



# **F.E.D.I.A.F.**

## **NUTRITIONAL GUIDELINES FOR COMPLETE AND COMPLEMENTARY PET FOOD FOR CATS AND DOGS**

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The glossary contains definitions of key words used in this Guideline followed by the source of the definition. Whenever appropriate, definitions are adapted to pet food.

## I. GLOSSARY

<b>Allowance</b>	An Allowance or Recommendation for daily intake (RDI) is the level of intake of a nutrient or food component that appears to be adequate to meet the known nutritional needs of practically all healthy individuals. It reflects the minimum requirement plus a safety margin for differences in availability between individual animals and for nutrient interactions. In practice this would be translated as the levels of essential nutrients that healthy individuals should consume over time to ensure adequate and safe nutrition.	FNB'94, Harper'90.
<b>Basal metabolic rate (BMR)</b>	Is the energy required to maintain homeostasis in an animal in a post-absorptive state (ideally after an overnight fast) that is lying down but awake in a thermo-neutral environment to which it has been acclimatised	Blaxter KL, 1989; in: Energy Metabolism in Animals and Man. Cambridge University Press ... Edit.
<b>Complementary pet food</b>	Pet food which has a high content of certain substances but which, by reason of its composition, is sufficient for a daily ration only if used in combination with other pet foods. See also FEDIAF explanation <a href="#">page 29</a>	Directive 79/373 on the circulation of compound feedingstuffs (art. 2(e)) adapted to pet food.
<b>Complete pet food</b>	Pet food which, by reason of its composition, is sufficient for a daily ration.	Directive 79/373 on the circulation of compound feedingstuffs (art. 2(d)) adapted to pet food.
<b>Daily ration</b>	The average total quantity of feedingstuffs, calculated on a moisture content of 12%, required daily by an animal of a given species, age category and yield, to satisfy all its needs.  The above-mentioned legal definition means the average total quantity of a specific pet food that is needed daily by a pet of a given species, age category and life style or activity to satisfy all its energy and nutrient requirements	D79/373 on the circulation of compound feedingstuffs (art. 2(c)).
<b>Digestible energy (DE)</b>	Is the gross energy less the gross energy of faeces resulting from the consumption of that pet food	McDonald <i>et al.</i> , 1995; in: Animal Nutrition 5 <sup>th</sup> Edit.
<b>DM</b>	Dry Matter	
<b>Dry pet food</b>	Pet food with a moisture content of less than 14%.	Hygienische productie en handel Huisdiervoeders 1997.
<b>Gross energy</b>	Is the total energy arising from complete combustion of a food in a bomb calorimeter.	McDonald <i>et al.</i> , 1995. Animal Nutrition. 5 <sup>th</sup> edition.
<b>Maintenance energy requirement (MER)</b>	Is the energy required to support energy equilibrium, (where ME equals heat production), over a long period of time.	Blaxter k. L., 1989. Energy Metabolism in Animals and Man. Cambridge University Press.
<b>Metabolizable energy (ME)</b>	Is the digestible energy less the energy lost in urine and combustible gases.	McDonald <i>et al.</i> , 1995; in: Animal Nutrition 5 <sup>th</sup> Edit.
<b>NRC</b>	National Research Council (USA) is a council is organised by the US National Academy of Sciences. The NRC ad hoc Committee on dog and cat nutrition has compiled the nutritional requirements for dogs and cats 2006.	www.national-academies.org
<b>Nutrient requirement</b>	Is the quantity of a nutrient that must be supplied to an animal in order to satisfy its metabolic needs. It reflects the minimum	Reeds PJ, PR Becket 1996; in: Present Knowledge in Nutrition 7 <sup>th</sup> Edit.

	average level of intake of a nutrient, which, over time, is sufficient to maintain the desired biochemical or physiological functions.	
<b>Nutritional maximum limit</b>	Highest known level of a nutrient in a complete pet food at which no adverse effects have been reported in the target animal when fed according to the feeding instructions.	FEDIAF 2006
<b>Pet food</b>	Any product produced by a Pet food manufacturer, whether processed, partially processed or unprocessed, intended to be ingested by pet animals after placing on the market.	FEDIAF 2006
<b>Pet food safety</b>	Is the assurance that, when eaten according to its intended use, the pet food will not cause harm to the pet animal.	EN ISO 22000:2005(E) adapted to pet food
<b>RA</b>	Recommended Allowance.	
<b>Recommendation</b>	See allowance	
<b>Semi-moist pet food</b>	Pet food with a moisture content of 14% or more and less than 60 %.	
<b>Wet pet food</b>	Pet food with a moisture content of 60% or more.	Hygienische productie en handel Huisdiervoeders 1997.
<b>Food allergy</b>	Immune-mediated reaction resulting in one or more of the clinical signs described in Annex V "Adverse reactions to food in dogs and cats" <a href="#">p.50</a> .	
<b>Anaphylaxis</b>	Anaphylaxis is an acute life-threatening multi-system allergic reaction resulting from exposure to an offending agent. In people, foods, insect stings, and medication are the most common causes. <sup>a,b,c</sup>	<sup>a</sup> Tang AW. A practical guide to anaphylaxis. Am Fam Physician 2003; 68 (7): 1325-1332. <sup>b</sup> Oswalt M, Kemp SF. Anaphylaxis: office management and prevention Immunol Allergy Clin North Am 2007; 27 (2): 177-191. <sup>c</sup> Wang J, Sampson HA. Food Anaphylaxis. Clin Exp Allergy. 2007; 37 (5): 651-660.
<b>Food intolerance / Food idiosyncrasy</b>	A reaction to a food component that is not immune mediated, but causes clinical signs resembling an immune-mediated reaction to food (food allergy).	
<b>Food toxicity</b>	A reaction to a toxic food component (e.g. onion poisoning) or a toxin released by contaminating organisms (e.g. mycotoxins).	
<b>Pharmacologic reaction</b>	An adverse reaction to food as a result of a naturally derived or added chemical that produces a drug-like or pharmacological effect in the host; e.g. methylxanthines in chocolate or a pseudo-allergic reaction caused by high histamine levels in not well-preserved scombroid fish such as tuna.	
<b>Dietary indiscretion</b>	An adverse reaction resulting from such behaviour as gluttony, pica, or ingestion of various indigestible materials or garbage.	

## II. INTRODUCTION

FEDIAF represents the national pet food industry associations in the EU and from Norway and Switzerland, representing in the region of 450 pet food factories across Europe.

One of FEDIAF's main objectives is to ascertain the well-being of pets by providing well balanced and nutritionally sound pet food through its member companies. Therefore FEDIAF has compiled the present **“Nutritional Guidelines for Complete and Complementary Pet Food for Cats and Dogs”**, which is based on the state of the art knowledge on cat and dog nutrition, providing pet food manufacturers with nutritional recommendations to ensure the production of well balanced and nutritionally sound pet food.

This document is reviewed yearly and updated whenever there are new relevant technological, scientific or legislative developments in pet nutrition.

### 1. OBJECTIVES

The objectives of FEDIAF's Guidelines for Complete and Complementary Pet Foods for Cats and Dogs are:

- i) To contribute to the production of nutritionally balanced pet food, while complying with relevant EU legislation on animal nutrition. To achieve this objective, the guidelines incorporate up-to-date scientific knowledge on cat and dog nutrition to:
  - Provide practical nutrient recommendations for pet food manufacturers when formulating their products for adult maintenance, growth and reproduction;
  - Help pet food manufacturers to assess the nutritional value of practical pet foods for healthy animals;
- ii) To be the reference document on pet nutrition in Europe for EU and local authorities, consumer organisations, professionals, and customers.
- iii) To enhance cooperation between pet food manufacturers, pet care professionals and competent authorities by providing scientifically sound information on the formulation and assessment of pet foods.
- iv) To complement FEDIAF's Guide to Good Practice for the Manufacture of Safe Pet Foods and the FEDIAF's Guide to Good Practice for Communication on Pet Food.

## 2. SCOPE

FEDIAF's Nutritional Guidelines provide:

- i) Recommendations for adequate and safe nutrient levels in practical foods for dogs and cats during growth, adult maintenance or reproduction;
- ii) Tools for the assessment of the nutritional value of pet foods;
- iii) Recommendations for energy intake.
- iv) Annexes with advice on specific topics (e.g. taurine, garlic, raisins, allergens etc.)

FEDIAF's Nutritional Guidelines give nutrient recommendations for healthy dogs or cats eating typical commercial pet food.

- The levels in this guide reflect the amounts of essential nutrients in commercial products that are required to ensure adequate and safe nutrition in healthy individuals when consumed over time.
- These guidelines relate to dog and cat foods manufactured from ingredients with normal digestibility (i.e.  $\geq 70\%$  DM digestibility;  $\geq 80\%$  protein digestibility) and average bioavailability.
- They include a safety margin for individual animal variation, and nutrient interactions.
- It follows from the above statements that adequate and high quality pet foods can be outside the recommendations based on the manufacturer's substantiation of nutritional adequacy.

Excluded from the FEDIAF's nutritional Guidelines are pet foods for particular nutritional purposes and some other specialised foods such as sporting dogs etc. Therefore specific products may have nutrient levels that are different from those stated in these guidelines.

### III. COMPLETE PET FOOD

#### 1 Guidance

Complete pet food means pet food which, by reason of its composition, is sufficient for a daily ration (Directive 79/373/EC adapted). When a complete pet food is fed for an extended period (i.e. covering the whole period of the life stage) as the only source of nutrients, it will provide all the nutritional needs of the particular animals of the given species and physiological state for which it is intended.

If a manufacturer labels a product as a complete pet food without specification of a determined life stage, it is assumed to be complete for all life stages, and should be formulated according to the levels recommended for **early** growth and reproduction. If the product is designed for a specific life stage, then the label must clearly state this. For example "Bloggo" is a complete pet food for breeding cats, or "Bloggo" is a complete pet food for growing puppies.

FEDIAF recommends to all members of each National Association that before a complete pet food is placed on the market:

- i) It should be formulated to take account of current nutritional knowledge and using the data compiled in this guide.
- ii) If certain nutrient levels are outside the values stated in this guide, manufacturers should be able to prove that the product provides adequate and safe intakes of all required nutrients.
- iii) Each family of products ([annex VII p. 76](#)) should be validated by chemical analysis of the finished product. It is recommended to use an officially recognised method (pp [31-32](#)).

#### 1.1 Nutrient recommendations for cat and dog foods

The nutrient requirements of cats and dogs are the subject of ongoing research. When formulating pet foods, manufacturers should not use a reference to minimum requirements but recommendations for adequate nutrient levels as contained in this guide. The nutritional tables are provided in "units/MJ ME", "units/1000 kcal ME" and "units/100 g DM".

This FEDIAF Guide is based on published scientific studies (including NRC 2006) and

unpublished data from the industry.

## 1.2 Energy contents of pet foods

Feeding trials are the most accurate way to measure the energy density of a cat and dog food (see pages [34-41](#) for the different methods).

A feeding trial normally measures digestible energy. By subtracting the energy lost in the urine, the same trials allow also for determining the metabolizable energy. The energy lost in the urine can be measured if urine is collected or, if urine is not collected, be calculated using the following correction factors: 1.25 kcal g<sup>-1</sup> digestible crude protein for dogs and 0.86 kcal g<sup>-1</sup> digestible protein for cats (see page [37](#)).

Alternatively, formulae given in annex I page [42](#) can be used by manufacturers to calculate the energy content of practical diets.

In addition, a bibliographic survey for calculating the energy needs of dogs and cats, in relation to body weight, physiological state and specific activities, is reported in annex I ([page 42](#)).

## 1.3 Maximum levels of certain substances in pet food for cats and dogs

FEDIAF has determined certain levels of nutrients which should not be exceeded (nutritional maximum levels) because they may be harmful in certain circumstances if present in excessive amounts.

In addition, maximum permitted levels have been determined by the legislator for several nutrients if added as a nutritional additive (i.e. trace-elements & vitamin D) (legal maximum). They are laid down in the Community Register of Feed Additives pursuant to Regulation 1831/2002/EC of the Parliament and the Council, concerning additives in feeding stuffs. They are listed in a separate column on the right, because they apply to all life stages.

Both groups of maximum values are reported in the FEDIAF tables on pages [12](#) to [17](#). The legal maximum only applies when the particular nutrient is deliberately added to the recipe as an additive, and relates to the '**total**' amount present in the finished product. If the nutrient comes entirely from feed materials, then the FEDIAF nutritional maximum applies.

A non-exhaustive list of scientifically recognised analytical methods that can be used to assess the nutrient levels in pet food is available [pp. 31-33](#).

## 1.4 Product validation

Before a product is placed on the market, it should have undergone the necessary

procedures to ensure its adequacy.

The following components should be taken into consideration for evaluation of nutritional adequacy.

NUTRIENTS			
<b>Major nutrients*</b>	Protein Fat		
<b>Essential fatty acids</b>	Linoleic acid	Arachidonic acid ( <b>cats</b> )	
	Alpha-linolenic acid	Eicosapentaenoic acid (EPA) Docosahexaenoic acid (DHA)	
<b>Amino acids</b>	Arginine	Histidine	Isoleucine
	Cystine	Tyrosine	Lysine
	Phenylalanine	Threonine	Tryptophan
	Leucine	Methionine	Valine
<b>Minerals</b>	Calcium	Phosphorus	Potassium
	Sodium	Copper	Iron
	Chloride	Magnesium	Iodine
	Manganese	Zinc	Selenium
<b>Vitamins</b>	Vitamin A	Vitamin D	Vitamin E
	Thiamine	Riboflavin	Pantothenic acid
	Niacin	Vitamin B6 (Pyridoxine)	Biotin
	Cobalamin	Folic acid	Vitamin K
<b>Vitamin-like substances</b>	Taurine ( <b>cats</b> )	Choline	
<b>Remarks</b>	See section on analytical method pp. for the appropriate method and other details.		
	Routine analysis for energy calculation includes moisture, crude protein, crude fat, crude ash, crude fibre (Weende analysis)		

### 1.5 Repeat analyses

Once a product has been passed and the formula remains essentially unchanged, there is no need for further analysis. However, bearing in mind the fluctuations in raw materials, it is recommended that regular analyses are conducted to make sure that the product still meets the appropriate nutritional standards and / or truly satisfies its claim of belonging to a family. The frequency of testing is the responsibility of the manufacturer.

If the manufacturer makes a major change in the formulation or processing, complete re-analysis is recommended / **required (QR)**.

### 1.6 Directions for use / feeding instructions

The manufacturer is required to provide, as part of the statutory statement, directions for the proper use of a pet food indicating the purpose for which it is intended. The **feeding instructions** should be clear and complete, and give an indication of the daily amounts to be fed. Feeding instructions could also provide information about the frequency of feeding and

possible need to adapt the amount according to activity. ANNEX 1 p. 42 can be used as basis for the feeding instructions.

**See also FEDIAF's "Guide to Good Practice for Communication on Pet Food"**

**2 Tables of Nutrient Recommendations**

**- How to read the tables -**

Values are expressed as follows: recommended value [nutritional maximum]

The legal maximum levels of nutrients are listed in a separate column (right column), because they apply to all life stages **(Reference? 1831/2003)**

For commercial dog and cat foods it is recommended that the nutrient levels are at or above the levels listed in the tables and do not exceed the nutritional or legal maximum. **If the protein digestibility of  $\geq 80\%$  mentioned in the scope on page 6 cannot be guaranteed, it is recommended to increase the essential amino acid values by a minimum of 10%.**

An asterix (\*) indicates that there is further information in the substantiation section which follows the nutrient recommendations

The nutritional tables provide nutrient allowances in "units/100 g dry matter (DM)", "units/1000 kcal ME" and "units/MJ ME".

**Conversion factors:**

units/1000kcal	←	x 2.5	units/100g DM	x 0.598	→	Units/MJ
units/100g DM	←	x 0.4	units/1000kcal	x 0.239	→	Units/MJ
units/100g DM	←	x 1.6736	Units/MJ	x 4.184	→	units/1000kcal

These conversions assume an energy density of 16.7kJ (4.0kcal) ME/g DM. For foods with energy densities different from this value, the recommendations should be corrected for energy density.

Specific recommendations for nutrient intake during reproduction are only available for a few nutrients. Hence, until more data become available, recommendations in the tables combine early growth and reproduction for dogs, and growth and reproduction for cats. Where there are proven differences between the two life stages both values are stated. They are declared as follows: **value for growth / value for reproduction.**

**TABLE A<sub>1,2,3</sub> – Nutrient Recommendations for Dogs**

A <sub>1</sub>	Nutrient recommendations for dogs: unit per 100g of dry matter (DM)
A <sub>2</sub>	Nutrient recommendations for dogs: unit per 1000 kcal of metabolizable energy (ME)
A <sub>3</sub>	Nutrient recommendations for dogs: unit per MJ of metabolizable energy (ME)

**TABLE B<sub>1,2,3</sub> – Nutrient Recommendations for Cats**

B <sub>1</sub>	Nutrient recommendations for cats: unit per 100 of dry matter (DM)
B <sub>2</sub>	Nutrient recommendations for cats: unit per 1000 kcal of metabolizable energy (ME)
B <sub>3</sub>	Nutrient recommendations for cats: unit per MJ of metabolizable energy (ME)

TABLE A<sub>1</sub> Nutrient Recommendations for Dogs – Unit per 100 g dry matter

Nutrient	UNIT	Adult	Early Growth ( < 14 weeks ) & Reproduction	Late Growth ( ≥ 14 weeks )	Legal maximum
Recommendation [nutritional maximum]					
<b>Protein*</b>	<b>g</b>	<b>18.0</b>	<b>25.0</b>	<b>20.0</b>	-
Arginine*	g	0.52	0.82	0.69	-
Histidine	g	0.23	0.39	0.25	-
Isoleucine	g	0.46	0.65	0.50	-
Leucine	g	0.82	1.29	0.80	-
Lysine*	g	0.42	0.88 [max. 2.80]	0.70 [max. 2.80]	-
Methionine*	g	0.31	0.35	0.26	-
Methionine-cystine*	g	0.62	0.70	0.53	-
Phenylalanine	g	0.54	0.65	0.50	-
Phenylalanine-tyrosine*	g	0.89	1.30	1.00	-
Threonine	g	0.52	0.81	0.64	-
Tryptophan	g	0.17	0.23	0.21	-
Valine	g	0.59	0.68	0.56	-
<b>Fat*</b>	<b>g</b>	<b>5.5</b>	<b>8.50</b>	<b>8.50</b>	-
Linoleic acid n-6*	g	1.32	1.30 [max. 6.50]	1.30	-
Arachidonic acid	mg	-	30.0	30.0	-
Alpha-linolenic acid*	g	-	0.08	0.08	-
EPA + DHA*	g	-	0.05	0.05	-
<b>Minerals</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>
Calcium*	g	0.50 [2.5]	1.00 [max. 1.6]	0.80 [max. 1.8]	-
Phosphorus	g	0.40 [1.60]	0.90	0.70	-
Ca / P ratio		1/1 – 2/1	1/1 – 1.6/1	1/1 – 1.8/1	-
Potassium	g	0.50	0.44	0.60	-
Sodium*	g	0.10 [max. 1.8]	0.22	0.22	-
Chloride	g	0.15 [max. 2.25]	0.29	0.33	-
Magnesium	g	0.07	0.04	0.04	-
<b>Trace elements*</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>
Copper*	mg	0.72	1.10	1.10	2.8
Iodine*	mg	0.11	0.15	0.15	1.1
Iron*	mg	3.60	8.80	8.80	142
Manganese	mg	0.58	0.56	0.56	17.0
Selenium*	µg	30.0	35.0	35.0	56.8
Zinc*	mg	7.2	10.0 [max. 100]	10.0 [max. 100]	28.4
<b>Vitamins</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>
Vitamin A*	IU	500 [max. 40,000]	500 [max. 40,000]	500 [max. 40,000]	-
Vitamin D*	IU	50.0 [max. 320]	55.2 [max. 320]	50.0 [max. 320]	227
Vitamin E*	IU	3.60	5.00	5.00	-
Thiamine	mg	0.23	0.14	0.14	-
Riboflavin*	mg	0.60	0.53	0.53	-
Pantothenic acid	mg	1.00	1.50	1.50	-
Vitamin B6 (Pyridoxine)	mg	0.15	0.15	0.15	-
Vitamin B12	µg	2.20	3.50	3.50	-
Niacin	mg	1.10	1.70	1.70	-
Folic acid	µg	18.0	27.0	27.0	-
Biotin*	µg	-	-	-	-
Choline	mg	120	170	170	-
Vitamin K*	µg	-	-	-	-

TABLE A <sub>2</sub> Nutrient Recommendations for Dogs – Unit per 1000 kcal of metabolizable energy (ME)					
Nutrient	UNIT	Adult	Early Growth (< 14 weeks) & Reproduction	Late Growth (≥ 14 weeks)	Legal maximum
<b>Protein*</b>	<b>g</b>	<b>45.0</b>	<b>62.5</b>	<b>50.0</b>	-
Arginine*	g	1.30	2.05	1.73	-
Histidine	g	0.58	0.98	0.63	-
Isoleucine	g	1.15	1.63	1.25	-
Leucine	g	2.05	3.23	2.00	-
Lysine*	g	1.05	2.20 [max. 7.0]	1.75 [max. 7.0]	-
Methionine*	g	0.78	0.88	0.65	-
Methionine-cystine*	g	1.55	1.75	1.33	-
Phenylalanine	g	1.35	1.63	1.25	-
Phenylalanine-tyrosine*	g	2.23	3.25	2.50	-
Threonine	g	1.30	2.03	1.60	-
Tryptophan	g	0.43	0.58	0.53	-
Valine	g	1.48	1.70	1.40	-
<b>Fat*</b>	<b>g</b>	<b>13.75</b>	<b>21.25</b>	<b>21.25</b>	-
Linoleic acid n-6*	g	3.30	3.25 [max. 16.25]	3.25	-
Arachidonic acid	mg	-	75.0	75.0	-
Alpha-linolenic acid*	g	-	0.20	0.20	-
EPA + DHA*	g	-	0.13	0.13	-
<b>Minerals</b>	-	-	-	-	-
Calcium*	g	1.25 [max. 6.25]	2.50 [max. 4.0]	2.00 [max. 4.5]	-
Phosphorus	g	1.00 [max. 4.00]	2.25	1.75	-
Ca / P ratio		1/1 – 2/1	1/1 – 1.6/1	1/1 – 1.8/1	-
Potassium	g	1.25	1.10	1.50	-
Sodium*	g	0.25 [max. 4.5]	0.55	0.55	-
Chloride	g	0.38	0.73	0.83	-
Magnesium	g	0.18	0.10	0.10	-
<b>Trace elements*</b>	-	-	-	-	-
Copper*	mg	1.80	2.75	2.75	7.1
Iodine*	mg	0.26	0.38	0.38	2.8
Iron*	mg	9.00	22.0	22.0	355
Manganese	mg	1.44	1.40	1.40	42.6
Selenium*	µg	75.0	87.5	87.5	142
Zinc*	mg	18.0	25.0 [max. 250]	25.0 [max. 250]	71.0
<b>Vitamins</b>	-	-	-	-	-
Vitamin A*	IU	1250 [max. 100,000]	1250 [max. 100,000]	1250 [max. 100,000]	-
Vitamin D*	IU	125 [max. 800]	138 [max. 800]	125 [max. 800]	568
Vitamin E*	IU	9.00	12.50	12.5	-
Thiamine	mg	0.56	0.35	0.35	-
Riboflavin*	mg	1.50	1.31	1.31	-
Pantothenic acid	mg	2.50	3.75	3.75	-
Vitamin B6 (Pyridoxine)	mg	0.38	0.38	0.38	-
Vitamin B12	µg	5.50	8.75	8.75	-
Niacin	mg	2.75	4.25	4.25	-
Folic acid	µg	45.0	67.5	67.5	-
Biotin*	µg	-	-	-	-
Choline	mg	300	425	425	-
Vitamin K*	µg	-	-	-	-

**TABLE A<sub>3</sub> Nutrient Recommendations for Dogs – Unit per MJ of metabolizable energy (ME)**

Nutrient	UNIT	Adult	Early Growth (< 14 weeks) & Reproduction	Late Growth (≥ 14 weeks)	Legal maximum
<b>Protein*</b>	<b>g</b>	<b>10.76</b>	<b>14.94</b>	<b>11.95</b>	-
Arginine*	g	0.31	0.49	0.41	-
Histidine	g	0.14	0.23	0.15	-
Isoleucine	g	0.27	0.39	0.30	-
Leucine	g	0.49	0.77	0.48	-
Lysine*	g	0.25	0.53 [max. 1.67]	0.42 [max. 1.67]	-
Methionine*	g	0.19	0.21	0.16	-
Methionine-cystine*	g	0.37	0.42	0.32	-
Phenylalanine	g	0.32	0.39	0.30	-
Phenylalanine-tyrosine*	g	0.53	0.78	0.60	-
Threonine	g	0.31	0.48	0.38	-
Tryptophan	g	0.10	0.14	0.13	-
Valine	g	0.35	0.41	0.33	-
<b>Fat*</b>	<b>g</b>	<b>3.29</b>	<b>5.08</b>	<b>5.08</b>	-
Linoleic acid n-6*	g	0.79	0.78 [max. 3.88]	0.78	-
Arachidonic acid	mg	-	17.9	17.9	-
Alpha-linolenic acid*	g	-	0.05	0.05	-
EPA + DHA*	g	-	0.03	0.03	-
<b>Minerals</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>
Calcium*	g	0.30 [max. 1.49]	0.60 [max. 0.96]	0.48 [max. 1.08]	-
Phosphorus	g	0.24 [max. 0.96]	0.54	0.42	-
Ca / P ratio		1/1 – 2/1	1/1 – 1.6/1	1/1 – 1.8/1	-
Potassium	g	0.30	0.26	0.36	-
Sodium*	g	0.06 [max. 1.08]	0.13	0.13	-
Chloride	g	0.09	0.17	0.20	-
Magnesium	g	0.04	0.02	0.02	-
<b>Trace elements*</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>
Copper*	mg	0.43	0.66	0.66	1.7
Iodine*	mg	0.06	0.09	0.09	0.68
Iron*	mg	2.15	5.26	5.26	84.9
Manganese	mg	0.34	0.33	0.33	10.2
Selenium*	µg	17.9	20.9	20.9	33.9
Zinc*	mg	4.30	5.98 [max. 60]	5.98	17.0
<b>Vitamins</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>
Vitamin A*	IU	299 [max. 23,900]	299 [max. 23,900]	299 [max. 23,900]	-
Vitamin D*	IU	29.9 [max. 191]	33.0 [max. 191]	29.9 [max. 191]	136
Vitamin E*	IU	2.20	1.80	3.00	-
Thiamine	mg	0.13	0.08	0.08	-
Riboflavin*	mg	0.36	0.31	0.31	-
Pantothenic acid	mg	0.60	0.90	0.90	-
Vitamin B6 (Pyridoxine)	mg	0.09	0.09	0.09	-
Vitamin B12	µg	1.31	2.09	2.09	-
Niacin	mg	0.66	1.02	1.02	-
Folic acid	µg	10.8	16.1	16.1	-
Biotin*	µg	-	-	-	-
Choline	mg	71.7	102	102	-
Vitamin K*	µg	-	-	-	-

TABLE B<sub>1</sub> Nutrient Recommendations for Cats – Unit per 100 g dry matter

Nutrient	UNIT	Adult	Growth and / Reproduction*	Legal maximum
Recommendation [nutritional maximum]				
<b>Protein</b>	<b>g</b>	<b>25.0</b>	<b>28.0 / 30.0</b>	
Arginine*	g	1.00	1.07 / 1.11 [max. kittens 3.5]	
Histidine	g	0.30	0.33	
Isoleucine	g	0.49	0.54	
Leucine	g	1.17	1.28	
Lysine*	g	0.39	0.85	
Methionine*	g	0.20	0.44 [max. kittens 1.3]	
Methionine-cystine*	g	0.39	0.88	
Phenylalanine	g	0.46	0.50	
Phenylalanine-tyrosine*	g	1.76	1.91	
Threonine	g	0.60	0.65	
Tryptophan*	g	0.15	0.16 [max. kittens 1.7]	
Valine	g	0.59	0.64	
Taurine (canned pet food)*	g	0.20	0.25	
Taurine (dry pet food)*	g	0.10	0.10	
<b>Fat</b>	<b>g</b>	<b>9.0</b>	<b>9.0</b>	
Linoleic acid n-6	g	0.50	0.55	
Arachidonic acid	mg	6.00	20.0	
Alpha-linolenic acid	g	-	0.02	
EPA + DHA*	g	-	0.01	
<b>Minerals</b>				
Calcium*	g	0.59	1.00	
Phosphorus	g	0.50	0.84	
Ca / P ratio*		0.65 – 2.0	0.65 – 1.5	
Potassium	g	0.60	0.60	
Sodium*	g	0.08 [max. 1.8]	0.16	
Chloride	g	0.11	0.24	
Magnesium*	g	0.04	0.05	
<b>Trace elements*</b>				
Copper*	mg	0.50	1.00	2.8
Iodine*	mg	0.06	0.18	1.1
Iron	mg	8.00	8.00	142
Manganese	mg	0.50	1.00	17.0
Selenium	µg	30.0	30.0	56.8
Zinc	mg	7.50 [max. 60.0]	7.50	28.4
<b>Vitamins</b>				
Vitamin A*	IU	333 [max 40,000]	900 [max 40,000/33,333]	
Vitamin D*	IU	25.0 [max.3,000]	75.0 [max.3,000]	227
Vitamin E*	IU	3.80	3.80	
Thiamine	mg	0.56	0.55	
Riboflavin	mg	0.40	0.40	
Pantothenic acid	mg	0.58	0.57	
Vitamin B6 (Pyridoxine)*	mg	0.25	0.40	
Vitamin B12	µg	2.25	2.00	
Niacin	mg	4.00	4.00	
Folic acid	µg	80.0	80.0	
Biotin*	µg	7.50	7.00	
Choline	mg	240	240	
Vitamin K*	µg	10.0	10.0	

**TABLE B<sub>2</sub> Nutrient Recommendations for Cats – Unit per 1000 kcal of metabolizable energy (ME)**

Nutrient	UNIT	Adult	Growth and Reproduction	Legal maximum
<b>Protein</b>	<b>g</b>	<b>62.5</b>	<b>70.0/75.0</b>	
Arginine*	g	2.50	2.68/2.78 [max. kittens 8.75]	
Histidine	g	0.75	0.83	
Isoleucine	g	1.24	1.35	
Leucine	g	2.93	3.20	
Lysine*	g	0.98	2.13	
Methionine*	g	0.49	1.10 [max. kittens 3.25]	
Methionine-cystine*	g	0.98	2.20	
Phenylalanine	g	1.15	1.25	
Phenylalanine-tyrosine*	g	4.40	4.78	
Threonine	g	1.50	1.63	
Tryptophan*	g	0.37	0.40 [max. kittens 4.25]	
Valine	g	1.47	1.60	
Taurine (canned pet food)*	g	0.50	0.63	
Taurine (dry pet food)*	g	0.25	0.25	
<b>Fat</b>	<b>g</b>	<b>22.5</b>	<b>22.5</b>	
Linoleic acid n-6	g	1.25	1.38	
Arachidonic acid	mg	15.0	50.0	
Alpha-linolenic acid	g	-	0.05	
EPA + DHA*	g	-	0.03	
<b>Minerals</b>				
Calcium*	g	1.48	2.50	
Phosphorus	g	1.25	2.10	
Ca / P ratio*		0.65 – 2	0.65 – 1.5	
Potassium	g	1.50	1.50	
Sodium*	g	0.19 [max 4.5]	0.40	
Chloride	g	0.29	<b>0.60</b>	
Magnesium*	g	0.10	0.13	
<b>Trace elements*</b>				
Copper*	mg	1.25	2.50	7.1
Iodine*	mg	0.15	0.45	2.8
Iron	mg	20.0	20.0	355
Manganese	mg	1.25	2.50	42.6
Selenium	µg	75.0	75.0	142
Zinc	mg	18.8 [max 150]	18.8	71.0
<b>Vitamins</b>	<b>-</b>	<b>-</b>	<b>-</b>	
Vitamin A*	IU	833 [max 100,000]	2250 [max 100,000/83,325]	
Vitamin D*	IU	62.5 [max 7,500]	188 [max 7,500]	568
Vitamin E*	IU	9.50	9.50	
Thiamine	mg	1.40	1.38	
Riboflavin	mg	1.00	1.00	
Pantothenic acid	mg	1.44	1.43	
Vitamin B6 (Pyridoxine)*	mg	0.63	1.00	
Vitamin B12	µg	5.63	5.00	
Niacin	mg	10.0	10.0	
Folic acid	µg	200	200	
Biotin*	µg	18.8	17.5	
Choline	mg	600	600	
Vitamin K*	µg	25.0	25.0	

**TABLE B<sub>3</sub> Nutrient Recommendations for Cats – Unit per MJ of metabolizable energy (ME)**

Nutrient	UNIT	Adult	Growth / Reproduction	Legal maximum
<b>Protein</b>	<b>g</b>	<b>14.94</b>	<b>16.73 / 17.93</b>	
Arginine*	g	0.60	0.64/0.66 [max. kittens 2.09]	
Histidine	g	0.18	0.20	
Isoleucine	g	0.30	0.32	
Leucine	g	0.70	0.76	
Lysine*	g	0.23	0.51	
Methionine*	g	0.12	0.26 [max. kittens 0.78]	
Methionine-cystine*	g	0.23	0.53	
Phenylalanine	g	0.27	0.30	
Phenylalanine-tyrosine*	g	1.05	1.14	
Threonine	g	0.36	0.39	
Tryptophan*	g	0.09	0.10 [max. kittens 1.02]	
Valine	g	0.35	0.38	
Taurine (canned pet food)*	g	0.12	0.15	
Taurine (dry pet food)*	g	0.06	0.06	
<b>Fat</b>	<b>g</b>	<b>5.38</b>	<b>5.38</b>	
Linoleic acid n-6	g	0.30	0.33	
Arachidonic acid	mg	3.59	11.95	
Alpha-linolenic acid	g	-	0.01	
EPA + DHA*	g	-	0.01	
<b>Minerals</b>				
Calcium*	g	0.35	0.60	
Phosphorus	g	0.30	0.50	
Ca / P ratio*		0.65 – 2	0.65 – 1.5	
Potassium	g	0.36	0.36	
Sodium*	g	0.05 [max 1.08]	0.10	
Chloride	g	0.07	0.14	
Magnesium*	g	0.02	0.03	
<b>Trace elements*</b>				
Copper*	mg	0.30	0.60	1.7
Iodine*	mg	0.04	0.11	0.68
Iron	mg	4.78	4.78	84.9
Manganese	mg	0.30	0.60	10.2
Selenium	µg	17.9	17.9	33.9
Zinc	mg	4.48 [max 35.9]	4.48	17.0
<b>Vitamins</b>				
Vitamin A*	IU	199 [max 23,901]	538 [max 23,901/19,917]	
Vitamin D*	IU	14.9 [max 1,793]	44.8 [max 1,793]	136
Vitamin E*	IU	2.30	2.30	
Thiamine	mg	0.33	0.33	
Riboflavin	mg	0.24	0.24	
Pantothenic acid	mg	0.34	0.34	
Vitamin B6 (Pyridoxine)*	mg	0.15	0.24	
Vitamin B12	µg	1.34	1.20	
Niacin	mg	2.39	2.39	
Folic acid	µg	47.8	47.8	
Biotin*	µg	4.48	4.18	
Choline	mg	143	143	
Vitamin K*	µg	5.98	5.98	

### III – COMPLETE PET FOOD (cont.)

#### 3 Substantiation of nutrient recommendations' tables

The following section provides substantiation and explanation for the recommended allowances (RA) (nutrient recommendations) for dogs and cats in the previous tables. These recommendations are based on scientific publications, NRC 2006 and data from the pet food industry.

**TABLE A<sub>4</sub> – Substantiation of nutrient recommendations for dogs**

GENERAL			
1	Amino acids, trace elements, vitamins (Adult dogs)	Unless indicated with an * and substantiated hereafter, the values recommended for adult dogs are the levels recommended by NRC 2006 increased by 20% to compensate for the lower energy requirement of household dogs (see Annex I, pp. 42) compared to the energy intake assumed by NRC.	<sup>a</sup> NRC Chapter 15. Nutrient Requirements and Dietary Nutrient Concentrations. In: Nutrient Requirements of Dogs and Cats. The National Academic Press, Washington, DC. 2006: pp. 359-360, table 15-4.
PROTEIN			
Total protein			
1	Total protein (Adult dogs)	NRC-2006 RA of 25g/1000kcal for adult dogs is based on Sanderson <i>et al.</i> (2001) <sup>a</sup> . However the diet in this study had a high protein digestibility and the energy intake was around 130kcal/KgBW <sup>0.75</sup> . FEDIAF has adjusted the protein value to take into account a digestibility of 75% and added a further 20% to account for lower energy intakes for pet dogs, giving a FEDIAF RA of 40g/1000kcal. This value has been increased to 45g/1000kcal to cover requirements of older dogs <sup>b,c,d</sup> . This is equivalent to 18g per 100g DM (10.8 g/MJ). If formulating below 18g protein/100g it is particularly important to ensure that the amino acid profile meets FEDIAF guidelines for adult maintenance.	<sup>a</sup> Sanderson SL, Gross KL, Ogburn PN, <i>et al.</i> (2001) Effects of dietary fat and L-carnitine on plasma and whole blood taurine concentrations and cardiac function in healthy dogs fed protein-restricted diets. <i>Am. J. Vet. Res.</i> 62: 1616-1623.  <sup>b</sup> Williams CC, Cummins KA, Hayek MG, Davenport GM. Effects of dietary protein on whole-body protein turnover and endocrine function in young-adult and aging dogs. <i>J. Anim. Sci.</i> 2001; 79: 3128-3136.  <sup>c</sup> Finco DR, Brown SA, Crowell WA, <i>et al.</i> Effects of aging and dietary protein intake on uninephrectomized geriatric dogs. <i>Am. J. Vet. Res.</i> 1994; 55: 1282-1290.
2	Total protein	The recommendation for protein assumes the	<sup>a</sup> Romsos DR, Palmer HJ, Muiruri KL, <i>et</i>

	(Reproduction)	diet contains some carbohydrate to decrease the risk of hypoglycaemia in the bitch and neonatal mortality. If carbohydrate is absent or at a very low level, the protein requirement is much higher, and may be double. <sup>a, b, c</sup>	<p><sup>a</sup> Influence of a low carbohydrate diet on performance of pregnant and lactating dogs. <i>J. Nutr.</i> 1981; 111: 678-689.</p> <p><sup>b</sup> Kienzle E, Meyer H, Lorie H. Einfluß kohlenhydratfreier Rationen mit unterschiedlichen Protein/Energierelationen auf foetale Entwicklung und Vitalität von Welpen sowie die Milchezusammensetzung von Hündinnen. <i>Fortschnitte in der Tierphysiologie und Tierernährung.</i> 1985; Suppl. 16: 73-99.</p> <p><sup>c</sup> Kienzle E, Meyer H. The effects of carbohydrate-free diets containing different levels of protein on reproduction in the bitch. In: <i>Nutrition of the dog and cat.</i> Burger IH, Rivers JPW edits. Cambridge University Press Cambridge, UK. 1989: pp. 229-242.</p>
3	Total protein (Growth)	For practical foods made from cereals and various animal by-products, the crude protein level needed for maximum nitrogen retention appears to be about 25 per cent dry matter for newly weaned puppies, whereas for puppies over 14 weeks of age it is 20 per cent dry matter. <sup>a</sup>	<p><sup>a</sup> NRC. Nitrogen (Crude Protein) minimum requirements, recommended allowances, and adequate intakes In: <i>Nutrient Requirements of Dogs and Cats.</i> The National Academic Press, Washington, DC. 2006: pp. 116-120.</p>

### Arginine

1	Arginine (All life stages)	The arginine requirement increases with increased protein content owing to its role as an intermediate in the urea cycle. For every gram of crude protein above the stated values, an additional 0.01g of arginine is required <sup>a</sup> . See ANNEX XX p. YY	<p><sup>a</sup> NRC Chapter 15. Nutrient Requirements and Dietary Nutrient Concentrations. In: <i>Nutrient Requirements of Dogs and Cats.</i> The National Academic Press, Washington, DC. 2006: pp. 357-363 tables 15-3, 15-5 and 15-8.</p>
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### Lysine

1	Lysine (nutritional maximum puppies) for	Czarnecki <i>et al.</i> (1985) <sup>a</sup> showed that excess dietary lysine (4.91% DM [basal diet 0.91% + 4% from a supplement]) decreases weight gain in puppies but not 2.91 % DM (basal diet + 2% from a supplement). It was concluded that the highest no-effect-level of lysine for puppies was 2.91% DM (energy density 4156 kcal/kg). This is equivalent to 7.0 g/1000 kcal or 2.8% DM (at 4 kcal/g DM) and this is therefore the FEDIAF maximum for puppy growth.	<p><sup>a</sup> Czarnecki GL, Hirakawa DA, Baker DH. (1985) Antagonism of arginine by excess dietary lysine in the growing dog. <i>J. Nutr.</i> 1985; 1115: 743-752.</p>
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### Methionine-cystine

1	Methionine-cystine (Adult dogs)	The recommended values are based on a dog food containing a very low taurine content, i.e. <100 mg/kg dry matter <sup>a</sup> . For products containing higher levels of taurine the RA for	<p><sup>a</sup> Sanderson SL, Gross KL, Ogburn PN, <i>et al.</i> (2001) Effects of dietary fat and L-carnitine on plasma and whole blood taurine concentrations and cardiac function in healthy dogs fed protein-restricted diets. <i>Am. J. Vet. Res.</i> 62:</p>
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		sulphur amino acids can be lower than the values quoted in the table. For further information see taurine section on page XX.	1616-1623.
2	Methionine	In the case of lamb and rice foods, the methionine level may have to be increased. <sup>a</sup>	<sup>a</sup> For details and references see annex II - taurine <a href="#">p. 44</a>

### Tyrosine

1	Tyrosine (All life stages)	For maximisation of black hair colour, the tyrosine content may need to be 1.5 to 2 times higher than the amount stated <sup>a,b</sup> .	<p><sup>a</sup> NRC Chapter 15. Nutrient Requirements and Dietary Nutrient Concentrations. In: Nutrient Requirements of Dogs and Cats. The National Academic Press, Washington, DC. 2006: pp. 357-363 tables 15-3, 15-5 and 15-8.</p> <p><sup>b</sup> Biourge V., and R. Sergheraert (2002). Hair pigmentation can be affected diet in dogs. Proc. Comp. Nutr. Soc. Number 4, Kirk-Baer, C.L., 103-104.</p>
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### FAT

#### Total fat

1	Total fat (All life stages)	Dogs fed foods containing normal levels of protein tolerate very high levels of fat (e.g. sled dogs). However very high fat foods with very low protein content have been linked with adverse effects in dogs. <sup>a</sup>	<sup>a</sup> Lindsay S, Entenman C, Chaikoff IL. Pancreatitis accompanying hepatic disease in dogs fed a high fat, low protein diet. Arch. Path. 1948; 45: 635-638.
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#### Omega 3 and 6 fatty acids

1	Omega-3 and Omega-6 poly-unsaturated long chain fatty acids (Growth & Reproduction)	<p>During gestation and early life after birth, DHA and arachidonic acid (AA) are selectively accumulated within the brain and retina. <sup>f</sup> Supplementation with <math>\alpha</math>-linolenic acid (ALA) and linoleic acid during gestation and lactation is an ineffective means of increasing the milk content of DHA and AA respectively <sup>a</sup>. Although very young puppies have the capacity to convert some ALA into DHA, after weaning puppies lose this capacity. <sup>c</sup></p> <p>Moreover, electroretinograms have revealed improved vision in puppies from mothers fed n-3 long chain poly-unsaturated fatty acids and fed the same food after weaning. <sup>b,d,e</sup> Consequently it is preferable to have small amounts of DHA and/or EPA, as well as AA in foods for growth and reproduction to supply enough for neonatal nutritional modifications.</p>	<p><sup>a</sup> Bauer JE, Heinemann KM, Bigley KE, <i>et al.</i> Maternal diet alpha-linolenic acid during gestation and lactation does not increase docosahexaenoic acid in canine milk. J. Nutr. 2004; 134 (8S): 2035S-2038S.</p> <p><sup>b</sup> Bauer J, Heinemann KM, Lees GE, Waldron MK. Retinal functions of young dogs are improved and maternal plasma phospholipids are altered with diets containing long-chain n-3 PUFA during gestation, lactation and after weaning. J. Nutr. 136: 1991S-1994S, 2006.</p> <p><sup>c</sup> Bauer JE, Heinemann KM, Lees GE, Waldron MK. Docosahexaenoic acid accumulates in plasma of canine puppies raised on <math>\alpha</math>-linolenic acid-rich milk during suckling but not when fed <math>\alpha</math>-linolenic acid-rich diets after weaning. J. Nutr. 2006; 136: 2087S-2089S.</p> <p><sup>d</sup> Heinemann KM, Waldron MK, Bigley KE, <i>et al.</i> Long-Chain (n-3) Polyunsaturated fatty acids are more efficient than <math>\alpha</math>-linolenic acid in improving electroretinogram responses of puppies exposed during gestation, lactation, and weaning. J. Nutr. 2005; 135: 1960-1966.</p> <p><sup>e</sup> Heinemann KM, Waldron MK, Bigley KE, Bauer JE. Improvement of retinal function in canine puppies from mothers</p>
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			<p>fed dietary long chain n-3 polyunsaturated fatty acids during gestation and lactation. J Vet Int Med 2005; 19 (3): 442-443, Abstr. 155.</p> <p><sup>f</sup> Heinemann KM, Bauer JE. Timely Topics in Nutrition - Docosahexaenoic acid and neurologic development in animals. J. Am Vet Med Assoc 2006; 228 (5): 700-705.</p>
2	Omega 3 fatty acids (Adult dogs)	Although there is increasing evidence of beneficial effects of omega-3 fatty acids, the current information is insufficient to recommend a <i>specific</i> level of omega-3 fatty acids for adult dogs.	NRC 2006
3	Omega 3 vs. 6 FA (Adult dogs)	The effects of omega-3 fatty acids depend on the level as well as on the ratio of omega-6 to omega-3 fatty acids. Very high levels of long chain omega-3 fatty acids can decrease cellular immunity, particularly in the presence of a low level of omega-6 fatty acids <sup>a, b</sup> .	<p><sup>a</sup> Hall JA, Wander RC, Gradin, Jewell DE. Effect of dietary n-6 to n-3 fatty acid ratio on complete blood and total white blood cell counts, and T-cell subpopulations in aged dogs. Am. J. Vet. Res. 1999; 60 (3): 319-327.</p> <p><sup>b</sup> Wander RC, Hall JA, Gradin JL, <i>et al.</i> The ratio of dietary (n-6) to (n-3) fatty acids influences immune system function, eicosanoid metabolism, lipid peroxidation and vitamin E in aged dogs. J Nutr 1997; 127: 1198-1997.</p>

## MINERALS

### Calcium

1	Calcium (Adult dogs)	As the calcium level approaches the stated nutritional maximum, it may be necessary to increase the levels of certain trace elements such as zinc and copper.	-
2	Calcium (RA for puppies)	A calcium level of 0.8g/100gDM has been shown to be adequate for growing dogs <sup>a-c, f</sup> . However, this level has been reported to be marginal for some breeds <sup>d,e</sup> particularly during the fast growing phase (particularly breeds with lower energy requirements). Therefore FEDIAF recommends a level of 1.0g/100g DM for early growth and 0.8g/100g DM for later growth.	<p><sup>a</sup> Jenkins KJ, Phillips PH. The Mineral Requirements of the Dog I. Phosphorus Requirement and Availability. J. Nutr. 1960; 70: 235-240.</p> <p><sup>b</sup> Jenkins KJ, Phillips PH. The Mineral Requirements of the Dog II. The Relation of Calcium, Phosphorus and Fat Levels to Minimal Calcium and Phosphorus Requirements. J. Nutr. 1960; 70: 241-246.</p> <p><sup>c</sup> Goodman SA, Montgomery RD, Fitch RB <i>et al.</i> Serial orthopaedic examinations of growing great Dane puppies fed three diets varying in calcium and phosphorus. In: Recent advances in canine and feline nutrition. Vol 2. Iams Nutrition Symposium Proceedings. G. Reinhardt &amp; D. Carye edits. Wilmington, Ohio, Orange Frazer Press. 1998; pp. 3-12.</p> <p><sup>d</sup> Alexander JE, Moore MP, Wood LLH. Comparative growth studies in Labrador retrievers fed 5 commercial calorie-dense diets. Modern Veterinary practice 1988; 31: 144-148.</p> <p><sup>e</sup> Laflamme DP. Effect of breed size on calcium requirements for puppies.</p>

			<p>Supplement to Compendium on Continuing Education for the Practicing Veterinarian 2001; 23 (9A): 66-69.</p> <p><sup>f</sup> Lauten SD, Cox NR, Brawner WR, et al. Influence of dietary calcium and phosphorus content in a fixed ration on growth and development of Great Danes. Am J Vet Res. 2002; 63 (7): 1036-1047.</p>
3	Calcium (Maximum puppies) for	<p>High intake of calcium has an adverse effect on skeletal development in large breed dogs, particularly during the early growth phase. <sup>a,b</sup></p> <p>Therefore a strict nutritional maximum is recommended for foods intended for large breed puppies.</p> <p>Weber <i>et al.</i> showed that when feeding a balanced food, a calcium level of 1.6 % DM from 9 weeks of age does not cause side effects. <sup>c,d</sup></p> <p>During later growth up to 1.8% DM can be fed to all breed dogs including giant breeds with the exception of great Danes. This breed may be more susceptible and it is preferable to continue with a food containing a maximum calcium content of 1.6%. <sup>c,d,e</sup></p>	<p><sup>a</sup> Hazewinkel HAW. Influences of different calcium intakes on calcium metabolism and skeletal development in young Great Danes. Thesis Utrecht University, 1985.</p> <p><sup>b</sup> Schoenmakers I, Hazewinkel HAW, Voorhout G, <i>et al.</i> Effect of diets with different calcium and phosphorus contents on the skeletal development and blood chemistry of growing grate Danes. Vet Rec. 2000; 147: 652-660.</p> <p><sup>c</sup> Weber M, Martin L, Dumon H, <i>et al.</i> Growth and skeletal development in two large breeds fed 2 calcium levels. J. Vet Int. Med 2000; 14 (May/June): 388 Abstr. 243.</p> <p><sup>d</sup> Weber M, Martin L, Dumon H, <i>et al.</i> Calcium in growing dogs of large breed: a safety range? ESVCN Congress Amsterdam, April 2000, Abstr.</p> <p><sup>e</sup> Laflamme DP. Effect of breed size on calcium requirements for puppies. Supplement to Compendium on Continuing Education for the Practicing Veterinarian 2001; 23 (9A): 66-69.</p>
<b>Sodium</b>			
1	Sodium (Adult dogs)	<p>Studies in dogs have demonstrated that 45.4 mg / MJ (0.19g / 1000kcal) sodium is adequate for all life stages. <sup>a</sup></p>	<p><sup>a</sup> Czarniecki-Maulden <i>et al.</i>, (1989) J. A. Vet. Med. Assoc. 195, 583-590].</p>
2	Sodium (Adult dogs)	<p>Studies in dogs have demonstrated that foods containing 2% of sodium (DM) may result in a negative potassium balance. <sup>a</sup> It is reasonable to set the safe nutritional maximum at 1.8% DM. <sup>b</sup></p>	<p><sup>a</sup> Boemke W, Palm U, Kaczmarczyk G, Reinhardt HW Effect of high sodium and high water intake on 24 h-potassium balance in dogs. Z. Versuchstierkd. 1990; 33 (4): 179-185.</p> <p><sup>b</sup> Kienzle E. Personal communication.</p>
<b>TRACE ELEMENTS</b>			
<b>General</b>			
1	General	<p>Manufacturers are reminded that the bioavailability of trace-elements is reduced by a high content of certain minerals (e.g. calcium), the level of other trace elements (e.g. high zinc decreases copper absorption) and sources of phytic acid (e.g. some soy products).</p>	
<b>Copper</b>			
1	Copper (General)	<p>Owing to its low availability copper oxide should not be considered as a copper source.</p>	<p><sup>a</sup> Fascetti AJ, Morris JG, Rogers QR. Dietary copper influences reproductive efficiency of queens. J. Nutr 1998; 128:</p>

			2590S-2592S
<b>Iodine</b>			
1	Iodine	From studies by Castillo <i>et al.</i> <sup>a, b</sup> a low nutritional maximum for iodine in dogs (0.4mg/100gDM) was recommended. However in these studies puppies were significantly overfed (approx. 75% above energy requirement) which resulted in a substantially increased intake of iodine. Furthermore the food was deficient in a number of key nutrients, e.g. Ca, P and K, and therefore inappropriate for puppies. Consequently, these results are irrelevant for normal commercial nutritionally balanced foods, and the existing legal maximum is safe for all dogs.	<p><sup>a</sup> Castillo VA, Pisarev MA, Lalia JC, <i>et al.</i> Commercial diet induced hypothyroidism due to high iodine. A histological and radiological analysis. <i>Veterinary Quarterly</i> 2001; 23 (4): 218-223.</p> <p><sup>b</sup> Castillo VA, Lalia JC, Junco M, <i>et al.</i> Changes in thyroid function in puppies fed a high iodine commercial diet. <i>Veterinary Journal</i> 2001; 161 (1): 80-84.</p>
<b>Iron</b>			
1	Iron	Because of very poor availability, iron from oxide or carbonate salts that are added to the diet should not be considered sources contributing to the minimum nutrient level.	<sup>a</sup> NRC Absorption and bioavailability of dietary iron in dogs and cats. In: <i>Nutrient Requirements of Dogs and Cats</i> . The National Academic Press, Washington, DC. 2006: pp. 168-169.
<b>Selenium</b>			
1	Selenium (Adult dogs)	There are no data available about the exact requirements for selenium of adult dogs. However, according to experts the availability of and requirement for selenium in dogs are similar to those in the cat. <sup>a</sup> Therefore, the recommended allowance for cats is used for dogs until more information becomes available.	<sup>a</sup> Wedekind K. Personal communication.
<b>Zinc</b>			
1	Zinc (Growth)	A pet food containing 5 mg zinc per 100g DM is sufficient to meet the requirements for growing puppies	<sup>a</sup> Booles D, Burger IH, Whyte AL, <i>et al.</i> Effects of two levels of zinc intake on growth and trace element status in Labrador puppies. <i>J Nutr</i> 1991; 121: S79-S80.
<b>VITAMINS</b>			
<b>Vitamin A</b>			
1	Vitamin A	The FEDIAF maximum is based on the studies reported by Hathcock <i>et al.</i> , Goldy <i>et al.</i> and Cline <i>et al.</i> in adult dogs <sup>a,b,c</sup> . The value is 80% of the dose that Goldy <i>et al.</i> identified "as may be approaching a level that challenges the dog's ability to maintain normal vitamin A homeostasis" and about 45% of the no-adverse-effect intake established by Cline <i>et al.</i> over one year (no detrimental effects on bone health). Furthermore Hathcock <i>et al.</i> reported	<p><sup>a</sup> Hathcock JN, D. G. Hattan, M. Y. Jenkins, <i>et al.</i> Evaluation of vitamin A toxicity. <i>Am. J. Clin. Nutr.</i> 1990;52: 183-202.</p> <p><sup>b</sup> Goldy GG, Burr JR, Longardner CN <i>et al.</i> Effects of measured doses of vitamin A fed to healthy dogs for 26 weeks. <i>Veterinary Clinical Nutrition</i> 1996; 3 (2): 42-49</p> <p><sup>c</sup> Cline JL, Czarniecki-Maulden, Losonsky JM, <i>et al.</i> Effect of increasing</p>

		<p>an intake at least three times the FEDIAF nutritional maximum as safe in adult dogs fed for ten months (body growth and haematological indices unaffected). In view of these data the FEDIAF maximum is considered appropriate for all life stages.</p>	<p>dietary vitamin A on bone density in adult dogs. J. Anim. Sci. 1997; 75: 2980-2985.</p>
2	Vitamin A (Puppies)	<p>There is no evidence so far that the nutritional maximum for puppies should be different from the current nutritional maximum for adults. This value has been used in this guide for at least 10 years and has never given rise to any problems in growing dogs. Nevertheless, the industry is actively investigating whether puppies behave similarly to adult dogs with regard to vitamin A metabolism. <sup>a-d</sup></p>	<p><sup>a</sup> Schweigert FJ, Raila J, Haebel S. Vitamin A excreted in the urine of canines is associated with a Tamm-Horsfall like protein. Vet Res. 2002; 33: 299-311. <sup>b</sup> Schweigert FJ, Ryder OA, Rambeck WA, Zucker H. The majority of vitamin A is transported as retinyl esters in the blood of most carnivores. Comp. Biochem. Physiol. A 1990; 95, 573-578. <sup>c</sup> Schweigert FJ, Thomann E, Zucker H. Vitamin A in the urine of carnivores. Int. J. Vitam. Nutr. Res. 1991; 61, 110-113. <sup>d</sup> Schweigert FJ, Bok V. Vitamin A in blood plasma and urine of dogs is affected by the dietary level of vitamin A. Int J Vitam Nutr Res 2000; 70, 84-91.</p>

**Vitamin D**

1	Vitamin D	<p>Studies in great Dane puppies showed that a dietary vitamin D level of 435 IU/100g DM can affect Ca absorption and may stimulate endochondral ossification disturbances. <sup>a, b</sup> Therefore, 320 IU per 100g DM should be the nutritional maximum for growing giant breed dogs. <sup>c</sup> Based on differences in cholecalciferol metabolism between giant breed and small breed puppies <sup>b</sup>, 425 IU/100g DM can be considered a safe nutritional maximum for small breed puppies. Since there is no information on maximum safe intakes for adult dogs and breeding bitches. FEDIAF recommends the same nutritional maximum for other life stages as those indicated for puppies.</p>	<p><sup>a</sup> Tryfonidou MA, Stevenhagen JJ, van den Bemd GJCM, <i>et al.</i> Moderate cholecalciferol supplementation depresses intestinal calcium absorption in growing dogs. J. Nutr. 2002; 132: 2644-2650. <sup>b</sup> Tryfonidou MA, Holl MS, Vastenburg M, <i>et al.</i> Chapter 7. Moderate vitamin D<sub>3</sub> supplementation mildly disturbs the endochondral ossification in growing dogs. In: PhD Thesis Utrecht University 19 December 2002: pp. 110-122. <sup>c</sup> NRC. Vitamin D In: Nutrient Requirements of Dogs and Cats. The National Academic Press, Washington, DC. 2006: pp. 200-205 and tables 15-10, 15-12 and 15-14 pp. 357-363.</p>
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**Vitamin E**

1	Vitamin E	<p>Requirement depends on intake of polyunsaturated fatty acids (PUFA) and other antioxidants. A fivefold increase may be required under conditions of high PUFA intake.</p>	<p>Reference? Origin: Guidelines 2005</p>
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**Vitamin K**

1	Vitamin K	Vitamin K does not need to be added unless diet contains antimicrobial or anti-vitamin compounds. <sup>a,b</sup>	<sup>a</sup> NRC 2006 <sup>b</sup> Kronfeld DS. Vitamin K. in: Vitamin & mineral supplementation for dogs & cats - A monograph on micronutrients Veterinary Practice Publishing Company 1989: p. 95.
<b>Riboflavin</b>			
1	Riboflavin	Based on erythrocyte glutathione reductase activity coefficient (EGRAC) Cline <i>et al.</i> determined that the riboflavin requirement for the adult dog at maintenance is 66.8µg/kg BW per day, when feeding a semi-purified diet. <sup>a</sup> This corresponds with about 0.6 mg/100g DM for practical pet foods by including a safety margin of 25%.	<sup>a</sup> Cline JL, Odle J, Easter RA. The riboflavin requirement of adult dogs at maintenance is greater than previous estimates J Nutr. 1996 Apr; 126 (4):984-988
1	Biotin	For healthy dogs biotin does not need to be added to the food unless the food contains antimicrobial or anti-vitamin compounds. <sup>a, b</sup>	<sup>a</sup> Kronfeld DS, Biotin and Avidin. In vitamin & Mineral Supplementation for dogs and cats – A monograph on micronutrients Veterinary Practice Publishing Company 1989: 71-72. <sup>b</sup> Kronfeld DS, Biotin. In vitamin & Mineral Supplementation for dogs and cats – A monograph on micronutrients Veterinary Practice Publishing Company 1989: 99.

**TABLE B<sub>4</sub> – Substantiation of nutrient recommendations for cats**

<b>PROTEIN</b>			
1	Glutamate (Kittens)	The level of glutamate should not exceed 6 per cent dry matter in foods for kittens. <sup>a, b</sup>	<sup>a</sup> Deady JE, Anderson B, O'Donnell III JA, <i>et al.</i> Effects of level of dietary glutamic acid and thiamine on food intake, weight gain, plasma amino acids and thiamin status of growing kittens. J. Nutr. 1981; 111: 1568-1579. <sup>b</sup> Deady JE, Rogers QR, Morris JG. Effect of high dietary glutamic acid on the excretion of <sup>35</sup> S-thiamin in kittens. J. Nutr. 1981; 111: 1580-1585.
<b>Arginine</b>			
1	Arginine (All life stages)	The arginine requirement increases with increased protein content owing to its role as an intermediate in the urea cycle. For every gram of crude protein above the stated values, an additional 0.02g of arginine is required <sup>a</sup> .	<sup>a</sup> NRC Chapter 15. Nutrient Requirements and Dietary Nutrient Concentrations. In: Nutrient Requirements of Dogs and Cats. The National Academic Press, Washington, DC. 2006: pp. 357-363 tables 15-10, 15-12 and 15-14.
2	Arginine (Kittens)	Taylor (1995) found that 45 g/kg diet (470 kcal/100 g) was associated with a small	<sup>a</sup> Taylor TP. MS thesis Univ California, Davis, CA USA. 1995

		decrease in growth rate. NRC therefore sets a prudent maximum of 3.5 g/100 g DM (400 kcal/100 g). <sup>a</sup>	
<b>Lysine</b>			
1	Lysine (Adult cats)	The recommended values are based on a study by Burger and Smith <sup>a</sup> showing that adult cats need 0.16 g lysine per MJ ME to maintain a positive N-balance. After adding a safety margin of 20% this corresponds to 0.34% DM or 0.85g per 1000 kcal ME.	<sup>a</sup> Burger IH, Smith PH. Aminosäurenbedarf erwachsener Katzen. In: Ernährung, Fehlernährung, und Diätetik bei Hund und Katze – Proceedings of the International Symposium Hannover (DE), September 3-4, 1987: pp. 93-97.
<b>Methionine-cystine</b>			
1	Methionine-cystine (Adult cats)	The recommended values are based on a study by Burger and Smith <sup>a</sup> showing that adult cats need 0.16 g methionine (without cystine) per MJ ME to maintain a positive N-balance. After adding a safety margin of 20% this corresponds to 0.34% DM or 0.85g per 1000 kcal ME methionine + cystine.	<sup>a</sup> Burger IH, Smith PH. Aminosäurenbedarf erwachsener Katzen. In: Ernährung, Fehlernährung, und Diätetik bei Hund und Katze – Proceedings of the International Symposium Hannover (DE), September 3-4, 1987: pp. 93-97.
<b>Tryptophan</b>			
1	Tryptophan (kittens)	Taylor <i>et al.</i> (1998) fed 15 g/kg in a diet containing 450 kcal/100 g with no ill effects. <sup>a</sup> Herwill (1994) fed levels up to 60 g/kg in a diet containing 470 kcal/100 g. Twenty was satisfactory but food intake decreased at 40 g/kg; much more severe effects were observed at 60 g/kg. Therefore the maximum can be set at 2 g per 470 kcal or 1.7g per 100g DM (400 kcal/100g). <sup>b</sup>	<sup>a</sup> Taylor TP, <i>et al.</i> <a href="#">Amino Acids 1998; 15, 221-234.</a> <sup>b</sup> Herwill A. <a href="#">MS thesis Univ California, Davis, CA USA. 1994</a>
<b>Phenylalanine-tyrosine</b>			
1	Phenylalanine-tyrosine (All life stages)	Diets with a moderate level of phenylalanine + tyrosine but higher than the minimum requirement for growth may cause discolouring of black hair in kittens. <sup>a,b</sup> This is corrected by feeding a food containing $\geq 1.8\%$ DM of phenylalanine or a combination of tyrosine and phenylalanine <sup>b</sup> . To maximise black hair colour, the tyrosine level should be equal or higher than that of phenylalanine. <sup>c</sup>	<sup>a</sup> Yu S, Rogers QR, Morris JG. Effect of low levels of dietary tyrosine on the hair colour of cats. <i>Journal of small Animal Practice</i> 2001; 42: 176-180. <sup>b</sup> Anderson PJB, Rogers QR, Morris JG. Cats require more dietary phenylalanine or tyrosine for melanin deposition in hair than for maximal growth. <i>J. Nutr.</i> 2002; 132: 2037-2042. <sup>c</sup> NRC Chapter 15. Nutrient Requirements and Dietary Nutrient Concentrations. In: <i>Nutrient Requirements of Dogs and Cats.</i> The National Academic Press, Washington, DC. 2006: pp. 357-363 tables 15-10, 15-12 and 15-14.
<b>Taurine</b>			

1	Taurine	Studies have shown that the bioavailability is lower when cats are fed a heated-processed canned food. <sup>a, b</sup> To maintain adequate taurine status, a heat-processed wet cat food needs to contain approximately 2 to 2.5 times more taurine than a dry extruded food; the latter should contain 0.1% DM taurine. <sup>c, d</sup>	<p><sup>a</sup> Hickman MA, Rogers QR, Morris JG. Effect of processing on fate of dietary [14C]taurine in cats. <i>J. Nutr.</i> 1990; 120: 995-1000.</p> <p><sup>b</sup> Hickman MA, Rogers QR, Morris J.G. Taurine Balance is Different in Cats Fed Purified and Commercial Diets. <i>J. Nutr.</i> 1992; 122: 553-559.</p> <p><sup>c</sup> Earle KE, Smith PM. The effect of taurine content on the plasma taurine concentration of the cat <i>Brit. J. Nutr.</i> 1991; 66: 227-235.</p> <p><sup>d</sup> Douglass GM, Fern EB, Brown RC. Feline plasma and whole blood taurine levels as influenced by commercial dry and canned diets. <i>J. Nutr.</i> 1991; 121: 179S-180S.</p>
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FAT			
Omega 3 and 6 fatty acids			
1	Omega 3 fatty acids (Growth & Reproduction)	The study by Pawlosky <i>et al.</i> suggests that juvenile felines it is important that the status of DHA in the nervous system is maintained for optimal retinal function. However, young felines have a low synthetic capacity to produce DHA. <sup>a</sup> Therefore it is recommended to have a small amounts of DHA and/or EPA in foods for growth and reproduction.	<p><sup>a</sup> Pawlosky RJ, Denkins Y, Ward G, <i>et al.</i> Retinal and brain accretion of long-chain polyunsaturated fatty acids in developing felines: the effects of corn oil-based maternal diets. <i>Am. J. Clin Nutr</i> 1997; 65 (2): 465-472.</p>
1	Omega 3 fatty acids (Adult cats)	Although there is increasing evidence of beneficial effects of omega-3 fatty acids, the current information is insufficient to recommend a specific level of omega-3 fatty acids for adult cats.	-

MINERALS			
Calcium			
1	Calcium	The FEDIAF value is higher than NRC 2006 including a safety margin to take into account the bioavailability of raw materials used.	-
2	Calcium-to-phosphorus ratio (Growth)	A calcium-to-phosphorus ratio of $\geq 0.65$ is appropriate for growing kittens, provided that the calcium and phosphorus levels in the food are at least 0.5% and 0.63% respectively. <sup>a, b</sup>	<p><sup>a</sup> Morris JG, Earl KE. Vitamin D and calcium requirements of kittens. <i>Vet. Clin. Nutr.</i> 1996; 3 (3): 93-96.</p> <p><sup>b</sup> Morris JG, Earl KE. Growing kittens require less dietary calcium than current allowances. <i>J Nutr.</i> 1999; 129: 1698-1704.</p>

**Sodium**

1	Sodium (Adult cats)	Based on plasma aldosterone concentration, Yu and Morris concluded that the minimum requirement of sodium for maintenance of adult cats is 0.08 % DM at 5.258 kcal ME/g (22kJ). <sup>a</sup> This corresponds with 0.076% at 4 kcal ME/g after adding a safety margin of about 25%.	<sup>a</sup> Yu S, Morris JG. Sodium requirement of adult cats for maintenance based on plasma aldosterone concentration. J. Nutr. 1999; 129: 419-423.
2	Sodium (Adult cats)	In one study with healthy adult cats, no adverse effects were seen when feeding a food with 1.5 % of sodium (DM). <sup>a</sup> The nutritional maximum should be set at 1.8% DM. <sup>b</sup>	<sup>a</sup> Burger I. Water balance in the dog and the cat. Pedigree Digest 1979; 6: 10-11. <sup>b</sup> Kienzle Personal communication
1	Sodium (Growth)	Based on plasma aldosterone concentration Yu and Morris recommended that a food for kittens should contain a minimum of 0.16% DM of sodium at 5.258 kcal ME/g (22kJ). <sup>a</sup> This corresponds with 0.16% at 4 kcal ME/g after adding a safety margin of about 30%.	<sup>a</sup> Yu S, Morris JG. The minimum sodium requirement of growing kittens defined on the basis of plasma aldosterone concentration. J. Nutr. 1997; 127: 494-501.
<b>Magnesium</b>			
1	Magnesium	Studies have demonstrated that 10mg/MJ will maintain adult cats. This value has been doubled to accommodate interactions with other dietary factors. <sup>a</sup>	<sup>a</sup> Pastoor <i>et al.</i> Doctoral Thesis, University of Utrecht 1993

**TRACE ELEMENTS**

**General**

1	General	Manufacturers are reminded that the bioavailability of trace-elements is reduced by a high content of certain minerals (e.g. calcium), the level of other trace elements (e.g. high zinc decreases copper absorption) and sources of phytic acid (e.g. some soy products).	
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**Copper**

1	Copper (General)	Owing to its low availability copper oxide should not be considered as a copper source.	<sup>a</sup> Fascetti AJ, Morris JG, Rogers QR. Dietary copper influences reproductive efficiency of queens. J. Nutr 1998; 128: 2590S-2592S
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**Iodine**

1	Iodine	Based on the Tc99m thyroid to salivary ratio, Wedekind <i>et al.</i> (2005) have shown that the minimum requirement of iodine for the cat is 0.51 mg/kg DM. <sup>a</sup>  The recommended allowance, therefore, can be set at 0.61 mg/kg DM taking into account a safety margin of about 20%.	<sup>a</sup> Wedekind KJ, Evans-Blumer MS, Spate V, Morris JS. Feline iodine requirement is much lower than the new NRC recommendation. The FASEB Journal 2005; 19 (5): A1705, Abstract 972.8
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**Iron**

1	Iron	Because of very poor availability, iron from oxide or carbonate salts that are added to the diet should not be considered sources contributing to the minimum nutrient level.	<sup>a</sup> NRC Absorption and bioavailability of dietary iron in dogs and cats. In: Nutrient Requirements of Dogs and Cats. The National Academic Press, Washington, DC. 2006: pp. 168-169.
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**VITAMINS**

**Vitamin A**

1	Vitamin A (Adult cats)	The FEDIAF maximum is based on the study reported by Seawright et al. in kittens <sup>a</sup> . The FEDIAF maximum of 40,000 IU/100g DM is about 50% of the maximum NOAEL reported by Sea Seawright et al. <sup>a</sup> in kittens from 6 to 8 weeks of age fed for 41 weeks. Since kittens are at least equally vulnerable as adults to hypervitaminosis A, this level should also be safe for adult cats.	<sup>a</sup> Seawright AA, English PB, Gartner RJW. Hypervitaminosis A and deforming cervical spondylosis of the cat. J. Comp. Path.1967; 77: 29-39.
2	Vitamin A (Growth and reproduction)	Seawright et al. <sup>a</sup> reported no adverse effects in kittens from 6 to 8 weeks of age fed for 41 weeks on a vitamin A intake of 50,000 IU/kg BW corresponding to about 90,000 IU per 100g DM. Therefore, FEDIAF's maximum of 40,000 IU/100g DM can be considered safe for growing kittens. Freytag <i>et al.</i> <sup>b</sup> reported that feeding a food with 100,000 IU/100g DM to pregnant queens caused fatal malformations in kittens. The next lowest value of 2000 IU/100g DM caused no adverse effects. From these data NRC 2006 recommended not to exceed 33,330 IU/100g DM in feeding stuffs intended for reproduction. <sup>c</sup> In view of these data, FEDIAF recommends a maximum vitamin A level of 33,330 IU/100g DM for products designed for reproducing queens.	<sup>a</sup> . Seawright AA, English PB, Gartner RJW. Hypervitaminosis A and deforming cervical spondylosis of the cat. J. Comp. Path.1967; 77: 29-39. <sup>b</sup> Freytag TL, Liu SM, Rogers AR, Morris JG. Teratogenic effects of chronic ingestion of high levels of vitamin A in cats. J. Anim Phys and Anim Nutr. 2003; 87: 42-51. <sup>c</sup> NRC Chapter 8. Vitamins - Hypervitaminosis A. In: Nutrient Requirements of Dogs and Cats. The National Academic Press, Washington, DC. 2006: p. 200.

**Vitamin D**

1	Vitamin D	Based on the study of Sih <i>et al.</i> (2001) a nutritional maximum of 3000 IU/100 DM (7500 IU/1000 kcal) can be considered safe for cats of all life stages. <sup>a</sup>	<sup>a</sup> Sih TR, Morris JG, Hickman MA. Chronic ingestion of high concentrations of cholecalciferol in cats. Am. J. Vet. Res. 2001; 62 (9): 1500-1506.
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**Vitamin E**

1	Vitamin E	10 IU of Vitamin E should be added above the level recommended in the table per gram of fish oil per kilogram of diet.	<b>Reference?</b> <b>Origin: Guidelines 2005</b>
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**Vitamin K**

1	Vitamin K	Vitamin K does not need to be added unless the diet contains antimicrobial or anti-vitamin compounds, or contains more than 25% fish on a DM basis. <sup>a</sup>	<sup>a</sup> Strieker MJ, Morris JG, Feldman BF, Rogers QR. Vitamin K deficiency in cats fed commercial fish-based diets. J Small Anim Pract. 1996; 37 (7): 322-326.
<b>Vitamin B6 (Pyridoxine)</b>			
1	Vitamin B6 (All life stages)	Requirements of vitamin B6 increase with increasing protein content of the food. <sup>a, b</sup>	<sup>a</sup> Bai SC, Sampson DA, Morris JG, Rogers QR. Vitamin B-6 requirement of growing kittens J. Nutr. 1989; 119: 1020-1027 <sup>b</sup> Bai SC, Sampson DA, Morris JG, Rogers QR. The level of dietary protein affects vitamin B-6 requirement of cats. J. Nutr. 1991; 121: 1054-1061.
1	Biotin	For healthy cats biotin does not need to be added to the food unless the food contains antimicrobial or anti-vitamin compounds. <sup>a, b</sup>	<sup>a</sup> Kronfeld DS, Biotin and Avidin. In vitamin & Mineral Supplementation for dogs and cats – A monograph on micronutrients Veterinary Practice Publishing Company 1989: 71-72. <sup>b</sup> Kronfeld DS, Biotin. In vitamin & Mineral Supplementation for dogs and cats – A monograph on micronutrients Veterinary Practice Publishing Company 1989: 99.

## IV. COMPLEMENTARY PET FOOD

Complementary pet food is legally defined as pet food which has a high content of certain substances but which, by reason of its composition, is sufficient for a daily ration only if used in combination with other pet foods (Directive 79/373/EC).

Complementary pet food covers a wide range of products including:

A. Products, such as a meat and biscuit combination, where each component contributes significantly to the energy content of the mixture.

B. Products, which contain certain nutrients but are not designed to contribute significantly to the energy content of the mixture.

- Treats and snacks are normally given to strengthen the human animal bond and as rewards during training.
- Tablets and conditioners often used to balance the diet when home made foods are given.
- Products, such as dog chews, that are not intended to contribute to the nutritional content of the daily ration, but are given to occupy the animal and can be eaten.

### 1. Recommended allowances

In view of the many different types of complementary pet foods, manufacturers are advised to base their feeding instructions on the intended role of the product in the total ration. The total ration should match the recommended allowances and nutritional and legal maximum values listed in the tables for complete pet food.

### 2 Validation procedure

FEDIAF recommends that for the purpose of nutrition validation, complementary pet food should be divided into three parts:

For products belonging to category A, the validation procedure should comply with that laid down for complete pet food in order to assess the nutritional adequacy of the total daily ration.

For products belonging to category B, the validation procedure should cover those nutrients that are relevant for the intended use of the product.

For occupational products belonging to category B, no specific validation procedure for nutritional adequacy is needed.

### **3. Repeat analysis**

When a validation procedure is recommended (see 2) the same rules should apply for complementary and complete pet food ([page 9](#))

## V. ANALYTICAL METHODS

In order to obtain representative results, samples have to be collected and treated according to the general principles laid down in Commission Directive 76/371/EEC of 1 March 1976 establishing Community methods of sampling for the official control of feeding stuffs, as amended, and in Commission Directive 71/250/EEC, establishing Community methods of analysis for the official control of feeding stuffs, as amended.

The analysis of only one sample may not reflect the level declared in the average analysis of the product. To obtain a representative analysis, multiple samples coming from different batches have to be analysed. A composite sample made from multiple samples is also valid. To evaluate the results of a single-sample analysis, minimum tolerances for deviation from the declared values, as foreseen in ANNEX PART A. 6 of Council Directive 79/373/EEC on the circulation of compound feeding stuffs should be permitted as well as tolerances for analytical latitudes.

### Non-exhaustive list of analytical methods

NUTRIENT	METHOD REFERENCE(S)
Sampling	EU method O.J. 1981 L 246 p.32 ISO/DIS 6491
Moisture	EU method O.J. 1971 L 279 p.7 ISO /DIS 6496
Protein (crude)	EU method O.J. 1993 L 179 p.8
Arginine	EU method O.J. 1998 L 257 p.14
Histidine	EU method O.J. 1998 L 257 p.14
Isoleucine	EU method O.J. 1998 L 257 p.14
Lysine	EU method O.J. 1998 L 257 p.14
Methionine	EU method O.J. 1998 L 257 p.14
Cyst(e)in	EU method O.J. 1998 L 257 p.14
Phenylalanine	EU method O.J. 1998 L 257 p.14
Tyrosine	EU method O.J. 1998 L 257 p.14
Threonine	EU method O.J. 1998 L 257 p.14
Valine	EU method O.J. 1998 L 257 p.14
Tryptophane	EU method O.J. 2000 L 174 p.32 2 <sup>nd</sup> ISO/CD 13904
Fat (crude)	EU method O.J. 1998 L 257 p.14
Linoleic Acid	VDLUFA method 5.6.2 B.S.I method BS684: section 2.34 : ISO 5509-1997 AOAC 15 <sup>th</sup> ed. (1990) 969.33 & 963.22
Arachidonic Acid	VDLUFA method 5.6.2 B.S.I method BS684: section 2.34 : ISO 5509-1997 AOAC 15 <sup>th</sup> ed. (1990) 969.33 & 963.22
Fiber (crude)	EU method O.J. 1993 L 344 p.35
Ash (crude)	EU method O.J. 1971 L 155 p.13
Calcium	EU method O.J. 1971 L 155 p.13 ISO/DIS 6869
Phosphorus	EU method O.J. 1971 L 279 p.7

	ISO/DIS 6491
Potassium	EU method O.J. 1971 L 155 p.13 ISO/DIS 6869
Sodium	EU method O.J. 1971 L 155 p.13 ISO/DIS 6869
Chloride	EU method O.J. 1971 L 155 p.7 §35 LMBG L06.00-5 AOAC 14th ed. (1984) 3.069-3.070 AOAC 15th ed. (1990) 920.155 & 928.04 AOAC 16th ed. (1998) potentiometric method 50.1.10
Magnesium	EU method O.J. 1973 L 83 p.21 ISO /DIS 6869
Iron	EU method O.J. 1978 L 206 p.43 ISO/DIS 6869
Copper	EU method O.J. 1978 L 206 p.43 ISO/DIS 6869
Manganese	EU method O.J. 1978 L 206 p.43 ISO/DIS 6869
Zinc	EU method O.J. 1998 L 257 p.13 ISO/DIS 6869
Iodine	Ministry of Agriculture, Fisheries and Food (1997). Dietary intake of iodine and fatty acids. Food Surveillance Information Sheet, 127. MAFF
Selenium	The Analyst 1979, 104, 784 VDLUFA, BD III method 11.6 (1993) AOAC 16 <sup>th</sup> ed. (1998) 9.1.01
Vitamin A	EU method O.J. 2000 L 174 p.32 VDLUFA method 13.1.2 2 <sup>nd</sup> ISO/CD 14565
Vitamin D *	VDLUFA method 13.8.1 D3 AOAC 15 <sup>th</sup> ed. (1990) 982.29 BS EN 12821 : 2000
Vitamin E	EU method O.J. 2000 L 174 p.32 2 <sup>nd</sup> ISO/CD 6867 VDLUFA method 13.5.4
Vitamin K	Analytical Proceedings, June 1993, Vol. 30, 266-267 (Vit. K3) J. of Chrom. 472 (1989) 371-379 (Vit. K1) BS EN 14148: 2003 (Vit. K1)
Thiamine	AOAC Int. <b>76</b> , (1993) 1156-1160 and 1276-1280 AOAC Int. <b>77</b> (1994) 681-686 The Analyst, 2000, No. 125, pp 353-360 EN 14122 (2003)
Riboflavin	AOAC Int. <b>76</b> (1993) 1156-1160 and 1276-1280 AOAC Int. <b>77</b> (1994) 681-686 AOAC 16 <sup>th</sup> ed. (1998) M 940.33 The Analyst, 2000, No. 125, pp 353-360 EN 14152 (2003)
Pantothenic Acid	AOAC 945.74 /42.2.05 (1990) USP XXIII, 1995, M 91
Niacin	AOAC 944.13 /45.2.04 (1990) USP XXIII, 1995, M 441
Vitamin B6 (Pyridoxine)	AOAC 16th ed. (1998) M 985.32 EN 14663: 2005
Folic Acid	AOAC 16th ed. (1998) M 944.12

	Biacore AB: Folic Acid Handbook; BR 1005-19
Biotin	USP XXI, 1986, M 88 Biacore AB: Biotin Kit Handbook; BR 1005-18
Vitamine B12	USP XXIII, 1995, M171 AOAC 952.20 Biacore AB: Vitamin B12 Handbook; BR 1004-15
Choline	AOAC Int. Vol 82, No. 5 1999 pp 1156-1162 EG-Draft 15.706/1/VI/68-D/bn
Taurine	AOAC Int. Vol. 82 No. 4, 2000 pp 784-788
Total dietary fibre (TDF)	AOAC Official Method 985.29 or 45.4.07 for Total Dietary Fibre in Food and Food Products
Insoluble fibre (IF)	AOAC Method 991.42 or 32.1.16 for the Insoluble Dietary Fibre in Food and Food Products
Soluble fibre (SF)	AOAC Official Method 993.19 or 45.4.08 for Soluble Dietary Fibre in Food and Food Products

Vitamin D analysis of pet foods containing levels which are approaching the minimum recommendation, say between 500 and 1000 IU/kg DM is difficult and unreliable. The detection limit for HPLC methods is approximately 3000 to 5000 IU/kg. Analysis is not required if supplementation is practised and it is unlikely that un-supplemented products with adequate levels of vitamins A and E will be deficient in vitamin D.

## VI – FEEDING TEST PROTOCOLS

### Recommended feeding trial protocol for the determination of metabolizable energy of cat and dog food

GE	Gross energy	CP	Crude protein
DE	Digestible energy	DP	Digestible protein
ME	Metabolizable energy	BW	Body weight
kJ	kilojoule	Cr <sub>2</sub> O <sub>3</sub>	Chromic oxide

#### 1 Indicator method

##### 1 Introduction

This feeding protocol has been designed in order to determine ME of cat & dog foods in a way not harmful for cats and dogs and is adapted from the "AAFCO dog and cat food metabolizable energy protocols - Indicator Method" (AAFCO 2007).

##### 2 Protocol

###### 2.1 Animals

A minimum of six fully grown animals at least one year of age shall complete the test. The animals shall be in good health and of known weight, sex and breed. Animals shall be individually housed during the trial (collection period).

###### 2.2 Feeding Procedures

Feeding procedures shall be standardized. The feeding shall consist of two phases.

The first phase shall be the pre-collection period of at least three days for dogs and five days for cats (Nott *et al.* 1994) with the objective of acclimatising the test animals to the diet and adjusting food intake, as necessary, to maintain body weight.

The second phase shall be the total collection period; faeces and possibly urine will be collected during at least four days (96 hours) for dogs and five days (120 hours) for cats.

###### 2.3 Food

Food type, flavour, and production codes representing the composite feed shall be recorded.

The food source shall remain constant throughout the test period.

The indicator shall be uniformly mixed in a quantity of food sufficient to feed all animals for the duration of the pre-collection and collection periods. If chromic oxide is used, approximately 0.25% of a high quality chromic oxide ( $\text{Cr}_2\text{O}_3$ ) free of soluble chromium shall be mixed with the food.

## **2.4 Food Allowances**

The amount of food presented to each animal may be based upon existing data on the quantity of food required to maintain body weight, or the estimated daily maintenance energy requirements (110-115 kcal ME per kg BW<sup>0.75</sup> for dogs or 60-70 kcal ME per kg BW for cats) (See ANNEX I on energy p. 42).

## **2.5 Times of Feeding**

Animals shall be fed at least once daily and at the same time each day. Water shall be available at all times. Food shall be fed as is, or per normal feeding instructions for the product. The excess food shall be weighed back after feeding.

## **2.6 Pre-trial Termination**

If, during the pre-collection phase, the food is continually rejected or results in minimal consumption by a majority of the animals, the trial shall not proceed into the collection phase.

## **2.7 Collection**

### **2.7.1 Faeces Collection**

It is imperative that all collection containers be clearly marked using double labels or any alternative adequate coding. The labels shall include the animal number, diet number, and dates of collection.

Aliquots of faeces from five separate days shall be collected. Every effort should be made to avoid collecting contaminants such as hair. The aliquots shall be dried and pooled per individual animal.

### **2.7.2 Urine collection**

During the collection period, all daily urine shall be collected for each animal and weighed, unless a correction factor is used to estimate metabolizable energy. Every effort should be made to avoid collecting contaminants such as hair.

## **2.8 Sample Preparation**

### **2.8.1 Food**

The food shall be blended to ensure a uniform consistency and an adequate quantity used for appropriate assays. Ample quantities of the remaining sample should be frozen and retained until assay results have been reviewed and found acceptable.

### **2.8.2 Faeces**

Faeces shall be analyzed using composite samples. The samples shall be blended to ensure a uniform consistency and an adequate quantity used for appropriate assays. Ample quantities of the remaining sample should be frozen and retained until assay results have been reviewed and found acceptable.

### **2.8.3 Urine**

Urine shall be collected in sulphuric acid containing receptacles to stabilize the urine and prevent loss of nitrogen. Aliquots of urine from the collection period shall be freeze dried and pooled per animal in sufficient amount for GE assay.

## **2.9 Analytical Determination**

Prepared samples shall be used for analysis. AOAC approved analytical methodology shall be used when available or one of the recommended analytical methods listed on p. 31. Food and faeces shall be assayed for gross energy (bomb calorimetry), crude protein, and the indicator. If urine is collected, gross energy and crude protein in the urine should also be determined.

If digestibility values of dry matter, fat or other nutrients are wanted, food and faeces should also be assayed for those substances.

Food and faeces are analysed for the indicator by the same method (Atomic absorption spectrophotometry is the preferred method if chromic oxide is used as the indicator (Arthur 1970). Since controlled sample digestion and oxidation of the chromic oxide to chromates is critical for reproducible results, colorimetric analysis of chromium is less reproducible than atomic absorption spectrophotometry.

Food, faeces and urine (if collected) are stored in the freezer in case of need for further analysis

## 2.10 Calculation of Digestible and Metabolizable Energy and Digestible Nutrients

### 1. Digestible energy & protein

The determination is based on assays of the gross energy or crude protein consumed minus the energy or crude protein in the faeces.

$$\text{DE (kcal/g)} = \left\{ 1 - \frac{(\text{GE of faeces} \times \% \text{Cr}_2\text{O}_3 \text{ in food})}{(\text{GE of food} \times \% \text{Cr}_2\text{O}_3 \text{ in faeces})} \right\} \times \text{GE of food}$$

$$\text{DP (\% food)} = \left\{ 1 - \frac{(\% \text{CP in faeces} \times \% \text{Cr}_2\text{O}_3 \text{ in food})}{(\% \text{CP in food} \times \% \text{Cr}_2\text{O}_3 \text{ in faeces})} \right\} \times \text{CP in food}$$

Digestible fat, ash and dry matter can be calculated in the same way as digestible protein.

### 2. Metabolizable energy

The determination is based on assays of the gross energy consumed minus the energy lost in faeces and in the urine.

If urine is collected

$$\text{ME (kcal/g)} = \text{DE} - \text{GE of urine ()}$$

If urine is not collected

$$\text{ME (kcal/g)} = \text{DE} - (\text{DP} \times \text{correction factor for energy lost in urine})$$

Correction factor for energy lost in urine (Kienzle *et al.* 1998):

1.25 kcal or 5.23 kJ/g for dogs

0.86 kcal or 3.60 kJ/g for cats

## 2 Quantitative collection method

### 1 Introduction

This feeding protocol has been designed in order to determine ME of cat & dog foods in a way not harmful for cats and dogs and is adapted from the "AAFCO dog and cat food metabolizable energy protocols – Quantitative Collection Method" (AAFCO 2007).

### 2 Protocol

#### 2.1 Animals

A minimum of six fully grown animals at least one year of age shall complete the test. The animals shall be in good health and of known weight, sex and breed. Animals shall be individually housed during the trial (collection period).

## **2.2 Feeding Procedures**

Feeding procedures shall be standardized. The feeding shall consist of two phases.

The first phase shall be the pre-collection period of at least three days for dogs and five days for cats (Nott *et al.* 1994) with the objective of acclimatizing the test animals to the diet and adjusting food intake, as necessary, to maintain body weight.

The second phase shall be the total collection period of at least four days (96 hours) for dogs and five days (120 hours) for cats.

The amount of food offered during the second phase shall remain constant. Food intake shall be recorded throughout both phases.

## **2.3 Food**

Food type, flavour, and production codes representing the composite feed shall be recorded. The food source shall remain constant throughout the test period.

## **2.4 Food Allowances**

The amount of food presented to each animal may be based upon existing data on the quantity of food required to maintain body weight or the estimated daily maintenance energy requirements (110-115 kcal ME per kg BW<sup>0.75</sup> for dogs or 60-70 kcal ME per kg BW for cats) (See ANNEX I on energy p. 42).

## **2.5 Times of Feeding**

Animals shall be fed at least once daily and at the same time each day. Water shall be available at all times. Food shall be fed as is, or per normal feeding instructions for the product. The excess food shall be weighed back after feeding.

## **2.6 Pre-trial Termination**

If, during the pre-collection phase, the food is continually rejected or results in minimal consumption by a majority of the animals, the trial shall not proceed into the collection phase.

## **2.7 Faeces Collection**

It is imperative that all collection containers be clearly marked using double labels or any alternative adequate coding. The labels shall include the animal number, diet number, and dates of collection. Faeces shall be collected daily for a minimum of four days for dogs and five days for cats. Every effort should be made to collect all of the faeces and avoid collecting contaminants such as hair. The methodology is as follows:

- i) Weigh collection container and record weight.
- ii) Place faeces in the respective animal's container for that day of collection. Collect faeces as quantitatively as possible.
- iii) Place collections in freezer for storage.
- iv) Faeces may be dried each day.
  - a. Weigh and record the weight of the faeces and container each day, and determine net weight of faeces. If the volume of faeces is large, an aliquot may be retained for drying.
  - b. Dry daily faeces collection (or aliquot). Faeces should be thin enough to dry quickly. Otherwise, nitrogen and carbon losses may occur due to fermentation products.
  - c. Pool the entire collection or proportional aliquots.

## **2.8 Sample Preparation**

### **2.8.1 Food**

The food shall be blended to ensure a uniform consistency and an adequate quantity used for appropriate assays. Ample quantities of the remaining sample should be frozen and retained until assay results have been reviewed and found acceptable.

### **2.8.2 Faeces**

Faeces shall be analyzed using composite samples. The samples shall be blended to ensure a uniform consistency and an adequate quantity used for appropriate assays. Ample quantities of the remaining sample should be frozen and retained until assay results have been reviewed and found acceptable.

### **2.8.3 Urine**

If urine collections are made, they shall be for the same period as the faeces collections. Urine shall be collected with a minimum of contamination, in a urine receptacle containing sulphuric acid to stabilize the urine and prevent nitrogen loss. After the total urine volume is determined, aliquot samples shall be freeze-dried in an appropriate container.

## **2.9 Analytical Determination**

Prepared samples shall be used for analysis. AOAC approved analytical methodology shall be used when available or one of the methods p.

Food, faeces and urine (if collected) shall be assayed for gross energy (bomb calorimetry). If urine is not collected, food and faeces also shall be assayed for crude protein.

If digestibility values of dry matter, fat or other nutrients are wanted, food and faeces should also be assayed for those substances.

### 2.10 Calculation of Digestible Energy and digestible nutrients

The determination is based on assays of the gross energy consumed minus the energy in the faeces.

DE (per g food) = (GE of food consumed - GE of faeces collected) / amount of food consumed.

DP (% of food) = (CP of food consumed - CP of faeces collected) x100/ amount of food consumed.

Digestible fat, ash and dry matter can be calculated in the same way as digestible protein.

### 2.11 Calculation of Metabolizable Energy

The determination is based on assays of the gross energy consumed minus the energy in the faeces and correction for energy lost in the urine (or energy lost in urine as determined by calorimetry).

#### 2.11.1 Without urine collection

ME = [(GE of food consumed - GE of faeces collected) – (grams protein consumed - grams protein in faeces) x correction factor for energy loss in urine] / amount of food consumed.

Correction factor for energy lost in urine (Kienzle et al. 1998):

1.25 kcal or 5.23 kJ/g for dogs

0.86 kcal or 3.60 kJ/g for cats

#### Example:

- a) gross energy of food 4.35 kcal/g or 18.19 kJ/g
- b) amount of food consumed 1250g
- c) gross energy of faeces 1.65 kcal/g or 6.90 kJ/g
- d) amount of faeces collected 600g
- e) protein in food 24%
- f) protein in faeces 9%
- g) correction factor (dog) 1.25 kcal/g or 5.23 kJ/g

$$\text{ME (kcal/kg)} = \frac{(\text{axb}) - (\text{cxd}) - \{(\text{bx}e / 100) - (\text{dx}f / 100)\} \times \text{g}}{\text{b}} \times 1000$$

$$\text{ME (kcal/kg)} = \frac{(a \times b) - (c \times d) - [(b \times e) - (d \times f)]}{b} \times 1000$$

$$\text{ME (kcal/kg)} = \frac{[(4.35 \times 1250) - (1.65 \times 600)] - [(1250 \times 24) - (600 \times 9)]}{1,250} \times 1000$$

$$\text{ME (kcal/kg)} = 3,312 \text{ kcal} \quad (\times 4.184 = 13,857.4 \text{ kJ})$$

### 2.11.2 With urine collection

ME = [(GE of food consumed - GE of faeces collected) - GE of urine collected] / amount of food consumed.

#### Example

- a) gross energy of food = 4.35 kcal/g
- b) amount of food consumed = 1250 g
- c) gross energy of faeces = 1.65 kcal/g
- d) amount of faeces collected = 600 g
- e) gross energy of urine = 0.25 kcal/ml
- f) volume of urine = 1230 ml

$$\text{ME (kcal/kg)} = \frac{[(a \times b - c \times d) - e \times f]}{b} \times 1000$$

$$\text{ME (kcal/kg)} = \frac{[(4.35 \times 1,250 - 1.65 \times 600) - (0.25 \times 1,230)]}{1,250} \times 1000$$

$$\text{ME (kcal/kg)} = 3,312 \text{ kcal} \quad (\times 4.184 = 13,857.4 \text{ kJ})$$

### References

1. AAFCO. AAFCO dog and cat food metabolizable energy protocols. In: Official Publication - Association of American Feed Control Officials Inc. 2007:160-165.
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3. Kienzle E, Opitz B, Earl KE, *et al.* The development of an improved method of predicting the energy content in prepared dog and cat food. J. Anim? Physiol. A. Anim. Nutr. 1998; 79: 69-79.
4. Nott HMR, Rigby SI, Johnson JV, *et al.* Design of digestibility trials for dogs and cats. J. Nutr. 1994; 124 (12S): 2582S-2583S.

## ANNEX I – Energy

### 1 Introduction

The feeding guide, more than anything else on a pet food label, draws the attention of the consumer, to who the amount to feed is certainly key.

Energy requirements vary considerably between individual dogs and cats, even between animals kept under the same conditions. This wide variation between individual animals can be the consequence of differences in age, breed, body size, body condition, insulation characteristics of skin and hair coat, temperament, health status or activity. It can also be caused by environmental factors such as ambient temperature and housing conditions (Meyer & Zentek 2005, NRC 2006).

No single formula will allow to calculate the energy requirements for all dogs or cats (Heusner 1991), and every equation only predicts a theoretical average for a specific group of animals.

Providing satisfactory feeding recommendations remains thus an ongoing challenge for pet food companies. The next section provides general recommendations for household dogs and cats and should be considered a starting point. The following discussion is intended to clarify some of the substantial differences seen between individual dogs or cats.

### 2 Abbreviations

BCS	Body condition score (lean, ideal, overweight, obese)	ME	Metabolizable energy
		MJ	Megajoule
BMR	Basal metabolic rate	MER	Maintenance energy requirements
BW	Body weight		
DE	Digestible energy	NFE	Nitrogen free extract
DER	Daily energy requirements	REE	Resting energy expenditure
DM	Dry matter	RER	Resting energy requirements
ECF	Extra cellular fluid	TNZ	Thermo-neutral zone
GE	Gross energy	UCT	Upper critical temperature

### 3 Energy density of the food

Energy is expressed either in kilocalories (kcal) or in kilojoules (kJ)

#### Conversions

1 kcal = 1000 cal = 4.184 kJ

1 MJ = 1000 kJ = 239 kcal

#### Gross energy

The gross energy (GE) of a food is defined as the total chemical combustible energy arising from complete combustion of a food in a bomb calorimeter (NRC 2006a). The predicted GE values of protein, fat and carbohydrate are listed in table 1.

**Table 1. Predicted gross energy values of protein, fat and carbohydrate**

Nutrient	Gross Energy	
Crude protein	5.7 kcal/g	23.8 kJ/g
Fat	9.4 kcal/g	39.3 kJ/g
NFE + Crude fibre	4.1 kcal/g	17.1 kJ/g

(Kienzle *et al.* 2002; NRC 2006a) NFE = nitrogen free extract

kcal GE/100g = 5.7 x % crude protein + 9.4 x % crude fat + 4.1 x (% NFE + % crude fibre)

kJ GE/100g = 23.8 x % crude protein + 39.3 x % crude fat + 17.1 x (% NFE + % crude fibre)

#### Metabolizable energy

Digestible energy and metabolizable energy are a more accurate way of expressing the energy density of a food. Metabolizable energy reflects better the energy that is utilised by the animal, but is more difficult to determine. The metabolizable energy (ME) of a pet food is measured most accurately by performing digestibility trials using one of the two methods described in [Section VI p. 35](#). The metabolizable energy can also be predicted by calculation from the average analysis using one of the equations hereafter. However, since the digestibility can differ between pet foods, a single equation can not be supposed to predict the ME of all pet food products.

##### a) Atwater factors

For processed pet foods modified Atwater factors can be used; they are based on an average digestibility of 90% for fat, 85% for carbohydrate (NFE) and 80% for protein (NRC 1985b).

Kcal ME /100g = % crude protein x 3.5 + % crude fat x 8.5 + % NFE x 3.5 (AAFCO 2008)

$$\text{KJ ME /100g} = \% \text{ crude protein} \times 14.65 + \% \text{ crude fat} \times 35.56 + \% \text{ NFE} \times 14.65$$

The factors developed by Atwater in 1902 work well for human food ingredients such as meat, fish and purified starch products) and can also be used for processed pet foods with a very high digestibility, milk substitutes and liquids for enteral nutrition (NRC 2006a).

$$\text{Kcal ME /100g} = \% \text{ crude protein} \times 4.0 + \% \text{ crude fat} \times 9.0 + \% \text{ NFE} \times 4.0$$

$$\text{KJ ME /100g} = \% \text{ crude protein} \times 16.74 + \% \text{ crude fat} \times 37.66 + \% \text{ NFE} \times 16.74$$

For canned cat food NRC 1986 had proposed the following more accurate equation:

$$\text{Kcal ME /100g} = \% \text{ crude protein} \times 3.9 + \% \text{ crude fat} \times 7.7 + \% \text{ NFE} \times 3.0 - 0.05$$

$$\text{KJ ME /100g} = \% \text{ crude protein} \times 16.32 + \% \text{ crude fat} \times 32.22 + \% \text{ NFE} \times 12.55 - 0.21$$

More accurate predictive equations for modern pet foods ~~for healthy animals~~ are discussed below:

#### **b) Predictive Equations for ME in foods for dogs and cats**

For calculation of ME in prepared pet foods for cats and dogs (dry and wet) the following 4-step-calculation can be used (NRC 2006a):

1. Calculate GE

$$\text{GE (kcal)} = (5.7 \times \text{g protein}) + (9.4 \times \text{g fat}) + [4.1 \times (\text{g NFE} + \text{g crude fibre})]$$

$$\text{GE (kJ)} = (16.74 \times \text{g protein}) + (37.66 \times \text{g fat}) + [16.74 \times (\text{g NFE} + \text{g crude fibre})]$$

2. Calculate energy digestibility (%):

$$\text{Dogs: \% energy digestibility} = 91.2 - (1.43 \times \% \text{ crude fibre in DM})$$

$$\text{Cats: \% energy digestibility} = 87.9 - (0.88 \times \% \text{ crude fibre in DM})$$

3. Calculate digestible energy:

$$\text{kcal DE} = (\text{kcal GE} \times \text{energy digestibility}) / 100$$

$$\text{kJ DE} = (\text{kJ GE} \times \text{energy digestibility}) / 100$$

4. Convert into metabolizable energy:

$$\text{Dogs: kcal ME} = \text{kcal DE} - (1.04 \times \text{g protein})$$

$$\text{kJ ME} = \text{kJ DE} - (1.04 \times \text{g protein})$$

$$\text{Cats: kcal ME} = \text{kcal DE} - (0.77 \times \text{g protein})$$

$$\text{kJ ME} = \text{kJ DE} - (0.77 \times \text{g protein})$$

This calculation is not suitable for milk substitutes and liquid preparations for enteral nutrition and may be inaccurate for foods with a crude fibre content of more than eight per cent.

#### **~~c) Equation for particular nutritional purposes (obesity, convalescence)~~**

~~The calculated energy density has to be declared on the pack of pet foods intended~~

~~for the following particular nutritional purposes “nutritional restoration, convalescence” and “reduction of excessive body weight”. To calculate the metabolizable energy density (MJ/kg) of those pet foods the following equations should be used:~~

~~All dog and cat foods except cat foods with moisture content exceeding 14 %:~~

$$\text{MJ/kg of ME} = 0.1464 \times \% \text{ CP} + 0.3556 \times \% \text{ CF} + 0.1464 \times \% \text{ NFE}$$

~~Cat foods with a moisture content exceeding 14 %:~~

$$\text{MJ/kg of ME} = (0.1632 \times \% \text{ CP} + 0.3222 \times \% \text{ CF} + 0.1255 \times \% \text{ NFE}) - 0.2092$$

~~CP = Crude protein; CF = Crude oils and fats; NFE = Nitrogen free extract~~

#### **d) Determination of ME content of foods by feeding trials**

Manufacturers should be aware that feeding trials are regarded as the gold standard for determination of the energy content of any pet food. In those trials described on pp. **XXY** the digestible energy (DE) can be measured accurately. An approximate factor to convert digestible into metabolizable energy is 0.9. Alternatively, NRC 2006 recommends subtracting 1.25 kcal g<sup>-1</sup> digestible crude protein for dogs and 0.9 kcal g<sup>-1</sup> for cats (NRC 2006a). FEDIAF recommends that members who wish to use feeding trials should employ the quantitative collection protocol published on pages **37-41**.

## **4 Literature review on energy requirements of dogs**

While the formulae give average metabolizable energy needs, actual needs of cats and dogs may vary greatly depending on various factors (Meyer & Zentek 2005, NRC 1985 & 2006). Energy allowances, recommended for maintenance of adult dogs, differ widely, with figures ranging from less than 90 kcal ME/kg<sup>0.75</sup> (377 kJ) to approximately 200 kcal ME/kg<sup>0.75</sup> (810 kJ). This diversity is not surprising when we consider the variation in adult size between the different breeds, which, with mature body weights ranging from one kg (Chihuahua) to 90 kg or more (St. Bernard), is the greatest diversity across mammalian species (Lauten 2006). ~~The amount of energy a particular dog will finally need is significantly influenced by factors such as age, breed, size, activity, environment, temperament, insulation characteristics of skin and hair coat, body condition or disease.~~

### **4.1 Maintenance Energy Requirements (MER) of adult dogs**

Energy requirements of animals with widely differing body weights are not correlated with kg body weight (BW) in a linear way (Meyer & Heckötter 1986, NRC 1985). Energy requirements are more closely related to BW raised to some power: The daily energy requirements of dogs most often are calculated in function of a metabolic weight, which

equals  $\text{kg}^{0.75}$ . Its accuracy for dogs has been questioned, and a valid alternative ( $\text{kg}^{0.67}$ ) is more related to body surface, and may thus better reflect heat production (Finke 1994, Kienzle & Rainbird 1991, Männer 1991). What such an equation tells you is the expected mean value for a “typical dog of the given size”. We will continue to use the  $\text{kg}^{0.75}$ , which is also recommended by NRC 2006. It is widely accepted and easy to calculate by cubing BW and then taking its square root twice (Lewis *et al.* 1987a).

MER is the amount of energy expended by a moderately active adult animal. It consists of the basal metabolic rate (BMR) plus the energy cost for obtaining, digesting and absorbing food in amounts that are necessary to maintain BW. It includes calories for spontaneous (inevitable) activity, and, in case of passing the critical temperature, energy needed to maintain normal body temperature (Meyer & Zentek 2005, Rainbird & Kienzle 1989). Independent from BW, MER is influenced by differences in age, type and breed, activity, temperament, environmental temperature, insulation characteristics of skin (i.e. hair length and subcutaneous fat), and social environment, among which “**age and activity**” appeared to be the most important contributors to individual energy needs (Burger 1994, Finke 1994, Kienzle & Rainbird 1991, Meyer & Heckötter 1986, NRC 1985).

Recommendations for MER may overestimate energy needs by 10 to 60 % (Männer 1991, NRC 2006a). They often include a reasonable amount for activity, whereas approximately 19 per cent of the owners never play with their dogs, and 22 per cent let their dogs out for exercise for less than three hours a week (Slater *et al.* 1995).

## 4.2 Activity

It is clear that spontaneous activity significantly influences MER; for example, standing up requires 40 per cent more energy than lying down (Meyer and Zentek 2005). However, recommendations for MER do not always mention the degree of activity included, whilst it is important that activity is taken into account when calculating the energy needs of an individual animal. Indeed, average recommendations could be too high for about one out of four dogs, since almost a quarter of the owners exercise their dogs less than **three hours a week** (Slater *et al.* 1995). To avoid overfeeding and the risk of obesity, it may be better to start from a lower calculated MER and add as needed to maintain optimal body weight.

## 4.3 Age

Apart from lactation and imposed activity during work or sport, age may be the single most-important factor influencing MER of most household dogs (Finke 1994). Three groups of adult dogs can be distinguished: dogs of one to two years old, the average adult dog (three to seven years old) and dogs of more than seven years of age (Finke 1994 & 1991, Kienzle & Rainbird 1991). Young adult dogs, under two years of age, require more energy because

they are more active and despite a body weight similar to that of older individuals of the same breed, may still be **growing developing** (Meyer & Zentek 2005, Rainbird & Kienzle 1989). Older animals need fewer calories because of decreased activity (Finke 1991, Meyer & Zentek 2005). In some dogs, however, calorie needs may further decrease as a consequence of an increase in subcutaneous fat and a decrease in body temperature (Meyer & Zentek 2005). Dogs over seven years of age may need 10 - 15 per cent less energy than at three to seven years (Finke 1994, Kienzle & Rainbird 1991). Therefore, practical recommendations should always be related to age (Finke 1994, Gesellschaft für Ernährungsphysiologie 1989a). The age at which a dog can be considered older and their activity decreases can differ according to breed and between individuals. Most of the assessed scientific work uses the age of seven years as a cut-off point, but this should not be regarded as a general rule.

#### **4.4 Breed & Type**

It has been shown that some breeds such as Newfoundland dogs and huskies have relatively lower energy requirements, while Great Danes have a MER above the average (Kienzle & Rainbird 1991, Rainbird & Kienzle 1989, Zentek & Meyer 1992). Breed-specific needs probably reflect differences in temperament, resulting in higher or lower activity, as well as variation in stature or insulation capacity of skin and hair coat, which influences the degree of heat loss. However, when data are corrected for age, inter-breed differences become less important (Finke 1994). Yet NRC 2006 reports Newfoundland dogs, Great Danes and terriers as breeds with energy requirements outlying the predictive range (NRC 2006a).

#### **4.5 Thermoregulation and Housing**

Cool environment increases animals' energy expenditure (Blaza 1982, Finke 1991, Meyer & Zentek 2005, NRC 1985, Walters et al. 1993). When kept outside in winter, dogs may need 10 to 90 per cent more calories than during summer.

Energy needed for maintaining body temperature is minimal at a temperature called the thermo-neutral zone (TNZ). The TNZ is species and breed specific and is lower when the thermal insulation is better. The TNZ has been estimated to be 15-20°C for long-haired dog breeds and 20-25°C for short haired dog breeds; it may be as low as 10-15°C for Alaskan Huskies (Kleiber 1961b, Männer 1991, Meyer & Zentek 2005, Zentek & Meyer 1992).

Besides insulation capacity, the energy expenditure also depends on differences in stature, behaviour and activity during cold weather, and degree of acclimatisation (Finke 1991, Meyer & Zentek 2005, NRC 1985, Zentek & Meyer 1992), as well as on air movement and air

humidity (McNamara 1989, Meyer & Zentek 2005). Animals kept together may decrease the rate of heat loss by huddling together; this phenomenon is very important for neonates (Kleiber 1961b).

During exposure to heat, the basal metabolic rate cannot be lowered (Ruckebusch *et al.* 1984). If the environmental temperature increases above the upper critical temperature (UCT), the animal has to get rid of the heat by either increasing blood flow to the surface (vasodilatation) or enhanced evaporation of water (panting), which also costs energy (Kleiber 1961b). Vasodilatation becomes ineffective when the environmental is equal to the rectal temperature (Kleiber 1961b). The UCT for adult dogs seems to be 30 to 35 °C (NRC 2006b). Individually housed dogs, with little opportunity to move, may have daily energy requirements (DER) as low as 70 kcal ME/kg<sup>0.75</sup>. When housed in kennels together with other dogs and a lot of mutual interaction, which stimulates activity, DER may rise to over 144 kcal ME/kg<sup>0.75</sup> (NRC 2006a).

Diet-induced thermogenesis plays a small role; it represents about 10 per cent of the daily energy expenditure in dogs. It increases with diets rich in protein and is greater in dogs fed four meals per day than in dogs fed once daily (NRC 2006a).

## 5. Practical recommendations for daily energy intake by dogs and cats in different physiological states

As mentioned before, it is impossible to have one equation which expresses the energy requirements for every individual animal. Since the energy requirement of an individual animal may differ from the average shown in the tables, these recommendations should only be used as starting points, and the owner has to adapt the amount when the animal tend to lose or gain weight.

### 5.1. Dogs

Tables 2-4 provide practical recommendations for maintenance energy requirements (MER) of adult dogs at different ages (TABLE 2), energy needed in relation to activity (TABLE 3) or for growth and reproduction (TABLE 4).

#### 5.1.1. Maintenance energy requirements

Table 2. Practical recommendations for MER in dogs at different ages		
Age	Average	Range
Years	kcal ME/kg <sup>0.75</sup>	kcal ME/kg <sup>0.75</sup>
1 – 2	132	125-140
3 – 7	115	100-130
> 7 (senior dogs)	100	80-120

<b>Obese prone adults</b>	≤ 90	
<b>Breed specific differences:</b>		
Great Danes	200	200-250
Newfoundlands	105	80-132

Männer K 1990 & 1991; Finke MD 1994 & 1991; Walters LM *et al.* 1993; NRC 2006a.

The values shown in Table 2 are only starting points, the amount of energy a particular dog will finally need is significantly influenced by other factors such as activity, environment, breed, temperament, insulation characteristics of skin and hair coat, body condition or disease.

Table 2 provides MER at different ages without taking into account the degree of activity. However, some young adult dogs may have a sedentary lifestyle and need fewer calories than the average shown in table 2, whereas older dogs (> 7 years of age) which are still playing and running will need more energy than indicated. Table 3 provides an example of the daily requirements of dogs at different levels of activity. Although mainly based on data from one breed (border collies), table 3 is a good alternative to table 2 to estimate the energy requirements of adult dogs in relation to their level of activity.

**Table 3. Recommendations for DER in relation to activity**

Activity level	kcal ME/kg <sup>0.75</sup>	kJ ME/kg <sup>0.75</sup>
Low activity (< 1 h/day) (e.g. walking on the lead)	100	418
Moderate activity (1 - 3 h/day) (e.g. playing, off the lead)	125	523
High activity (3 - 6 h/day) (working dogs, e.g. sheep dogs)	150 -175	628 – 732
High activity under extreme conditions (racing sled dogs 168 km/d in extreme cold)	860-1240	3600-5190

Burger 1994 and NRC 2006b.

In addition, when dogs are housed at an ambient temperature, which is below or over their specific thermo-neutral zone, MER increases by 2-5 kcal (8-21 kJ) per kg<sup>0.75</sup> for every degree centigrade (NRC 2006b).

### 5.1.2. Growth and reproduction

Energy requirements for lactation depend on the litter size. Except for bitches with only one or two puppies, lactating bitches should be fed ad libitum. Table 4 provides equations to calculate the average energy needs of lactating bitches at different stages of lactation.

**Table 4. Average energy requirements during growth and reproduction in dogs**

Puppies	Age	Energy requirement
	Newborn puppies	25 kcal/100 g BW
	Up to 50 % of adult weight	210 kcal/kg <sup>0.75</sup>
	50 to 80 % of adult weight	175 kcal/kg <sup>0.75</sup>
	80 to 100 % of adult weight	140 kcal/kg <sup>0.75</sup>
Bitches	Reproduction phase	Energy requirement
<b>Gestation*</b>	first 4 weeks of gestation	130 kcal/kg BW <sup>0.75</sup>
	last 5 weeks of gestation	130 kcal/kg BW <sup>0.75</sup> + 26 /kg BW
<b>Lactation**</b>		
	1 to 4 puppies	132/kg BW <sup>0.75</sup> + 24n x kg BW x L
	Lactating bitch, 5 to 8 puppies	132/kg BW <sup>0.75</sup> + (96 + 12m) x kg BW x L

\* Gesellschaft für Ernährungsphysiologie 1989a; \*\* NRC 2006a & 2006c, n = number of puppies; m = number of puppies between 5 and 8; L = 0.75 in week 1 of lactation; 0.95 in week 2; 1.1 in week 3 and 1.2 in week 4

Overfeeding puppies can result in skeletal deformities especially in large and giant breeds (Dämmrich 1991, Kealy *et al.* 1992; Meyer & Zentek 1992; Richardson & Toll 1993). Therefore, puppies should never be fed ad libitum and weight gain closely monitored.

## 5.2. Cats

Owing to the small variation in adult body weights, the energy needs of cats can be expressed per kg BW instead of per kg metabolic weight. In addition, if metabolic weight is used to calculate MER with the intra-specific allometric coefficient of 0.67 proposed by Heusner in 1991 should be used (NRC 2006a), which has recently been confirmed to be a more accurate than the 0.75 (Nguyen *et al.* 2001; Edtstadtler-Pietsch 2003).

The equation of 100 kcal (418 kJ) ME per kg<sup>0.67</sup> proposed by NRC 2006 corresponds with a daily energy intake of about 60-70 kcal (250-293 kJ) ME per kg body weight. Although NRC specifies that this equation is only valid for cats with a lean body condition, many lean cats may need less energy (Riond *et al.* 2003, Wichert *et al.* 2007). Particularly for neutered cats and cats living indoors energy requirements may be substantially lower. Therefore it is justified to recommend a range that starts at 80 kcal (335 kJ) ME per kg<sup>0.67</sup> [about 50-60 kcal (209-250 kJ) ME per kg body weight].

**Table 5. Average daily energy requirements of adult cats\***

Gender / Age	Kcal ME / kg <sup>0.67</sup>	kcal ME / kg BW
Intact male & female	80-100	50-70
Neutered male & female		35-55

\* NRC 2006 a & c, [Riond et al. 2003](#), [Wichert et al. 2007](#)

**Table 6: Average energy requirements during growth and reproduction in cats**

Kittens	Age	Times MER
	Up to 4 months	2.0-2.5
	4 to 9 months	1.75-2.0
	9 to 12 months	1.5
Queens	Reproduction phase	
<b>Gestation</b>		140 kcal/kg <sup>0.67</sup> BW
<b>Lactation</b>	< 3 kittens	MER + 18 x kg BW x L
	3-4 kittens	MER + 60 x kg BW x L
	> 4 kittens	MER + 70 x kg BW x L

L = 0.9 in weeks 1-2 of lactation; 1.2 in weeks 3-4; 1.1 in week 5; 1 in week 6; and 0.8 in week 7 (Loveridge 1986 and 1987, Rainbird 1988, Kienzle 1998, Dobenecker *et al.* 1998, Debraekeleer 2000; Nguyen *et al.* 2001, NRC 2006a & c)

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## ANNEX II – TAURINE

### Introduction

Taurine (2-Aminoethanesulfonic acid =  $\text{NH}_2\text{CH}_2\text{-CH}_2\text{-SO}_3\text{H}$ ) is a  $\beta$ -aminosulfonic acid rather than an  $\alpha$ -carboxylic amino acid (Huxtable '92). It was first isolated from the bile of the ox "Bos Taurus" and was named after it (Huxtable '92).

Dogs and cats exclusively use taurine to conjugate bile acids. In dogs the rate of taurine synthesis appears to be adequate to meet the needs, if their food contains adequate amounts of sulphur-containing amino acids. In cats, the ability to synthesize taurine is limited and insufficient to compensate for the natural losses via the conjugated bile acid (taurocholic acid) in the gastrointestinal tract. Hence taurine is an essential nutrient for the cat.

### 1 Cat

Taurine deficiency can lead to feline central retinal degeneration, dilated cardiomyopathy and reproductive failure. Taurine intake is considered to be adequate when plasma levels are greater than 50-60  $\mu\text{mol/L}$  (Pion *et al.* '87, Douglas *et al.* '91, ) or the whole blood concentration 200  $\mu\text{mol/L}$  or higher (Fox '00).

In the late 1980s, the feeding of commercial cat foods containing levels of taurine that were considered to be adequate [based on studies with purified diets (Burger *et al.* '82, NRC '86)] resulted in low plasma taurine levels in cats, and were associated with retinal degeneration and dilated cardiomyopathy (Pion *et al.* 1987).

Taurine is not degraded by mammalian enzymes, but is excreted as such in the urine or in the form of taurocholate or related bile acids via the gastrointestinal tract (Huxtable '92, Odle *et al.* '93). However, balance studies have indicated that taurine is can be degraded by the intestinal microflora (Morris *et al.* '94). The composition of the cat food, as well as the type of production process influence this intestinal degradation (Morris *et al.* '94). Hickman *et al.* showed that heat-processed cat foods resulted in lower taurine plasma levels and greater losses compared to the same food but frozen-preserved (Hickman *et al.* '90 & '92). This was the consequence of increased sensitivity of taurine to intestinal bacterial degradation owing to the heat processing (Morris *et al.* '94). For this reason the recommendation for taurine in canned cat food is higher than that for dry food or purified diets.

### 2 Dog

Healthy dogs synthesize sufficient taurine from dietary sulphur-containing amino acids such as methionine and cysteine. Nevertheless, low plasma or low whole-blood taurine levels may be seen in dogs fed non-supplemented very-low protein diets, or foods that are low in

sulphur-containing amino acids or with poor availability of the sulphur-containing amino acids (Sanderson *et al.* '01, Backus *et al.* '03). Feeding certain lamb and rice foods may increase the risk of a low-aurine status, because of lower bioavailability of sulphur-containing amino acids and increased faecal losses of taurine possibly caused by rice bran (Backus *et al.* '03, Delaney *et al.* '03, Fascetti *et al.* '03, Torres *et al.* '03).

In dogs, low plasma levels of taurine (<40µmol/L) may also predispose to dilated cardiomyopathy (Pion *et al.* '98). However, some breeds seem to be more sensitive to develop such side effects (Pion *et al.* '98), particularly Newfoundland dogs, in which the rate of taurine synthesis is decreased (Backus *et al.* '06). The addition of taurine to such foods or increasing the intake of the precursors of taurine (methionine and cysteine) can prevent such a decrease (Backus *et al.* '03, Torres *et al.* '03). In dogs, adequate levels of taurine are values greater than 40µmol/L in plasma and greater than 200µmol/L in whole blood (Elliott *et al.* 2000).

### 3 Conclusion

The taurine values for cats, stated in the tables on **pages 13, 14 and 15**, are starting points. Individual companies can have different levels of taurine in their products as long as they ensure that the products maintain adequate blood value in the cat's body (plasma levels should be greater than 50/60 µmol/l, > 200 µmol/L in whole blood). For dogs dietary taurine is not essential, since dogs can synthesize taurine from sulphur amino acids, therefore dog foods should be formulated to maintain adequate body reserves of taurine (> 40µmol/L in plasma and >200µmol/L in whole blood).

Analytical methods for taurine are given on page 28 (to be checked in the end).

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### ANNEX III – ARGININE

The arginine requirement increases with increased protein content owing to its role as an intermediate in the urea cycle. The NRC 2006 advises an extra 0.01 g arginine for every 1% increase in protein (% DM) above the recommended allowance for all life stages in dogs, and an extra 0.02 g arginine (dogs) for every 1% increase in protein for cats.

The following tables outline the arginine recommendations for various protein contents. All values are stated as g/100 g DM.

#### DOGS

Protein content	Arginine level			
	adult	Growth	Early growth	Reproduction
g/100g DM	g/100g DM	g/100g DM	g/100g DM	g/100g DM
18	0.52			
20	0.54	<b>0.69</b>		
<b>22</b>	<b>0.56</b>	<b>0.71</b>		
<b>22.5</b>	<b>0.57</b>	<b>0.72</b>	<b>0.79</b>	<b>0.79</b>
25	0.59	0.74	0.82	0.82
30	0.64	0.79	0.87	0.87
35	0.69	0.84	0.92	0.92
40	0.74	0.89	0.97	0.97
45	0.79	0.94	1.02	1.02
50	0.84	0.99	1.07	1.07
55	0.89	1.04	1.12	1.12

#### CATS

Protein content	Arginine level
	All life stages
g/100g DM	g/100g DM
25	<b>1.00</b>
28	<b>1.06</b>
30	<b>1.10</b>
35	<b>1.20</b>
40	<b>1.30</b>
45	<b>1.40</b>
50	<b>1.50</b>
55	<b>1.60</b>
60	<b>1.70</b>

## ANNEX IV – VITAMINS

Conversion factors - Vitamin source to activity					
Vitamin	Unit declared	Vitamin source used		Vitamin activity	
<b>Vitamin A</b>	<b>IU</b>			<b>Retinol activity</b>	
		vitamin A alcohol (retinol) <sup>2,3</sup>	0.3 µg	=	1 IU
			1.0 mg	=	3,333 IU
		vitamin A acetate	0.344 µg	=	1 IU
		vitamin A propionate	0.359 µg	=	1 IU
		vitamin A palmitate	0.55 µg	=	1 IU
		vitamin A alcohol (retinol)	1.0 µg	=	1 RE
		RE = Retinol Equivalent			
		Provitamin A (β-carotene) (dogs) <sup>4</sup>	1.0 mg	=	833 IU
<b>Vitamin D Cholecalciferol</b>	<b>IU</b>			<b>Vitamin D activity</b>	
		vitamins D <sub>3</sub> & D <sub>2</sub> <sup>1,3</sup>	0.025 µg	=	1 IU
			1 µg	=	40 IU
<b>Vitamin E - Tocopherol</b>	<b>IU</b>			<b>Vitamin E activity</b>	
		dl-α-tocopheryl acetate (all-rac-α-tocopheryl acetate)	1 mg	=	1 IU
		Bio-equivalence of various tocopherols:			
		d-α-tocopherol	1 mg	=	1.49 IU
		d-α-tocopherol acetate <sup>1</sup>	1 mg	=	1.36 IU
		dl-α-tocopherol	1 mg	=	1.10 IU
		dl-α-tocopheryl acetate	1 mg	=	1.00 IU
		dl-β-tocopherol	1 mg	=	0.33 IU
		dl-δ-tocopherol	1 mg	=	0.25 IU
		dl-γ-tocopherol	1 mg	=	0.01 IU
<b>Vitamin B1 - Thiamine</b>	<b>mg</b>			<b>Thiamine</b>	
		thiamine mononitrate	1 mg	=	0.92 mg
		thiamine hydrochloride	1 mg	=	0.89 mg
<b>D-Pantothenic acid</b>	<b>IU</b>			<b>Pantothenic acid</b>	

		calcium D-pantothenate	1 mg	=	0.92 mg
		calcium DL-pantothenate	1 mg	=	0.41 - 0.52mg
<b>Vitamin B6 - Pyridoxine</b>	<b>mg</b>				<b>Pyridoxine</b>
		pyridoxine hydrochloride	1 mg	=	0.89 mg
<b>Niacin</b>	<b>mg</b>				<b>Niamin</b>
		nicotinic acid	1 mg	=	1 mg
		nicotinamide	1 mg	=	1 mg
<b>Choline</b>	<b>mg</b>				<b>Choline</b>
		choline chloride (basis choline ion)	1 mg	=	0.75 mg
		choline chloride (basis choline hydroxyl-analogue)	1 mg	=	0.87 mg
<b>Vitamin K3 - Menadione</b>	<b>mg</b>				<b>Menadione</b>
		menadione sodium bisulphite (MSB)	1 mg	=	0.51 mg
		menadione pyrimidinol bisulphite (MPB)	1 mg	=	0.45 mg
		menadione nicotinamid bisulphite (MNB)	1 mg	=	0.46 mg

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## ANNEX V – ALLERGENS IN PET FOOD

### I. Introduction

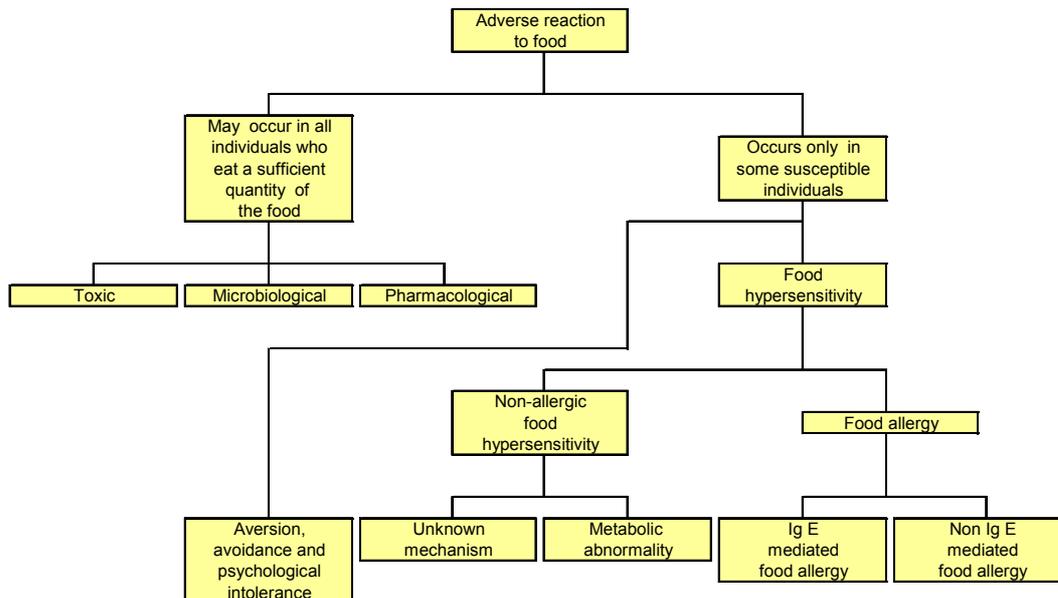
Adverse food reactions in cats and dogs are mainly expressed by pruritus and gastrointestinal signs. Acute anaphylactic reactions such as those seen in a minority of people who are allergic to nuts and some other foods have not been reported in relation to pet food.

### II. Definitions

#### 1. Adverse reactions to food

An adverse reaction to a food is an abnormal or exaggerated clinical response to the ingestion of a food or food additive. It may be immune mediated (called food allergy or hypersensitivity) or not immune mediated (called food intolerance) (Reedy *et al.* 1997).

A classification of adverse reactions to food



Source: ILSI Monograph Food Allergy 2003

#### 2. Food allergy

Allergy

immune-mediated reaction resulting in one or more of the clinical signs described under “IV. Adverse reactions to food in dogs and cats“

**Anaphylaxis** Anaphylaxis is an acute life-threatening multi-system allergic reaction resulting from exposure to an offending agent. In people, foods, insect stings, and medication are the most common causes (Tang 2003, Oswalt *et al.* 2007, Wang *et al.* 2007). The term has been variably employed to denote a defined IgE-mediated antigen-induced reaction or as a descriptive term delineating a severe, abrupt, untoward event of un-stated immunologic significance (Wasserman 1983).

### 3. Non-allergic food hypersensitivity

**Food idiosyncrasy:** a non-immune mediated reaction to a food component that causes clinical signs resembling an immune-mediated reaction to food (food allergy)

**Metabolic reaction:** Food intolerance. An adverse reaction caused by a metabolic defect (e.g. lactose intolerance).

### 5. All individuals susceptible if sufficient quantity eaten

**Toxic reaction:** Reaction to a toxic food component (e.g. onions)

**Microbiological reaction:** Reaction to a toxin released by contaminating organisms (e.g. mycotoxins)

**Pharmacologic reaction:** adverse reaction to a food as result of a naturally derived or added chemical producing a drug-like or pharmacological effect in the host such as methylxanthines in chocolate or pseudo-allergic reactions caused by high histamine levels in not well-preserved scromboid fish (tuna or salmon).

**Dietary indiscretion:** Adverse reaction resulting from such behaviour as gluttony, pica or ingestion of various indigestible materials or garbage.

## III. Food allergy in man

Food allergies are the single most common cause of generalised anaphylaxis seen in hospital emergency departments, accounting for about one third of cases seen (twice the number of cases seen for bee stings) (Sampson '99). It is estimated that about 100 fatal cases of food-induced anaphylaxis occur in the US each year (Sampson '99). The most common allergens causing anaphylaxis in people are nuts, shellfish, milk, egg white, legumes, certain fruits, grains, chocolate, and fish (Wasserman '83).

As far as we are aware of, cases of allergies in humans related to ingestion or contact with

pet foods are not reported in the literature.

#### **IV. Adverse reactions to food in cats and dogs**

The predominant clinical sign in dogs and cats (almost 100% of the cases) is pruritus (itching) (Rosser '90, White '86, White '89, Scott *et al.* 2001). The pruritus can be generalised or localised, sometimes being restricted to recurrent otitis. Other dermatological changes such as seborrhoea, recurrent pyoderma or *Malassezia* can be seen in allergic dogs (White '86, Scott *et al.* 2001). In allergic cats eosinophilic plaque, miliary dermatitis or alopecia caused by excessive grooming can be the only clinical sign present (White '86, Scott *et al.* 2001).

An estimated 10 to 15 % of the cases of food allergy in dogs and cats are believed to result into gastrointestinal (GI) signs such as: diarrhoea and vomiting (Scott *et al.* 2001). However, the GI signs can be very discrete (e.g. more frequent bowel movements (Scott *et al.* 2001) and their prevalence may be underestimated (Loeffler ).

In cats and dogs immune mediated reactions are seldom confirmed in practice. Therefore, the term adverse reactions to food is generally accepted and used for cats and dogs.

In dogs and cats, adverse reactions to food are only diagnosed through the elimination of the food component (eviction diet) following either dermatological or digestive symptoms (or both). Ideally this should be confirmed by a challenge (reintroduction of the suspected component) after clinical signs have disappeared when feeding the eviction diet. (Wills J. '94, Helm 2002)

Adverse reactions to food are deemed to account for about 1-5 % of all skin conditions in dogs and 1-6% of all feline dermatoses (animal presented to veterinary practices) (Reedy *et al.* '97). Most food ingredients have the potential to induce adverse reactions because they contain intact proteins.

Now, intact proteins are part of all products made by our industry including all pet foods (except special diets with hydrolysed proteins as the sole source of protein). All products containing intact protein can potentially cause allergic/adverse reactions in predisposed animals (ref 13). There are proteins against which dogs and cats seem to react more often (Wills '94). Milk, beef, eggs, cereals and dairy products are mentioned most often whereas more controlled studies mentioned wheat, soy, chicken and maize as the most important allergens. However, it is not always clear whether these data are taken over from human literature or not. In addition, the data do not always enable to see whether the high incidence is not simply the consequence of the fact that those proteins have been eaten more frequently by dogs and cats.

Through veterinarians, special diets made with selected protein sources or hydrolysed

proteins are available for dogs and cats suffering of adverse reactions to food; the formulation and the label declarations for those foods are regulated by the specific EU legislation on dietetic foods for animals.

## V. Conclusions

- Most protein containing ingredients have the potential to induce allergic reactions if they are regularly fed to dogs and cats.
- Anaphylactic reactions to food as seen in humans are not, as far as we know, reported in literature relating to cats and dogs. The hallmark of adverse reaction in dogs and cats to food is pruritus.

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## ANNEX VI – SPECIFIC FOOD HAZARDS

Annex VI provides some practical information about some common human foods (*such as raisins, grapes, onions, garlic and chocolate*) with documented adverse effects when given to dogs or cats either as a treat or when left over from the table are shared with pets. This annex lists signs that should alert pet owners and combines information that is not easily found in one place or has only been available recently. There may be other foods that are potentially hazardous when fed to dogs or cats, but they are not yet documented.

### 1. GRAPE AND RAISIN TOXICITY IN DOGS

#### Background

Since 1989 the Animal Poison Control Centre (APCC) of the American Society for the Prevention of Cruelty to Animals has recorded cases of poisoning in dogs that had eaten grapes (*Vitis* spp) or raisins. From April 2003 to April 2004 the APCC managed 140 cases, of which 50 dogs developed clinical signs and seven died (ASPCA, 2004). Cases have been reported in the USA and the UK (Eubig *et al.* 2005, Penny *et al.* 2003)

#### Clinical signs and pathology

Affected dogs typically suffer gastrointestinal upset followed by acute renal failure (ARF). The initial signs of grape or raisin toxicity are vomiting (100% of reported cases) followed by lethargy, anorexia, diarrhoea, abdominal pain, ataxia, and weakness (Eubig *et al.* 2005). In the majority of dogs, vomiting, anorexia, lethargy and diarrhoea occur within the first 24 hours of exposure, in some cases vomiting starts as early as 5 to 6 hours after ingestion (Eubig *et al.* 2005). The vomit and or faeces may contain partially digested grapes or raisins or swollen raisins. Classic signs of ARF can develop within 24 hours or up to several days later. These include substantial increases in blood urea and serum creatinine, as well as in the calcium x phosphorus product, serum phosphorus and later in total calcium level (Eubig *et al.* 2005). If the condition progresses, the dog eventually is unable to pass urine. At this stage the prognosis is generally poor and usually a decision is taken to euthanize the animal. The most consistent histopathological lesions reported were diffuse renal tubular degeneration, especially in the proximal tubules (Eubig *et al.* 2005). Mineralization of necrotic renal structures has been reported, but also tubular cell regeneration in some cases. Mineralization and/or congestion of extra-renal tissues and organs have also been observed (Eubig *et al.* 2005). It has to be pointed out, however, that many dogs never develop AFR

after ingestion of raisins or grapes.

### **Toxic agent**

The toxic agent (or agents) has so far defied detection. Analysis for a variety of substances has proved negative, including mycotoxins, heavy metals, pesticides and vitamin D<sub>3</sub> (AFIP 2003, Eubig *et al.* 2005). It is postulated that the cause may be a nephrotoxin or anaphylactic shock leading to renal problems (AFIP 2003). Excess sugar intake has also been suggested, resulting in a disturbance of sugar metabolism, but this seems unlikely as dogs are not known for susceptibilities to high sugar intake.

The poisoning seems to occur with grapes and raisins of all types: those purchased from a store or grown at home, grape pressings from wineries and seedless and seeded varieties (Eubig *et al.* 2005). Grape extract is not considered a threat; the grape or raisin itself has to be eaten for poisoning to occur (McKnight, 2005).

The lowest intake that has so far been reported to cause poisoning is around 2.8g of raisins per kg bodyweight (BW) and 19.6 g of grapes per kg BW; one dog became ill after only eating 10 to 12 grapes (Eubig *et al.* 2005). The severity of the illness does not seem to be dose-related (Eubig *et al.* 2005). Even a large dog of 40 kg may need to eat only 120 g to be at risk and as cartons of raisins typically contain 500g this amount could be ingested in one session. At present it appears that only dogs are affected – the susceptibility of other species is unknown.

### **Treatment**

Immediate treatment consists of inducing emesis and lavage of the stomach to remove the poison, followed by decontamination using activated charcoal to inactivate the remaining poison. Aggressive fluid therapy is essential to increase the chances of survival, and should be maintained long enough (at least 48 hours). Haemodialysis and diuretics such as furosemide have been recommended to treat the ARF and oliguria (McKnight, 2005), but do not seem to increase survival substantially (Eubig *et al.* 2005).

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## 2. CHOCOLATE TOXICITY

### Background

Cocoa poisoning was highlighted during the Second World War, when pigs, calves, dogs and horses were poisoned because by-products of cacao beans were used to supplement feeds as a result of a surplus.

Chocolate is palatable to most dogs, but it is not an innocent snack being relatively toxic. In dogs signs of toxicity may develop within hours after consumption.

In addition, chocolate cakes and other cocoa containing human foods are best avoided. It is not surprising that most accidents are reported during holiday periods such as Christmas and Easter (Campbell 2001). Chocolate treats specially developed for dogs are not toxic as they are made from ingredients that contain low or no theobromine.

No reports of chocolate poisoning in cats have been published to our knowledge, probably as a consequence of their different eating habits.

### Toxic agent

The principle toxic components of chocolate and cocoa products are the methylxanthine alkaloids, of which theobromine is the major toxin (Campbell 2001). As long ago as 1917, cacao bean shell intoxication in horses was attributed to theobromine by French researchers. Theobromine is particularly toxic to dogs, because its elimination is very slow compared with the rate in other species such as man (Hooser '84, Glauberg '83). The half life of theobromine in dogs is about 17.5 hours (Farbman 2001, Hooser & Beasley '86). Theobromine undergoes enterohepatic recirculation resulting in an accumulative effect (Campbell 2001, Farbman 2001). As a consequence, repeated intakes of smaller (non-toxic) quantities may still cause intoxication. The slow elimination of theobromine is also responsible for decreased survival rate in affected dogs and death may still occur at a stage when clinical signs are already attenuating (Strachan & Bennett '94).

Caffeine is another methylxanthine present in cocoa products, and may contribute to the toxicity. However, the levels of caffeine in cocoa products are much lower than those of theobromine and the half life is much shorter (4.5 hours) (Farbman 2001, Hooser & Beasley '86).

The LD<sub>50</sub> of theobromine has been reported to be between 250mg and 500mg per kg body weight (BW); lethal cases have been seen when dogs ingested amounts of chocolate that reflect an estimated theobromine intake of 90-115 mg/kg BW (Glauberg '83, Hooser & Beasley '86, Carson TL 2001).

The level of theobromine content of chocolate varies, with dark chocolate containing the highest level (TABLE 1). Unsweetened baking chocolate should definitely be kept out of reach of dogs, since it contains up to 20 mg of theobromine per gram. Dogs also voluntarily eat cocoa powder, in which the average theobromine level varies from 10 to 30 mg/g (Sutton '81). About four grams of cocoa powder per kg BW may be sufficient to kill a dog (Faliu '91). Increasingly cocoa shell mulches are used to prevent weeds and for landscaping in gardens. They are often attractive to dogs because of the chocolate smell and therefore may be a potential cause of theobromine poisoning (Hansen *et al.* 2003).

**Table 1.** Theobromine content of different types of chocolate and cocoa products (mg/g) (Farbman DB 2001, Gwaltney-Brant S. 2001, Hansen *et al.* 2003, Shively *et al.* 1984, Carson 2001)

White chocolate	0.009 - 0.035	Cocoa powder	4.5 – 30
Milk chocolate	1.5 – 2.0	Cocoa beans	10 – 53
Sweet to Semisweet dark chocolate	3.6 – 8.4	Cocoa shell mulches	2 – 30
Bitter chocolate, chocolate liquor, baking chocolate	12 – 19.6	Coffee beans	0

### Clinical signs

In dogs methylxanthines cause stimulation of the central nervous system with tachycardia (fast heart beating), respiratory stress and hyperactivity (Campbell 2001, Farbman 2001). The clinical signs include vomiting, diarrhoea, agitation, muscular tremors and weakness, cardiac arrhythmias, convulsions, and, in severe cases, renal damage, coma and death (Glauberg '83, Decker '72, Nicholson '95, Farbman 2001, Hooser & Beasley '86). Death may occur within six to 15 hours after intake of excessive amounts of chocolate or cocoa products (Glauberg '83, Decker '72, Drolet *et al.* '84).

At necropsy, congestion in liver, kidneys, pancreas and the gastro-intestinal tract are seen, as well as unclotted haemorrhagic fluid in peritoneal and thoracic cavities (Sutton '81, Strachan & Bennett '94).

### Treatment

No specific antidote is available for theobromine, only symptomatic treatment. In order to minimise the absorption of theobromine vomiting can be induced immediately after ingestion. Subsequently lavage can be applied with warm water to keep the chocolate liquid. Repeated

doses of activated charcoal can then be used to bind the remaining material and prevent further absorption and increase excretion (Glauber '83, Hooser & Beasley '86, Farbman 2001, Carson 2001).

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### 3. TOXICITY OF ONIONS AND GARLIC IN CATS & DOGS

#### Background

It has been known since 1930 that dogs are very sensitive to onions (*Allium* spp) whether raw, cooked or dehydrated.

#### Clinical signs and pathology

Regenerative anaemia with marked Heinz body formation has been reported in cats and dogs after eating onions or onion containing foods (Harvey *et al.* '85, Kaplan '95, Robertson *et al.* '98, Spice '76, Tvedten *et al.* '96,). Consumption of a sufficient amount of onions leads to oxidative injury of the lipid membrane of the erythrocytes and irreversible oxidative denaturation of haemoglobin. This results in formation of Heinz bodies, eccentrocytes (red blood cells with haemoglobin clustering at one side of the cell), haemolytic anaemia, haemoglobinuria, increased serum bilirubin and possibly methaemoglobinaemia (Faliu '91, Cope '05, Harvey *et al.* '85, Kaplan '95, Lee *et al.* '00, Robertson *et al.* '98, Means '02). Relatively small amounts of fresh onions (5 to 10 g/kg BW) can already be toxic (Faliu '91, Cope '05). Robertson *et al.* '98 showed that effect was dose dependent.

The clinical signs are secondary to the anaemia and include pale mucous membranes, tachycardia, tachypnoea, lethargy and weakness (Gfeller & Messonier '98, Cope '05). Vomiting, diarrhoea and abdominal pain may also be present. If only a moderate amount of onions has been eaten, the Heinz body anaemia resolves spontaneously after discontinuing the onions (Kaplan '95, Robertson *et al.* '98). In more severe cases, icterus and renal failure can be seen as a consequence of the haemolysis and haemoglobinuria respectively, and possibly death (Ogawa *et al.* '86, Cope '05).

Although onion ingestion has been reported as being the most common cause of Heinz body haemolysis in dogs (Weiser '95), it may be difficult to correlate clinical signs with the onion ingestion because of the lag of several days before the onset of clinical signs (Weiser '95, Cope '05).

Although onion poisoning is more common in dogs, cats are more sensitive to onion and garlic poisoning owing to their specific haemoglobin structure, making them more susceptible to oxidative stress (Giger '00).

Garlic and Chinese chives have also been reported to cause the development of Heinz bodies, eccentrocytes<sup>a</sup>, haemolytic anaemia and increases in methaemoglobin levels in dogs (Lee *et al.* '00, Yamato *et al.* '05). Lee *et al.* reported toxic effects after administration 1.25 ml of garlic extract per kg BW (equivalent to 5g/kg BW of whole garlic) for 7 days, this is similar to the amounts reported in onion poisoning.

The increase in reduced glutathione (G-SH), which has been reported after ingestion of

onions and garlic, may seem inconsistent with oxidative damage, but the increase can be a compensatory rebound reaction after an initial decrease in G-SH and other body antioxidants, and an increase in oxidised glutathione (GSSG) within the first few days (Yamato '92, Ogawa *et al.* '86).

Dogs with hereditary high erythrocyte concentrations of reduced glutathione and potassium appear to be more sensitive to onion and garlic poisoning (Yamato *et al.* '92).

Wild onions (*A. validum* & *A. Canadense*), and wild garlic (*A. ursinum*) have caused haemolytic anaemia in horses and ruminants (Lee *et al.* '00) and are potentially toxic for dogs and cats as well.

### Toxic agents

Several organo-sulfoxides have been implicated in toxicity induced by onions and garlic (TABLE 2). Miyata reported the extraction from onions of an unnamed phenolic compound causing similar effects on red blood cells "*in vitro*" (Miyata '90). Allicin, a compound found in garlic, is similar to *n*-propyl disulfide found in onions (Gfeller & Messonier '98).

These organosulfur compounds are readily absorbed in the gastrointestinal tract and metabolised to highly reactive oxidants (Cope '05).

**Table 2.** Compounds isolated from onions and garlic and reported to oxidise canine erythrocytes. (Chang *et al.* '04, Fenwick '84, Hu *et al.* '02, Yamato *et al.* '98, Yamato *et al.* '03)

Onions	Garlic
<i>n</i> -propyl disulfide	sodium 2-propenyl thiosulfate
<i>n</i> -propyl	bis-2-propenyl trisulfide
3 different sodium alk(en)yl thiosulfates	bis-2-propenyl tetrasulfide
e.g. sodium <i>n</i> -propyl thiosulfate	bis-2-propenyl pentasulfide
<i>trans</i> -1-propenyl thiosulfate	bis-2-propenyl thiosulfonate
<i>cis</i> -1-propenyl thiosulfate	several sulphur containing esters

### Treatment

No specific antidote exists, and the treatment is supportive and is intended to reduce the oxidative effects and to prevent renal damage caused by haemoglobinuria. Oxygen therapy, fluid therapy (particularly crystalloids) and blood transfusion have been recommended (Gfeller & Messonier '98). Induction of vomiting can be useful within the first hour after ingestion of onions if the patient does not yet show clinical signs (Gfeller & Messonier '98). Anti-oxidant vitamins such as vitamins E and C may have subclinical beneficial effects that

help in milder cases, but a study in cats did not show a significant effect on the formation of Heinz bodies (Hill *et al.* '01).

<sup>a</sup> Eccentrocytes are red blood cells with haemoglobin clustering at one side of the cell, which makes these cells more susceptible to lysis than normal red blood cells.

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## ANNEX VII – PRODUCT FAMILIES

1. Product families are considered within a company.
2. Product families are defined by animal species (dogs/cats).
3. All products within a family must be of the same processing type (extruded, baked, pelleted, canned, fermented, etc.) and within the same moisture content category (*dry, semi-moist and wet*).
4. A product family refers to complete or complementary foods.
5. A product family has to refer to a specific life stage, a specific life style or a specific animal size.
6. The product family members must meet the metabolizable energy (ME) density (as it is described in the specific chapter of these Guidelines) of the lead product members and be formulated on an ME basis to :
  - a. meet the nutrient levels of the lead family product for key nutrients, and
  - b. not exceed the maximum levels of any nutrient or nutrient ratio established in the fediaf Nutritional Guideline or by law.

**N.B.** When analyses are performed, the same analytical methods must be used for all products belonging to the product family.