly to provide a reference for the minimally invasive 13C breath test approach for the measurement of passage rate kinetics in calves, described in the study of annex 2. Using the same calves of the study described in annex 2, we reduce the total number of calves needed. Refinement: After careful consideration, it is decided to avoid the use of metabolism cages in this study. Therefore, the calves will be housed in pairs. We intend to house the calves in pairs while they are fitted with harnasses. If is questionable whether this possible, because it might be that the calves will start interacting with the harnass of the other calf. If all goes well, we continue housing them in pairs. If the housing in pairs in combination with the harnasses does not work, we will place a fence within their pen. Calves will be harnessed but will only be fixed when the faecal collection bags need to be replaced. We intend to use free housing, but if needed we restrain the calves during feeding. Furthermore, experienced animal care takers will monitor the calves to be sure a potential case of illness or reduced welfare will be diagnosed in an early stage. Handling of the calves will be performed by experienced personnel carefully and quietly, preferably in rest to prevent unnecessary distress. Analgesia and medication are applied where needed. If (in rare cases) analgesia or medication may influence trial results, calfs are treated and excluded from the trial.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

No adverse effects are foreseen.

Repetition and Duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Based on a literature search within the Pubmed and Scopus database using 'calf, passage rate kinetics, milk replacer, roughage, and concentrate' as searching criteria, we conclude that no study has been published in which digesta passage rate kinetics has been measured in calves fed combination of MR and solid feed are available. Hence this is not a repeat of research of others.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

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

Calves will be housed in pairs in a pen size meeting the EU directive. Calves will be harnessed, but will only be fixed when the faecal collection bags need to be replaced. If housing in pairs with calves that are harnessed is not possible, a fence will be placed in between the calves. We intend to use free housing, but if needed we restrain the calves during feeding. Bedding material cannot be used as the possible consumption can alter digesta passage rates.

G. Location where the animals procedures are performed

G. 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 H.
[] 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.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

[X] No > Continue with question I.
[] Yes > Will anaesthesia, analgesiaor other pain relieving methods be used?

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

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

I. Other aspects compromising the welfare of the animals

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

Harnasses to fix faecal collection bags, fixing the calves when switching the faecal collection bags, the possible restrainment during feeding, and the possible individual housing if calves interfere with eachothers harnass.

Explain why these effects may emerge.

Some discomfort from wearing harnesses cannot be prevented. In addition, fixing the calves at times of changing the faecal collection bags as well as restraining the calves during feeding will cause some discomfort. Moreover, the possible restrainment during feeding (when eating from eachothers food) and the possible individual housing (when interfering with eachothers harnass), will cause some discomfort.

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

Harnesses will be checked daily and adjusted if needed. Fixing calves at the time of changing faecal collection bags is needed, but as opposed to housing on metabolism cages, it allows calves to move around freely when not fixed.

J. 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 K.
[X] Yes > Describe the criteria that will be used to identify the humane endpoints.

• a calf has feed refusals exceeding 20% of the amount of MR offered for a period exceeding 3 days. • a calf has a fever for 3 successive days, not responding to medical treatments proposed by a veterinarian. • a calf suffers from problems related to the harnass, showing wounds at places the harness is connected. • in the expert judgement of the veterinarian, future observations on a calf will not provide reliable results. • in the expert judgement of the veterinarian, a calf is not fit/happy/healthy (reduced welfare) without affecting experimental results.

Indicate the likely incidence.

The likely incidence of calves to be removed from the experiment is estimated at less than 10% during the duration of the trial. The major portion of this 10% is expected to origin from feed refusals due to health problems unrelated to experimental procedures. In addition, problems arising from the harnesses will have a very short duration, as calves will then be removed from the trial

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned (non-recovery, mild, moderate, severe).

The level of discomfort is expected to be as listed below: • Housing in pairs, or individually, fixing at the time of changing faecal collection bags during 7 days: mild • Wearing of harnasses for the collection of faecal collection bags: mild Hence the cumulative discomfort in this trial is estimated as mild.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

[X] No > Continue with Section 3: 'Signatures'.
[] 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 > Descripe the method of killing that will be used and provide justifications for this choice.

[] Yes

14.

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, see our website www.zbo-ccd.nl.
- Or contact us by phone. (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10400	
1.2	Provide the name of the licenced establishment.	Wageningen University	
1.3	List the different types of animal procedures. Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.	Serial number 4	Type of animal procedure P requirements in veal calves fed different rations

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 objective of this trial is to estimate minimal P requirements for growth of calves fed different mixtures of solid feed and milk replacer. There are two options for this trial, depending on the outcome of the pilot (annex 1). When the rate of adaptation of urinary P excretion following a substantial reduction in P intake via the MR stabilizes within 72 h after the reduction in dietary P supply, the technique can be considered applicable for individual calves. If this is the case, a within-calf response dose technique can be used to estimate P requirement for individual calves, in analogy to the dose response approach to estimate amino acid requirements, described by Kampman-van de Hoek (2013). If the results of the pilot demonstrate that more time is needed for urinary P excretion to stabilize, a more traditional, between animal, dose-response approach will be used (see e.g. Erickson et al., 2002), comparing P excretion at different levels of intake between animals.
The age of the calves at the start of the measurements in this experiment will range from 13 weeks of age to 18 weeks of age, depending on the batch of calves. This is needed because of the simultaneous availability of 4 climate-respiration chambers for the study described in annex 2 (calves are re-used). In each batch, 8 calves will be used. During the 6 batches, measurements on 48 calves are obtained. Hence differences between batches represent a composite effect of time, age and batch of calves.

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

Option 1: within-calf dose response approach
Upon completion of the study described in annex 3 of this project, calves will remain on their 4 nutritional treatments, but transferred to a MR with a high P level. All calves will be housed individually on metabolism cages. Calves will be fitted with harnesses to allow quantitative collection of faeces and collection of clean urine from funnels mounted underneath the cage. P intake via the MR will be reduced in a step-wise manner in 7 steps of 3d each (21d in total). Faecal P excretion will be measured daily, and urinary excretion in 12h periods. The response of total and urinary P excretion to a reduction in P intake will be analyzed using nonlinear regression techniques to estimate the inflection point (see Kampman-van de Hoek et al., 2013), which can be considered the minimum P requirement for each calf. Effects of the nutritional treatments on the estimated P requirements will be analyzed.
With this option, the calves will be followed for 21d in total. It could be that the minimum P requirement of a calf is reached before that (e.g., step 5 at 15 days or with step 6 at 18 days). However, the faecal and urine samples will be analyzed in the laboratory after the experiment. Therefore, we are unable to determine the minimum P requirement while the experiment is running, thus each calve has to be followed for 21d.

Option 2: between calf dose response approach
Upon completion of the study described in annex 3 of this project, calves will remain housed in pairs for two weeks and transferred to two dietary treatments: i.e. two levels of SF intake at a fixed roughage: concentrate ratio. Hence there will be 24 calves on each SF treatment. Within each SF treatment, calves will be assinged to a MR with one of 6 P concentrations, varying from low (at 50% of the estimated requirements) to high (at 150% of the estimated requirements). Thus, there will be 4 calves assigned to each P level within the SF treatment. Calves will then be housed individually on metabolism cages. Calves will be fitted with harnesses to allow quantitative collection of faeces and collection of clean urine from funnels mounted underneath the cage. Faecal and urinary P excretion will be quantified of the complete 7d collection period. The response of total and urinary P excretion to a increasing P intake will be analyzed using nonlinear regression techniques to estimate the inflection point. In this way, one estimate will be obtained for each SF treatment,

Literature

Erickson GE, Klopfenstein TJ, Milton CT, Brink D, Orth MW and Whitted KM. 2002. Phosphorus requirements of finishing feedlot calves. J. Anim,. Sci. 80(6) 1690-1695.
Kampman-van de Hoek, E, Gerrits, WJJ, van der Peet-Schwering, CMC Jansman, AJM and van den Borne, JJGC 2013. A simple amino acid dose-response technique to quantify amino acid requirements of individual meal-fed pigs Journal of Animal Science. 91, 10, p. 4788-4796

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

Depending on the option chosen, there will be one estimate of P requirements per calf, or one per SF treatment. Between-calf variation of such estimates are unknown, but previous experience using a similar dose response approach for amino acid requirements in pigs (Kampman van de Hoek et al, 2013; Bruininx et al., 2015) and for responses to graded levels of starch or starch products in calves (Gilbert et al., 2015) demonstrate sensitive responses to step-wise changes in the driving variable using 10 animals/treatment.
For estimating P requirements using a between-calf approach, 24 calves per treatment assigned to graded levels of P intake is an approach

previously shown to provide accurate P requirements, and can be expected to be adequate to develop reliable requirement estimates for our two nutritional conditions.

Literature

Bruininx, E. M. A. M., van den Borne, J. J. G. C., Eising, I., Vervenne, P., Sakkas, P. & Gerrits, W. J. J. 2015. Optimal lysine:DE ratio in growing pigs is independent of starch or fat as main energy source at two energy intake levels Journal of Animal Science. 93, 10, p. 4774-4780

Gilbert, M. S., van den Borne, J. J. G. C., Berends, H., Pantophlet, A. J., Schols, H. A. & Gerrits, W. J. J. 2015. A titration approach to identify the capacity for starch digeston in milk-fed calves. Animal. 9, 2, p. 249-257

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

In this experiment, 48 male Holstein Friesian calves will be re-used from the study described in annex 2 and 3 of this project. Male calves will be used to ease separate collection of faeces and urine. At the beginning of this experiment, the age of the calves will range from 13 weeks to 20 weeks. At the end of the experiment, the age of the calves will range from 16 weeks to 23 weeks.

Species	Origin	Maximum number of animals	Life stage

calves	moderate	48

C. Re-use

Will the animals be re-used?

[] No, continue with question D.
[X] Yes > Explain why re-use is considered acceptable for this animal procedure.

Discomfort during the previous experiment was considered mild. The interest in this project is in the same treatments imposed on calves of annex 2, 3, and 4, facilitating repeated measurements.

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.

D. 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: The male Holstein Friesian calf is the target animal of this project. Effects of SF intake and composition on P requirements of calves cannot be estimated in vitro or by the use of mathematical models. Reduction: Depending on the results of the pilot study (i.e. when a within-calf titration approach appears feasible), this study contributes to a reduction in the number of calves needed to obtain estimates of P requirements under various nutritional conditions. Refinement: The duration of the dose response study depends on the rate of adaptation of urinary P excretion to changes in P intake via MR. If changes in urinary P excretion following a reduction in P intake via the MR adapt within 48 hours, the duration of the dose response study can be reduced from 21 to 14 d, and thus reducing the duration of the individual housing of these calves. As indivual housing on metabolism cages causes discomfort, we'll take precautions to minimize the impact. We place pairs of metabolic cages close to each other to facilitate physical contact between two calves. Additionally, we enrich the metabolic cages with brushes, a dry teat for suckling needs, and provide a ball inside the cage. Additionally, the use of chains is minimized (to avoid unneccesary noice). Furthermore, experienced animal care takers will monitor the calves to be sure a potential case of illness or reduced welfare will be diagnosed in an early stage. Handling of the calves will be performed by experienced personnel carefully and quietly, preferably in rest to prevent unnecessary distress. Analgesia and medication are applied where needed. If (in rare cases) analgesia or medication may influence trial results, calfs are treated and excluded from the trial.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

The following measures will be taken to reduce the impact of housing in metabolism cages on welfare of the calves: - use of chains will be minimized as the noise can disturb the calves - availability of brushes, mounted on the cage and a dry teat to satisfy sucking needs - a ball inside the cage. - cages will be organized in pairs close to each other to facilitate some physical contact between calves

Repetition and Duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Based on a literature search within the Pubmed and Scopus database using 'calf, phosphorous requirement, milk replacer, roughage, and concentrate' as searching criteria, we conclude that no study has been published that measured P requirements in calves fed combination of MR and solid feed are available. Hence this is not a repeat of research of others. P requirement studies in beef calves are available, but these calves are fed a ration based on concentrates and roughages without MR, and therefore do not take the potential to recycle P from the MR into account.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

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

Calves will be housed individually in metabolism cages. Fecal collection bags will be harnassed to the calves, to facilitate the quantitative collection of faeces and thereby the quantitative collection of clean urine in buckets underneath funnels, mounted underneath the cage. The dimensions of the cage will be 0.8 x 2m, and calves will be fixed to the front of the cage, allowing them to stand or lie freely.

G. 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 H.
[] 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.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

[X] No > Continue with question I.
[] Yes > Will anaesthesia, analgesiaor other pain relieving methods be used?

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

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

I. Other aspects compromising the welfare of the animals

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

Prolonged individual housing, restricted movements, no bedding material on the floor, harnasses to fix faecal collection bags. This might result in increased occurence of abnormal oral behaviours (e.g., repetitive self-licking, flank or object sucking, tongue rolling). Please note: we are aware that

in some occasions the calves receive a diet well below the P requirement. In comparison with studies performed with pigs, as well as the duration of the experiment, we do not consider this as a cause of discomfort. We do however acknowledge the risk, and will assess the calves daily to see the development of P deficiency symptoms in an early stage. After this experiment had ended, all calves will be fed the high P MR.

Explain why these effects may emerge.

The calves are deprived from exploring their environment due to a relative long period of individual housing in metabolic cages. Some discomfort from wearing harnesses cannot be prevented.

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

Housing on bedding material is not possible as clean urine needs to be collected from underneath the cages. The following measures will be taken to reduce the impact of housing in metabolism cages on welfare of the calves: - use of chains will be minimized as the noise can disturb the calves - availability of brushes, mounted on the cage and a dry teat to satisfy sucking needs - a ball inside the cage. - cages will be organized in pairs close to each other to facilitate some physical contact between calves After this experiment had ended, all calves will be fed a diet which had a P content equal or above P requirements.

J. 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 K.
[X] Yes > Describe the criteria that will be used to identify the humane endpoints.

• a calf has feed refusals exceeding 20% of the amount of MR offered for a period exceeding 3 days. • a calf has a fever for 3 successive days, not responding to medical treatments proposed by a veterinarian, and signs of infection and inflammation. • a calf suffers from problems related to the harnass, showing wounds at places the harness is connected. • a calf which clearly suffers from P deficiency (occurance of symptoms like bone fractures). • in the expert judgement of the veterinarian, future observations on a calf will not provide reliable results. • in the expert judgement of the veterinarian, continuation the experiment for a calf will cause more discomfort than foreseen.

Indicate the likely incidence.

The likely incidence of calves to be removed from the experiment is estimated at about 15% during the duration of the trial. The major portion of this 15% is expected to origin from feed refusals due to health problems unrelated to experimental procedures. In addition, problems arising from the harnesses will have a very short duration, as calves will then be removed from the trial. The higher incidence compared with the study described in annex 1 is related to the longer duration of this trial.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned (non-recovery, mild, moderate, severe).

The level of discomfort is expected to be as listed below: • Individual housing on balance cages, in absence of bedding material during 21 days: moderate • Wearing of harnasses for the collection of faecal collection bags: mild • Exposure to a P deficient diet for a period of about 10 days: moderate Hence the cumulative discomfort in this trial is estimated at moderate.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

[X] No > Continue with Section 3: 'Signatures'.
[] 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 > Descripe the method of killing that will be used and provide justifications for this choice.

[] Yes

A. Algemene gegevens over de procedure

1. Aanvraagnummer: **AVD104002017874**

2. Titel van het project: Influence of solid feed intake on digesta passage rate and phosphorus requirements of veal calves

3. Titel van de NTS: Invloed van ruw- en krachtvoer op fosforbehoefte vleeskalveren

4. Type aanvraag: nieuwe aanvraag projectvergunning

5. Contactgegevens DEC:
   DEC-WUR
   [redacted]
   Secretaris: dec@wur.nl

6. Adviestraject
   Ontvangen door DEC: 20-02-2017
   Aanvraag compleet:
   In vergadering besproken: 20-02-2017
   Anderszins behandeld: n.v.t.
   Termijnonderbreking(en) van 24-02-2017 tot 01-03-2017
   Besluit van CCD tot verlenging van de totale adviestermijn met max. 15 werkdagen: n.v.t.
   Aanpassing aanvraag: 01-03-2017
   Advies aan CCD: 21-03-2017

7. De Instantie voor Dierenwelzijn heeft een positief oordeel over de kwaliteit van de aanvraag uitgebracht en de DEC heeft dit in haar overweging betrokken.

8. Eventueel horen van aanvrager: n.v.t.

9. Correspondentie met de aanvrager:
   Datum vragen: 24-02-2017
   Datum antwoord: 01-03-2017
   Gestelde vragen *en antwoorden*:
   M.b.t. het projectvoorstel:
   De DEC verzoekt u de aangekruiste doelcategorieën te wijzigen, aangezien zij van mening is, dat 'Basic Research' hier niet aan de orde is. Het gaat in de ogen van de DEC om het ontwikkelen van een methode en het vaststellen van variabelen.
   Dit is aangepast. De doelcategorie 'Basic Research' is niet meer aangekruist. Dit onderzoek heeft nu alleen 'Translational and applied research' als doelcate-gorie.
   M.b.t. alle appendices:
   De DEC verzoekt u bij D. (vervanging) ook in te gaan op de mogelijkheden voor het gebruik van modellen.
   We hebben in alle appendices bij sectie D de mogelijkheid voor het gebruik van modellen beschreven. Specifieker, modellen zijn wel beschikbaar voor varkens, melkvee en vleeskalveren die alleen kalvermelk ontvangen, maar nog niet voor vleeskalveren die zowel kalvermelk als ruwvoer en krachtvoer ontvangen. Daardoor bieden mathematische modellen geen mogelijkheid tot vervanging.
   Daarnaast verzoekt zij u alle appendices toe te spitsen op en te beperken tot de proefbehandelingen en de context, waar de betreffende appendix betrekking op heeft. Zij heeft de indruk dat er nu soms teksten staan, die niet aan de orde zijn (bijv. in Appendix 1 onder D.: "rumination is essential" is hier niet relevant).
   Alle appendices zijn nagelezen en de nodige tekst is verwijderd. Bijvoorbeeld de

tekst die jullie aangeven en '13CO2 excretion...of Wageningen University', de laatste zin van Appendix 2 sectie A.
Bovendien verzoekt ze u bij B: (Specify the species, origin, estimated numbers, and life stages) op alle gevraagde aspecten in te gaan en ook in appendices 2 en 3 de keuze voor mannelijke dieren te onderbouwen.
Bij alle Appendices hebben we nu onderbouwing gegeven voor alle aspecten.
Tevens verzoekt ze u bij I. (Describe which other adverse effects on the animals welfare may be expected?) consequent alle bronnen van ongerief (die niet onder H. worden genoemd) te vermelden (bijv. als gevolg van individuele huisvesting, ontbreken van bedding etc.).
Bij alle Appendices staat nu beschreven waaruit het ongerief bestaan (bijvoorbeeld individueel huisvesten, ontbreken bedding, mogelijk vastbinden tijdens voermomenten, IV injectie, etc.).
M.b.t. appendix 1:
De DEC verzoekt u bij 2.A., de verschillende stappen duidelijker te beschrijven, aangezien in haar ogen het aantal dagen in de eerste alinea ("P intake will be step-wise reduced via MR (in 7 steps with every step lasting 72h)") niet overeenkomt met de tweede alinea ("Calves will be fed a normal ration with a high P milk replacer for the 4-d adaptation period and for the first 3 days of the experimental period, after which they will be switched to a MR lowering the P content of the MR by 20%, which will be fed the remaining 4 days").
Het aantal dagen dat beschreven was in de eerste alinea (i.e., 7 steps of 72 hours) was incorrect. Het betreft geen 7 stappen van 72 uur. Het betreft slechts 1 stap: na 3 dagen wordt de P concentratie in de kalvermelk met 20% verlaagd en wordt dit nog gedurende 4 dagen gevoerd. Met andere woorden, het tijdsbestek is 7 dagen in totaal. De tekst in de eerste alinea is aangepast.
M.b.t. appendix 3:
De DEC verzoekt u bij 2.A de "statistical methods" uitgebreider te beschrijven en niet alleen te verwijzen naar de andere appendices, aangezien elke appendix op zichzelf moet staan.
De statische onderbouwing van Appendices 2 en 4 zijn nu uitgebreider beschreven in Appendix 3 in plaats van er alleen naar te verwijzen.
M.b.t. de NTS:
De DEC verzoekt u de NTS zo aan te passen conform aanpassingen in de overige documenten.
Gedaan. Zo zijn bijvoorbeeld de wiskundige modellen opgenomen bij 'vervanging' en worden verschillende vormen van ongerief uitgebreider beschreven.

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 is een nieuwe aanvraag.
3. De DEC is competent om over de aanvraag te adviseren vanuit het oogpunt van onafhankelijkheid, onpartijdigheid en beschikbare expertises.
4. Vanwege betrokkenheid bij het betreffende project is een DEC-lid, met het oog op onafhankelijkheid en onpartijdigheid, niet betrokken bij de behandeling van de aanvraag en het opstellen van het advies. Deze betrokkenheid bestaat in het uitvoeren van praktische handelingen en ondersteuning in de organisatie tijdens dit experiment.

C. Beoordeling (inhoud)

1. De DEC heeft vastgesteld dat de aanvraag toetsbaar is en voldoende samenhang heeft. De DEC vindt het een goed georganiseerd en slim opgezet, goed doordacht project, waar met betrekkelijk weinig dieren veel informatie kan worden verkregen. Het kan op termijn bijdragen aan vermindering van proefdiergebruik. Hoewel drie typen dierproef betrekking hebben op fosfor en een type dierproef (app. 2) daar los van staat, ziet de DEC wel de samenhang, aangezien appendix 2 wel ook betrekking heeft op de 'passage rate', wat de kern is van de fosforbenutting.
2. De DEC heeft geen tegenstrijdige wetgeving, gericht op de gezondheid en welzijn van het dier of het voortbestaan van de soort, gesignaleerd die het uitvoeren van de proef in de weg kan staan.
3. De DEC heeft vastgesteld dat de in de aanvraag aangekruiste doelcategorie in overeenstemming is met de hoofddoelstelling. Het gaat om fundamenteel onderzoek: het ontwikkelen van een methode en het vaststellen van variabelen.

Belangen en waarden

4. Het directe doel van de aanvraag is: het ontwikkelen van technieken om de voedselpassagesnelheid non-invasief te meten, het bepalen van de invloed van solid food daarop en het bepalen van de P-benutting.
   Het uiteindelijke doel van de aanvraag is: het realiseren van een optimale benutting van P door aanpassing van het dieet en van verminderde uitstoot naar het milieu.
   De DEC heeft vastgesteld dat er een directe en reële relatie is tussen beide doelstellingen en dat het directe doel gerechtvaardigd is binnen de context van het onderzoeksveld.
5. De belanghebbenden en hun morele waarden in het project zijn: kalveren (proefdieren): ze ervaren ongerief als gevolg van de proefbehandelingen en hun gezond-heid kan in het geding zijn als ze te weinig P krijgen; kalveren (doeldieren): gezondheidswinst door betere afstemming van P op de behoefte; de boeren: economisch voordeel; het milieu: vermindering van de P-uitstoot naar het milieu; kalvervoerfabricanten: economisch voordeel.
6. Voor zover de DEC dat kan inschatten is er geen aanleiding voor de DEC om de in de aanvraag beschreven effecten op het milieu in twijfel te trekken.

Proefopzet en haalbaarheid

7. De DEC heeft vastgesteld dat de kennis en kunde van de onderzoeksgroep en andere betrokkenen bij de dierproeven, afgaande op het geschreven voorstel en het oordeel van de IvD, voldoende gewaarborgd zijn. De leden die bij het project zijn betrokken beschikken over inhoudelijke expertise en kennis over zowel dit onderwerp als over de praktische uitvoerbaarheid van de experimenten.
8. De DEC heeft vastgesteld dat het project goed is opgezet, de voorgestelde experimentele opzet en uitkomstparameters logisch en helder aansluiten bij de aangegeven doelstelling. De gekozen strategie en experimentele aanpak kunnen in de ogen van de DEC leiden tot het behalen van de doelstelling binnen het kader van het project. Er wordt in eerste instantie een pilot uitgevoerd, waardoor dierproeftype 4 beter ingeschat kan worden. De andere twee experimenten zijn ter ondersteuning van wat het beste in experiment 4 getest kan worden, dat is logisch en navolgbaar. Het project is goed georganiseerd, met een logische opbouw in de tijd. Alle 4 de typen dierproef leiden volgens de DEC tot het uiteindelijk behalen van de doelstelling.

Welzijn dieren

9. Er is sprake van de volgende bijzonderheid op het gebied van categorieën van dieren, omstandigheden of behandeling van de dieren: in diverse typen dierproef worden dieren hergebruikt uit vorige proeven. De keuze hiervoor is realistisch ingeschat en geclassificeerd. Er is in die gevallen altijd sprake van gering ongerief in de voorgaande proef.
10. De dieren worden niet gehuisvest en verzorgd op een wijze die voldoet aan de eisen die zijn opgenomen om bijlage III van richtlijn 2010/63/EU. De dieren worden (af-

hankelijk van het type dierproef) tijdelijk individueel gehuisvest, zonder bedding. Daarnaast worden de dieren tijdens de metingen in de klimaatrespiratiecellen in paren gehuisvest zonder bedding.

11. De DEC stelt vast dat het cumulatieve ongerief als mild of matig (afhankelijk van het type dierproef) voor elk dier realistisch is ingeschat en geclassificeerd. Ongerief in de experimenten zal bestaan uit: een enkele injectie of bloedafname, gedurende geen enkele periode zijn de dieren alleen. In een deel van de proeven worden de kalveren aangebonden en worden ze individueel gehuisvest, waarbij een deel een voer met een erg laag P-gehalte aangeboden krijgt.
12. Naast ongerief is er geen sprake van aantasting van integriteit van het dier, anders dan voortvloeiend uit de proefbehandelingen.
13. De DEC heeft vastgesteld dat de criteria voor humane eindpunten (HEPs) goed zijn gedefinieerd en dat goed is ingeschat welk percentage van de dieren een humaan eindpunt zal bereiken. Er wordt niet verwacht dat als gevolg van de proefbehandelingen HEPs worden bereikt. Bij lage P-waarden wordt er expliciet naar gedrag gekeken en er vindt behandeling plaats bij interferente ziektes (zie ook 18.).

3 V's

14. De DEC heeft vastgesteld dat de onderzoeker voldoende aannemelijk heeft gemaakt dat er geen alternatieven zijn om de doelstelling van het project te realiseren. De behoefte aan fosfor voor kalveren die een combinatie van kalvermelk, ruwvoer en krachtvoer krijgen verstrekt kan niet bij andere dieren worden vastgesteld omdat de uitscheiding van fosfor bij kalveren anders werkt dan bij andere diersoorten. Er zijn geen proefdiervrije methoden beschikbaar, zoals wiskundige modellen.
15. De DEC heeft vastgesteld dat de onderzoeker voldoende aannemelijk heeft gemaakt dat er optimaal tegemoet gekomen wordt aan de vereiste van vermindering van dierproeven. De onderzoekers geven blijk van hun kennis over regressieanalyses en geven aan dat de opzet deels gebaseerd is op eerder uitgevoerde experimenten. Daarnaast vindt er hergebruik plaats, waardoor men met minimale aantallen kan volstaan.
16. De DEC heeft vastgesteld dat het project in overeenstemming is met de vereiste van verfijning van dierproeven. Er worden niet-invasieve methoden gebruikt. Tijdens de studies worden de kalveren, indien mogelijk sociaal gehuisvest (in groepen of in paartjes). Individuele huisvesting wordt zo kort mogelijk gehouden. Hierbij wordt gebruik gemaakt van afleidingsmateriaal/ kooiverrijking en wordt fysiek contact mogelijk gemaakt. De DEC ziet geen extra mogelijkheden voor verfijning, anders dan die de onderzoeker nu toepast.

Naast bovengenoemde punten merkt de DEC op, dat de te ontwikkelen techniek mogelijk kan bijdragen aan verminderd proefdiergebruik in de toekomst. Dit heeft zowel betrekking op verfijning, vervanging, als op vermindering.

Dieren in voorraad gedood en bestemming dieren na afloop proef

17. De dieren worden niet van beide geslachten in gelijke mate ingezet in de proeven. De DEC heeft vastgesteld dat de aanvrager in voldoende mate wetenschappelijk heeft onderbouwd waarom dit noodzakelijk is. Er worden alleen mannelijke dieren gebruikt. Enerzijds is het nodig mest en urine op te vangen, wat makkelijker is bij mannelijke kalveren. Daarnaast zijn de mannetjeskalveren in principe onbruikbaar voor de melkveehouderij en ze worden derhalve opgefokt voor hun vlees. Dit onderzoek laten uitvoeren met vrouwelijke dieren heeft geen enkele toegevoegde waarde voor het doel, noch zorgt het voor enige reductie in het totale aantal dieren, eerder een vermeerdering.
18. De dieren worden niet gedood in het kader van het project.
    De DEC acht het wenselijk de dieren, die voer met een laag P-gehalte krijgen na afloop van het project te monitoren, om te voorkomen, dat ze te snel worden hergebruikt.

NTS

19. De NTS is naar het oordeel van de DEC een evenwichtige weergave van het project, begrijpelijk geformuleerd en voldoet aan de vereisten in de herziene Wod Art. 10.a.1.7.

D. Ethische afweging

1. De centrale morele vraag van het project is: Weegt het vergaren van kennis omtrent voedings- en verteringfysiologie van het voer, een efficiënter gebruik van fosfor en het verlagen van de milieubelasting op tegen het maximaal matige ongerief voor 48 kalveren?
2. In de afweging heeft de DEC geconstateerd dat het hier gaat om een aanvraag met voldoende samenhang. In haar afweging heeft de DEC meegewogen dat als het project zijn uiteindelijke doel haalt, dat er m.b.t. het milieu sprake is van een substantieel belang door een vermindering van de P-uitstoot naar het milieu. Ook voor de kalveren (doeldieren) is er in dat geval sprake van een reële gezondheidswinst door betere afstemming van P op de dierbehoefte. Voor zowel de boeren als de veevoedersector speelt daarbij ook een economische waarde. Die heeft de DEC als een beperkt belang ingeschat. Tot slot zijn de waarden van de proefdieren in het geding. Zij ervaren maximaal matig ongerief als gevolg van de handelingen binnen dit project.
   De DEC heeft in de beoordeling van het doel meegewogen dat uitgebalanceerde voeding van belang is voor alle vormen van rundveehouderij en dat dit project zich niet enkel richt op de intensieve veehouderij, maar ook relevantie heeft daarbuiten (opfok van melkvee, de biologische houderij).
   Aanvullend is de bevordering van kennis van voedings- en verteringsfysiologie en het verbruik van fosfor in vleeskalveren interessant als onderzoeksdoelstelling omdat er vanuit de (Europese) politiek druk is om de fosforuitstoot te verminderen. Door het gehele proces rondom de opfok te optimaliseren, kan dit mogelijk bereikt worden. De DEC heeft de aanvraag beoordeeld binnen de context dat de vleesindustrie maatschappelijk is geaccepteerd en zij vindt het ethisch verantwoord hier onderzoek naar te doen met 48 dieren die gedurende 21 dagen maximaal matig ongerief ondergaan.
   De DEC ziet in dit stadium geen mogelijkheden op het terrein van vervanging, vermindering van het aantal dieren en verfijning van de aanvraag en is van mening dat dit onderzoek zelf een bijdrage kan leveren aan de 3V's bij toekomstig onderzoek.
3. De centrale morele vraag kan met "ja" beantwoord worden.

E. Advies

1. Advies aan de CCD:
   De DEC adviseert de vergunning te verlenen.
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.

17.

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

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Onze referentie
Aanvraagnummer
AVD104002017874
Bijlagen
1

Datum 11 april 2017
Betreft Beslissing aanvraag projectvergunning Dierproeven

Geachte [redacted]

Op 17 februari 2017 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Influence of solid feed intake on digesta passage rate and phosphorus requirements of veal calves" met aanvraagnummer AVD104002017874. Wij hebben uw aanvraag beoordeeld.

Beslissing
Wij keuren uw aanvraag goed op grond van artikel 10a van de Wet op de Dierproeven (hierna: de wet). Hierbij gelden de voorwaarden zoals genoemd in de vergunning.
De algemene voorwaarde(n) zijn opgenomen op grond van artikel 1d lid 4, artikel 10a1 lid 2, artikel 10 lid 2 en/of artikel 10a3 van de wet.

U kunt met uw project "Influence of solid feed intake on digesta passage rate and phosphorus requirements of veal calves" starten. De vergunning wordt afgegeven van 1 mei 2017 tot en met 30 november 2017.

Overige wettelijke bepalingen blijven van kracht.

Procedure
Wij hebben advies gevraagd bij de Dierexperimentencommissie DEC WUR. Dit advies is opgesteld op 21 maart 2017. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a, lid 3 van de wet. Wij hebben de DEC om aanvullende informatie gevraagd. Op 27 maart 2017 heeft de DEC gereageerd op onze vragen. De DEC is gevraagd een toelichting te geven op de afwijkende huisvesting.

Wij kunnen ons vinden in de inhoud van het advies van de Dierexperimentencommissie. Dit advies van de commissie nemen wij over, inclusief de daaraan ten grondslag liggende motivering. Er worden aanvullende algemene voorwaarde(n) gesteld.
Het DEC-advies en de in de bijlage opgenomen beschrijving van de artikelen van de wet- en regelgeving zijn de grondslag van dit besluit.

Bezwaar
Als u het niet eens bent met deze beslissing, kunt u binnen zes weken na verzending van deze brief schriftelijk een bezwaarschrift indienen.
Een bezwaarschrift kunt u sturen naar Centrale Commissie Dierproeven, afdeling Juridische Zaken, postbus 20401, 2500 EK Den Haag.

Bij het indienen van een bezwaarschrift vragen we u in ieder geval de datum van de beslissing waartegen u bezwaar maakt en het aanvraagnummer te vermelden. U vindt deze nummers in de rechter kantlijn in deze brief.

Bezwaar schorst niet de werking van het besluit waar u het niet mee eens bent. Dat betekent dat dat besluit wel in werking treedt en geldig is. U kunt tijdens deze procedure een voorlopige voorziening vragen bij de Voorzieningenrechter van de rechtbank in de woonplaats van de aanvrager. U moet dan wel kunnen aantonen dat er sprake is van een spoedeisend belang.

Voor de behandeling van een voorlopige voorziening is griffierecht verschuldigd. Op http://www.rechtspraak.nl/Organisatie/Rechtbanken/Pages/default.aspx kunt u zien onder welke rechtbank de vestigingsplaats van de aanvrager valt.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Centrale Commissie Dierproeven
namens deze:

[redacted]

plv. Algemeen Secretaris

Bijlagen:
- Vergunning
  Hiervan deel uitmakend:
  - DEC-advies
  - Weergave wet- en regelgeving

Centrale Commissie Dierproeven

Projectvergunning

gelet op artikel 10a van de Wet op de Dierproeven

Verleent de Centrale Commissie Dierproeven aan

Naam:	Wageningen University
Adres:	Postbus 59
Postcode en plaats:	6700 AB WAGENINGEN
Deelnemersnummer:	10400

deze projectvergunning voor het tijdvak 1 mei 2017 tot en met 30 november 2017, voor het project "Influence of solid feed intake on digesta passage rate and phosphorus requirements of veal calves" met aanvraagnummer AVD104002017874, volgens advies van Dierexperimentencommissie DEC WUR. Er worden aanvullende algemene voorwaarde(n) gesteld.
De functie van de verantwoordelijk onderzoeker is Onderzoeker.
De aanvraag omvat de volgende bescheiden:

1 een aanvraagformulier projectvergunning dierproeven, ontvangen op 17 februari 2017
2 de bij het aanvraagformulier behorende bijlagen:
a Projectvoorstel, zoals ontvangen per digitale indiening op 21 maart 2017;
b Niet-technische Samenvatting van het project, zoals ontvangen per digitale indiening op 21 maart 2017;
c Advies van dierexperimentencommissie d.d. 21 maart 2017, ontvangen op 21 maart 2017, aanvullend advies, zoals ontvangen op 27 maart 2017.

Aanvraagnummer:
AVD104002017874

Naam proef	Diersoort/ Stam	Aantal dieren	Ernst	Opmerkingen
3.4.4.1 P requirements in veal calves - a pilot study				
	Runderen (Bos taurus) / Kalveren	6	Matig	
3.4.4.2 Passage rate kinetics in veal calves - 13C tracer technique				
	Runderen (Bos taurus) / Kalveren	48	Licht	
3.4.4.3 Passage rate kinetics in veal calves - faecal excretion curves				Dit zijn dezelfde dieren als 3.4.4.2
	Runderen (Bos taurus) / Kalveren	48	Licht	
3.4.4.4 P requirements in veal calves fed different rations				Dit zijn dezelfde dieren als 3.4.4.2
	Runderen (Bos taurus) / Kalveren	48	Matig	

Voorwaarden

Op grond van artikel 10a1 lid 2 van de Wet op de dierproeven zijn aan een projectvergunning voorwaarden te stellen

De vergunning wordt verleend onder de voorwaarde dat go/no go momenten worden afgestemd met de IvD.

In artikel 10, lid 1 sub a van de wet, wordt bepaald dat het verboden is een dierproef te verrichten voor een doel dat, naar de algemeen kenbare, onder deskundigen heersende opvatting, ook kan worden bereikt anders dan door middel van een dierproef, of door middel van een dierproef waarbij minder dieren kunnen worden gebruikt of minder ongerief wordt berokkend dan bij de in het geding zijnde proef het geval is. Nieuwe onderzoeken naar alternatieven kunnen tot gevolg hebben dat inzichten en/of omstandigheden van het aangevraagde project in de vergunningsperiode wijzigen, gedurende de looptijd van deze vergunning. Indien bovenstaande zich voordoet dient aanvrager dit in afstemming met de IvD te melden bij de CCD. De CCD kan in een dergelijke situatie aan de vergunning nieuwe voorwaarden verbinden en gestelde voorwaarde wijzigen of intrekken.

Centrale Commissie Dierproeven

Aanvraagnummer:
AVD104002017874

Weergave wet- en regelgeving

Dit project en wijzigingen
Volgens artikel 10c van de Wet op de Dierproeven (hierna de wet) is het verboden om andere dierproeven uit te voeren dan waar de vergunning voor is verleend. De dierproeven mogen slechts worden verricht in het kader van een project, volgens artikel 10g. Uit artikel 10b volgt dat de dierproeven zijn ingedeeld in de categorieën terminaal, licht, matig of ernstig. Als er wijzigingen in een dierproef plaatsvinden, moeten deze gemeld worden aan de Centrale Commissie Dierproeven. Hebben de wijzigingen negatieve gevolgen voor het dierenwelzijn, dan moet volgens artikel 10a5 de wijziging eerst voorgelegd worden en mag deze pas doorgevoerd worden na goedkeuren door de Centrale Commissie Dierproeven.
Artikel 10b schrijft voor dat het verboden is een dierproef te verrichten die leidt tot ernstige mate van pijn, lijden, angst of blijvende schade die waarschijnlijk langdurig zal zijn en niet kan worden verzacht, tenzij hiervoor door de Minister een ontheffing is verleend.

Verzorging
De fokker, leverancier en gebruiker moeten volgens artikel 13f van de wet over voldoende personeel beschikken en ervoor zorgen dat de dieren behoorlijk worden verzorgd, behandeld en gehuisvest. Er moeten ook personen zijn die toezicht houden op het welzijn en de verzorging van de dieren in de inrichting, personeel dat met de dieren omgaat moet toegang hebben tot informatie over de in de inrichting gehuisveste soorten en personeel moet voldoende geschoold en bekwaam zijn. Ook moeten er personen zijn die een eind kunnen maken aan onnodige pijn, lijden, angst of blijvende schade die tijdens een dierproef bij een dier wordt veroorzaakt. Daarnaast zijn er personen die zorgen dat een project volgens deze vergunning wordt uitgevoerd en als dat niet mogelijk is zorgen dat er passende maatregelen worden getroffen.
In artikel 9 staat dat de persoon die het project en de dierproef opzet deskundig en bekwaam moet zijn. In artikel 8 van het Dierproevenbesluit 2014 staat dat personen die dierproeven verrichten, de dieren verzorgen of de dieren doden, hiervoor een opleiding moeten hebben afgerond.
Voordat een dierproef die onderdeel uitmaakt van dit project start, moet volgens artikel 10a3 van de wet de uitvoering afgestemd worden met de instantie voor dierenwelzijn.

Pijnbestrijding en verdoving
In artikel 13 van de wet staat dat een dierproef onder algehele of plaatselijke verdoving wordt uitgevoerd tenzij dat niet mogelijk is, dan wel bij het verrichten van een dierproef worden pijnstillers toegediend of andere goede methoden gebruikt die de pijn, het lijden, de angst of de blijvende schade bij het dier tot een minimum beperken. Een dierproef die bij het dier gepaard gaat met zwaar letsel dat hevige pijn kan veroorzaken, wordt niet zonder verdoving uitgevoerd. Hierbij wordt afgewogen of het toedienen van verdoving voor het dier traumatischer is dan de dierproef zelf en het toedienen van verdoving onverenigbaar is met het doel van de dierproef. Bij een dier wordt geen stof toegediend waardoor het dier niet meer of slechts in verminderde mate in staat is pijn te tonen, wanneer het dier niet tegelijkertijd voldoende verdoving of pijnstilling krijgt toegediend, tenzij wetenschappelijk gemotiveerd. Dieren die pijn

kunnen lijden als de verdoving eenmaal is uitgewerkt, moeten preventief en postoperatief behandeld worden met pijnstillers of andere geschikte pijnbestrijdingsmethoden, mits die verenigbaar zijn met het doel van de dierproef. Zodra het doel van de dierproef is bereikt, moeten passende maatregelen worden genomen om het lijden van het dier tot een minimum te beperken.

Einde van een dierproef
Artikel 13a van de wet bepaalt dat een dierproef is afgelopen wanneer voor die dierproef geen verdere waarnemingen hoeven te worden verricht of, voor wat betreft nieuwe genetisch gemodificeerde dierenlijnen, wanneer bij de nakomelingen niet evenveel of meer, pijn, lijden, angst, of blijvende schade wordt waargenomen of verwacht dan bij het inbrengen van een naald. Er wordt dan door een dierenarts of een andere ter zake deskundige beslist of het dier in leven zal worden gehouden. Een dier wordt gedood als aannemelijk is dat het een matige of ernstige vorm van pijn, lijden, angst of blijven schade zal blijven ondervinden. Als een dier in leven wordt gehouden, krijgt het de verzorging en huisvesting die past bij zijn gezondheidstoestand.
Volgens artikel 13b moet de dood als eindpunt van een dierproef zoveel mogelijk worden vermeden en vervangen door in een vroege fase vaststelbare, humane eindpunten. Als de dood als eindpunt onvermijdelijk is, moeten er zo weinig mogelijk dieren sterven en het lijden zo veel mogelijk beperkt blijven.
Uit artikel 13d volgt dat het doden van dieren door een deskundig persoon moet worden gedaan, wat zo min mogelijk pijn, lijden en angst met zich meebrengt. De methode om te doden is vastgesteld in de Europese richtlijn artikel 6.
In artikel 13c is vastgesteld dat proefdieren geadopteerd kunnen worden, teruggeplaatst in hun habitat of in een geschikt dierhouderijsysteem, als de gezondheidstoestand van het dier het toelaat, er geen gevaar is voor volksgezondheid, diergezondheid of milieu en er passende maatregelen zijn genomen om het welzijn van het dier te waarborgen.
De Minister heeft vrijstelling ontheffing verleend volgens artikel 13c, die de afwijkende methode van doden op basis van wetenschappelijke motivering ten minste even humaan acht als de in de richtlijn opgenomen passende methoden.