CONNECTING. LEARNING. SUCCEEDING.

PETFOOD FORUM EUROPE

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Advancing in-home protocols to assess digestibility of dog and cat foods

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Digestibility testing

Regularly used guidelines

<table>
<thead>
<tr>
<th>AAFCO¹</th>
<th>FEDIAF²</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 healthy adult animals</td>
<td></td>
</tr>
<tr>
<td>Individual housing</td>
<td></td>
</tr>
<tr>
<td>Standardized condition</td>
<td></td>
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</tbody>
</table>

5 adaptation days
5 total fecal collection days
1 pooled sample for analyses

3 adaptation days
4 total fecal collection days
1 pooled sample for analyses

Nutritional value¹,²
Fecal output³
Food acceptance³

No differences between pooled fecal samples of 6 beagle dogs over day 4-7, 8-14, 15-21⁴

Digestibility test conditions

Animal facilities
- Low variation in animals
- Standardized conditions

In-home situation
- High variation in animals
- Variable conditions

Potential impact on digestibility values

5-21. See reference list at the end of the presentation
### Aim of the research

<table>
<thead>
<tr>
<th>Challenges to overcome</th>
<th>Gain insight in the protocol requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>No guidelines</td>
<td>Number of adaptation days</td>
</tr>
<tr>
<td>More variation</td>
<td>Number of fecal collection days</td>
</tr>
<tr>
<td>Logistics</td>
<td>Number of pets</td>
</tr>
<tr>
<td>Owner compliance</td>
<td>Pet characteristics</td>
</tr>
</tbody>
</table>

Challenges to overcome:
- No guidelines
- More variation
- Logistics
- Owner compliance

Gain insight in the protocol requirements:
- Number of adaptation days
- Number of fecal collection days
- Number of pets
- Pet characteristics
Study design

Dogs

- N=60
- Days: 1 to 14
- Food: Own food, relatively high and low digestible food
- Feces collection by owner

Cats

- N=30
- Days: 1 to 16
- Food: Own food, relatively high and low digestible food
- Feces collection by owner
## Test foods

<table>
<thead>
<tr>
<th></th>
<th>Relatively high digestible</th>
<th>Relatively low digestible</th>
<th>Relatively high digestible</th>
<th>Relatively low digestible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>26%</td>
<td>22%</td>
<td>29%</td>
<td>31%</td>
</tr>
<tr>
<td>Crude fat</td>
<td>15%</td>
<td>10%</td>
<td>16%</td>
<td>11%</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>2%</td>
<td>3%</td>
<td>2%</td>
<td>3.5%</td>
</tr>
<tr>
<td>Crude ash</td>
<td>8%</td>
<td>6.5%</td>
<td>6.5%</td>
<td>6.8%</td>
</tr>
<tr>
<td>Energy value</td>
<td>3720 kcal/kg ME</td>
<td>3460 kcal/kg ME</td>
<td>3816 kcal/kg ME</td>
<td>3403 kcal/kg ME</td>
</tr>
<tr>
<td>Composition</td>
<td>Disclosed corn, dried chicken, dried beef/pork, animal protein extract, poultry fat, corn, rice, minerals</td>
<td>Barley, rice, dried lamb, dried poultry, poultry fat, dried beet pulp, brewer’s yeast, dried fish, flax seed, egg powder, lecithin</td>
<td>Dried salmon (31%), rice, dried potato, poultry fat, rice protein, dried beet pulp, salmon oil, psyllium, linseed oil</td>
<td>Wheat, meat bone meal, greaves, wheat grits, barley, poultry fat, lignocellulose, hydrolysed chicken liver</td>
</tr>
</tbody>
</table>

*TiO₂ as indigestible marker*
Procedure

- > 1 year
- Healthy
- Not pregnant/lactating
- Only test food
- Faecal collection

Online survey → Selected → Information brochure → Appointment 1 → Study participation → Appointment 2 → Evaluation
Materials & daily study activities
Participants

- N = 146
- N = 107

Online survey
- Owner/pet not eligible
- Selected
- No response
- Contact established
- Owner withdraws
- Cat tastes food samples
- Cat does not eat both foods

N = 60
N = 30

Started
- Pet does not eat one or both foods
- Owner withdraws
- Pet has non-food related health issues
- Cat does not use cat litter
- Severe non-compliance of the owner

N = 53
N = 27

Finished
Sample collection

Defecations per day (owner)

Presence of daily fecal sample (researcher)

Reason of absence of fecal sample

728 study days

432 study days
Chemical analyses

**DM**

**Ash**

**N**

**CFat_{HCl}**

**Starch**

**GE**

OM (calculated as 1000 – ash)

CP (calculated as N*6.25)
Calculations and statistical analyses

The required adaptation period
- Compare Ti values over the week
- Compare digestibility values over the week

The required study length
- Compare digestibility values over the week
- Check confidence interval per number of fecal collection days

The required sample size
- Check confidence interval per number of dogs/cats
- Check the accepted margin of error

Apparent nutrient digestibility (%) = 100 - \( \frac{N_{faeces} \times T_{food}}{N_{food} \times T_{faeces}} \) x 100%

Repeated measures ANOVA followed by Tukey-Kramer multiple comparison test
Bootstrap sampling including 10,000 replicates
The number of adaptation days

Determined by fecal marker concentrations (mean + SE)

Period 1
For both foods: day 1 different (P<0.05)

Period 2
For both foods: no differences (P<0.10)
The number of adaptation days
Determined by fecal marker concentrations (mean + SE)

Period 1

Period 2

Both periods, Food C:
Day 1+2 different (P<0.05)

Both periods, Food D
Day 1 different (P<0.05)

Relatively high digestible
Relatively low digestible
The number of adaptation & fecal collection days
Determined by digestibility values (mean + SE)

Day 2 is the same as a pooled sample from day 4-7 (P>0.10)

Period 1
For both foods: day 1 different (P<0.05)

Period 2
For both foods: no differences (P<0.10)
The number of adaptation &
fecal collection days
Determined by digestibility values (mean + SE)

Period 1
Food C
Day 1+2 different (P<0.05)

Period 2
Food D
Day 1 different (P<0.05)

Period 1
Day 1 different (P<0.05)

Period 2
No differences (P>0.10)
The number of fecal collection days & sample size

Determined by simulated pooling of samples & bootstrapping (estimates ± 95% CI)

1 day = day 2 after first food consumption
2 days = day 2+3
3 days = day 2+3+4

Increasing number of days does not increase precision
Increasing number of dogs does increase precision
The number of fecal collection days & sample size
Determined by simulated pooling of samples & bootstrapping (estimates ± 95% CI)

Increasing number of days does not increase precision
Increasing number of cats does increase precision

1 day = day 3 after first food consumption
2 days = day 3+4
3 days = day 3+4+5
....
Sample size & margin of error

Determined by bootstrap estimates, 0.5*confidence intervals and former studies\textsuperscript{22, 23}

- Hall et al. 2013\textsuperscript{22}
  - 177 dog foods
  - 129 cat foods

- Carciofi\textsuperscript{23}
  - 23 dog foods
Conclusions & remarks

The required adaptation period

- **1 day**
- **2 days**

The required study length

- **1 day**
- **1 day**

The required sample size

- **N**: 22 & 25, 7 & 21
- **DM**: 21 & 35, < 5 & 6
- **GE**: 24 & 43, < 5 & 8

### Variation due to animal characteristics and specific foods?

- AAFCO
- FEDIAF

### Practicality & feasibility

- Practicality & feasibility

### Dependent on food, nutrient and study population

- 2 days
- 3 days

Variation due to animal characteristics and specific foods?

Practicality & feasibility

Dependent on food, nutrient and study population

- 22 & 25
- < 5 & 6
- 21 & 35
- < 5 & 8
- 24 & 43
- 7 & 21
Take home messages

- In-home testing would include: 3 days for dogs and 5 days for cats
- The sample size depends on margin of error deemed acceptable
- Future research should focus on the identification & controlling of sources of variation
- Protocol adjustments might be needed based on animal and food characteristics
References

22. Personal communication with Dr. Carciofi
Extra slides – different variation per nutrient

Example

5 dogs

Number of fecal collection days

GE digestibility (%)  

Starch digestibility (%)  

5 dogs

Number of fecal collection days
Extra slides - sample size & margin of error
Determined by bootstrap estimates, 0.5*confidence intervals and former studies\textsuperscript{22, 23}

- N = 22 & 25 dogs
- DM = 21 & 35 dogs
- GE = 24 & 43 dogs

Hall et al. 2013\textsuperscript{22} 177 dog foods
Carciofi\textsuperscript{23} 23 dog foods
Extra slides - sample size & margin of error

Determined by bootstrap estimates, 0.5*confidence intervals and former studies\textsuperscript{22}

\begin{itemize}
  \item \textbf{N} = 7 & 21 cats
  \item DM = \(< 5 & 6\) cats
  \item GE = \(< 5 & 8\) cats
\end{itemize}

Hall et al. 2013\textsuperscript{22}

129 cat foods

\begin{itemize}
  \item N = \text{Average margin of error} (%)
  \item DM = \text{Average margin of error} (%)
  \item GE = \text{Average margin of error} (%)
\end{itemize}
Extra slides – Other ingestions

- **Snacks:**
  - Yes: 3
  - No: 87
  - Not indicated: 10

- **Other ingestions:**
  - Yes: 9
  - No/unknown: 91
  - Total: 91

- **Total other ingestions:**
  - Yes: 18
  - No: 82
  - Total: 100

- **Type of snacks:**
  - Unclear
  - Not specified
  - Bones
  - Dog cookies
  - Dog kibbles
  - Human food
  - Cat kibbles

- **Type of other ingestions:**
  - Animal faeces
  - Grass
  - Sand/earth
  - Wood
  - Animal feed
  - Something from the street
  - Worm treatment
Extra slides – Frequency of other ingestions

**Frequency of 'snack days'**

Number of dogs

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

Number of snack days during the 14-day study period

**Frequency of 'other ingestion days'**

Number of dogs

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

Number of other ingestion days during the 14-day study period
Extra slides – Other ingestions

Total other ingestions

- yes: 88
- no: 4
- not indicated: 8

Type of other ingestions

- not specified
- unknown
- human food
- cat food
- cat snacks
- dog food
Extra slides – Sample contamination

Contaminants in sample
- yes: 70%
- no: 30%

Type of contaminants
- branches/wood
- grass
- hair
- plastic (poop back)
- straw
- sand
Extra slides – Sample contamination