Received: 18 February 2020 | Accepted: 1 February 2021

DOI: 10.1111/jpn.13510

ORIGINAL ARTICLE

Journal of Animal Physiology and Animal Nutrition WILEY

Relationship between a plant-based 'vegan' pet food and clinical manifestation of multiple nutrient deficiencies in two cats

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Abstract

A topical subject in human nutrition is the steadily growing number of people choosing to limit or completely avoid all animal-derived food products either for moral dilemma, health concerns or both. To meet people's will of applying their dietary choices to their domestic animals, the pet food industry answered by launching on the market some plant-based diets. This leads to concerns about whether these diets are adequately formulated to satisfy the target species nutritional requirements, especially for cats which are still considered strict carnivores. This case report follows a 2-year-old male neutered Main Coon and a 1-year-old female spayed Domestic Shorthair cat, presented to the nutrition service of the University of Toulouse, France. Reason for consultation was lethargy with in anamnesis a recent dietary transition to a plant-based pet food. Dysorexia, lethargy and muscle waste were present at first consultation. Progressive weight loss developed during follow-ups. A macrocytic, non-regenerative anaemia with serum folates below reference were the main clinical features. Analysis of pet food showed multiple nutrients below minimum recommendation at the average daily intake of both cats. Folic acid supplementation improved dysorexia, and subsequent reintroduction of animal-derived ingredients in the diet restored appetite, weight and a normal mentation in both cases.

KEYWORDS
cats, nutrient deficiency, nutrition, pet food, plant-based diet, vegan

1 | INTRODUCTION

Environmental issues, health concerns and the increasing empathy for livestock animals are some of the main reasons driving the growing wave of people deciding to modify their dietary regimen towards the restriction (ovo-lacto vegetarianism and vegetarianism) or complete avoidance (veganism) of animal-derived food products (Fox & Ward, 2008). True to their lifestyle choices, many pet-owners struggle with the dilemma of feeding their dogs and cats with diets containing meat and other ingredients of animal origin (Dodd et al., 2019). In a recent internet-based survey, half of the questioned vegan pet-owners were already giving a plant-based diet to their pets, while the other half was ready to do the same if these diets were easier to find on the market (Dodd et al., 2019).

Answering the owners call, the pet food industry readily filled this novel market niche with plant-based diets, both for dogs and cats, labelled as balanced and complete (Parr & Remillard, 2014). This situation raises some concerns about whether these pet foods are adequately formulated to satisfy the target species nutritional requirements, knowing how certain nutrients are not abundant in plant sources (Spitze et al., 2003) and also that some species-specific enzymatic pathways must be taken into consideration (James G Morris, 2002).

While domesticated dogs evolved on an omnivorous starch-rich diet, putting some distance from their carnivore wild ancestors (Axelsson et al., 2013), modern cats still share the obligate carnivore nature of their descendants (Morris, 2002a). Data on the dietary nutrient profile of feral cats showed how their daily energy intake comes from 52% crude protein, 46% crude fat and only 2% nitrogen-free extract (NFE) (Plantinga et al., 2011). This dietary background defined this species metabolic pathways with higher needs for protein, arginine, sulphur-containing amino acids, taurine, arachidonic acid, vitamin A and D, niacin and pyridoxine (MacDonald et al., 1984; Morris, 2002a; Zoran, 2002).

Keeping in mind that animals have nutrient and not ingredient requirements (Laflamme et al., 2014), the possibility of feeding cats on a plant-based diet should not be discharged as impossible: critical attention should be put on its correct formulation and furthermore on how feline's physiology answers to it.

Available literature on the subject of plant-based diets has been reviewed by Knight and Leitsberger and is mainly focused on the analytical approach of pet foods' composition in comparison with nutrient requirements, with just a few studies with a limited clinical approach (Knight & Leitsberger, 2016). On the diets' composition adequacy, Kanakubo and colleagues analysed crude protein and amino acids' levels in 11 canned and 13 dry plant-based pet foods finding that 25% of these were not meeting the Association of American Feed Control Officials (AAFCO) minimum amino acids' requirements (Kanakubo et al., 2015). More recently, the study group of the University of São Paulo analysed macronutrient composition, fatty acids and amino acids profile, and essential minerals content of all dry vegan pet food available on the market in Brazil, finding that all food tested had one or more nutrients below industries' recommendation (Zafalon et al., 2020).

While the online community claims a plethora of beneficial effects of transitioning pets to vegan or vegetarian diets, no randomised controlled clinical trials have yet been published as scientific proof (Sackett et al., 1996). To the authors' knowledge, Wakefield and colleagues published the only available study on cats fed long term (more than 1 year) a vegetarian diet: blood and plasma taurine, and plasma cobalamin of 17 cats were found in physiologic ranges (Wakefield et al., 2006). Neither follow-ups nor information about physical examination or other laboratory parameters were collected due to study's design (Wakefield et al., 2006). More recently, in an unpublished master's thesis of the University of Vienna, 20 dogs and 15 cats fed a plant-based diet were physically examined followed by haemato-biochemical analysis, magnesium, calcium, iron, total protein, folic acid, cobalamin and carnitine measurements. No abnormalities were found during physical evaluation, while a folate deficiency was found in 29% of dogs and 53% of sampled cats (Semp, 2014). No information was given on when the plant-based vegan regimen was started prior to blood analysis, nor follow-ups were made.

Paucity of literature on the long-term feasibility of plant-based diets in cats still demands answers. Concerns on the metabolic response of this obligate carnivore species require focus by the veterinary community.

To the authors' knowledge, this is the first published case report following over a long period the clinical evolution of two cats transitioned to a plant-based commercially available diet.

TABLE 1 Guaranteed nutrients concentrations and composition of the plant-based pet food given by the manufacturer

Analytical constituents (as fed)	
Crude protein (%)	33
Crude fat (%)	14
Crude fibre (%)	3
Crude Ash (%)	5.9
Moisture (%)	8
Magnesium (%)	0.09
Omega−3 (%)	0.8
Omega−6 (%)	6.5
Additives	
Vitamin A (IU/kg)	27,500
Vitamin D_3 (IU/kg)	1600
Vitamin E (mg/kg)	270
Copper (mg/kg)	10
Iron (mg/kg)	186
Iodine (mg/kg)	4
Selenium (mg/kg)	10
Zinc (mg/kg)	100
Manganese (mg/kg)	12
Taurine(mg/kg)	1500
L-carnitine (mg/kg)	300

Ingredients list: Corn, corn gluten, corn oil, rice, pea protein, pea fibre, brewer's yeast, dicalcium phosphate, linseed, hydrolysed vegetable protein, potato protein, sodium chloride, calcium carbonate, rapeseed oil.

2 | CASE HISTORY

A 2-year-old male neutered Maine Coon (MC) and a 1-year-old female spayed Domestic Shorthair cat (DSH) by the same caregivers, were referred to the nutrition service of the University of Toulouse (École Nationale Vétérinaire de Toulouse, France). Both felines were already known to the clinic for regular vaccination. Reason for consultation was the development of lethargy with in anamnesis a recent (5 months prior to symptoms) dietary transition to a plant-based pet food (Table 1). The owners had embraced veganism for several years because of increased empathy for livestock animals. Information about the plant-based diet was gathered on the internet. Positive feedback by the online community was enough to make the decision.

The MC and DSH lived strictly indoor, had been thoroughly dewormed, correctly vaccinated for core vaccines and already tested negative for Feline Immunodeficiency (FIV) and Feline Leukaemia (FeLV) viruses.

Clinical examination showed a dull and dry coat, abdominal distension (bloating) with augmented borborygmi, lethargy and an altered mentation paralleled by a slowed menace response with normal cranial nerve function. Both MC and DSH were found slightly under ideal body condition and therefore scored 4 on the Laflamme's BCS scale (Laflamme, 1997); a mild muscle waste was present, especially on the hindlimbs' muscle masses (Michel et al., 2011). Based on both BCS and muscle mass score (MMS), on present weight in kilograms (MC weighed 6.3 kg, while the DSH weighed 3.9 kg), and on published body weight (BW) references (Kienzle & Moik, 2011), MC's ideal BW was estimated 7 kg, while 4.5 kg was deemed ideal for the DSH.

3 | DIAGNOSTICS AND CLINICAL NUTRITIONAL ASSESSMENT

Diagnostics included haemato-biochemical analysis, serum cobalamin (vitamin B_{12}) and folate (vitamin B_9), plasma taurine and thiamine (vitamin B_1), iron and serum ammonia levels. All biochemical parameters, including ammonia, were in the laboratory's reference ranges, while both felines presented a mild macrocytic, hypochromic, non-regenerative anaemia (based on increased mean corpuscular volume, decreased mean corpuscular haemoglobin concentration, reticulocyte count and haematocrit) at the complete blood count, with slight hypernatremia (MC, 164 mM; DSH, 165 mM; [range 148–157 mM]) as single anomaly found in the electrolytes' panel. Neutropenia and hyperproteinaemia (81.1 g/L [range 55–71 g/L]) were features present only in the MC analyses. Results of the haemato-biochemical analyses at first consultation are resumed in Table 2.

Neither taurine nor cobalamin was below limits despite the present plant-based dietary regimen, while both cats presented a remarkable folate deficiency (MC, 3.69 ng/ml; DSH, 3.86 ng/ml; [range 10–25 ng/ml]). Thiamine was perfectly in laboratory's range; iron was near the lower limit in the DSH (12.3 μM [range 12–38 μM]), and just below in the MC (11.5 μM). Results of analysis performed to inquire nutritional status are detailed in Table 3.

To evaluate feeding behaviour and food intake with the purpose of assessing if energy and nutrient requirements were met, it was asked to measure (g/day) the daily amount of kibbles eaten per cat. To this end, it was used an electronic pet feeder (SureFeed®, Feeder Connect), which allowed to keep the *ab libitum* feeding behaviour while still knowing how much each cat was eating: collar-microchip devices allowed cats to be recognised so that both could eat from their own dedicated bowl without competition for food. Chronological record of food intake by each cat was registered thanks to the feeder's integrated scale (1 g accuracy and 400 ml of bowls' capacity). Details were readily accessible via a smartphone application (Sure Petcare®). Values in grams per day were then converted in terms of as-fed energy intake based on the pet food metabolisable energy (ME = 15.98 MJ/kg) calculated via the NRC (2006) equation (Calvez et al., 2019). Considering that, according to the owners, the MC and DSH did not receive any other pet food, treat or table food, it was possible to presume their overall daily energy intake.

TABLE 2 Results of the haemato-biochemical parameters at first consultation

Parameter	MC	DSH	Reference range
HCT (%)	21.8	22.4	25–45
RBC ($\times 10^{12}$/L)	5.6	6.2	5.5–10
MCV (fL)	68.3	61.5	40–55
MCH (pg)	13.4	14.0	12.5–17
MCHC (g/dL)	28.4	28.6	30–35
Hb (g/dL)	12	10	8–15
WBC ($\times 10^9$/L)	8.7	7.6	4.9–19.0
Neutrophil count ($\times 10^9$/L)	2.1	3.6	2.4–12.5
Lymphocyte count ($\times 10^9$/L)	4.2	4.3	1.4–6.0
Monocyte count ($\times 10^9$/L)	0.5	0.4	0.1–0.7
Eosinophil count ($\times 10^9$/L)	0.4	0.6	0.1–1.6
Reticulocytes (%)	<0.1	<0.1	–
Glucose (mM)	5.61	7.7	4.2–11.0
Urea (mM)	7.1	7.8	5.4–10.4
Creatinine (μM)	89.4	91.5	80–229
Sodium (mM)	164	165	148–157
Potassium (mM)	4.3	3.9	3.5–5.1
Chloride (mM)	124	120	115–128
tCa (mM)	2.4	2.7	2.3–2.9
Mg (mM)	1.0	0.9	0.8–1.1
Phosphate (mM)	1.38	1.1	0.8–3.0
Cholesterol (mM)	2.76	2.04	2.0–7.9
TG (mM)	0.31	1.25	0.2–1.8
AST (U/L)	28	27	6–44
ALT (U/L)	59	41	20–107
CK (U/L)	389	361	49–688
ALP (U/L)	39	49	23–107
GGT (U/L)	<5	<5	0–5
TBIL (μM)	4	2.5	1.7–8.4

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; GGT, gamma-glutamyl transpeptidase; Hb, haemoglobin; HCT, haematocrit; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell count; TBIL, total bilirubin; tCa, total calcium; TG, triglycerides; WBC, white blood cell count.

Recording of BW, starting from first consultation up to the last follow-up, was routinely done in the morning after an overnight fasting of at least eight hours using a portable scale (Marsden® V-22 veterinary scale, 20 kg capacity and graduations to 5 g). Patients BW was measured weekly.

TABLE 3 Results of blood parameters used for nutritional assessment at the first consultation

Parameter	MC	DSH	Reference range
Thiamine (µg/L)	44.10	64.60	20–90
Folate (ng/ml)	3.69	3.86	10–25
Cobalamin (pg/ml)	805	717	300–1000
Taurine (µM)	71.91	81.53	44–224
Iron (µM)	11.50	12.30	12–38
Total protein (g/L)	81.10	64.20	55–71
Albumin (g/L)	37.30	35.40	27–39
Ammonia (µM)	24	26	<98

Analysis of the daily food intake allowed to picture a fluctuating dysorectic feeding pattern for both cats. Results were compared with the FEDIAF's minimum (215 kJ/kg$^{0.67}$) and maximum (314 kJ/kg$^{0.67}$) daily energy intake for neutered and/or indoor cats (Figure 1).

Pet food's nutrients were analysed (laboratory Eurofins Analytics France, Nantes, France) to compare with guaranteed concentrations and industry's recommendations (FEDIAF, 2019). Analysis was limited to those nutrients which could explain the clinical picture and to some that could be deficient in a plant-based pet food. Differences between macronutrients declared on labelling and analysis were small and in the allowed tolerance. Results of the analysis for crude protein, amino acid profile, B-complex vitamins and trace elements are detailed and compared with FEDIAF requirements for indoor/neutered cats in Table 4.

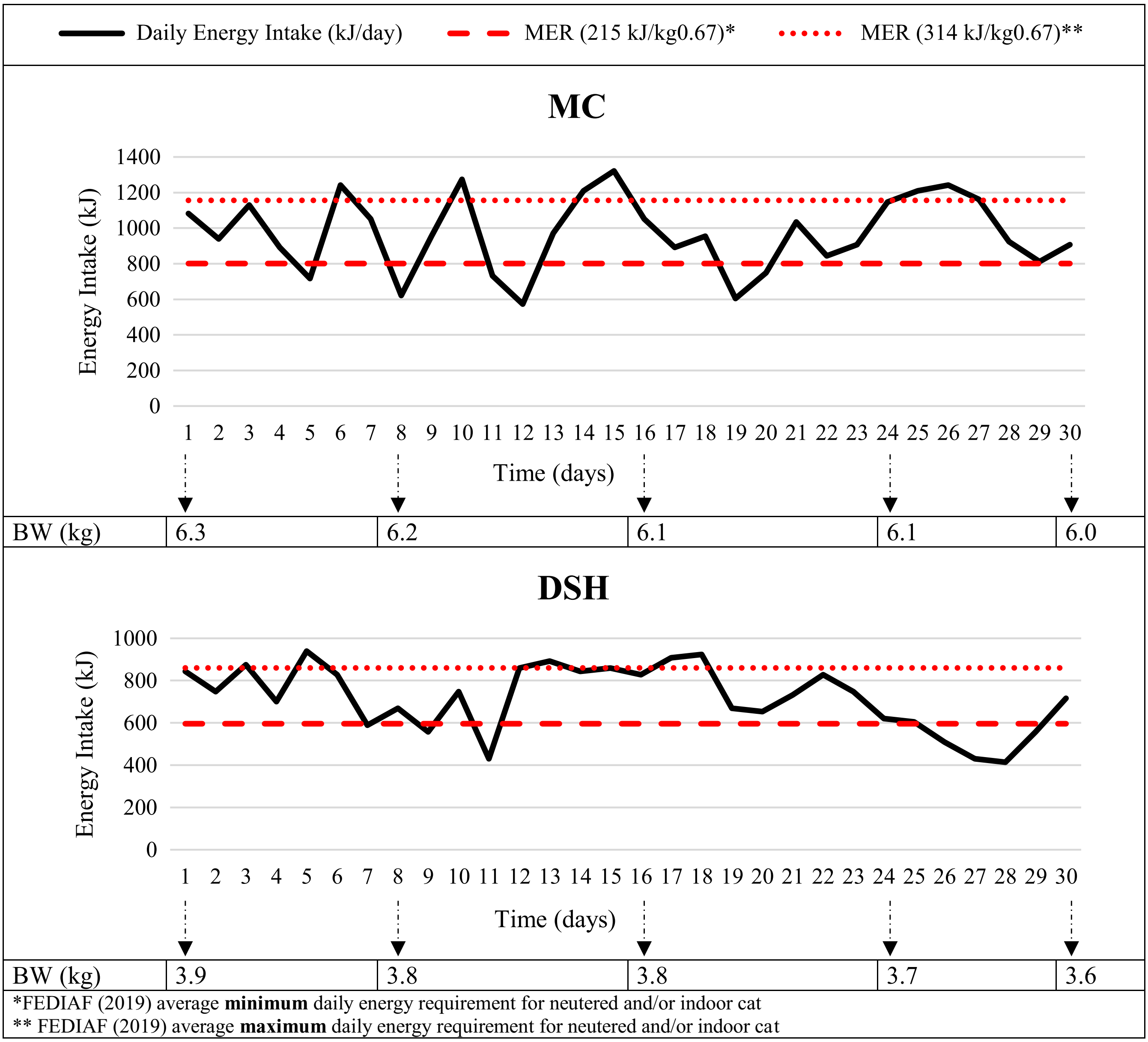

FIGURE 1 Main Coon (MC) and Domestic Shorthair cat (DSH) feeding curves (kJ/day) and weekly body weight (kg) measurement during the first month after nutritional assessment

TABLE 4 Plant-based pet food analytical constituents in comparison to FEDIAF's minimum recommendations for adult neutered and/or indoor cats

	FEDIAF minimum recommendation (314 kJ/kg$^{0.67}$)	Pet Food analysis[b]	Analytical method (Reference)
MACRONUTRIENTS	g/MJ	g/MJ	
Crude protein	19.92	20.21	Kjeldahl method
AMINO ACIDS	g/MJ	g/MJ	
Arginine	0.80	0.86	AH (ISO 13903:2005; EU 152/2009)
Histidine	0.21	0.44	AH (ISO 13903:2005; EU 152/2009)
Isoleucine	0.35	0.82	AH (ISO 13903:2005; EU 152/2009)
Methionine	0.14	0.43	AH (ISO 13903:2005; EU 152/2009)
Leucine	0.81	2.90	AH (ISO 13903:2005; EU 152/2009)
Lysine	0.27	0.62	AH (ISO 13903:2005; EU 152/2009)
Phenylalanine	0.32	1.19	AH (ISO 13903:2005; EU 152/2009)
Threonine	0.41	0.76	AH (ISO 13903:2005; EU 152/2009)
Tryptophan	0.11	0.16	AH (EU 152/2009)
Valine	0.41	0.98	AH (ISO 13903:2005; EU 152/2009)
Taurine	0.08	0.15	AH (ISO 13903:2005)
VITAMINS B	mg/MJ	mg/MJ	
Thiamine	0.35	0.30[a]	HPLC (EN 14122:2003)
Riboflavin	0.25	0.17[a]	HPLC (EN 14152:2003)
Niacin	2.52	3.79	HPLC (EN 15652:2009)
Pantothenic Acid	0.46	0.48	UPLC-MS/MS (AOAC 2012.16)
Pyridoxine	0.20	0.26	HPLC (EN 14164:2014)
	µg/MJ	µg/MJ	
Biotin	4.78	15.89	Turbidimetric (LST AB 266.1,1995)
Folic Acid	60.50	37.24[a]	Microbiological (NMKL 111:1985)
Cobalamin	1.40	1.68	HPLC (J. AOAC 2008, vol. 91 no. 4)
TRACE ELEMENTS	mg/MJ	mg/MJ	
Iron	6.37	14.58	ICP/MS (internal[b])
Copper	0.40	1.06	ICP/MS (internal[b])
Zinc	5.98	6.02	ICP/MS (internal[b])
Iodine	0.1	0.03[a]	ICP/MS (EN 15111)

Abbreviations: AH, acid hydrolysis; HPLC, high-performance liquid chromatography; ICP/MS, inductively coupled plasma mass spectrometry; UPLC-MS/MS, ultra-performance liquid chromatography tandem mass spectrometry.

[a]Below minimum recommendation.

[b]Laboratory Eurofins Analytics France, Nantes, France.

Protein and amino acid profile met requirements. In the analysed vitamin B-group, thiamine (0.3 mg/MJ), riboflavin (0.17 mg/MJ) and folic acid (37.24 µg/MJ) did not meet FEDIAF minimum recommended nutrient levels for indoor/neutered cats at the upper end of the proposed daily energy requirement. As for trace minerals, iodine (0.03 mg/MJ) was the only nutrient below recommendation.

4 | CASE PROGRESSION

A dietary change was proposed as non-pharmacological treatment, justified by the multiple nutrients found below and/or just above minimum recommendation. Pet owners showed reluctance to the idea of going back to a diet with animal-derived ingredients. Due to the unwillingness to change the dietary regimen, follow-ups were programmed to closely assess clinical evolution. Each consultation was performed by the same operators starting with complete physical examination, weighing, scoring of adipose tissue (1–9 scale) (Laflamme, 1997) and muscle masses (0–3 scale) (Michel et al., 2011), assessment of mentation, menace response and cranial nerve reflexes. Body weight (kg) and daily metabolisable energy intake (kJ/day) were continuously measured.

During first follow-up (30 days after first consultation), serum folates were still way below laboratory reference for both cats (MC, 2.94 ng/ml; DSH, 3.74 ng/ml; [range 10–25 ng/ml]) while, despite being virtually absent in plant products, serum levels of cobalamin were above the lower limit (MC, 351 pg/ml; DSH, 346 pg/ml; [range 300–1000 pg/ml]). No obvious neurological degradation was observed for the DSH, while the MC appeared more lethargic than previously assessed. Thiamine blood levels, while lowered since first measurement, were still in reference limits (MC, 38.2 μg/L; DSH, 48.7 μg/L; [range 20–90 μg/L]); progression of weight loss was similar for both felines (0.3 kg in 30 days).

Other diagnostics performed in the follow-up period, allowed to focus mainly on the nutritional aspect, dismissing other differential diagnosis. Thyroid profile (total thyroxine [TT4], free thyroxine [fT4] and thyroid-stimulating hormone [TSH]), despite low dietary iodine concentrations, did not show abnormalities. Abdominal ultrasonography was inconclusive, with no signs of small intestine's disease suggesting nutrients' malabsorption. A mild macrocytic, non-regenerative anaemia was present in the haematological profile. While always near the lower limit, iron was now in laboratory's range for both DSH and MC.

5 | TREATMENT AND OUTCOME

To address the presence of multiple B-complex vitamins and trace element deficiencies found in the pet food analytical composition, in light of clinical picture and blood analyses results, a supplement containing vitamins, trace elements and amino acids was proposed to the owner as alternative to the unwanted dietary change. Despite continuing to refuse any dietary modification or introduction of a supplement to compensate for deficiencies, stressing the importance of a complete and balanced diet brought to a compromise for folic acid supplementation, having for this nutrient a strong

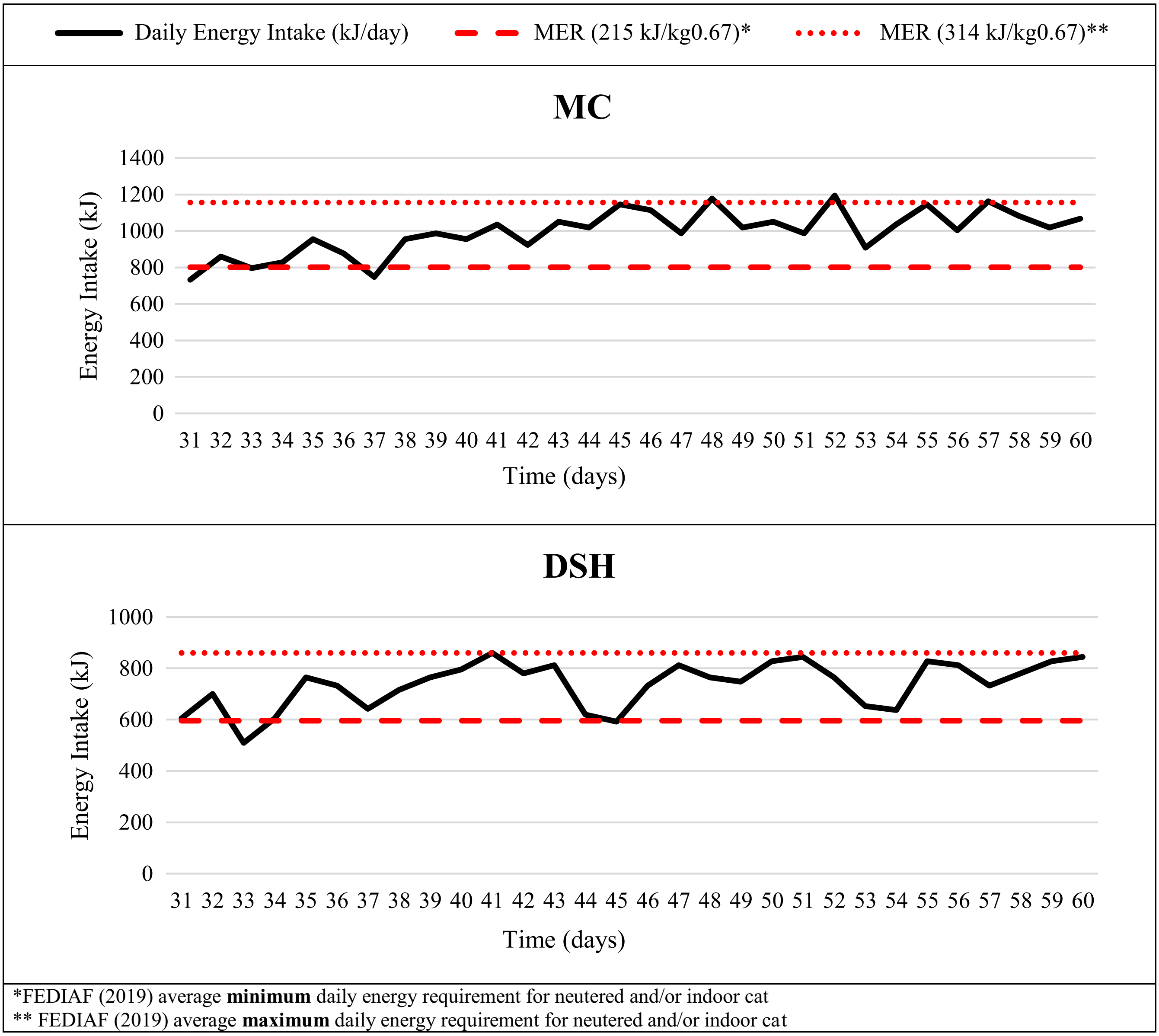

FIGURE 2 Main Coon (MC) and Domestic Shorthair cat (DSH) feeding curves (kJ/day) after folic acid supplementation

correlation between its concentration in the diet and in the serum. Supplementation began immediately after first follow-up, at a total daily dose of 0.4 mg/cat PO (Ruax, 2013), using a folic acid water-soluble tablet (Acide Folique CCD 0.4 mg®, CCD Laboratory, Paris, France). Metabolisable energy intake (kJ/day) after folic acid supplementation is shown in Figure 2.

Cats showed partial improvement of feeding behaviour: mean daily energy intake of MC during first 30-day measurement was 972 kJ/day (prior to supplementation), accounting for 84.1% of the calculated MER, which improved to an average of 994 kJ/day (86.0% MER) in the next month. It should be noted that daily energy intake stabilised after initial 10 days of oral vitamin supplementation, making for a mean of 1052 kJ/day (91.0% MER) in 20 days after; DSH consumed a mean of 718 kJ/day (83.4% MER) in the first 30-day measurement (prior to supplementation), augmenting to an average of 747 kJ/day (86.8% MER) in the second month. As for the MC, food intake really improved after 10 days of oral folic acid, making the mean energy intake 777 kJ/day (90.4% MER). Overall, introduction of oral folic acid supplementation brought to a 7% increase of daily energy intake.

Follow-up of serum folate at 30-days was 17.7 and 19.3 ng/ml for the MC and DSH respectively (range 10–25 ng/ml). Cats' feeding behaviour was less dysorectic, but nonetheless food

TABLE 5 Comparison between the FEDIAF's minimum recommended nutrient level per $kg^{0.67}$ and the amount of nutrient per $kg^{0.67}$ provided by the vegan pet food when eating FEDIAF's minimum daily energy requirement for neutered/indoor cats and at the average (±SD) energy intake of the MC and DSH

	Nutrient level per $kg^{0.67}$ in the vegan feed considering minimum FEDIAF energy intake [215 kJ/$kg^{0.67}$]	FEDIAF's minimum recommended nutrient level per $kg^{0.67}$	Nutrient level per $kg^{0.67}$ in the vegan feed considering the average energy intake of the MC [286(±39) kJ/$kg^{0.67}$]	Nutrient level per $kg^{0.67}$ in the vegan feed considering the average energy intake of the DSH [294(±39) kJ/$kg^{0.67}$]
MACRONUTRIENTS	g			
Crude Protein	4.35[a]	6.25	5.79 (±0.78)[a]	5.94 (±0.78)[a]
AMINO ACIDS	g	g		
Arginine	0.18[a]	0.25	0.246 (±0.033)[a]	0.253 (±0.033)
Histidine	0.09	0.08	0.13 (±0.02)	0.13 (±0.02)
Isoleucine	0.18	0.12	0.23 (±0.03)	0.24 (±0.03)
Methionine	0.09	0.04	0.12 (±0.02)	0.13 (±0.02)
Leucine	0.62	0.29	0.83 (±0.11)	0.85 (±0.11)
Lysine	0.13	0.09	0.18 (±0.02)	0.18 (±0.02)
Phenylalanine	0.26	0.12	0.34 (±0.05)	0.35 (±0.05)
Threonine	0.16	0.15	0.22 (±0.03)	0.22 (±0.03)
Tryptophan	0.03[a]	0.04	0.05 (±0.01)	0.05 (±0.01)
Valine	0.21	0.15	0.28 (±0.04)	0.29 (±0.04)
Taurine	0.03	0.03	0.04 (±0.01)	0.04 (±0.01)
VITAMINS B	mg			
Thiamine	0.06[a]	0.11	0.09 (±0.01)[a]	0.09 (±0.01)[a]
Riboflavin	0.04[a]	0.08	0.05 (±0.01)[a]	0.05 (±0.01)[a]
Niacin	0.81	0.79	1.09 (±0.15)	1.11 (±0.15)
Pantothenic Acid	0.10[a]	0.14	0.137 (±0.019)[a]	0.141 (±0.019)
Pyridoxine	0.06	0.06	0.07 (±0.01)	0.08 (±0.01)
	μg			
Biotin	3.42	1.50	4.55 (±0.62)	4.67 (±0.62)
Folic Acid	12.98[a]	19.00	17.29 (±2.34)[a]	17.76 (±2.34)[a]
Cobalamin	0.15[a]	0.44	0.19 (±0.03)[a]	0.20 (±0.03)[a]
TRACE ELEMENTS	mg			
Iron	3.13	2.00	4.18 (±0.56)	4.29 (±0.56)
Copper	0.23	0.13	0.30 (±0.04)	0.31 (±0.04)
Zinc	0.95[a]	1.88	1.27 (±0.17)[a]	1.31 (±0.17)[a]
Iodine	0.01[a]	0.03	0.007 (±0.001)[a]	0.007 (±0.001)[a]

[a]Below minimum recommendation.

intake was insufficient to meet energy requirements and achieving ideal BW. No weight loss was measured for the DSH, while the MC now weighed 5.9 kg (0.1 kg less than previous consultation). Haematological analysis was in the limits, with no signs of anaemia or macrocytosis.

Looking at the food intake over a 60-day follow-up period, it was possible to better analyse the daily energy and nutrient intake of both cats. Although FEDIAF's (2019) recommendations for cats are detailed only for the maximum daily requirement of neutered/indoor (314 kJ/$kg^{0.67}$) and active (418 kJ/$kg^{0.67}$) cats, the Federation also acknowledges that some felines may have daily energy requirements, proposing a minimum of 215 kJ/$kg^{0.67}$ (FEDIAF, 2019) based on published data (Riond et al., 2003; Wichert et al., 2007). Because both cats in the present work exhibited an energy intake below 314 kJ/$kg^{0.67}$, it was assessed if the nutrients concentrations of the vegan pet food were adequate to meet requirements at the minimum daily energy intake for neutered/indoor cats proposed by FEDIAF, and of course at the average (±SD) daily energy intake (over the study 60-day period) of the MC and DSH (Table 5). Results showed that the analysed vegan pet food did not ensure the minimum requirement for several nutrients if considering the lower end of the daily energy intake. Multiple nutrient deficiencies were also present when the average daily energy intake of the study's felines was considered: only 92.6% (±12.5) and 95.1% (±12.5) of the minimum crude protein requirement was met for the MC and DSH respectively. Looking at the plant-based diet aminogram, the average intake of arginine was below recommendation only for the MC [98.5% (±13.3) of the minimum requirement]. Of the vitamin B-complex, thiamine [MC 78.1% (±10.6); DSH 80.2%(±10.6)], riboflavin [MC 60.9% (±8.2); DSH 62.5% (±8.2)], pantothenic acid [MC 98.2% (±13.3); DSH 93.5% (±12.3)], folic acid [MC 91.0% (±12.3); DSH 93.5% (±12.3)] and cobalamin [MC 44.3% (±6.0); DSH 45.5% (±6.0)] were below the minimum recommended intake for $kg^{0.67}$ in both cats. Finally, deficiency of zinc [MC 67.6% (±9.2); DSH 69.5% (±9.2)] and iodine [MC 23.9% (±3.2); DSH 24.5% (±3.2)] was also common to the MC and DSH at the measured daily intake.

At 3 months from initial nutritional assessment, discouraged by the lack of weight gain and complete resolution of the clinical picture, proposition of a diet change was accepted. The thorough diagnostic work-up paralleled by feed analyses and food intake diagrams made for stronger argumentations, and finally led the pet-owners to open up to the idea of transition to an animal-based standard pet food.

To avoid perturbation of the microbiota, because of the novel ingredients' introduction, a 10-day dietary transition was advised maintaining *ab libitum* feeding. For the newly proposed diet, analytical constituents declared by manufacturer are detailed in Table 6.

Telephone follow-ups were made every month. Proposed diet was well accepted and, according to owner-perception, both cats regained vitality and appetite. At 4 months since reintroduction of animal-derived ingredients, MC and DSH weighed 6.8 and 4.2 kg, respectively, accounting for approximately an average 1% BW gain per week.

TABLE 6 Guaranteed nutrients concentrations and composition of the proposed pet food with animal-based protein sources

Analytical constituents (as fed)	
Crude protein (%)	**31.2**
Crude fat (%)	8.6
Crude fibre (%)	7.7
Nitrogen-Free Extract (%)	39.5
Moisture (%)	8.0
Calcium (%)	0.83
Phosphorus (%)	0.65
Potassium (%)	0.63
Magnesium (%)	0.07
Omega−3 (%)	0.22
Omega−6 (%)	2.09
Additives	
Vitamin A (IU/kg)	9,700
Vitamin D_3 (IU/kg)	410
Vitamin E (mg/kg)	550
Vitamin C (mg/kg)	70
Taurine (%)	0.21
L-carnitine (mg/kg)	475

Ingredients list: Chicken, brewers rice, chicken meal, corn gluten meal, whole grain wheat, powdered cellulose, chicken fat, chicken liver flavour, potassium chloride, flaxseed oil, L-carnitine, calcium sulphate, DL-methionine, taurine, vitamins and trace elements.

6 | DISCUSSION

This case report describes the clinical signs and anomalies in laboratory findings in two cats on a plant-based diet. Analysis of the plant-based vegan pet food showed several nutrients below, or just above, minimum recommendation. This finding, paralleled by clinical manifestations and results of the diagnostic work-up, brought to suspect multiple nutrient deficiencies. Main haematological feature was the presence of hypofolatemia paralleled by signs of anaemia. Supplementation of folic acid had a positive effect on feeding behaviour, with lesser variation in daily food intake. The nutrient composition of the plant-based diet was found to be unsuitable for meeting protein, arginine, thiamine, riboflavin, pantothenic acid, folic acid, cobalamin, zinc and iodine at the MC and DSH average daily energy intake measured over 60 days.

Complete resolution of the clinical picture was achieved only after dietary change, with reintroduction of animal-derived ingredients, showing that, as suspected, multiple nutrient deficiencies played a role.

Despite many years have now passed since the appearance on the market of plant-based 'vegan' pet foods, published knowledge on the consequences of long-term feeding in the feline species is still lacking. These diets are designed to answer the same health, environmental and ethical concerns that drive the growing popularity of veganism in people (Dodd et al., 2019), and usually consumers buying this specific diets, are just trying to apply their own beliefs to their cats as it was the case for the owner of the two patients followed in the present work.

It may seem strange, but, sometime, prescribing a pharmacological solution, in face of clinical manifestations, can be easier than advising a dietary change. Increased awareness of pet-owners of the importance of nutrition, and access to the unfiltered information provided by the web, makes it extremely important for the veterinary practitioner to have the proper tools to provide scientific-based knowledge to justify its dietary councils. This lack of confidence in veterinarians when it comes to nutrition, has been recently highlighted in a survey done by a study group in Italy about non-conventional diets (e.g. raw meat-based diets), where 60% of the respondents had chosen their pets' diets based on information gathered on the internet, in contrast to only a 9% who followed the advice of their veterinarian (Morelli et al., 2019).

The strict carnivore nature of cats is defined by several anatomical and physiological features. The metabolic idiosyncrasies characterising felines, make it clear that animal-derived foods are more suitable sources for answering its nutrient requirements arising concern on the feasibility and risks of plant-based diets in this species. Cats have, in fact, higher protein (i.e. nitrogen) requirements are more susceptible to arginine deficiency and, contrary to dogs, count taurine, which is virtually non-existent in plants, as the eleventh essential amino acid (Morris, 2002a). They also have higher thiamine (at least 3-fold dogs) and niacin (2.5-fold dogs) requirements (FEDIAF, 2019), and use more efficiently the animal-derived vitamin D_3 (cholecalciferol) instead of the form actually present in pants (ergocalciferol) (Morris, 2002b). Cats are unable to transform carotenoids (provitamin A) from plants into vitamin A, which has to be consumed from animal sources, and have an essential dietary need for arachidonic acid, a fatty acid typically found in animal-derived fats (Morris, 2002a). Moreover, carbohydrate metabolism has some distinguish features too (e.g. lower activity of hepatic *glucokinase* and *glycogen synthase*), which allow a preferential use of amino acids and fatty acids instead of glucose for energy production (Verbrugghe & Hesta, 2017).

At first examination, cats showed non-specific symptoms, such as a fluctuating appetite (dysorexia) and lethargy, ascribable to a plethora of clinical conditions, which warranted the thorough diagnostic work-up, fundamental to rule out other underlying diseases.

A mild muscle waste was detected during physical examination. The assessment of the latter via muscle mass scoring could not be confirmed via more advanced diagnostic techniques, such as the dual-energy X-ray absorptiometry (DEXA), which is currently considered as the *gold standard* (Michel et al., 2011). However, it could be argued that the found suboptimal (below minimum recommendation) intake of protein guaranteed by the vegan pet food at the average daily energy intake of both cats, could explain the loss of muscle mass. In fact, as previously said, one of the defining metabolic features of the strict carnivore nature of cats, is the species higher protein (i.e. nitrogen) requirement. Cats have little adaptation in the activity of their hepatic aminotransferases and urea cycle enzymes, meaning that in situations of fasting or low protein intake, these enzymes readily catabolise protein from muscles, potentially causing muscle waste (Morris, 2002a).

Despite this hypothesis, it should be said that the authors could not prove that the muscle atrophy developed consequently to the dietary transition. Potentially the process could have started before, although no loss of lean mass was recorded in the University's database at the moment of the felines vaccination just before the introduction of the plant-based diet.

No severe neurological sign was observed by the authors, still, both cats showed lethargy and a dull mentation. When considering the amount of food consumed per day during the first 2 months of follow-ups, the MC showed suboptimal intake of the essential amino acid arginine in comparison with the minimum recommendation proposed by FEDIAF per kilogram of metabolic weight (g nutrient/$kg^{0.67}$). Arginine intake for the DSH was just above the minimum.

The higher requirement for arginine in cats is also considered as one of its metabolic idiosyncrasies. Cats have, in fact, a low capacity of synthesising arginine from its precursors ornithine and citrulline (low enzymatic activity of *pyrroline-5-carboxylase synthase* and *ornithine aminotransferase* in the intestinal mucosa) coupled with the aforementioned inability to downregulate the liver-enzymes activity; hence, they need a high dietary concentration of arginine (Carvalho et al., 2020; Morris, 2002a). Near-adult cats fed a single arginine-free meal quickly develop hyperammonaemic encephalopathy due to the role of this amino acid in the conversion of ammonia into the less toxic urea (urea cycle inside liver's mitochondria) (Morris & Rogers, 1978).

Circulating blood ammonia was found to be in the physiological range for both cats at first consultation but no follow-ups were made for assessing its evolution. Moreover, plasma amino acid profile was not obtained to confirm possible low circulating arginine levels. The MC, which had arginine intake below minimum recommendation, showed an increasingly obtunded mentation, still, no severe neurological manifestation (e.g. hyperaesthesia, ataxia, extended limbs) typically linked to arginine deficiency was seen (Morris & Rogers, 1978).

Amongst the differential diagnosis for lethargy and altered mentation, thiamine (vitamin B_1) deficiency was also considered. This vitamin is involved in several vital pathways, namely the tricarboxylic acid cycle and the pentose phosphate pathway in the carbohydrate metabolism (Markovich et al., 2013). Cats have a greater species-specific requirement for thiamine (FEDIAF, 2019). This aspect has to be considered during feline pet food formulation, knowing also that thiamine is extremely heat labile and a high percentage is usually lost during processing (Markovich et al., 2014).

Dietary intake was found below minimum recommendation in both cats. Even though no vomiting was described by the owner, lethargy and reduced appetite were present, which are signs linked to the first (induction) of the three progressive stages of thiamine deficiency described in cats (Chang et al., 2017).

Thiamine was also measured in the plasma of the MC and DSH via the direct method using high-performance liquid chromatography (HPLC) (Kritikos et al., 2017). Little is known about this vitamin status in cats, and an accurate reference range has not yet been validated (Kritikos et al., 2017). Nonetheless, results for plasma thiamine in the study's felines were always in the laboratory reference range, with follow-up concentrations slightly lower in comparison with first analysis.

In the vitamin B-complex, also the minimum requirement of riboflavin (vitamin B_2), pantothenic acid (vitamin B_5), cobalamin (vitamin B_{12}) and folic acid (vitamin B_9) was not met by the plant-based vegan pet food at the MC and DSH daily intake.

Both cats daily intake of riboflavin was below FEDIAF minimum recommendation, but none of the described clinical features of its deficiency, such as periauricular alopecia, cataracts and fatty liver, were found during the diagnostic process (NRC, 2006; Scott et al., 1964).

Only the MC did not meet the minimum intake for pantothenic acid. The latter is essentially an integral component of coenzyme A (CoA) and plays a role in acyl group transfers and condensation reactions (NRC, 2006). Deficiencies are rare in cats, with one dated study by Gershoff and Gottlieb (1964) reporting failure to grow and histological changes in liver and small intestine. The hypothesis that this nutrient deficiency played an important role in the clinical manifestation of the MC was rapidly discarded by the authors, considering that the daily intake was only slightly below minimum recommendation.

Less than half of the minimum recommended intake for cobalamin was met by both cats during the observed period. The major dietary sources of this vitamin are animal products, even though cobalamin-containing plant food with high concentrations do exist (e.g. *Enteromorpha* spp and *Porphyra* spp algae) (Watanabe et al., 2014).

This essential nutrient is involved as a cofactor in a plethora of enzymatic reactions, and encephalopathy, vomiting, diarrhoea, anaemia, anorexia and failure to thrive have been described as clinical manifestations of its severe deficiency in cats (NRC, 2006).

Serum cobalamin concentration was measured in both cats without finding any result outside the laboratory reference range. Curiously, when comparing serum levels at first consultation with follow-up (30 days after), in both cats circulating levels had lowered by more than 40%. This decline in such a short period of time may imply that initial concentrations were kept higher by the limited liver-storage capacity of this water-soluble vitamin (Jerzy Glass, 1959), possibly confirming the suboptimal intake.

Dietary intake of folate was also below minimum recommendation. Folate is an essential water-soluble vitamin which can be naturally found (pteroylpolyglutamate derivatives) in foods, especially leafy green vegetables and organs. Biological mechanisms involving folate, concern amino acid and nucleotide metabolism, disposal of one-carbon units, mitochondrial protein synthesis and haematopoiesis (Crider et al., 2012).

In industrial pet food manufacturing, folates are routinely included in vitamin premixes, taking into consideration that processing can produce different levels of folate losses (Tran et al., 2008).

In case of its deficiency, a known haematological finding in people is megaloblastic anaemia, where the defective DNA synthesis and haematopoiesis manifests in a macrocytic, non-regenerative anaemia which can be rapidly reversible with vitamin oral supplementation (McNulty & Scott, 2008). Although reports of similar alterations in haematological parameters have been described in cats, the same relationship with vitamin B deficiencies has not been as clearly explored in veterinary medicine, but findings suggest a similar pathophysiology (Thenen & Rasmussen, 1978; Yu & Morris, 1998).

In the present case report, both felines had folates concentrations below laboratory reference, paralleled by normal serum cobalamin. This same pattern was reported in a thesis by the veterinary University of Vienna, where 46% (n = 7/15) of the examined cats on a commercial plant-based diet showed hypofolatemia (Semp, 2014). Furthermore, both the MC and DSH showed a non-regenerative macrocytic anaemia in the haematological profile, which improved and disappeared after supplementation of folic acid.

Other differential diagnosis should be considered for hypofolatemia, but no biological parameter or other performed diagnostic suggested a concomitant metabolic disorder: ultrasound imaging was inconclusive, as for biochemistry and thyroid profile. Currently, there is no clinical trial showing that a lower serum folate concentration is associated with a functional folate deficiency, but the relationship between dietary folate's intake and serum folate concentration has been demonstrated (Yu & Morris, 1998).

Amongst trace minerals, iodine and zinc were found below minimum recommendation at the average daily intake of the MC and DSH.

Despite iodine dietary low intake, no abnormalities were shown in both felines' thyroid profile. Iodine concentration was 0.43 mg/kg (DM basis), which is way below FEDIAF [1.3 mg/kg (DM basis)] and NRC [1.4 mg/kg (DM basis)] recommendations (FEDIAF, 2019; NRC, 2006). There is still some controversy around this nutrient dietary need, and discrepancies in requirements can be important: AAFCO recently augmented from 0.35 to 0.6 mg/kg (DM basis), while according to the study by Wedekind et al. (2009), iodine requirement for adult cats should be 0.46 mg/kg (DM basis). Theoretically, suboptimal dietary iodine levels should lead to its insufficient availability in thyroid's hormone synthesis ensuing TSH activity, which could promote thyroid hyperplasia which was not found in the studies patients (Loftus et al., 2019).

Concentration of zinc in the plant-based pet food was also found to be deficient at the dietary intake of the two felines. An opposite result was recently showed in the nutrient analysis of the only vegan pet food for cats present on the market in Brazil, where zinc concentration was actually above declaration (Zafalon et al., 2020).

This nutrient plays a role as cofactor or catalyst in over two hundred enzymes involved in carbohydrate and protein metabolism, cellular replication, skin function, wound healing and other biological functions (NRC, 2006). Poor coat was described during first examination in both cats. No other cutaneous sign, such as ulceration of the buccal margins, was indicating a stronger link with a possible deficiency of this trace mineral (Kane et al., 1981).

Skin and coat quality may also be affected by the diet's fatty acid composition. The latter was not analysed in the study's pet food: concentration of the essential linoleic acid and arachidonic acid could not be evaluated.

Synthesis of arachidonic acid from linoleic acid is limited in cats due to a low *Δ6-desaturase* activity, (Morris, 2002a). Despite there is scientific proof that it can be sourced from some algae (van Ginneken et al., 2011), it is commonly added in standard feline pet food via animal-derived fats. Several studies on the adequacy of industrial plant-based diets for cats highlighted how this nutrient is frequently 'forgotten' during formulation (Gray et al., 2004; Kienzle & Engelhard, 2001; Zafalon et al., 2020), questioning the knowledge of plant-based diets' formulators around the essentiality of this nutrient in cats.

Overall several nutrients were found below minimum recommended intake considering the daily average amount of food consumed by both cats. This fluctuating feeding behaviour, which brought to exacerbating the suboptimal intake of nutrients, opens up to questioning the palatability of plant-based diets in strict carnivore felines.

Palatability is a complex concept and has been defined as the subjective temporary pleasant oro-sensory feeling of food consumption (Stubbs & Whybrow, 2004). When given the choice, cats tend to choose protein-rich diets (Hewson-Hughes et al., 2011, 2016; Salaun et al., 2017) with a nutrient profile of the overall diet resembling that of free-roaming feral cats feeding on whole preys (Plantinga et al., 2011). This feeding pattern has been recently questioned in a study by Hall et al. (2018): according to the 28-day study, when offering diets with similar palatability, cats chose to consume most of their calories from carbohydrates (43%), contradicting the previously introduced concept of *carbohydrate ceiling effect*. Nonetheless, despite the study's opposite results in terms of macronutrient selection, balancing ingredients and palatants (natural chicken flavour) to avoid 'palatability' as a confounding factor, acknowledged by itself that animal-derived ingredients affect intake in cats (Hall et al., 2018).

Having said so, some of the most important ingredients which positively affect palatability in felines are known to be of animal origin: salmon oil, animal-derived proteins and fats (Pekel et al., 2020). For this reason, following the concept of cats as strict carnivores, animal-derived flavour enhancers (*palatants*) have been used since long time by the pet food industry to increase palatability of feline diets. Hydrolysed animal-proteins (e.g. liver, chicken) (Martínez-Alvarez et al., 2015) and spray-dried animal plasma (in canned diets) (Polo et al., 2005) are some of those routinely added for this purpose.

As previously said, it is clear that sourcing the nutrients required by carnivore cats in plant-based ingredients it is not an easy task. For this reason, the scientific community raises significant doubt about those manufacturers which advertise complete plant-based diets as 'vegan'.

When considering the declared composition of the study plant-based diet, some questions came up concerning their origin. Taurine was present in the list of additives. This amino acid was thought to be non-existent in plant tissues, but significant amounts have been discovered in some algae (e.g. *Mazaella* spp) (McCusker et al., 2014). Vitamin A has to be added to by-pass the inability of cats to transform plant carotenoids, and synthetic forms can be used in premixes (Parker et al., 2016). Plant-based vitamin D_2 is not present in the European Union register of feed additives. The only legal additive left for vitamin D is cholecalciferol (vitamin D_3), which was the one declared in the study's diet. Synthesis of cholecalciferol as an additive comes from irradiation of 7-dehydrocholesterol extracted from lanolin in sheep fleeces (Taofiq et al., 2017) and therefore does not embrace the definition of 'vegan', making the pet food mislabelled as such.

All these considerations taken together raise concern on the adequacy of these diets, and on the knowledge of plant-based manufacturers of the species-specific requirements of cats. It can be argued that from an ethical point of view these diets do not embrace the physiology and ethogram of this species. The « *code rural et de la pêche maritime*» is a French national code of law which at the Article L214-1 states that 'animals must be placed by their owners in conditions compatible with the biological imperatives of the species'. A similar statement can be found in the UK Animal Welfare Act where it is clearly underlined that owners must ensure a 'suitable diet' for their pets. Therefore, without disrespecting the moral dilemma of vegan people feeding their cats with animal-derived ingredients, concern should be raised on the ethics of a strict carnivore fed a plant-based diet.

7 | CONCLUSION

Feeding strict carnivore cats plant-based diets raises several concerns, namely about nutritional adequacy, palatability and also about the ethic of doing so. Results of the analyses performed on the study diet highlight several nutrient deficiencies, and the unsuitable composition for felines with lower energy intake, as it was the case for the study patients. Lethargy, dysorexia, muscle waste and weight loss developed in two cats after 5 months on an industrial plant-based diet. At the measured average food intake of both felines, protein, arginine, thiamine, riboflavin, pantothenic acid, folic acid, cobalamin, zinc and iodine were below minimum recommendation. Dietary transition to a standard diet with animal-derived ingredients brought to progressive amelioration of the clinical picture.

CONFLICT OF INTEREST

Authors have no conflicts of interest to disclose.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to.

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How to cite this article: Fantinati M, Dufayet R, Rouch-Buck P, Priymenko N. Relationship between a plant-based 'vegan' pet food and clinical manifestation of multiple nutrient deficiencies in two cats. *J Anim Physiol Anim Nutr*. 2021;00:1–13. https://doi.org/10.1111/jpn.13510