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## Evaluation of plant-based recipes meeting nutritional requirements for dog food: The effect of fractionation and ingredient constraints

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### ABSTRACT

Nowadays, plant-based human food products are considered to be more sustainable than animal-derived products. This could also be true for pet food, explaining a potential demand for plant-based pet food. Here, the concept of the degree of crop protein utilization was introduced for complete plant-based foods for dogs to understand the balance between nutrition and sustainability. The first step was to investigate which level of refinement, i.e. fractionation, of plant protein sources was necessary to fulfil the FEDIAF recommended nutrient levels for adult dogs. Moreover, the consequences of excluding certain ingredients were investigated. Thus, a database of plant-based ingredients, including the fractions of flours, protein concentrates and protein isolates, was created. Linear programming was used to formulate nutritionally complete recipes, minimizing either the number or the amount of nutrients that need to be added. Constraints of certain ingredients were translated to exclusion of soy, gluten, and/or tomato and potato. The recipe with the highest degree of crop protein utilization was the recipe in which isolates, concentrates, soy, gluten, tomato, and potato were excluded. The outcome of the optimization calculations showed that the level of refinement of ingredients, i.e. fractionation, has a direct influence on the sustainability of a recipe. Further, it was possible to obtain efficient recipes without the use of heavily processed ingredients and excluding certain undesired ingredients, while fulfilling the nutrient requirements of foods for adult dogs. Fortification to account for missing nutrients remained in line with the fortification used in conventional animal-based pet food. Thus, the design of plant-based dog food that is complete, in the most sustainable way, is achieved with recipes formulated with minimally refined ingredients. The results further show that the concept of calculating the degree of crop protein utilization when formulating recipes provides valuable information on which combination of raw materials to use.

**Abbreviations:** LCA, life cycle analysis; CMU, crop mass utilization; CPU, crop protein utilization.

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## 1. Introduction

Sustainable food choices are attracting increasing interest among consumers. This is also reflected in the pet food sector, where sustainability is being discussed not only in companies but also in scientific and governmental organizations. Recommendations for sustainable food choices for human food suggest that plant-based food choices for pets is the next step (Alexander et al., 2020). However, extensive evaluations of the sustainability of pet food recipes, such as life cycle analysis (LCA), are not yet done routinely by manufacturers and present many hurdles; for example, the availability of accurate datasets and varying outcomes with the choice of different allocations (Majeau-Bettez et al., 2018). In addition, the requirements for a complete pet food, i.e. providing all the daily nutritional needs, present a challenge with regard to plant-based pet foods. Several studies have shown that commercial plant-based pet foods are often not complete and balanced, even though they claim to be (Kanakubo et al., 2015; Zafalon et al., 2020). A select few minerals and amino acids are among the macronutrients insufficient in these products. However, when designing plant-based pet foods, careful selection of ingredients can overcome possible limitations in nutrients and the remaining limitations can be solved through fortification of nutrients such as methionine or calcium. Reported nutritional deficiencies in plant-based pet food lead to the question of the level of fortification. The severity of the nutritional deficiencies will depend on the type of plant-based ingredients used. The use of protein isolates has potential to provide ample amino acids to fulfil the requirements of dogs and cats, making it easier to produce a complete pet food (Reilly et al., 2020) due to the high protein content in isolates. However, less refined ingredients, namely flours and concentrates, have a lower environmental impact generally and therefore align more with consumer preferences. Soy and wheat gluten have advantageous properties both nutritionally and from the point of view of technology. Soy provides a wide range of essential amino acids and is already used in pet food (Cerundolo et al., 2004). Gluten is unmatched with regard to its binding properties and is thus widely used in food (Day et al., 2011). However, both soy and gluten do not have the best reputation among consumers (Beaton, 2019; Schleicher et al., 2019) due to perceived issues related to health and wellness, the association of soy with phytoestrogens and deforestation, and gluten with celiac disease. Although negative health consequences of feeding soy to dogs or cats have not been reported to the authors knowledge, experiments on other species have shown behavioural effects of long-term consumption (Simon et al., 2004). Sensitivity to gluten has been reported as a genetic trait in Irish Setters (Daminet, 1996) and avoiding gluten was reported to be beneficial for Border Terriers with canine epileptoid cramping syndrome (Lowrie et al., 2015). Whether allergy or

**Table 1**  
Ingredients and essential nutrients chosen to formulate recipes for plant-based foods for adult dogs.

Ingredients	Nutrients
Black bean	Arginine
Dehydrated carrot	Histidine
Faba bean	Isoleucine
Faba bean concentrate	Leucine
Navy bean	Lysine
Oats	Methionine
Pea	Cystine
Pea protein concentrate	Phenylalanine
Pea protein isolate	Tyrosine
Potato flour	Threonine
Potato protein	Tryptophan
Rice	Valine
Soy flour	Linoleic acid
Soy protein concentrate	Calcium
Soy protein isolate	Phosphorus
Sunflower oil	Potassium
Sweet potato	Sodium
Tomato powder	Chloride
Wheat gluten	Magnesium
	Copper
	Iodine
	Iron
	Manganese
	Selenium
	Zinc
	Vitamin A
	Vitamin D
	Vitamin E
	Thiamine
	Riboflavin
	Pantothenic acid
	Vitamin B6
	Vitamin B12
	Niacin
	Folic acid
	Choline

intolerance to gluten exists in the general dog population is unknown because of difficulty with the diagnosis (Verlinden et al., 2006). Still, consumer preferences need to be considered when formulating pet food. Another consumer preference is to avoid ingredients from the nightshade (Solanaceae) family containing the toxic compound solanine (Clipsham, 2012). Solanine can potentially be found in tomatoes and potatoes; for example, in unripe tomatoes or after prolonged exposure of potatoes to bright light. Some commercially available plant-based pet foods do contain tomatoes and potatoes. It is therefore interesting to analyse the consequences of exclusion of those ingredients when designing a complete dog food.

An indication of the sustainability of a recipe can be obtained using crop protein utilization (CPU), defined as the amount of protein in the final product per kilogram of protein in the raw material used. The basis of this sustainability approximation is a mass balance, which in contrast to commonly used LCA, does not include the energy, resource flows and emissions, and therefore needs fewer assumptions. This makes it simple and objective but still gives a good representation of the sustainability of a recipe without having to perform a full LCA. It is known that the CPU of meat is in the order of 4–26 % depending on the type of meat (Šebek and Temme, 2009). Generally, the use of plant-based ingredients leads to a higher CPU, but the use of highly refined ingredients, such as protein isolates, hampers potential improvement in the CPU due to protein losses as a result of fractionation. Fractionation is the process of separating a material into different fractions; for example, the protein fraction and the fraction containing the remainder of the material. The impact of ingredient fractionation on the CPU depends on the refinement method used to obtain the product (Felix et al., 2018) and the type of raw material used (Schutyser et al., 2015). Minimally processed flours could be used to achieve a high CPU. However, flours have relatively low protein, high fibre and high antinutrient levels. High fibre and high levels of antinutritional factors (ANFs) might decrease digestibility and thereby increase nutrient requirements (Kienzle et al., 2017). The latter holds mainly for the ANF phytate; plant breeding, fractionation and petfood processing reduce or eliminate most ANFs (van der Poel et al., 1991; van der Poel et al., 1992; Cardador-Martínez et al., 2012; Luo and Xie, 2013). High nutrient requirements can lead to inefficiencies. In addition, consumption of excess protein from highly processed ingredients in the diet can also be considered unsustainable (Alexander et al., 2017).

The aim of the study was to better understand how set formulation limitations translate into the composition of complete plant-based foods for dogs by calculating the CPU values for these foods. In this way, plant-based dog foods were evaluated based on the nutritional value and the use of raw materials, demonstrating the trade-offs when designing a fully plant-based dog food.

## 2. Materials and methods

### 2.1. Establishment of an ingredient database

Nineteen ingredients were chosen based on current use in commercial plant-based diets, commercial availability, and the availability of data from different fractionation levels of the raw materials (Table 1). A database with information on the nutritional composition of ingredients was established from various reference tables (Sheffy et al., 1985; Botems et al., 2004; Souci et al., 2008; CVB, 2019; USDA, 2019). In the case of missing data, information was obtained from the scientific literature (Bhatty and Christison, 1984; Sheffy et al., 1985; Wang et al., 2004; Simons et al., 2015). Sufficient data were not available in the literature for faba bean concentrate, pea protein isolate and sweet potato flour. Therefore, the raw materials were analysed to obtain the missing data using internationally accepted standard methodologies in an accredited laboratory (Intertek Food Services GmbH, Bremen, Germany). Faba bean concentrate (Vitessence Pulse 3600, Ingredient, Hamburg, Germany) was analysed for pantothenic acid, folic acid,  $\alpha$ -tocopherol acetate (to calculate vitamin E), copper, magnesium, manganese, sodium, selenium, iodine, chloride, choline, linoleic acid, moisture, nitrogen (to calculate crude protein by multiplying the nitrogen level by 6.25), crude fat, crude ash and crude fibre. Pea protein isolate (Vitessence Pulse 1803, Ingredient, Hamburg, Germany) was analysed for thiamine, riboflavin, niacin, pantothenic acid, pyridoxine,

No constraint base formulation	Refinement constraint	Ingredient constraint	Refinement and ingredient constraint
= Recipe N	<u>Low refinement:</u> Isolates and concentrates excluded = Recipes I a, I b	<u>No soy, gluten:</u> Soy, gluten excluded = Recipes IV a, IV b	<u>Low refinement;</u> <u>no soy, gluten, tomato, potato:</u> Isolates, concentrates, soy, gluten, tomato, potato, excluded = Recipes VII a, VII b
Amount of nutrients added minimized = a	<u>Medium refinement:</u> Isolates excluded = Recipes II a, II b	<u>No tomato, potato:</u> Tomato, potato excluded = Recipes V a, V b	
Number of nutrients added minimized = b	<u>High refinement:</u> Flours excluded = Recipes III a, III b	<u>No soy, gluten, tomato, potato:</u> Soy, gluten, tomato, potato excluded = Recipes VI a, VI b	

Minimizations a and b applied on every constraint

Fig. 1. Ingredient and nutrient constraints of the scenarios defining the recipes for plant-based foods for adult dogs.

folic acid, vitamin E, iodine,  $\beta$ -carotene, choline, linoleic acid, moisture, nitrogen (to calculate crude protein by multiplying the nitrogen level by 6.25), crude fat, crude ash and crude fibre. Sweet potato flour without skin (nu3, Berlin, Germany) was analysed for thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, folic acid,  $\alpha$ -tocopherol acetate (to calculate vitamin E), choline, moisture, nitrogen (to calculate crude protein by multiplying the nitrogen level by 6.25), crude fat, crude ash and crude fibre. The conversion factor for  $\beta$ -carotene according to FEDIAF (FEDIAF, 2019) was used to calculate the amount of vitamin A in international units.

## 2.2. Recipe formulation, constraints and daily serving size

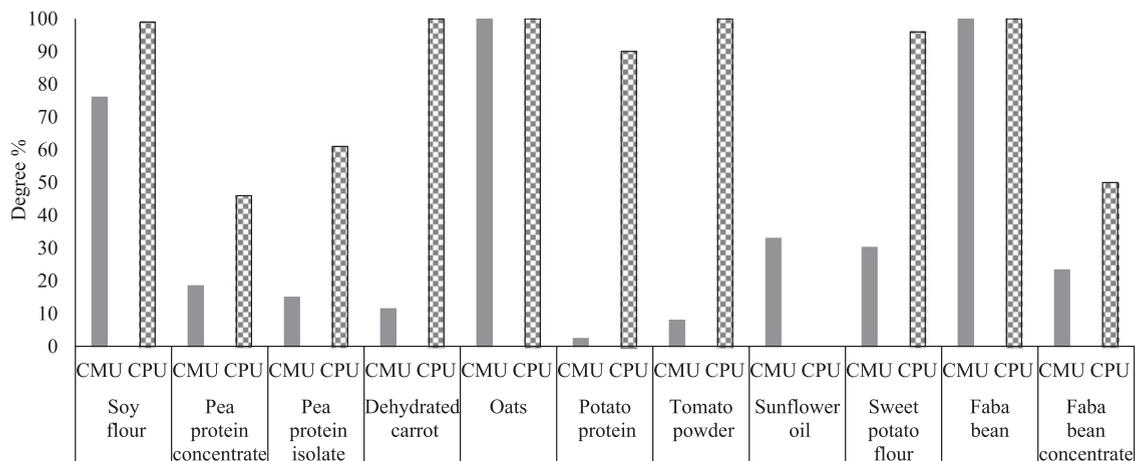
Recipe formulation was based on the recommendations of the FEDIAF Nutritional Guidelines, freely available internationally accepted nutritional guidelines for the manufacture of pet food (FEDIAF, 2019). The basic (base) recipe was formulated according to FEDIAF recommended nutrient levels for food for adult dogs, with a maintenance energy requirement of 95 kcal (0.4 MJ) metabolizable energy (ME)/kg body weight<sup>0.75</sup>. Safety margins to account for reduced amounts of bioavailable nutrients through food processing (e.g. extrusion or sterilization) and storage were 100 % (i.e. twice the recommended level) for vitamins and 10 % for protein, amino acids, fat, linoleic acid, minerals and trace elements. The Ca to P ratio was set at 1.3–1. The recipes were calculated with linear programming using FICO Xpress IVE (8.9, Fair Isaac Corporation, San Jose, CA, USA). The objectives for the calculations were to analyse the consequences of (a) minimizing the total amount of nutrients (relative to the recommended minimum nutrient level) to be added to the plant-based dog food recipes and (b) minimizing the number of nutrients to be added to the plant-based dog food recipes (Fig. 1). The effects of constraints regarding the refinement level of ingredients (e.g. fractionation and dog owner preferences) were also investigated. Three levels of ingredient refinement were analysed: low refinement (i.e. excluding the use of isolates and concentrates), medium refinement (i.e. excluding the use of isolates), and high refinement (i.e. excluding the use of flours). Two types of ingredient constraints were applied: the consumer trend to exclude soy ingredients and gluten (Banton et al., 2021) and solanine-related exclusion of potato and tomato, and their combination. Finally, the ingredient refinement level and ingredient constraints were combined, excluding isolates, concentrates, soy, gluten, potato and tomato. The composition of the recipe was modelled by the amount of energy provided by each ingredient. The energy density of the ingredients was calculated using the formula of the National Research Council (NRC, 2006). Modified Atwater factors were used for ingredients where the crude fibre level was > 80 g/kg, (AAFCO, 2019). The energy density of the ingredients was used to calculate the weight of all ingredients, the sum of which gave the mass of the recipe. The weight of the fortifiers in the complete recipe was added to the recipe mass. Thus, the total recipe mass or daily serving size is (unlike energetic content) not equal for all recipes.

The recommended Ca/P ratio for dry and wet foods for adult dogs is between 1:1 and 1:2 (FEDIAF, 2019). Use of a constraint to reach this Ca/P ratio (Ca/P min = 1, Ca/P max = 2), would have required P to be suppressed to minimize the Ca. In plant material, 60–82 % of P is phytate-bound (Ravindran et al., 1994), therefore we decided to achieve the Ca/P ratio as a second step through fortification.

## 2.3. Crop protein utilization

The CPU of the recipes was calculated to provide a measure of the sustainability of the formulations. The protein supplied by selected ingredients was compared with the protein present in the respective original, unprocessed crop. The degree of crop mass utilization (CMU) of the ingredients selected by the programming was used to calculate the CPU of the recipe (Fig. 2).

The CPU of each recipe was calculated according to the following formula:



**Fig. 2.** The degree of crop mass utilization (CMU<sub>*i*</sub>) defined as g of purified fraction/100 g of crop and degree of crop protein utilization (CPU<sub>*i*</sub>) as g of protein in purified fraction/100 g of protein in the crop with ingredients *i* selected by the programming. CMU<sub>*i*</sub> and CPU<sub>*i*</sub> are a measures for the amount of mass and amount of protein, respectively, that is preserved when processing a crop into a purified ingredient.

**Table 2**  
Ingredients of dog food recipes minimizing either the amount (g/kg) or the number of added nutrients.

	Base		Low refinement		Medium refinement		High refinement		No tomato, potato	No soy, gluten, tomato, potato	Low refinement; no soy, gluten, tomato, potato
	a	b	a	b	a	b	a	b	a,b	a,b	a,b
Soy flour	–	–	496.50	546.11	383.63	125.06	–	–	390.98	–	–
Pea protein concentrate	–	–	–	–	–	–	–	27.70	–	–	–
Pea protein isolate	45.62	44.66	–	–	–	–	113.63	114.76	87.64	89.54	–
Dehydrated carrot	–	–	–	–	–	–	–	–	69.03	72.87	247.79
Oats	–	–	–	–	–	–	–	–	–	–	155.98
Potato protein	143.80	254.07	–	–	–	–	96.83	238.93	–	–	–
Tomato powder	412.56	423.77	368.40	370.36	372.72	589.75	–	–	–	–	–
Sunflower oil	33.72	34.74	36.74	36.74	36.68	37.24	23.71	26.42	25.75	25.15	28.92
Sweet potato	–	–	91.36	39.72	–	49.19	–	–	–	–	69.72
Faba bean	–	–	–	–	–	–	–	–	–	–	487.85
Faba bean concentrate	356.03	235.21	–	–	198.26	186.60	753.90	581.44	416.83	798.54	–
Nutrients fortified	8.27	7.56	7.00	7.07	8.72	12.15	11.93	10.75	9.77	13.90	9.74

All values are g/kg. Low refinement, isolates and concentrates excluded; medium refinement, isolates excluded; high refinement, flours excluded; a, total amount of nutrients to be added minimized; b, total number of nutrients to be added minimized; results when soy and gluten were excluded were identical to those for the base formulation.

**Table 3**  
Added nutrients in dog food recipes minimizing either the amount (per MJ ME) or the number of added nutrients.

Nutrient		Base		Low refinement		Medium refinement		High refinement		No tomato, potato	No soy, gluten, tomato, potato	Low refinement; no soy, gluten, tomato, potato
		a	b	a	b	a	b	a	b	a, b	a, b	a, b
Methionine	g			0.02		0.05	0.16				0.11	0.19
Methionine and cystine	g						0.24				0.05	0.28
Calcium	g	0.40	0.32	0.29	0.31	0.39	0.32	0.64	0.53	0.57	0.67	0.28
Sodium	g			0.01	0.02	0.03						
Chloride	g	0.03	0.03	0.09	0.09	0.06	0.07			0.03		0.07
Iodine	mg	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Selenium	µg	6.39	0.00	17.16	17.69	17.08	16.76	7.20		14.41	12.82	17.99
Zinc	mg	2.44	3.18	2.94	2.81	2.20	2.81	0.63	1.51	1.10	0.31	3.49
Vitamin A	IU							838.41	838.41			
Vitamin D	IU	76.00	76.00	76.00	76.00	76.00	76.00	76.00	76.00	76.00	76.00	76.00
Vitamin E	IU							3.70	3.80	3.48	3.34	2.66
Riboflavin	mg			0.50	0.49							0.58
Pantothenic acid	mg	0.80	0.79	0.27	0.26	0.47		1.88	1.96	1.47	1.80	1.12
Vitamin B12	µg	4.63	4.63	4.63	4.63	4.63	4.63	4.63	4.63	4.63	4.63	4.63
Niacin	mg							0.23	0.61			
Folic acid	µg			34.18	35.05							30.22
Choline	mg	166.45	184.77	127.54	119.12	123.02	170.71	108.56	132.60	81.21	100.09	165.29

Low refinement, isolates and concentrates excluded; medium refinement, isolates excluded; high refinement, flours excluded; a, total amount of nutrients to be added minimized; b, total number of nutrients to be added minimized; results when soy and gluten were excluded were identical to those for the base formulation.

$$\text{CPU} = \frac{\sum_i (X_i \bullet P_i)}{\sum_i (P_{i,0} \bullet \text{CMU}_i)}$$

where  $X_i$  is the contribution of ingredient  $i$  in the recipe (g fraction/100 g product),  $P_i$  is the protein content in the fraction used (g protein/100 g fraction),  $P_{i,0}$  is the protein content of the original crop (g protein/100 g crop) and  $\text{CMU}_i$  is the CMU going from the original crop to the purified fraction (g fraction/100 g crop).  $\text{CMU}_i$  was calculated from mass balances and yield data from publications (Bradbury et al., 1984; Fredrikson et al., 2001; Deng et al., 2013). To account for excess protein in the recipes, the CPU was corrected for protein exceeding the recommended minimum protein levels needed to fulfil the amino acid requirements (FEDIAF, 2019) plus the 10 % safety margin that was also applied in the recipe calculations. The corrected [[CCPU]] is defined as the CPU corrected by the excess protein in the recipe:

$$\text{CCPU} = \text{CPU} \bullet \frac{P_{\text{tot. rec}}}{\sum_i (X_i \bullet P_i) - P_{\text{tot. rec}}}$$

$P_{\text{tot. rec}}$  was calculated according to (FEDIAF, 2019), including a 10 % safety margin. The total protein in the recipe was not allowed to go below this level, only above.

### 3. Results

Linear programming was used to calculate the recipes and revealed that it is possible to make complete formulations using the chosen plant-based ingredients in combination with conventional fortification. Recipes mostly had a high protein content, even when isolates and concentrates were excluded. High CUP was related to a low refinement level of ingredients. The base formulation had tomato powder (413 g/kg), faba bean concentrate (356 g/kg) and potato protein (144 g/kg) as the main ingredients (Table 2). Pea protein isolate and sunflower oil were also included in the recipe but soy was not selected.

#### 3.1. Effect of constraints on the recipes

The next step was to evaluate the effect of extra constraints. The exclusion of more refined protein sources (i.e. the isolates and concentrates), the so-called low (ingredient) refinement scenario, resulted in recipes that still had tomato powder (a, 368 g/kg; b, 370 g/kg) as one of the main ingredients, but now soy flour (a, 497 g/kg; b, 546 g/kg) also became a main ingredient. In the medium (ingredient) refinement recipes, soy flour (a, 384 g/kg) and tomato powder (b, 590 g/kg) were the main ingredients. In recipes that allowed use of isolates and concentrates, the so-called high (ingredient) refinement level, faba bean concentrate (a, 754 g/kg; b, 581 g/kg) became the main ingredient. Exclusion of soy and gluten resulted in the same recipes as the base formulation, which was understandable because the base formulation did not include those ingredients. When tomato and potato were excluded, faba bean concentrate (a, b, 417 g/kg) and soy flour (a, b, 391 g/kg) were the main ingredients. The combination of ingredient constraints resulted in a recipe based on faba bean concentrate (a, b, 799 g/kg). When both the ingredient constraints and low refinement level combined were applied (recipe VI), the main ingredients were faba bean (a, b, 488 g/kg) and dehydrated carrot (a, b, 248 g/kg).

**Table 4**

Essential amino acids in plant-based food recipes for adult dogs minimizing either the amount or the number of added nutrients relative to FEDIAF (FEDIAF, 2019) (%) recommended minimum levels.

Amino acid	Base		Low refinement		Medium refinement		High refinement		No tomato, potato	No soy, gluten, tomato, potato	Low refinement; no soy, gluten, tomato, potato
	a	b	a	b	a	b	a	b	a, b	a, b	a, b
Arginine	476	471	405	442	468	288	738	736	699	685	335
Histidine	385	403	349	379	384	251	530	554	515	478	206
Isoleucine	371	422	291	317	323	204	486	546	442	416	175
Leucine	366	422	270	294	302	189	484	549	428	406	175
Lysine	591	662	430	468	491	318	790	879	697	680	280
Methionine	110	153	110	110	109	110	110	160	110	110	109
Methionine and cystine	116	146	110	119	110	110	131	166	130	110	110
Phenylalanine	347	401	280	304	303	188	449	513	413	376	153
Phenylalanine and tyrosine	383	446	289	314	319	199	493	568	440	410	161
Threonine	293	352	225	243	240	156	353	423	317	285	137
Tryptophan	267	298	233	249	253	167	345	380	322	304	110
Valine	321	372	237	257	266	170	413	473	363	348	154

Low refinement, isolates and concentrates excluded; medium refinement, isolates excluded; high refinement, flours excluded; a, total amount of nutrients to be added minimized; b, total number of nutrients to be added minimized; results when soy and gluten were excluded were identical to the base formulation.

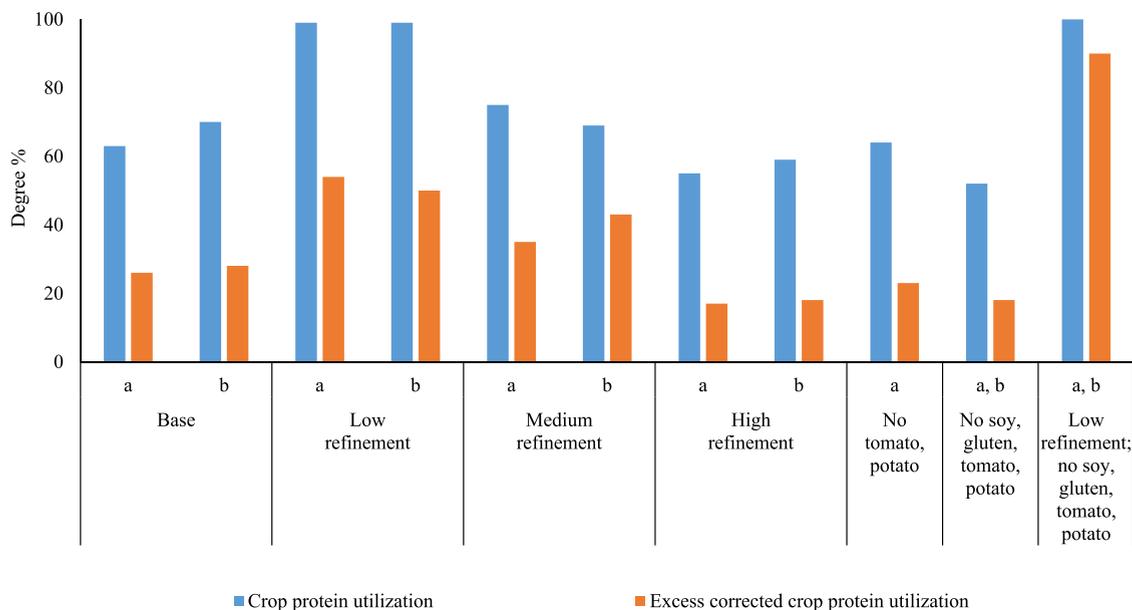
### 3.2. Nutritional value of the recipes

All the recipes needed fortifiers to fulfil the nutritional guidelines, but the number of nutrients added ranged from 8 to 14 (Table 3). Based on weight, the added nutrients ranged from 0.54 to 0.96 g/MJ ME (7–12 g/kg of total recipe; Table 2). Calcium, iodine, zinc, vitamin D, vitamin B12 and choline were always added. Methionine and cystine had to be added to the recipe for the medium refinement scenario (recipe II), when both the ingredient constraints were combined (recipe VI), and when both the ingredient constraints and low refinement level were combined (recipe VII) (Table 3). In the scenario of a low level of processed nutrients and minimal total amount of nutrients (recipe I a), addition of methionine was necessary. For the latter recipe and when both the ingredient constraints were combined (recipe VI), these fortifiers were only necessary to fulfil the safety margin. Recipes based on low refinement, and thus reliant on flours relatively high in fibre, required a higher number of fortified nutrients as expected, but the total amount of fortified nutrients by weight did not increase.

Evaluation of the recipes showed that they all exceeded the FEDIAF (FEDIAF, 2019) recommended minimum of crude protein content (12.5 g/MJ ME). When both the ingredient constraints and low refinement level were combined (recipe VII), the recipe exceeded the FEDIAF (FEDIAF, 2019) recommended minimum for crude protein by 1.43 g/MJ ME, whereas in recipes with high refinement level, excess crude protein (recipe III a, 27.0 g/MJ ME; III b, 28.4 g/MJ ME) was considerably larger than for the other formulations (minimum recipe II b, 7.41 g/MJ ME; maximum recipe VI a, b, 23.2 g/MJ ME). Recipes varied not only in crude protein but also in crude fibre content. When comparing recipes, those high in crude protein had lower crude fibre, and those with lower crude protein tended to have higher crude fibre. The recipes exceeded the required level of most essential amino acids (Table 4). Comparison of the amino acid content of the recipes calculated to the FEDIAF recommended levels showed that lysine supplied around 3- to 8-fold the recommended levels and other amino acids supplied 3- to ~9-fold the levels, except when both the ingredient constraints and low refinement level were combined (recipe VII). Hence, lysine is not the first limiting amino acid. Methionine is the first limiting amino acid; this is not only the case for plant-based ingredients but applies to most proteinaceous ingredients in general. Despite differences in crude protein and crude fibre content, serving sizes remained similar. Daily serving sizes of the calculated recipes required for a dog ranged from 65 to 82 g per MJ ME.

### 3.3. Crop protein utilization

The CPU ranged from 28 % to 100 % (Fig. 3), with an efficiency of 63 % (a) and 70 % (b) for the base formulation. The application of constraints related to the refinement level, excluding isolates and isolates and concentrates (recipe II, recipe I) increased the CPU to 99 %. Excluding flours reduced the CPU to 55 % (recipe I) or 59 % (recipe II). Excluding soy and gluten only led to a decrease in efficiency (by 11 %) when both the ingredient constraints were combined (recipe VI). When both the ingredient constraints and low refinement level were combined (recipe VII), the resulting recipe had a CPU of 100 %. When the CPU was corrected for the minimum protein level supporting the amino acid requirements, the CPU ranged from 17 % to 90 %. The decrease in CPU as a result of this



**Fig. 3.** The effect of formulation constraints on the degree of crop protein utilization compared with the average protein efficiency of meat. Low refinement, isolates, concentrates excluded; medium refinement, isolates excluded; high refinement, flours excluded; a, total amount of nutrients to be added minimized; b, total number of nutrients to be added minimized; results for excluding soy and gluten were identical to the those for the base formulation.

correction was profound in most recipes, because it was more than half of their initial CPU. The exceptions were when isolates were excluded and the recipe was minimized for number of nutrients added (recipe II b) and when both the ingredient constraints and low refinement level combined were applied (recipe VII). For the latter, a decrease of 10 % was observed. Nevertheless, all the recipes had a higher CPU than the average protein efficiencies normally reported for production of beef, pork and chicken (Sebek and Temme, 2009).

#### 4. Discussion

The results indicate that a range of plant-based recipes calculated based on different constraints related to the degree of refinement of the ingredients and the exclusion of specific ingredients (e.g. those not preferred by pet owners) can meet the FEDIAF recommended nutrient levels for complete adult dog food. The constraints resulted in specific selection of the main ingredients in two categories of nutrient profiles, and in differences in the types and amounts of fortifiers. Tomato powder was included in the base formulation from 370 to 600 g/kg, in the scenarios with low and medium refinement and in the scenario that excluded soy and gluten from the formulation. Tomato powder was likely selected by the programme because of its high levels of potassium, pantothenic acid, riboflavin, vitamin E, and  $\beta$ -carotene compared with the other ingredients in the database. Faba bean concentrate was also selected frequently likely because of its high content of zinc, potassium, riboflavin, vitamin E, niacin and folic acid. Of course, the outcomes depended on our ingredient database. If the ingredient database (Table 1) had been larger, other ingredients and nutrient options would have been available for selection by the programme. Data on chloride levels, for example, were not available for all the ingredients, which could explain why chloride needed to be added to eight recipes. Also the high carrot content in the diets obtained after applying all the constraints could have been avoided. However, ingredients and nutritional data were sufficient to demonstrate the concept of CPU. Generally speaking, the nutritional profiles of the recipes fall into two categories: (1) high in protein; (2) high in fibre. The high-protein recipes are mostly based on inclusion of highly processed (i.e. refined) ingredients. The high-fibre recipes are mostly based on the use of less processed ingredients. The high-protein diets contained more protein than needed to support the amino acid requirements. It should therefore be explored whether the protein content in high-protein diets can be lowered, for example, by adding lipid or starch to the diet. Another reason to avoid excess protein is that it leads to excess excretion of nitrogen (Aarnink and Versteegen, 2007). Excretions from faeces and urine increase the nitrate concentration in groundwater and pollute the environment (Fangmeier et al., 1994). However, a clear advantage of high-protein diets is the lower number of fortifiers needed. A low number of fortifiers is preferred by consumers because they prefer more natural foods without these additives. Although high in fibre, all the high-fibre recipes fulfilled the nutritional requirements without excessive nutrient fortifiers, except for one recipe. CPU is highest for recipes using flours, including high-fibres recipes. Thus, when considering CPU, recipes based on low refinement seem to be the best option. Compared with high-protein recipes, the number of fortifiers had to be increased from 10 to 14 in high-fibre diets. Fortification with about 14 nutrients is also standard practice in animal-based pet food production (Thompson, 2008).

A next step would be to investigate whether those recipes can be used to make products that are digestible, palatable and acceptable for dogs and fulfil the nutritional needs of the animals in practice through feeding tests.

##### 4.1. Recipes high in protein

The calculations revealed that protein contents of plant-based dog food recipes (13.86–40.87 g/MJ ME) were comparable with or higher than most dry pet food (11.23–22.47 g/MJ ME) and most wet pet food (16.25–40.15 g/MJ ME) (Hill et al., 2009; Davies et al., 2017). The protein levels were up to 3.3 times the FEDIAF recommended minimum for dog foods (12.5 g/MJ ME). At present, there are no data indicating that excess protein levels overload the metabolism, meaning that no increased liver or kidney health risk in healthy dogs is foreseen (Ephraim et al., 2020) (Leriche et al., 2020), despite the fact that excess protein can lead to increased hindgut protein fermentation. However, in diets with protein levels up to 23.18 g/MJ ME, the actual protein source in the diet had a higher impact on the extent of fermentation than the protein level (Nery et al., 2012). Therefore, digestibility of proteins should be considered when formulating plant-based pet food. Faba bean concentrate, a main protein source in recipes N, III, V, and VI, showed 88.0–94.6 % ileal digestibility for essential amino acids in precision-fed cecectomized roosters (Reilly et al., 2020). Recipes were minimized for added nutrients, i.e. also for the limiting amino acids, methionine and cystine. Except for the recipe when both the ingredient constraints and low refinement level were combined (recipe VII), simulations resulted in recipes with excess amino acid levels, therefore it would be valuable for manufacturers to design recipes without minimization for added nutrients, especially the limiting amino acids (methionine and cystine) to avoid excess protein.

##### 4.2. Recipes high in fibre

The average crude fibre content of most commercial dry pet foods ranges from 0.48 to 3.59 g/MJ ME and ranges from 0.24 to 6.21 g/MJ ME in most wet pet foods (Hill et al., 2009; Davies et al., 2017). Recipes 0, I, II, IV and VII had a larger calculated crude fibre content than commercial pet foods, whereas the crude fibre content of recipes III, V and VI was still within the crude fibre range of commercial pet foods. Compared with weight loss diets, which often have higher crude fibre levels (7.89–15.30 g/MJ ME) (Flanagan et al., 2017), the calculated crude fibre content was the same (7.88 g/MJ ME) for recipe II b, and for the rest of the formulations, the crude fibre content was lower. Therefore, considering the crude fibre levels of the resulting recipes, it can be expected that those recipes are digestible and nutritious. However, crude fibre analysis underestimates the actual content of dietary fibre (de-Oliveira et al., 2012). Consequently, in nitrogen-free extracts, the content of supposedly digestible carbohydrates is overestimated. Moreover,

the energy content of the raw materials was calculated using the formula of the National Research Council (NRC, 2006). Modified Atwater (AAFCO, 2019) factors were used for raw materials where the crude fibre content was > 80 g/kg. In this case, both methods do not represent the energy content of plant material accurately because crude fibre lowers digestibility. Thus, the modified Atwater factors overestimate low-energy fibre-rich plant-based foods (Castrillo et al., 2009). This outcome has consequences for the requirements of fortifiers, because the nutrient levels in the recommendations are bound to the energy density of the food. In plant-based recipes, the energy density is lower than calculated—the animal needs to eat more to fulfil its energy requirements—and therefore the required nutrient density can be lower than assumed. Hence, the present recipes might also be oversupplying micronutrients and probably less fortifiers might actually be needed. Especially for limiting nutrients (e.g. methionine, cystine), it is important to know the exact level that is required in the pet food.

Therefore, when designing plant-based pet food, how ME, energy and protein digestibility are determined should be reconsidered. Calculations might not be accurate enough to ensure nutritional adequacy and should be complemented by feeding trials. As a consequence, instead of minimizing the added nutrients, a valuable formulation constraint is to limit crude fibre. However, because lower crude fibre results in lower CPU, a balance between the crude fibre content and CPU needs to be found.

#### 4.3. Crop protein utilization of the recipes

CPU was used as a measure to evaluate the environmental impact of the various recipes. This approach to sustainability is very simplified and focuses on protein only. A full LCA takes into account all resources and emissions throughout the life cycle, but this assessment is condensed to only consider the utilization of protein. This potentially underestimates or omits environmental consequences of, for example, the production processes of specific ingredients. In addition, differences in the environmental impacts of cultivation of different crops are not considered, which could be significant mainly when land-use change effects are included for global warming potential. However, the CPU approach greatly reduces the need for data to be available, LCA expertise for decision-making from the practitioner and evaluation of the trade-offs between various environmental impact indicators. The CPU can be rationalized, because preparation of protein isolates and concentrates leads to losses and requires resources during production, such as energy and water. A lower CPU would also mean that more raw material has to be produced on the land. Generally, the agricultural stage has the highest impact in the LCA of agri-food products (Notarnicola et al., 2015). Therefore, better use of the primary materials and generating less material waste are the most important measures to reduce the associated environmental impacts (Springmann et al., 2018). Hence, product and protein yield per kilogram of raw material could serve as an efficiency indicator of a plant protein ingredient in a recipe. This explains why recipes containing concentrates (recipe II) rather than isolates (recipes III, V, VI) are more efficient. This finding aligns with an LCA comparing faba bean isolate and faba bean protein-enriched flour (Vogelsang-O'Dwyer et al., 2020). However, as can be seen in the results of the recipe simulations, when formulating a complete pet food, isolates are not the only ingredients; the foods consist of a broad range of concentrates, isolates, and flours. Therefore, total average ingredient yields were used to calculate the CPU of the recipes. It was found that the refinement level of the ingredients was the determining factor for the CPU of the recipes: decreasing the refinement level led to a higher CPU. Even when ingredients were excluded, the refinement level remained the determining factor for the CPU of the recipes.

The levels of fibre and protein could be an additional decision criterion for choosing sustainable recipes, because after correction for the protein level above the amount that supports the amino acid requirements, the CPU decreases considerably (Fig. 3). As mentioned earlier, based on the current selection of ingredients, the recipes have a considerable excess of protein and thereby amino acids, except when both the ingredient constraints and low refinement level were combined (recipe VII). Here, the decrease in the CPU is relatively small (100 % vs 90 %). Even though excess protein is acceptable from a nutritional point of view, excess protein leads to nitrogen pollution and should therefore be avoided when aiming for sustainable recipes. In the formulations presented here, high-protein low-fibre recipes had low CPU when corrected for the minimum recommended level of protein in dog food. This could mean that not only excess protein is an indicator of a non-sustainable diet but also the combination with low crude fibre content. Even when the CPU was corrected for excess protein content and thus decreased compared with the non-corrected values, the CPU of all plant-based recipes was higher than the reported animal protein efficiency of 4 % for beef, 8 % for pork and 23 % for chicken (Šebek and Temme, 2009). This is in line with the general notion that plant protein consumption is more efficient than animal protein consumption. Inefficiencies with animal protein mainly come from conversion inefficiencies of plant materials during animal production, whereas the inefficiencies in plant protein come from losing material during refinement into higher value fractions. Even though (ruminant) animals are able to transform material unsuitable for human consumption into high-quality edible protein, this is not how most cattle is produced in practice at present (van Zanten et al., 2016). Despite this general difference between animal and plant CPU, one could argue that most pet food is produced from animal side streams and therefore allocation of inefficiencies and environmental impacts can be debatable. However, because the definition of side streams and human edibility of animal by-products are subjective and the meat market and its economics are volatile and constantly changing, these definitions are precarious. With the current research, we do not aim to contribute to that discussion; rather, we present plant-based options for nutritionally complete dog food and the effects of the refinement level and consumer preferences on their CPU. These are the factors that need to be considered by pet food manufacturers when declining meat markets and side stream availability or changing consumer preferences create a demand for plant-based dog food.

## 5. Conclusion

Linear programming aimed at minimizing the nutrients added while using constraints regarding the ingredients used was applied to

calculate a range of plant-based recipes that fulfilled the minimum nutritional requirements (i.e. the recipes are complete). Soy protein isolates and concentrates were not the first-choice ingredients and faba bean concentrate and tomato powder were often selected. Recipes using ingredients with a high protein concentration had a higher environmental impact, as demonstrated by a lower CPU. Medium and low refinement levels (i.e. fractionation) combined with ingredient constraints led to better outcomes compared with the base model in which no constraints were applied. Fortification with nutrients to make complete recipes was comparable with or lower than the level of fortification used in conventional pet food. The protein content of the recipes was generally high. The inclusion of other ingredients to provide nutrients that were limited or directly adding these nutrients might reduce the fibre and protein content and increase the CPU. To date, sustainability is not included in recommendations for nutritional requirements for dogs, but we encourage this to be included in future recommendations, e.g. by calculating the CPU. The use of the CPU reveals that recipes using less refined ingredients are most sustainable, especially when corrected for a protein level above the recommended required minimum in dog foods. Most likely, a full LCA would arrive at similar conclusions.

### CRedit authorship contribution statement

**Ariane Wehrmaker:** Conceptualization, Methodology, Investigation, Writing – original draft, Visualization, Project administration. **Nynke Draijer:** Conceptualization, Methodology, Software, Formal analysis, Data curation, Writing – review & editing. **Guido Bosch:** Conceptualization, Writing – review & editing. **Atze Jan van der Goot:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition.

### Declaration of interest

Ariane Wehrmaker is employee of saturn petcare gmbh. Nynke Draijer is employee of Blonk Consultants.

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### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.anifeedsci.2022.115345](https://doi.org/10.1016/j.anifeedsci.2022.115345).

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