Is the use of legumes safe in pet food?

Anna Kate Shoveller, PhD, PAS
Champion Petfoods Chair in Nutrition, Physiology
University of Guelph Research Leadership Chair

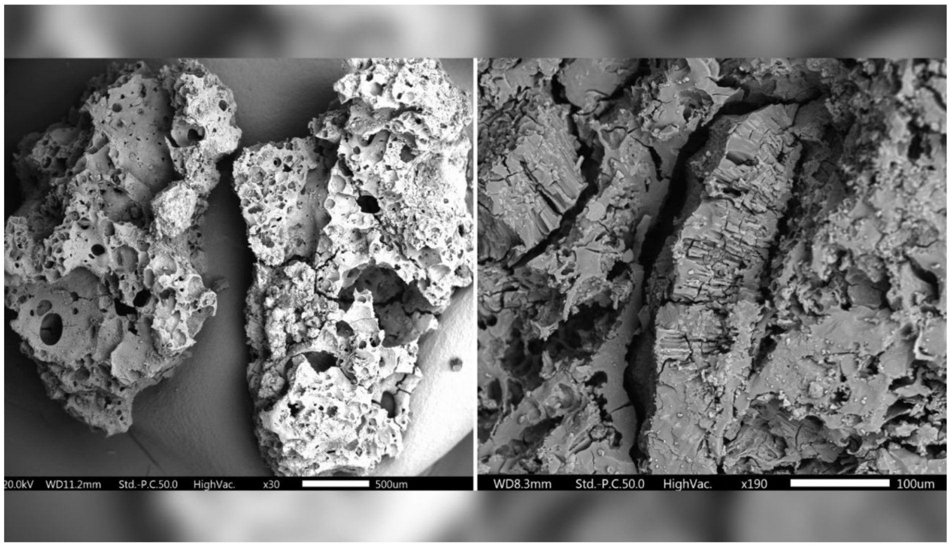
UNIVERSITY OF GUELPH

You can have definitive nutrient targets, but you need ingredients in order to meet these.

GLOBAL INGREDIENT SUPPLY

Neanderthals cooked meals with pulses 70,000 years ago

By Katie Hunt, CNN
Updated 7:46 PM EST, Tue November 22, 2022

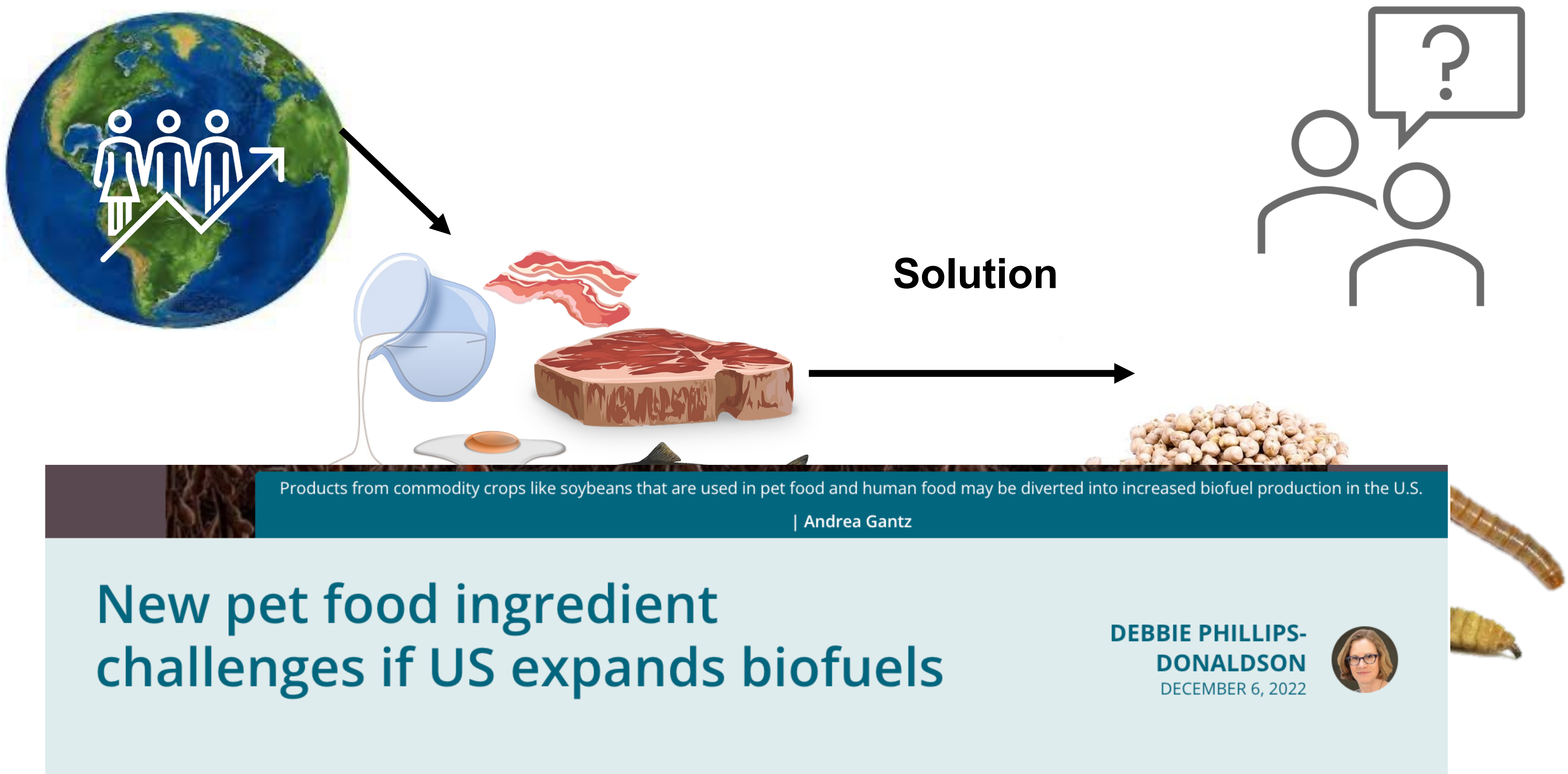


Ceren Kabukcu

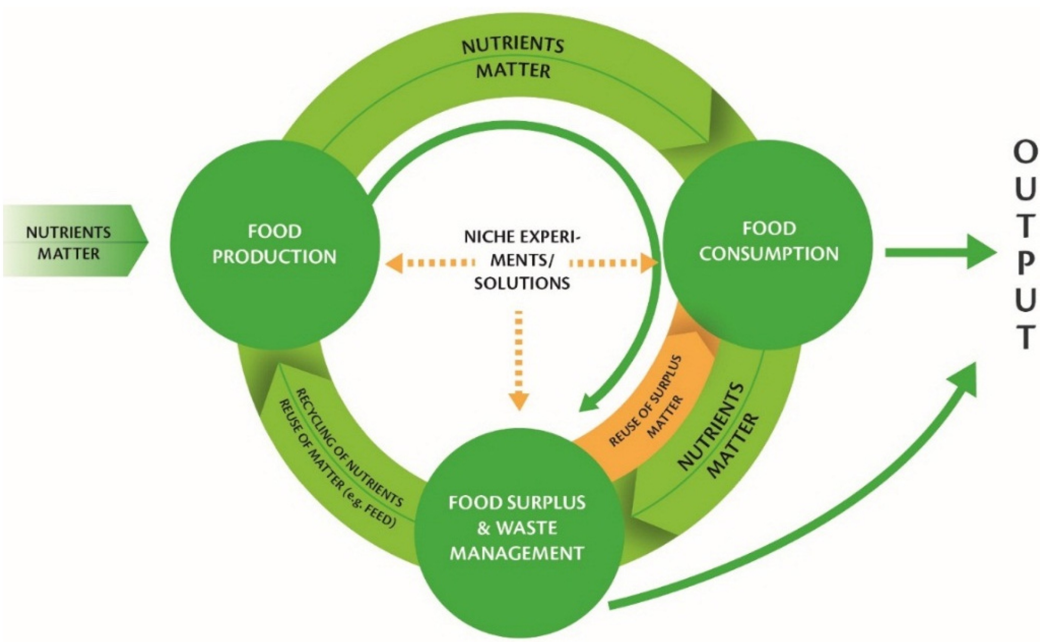
(From left) Breadlike food was found in Franchthi Cave in Greece; pulse-rich food with wild peas was uncovered in Shanidar Cave in northern Iraq.

UPDATED 5 MARCH, 2022 - 18:00 JAKE LEIGH-HOWARTH

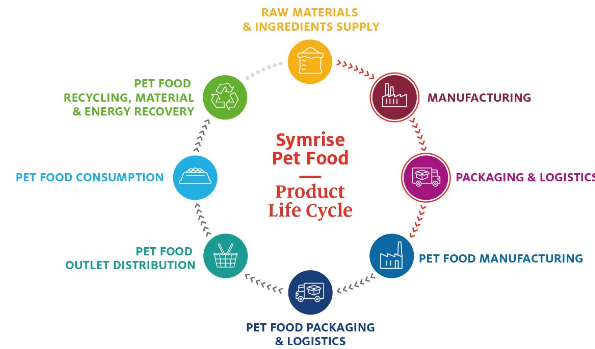
Eating Insects: The History of the Human Hunger for Bugs


Solution
Products from commodity crops like soybeans that are used in pet food and human food may be diverted into increased biofuel production in the U.S.
| Andrea Gantz
New pet food ingredient challenges if US expands biofuels
DEBBIE PHILLIPS-DONALDSON
DECEMBER 6, 2022

New ingredients are needed to support a circular economy



Jurgilevich et al. 2016. Transition towards a circular economy in the food system. *Sustainability* https://doi.org/10.3390/su8010069



Symrise Pet Food Product Life Cycle

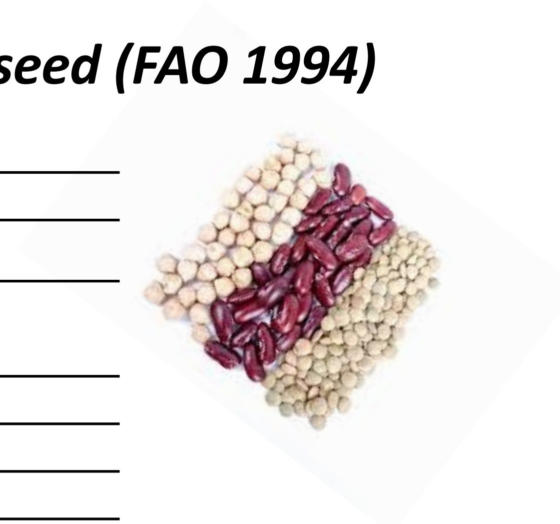
https://petfood.symrise.com/emea-en/approach/sustainability

The product life cycle approach aims to consider a product over its whole life at every stage, from extraction of raw materials to usage and recycling.

Symrise Pet Food is born from the valorization of co-products of agro-agri industries and is therefore based on a circular economy model. It strives to provide sustainable added-value at each stage of the products' life cycle.

Pulses: what are they?

Leguminous crops harvested solely for the dry seed (FAO 1994)



Pulse Class	Example
Beans, Dry	Kidney and Adzuki bean
Broad Beans (Faba), Dry	Horse bean and Broad bean
Peas, Dry	Field pea
Chickpeas	
Cowpeas, Dry	Black eye pea
Pigeon Peas	Cajan pea
Lentils	
Bambara Beans	Bambabra ground nut
Vetches	
Lupins	
Pulses NES (minor pulses)	Velvet bean

Adapted from FAO (1994)

WHY **PULSES?**

Pulses are climate smart as they simultaneously adapt to climate change and contribute towards mitigating its effects.

Pulses can fix atmospheric nitrogen and provide it to the soil

This **reduces the need for synthetic nitrogen fertilizers** and contributes in reducing greenhouse gas emissions.

85 million ha of pulses have contributed globally to fixating **3 - 6 million tonnes** of nitrogen in soils.*

Better varieties

Pulses have a **broad genetic diversity.**

This diversity is a particularly important attribute because **more climate-resilient pulse varieties can be developed.**

INCREASING **RESILIENCE**

Pulse-based cropping systems

Including pulses in crop rotations **exploits symbiotic microbes to fix nitrogen**, partly transferring it to subsequent crops, increasing their yields.

Intercropping has a **higher soil carbon sequestration** potential than monocrop systems.

Pulses and agroforestry systems

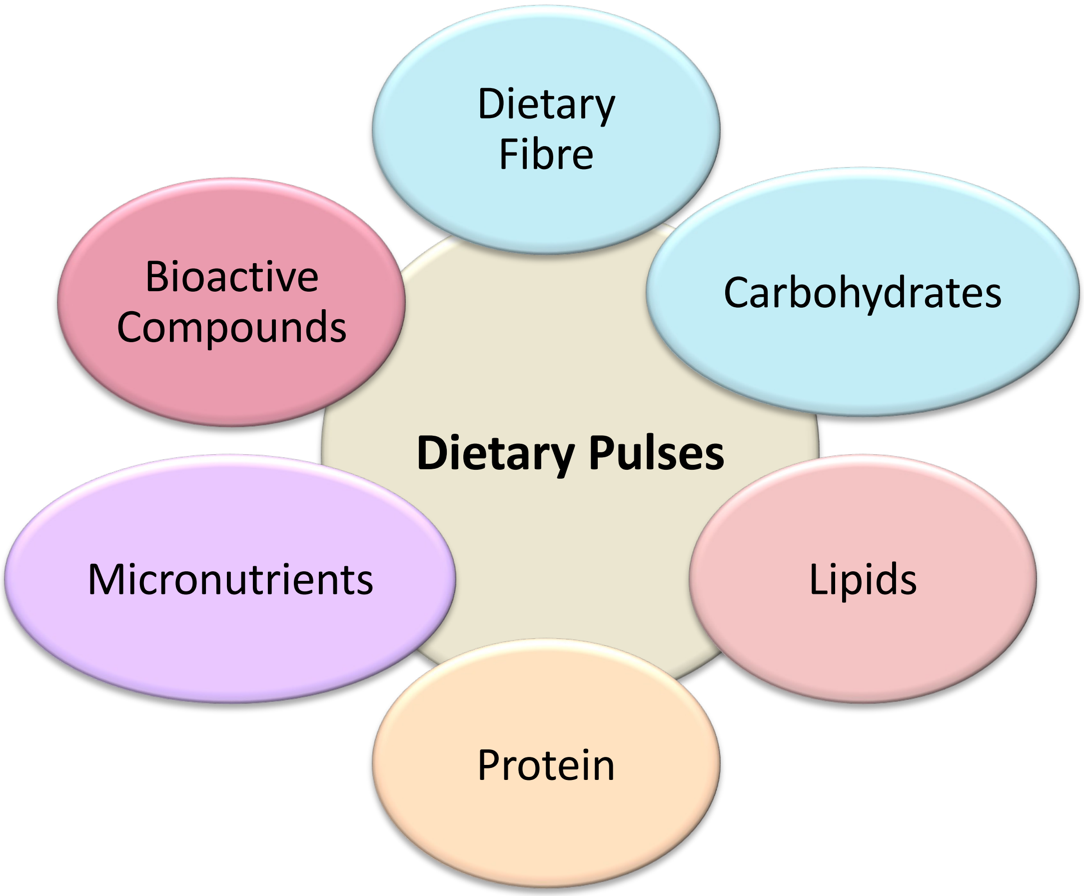
Growing pulses such as pigeon peas simultaneously with other crops, **improve farmers' food security**, by helping them to diversify their nutrition and sources of income.

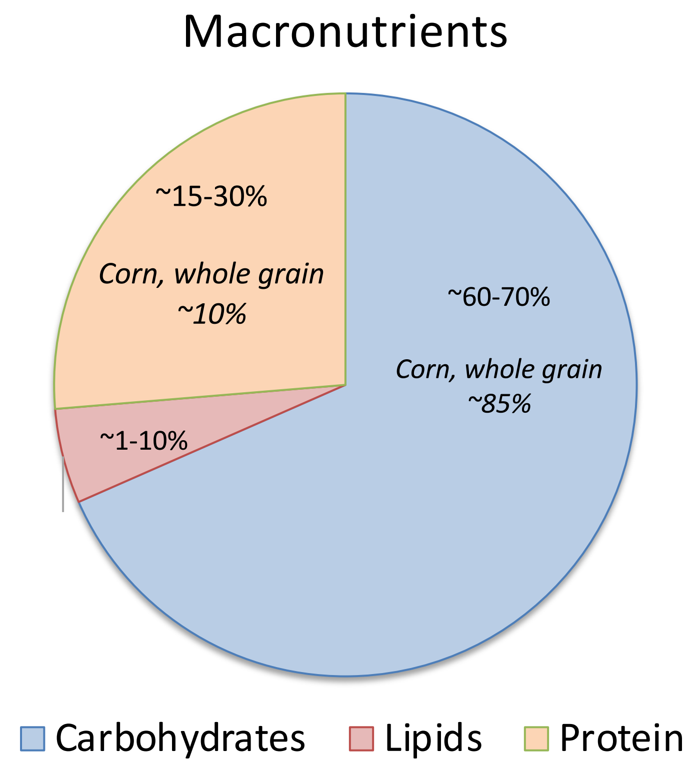
Pulses in animal nutrition

When included in livestock feed, pulse by-products **contribute to improve feed conversion ratio** while, reducing greenhouse gas emissions at the same time.

Decreased methane emissions from ruminants.

*FAOSTAT, 2014





(Hall et al., 2017)

Nutrient content protein & amino acid

- Variety and cultivar can influence protein quality
- Amino Acid (AA) content complementary to cereal grains
- Without complementation, sulfur AA may be low

Pulses

↑ Lysine

↓ Sulfur AA (Methionine)

Cereal Grains

↑ Sulfur AA (Methionine & Cysteine)

↓ Lysine

How do we think about novel ingredients?

Basic tenant: Animals require nutrients and we use ingredients to meet these requirements

- Characterize the chemical composition of an ingredient.
- Understand how processing affects the chemical composition.

Characterization of an ingredient, ie. pulses

Swine Innovation Porc

CANADIAN AGRICULTURAL PARTNERSHIP | PARTENARIAT CANADIEN pour l'AGRICULTURE

OPEN ACCESS | Article

The effects of grinding and pelleting on nutrient composition of Canadian pulses

Authors: Cara L. Cargo-Froom, Rex W. Newkirk, Christopher P.F. Marinangeli, Anna K. Shoveller, Yongfeng Ai, Elijah G. Kiarie, and Daniel A. Columbus | AUTHORS INFO & AFFILIATIONS

Publication: Canadian Journal of Animal Science • 21 April 2022 • https://doi.org/10.1139/cjas-2021-0111

Research Article

The effects of extrusion on nutrient content of Canadian pulses with a focus on protein and amino acids

Authors: Cara L Cargo-Froom, Rex W. Newkirk, Christopher PF Marinangeli, Anna K Shoveller, Yongfeng Ai, and Daniel Allan Columbus | AUTHORS INFO & AFFILIATIONS

Publication: Canadian Journal of Animal Science • 21 October 2022 • https://doi.org/10.1139/CJAS-2022-0088

Research Article

Processing effects on the starch and fibre composition of Canadian pulses

Authors: Olufemi O Babatunde, Cara L Cargo-Froom, Yongfeng Ai, Rex W. Newkirk, Christopher PF Marinangeli, Anna K Shoveller, and Daniel Allan Columbus | AUTHORS INFO & AFFILIATIONS

Publication: Canadian Journal of Animal Science • 14 December 2022 • https://doi.org/10.1139/CJAS-2022-0108

Methods for Processing Pulses to Optimize Nutritional Functionality and Maximize Amino Acid Availability in Foods and Feeds

Cara Cargo-Froom,[1,2] Anna-Kate Shoveller,[1,3] Christopher P. F. Marinangeli,[4] and Daniel A Columbus[5,6]

CEREAL FOODS WORLD, NOVEMBER-DECEMBER 2020, VOL. 65, NO. 6 / DOI: https://doi.org/10.1094/CFW-65-6-0068

How do we think about novel ingredients?

Basic tenant: Animals require nutrients and we use ingredients to meet these requirements

- Chemical composition of an ingredient.
- Understand how processing affects the chemical composition.
- *In vivo* to determine digestibility.

Characterization of an ingredient, ie. pulses

Swine Innovation Porc

CANADIAN AGRICULTURAL PARTNERSHIP | PARTENARIAT CANADIEN pour l'AGRICULTURE

O54 Comparison of ileal digestibility and indicator amino acid oxidation as methodological assessments of protein quality in growing swine

C.L. Cargo-Froom[a], F. Tansil[a,b], D.A. Columbus[c,d], L. Huber[a], E.G. Kiarie[a], C.P.F Marinangeli[e], C. Levesque[f], G. Courtney-Martin[g], A.K. Shoveller[a]

[a] University of Guelph, Guelph, Canada
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[c] Prairie Swine Centre, Saskatoon, Canada

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Abstracts / Animal - Science Proceedings 13 (2022) 241–510

[d] University of Saskatchewan, Saskatoon, Canada
[e] Pulse Canada, Winnipeg, Canada
[f] South Dakota State University, Brookings, USA
[g] Sick Kids, Toronto, Canada

Keywords: Standardized ileal digestibility; Indicator amino acid oxidation; Swine; Pulses; Black soldier fly larvae

JOURNAL ARTICLE

166 A Comparison of key Methodologies Used to Quantify Protein Quality in Mammals: Ileal Digestibility, Indicator Amino Acid Oxidation, and in Vitro Digestibility

Cara Cargo-Froom, Daniel A Columbus, Elijah Kiarie, Christopher Marinangeli, Anna-Kate Shoveller

Journal of Animal Science, Volume 100, Issue Supplement_3, October 2022, Page 77,
https://doi.org/10.1093/jas/skac247.151

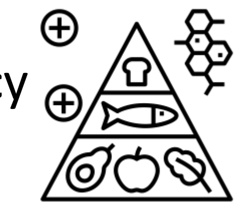
Table 1 Standardized ileal digestibility (% DM basis) of crude protein and indispensable amino acids of pulses

	Faba	Lentil	Pea	SEM	P value
Crude Protein	76.48	79.16	80.53	4.28	0.66
Histidine	92.80	89.98	87.27	2.34	0.20
Isoleucine	91.19	91.26	85.19	2.96	0.12
Leucine	92.52	91.68	88.22	2.30	0.38
Lysine	93.42	91.99	89.91	1.91	0.31
Methionine	89.69	89.78	89.03	2.00	0.95
Phenylalanine	91.45	90.94	88.52	2.18	0.53
Threonine	88.02[a]	86.88[a]	76.11[b]	3.46	0.02
Valine	88.26	90.00	82.99	3.31	0.24

[a-b]Means within the same row with differing superscripts are different from one another

How do we think about novel ingredients?

Basic tenant: Animal require nutrients and we use ingredients to meet these requirements

- Chemical composition of ingredient and under different processes 
- *In vivo* 
- Longer term feeding to determine gross safety and efficacy 

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,[‖] Jessica Varney,[‖] Christine Pendlebury,[$] and Anna K. Shoveller[†,1]

- Fecal bile acid excretion increased after 26 wk of feeding Acana to dogs
- Despite the higher fecal excretion of bile acids (largely primary bile acids), plasma and whole blood taurine increased over the 26-wk feeding study
- Plasma His, Met, Trp, and taurine and whole blood taurine concentrations increased over the course of the study
- Urinary taurine to creatinine ratio was not affected by diet

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

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. (2021) fed Beagles diets containing 45% green lentils for 90 days. Dogs experienced no changes in plasma methionine, cysteine or taurine and greater total primary bile acids excretion than control.

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.

Dietary protein, fat and fiber increase bile acid losses

SYSTEMATIC REVIEW article

Front. Vet. Sci., 07 October 2021

Sec. Animal Nutrition and Metabolism

https://doi.org/10.3389/fvets.2021.748803

Examining the Effects of Diet Composition, Soluble Fiber, and Species on Total Fecal Excretion of Bile Acids: A Meta-Analysis

Julia Guazzelli Pezzali*, Anna K. Shoveller and Jennifer Ellis

Department of Animal Biosciences, Centre for Nutrition Modelling, University of Guelph, Guelph, ON, Canada

- Increased concentrations of dietary protein and fat resulted in greater fecal excretion of BA
- Increasing levels of dietary carbohydrate led to lower excretions of BA
- Increased dietary soluble fiber containing ingredients resulted in greater excretion of BA in all models
- Not only soluble fiber, but also increasing levels of dietary fat and protein may result in greater fecal excretion of BA, potentially altering taurine and/or glycine metabolism and affecting the need for diet delivery of these AA

How do we think about novel ingredients?

Basic tenant: Animal require nutrients and we use ingredients to meet these requirements

- Chemical composition of ingredient and under different processes 
- *In vivo* 
- Longer term feeding
- We need to empirically measure nutrient requirements and understand what can increase or decrease nutrient requirements and the secondary metabolites.

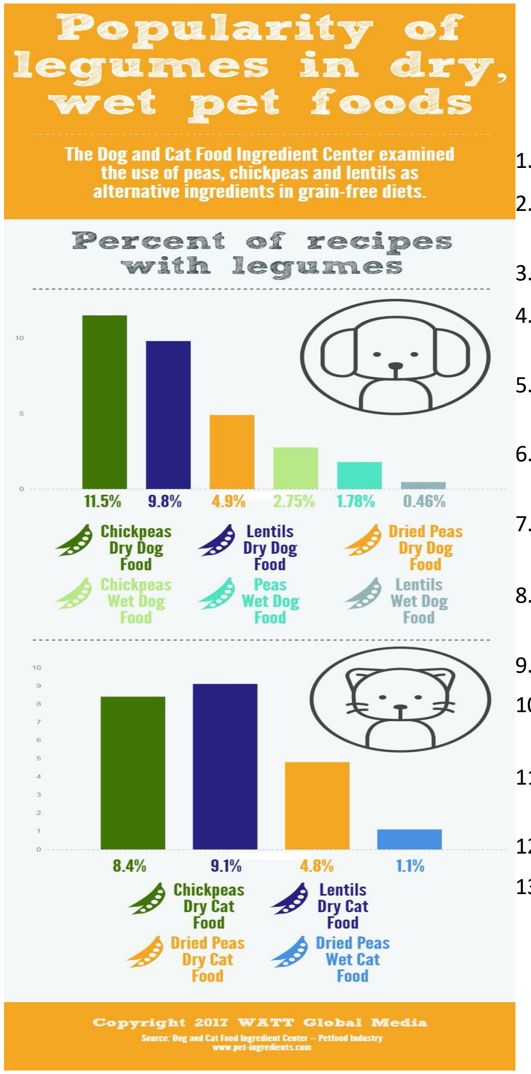
Minimum methionine requirements (Mansilla et al. 2020)

P&G Pet Care

	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

Taurine concentrations (Mansilla et al. 2020)

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			



Legume research in dogs before 2018

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11. Adolphe JL, et al. 2015 Effect of an extruded pea or rice diet on postprandial insulin and cardiovascular responses in dogs. J Anim Physiol Anim Nutr (Berl) 99:767-76. ***12 week study that showed no effect of pea inclusion on cardiac health**
12. Teixeira FA, et al. 2018 Effects of pea with barley and less-processed maize on glycaemic control in diabetic dogs. Br J Nutr120:777-86.
13. Reilley L. et al. 2018 Determineation of macronutrient composition and amino acid digestibility of plant-based proteins for use in canine and feline diets. J Anim Sci. 96(3): 147-148.

Research summary

- Digestible
- Pulses, due to their fiber, improve glycemic control in dogs
- Pulses may also help dogs prone to weight gain as they support satiety

Breaking News: FDA Releases List of Dog Food Brands Associated with Heart Disease Reports (DCM)

Adapted from: Robinson, Saldanhea &McCoy (2011); Muller-Block & Kranz (2015); Miles (2017)

Clinical/epidemiological studies

Kaplan et al. 2018, Adin et al. 2019, Fried et al. 2020 all saw echocardiographic improvements in dogs with DCM who had been eating a GF diet and were switched to a grain-inclusive diet.

Freeman et al. 2022 found improvement in FS in dogs fed GF diet and switched to 1 of 6 TD diets and Haimovitz et al. 2022 found a small decrease in hs-cTn1 and LVIDsN in dogs that had been fed GF diet and were switched to TD.

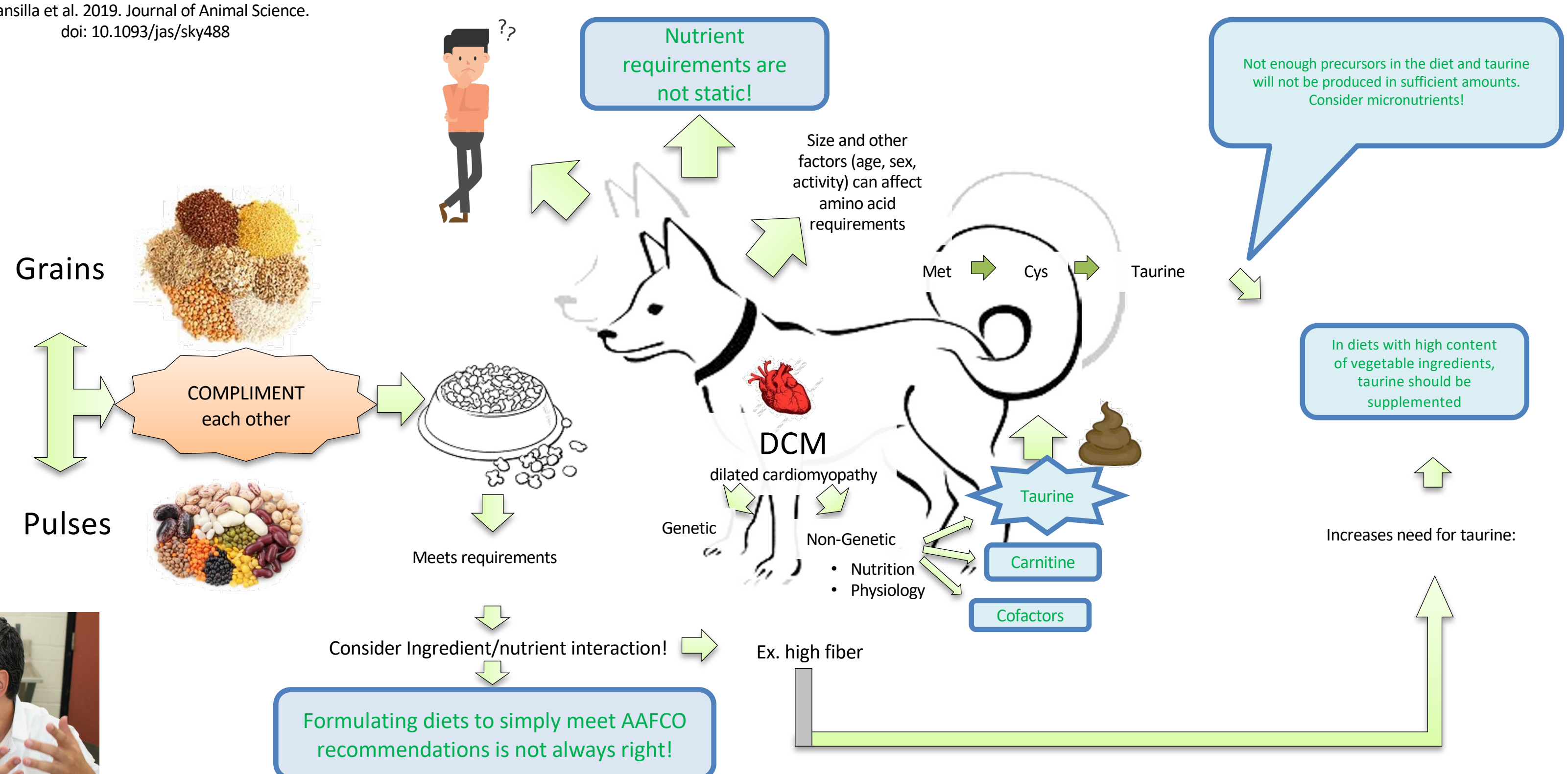
Limitations

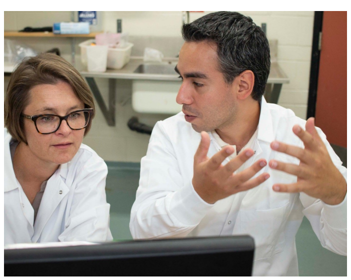
- Diet change alone will create hormetic responses and metabolism is dynamic
- Nutrient composition and intake of dogs' diets = unknown
- +/- Cardiac medications
- +/- Taurine supplementation

Special topic: The association between pulse ingredients and canine dilated cardiomyopathy: addressing the knowledge gaps before establishing causation[1]

Wilfredo D. Mansilla,[†] Christopher P.F. Marinangeli,[‡] Kari J. Ekenstedt,[‖] Jennifer A. Larsen,[$]
Greg Aldrich,[¶] Daniel A. Columbus,[††] Lynn Weber,[‡‡] Sarah K. Abood,[‖‖] and Anna K. Shoveller[†,2]

Mansilla et al. 2019. Journal of Animal Science.
doi: 10.1093/jas/sky488





The pulse of it: Dietary inclusion of up to 45% whole pulse ingredients with chicken meal and pea starch in a complete and balanced diet does not affect cardiac function, fasted sulfur amino acid status, or other gross measures of health in adult dogs

https://doi.org/10.1016/j.tjnut.2023.03.27

Champion Petfoods®
World's Best Petfood

Objectives

Assess the effects of pulses on cardiac function in dogs

To quantify the effects of varying levels of pulse inclusion from zero to forty-five percent, specifically, **whole green and yellow peas, pinto beans, chickpeas and lentils** in canine diets on cardiac health when fed to healthy dogs for 5 months, using

- Echocardiographic assessment
- Serum cardiac Troponin I concentrations
- Plasma NT-proBNP concentrations
- CBC and serum chemistry
- Plasma AA
- Body composition

Study Design

28 adult Siberian huskies housed (1-10yrs; 13 females, 15 males)

No genetically predisposed

Blocked by: echo EF %, age, weight & sex

4 experimental diets: 0% 15% 30% 45%

Green/yellow peas pinto beans, chickpeas and lentils

1-month wash-in
5-month on treatment

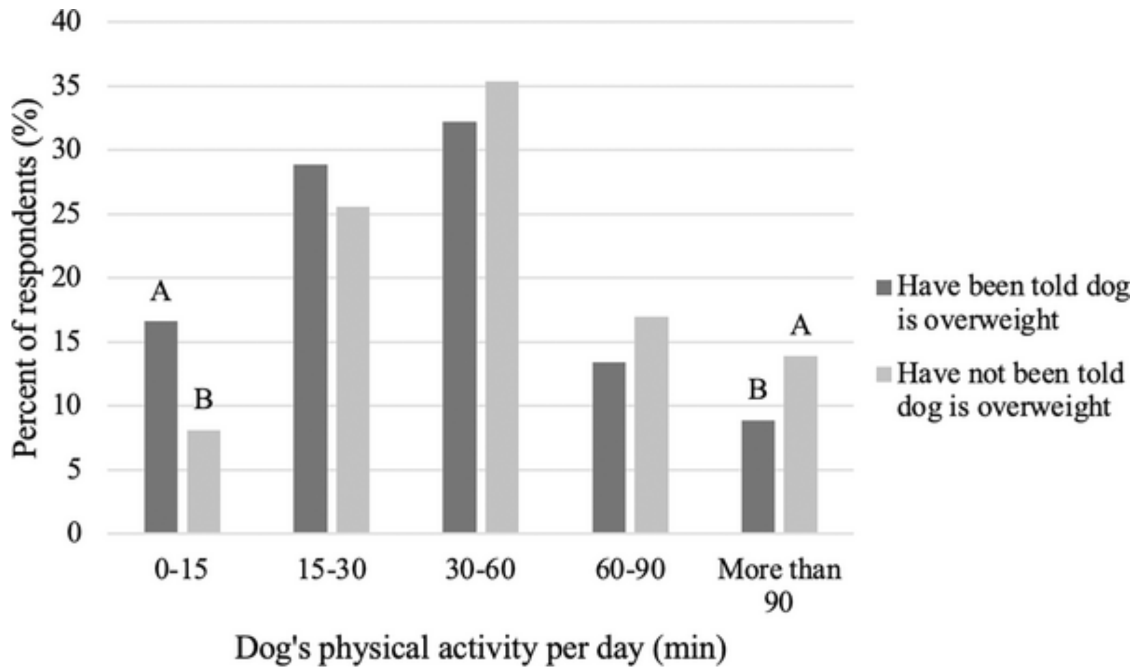
- Echocardiogram
- Serum cTnI
- NT-proBNP
- CBC
- Serum biochemistry

Grains on the brain: A survey of dog owner purchasing habits related to grain-free dry dog foods

(Banton et al, 2021 *DOI:* https://doi.org/10.1371/journal.pone.0250806*)*

- Follow 5 or more of their own dietary regimens
- Look for more protein options in pet food, no fillers, no by-products
- Give more other foods on a daily basis

Difference in time spent exercising their dog among those who have or have not been told their dog is overweight (n = 3,298).



Banton S, von Massow M, Pezzali JG, Verbrugghe A, Shoveller AK (2022) Jog with your dog: Dog owner exercise routines predict dog exercise routines and perception of ideal body weight. PLOS ONE 17(8): e0272299. https://doi.org/10.1371/journal.pone.0272299
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0272299

Study Design

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Blocked by: echo EF %, age, w

Similar physiology per treatment group

4 experimental diets:

0% 15% 30% 45%

Green/yellow peas
pinto beans,
chickpeas and
lentils

1-month wash-in
5-month on treatment

- Echocardiogram
- Serum cTnI
- NT-proBNP
- CBC
- Serum biochemistry

Data collection timeline

BLINDED CARDIOLOGIST
Dr. Shari Raheb
BSc, DVM, DACVIM (Cardiology)
Ontario Veterinary College, University of Guelph

Accreditation

The Animal Health Laboratory (AHL), a unit of the Laboratory Services Division of the University of Guelph, employs a comprehensive quality program. The AHL complements the regulatory and analytical testing carried out by the Division. The AHL is a full-service, fully computerized veterinary diagnostic lab that boasts an extensive cadre of laboratorians with post-graduate training and specialty board certification.

The expertise of AHL laboratorians and University of Guelph personnel is linked to provide a single source of laboratory services in the field of animal health, encompassing both food-producing and companion animals.

The AHL is accredited by:

- American Association of Veterinary Laboratory Diagnosticians (AAVLD) – full accreditation, all species
- Standards Council of Canada (SCC) to ISO/IEC 17025 standard for accredited techniques and specific tests listed on our scope of accreditation

Week -2: Baseline echo

Week 0: Baseline blood NTProBNP cTn1

Week 3: blood NTProBNP cTn1

Week 6: blood NTProBNP cTn1

Week 10: blood NTProBNP cTn1

Week 14: blood NTProBNP cTn1

Week 18: blood NTProBNP cTn1

Week 20: Final echo

Repeated measures allow for an understanding of whether physiology is changing over the 5 months and may be suggestive of further changes over time.

Study Design

28 adult Siberian huskies housed
(1-10yrs; 13 females, 15 males)

No genetically predisposed

Blocked by: echo EF %, age, w

Similar physiology per treatment group

4 experimental diets:

0% 15% 30% 45%

Green/yellow peas
pinto beans,
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lentils

1-month wash-in
5-month on treatment

- Echocardiogram
- Serum cTnI
- NT-proBNP
- CBC
- Serum biochemistry

Materials & methods: Diet Composition

Ingredient	Ctl	Pulse15	Pulse30	Pulse45
Whole grain corn	33.00			
Corn gluten meal	12.00			
Chicken Meal	25.00	33.00	27.25	25.00
Whole Green & Yellow Peas flours		5.00	10.00	15.00
Whole Pinto Beans flours		5.00	10.00	15.00
Whole Chickpeas & Lentils (50:50) flours		5.00	10.00	15.00
Pea starch	2.20	24.20	14.94	2.20
Fresh Chicken	10.00	10.00	10.00	10.00
Chicken Fat	7.50	7.50	7.50	7.50
Potassium Chloride	0.75	0.75	0.75	0.75
Ground Miscanthus Grass	2.00	2.00	2.00	2.00

Analyzed nutrient composition

Nutrient	Ctl	Pulse15	Pulse30	Pulse45
Proximate analysis, %				
Moisture	9.58	10.60	11.20	10.20
Protein	31.92	32.72	31.52	32.42
Fat	15.46	13.50	13.59	14.28
Fibre	2.12	3.06	3.28	3.44
Nitrogen-free extract (NFE, calculated)[1]	33.83	31.50	31.71	30.83
Calculated Metabolizable energy, kcal/kg[2]	3615.31	3395.32	3368.05	3427.26
Total Dietary Fibre, mg/g	5.50	6.65	8.21	8.31
Total Insoluble Fibre, %	4.75	5.78	6.19	6.36
Total Soluble Fibre, %	0.75	0.87	2.02	1.95
Oligosaccharides	0.41	1.68	1.74	1.74
Total Starch, %	22.03	16.95	16.77	15.89
Sulfur amino acids, %				
Methionine	0.76	0.63	0.59	0.58
Cysteine	0.48	0.46	0.45	0.45
Taurine (mg/100g)	0.09	0.14	0.13	0.12
Carnitine (mg/100g)	1.86	2.16	1.96	1.81

Analyzed nutrient composition

Nutrient	Ctl	Pulse15	Pulse30	Pulse45
Proximate analysis, %				
Moisture	9.58	10.60	11.20	10.20
Protein	31.92	32.72	31.52	32.42
Fat	15.46	13.50	13.59	14.28
Fibre	2.12	3.06	3.28	3.44
Nitrogen-free extract (NFE, calculated)[1]	33.83	31.50	31.71	30.83
Calculated Metabolizable energy, kcal/kg[2]	3615.31	3395.32	3368.05	3427.26
Total Dietary Fibre, mg/g	5.50	6.65	8.21	8.31
Total Insoluble Fibre, %	4.75	5.78	6.19	6.36
Total Soluble Fibre, %	0.75	0.87	2.02	1.95
Oligosaccharides	0.41	1.68	1.74	1.74
Total Starch, %	22.03	16.95	16.77	15.89
Sulfur amino acids, %				
Methionine	0.76	0.63	0.59	0.58
Cysteine	0.48	0.46	0.45	0.45
Taurine (mg/100g)	0.09	0.14	0.13	0.12
Carnitine (mg/100g)	1.86	2.16	1.96	1.81

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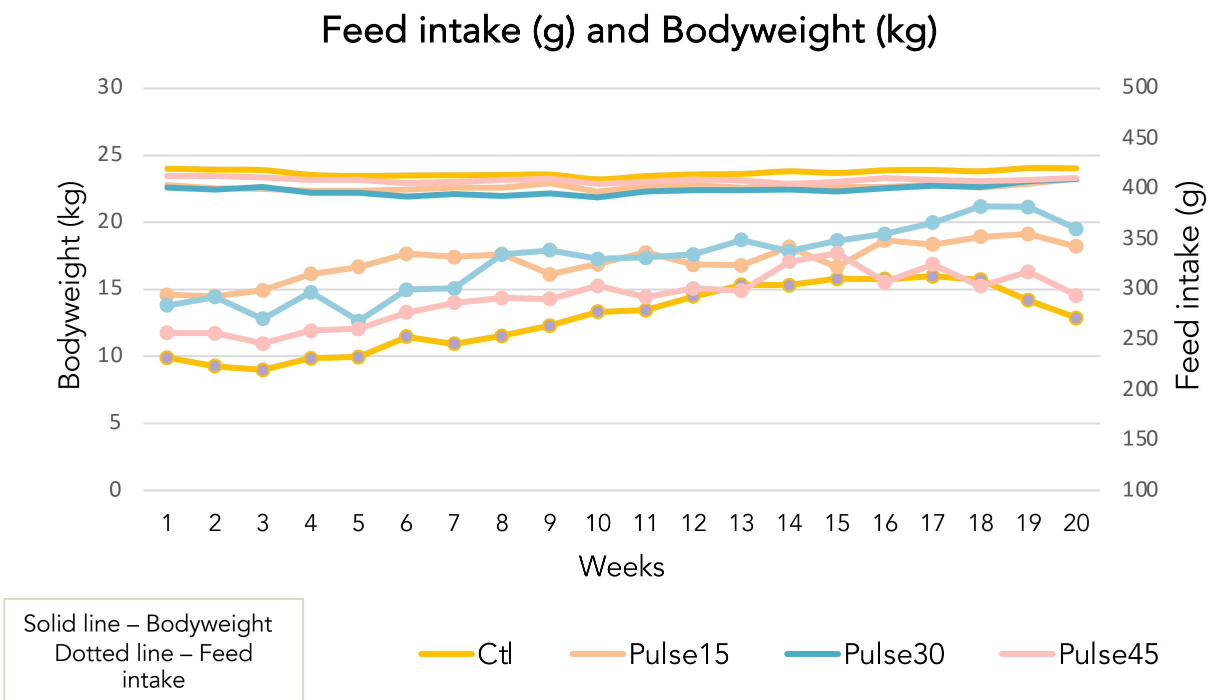
The cardiometabolic effects of diets with pulse inclusion in contrast to grain inclusion in dogs that are not genetically pre-disposed to DCM.

KEY RESULTS

Results – Feed intake & bodyweight



NO changes in BW among treatment groups or overtime (P>0.05)

Natural fluctuations coinciding with seasonal changes in temperature & humidity (May – October).

NOTE: We acknowledge the timing of the study, different results may occur if this study was ran winter to summer.

Results - DEXA

	Week	Treatments (LSM±SE)				P-values			
		Ctl	Pulse15	Pulse30	Pulse45	Trt	L	Q	C
Fat (g)	0	6041 ± 1026	5199 ± 950	4961 ± 950	4835 ± 950	0.4432	0.604	0.2749	0.4441
	20	5455 ± 924	4843 ± 855	5623 ± 855	4841 ± 855				
Lean (g)	0	16851±1074	15852 ± 995	15830 ± 995	16459 ± 995	0.5662	0.9025	0.3482	0.5127
	20	16089 ± 1074	15493 ± 995	15799 ± 995	15720 ± 995				
Bone mineral composition (g)	0	764 ± 69	670 ± 64	665 ± 64	750 ± 64	0.3739	0.1046	0.4457	0.8132
	20	780 ± 43	684 ± 40	672 ± 40	680 ± 40				

No changes to DEXA measurements between treatment groups nor over time (P>0.05).
No trends in data correlated to increasing pulse inclusion.

Results: Echocardiographic parameters after 5 months

No changes in echocardiographic measurements from baseline across diet groups.

	Treatments				P-values			
	Ctl	**Pulse15**	**Pulse30**	**Pulse45**	**Trt**	**Linear**	**Quadratic**	**Cubic**
LVIDd (mm)	37.0 ± 0.9	35.7 ± 0.8	35.7 ± 0.8	36.7 ± 0.9	0.64	0.86	0.21	0.91
LVIDs (mm)	26.1 ± 0.9	25.2 ± 0.8	25.0 ± 0.8	25.3 ± 0.8	0.81	0.53	0.47	0.93
LVEDV (mL)	58.8 ± 2.4	54.3 ± 2.2	52.80± 2.2	57.2 ± 2.3	0.24	0.56	0.06	0.77
LVESV (mL)	29.7 ± 1.8	28.2 ± 1.7	28.4 ± 1.7	28.2 ± 1.7	0.92	0.59	0.71	0.76
EF %	50 ± 2.0	49 ± 1.9	46 ± 1.9	50 ± 1.9	0.38	0.32	0.60	0.73
FS %	29.2 ± 1.8	29.58 ± 1.7	29.60 ± 1.7	31.80 ± 1.7	0.69	0.32	0.60	0.73
SI (cm)	1.88 ± 0.05	1.95 ± 0.04	1.88 ± 0.05	1.88 ± 0.05	0.67	0.77	0.53	0.36

Results: Echocardiographic parameters after 5 months

No changes in echocardiographic measurements from baseline across diet groups.

Treatments

	Ctl	Pulse15	Pulse30	Pulse45
LVIDd (mm)	37.0 ± 0.9	35.7 ± 0.8	35.7 ± 0.8	36.7 ± 0.9
LVIDs (mm)	26.1 ± 0.9	25.2 ± 0.8	25.0 ± 0.8	25.3 ± 0.8
LVEDV (mL)	58.8 ± 2.4	54.3 ± 2.2	52.80± 2.2	57.2 ± 2.3
LVESV (mL)	29.7 ± 1.8	28.2 ± 1.7	28.4 ± 1.7	28.2 ± 1.7
EF %	50 ± 2.0	49 ± 1.9	46 ± 1.9	50 ± 1.9
FS %	29.2 ± 1.8	29.58 ± 1.7	29.60 ± 1.7	31.80 ± 1.7
SI (cm)	1.88 ± 0.05	1.95 ± 0.04	1.88 ± 0.05	1.88 ± 0.05

Common DCM features:

- $\boxed{\uparrow}$ Left ventricular internal diameter in diastole and systole (LVIDd/s)
- $\boxed{\uparrow}$ Left ventricular end-diastole or end-systole volume (LVEDV, LVESV)
- $\boxed{\downarrow}$ Ejection fraction % (EF)
- $\boxed{\downarrow}$ Fractional shortening % (FS)
- $\boxed{\downarrow}$ Sphericity index (SI)

Results: cTnI concentration

- All dogs cTnI concentrations remained below 0.2 ng/mL for the duration of the trial.

- [Serum cTnI]

<0.2ng/mL = normal

>0.2ng/mL = high, cardiac damage

Limitation: high sensitivity cTNI should be used in subsequent studies to detect modest changes over time

Fasted taurine concentrations

(nmol/mL)	Treatment Mean
Plasma Taurine	
Ctl	115 ± 9.69
Pulse15	113 ± 9.84
Pulse30	118 ± 8.90
Pulse45	112 ± 9.04
WB Taurine	
Ctl	173 ± 10.20
Pulse15	188 ± 9.78
Pulse30	173 ± 9.03
Pulse45	181 ± 8.78

Concentrations of taurine associated with DCM

Plasma taurine ≤ 60 nmol/mL

WB taurine ≤ 155 nmol/mL Backus 2003

Freid =low taurine concentration was defined as <60 nmol/mL (plasma) and <200 nmol/mL (whole blood

Concentration of bile acids in fecal samples

Bile Acid	Treatment							
(µmol/g)	Ctl	Pulse15	Pulse30	Pulse45	P-values			
n	7	6	7	6	TRT[2]	L	Q	C
Primary BA[1]								
Cholic acid	0.149	0.147	0.121	0.182	0.992	0.920	0.998	0.791
Chenodeoxycholic acid	0.086	0.267	0.261	0.242	0.619	0.322	0.504	0.783
Total primary BA	0.259	0.452	0.451	0.515	0.927	0.690	0.689	0.799
Secondary BA								
Deoxycholic acid	3.304	8.213	6.403	4.062	0.261	**0.054**	0.951	0.774
Lithocholic acid	**0.882[b]**	**3.694[a]**	**2.826[a]**	**1.812[ab]**	**0.008**	**0.001**	0.408	0.935
Ursocholic acid	0.038	0.024	0.038	0.008	0.209	0.965	0.343	0.058
Total secondary BA	4.701	12.966	10.074	6.431	0.131	**0.022**	0.922	0.814
Total BA*	5.384	14.372	11.831	8.451	0.209	0.045	0.711	0.986

Fecal short-chain fatty acid and branch-chain fatty acid concentrations

Fecal metabolites	Treatment					P-value		
(umol/g)	Ctl	Pulse15	Pulse30	Pulse45	SEM	TRT[1]	Week	TRT * Week
Acetic acid	67.25	85.65	78.5	78.5	5.01	0.086	0.080	0.893
Propionic acid	29.8[b]	40.44[ab]	44.80[a]	40.93[ab]	3.33	0.017	0.007	0.934
Butyric acid	41.41	45.2	44.46	44.17	2.01	0.564	0.835	0.131
Formic acid	14.82	16.45	16.06	15.69	2.74	0.975	0.129	0.894
Iso-butyric acid	23.8[a]	20.27[b]	20.81[b]	20.72[b]	0.48	<0.0001	0.019	0.0630
Iso-valeric acid	2.89	2.77	2.91	1.81	0.22	0.280	0.424	0.488
Lactic acid	19.88	17.62	25.45	14.26	5.39	0.533	0.812	0.5149
Total SCFA[2]	138.52	171.00	167.78	163.60	9.07	0.057	0.043	0.870
Total BCFA[3]	25.51[a]	22.93[b]	21.91[b]	21.23[b]	0.66	0.001	0.126	0.004

Controlled studies of DCM risks

Bakke et al. (2022): Labrador retrievers fed grain-free diet with 20% split pea and 40% lentils for 30 days had reduced red blood cells and hemoglobin and increased plasma inorganic phosphate compared to baseline.

- Reis et al. (2021): Beagles fed HP (41%) for 7 days had reduced RBC compared with NP (27%) and fermentation of fava bean improved RBC. LVESV and cardiac output were increased in NP compared with HP-fed dogs, but fava bean did not affect either.
- Reis et al., (2021): Beagles fed *fermented* fava bean (30% inclusion) diets for 28 days had higher RBC than dogs consuming *unfermented* fava bean.

Cavanaugh et al. (2021): Fed a commercial plant-based diet (pea-protein based) for 12 weeks and observed no changes in echocardiographic parameters compared to dogs fed traditional animal protein and grain-inclusive diets.

Conclusions

- Feeding healthy dogs grain-free, pulse inclusive diets with up to 45% inclusion of green peas, pinto beans, chickpeas, and lentils and exceeded AA and micronutrient requirements for 5 months did not:
  - Affect plasma or whole blood taurine concentrations
  - Result in negative repercussions on canine cardiac health as assessed by echocardiogram, cTnI and NT-proBNP concentrations
  - Support gastrointestinal health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Dr. Guido Bosch, Wageningen University

"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