Aanmeldingsformulier voor proeven met gewervelde dieren.

Secretariaat DEC
[redacted]

Aanvrager: [redacted]
Afdeling:

Titel dierproef: The effect of dietary hydroxyproline on oxalate synthesis and the excretion of oxalate in faeces and urine of healthy adult cats

Aanmeldcode / Protocol: 2011113.a
Stadia van de proef:

06-12-2011	Aangemeld	[redacted]
21-12-2011	Wijzigen	Secretaris van de DEC
06-01-2012	Gekopieerd	[redacted]

Is deze proef wetenschappelijk getoetst en goedgekeurd? Ja
Toelichting:
In het kader van AIO procedure.

1.a. Met dit onderzoek te beantwoorden concrete vraag:
B. Ontwikkeling van veterinaire (hulp)middelen ontw. med. hulpmiddelen / toepassingen
The main objective of this study:
- To determine the influence of dietary hydroxyproline on the urinary oxalate excretion as an indicator for endogenous oxalate synthesis in healthy cats in a controlled environment.

Sub objectives of this study
- To determine the apparent secretion of oxalate into the intestines in cats fed oxalate-free diets.
- To determine the apparent absorption of dietary oxalate in cats fed the diet containing oxalate.
- To determine the contribution of dietary oxalate to the oxalate excreted in the urine in cats fed the diet containing oxalate.

Het uiteindelijke doel (Maatschappelijke en wetenschappelijke relevantie):

Urolithiasis is the second most common cause of lower urinary tract disease (LUTD) in cats (1;2) (symptoms: difficult and painful urination, blood in the urine, partial or complete urethral obstruction) and different urolith (stone) types are known to cause urolithiasis. Over the past decades, a progressive increase in the prevalence of calcium oxalate (CaOx) uroliths (from 2% to 55-60%) has been reported in cats with LUTD in the United States of America (3) and Benelux (4). In dogs, the same general trend in urolith composition can be observed but is less extreme than in cats (3;4). Nutrition is thought to play a major role in this increase in prevalence of CaOx urolithiasis. Today's nutritional interventions are mainly based on human medicine studies as only a few studies have been conducted in cats.
The current treatment of choice for severe CaOx urolithiasis is surgical urolith removal, followed by methods to prevent urolith recurrence. At present, the standard method for preventing CaOx urolith recurrence is to feed a urolith reducing therapeutic diet and encourage water intake. Despite these preventative measures, the prevalence and recurrence rate of CaOx uroliths remains high in cats.

Therefore, more knowledge regarding the etiopathogenesis of CaOx urolithiasis should be generated to improve current preventative measures and to decrease the prevalence of CaOx urolithiasis.
Urinary oxalate and calcium excretion are essential factors in CaOx urolith formation as they are the two elements to precipitate with each other in the urine. An increase in the excretion of either calcium or oxalate or both could lead to CaOx urolith formation. Since calcium metabolism is of interest for more diseases, several studies have been conducted to determine the nutrients that influence the urinary calcium excretion. This is however not the case for urinary oxalate excretion and its origin.

Oxalate in the urine can be of dietary or endogenous origin. In humans it is known that both sources contribute equal to oxalate excreted in the urine (5;6). In cats, no exact data is available yet on this subject, but only a data mining study suggesting that the intestinal absorption of dietary oxalate and therefore the contribution of dietary oxalate to urine oxalate may be minimal ([redacted] et al, unpublished results). In dogs, the urinary oxalate excretion could not be affected by changing dietary oxalate content (7). When indeed the dietary contribution to oxalate excreted in the urine is minimal, the preventative measures extrapolated from human medicine on this aspect does not hold true for cats. The contribution of oxalate to oxalate in the urine can be studied by feeding an oxalate-free diet vs. a diet supplemented with oxalate. The absorption can be determined by subtracting the dietary oxalate intake by the fecal oxalate content.
As the contribution of dietary oxalate may be minimal, the oxalate excreted in the urine is likely to be of endogenous origin. Endogenous oxalate synthesis has been studied extensively in human medicine. In these studies, oxalate synthesis could be affected by varying the intake of pyridoxine (vitamin B6), ascorbic acid (vitamin C), glucose, fructose and certain amino acids. Of the amino acids, hydroxyproline was the most potent amino acid in inducing oxalate synthesis (8-10). Hydroxyproline is an amino acid abundant in the protein collagen. In a study with cats fed different protein sources, collagen resulted in 2 to 3 fold higher oxalate excretions in the urine than soya and horse meat (11). However, unknown is whether these observed differences can be ascribed to differences in oxalate synthesis or oxalate intake as the oxalate content of these diets were not tested. The effect of hydroxyproline on oxalate synthesis remains to be determined in cats and can be achieved by feeding diets equal in oxalate content. This can be achieved by feeding diets differing in hydroxyproline content.
Oxalate synthesis can be underestimated when only the oxalate excreted in the urine is measured, since oxalate could also be measured in fecal samples after feeding rats an oxalate-free diet (9). All oxalate secreted in the intestines instead of in the urine is beneficial as this oxalate cannot contribute to calcium oxalate urolith formation anymore. Investigating the fraction of endogenous oxalate in feces (relative to urine) of cats fed an oxalate-free diet may provide useful information.

Information on the contribution of dietary and endogenous oxalate on the oxalate excreted in the urine, hydroxyproline as an inducer of endogenous oxalate and the contribution of oxalate-degrading bacteria in the intestines may provide new insights for dietary strategies that prevent urolithiasis.

References
(1) Bartges JW, Kirk C, Lane IF. Update: Management of calcium oxalate uroliths in dogs and cats. Vet Clin North Am Small Anim Pract 2004 Jul;34(4):969-87, vii.
(2) Gerber B, Boretti FS, Kley S, Laluha P, Muller C, Sieber N, et al. Evaluation of clinical signs and causes of lower urinary tract disease in European cats. J Small Anim Pract 2005 Dec;46(12):571-7.
(3) Osborne CA, Lulich JP, Kruger JM, Ulrich LK, Koehler LA. Analysis of 451,891 canine uroliths, feline uroliths, and feline urethral plugs from 1981 to 2007: perspectives from the Minnesota Urolith Center. Vet Clin North Am Small Anim Pract 2009 Jan; 39(1):183-97.
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(5) Holmes RP, Goodman HO, Assimos DG. Contribution of dietary oxalate to urinary oxalate excretion. Kidney Int 2001; 59, 270-276.
(6) von Unruh GE, Voss S, Sauerbruch T. Reference range for gastrointestinal oxalate absorption measured with a standardized [3C2]oxalate absorption test. J Urol 2003; 169, 687-690.
(7) Stevenson AE, Hynds WK, Markwell PJ. The relative effects of supplemental dietary calcium and oxalate on urine composition and calcium oxalate relative supersaturation in healthy adult dogs. Res Vet

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(8) Knight J, Jiang J, Assimos DG, Holmes RP. Hydroxyproline ingestion and urinary oxalate and glycolate excretion. Kidney Int 2006 Dec; 70(11):1929-34.
(9) Ribaya JD, Gershoff SN. Effects of hydroxyproline and vitamin B-6 on oxalate synthesis in rats. J Nutr 1981; 111, 1231-1239.
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(11) Zentek J, Schulz A. Urinary composition of cats is affected by the source of dietary protein. J Nutr 2004 Aug; 134(8 Suppl):2162S-5S.

1.b. Het uiteindelijk doel (Maatschappelijke en wetenschappelijke relevantie):
Doel:
Zie 'Direct doel'

1.c. Lekensamenvatting:

2. Gepland vanaf: 15-01-2012 **tot** 15-04-2012

3. Diersoort: katten **; Totaal aantal:** 8

4.a. Nadere aanduiding gebruikte dieren:
Intact adult Domestic Shorthair cats, all females, bodyweight 2-4 kg, age between 1 and 6 years.

4.b. Motivatie waarom is gekozen voor deze diersoort:
The occurrence of CaOx urolithiasis is known to be more common in cats than in dogs. This implicates that cats are more sensitive for risk factors leading to CaOx urolith formation.

The basal urinary oxalate excretion is a little higher in male cats which may be due to the influence of testosterone on endogenous oxalate synthesis. Intact females and neutered males and females show a more similar urinary oxalate excretion. When using intact female cats, the results can be extrapolated to majority of the cat population as most privately-owned male cats are neutered. In addition, as most male cats are spraying urine for marking, quantitatively urine collection is less accurate using male cats.

4.c. Toelichting voor het aantal gebruikte dieren:
Based on a sample size calculation the conclusion could be drawn that a minimum of 6 observations of different cats per diet is required to obtain statistical significant results in an experiment determining the effect of diet on urinary oxalate excretion. This sample size calculation was conducted using the following parameters: SD=5 µmol oxalate/kg BW/day, which is compiled from data of the study of Zentek and Schultz (11) and our unpublished data, biological relevant difference is 10% and statistical power is 90%. In case some cats cannot complete the experiment due to reasons such as illness, diet refusal, urination outside the litter box, an increase of the sample size with 2 cats would be rational. This means that each diet will be tested on 8 cats.

References
(11) Zentek J, Schulz A. Urinary composition of cats is affected by the source of dietary protein. J Nutr 2004 Aug; 134(8 Suppl):2162S-5S.

4.d. Herkomst: G. dier is in reg.jr. meer dan een keer hergebr.

Toelichting:
The cats are housed at the laboratory animal facility of [redacted].

5.a. Accommodatie: [redacted]
The cats are housed in a group accommodation with a surface of 25 m2. During the adaptation period of the experiment (day 1 to 7), the cats will be kept in the group during the day. The cats involved in the experiment will be housed individually in the metabolism cage during feeding time and overnight. During the sampling period (day 8 to 12) the cats will be housed individually in the metabolism cages for 23 hours per day and 1 hour in small groups under supervision to ensure no urine will be voided. The metabolism cages are constructed of Trespa panels and aluminium front frame and are 0.80x1.00x0.75 m. The front contains a feeding and water bowl and in the back corners of the cage a removable litter tray (29x29x12 cm) is securely positioned.

5.b. Huisvesting & Verzorging:
The daily care during the experiments will be performed by the persons involved in the experiment (see also point 10) and will be in close contact with the animal caretakers/staff members of [redacted]

5.c. Voeding:
During the experiment four experimental diets will be fed. These experimental diets consist of a basal balanced and complete basal diet (according the recommended nutrient requirements for adult cats of the National Research Council (2006) (12)) supplemented with hydroxyproline or alanine, and sodium oxalate (table 1). As the supplements do only account for 5.2% of the diet (i.e. 95% remains the same), no problems are expected regarding the adaptation from one to other diet. As the diets will be extruded and will be coated with digest (i.e. a substance giving a good taste to a kibble), it is expected the palatability of the diets will be good.

Table 1: Composition of the experimental diets.

Diet	Supplement (%)		
	Hyp	Ala	sodium oxalate
A: Hyp - Fox	5.2*	0	0
B: Hyp/Ala - Fox	2.6	2.6	0
C: Ala - Fox (control)	0	5.2	0
D: Ala - Hox	0	5.2	0.13**

*Based on the amount of Hyp supplemented in a similar study conducted in rats (9) and equals to 1.27 g/100 kcal metabolizable energy (ME).
** This amount equals to 21 mg/100 kcal ME, which is an oxalate content within the observed range of oxalate in commercially available diets for adult cats ([redacted] et al., unpublished results).
Hyp, hydroxyproline; Ala, alanine; Fox, free of oxalate, Hox, high in oxalate.

The amount of food per cat will be determined based on energy requirement of the individual cat by using 100 kcal/kg BW0.67/day (12). To assure the cats receive enough food, food intake will be monitored and the cats will be weighed weekly at the start of each adaptation and sampling period (i.e. day 1 and 8). Water is available ad libitum.

6.a. Proefschema / proefbehandelingen:
The four experimental diets will be fed according a repeated 4*4 latin square design to obtain more statistical power. The repetition, i.e. 2 cats per sequence, is incorporated to reduce the chance the Latin square will be incomplete as a result of missing values due to for example illness in cats.

Table 2: Feeding schedule of the experimental diets according a repeated Latin Square design.

	Period			
Cat	I	II	III	IV
1 and 2	A	B	C	D
3 and 4	B	A	D	C

5 and 6	C	D	B	A
7 and 8	D	C	A	B

Each treatment period consists of an adaptation period of 7 days (day 1 to 7), and a sampling period of 5 days (day 8 till 12) (table 3). The choice for a 7-day adaptation period is based on the result of a previous conducted experiment. In this experiment with 4 cats, we found that the urinary oxalate excretion was stabilized after 5.40 ± 0.39 days (mean ± SD) after a (gradual) change of diet ([redacted]). Based on these results we conclude that an adaptation period of a minimum of 6-7 days is required to adapt to their new diet.
To answer the research questions accurately, it is essential to quantify the oxalate in the faeces and urine (µmol oxalate/kg BW0.75/day) (for arguments to collect fecal and urine samples, see doel). To cancel out the effect of day-to-day variation, the pooling of all the faeces and urine collected on 5 consecutive days is required (13). Faeces and urine sample collection will be achieved using a modified litter box as described by Hendriks and co-authors (14), in which the stainless steel wire mesh was replaced by a solid plastic bottom containing two rows of 1.5-mm holes at the lowest point. The top tray, with contained polyethylene grains (Ø 2-4 mm) to prevent contamination of the urine with feces and allow cats to bury faeces. The bottom tray of the litter box will contain 5 ml of 3N HCl to acidify the urine immediately for conservation. The urine needs to be acidified for the analysis of oxalate and calcium.
The food intake will be recorded daily and body weight will be recorded at day 1 and 8 of every period. When the food intake is be below 50 kcal/kg BW0.67/day and the body weight reduced more than 5% during a period, the cat will taken out of the experiment.

Table 3: Schedule for the training and the experiments with the cats plus the sampling scheme of the main study.

Period	Food intake recording	Weighing of cats	Fecal and urine sampling
I	daily	day 1 and 8	day 8 to 12
II	daily	day 1 and 8	day 8 to 12
III	daily	day 1 and 8	day 8 to 12
IV	daily	day 1 and 8	day 8 to 12

Previous to the study the cats will get acquainted gradually to the procedures such as weighing, individually housing in the metabolism cages and the use of the modified litter box. Before the start of the experiment, all cats will receive a general health check by a veterinarian [redacted]

References
(12) National Research Council (U.S.) and Committee on Dog and Cat Nutrition. Nutrient Requirements of Dogs and Cats. Washington: The National Academies Press; 2006.
(13) Pastoor FJ, van 't Klooster AT, Beynen AC. An alternative method for the quantitative collection of faeces and urine of cats as validated by the determination of mineral balance. Z Versuchstierkd 1990; 33(6):259-63.
(14) Hendriks WH, Wamberg S, Tarttelin MF. A metabolism cage for quantitative urine collection and accurate measurement of water balance in adult cats (Felis catus). J Anim Physiol Anim Nutr (Berl) 1999 Feb 1;82:94-105.

6.b. Mate van ongerief: B. Gering/Matig

6.c. Waaruit bestaat het ongerief en hoe bent u tot uw inschatting van de mate van ongerief gekomen?
The individual housing of the cats in the metabolism cage during 5 consecutive days can result to inconvenience for the cat as the cats are used to be housed as a group. In addition, the weighing procedure can also lead to little inconvenience.

7. Welke maatregelen heeft u getroffen om het ongerief tot een minimum te beperken?
Anesthesie: A. Niet toegepast (geen aanleiding).
Pijnbestrijding: A. Wordt niet toegepast omdat hiertoe geen aanleiding bestaat.

o The cats will be trained/adapted step-by-step to be housed in the metabolism cage for a prolonged period and for learning to urinate and defecate on the special designed litter box. In this way the stress response of cats to these new procedures will be minimized.
o The cats are trained/adapted to the weighing procedure as they're regularly weighed throughout the year.
o During the adaptation periods of the experiment the cats will be only housed in the cages during the night and feeding times. The rest of the day they can walk around freely and interact with each other.
o The metabolism cages are positioned in such a way that when the cats are housed in the cage they can make eye contact with each other, hear each other, etc.
o During daytime almost continuously researchers and/or caretakers will be present in the room were cats are housed in the metabolism cages. Also time is scheduled to socially interact with the cats.
o During the sampling period, the cats will be kept in small groups for one hour per day, under supervision to ensure no urine will be voided.
o Additional to the set-up of the litter box described in Hendriks and co-authors (14) non-absorbable cat litter will be added. In this way the cat will be less disturbed in expressing its natural behaviour previous to urinating and defecating, i.e. scratching/digging into the 'soil'.

8. Toestand van dieren na einde van de proef: Het dier is na de proef in leven gelaten.
Toelichting:
The cats will stay in the (group accommodation of the) cat facility of the [redacted] after the experiment.

9. Welke alternatieven (vervanging, verfijning, vermindering) zijn voor de beschreven experimenten overwogen en waarom zijn deze verworpen?
Replacement is not possible since the cat is the target species for this study. Furthermore, it is not possible to answer the research questions by conducting an in vitro-experiment.

Reduction of the number of animals is not possible as the indicated amount of animals is based on a power analysis

Refinement of the duration of the experiment is not possible since the adaptation/pre-period is already minimalized by a previous experiment conducted to determine the minimal time needed for oxalate excretion to reach its steady state/plateau phase.

10. Namen van direct betrokkenen bij de dierproef (artikel 9- en 12-functionarissen):
[redacted]

Tabel registratiecode opties voor aanvraag 2011113.a (K14):

1	2	3	4	5	6	7	8	9	10	11	12	13
1	**Ot**	**7**	**8**	**12**	**1**	**1**	**01**	**01**	**1**	**1**	**2**	**3**

Aanmeldingsformulier voor proeven met gewervelde dieren.

Secretariaat DEC
[redacted]

Aanvrager: [redacted]
Afdeling:

Titel dierproef: The effect of dietary hydroxyproline on oxalate synthesis and the excretion of oxalate in faeces and urine of healthy adult cats

Aanmeldcode / Protocol: 2011113.b
Stadia van de proef:

06-01-2012	Aangemeld [redacted]	
24-01-2012	Positief advies na behandeling DEC	Secretaris van de DEC
25-09-2012	Opmerkingen [redacted]	
27-09-2012	Welzijnsevaluatie aangemaakt [redacted]	

Is deze proef wetenschappelijk getoetst en goedgekeurd? Ja
Toelichting:
In het kader van AIO procedure.

1.a. Met dit onderzoek te beantwoorden concrete vraag:
B. Ontwikkeling van veterinaire (hulp)middelen ontw. med. hulpmiddelen / toepassingen
The main objective of this study:
- To determine the influence of dietary hydroxyproline on the urinary oxalate excretion as an indicator for endogenous oxalate synthesis in healthy cats in a controlled environment.

Sub objectives of this study
- To determine the apparent secretion of oxalate into the intestines in cats fed oxalate-free diets.
- To determine the apparent absorption of dietary oxalate in cats fed the diet containing oxalate.
- To determine the contribution of dietary oxalate to the oxalate excreted in the urine in cats fed the diet containing oxalate.

Het uiteindelijke doel (Maatschappelijke en wetenschappelijke relevantie):

Urolithiasis is the second most common cause of lower urinary tract disease (LUTD) in cats (1;2) (symptoms: difficult and painful urination, blood in the urine, partial or complete urethral obstruction) and different urolith (stone) types are known to cause urolithiasis. Over the past decades, a progressive increase in the prevalence of calcium oxalate (CaOx) uroliths (from 2% to 55-60%) has been reported in cats with LUTD in the United States of America (3) and Benelux (4). In dogs, the same general trend in urolith composition can be observed but is less extreme than in cats (3;4). Nutrition is thought to play a major role in this increase in prevalence of CaOx urolithiasis. Today's nutritional interventions are mainly based on human medicine studies as only a few studies have been conducted in cats.
The current treatment of choice for severe CaOx urolithiasis is surgical urolith removal, followed by methods to prevent urolith recurrence. At present, the standard method for preventing CaOx urolith recurrence is to feed a urolith reducing therapeutic diet and encourage water intake. Despite these

preventative measures, the prevalence and recurrence rate of CaOx uroliths remains high in cats. Therefore, more knowledge regarding the etiopathogenesis of CaOx urolithiasis should be generated to improve current preventative measures and to decrease the prevalence of CaOx urolithiasis.
Urinary oxalate and calcium excretion are essential factors in CaOx urolith formation as they are the two elements to precipitate with each other in the urine. An increase in the excretion of either calcium or oxalate or both could lead to CaOx urolith formation. Since calcium metabolism is of interest for more diseases, several studies have been conducted to determine the nutrients that influence the urinary calcium excretion. This is however not the case for urinary oxalate excretion and its origin.

Oxalate in the urine can be of dietary or endogenous origin. In humans it is known that both sources contribute equal to oxalate excreted in the urine (5;6). In cats, no exact data is available yet on this subject, but only a data mining study suggesting that the intestinal absorption of dietary oxalate and therefore the contribution of dietary oxalate to urine oxalate may be minimal ([redacted] et al, unpublished results). In dogs, the urinary oxalate excretion could not be affected by changing dietary oxalate content (7). When indeed the dietary contribution to oxalate excreted in the urine is minimal, the preventative measures extrapolated from human medicine on this aspect does not hold true for cats. The contribution of oxalate to oxalate in the urine can be studied by feeding an oxalate-free diet vs. a diet supplemented with oxalate. The absorption can be determined by subtracting the dietary oxalate intake by the fecal oxalate content.
As the contribution of dietary oxalate may be minimal, the oxalate excreted in the urine is likely to be of endogenous origin. Endogenous oxalate synthesis has been studied extensively in human medicine. In these studies, oxalate synthesis could be affected by varying the intake of pyridoxine (vitamin B6), ascorbic acid (vitamin C), glucose, fructose and certain amino acids. Of the amino acids, hydroxyproline was the most potent amino acid in inducing oxalate synthesis (8-10). Hydroxyproline is an amino acid abundant in the protein collagen. In a study with cats fed different protein sources, collagen resulted in 2 to 3 fold higher oxalate excretions in the urine than soya and horse meat (11). However, unknown is whether these observed differences can be ascribed to differences in oxalate synthesis or oxalate intake as the oxalate content of these diets were not tested. The effect of hydroxyproline on oxalate synthesis remains to be determined in cats and can be achieved by feeding diets equal in oxalate content. This can be achieved by feeding diets differing in hydroxyproline content.
Oxalate synthesis can be underestimated when only the oxalate excreted in the urine is measured, since oxalate could also be measured in fecal samples after feeding rats an oxalate-free diet (9). All oxalate secreted in the intestines instead of in the urine is beneficial as this oxalate cannot contribute to calcium oxalate urolith formation anymore. Investigating the fraction of endogenous oxalate in feces (relative to urine) of cats fed an oxalate-free diet may provide useful information.

Information on the contribution of dietary and endogenous oxalate on the oxalate excreted in the urine, hydroxyproline as an inducer of endogenous oxalate and the contribution of oxalate-degrading bacteria in the intestines may provide new insights for dietary strategies that prevent urolithiasis.

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1.b. Het uiteindelijk doel (Maatschappelijke en wetenschappelijke relevantie):
Doel:
Zie 'Direct doel'

1.c. Lekensamenvatting:

2. Gepland vanaf: 15-01-2012 **tot** 15-04-2012

3. Diersoort: katten ; **Totaal aantal:** 8

4.a. Nadere aanduiding gebruikte dieren:
Intact adult Domestic Shorthair cats, all females, bodyweight 2-4 kg, age between 1 and 6 years.

4.b. Motivatie waarom is gekozen voor deze diersoort:
The occurrence of CaOx urolithiasis is known to be more common in cats than in dogs. This implicates that cats are more sensitive for risk factors leading to CaOx urolith formation.

The basal urinary oxalate excretion is a little higher in male cats which may be due to the influence of testosterone on endogenous oxalate synthesis. Intact females and neutered males and females show a more similar urinary oxalate excretion. When using intact female cats, the results can be extrapolated to majority of the cat population as most privately-owned male cats are neutered. In addition, as most male cats are spraying urine for marking, quantitatively urine collection is less accurate using male cats.

4.c. Toelichting voor het aantal gebruikte dieren:
Based on a sample size calculation the conclusion could be drawn that a minimum of 6 observations of different cats per diet is required to obtain statistical significant results in an experiment determining the effect of diet on urinary oxalate excretion. This sample size calculation was conducted using the following parameters: Mean=19 and SD=5 µmol oxalate/kg BW/day, which is compiled from data of the study of Zentek and Schultz (11) and our unpublished data, biological relevant difference is 25% and statistical power is 90%. In case some cats cannot complete the experiment due to reasons such as illness, diet refusal, urination outside the litter box, an increase of the sample size with 2 cats would be rational. This means that each diet will be tested on 8 cats.

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4.d. Herkomst: G. dier is in reg.jr. meer dan een keer hergebr.

Toelichting:

The cats are housed at the laboratory animal facility of [redacted].

5.a. Accommodatie: [redacted]

The cats are housed in a group accommodation with a surface of 25 m2. During the adaptation period of the experiment (day 1 to 7), the cats will be kept in the group during the day. The cats involved in the experiment will be housed individually in the metabolism cage during feeding time and overnight. During the sampling period (day 8 to 12) the cats will be housed individually in the metabolism cages for 23 hours per day and 1 hour in small groups under supervision to ensure no urine will be voided. The metabolism cages are constructed of Trespa panels and aluminium front frame and are 0.80x1.00x0.75 m. The front contains a feeding and water bowl and in the back corners of the cage a removable litter tray (29x29x12 cm) is securely positioned.

In the metabolism cage a shelf (verhoging) is available to lie or sleep on. There is no box or clothing etc placed in the cage as cats may use it to urinate of defecate, which would interfere with interpretation of the results. To minimize discomfort it was proposed to daily offer the cats time out of the metabolism cages for at least an hour a day in small groups, accompanied by the researchers.

5.b. Huisvesting & Verzorging:

The daily care during the experiments will be performed by the persons involved in the experiment (see also point 10) and will be in close contact with the animal caretakers/staff members of [redacted].

5.c. Voeding:

During the experiment four experimental diets will be fed. These experimental diets consist of a basal balanced and complete basal diet (according the recommended nutrient requirements for adult cats of the National Research Council (2006) (12)) supplemented with hydroxyproline or alanine, and sodium oxalate (table 1). As the supplements do only account for 5.2% of the diet (i.e. 95% remains the same), no problems are expected regarding the adaptation from one to other diet. As the diets will be extruded and will be coated with digest (i.e. a substance giving a good taste to a kibble), it is expected the palatability of the diets will be good.

Table 1: Composition of the experimental diets.

Diet	Supplement (%)		
	Hyp	Ala	sodium oxalate
A: Hyp - Fox	5.2*	0	0
B: Hyp/Ala - Fox	2.6	2.6	0
C: Ala - Fox (control)	0	5.2	0
D: Ala - Hox	0	5.2	0.13**

*Based on the amount of Hyp supplemented in a similar study conducted in rats (9) and equals to 1.27 g/100 kcal metabolizable energy (ME).

** This amount equals to 21 mg/100 kcal ME, which is an oxalate content within the observed range of oxalate in commercially available diets for adult cats ([redacted] et al., unpublished results).

Hyp, hydroxyproline; Ala, alanine; Fox, free of oxalate, Hox, high in oxalate.

The amount of food per cat will be determined based on energy requirement of the individual cat by using 100 kcal/kg BW0.67/day (12). To assure the cats receive enough food, food intake will be monitored and the cats will be weighed weekly at the start of each adaptation and sampling period (i.e. day 1 and 8). Water is available ad libitum.

6.a. Proefschema / proefbehandelingen:

The four experimental diets will be fed according a repeated 4*4 latin square design to obtain more statistical power. The repetition, i.e. 2 cats per sequence, is incorporated to reduce the chance the Latin square will be incomplete as a result of missing values due to for example illness in cats.

Table 2: Feeding schedule of the experimental diets according a repeated Latin Square design.

	Period			
Cat	I	II	III	IV
1 and 2	A	B	C	D
3 and 4	B	A	D	C
5 and 6	C	D	B	A
7 and 8	D	C	A	B

Each treatment period consists of an adaptation period of 7 days (day 1 to 7), and a sampling period of 5 days (day 8 till 12) (table 3). The choice for a 7-day adaptation period is based on the result of a previous conducted experiment. In this experiment with 4 cats, we found that the urinary oxalate excretion was stabilized after 5.40 ± 0.39 days (mean ± SD) after a (gradual) change of diet ([redacted]). Based on these results we conclude that an adaptation period of a minimum of 6-7 days is required to adapt to their new diet.
To answer the research questions accurately, it is essential to quantify the oxalate in the faeces and urine (µmol oxalate/kg BW0.75/day) (for arguments to collect fecal and urine samples, see doel). To cancel out the effect of day-to-day variation, the pooling of all the faeces and urine collected on 5 consecutive days is required (13). Faeces and urine sample collection will be achieved using a modified litter box as described by Hendriks and co-authors (14), in which the stainless steel wire mesh was replaced by a solid plastic bottom containing two rows of 1.5-mm holes at the lowest point. The top tray, with contained polyethylene grains (Ø 2-4 mm) to prevent contamination of the urine with feces and allow cats to bury faeces. The bottom tray of the litter box will contain 5 ml of 3N HCl to acidify the urine immediately for conservation. The urine needs to be acidified for the analysis of oxalate and calcium.
The food intake will be recorded daily and body weight will be recorded at day 1 and 8 of every period. When the food intake is be below 50 kcal/kg BW0.67/day and the body weight reduced more than 5% during a period, the cat will taken out of the experiment.

Table 3: Schedule for the training and the experiments with the cats plus the sampling scheme of the main study.

Period	Food intake recording	Weighing of cats	Fecal and urine sampling
I	daily	day 1 and 8	day 8 to 12
II	daily	day 1 and 8	day 8 to 12
III	daily	day 1 and 8	day 8 to 12
IV	daily	day 1 and 8	day 8 to 12

Previous to the study the cats will get acquainted gradually to the procedures such as weighing, individually housing in the metabolism cages and the use of the modified litter box. Before the start of the experiment, all cats will receive a general health check by a veterinarian [redacted]

References

(12) National Research Council (U.S.) and Committee on Dog and Cat Nutrition. Nutrient Requirements of Dogs and Cats. Washington: The National Academies Press; 2006.
(13) Pastoor FJ, van 't Klooster AT, Beynen AC. An alternative method for the quantitative collection of faeces and urine of cats as validated by the determination of mineral balance. Z Versuchstierkd 1990; 33(6):259-63.
(14) Hendriks WH, Wamberg S, Tarttelin MF. A metabolism cage for quantitative urine collection and accurate measurement of water balance in adult cats (Felis catus). J Anim Physiol Anim Nutr (Berl) 1999 Feb 1;82:94-105.

The cats need to be housed in a metabolism cage for 5 days to obtain a representative fecal sample. As cats generally do not defecate daily, the day-to-day variation in fecal samples is large. Pastoor et al. (1990) determined that a minimal collection period of 5 days was required to average out the day-to-day variance on mineral retention. The reasons why we want to obtain quantitative faeces samples is explained in 1.a..

6.b. Mate van ongerief: B. Gering/Matig

6.c. Waaruit bestaat het ongerief en hoe bent u tot uw inschatting van de mate van ongerief gekomen?
The individual housing of the cats in the metabolism cage during 5 consecutive days can result to inconvenience for the cat as the cats are used to be housed as a group. In addition, the weighing procedure can also lead to little inconvenience.

7. Welke maatregelen heeft u getroffen om het ongerief tot een minimum te beperken?
Anesthesie: A. Niet toegepast (geen aanleiding).
Pijnbestrijding: A. Wordt niet toegepast omdat hiertoe geen aanleiding bestaat.

o The cats will be trained/adapted step-by-step to be housed in the metabolism cage for a prolonged period and for learning to urinate and defecate on the special designed litter box. In this way the stress response of cats to these new procedures will be minimized.
o The cats are trained/adapted to the weighing procedure as they're regularly weighed throughout the year.
o During the adaptation periods of the experiment the cats will be only housed in the cages during the night and feeding times. The rest of the day they can walk around freely and interact with each other.
o The metabolism cages are positioned in such a way that when the cats are housed in the cage they can make eye contact with each other, hear each other, etc.
o During daytime almost continuously researchers and/or caretakers will be present in the room were cats are housed in the metabolism cages. Also time is scheduled to socially interact with the cats.
o During the sampling period, the cats will be kept in small groups for one hour per day, under supervision to ensure no urine will be voided.
o Additional to the set-up of the litter box described in Hendriks and co-authors (14) non-absorbable cat litter will be added. In this way the cat will be less disturbed in expressing its natural behaviour previous to urinating and defecating, i.e. scratching/digging into the 'soil'.

8. Toestand van dieren na einde van de proef: Het dier is na de proef in leven gelaten.
Toelichting:
The cats will stay in the (group accommodation of the) cat facility of the [redacted] after the experiment.

9. Welke alternatieven (vervanging, verfijning, vermindering) zijn voor de beschreven experimenten overwogen en waarom zijn deze verworpen?
Replacement is not possible since the cat is the target species for this study. Furthermore, it is not possible to answer the research questions by conducting an in vitro-experiment.

Reduction of the number of animals is not possible as the indicated amount of animals is based on a power analysis

Refinement of the duration of the experiment is not possible since the adaptation/pre-period is already minimalized by a previous experiment conducted to determine the minimal time needed for oxalate excretion to reach its steady state/plateau phase.

10. Namen van direct betrokkenen bij de dierproef (artikel 9- en 12-functionarissen):
[redacted]

Tabel registratiecode opties voor aanvraag 2011113.b (K14):

1	2	3	4	5	6	7	8	9	10	11	12	13
1	**Ot**	**7**	**8**	**12**	**1**	**1**	**01**	**01**	**1**	**1**	**2**	**3**

Uw aanvraag 2011113.a, door u aangemeld vanuit DRS heeft van de DEC de status: 'Wijzigen' gekregen.

De DEC is van mening dat het doel van de proef opweegt tegen het te verwachten gering/ matige ongerief dat de dieren ondergaan. Voorafgaand aan een definitief advies heeft de DEC de volgende vragen en opmerkingen ter verheldering:
De DEC verzoekt u bij 4.c. (Toelichting aantal dieren) ook het gemiddelde te vermelden, ten opzichte waarvan de vermelde standaardafwijking geldt.
Daarnaast verzoekt de DEC u bij 6.a. (proefschema) duidelijker te onderbouwen, waarom het nodig is de dieren gedurende 5 dagen in de metabolismekooien te huisvesten.
Bovendien verzoekt de DEC u bij 5.a. (Accommodatie) te vermelden, of er in de metabolismekooien een slaapplaats aanwezig is voor de katten.
Tenslotte wil de DEC u er op wijzen, dat proefplannen in het Nederlands dienen te worden opgesteld, tenzij een van de betrokken art.9- functionarissen de Nederlandse taal niet machtig is.

Na aanpassing zal de proef door de secretaris van de DEC worden afgehandeld.

Uw aanvraag 2011113.b, door u aangemeld vanuit DRS heeft van de Secretaris DEC de status: 'Positief advies na behandeling DEC' gekregen.

De DEC is van mening dat het doel van de proef opweegt tegen het te verwachten gering/ matige ongerief dat de dieren ondergaan en dat de vraag m.b.t. alternatieven voldoende is beantwoord.

Met vriendelijke groet,

[redacted]
Secretaris DEC