Protein to dogs and cats: the minimum and the ideal?

What have we learned and how it informs change

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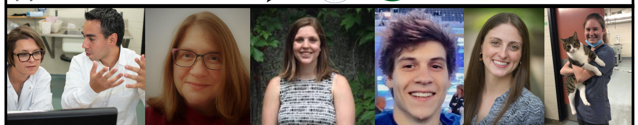
My funders and team



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Rethinking guidance on protein and amino acid content in diets intended for dogs and cats

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Protein quality

Protein quality is dependent upon:

1. Protein content
2. Amino acid composition
3. Protein digestibility and amino acid availability

compared to the requirements of the species of interest.

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Protein quality

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Range in CP and AA content

Source	% CP	LYS	MET	ARG
Milk (skim dried)	~35	2.86	0.92	1.24
Poultry BP meal	~64	3.32	1.11	3.94
Meat and bone meal	~52	2.51	0.68	3.45
Fish meal (white meal)	~63	4.51	1.76	4.04
Soybean meal	~44	2.83	0.61	2.83
Rice (grain, polished and broken)	~7.5	0.30	0.18	0.30
Corn gluten meal	~60	1.02	1.43	1.02
RANGE	**7.5-64%**	**0.30-4.51**	**0.18-1.76**	**0.30-4.04**

% of ingredient on an as fed basis.

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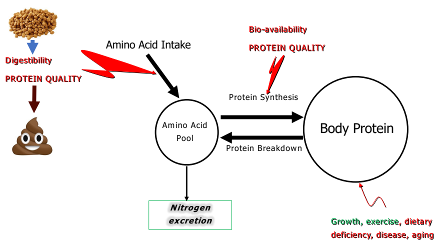
Model of EAA metabolism and protein turnover



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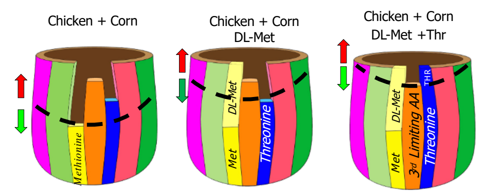
The limiting amino acid theory



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Protein quality

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compared to the requirements of the species of interest.

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What can affect protein digestibility and availability?

- Although an ingredient may have sufficient total (crude) protein and a good amino acid profile to support whole body protein turnover, the use of protein from that ingredient may also be limited by:
  - Digestibility
  - Metabolic availability
  - Competitive inhibition

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Digestibility of current ingredients in pet food

- Animal proteins are not necessarily superior to plant proteins
- Processing affects bioavailability of ingredients differently
- We need to consider more than the digestibility of protein and ratio of amino acids, but secondary outcomes
- While other animal nutrition sectors have values for ingredient digestibility that are additive, we do not have the same in pet.

We need to understand how ingredients interact and what the bioavailability of the final product is.

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Protein quality

Protein quality is dependent upon:

1. Protein content
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3. Protein digestibility and amino acid availability

compared to the requirements of the species of interest.

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Study	Animals	Diet approach	Outcome	> 14 weeks NRC (MR)	Adult maintenance AAFCO (min)
Methionine and cysteine	Puppies and/or growing dogs = **OVER**	Titration with synthetic or semi-synthetic diets = **UNDER**	Weight gain or nitrogen retention = **UNDER**	2.1 g/kg for both Met and Cys	3.3 g/kg Met and 3.2 g/kg Cys
Lysine				5.6 g/kg	6.3 g/kg
Isoleucine				4.0 g/kg	3.8 g/kg
Leucine				6.5 g/kg	6.8 g/kg

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Which life stage? Which life style? Which breed?

Begging the question: Who do we target? What do we need to know to make good decisions?

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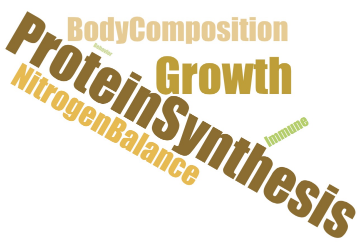
We need multiple approaches and outcomes when considering AA



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Minimum methionine requirements

Consideration #1: Met requirements will differ among small, medium and large breed dogs

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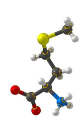
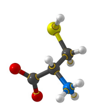
Minimum methionine requirements

- Methionine
  - A dietary indispensable amino acid
  - Minimum Met requirement must be met
  - Needed for protein synthesis, as a methyl (CH_3) donor (L-carnitine, creatine, choline etc.), and for the synthesis of cysteine and further metabolites



- Cysteine
  - A conditionally indispensable amino acid
  - Spares the requirement for methionine
  - Needed for protein, taurine, glutathione and sulfate synthesis



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Study	Animals	Diet approach	Outcome	> 14 weeks NRC (MR)	Adult maintenance AAFCO (min)
Methionine and cysteine	1. Immature Beagles 2. Immature Beagles 3. English pointer puppies	Simple titration	Weight gain: 2.1 g/kg Met and 3.5 g/kg Cys Weight gain: 2.0 g/kg Met and 1.9 Cys Weight gain: 2.3 g/kg Met and 2.2 Cys	2.1 g/kg for both Met and Cys = 4.2 g/kg TSAA Long term commercial diet sufficiency= 3.2 g/kg Met and 3.0 g/kg Cys= 6.2 g/kg	3.3 g/kg Met and 3.2 g/kg Cys = 6.5 g/kg

No studies to examine whether requirements differ due to:
- Breed differences in sulfur amino acid metabolism
- Interaction among nutrients, most importantly how different ratios of SAA and amounts of cofactors may alter Met and Cys requirements or how they alter taurine synthesis
- Emerging data on how dietary fibre may alter SAA requirements and secondary metabolites

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Minimum methionine requirements (Mansilla et al. 2020)

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	AAFCO	FEDIAF (110 kcal/kg$^{0.75}$)	NRC		Miniature Dachshunds		Beagles		Labrador Retrievers		Beagles and Labradors (pooled data)	
			MR	RA	MR	CL	MR	CL	MR	CL	MR	CL
g/100 g DM	0.33	0.40	0.26	0.33	[0.21-0.26]	0.304	0.338	0.458	0.360	0.517	0.360	0.482
g/Mcal ME	0.83	1.00	0.65	0.83	[0.57-0.70]	0.822	0.914	1.238	0.973	**1.397**	0.973	1.303
mg/kg BW					[35.7-45.0]	51.6	57.5	77.9	50.4	72.4	56.0	75.8
mg/kg BW^0.75			85	110	--	--	107.7	147.8	121.8	159.6	118.4	150.5
g/Mcal ME										0.787[1]		

[1] Harrison et al. 2020

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Minimum methionine requirements (Mansilla et al. 2020)

TAKE HOME:
- Beagles and Labrador retrievers have greater SAA requirements than Miniature Dachshunds
- The SAA requirement may be greater than recommendations from AAFCO and FEDIAF, but the total SAA requirement needs to be measured
- Greater diet consumption may reduce the SAA requirement

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Plasma SAA concentrations (Mansilla et al. 2020)

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AA, µM	Breed	Dietary Met, % (n=4)										
		0.21	0.26	0.31	0.36	0.41	0.46	0.66	SEM[1]	Breed	Met	Interaction
Cystine	Dachshunds	84.6	67.6	93.1	49.0	66.2	23.3	45.7	34.0	0.276	0.511	0.794
	Beagles	46.1	43.2	41.7	34.5	42.4	44.2	36.2	6.9			
	Labradors	45.4	52.4	33.5	39.5	29.8	33.0	40.2	7.1			
Methionine	Dachshunds	26.1	23.1	34.7	43.9	52.4*	63.4*	63.2*	7.0	<0.001	0.016	0.125
	Beagles	141.0	220.6	199.3	238.5	304.3	222.8	344.9*	51.0			
	Labradors	280.6	285.0	147.6	224.8	194.1	252.2	347.6	41.0			
Taurine	Dachshunds	266.1	227.0	195.6	238.5	226.0	217.6	208.7	25.5	<0.001	0.243	0.882
	Beagles	175.9	159.4	141.4	176.6	184.5	192.2	177.1	24.0			
	Labradors	133.8	131.3	110.1	126.9	119.3	116.2	127.4	12.8			

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Plasma SAA concentrations (Mansilla et al. 2020)

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TAKE HOME:
- Despite being fed the EXACT same diets at similar intakes, fed state plasma amino acid concentrations of methionine and taurine differed significantly among breeds and did not differ among dietary treatments
  - Note: This was a short-term diet acclimation and may not have been sufficient to upregulate taurine synthesis
- Labrador retrievers have significantly greater methionine concentrations and lower taurine concentrations compared with Beagles and Miniature Dachshunds, suggesting fundamental differences in SAA metabolism among breeds

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Amino acid requirements (g/100g DM) are likely greater than AAFCO minimum requirements

	AAFCO/NRC RA	Small	Medium	Large
Phenylalanine (Shoveller et al, 2018 JAS)	0.44/ 0.45	0.39	0.40	0.50 ↑
Tryptophan (Templeman et al, 2019 JAS)	0.16/0.14	0.18	0.26	0.20 ↑
Threonine (Mansilla et al, 2020 JAS)	0.48/0.43	--	0.60	0.57 ↑
Lysine (Sutherland et al, 2020, TAS)	0.63/0.35	--	0.58	↑
Methionine (Mansilla et al, 2020, JAS)	0.33/0.33	0.34	0.50	0.57 ↑

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Taurine losses

Consideration #2: Inclusion of pulse ingredients will increase fecal taurine losses due to losses of bile acids

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A commercial grain-free diet does not decrease plasma amino acids and taurine status but increases bile acid excretion when fed to Labrador Retrievers

Renan A. Donadelli,[†,ⓘ] Julia G. Pezzali,[†] Patricia M. Oba,[‡,ⓘ] Kelly S. Swanson,[‡,ⓘ] Craig Coon,[ǁ] Jessica Varney,[ǁ] Christine Pendlebury,[$] and Anna K. Shoveller[†,1,ⓘ]

Similar data with feeding pulses:

Pezzali et al. (2020) Dogs fed GF (potatoes, peas and tapioca starch) or GB (sorghum, spelt, millet) had similar SAA status, but GF had greater fecal losses of primary bile acids

Reilly et al. (2020) Precision-fed rooster was used to quantify nutrient digestibility of black beans, grits, garbanzo beans, green lentils, navy bean powder, and yellow peas. The AA were highly digestible with the exception of methionine, which was commonly the limiting AA in all pulses.

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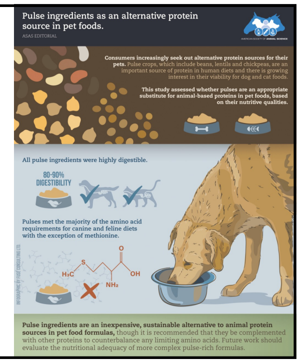
Infographic prepared after the comprehensive assessment of digestibility across pulses ingredients using the caecectomized rooster and the previous work looking at digestibility of animal and protein based ingredients.

Summary: Pulses are highly digestible protein sources, but are limited and unbalanced in some AA, particularly methionine.



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The effects of supplemental taurine, methionine and methyl compounds on SAA metabolism

Consideration #3: Dietary supplementation of taurine, methionine or methyl compounds will alter SAA metabolism

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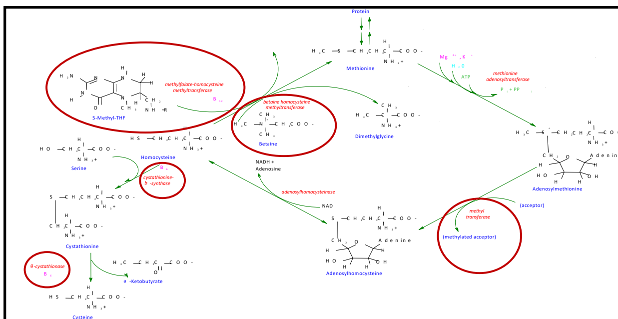

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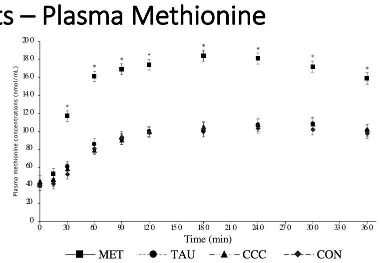
Results – Plasma Methionine



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Results – WB Taurine

- WB Tau concentrations tended to be higher in dogs fed TAU (255 ± 6.1 nmol/mL) compared to dogs fed CCC (241 ± 6 nmol/mL ; P<0.10)

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Results – Plasma Taurine

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Summary:

Methionine is equally effective as a combination of L-carnitine, choline and creatine at supporting taurine status, and less effective than taurine. The methyl compounds likely spare the use of methionine for methylation and allow methionine to enter the transsulfuration pathway.

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Therefore, if we had data on:

- The amount of protein present,
- The amino acid profile of that protein,
- The relative amounts of anti-nutritional factors and their effects on digestibility and metabolic availability,
- The AA requirement of dogs and cats that we want to target;

then we could determine the exact amount of available protein in a given ingredient and use these to formulate optimum diets for dogs and cats.

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How could we add protein quality metrics? EASY, we are doing this for human food, so let's do it for pet food!

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Predictions of protein quality using PDCAAS approaches for raw meat (Kerr et al.2013)

Amino acid score (AAS), protein digestibility-corrected AA score (PDCAAS), and first limiting AA (LAA) of beef-, bison-, elk-, and horsemeat-based raw diets fed to domestic and captive exotic felids using domestic cat life stage minimal requirements for growth of kittens as reference values[1]

Diet	AAS	PDCAAS	LAA
Beef	89	85	Met + Cys
Bison	81	75	Met + Cys
Elk	95	90	Met + Cys
Horse	87	80	Met + Cys

1 NRC (2006).

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Predictions of protein quality using PDCAAS approaches for insects (Bosch et al.2013)

Table 1. Proximate composition (percentage of DM), indispensable amino acid composition (percentage of CP) and amino acid (AA) score of insect and reference substrates

	Insect substrates										Reference substrates		
Parameter	HFp	BSFl	BSFp	HC	YMW	LMW	MW	SSR	DHCR	ACR*	PMM	FM	SBM
CP	62·5	56·1	52·1	70·6	52·0	64·8	47·0	66·3	65·0	64·4	69·1	71·0	51·6
Fat	19·2	12·8	19·7	17·7	33·9	22·2	39·6	25·1	22·0	24·5	12·8	9·2	2·5
Ash	5·6	12·6	13·9	5·3	3·9	4·1	3·0	3·6	3·9	4·4	15·4	19·9	6·8
AA													
Arg	4·2	3·7	4·2	5·7	4·6	4·8	4·6	3·6	3·9	3·5	5·8	4·5	6·3
His	4·8	4·4	4·7	3·4	5·1	4·9	4·8	4·3	4·6	4·5	3·7	3·4	3·1
Ile	4·0	4·0	4·2	4·0	4·6	4·6	5·0	3·4	3·7	3·2	3·8	4·8	5·0
Leu	6·1	6·1	6·5	6·6	7·3	6·7	7·2	5·4	5·9	5·3	6·4	7·1	7·8
Lys	6·2	5·4	5·4	5·8	5·5	6·5	5·3	4·3	4·7	4·0	5·6	7·4	6·2
Met	2·6	1·4	1·7	1·6	1·4	1·3	1·6	1·3	1·2	1·3	1·0	1·9	2·0
Phe	5·2	3·1	3·3	3·2	3·4	3·9	3·7	2·6	2·7	2·7	3·3	3·5	5·2
Thr	3·8	3·6	3·6	3·6	4·0	4·0	4·1	3·1	3·3	3·1	3·6	4·0	3·9
Val	5·0	5·5	5·7	5·7	6·3	5·9	6·5	5·6	6·1	5·4	4·6	5·0	5·0
tIAA	41·8	37·1	39·3	39·6	42·3	42·7	42·7	33·5	36·2	33·1	37·8	41·5	44·4
AA scores†													
Dog	94·0	63·4	74·4	69·3	68·4	60·4	73·8	53·0	55·5	59·7	44·6	73·1	89·1
Cat	106·1	79·2	93·0	86·6	85·5	75·5	92·2	66·2	69·4	74·6	55·8	91·6	107·5

CP, crude protein; HFp, housefly pupae; BSFl and BSFp, black soldier fly larvae and pupae; HC, house cricket; YMW, yellow mealworm; LMW, lesser mealworm; MW, Morio worm; SSR, six spot roach; DHC, death's head cockroach; ACR, Argentinean cockroach; PMM, poultry meat meal; FM, fish meal; SBM, soyabean meal; tIAA, total indispensable amino acids.
*Females.
†Calculated as described in Kerr *et al.*[13] using minimal requirements for growth of kittens and puppies[14] as reference values.

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Table 1. Amino acid score[1], most limiting amino acid, and nitrogen excess[2] for a selection of **animal**-based protein-containing ingredients potentially used for companion animal diet formulation.

Species	Ingredient	Product type	Category	AAS[3]	LAA	N excess (g)
Beef	Tripe	Primary	Viscera	122.78	Thr[4]	5.34
Beef	Heart	Primary	Viscera	124.76	SAA	4.80
Beef	Kidney	Primary	Viscera	108.08	Trp	4.29
Beef	Liver	Primary	Viscera	91.44	Trp	3.85
Beef	MBM[5]	By-product	Meal	80.92	Trp	2.66
Beef	MS[6] Meat	By-product	Meat	97.43	Thr	1.81
Beef	Blood meal	By-product	Meal	82.99	Met	8.63
Beef	Loin	Primary	Meat	100.15	Thr	4.31
Beef	Shank	Primary	Meat	111.59	Trp	5.45
Chicken	Gizzard	Primary	Viscera	124.23	Trp	5.49
Chicken	Poultry Meal	By-product	Meal	109.13	Trp	4.15
Chicken	Liver	Primary	Viscera	95.34	Trp	3.71
Chicken	Heart	Primary	Viscera	105.42	Trp	3.39
Chicken	MS Meat	Primary	Meat	105.66	AAA	1.85
Chicken	Drumstick	Primary	Meat	108.15	Trp	4.42
Chicken	Thigh	Primary	Meat	101.48	Trp	2.90
Chicken	Breast	Primary	Meat	93.56	Phe	6.20
Chicken	Feather meal	By-product	Meal	97.30	Trp	6.27
Chicken	Necks and backs	Primary	Viscera	91.09	Thr	0.78
Pork	Kidney	Primary	Viscera	114.73	Thr	5.78
Pork	Liver	Primary	Viscera	117.65	Thr	5.14
Pork	Meat and bone meal	By-product	Meal	82.40	Trp	3.00
Pork	MS Meat	Primary	Meat	103.30	SAA	1.78
Pork	Loin	Primary	Meat	105.45	Trp	6.24
Pork	Shank	Primary	Meat	118.70	Trp	4.20
Lamb	Loin	Primary	Meat	110.55	Trp	5.36
Lamb	Shank	Primary	Meat	110.18	Trp	3.56
Lamb	Kidney	Primary	Viscera	101.80	SAA	4.72
Lamb	Liver	Primary	Viscera	103.19	SAA	4.19
Lamb	MS Meat	Primary	Meat	132.16	Trp	2.21
Lamb	Meal	By-product	Meal	79.54	SAA	2.45

[1]Amino acid score calculated as described in Kerr et al., (2013) using minimal requirements (mg/g crude protein) for growth of puppies <14 weeks of age (NRC, 2006) as reference protein pattern.
[2]Nitrogen excess (g) calculated using recommended allowance (g/100g dry matter) for growth of puppies <14 weeks of age (NRC, 2006) as reference protein pattern.
[3]AAS, amino acids score; LAA, limiting amino acid; N, nitrogen.
[4]Thr, threonine; SAA, sulphur amino acids (methionine + cystine); Trp, tryptophan; Met, methionine; AAA, aromatic amino acids (phenylalanine + tyrosine); Phe, phenylalanine.
[5]MBM, meat and bone meal.
[6]MS, mechanically separated.

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Table 2. Amino acid score[1], most limiting amino acid, and nitrogen excess[2] for a selection of **plant**-based protein-containing ingredients potentially used for companion animal diet formulation.

Variety	Ingredient	Product type	Category	AAS[3]	LAA	N excess (g)
Grain	Whole barley	Primary	Intact	95.42	Thr[4]	0.28
Grain	Canola meal	By-product	Meal	65.76	Phe	1.93
Grain	DGS[5] corn	By-product	Milled	72.46	Trp	1.10
Grain	Corn germ meal	By-product	Meal	97.51	Thr	0.99
Grain	Corn gluten meal	By-product	Meal	41.76	Lys	4.45
Grain	Whole corn	Primary	Intact	73.18	Lys	0.42
Grain	Corn meal	By-product	Meal	74.04	Lys	0.36
Grain	Whole flaxseed	Primary	Intact	65.13	AAA	0.95
Grain	Flaxseed meal	By-product	Meal	70.36	AAA	1.75
Grain	Whole millet	Primary	Intact	82.21	Lys	0.50
Grain	Whole oat	Primary	Intact	96.36	Thr	0.38
Grain	Whole brown rice	Primary	Intact	112.47	Trp	0.33
Grain	Whole rye	Primary	Intact	47.78	Phe	0.50
Grain	Whole sorghum	Primary	Intact	61.04	Lys	0.41
Grain	Whole wheat flour	By-product	Milled	70.73	Lys	0.44
Grain	Wheat germ	By-product	Milled	115.65	Thr	0.96
Grain	White wheat flour	By-product	Milled	57.19	Lys	0.38
Grain	Wheat middlings	By-product	Milled	74.64	Thr	0.54
Legume	Whole green lentils	Primary	Intact	36.76	Trp	1.22
Legume	Soybean hulls	By-product	Milled	78.68	Met	0.23
Legume	Soybean meal	By-product	Meal	92.50	Met	3.34
Legume	Whole lupin bean	Primary	Intact	19.55	Phe	2.17
Legume	Whole fava bean	Primary	Intact	24.88	Phe	1.73
Legume	Whole kidney bean	Primary	Intact	44.97	AAA	1.40
Legume	Whole garbanzo bean	Primary	Intact	88.50	Trp	0.83
Legume	Whole green pea	Primary	Intact	65.32	SAA	0.92
Legume	Whole yellow pea	Primary	Intact	66.60	Met	0.61
Legume	Whole field pea	Primary	Intact	30.63	Phe	1.57

[1]Amino acid score calculated as described in Kerr et al., (2013) using minimal requirements (mg/g crude protein) for growth of puppies <14 weeks of age (NRC, 2006) as reference protein pattern.
[2]Nitrogen excess (g) calculated using recommended allowance (g/100g dry matter) for growth of puppies <14 weeks of age (NRC, 2006) as reference protein pattern.
[3]AAS, amino acids score; LAA, limiting amino acid; N, nitrogen.
[4]Thr, threonine; Phe, phenylalanine; Trp, tryptophan; Lys, lysine; AAA, aromatic amino acids (phenylalanine + tyrosine); Met, methionine; SAA, sulphur amino acids (methionine + cystine).
[5]DGS, distiller grain with solubles.

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As an industry, we can also apply a PDCASS or DIAAS type approach for complete diets and set criteria to categorize diets as a source of protein, a good source of protein or an excellent source of protein based off of nitrogen digestibility and protein and amino acid content.

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Foods	Canada claim	US Claim
Milk, whole	Excellent Source	Good Source
Eggs, hard-boiled	Good Source	Good Source
Chicken breast	Excellent Source	Excellent Source
Baked beans	No claim	No claim
Black beans	No claim	Good Source
Chickpeas	No claim	Good Source
Green Lentils	No claim	Good Source
Split Yellow Peas	No claim	No claim
Soy-based Tofu	Good Source	Good Source

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What should we do?

Data needed:

- Protein and AA requirements of dogs and cats under a variety of conditions to establish a benchmark
- The effects of essential and non-essential nitrogen on health and well being
- The interaction among dietary nutrients * species/breed * physiological state

A path forward with the consumer:

- What is the obligation of marketing and advertising in next generation products?
- *What will be the food industry's story in 10 years?*

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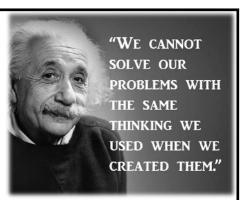
"If I had an hour to solve a problem, I would spend 55 minutes thinking about the problem and 5 minutes thinking about solutions."
Albert Einstein



Fall in love with the problem, not the solution.
Don White

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