2023

Nutrition-Based Interventions in Dogs: A Comprehensive Review of Health Outcomes and Implications for Dog Food Recipes

N.R. Medforth (MSc) & R. Wiener

Abstract

Introduction: Concerns about mineral imbalances and inadequate adherence to nutritional guidelines in commercial dog foods, prompted us to review nutritional research in dogs to develop a dog food recipe that would provide optimal nutrition.

Methods: We reviewed 117 studies categorized into six groups: 'Protein,' 'Fats & oils,' 'Carbohydrates,' 'Fibre,' 'Probiotics,' and 'Vitamins, Minerals & Bioavailability.' Due to heterogeneity, a narrative analysis approach was employed to synthesize and explain the data.

Results: Animal protein was found to be superior to plant-based protein due to its complete amino acid profile, although combining complementary plant-based proteins with supplements can provide adequate nutrition. Sous vide cooking improves nutrient bioavailability in meat. High protein diets aid weight management and glycaemic control. Optimal n-6 to n-3 fatty acid ratios remain elusive, and high n-3 doses require vitamin E supplementation. Complex carbohydrates can provide extra energy, though careful preparation is necessary for optimal digestibility. Grain-free diets are not inherently healthier, and a link between dilated cardiomyopathy and grain-free diets was noted. Fibre supports gut health and weight management, with small and large breed dogs having slightly different fibre needs. Probiotics improve gut microbial composition, stool quality and nutrient digestibility. Considering nutrient interactions, avoiding excessive synthetic nutrient additives, and utilising bioavailable forms of essential nutrients are vital in dog food formulation.

Conclusion: Our systematic review highlights the importance of developing dog foods with a comprehensive understanding of nutrient needs and interactions. Animal protein, real food sources, and bioavailable nutrients are preferred over high carbohydrates and synthetic additives.

Abbreviations: EAA, essential amnio acids; **AA**, amino acids; **PUFA**, polyunsaturated fatty acids; **n-3**, omega 3; **n-6**, omega 6; **EPA**, eicosapentaenoic acid; **LA**, Linoleic acid; **AA**, arachidonic acid; **ALA**, alpha linoleic acid; **OA**, osteoarthritis; **AD**, atopic dermatitis; **SCFA**, short chain fatty acids; **GF**, grain free; **HFF**, high fermentable fibre; **LFF**, low fermentable fibre; **BCS**, body condition score; **OM**, organic matter; **DM**, dry matter; **CP**, crude protein; **CF**, crude fibre; **CFU**, colony forming units; **FDA**, food and drug agency

Introduction

Following a preliminary investigation into research relating to dog food and nutrition, two notable studies emerged. These included studies conducted by Davies et al. (2017) and Pereira et al. (2018) which focused on analysing the mineral composition of numerous widely consumed wet and dry dog food brands, including veterinary diets, available in Europe and the UK. Their findings revealed that most of these foods exhibited mineral imbalances, failing to adhere to the nutritional guidelines set out by the European Pet Food Industry Federation (FEDIAF). In particular, essential minerals were often found to be either below the minimum or above the maximum recommended levels. Pereira et al. (2018) attributed the excessive levels of minerals found in the tested foods to the overuse of mineral additives, with additives responsible for up to 55% of the total mineral content in the dog foods tested. Davies et al. (2017) concluded that if these foods are fed exclusively for prolonged periods of time, this could compromise the health of our pets.

The above studies concerned us, which prompted our decision to develop a dog food recipe that would provide the appropriate level and balance of nutrients. Prior to embarking on the formulation of the Proper Dog Food recipe, we conducted an extensive review of scientific literature. This review aimed to comprehensively examine research focused on nutritional interventions in dogs, allowing us to gain a thorough understanding of the subject.

FEDIAF is the trade body which represents the European pet food industry. FEDIAF pet food nutritional guidelines, provides pet food manufacturers with nutritional recommendations including minimum levels for essential nutrients dogs require from their diet. While they are only recommendations, legal maximums do apply for several nutrients if added as a nutritional additive. While this is a good starting point for formulating a dog food, we felt the minimum protein recommendations were low and there was also little information on fatty acids. Additionally, we did not want to simply produce a dog food which complies with recommended minimums, we sought to create a dog food that could provide optimal nutrition. This review therefore allowed us to gain thorough insight into not only what nutrients dogs need, but also why they need them, the best sources of these nutrients, and their effects on health parameters in dogs.

Review Objective

To gain a comprehensive overview of research investigating nutritional interventions in healthy dogs or dogs with mild health ailments. To understand the nutritional needs of dogs.

Methods

See protocol for full methods including literature searches, data extraction, risk of bias tools and data synthesis methods.

Analysis: We were initially intending to pool studies into multiple meta-analyses, as we knew there be heterogeneity among studies. However, differences in the methods employed, outcomes studied, and summary data varied too significantly across studies for a metanalysis to be appropriate. Instead, we took a narrative analysis approach in synthesising and explaining the data.

Results

Of the 639 studies screened 103 were included in the review plus 17 hand searched studies were added. 478 studies were excluded due to irrelevance during title abstract screening. 58 studies were excluded in full text screening, reasons for exclusion included: wrong subject population, wrong study design, wrong outcomes, and 2 studies were excluded due to obvious conflict of interest and poor study design.

Following tabulation of the literature, 6 broad categories emerged, namely ‘Protein’, ‘Fats & oils’, ‘Carbohydrates’, ‘Fibre’, ‘Probiotics’, and ‘Vitamins, Minerals & Bioavailability’. We synthesised the data further into subcategories of specific interventions and outcomes. For example, studies grouped under ‘Protein’ have been further divided into studies investigating the ‘quality of protein’; alternative ‘non-meat protein’ and ‘protein levels’ in diets for dogs. The findings for each study have been summarised and reported below, along with summary tables of the literature.

Protein

Dietary protein is a source of essential and non-essential amino acids. Essential amino acids (EAA) must be provided in a dog's diet as they do not produce these on their own. EAA dogs require include Arginine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan and Valine. Non-essential amino acids are not necessarily required through diet as they are generated endogenously. Endogenous amino acids are predominantly produced through catabolism of proteins in lean body mass, new proteins are then synthesised, catabolised and the cycle, known as protein turnover, continues. Amino acids (AAs) are required for protein synthesis, thus deficiency in these leads to a reduction in protein turnover and subsequent impairments in many important biological processes, such as the metabolism of nutrients and synthesis of neurotransmitters.[3,4]

Quality of protein

Research in this area has revealed that not all sources of dietary protein are equal in nutritional value. For example, animal protein sources are deemed higher quality than vegetable protein sources as they have a more complete amino acid profile.[5] A dog food which only contains vegetable or plant-based foods as the main source of dietary protein is often lacking in one or more essential amino acids including Lysine, Methionine, Threonine, and Tryptophan, thus would have to be supplemented with the missing amino acids.[3] Additionally, the processing or cooking of a protein source will also dictate its nutritional value. For example, high temperature high pressure (HTHP) processing (135 degrees Celsius with 3 bars pressure) of meat and bone meal, reduces the bioavailability of absorbed amino acids.[6] HTHP processing induces the Maillard reaction, in which sugar reacts with amino acids creating amino acid complexes that can be absorbed by the animal, but not actually utilised for protein synthesis.[7] In a comprehensive review exploring the impact of cooking methods on meat protein, it was found that sous vide cooking demonstrated advantages in enhancing protein digestion from meat. Conversely, it has been observed that high-temperature cooking methods, such as boiling, can lead to protein aggregation, which subsequently decreases the availability of cleavage sites. As a result, the digestion of protein from meat becomes more challenging as the reduced accessibility of these cleavage sites hinders the action of enzymes responsible for breaking down proteins during digestion.[8]

Non-meat protein

Some of the research we reviewed investigated dog food containing alternative protein sources to meat, including soya meal, corn gluten meal, wheat gluten and maize gluten.[9,10,11,12] We note that the plant-based food used in Brown et al. (2009) was supplemented with a higher proportion of vitamin-mineral premixes than the meat-based diet (see table 1.) This is most likely due to the lack of B vitamins, zinc and iron in plant-based proteins, which are essential nutrients for dogs. Hill et al. (2001)[13] found that increasing the soy content in their food to replace beef protein resulted in decreased digestion of nutrients and increased faecal moisture. Other studies reviewed here show that plant-based proteins do seem to be able to provide adequate nutrition when they are combined (i.e. soya meal and corn meal-),[12,14] mixed with animal protein such as chicken meal, or supplemented with crystalline amino acids.[9,10,11] Further study is warranted however, to see how plant-based meals affect dogs' body condition long-term. Additionally, owners feeding vegetarian or vegan diets to their dogs should regularly check urine PH to avoid Urinary Alkalinisation caused by the lack of acidifying amino acids in plant protein, which can lead to the development of kidney stones.[15]

Table 1. Studies using alternative protein sources.

Reference	Diets/intervention	Dog breed and sample size	Duration	Findings
Hill et al (2001)	Canned food containing Texturized vegetable protein (TVP) from soy flour to replace beef: 8% moisture, 51% CP, 1% fat, 31% nitrogen-free extract, and 7% ash. – graded levels replaced beef Beef diet contained 69% moisture, 19% CP, 12% fat, and 1% ash	8 hound dogs	3 weeks per diet	As TVP increased, CP digestibility decreased $P < 0.001$). Amino acid digestibility also decreased. Small but statistically significant decrease in carbohydrate digestibility with increased TVP (89 to 84%; $P < 0.001$). Digestibility of sodium also decreased markedly $P < 0.001$. Phosphorus digestibility decreased on 14 or 57% TVP compared to the all-beef diet $P < 0.05$. Faecal moisture increased when TVP increased $p <0.00$.
Brown et al. (2009)	Control: Meat based diet 43% poultry meal vit-min mix 50g/kg Test: meat-free diet 43% maize gluten and soya meal; vit-min mix 80g/kg	Sprint racing Siberian huskies (12)	16 weeks	All haematology parameters were within normal ranges for dogs on either diet. Erythrocyte counts Hb PCV increased significantly over time for both groups probably due to high crude protein content (approx. 30%). These were higher in meat-diet group, but this did not reach significance.
Nery et al. (2010)	Cross over design: 5 dry extruded diets: Meat free- wheat gluten (WG) Meat-diet – poultry meal (PM) Mixed diet WG & PM Either High protein (HP), low protein (LP) or medium protein (MP)	5 Miniature Poodles 1 Jack Russell 1 Miniature Schnauzer 6 Standard Schnauzers 6 Giant Schnauzers 8 German Shepherds	14 days on each diet	Poorest faecal quality on PMHP. Higher faecal score for PMHP compared with WGLP diet $P < 0.001$. Greater digestibility of: DM: WG (86.6 ± 0.3%) than PM (84.0 ± 0.3%) $P < 0.001$; CP: WG (89.9 ± 0.4%) than PM (82.9 ± 0.3%) $P < 0.001$; And Fat: - WG (96.3 ± 0.2%) than PM (95.6 ± 0.1%; $P < 0.001$)
Menniti et al. (2014)	Diets were formulated with commercial grade Soy bean meal (SBM) (48% CP) to replace 0, 10, 20, or 30% of the	Female purpose-bred hounds adults (36) 12 in cortrol group	24 weeks	Positive linear responses were noted for the apparent digestion of protein ($P < 0.05$) and fat ($P < 0.05$) with increased dietary SBM inclusion.

	protein provided by dried chicken protein resulting in final SBM inclusion of 0, 6.0, 11.5, and 17.0% (as-fed basis) respectively.	8 in each test group		CP intake, also increased linearly ($P < 0.05$) with increasing dietary SBM inclusion
Cargo-Froom et al. (2019)	Animal based (diet 1) containing fresh beef, fresh chicken and chicken meal. Vegetable based (diet 2) contain SBM and corn gluten as main protein plus chicken meal	8 Beagles	10 days per diet cross over design. 6 days acclimation to diet followed by 4 days faeces collection	AD: DM and OM digestibility was higher for diet 2 compared with all other diets ($P = 0.02$). Mineral digestibility was similar except phosphorus digestibility was greater for diet 2 compared with diet 1 ($P = 0.01$). Alanine and leucine digestibility was greater for diet 2 compared with all other diets ($P = 0.0003$) TD: P, magnesium (Mg), zinc (Zn), and manganese (Mn) digestibility was greater for diet 2 compared with diet 1 ($P < 0.05$). No difference in TD of other minerals ($P \geq 0.10$).

Vit-min = vitamin and mineral; PCV = packed cell volume; Hb = haemoglobin; DM = dry matter; CP = crude protein; OM = organic matter AD = apparent digestibility; TD = true digestibility

Protein Levels

Even when EAA are adequately provided in a dog's diet, the minimum amount of protein required must be met to avoid health issues. For example, Sanderson et al. (2001) showed that protein restricted diets (approx. 10% protein on a dry matter basis), containing methionine-cystine concentrations at American Feed Control Officials (AAFCO) recommended minimums, caused plasma taurine and whole blood taurine to drop below reference ranges in beagle dogs after 48 months, with one developing dilated cardiomyopathy.[16] The authors note that taurine may become conditionally essential to a dog's diet if it is low in protein, even when supplemented with the necessary amino acids.[16] Protein is an essential macronutrient crucial for the overall health of dogs. FEDIAF has therefore established minimum recommended protein levels for dogs at between 18% to 21% based on the dry matter content .[17]

Research reviewed here however, has shown the benefit of higher protein diets for dogs in relation to weight management and metabolic health. A combination of a high protein, high fibre diet provides a satiating effect which aids weight loss whilst retaining lean body mass.[18,19,20] High protein diets have also been associated with improvements in immune metabolic health. For example, Blees et al. (2019) found that a high protein diet, compared to high fibre and high carb diets, resulted in lowest post prandial leptin concentrations and the least decreases in adiponectin concentrations.[21]

Table 1.2 Studies investigating high protein diets on weight management.				
Reference	**Diet/intervention**	**Breed**	**Duration**	**Findings**
Diez et al. (2002)	HP diet: crude protein 47.5%; starch 5.3%; TDF 30.8% Control Diet: crude protein, 23.8%; starch, 23.9%; TDF, 38.6%;	8 Beagles	Until target weight 164.5 ± 7.3 days for HP 127.8 ± 17.7 days for control	Total body weight loss for HP diet: 6.3 ± 0.9 and control diet: 5.7 ± 0.4 kg. total fat loss: 80.4 ± 3.1% for HP; and 70.0 ± 3.1% for control, was significantly different (P< 0.056). Less losses of LBM after weight loss on HP diet.
Weber et al. (2007)	HPHF diet: protein = 30g/100g HP diet: protein =34g/100g HF diet = protein 22.8g/100g	2 Shetland Sheepdogs, 2 Brittany Spaniels, and 2 Labrador Retrievers.	Amount of energy consumed (in kilocalories per kilogram metabolic body weight [kg0.73]; kcal kg BW0.73) during a 15-minute period of ad libitum access to food.	Spontaneous food intake was higher for HP diet compared with HF and HPHF P 5 .0057) Also more energy consumed on HP compare with HPHF P < .0025 and HF P < .016, Medium satiety effect showed significantly less energy was consumed on the HPHF than on the HP diet (P 5 .041). Following an energy restricted meal, energy intake was significantly higher at second meal in dogs on the HF (+146%, P < .001) and the HP diet, (+75%, P < .001) but not for the HPHF diet (+15%, P=.27). Also less energy consumed on HPHF compared with HP (P < .0051) or HF (P < .014) diets.
German et al. (2010)	HPHF: protein = 30g/100g DM fibre= 17.5g/100g DM HPMF: protein = 34g/100g DM fibre = 11.5g/100g DM	42 dogs - mixture of different breeds Matched groups	Until target weigh reach with assessments every 7-21 days	Overall percentage weight loss was greater for HPHF (median 31.8% range 12.0–41.2%) than for HPMF (median 20.0% range 5.9–45.0%), *P* = 0.016). Body fat percentage was lower at the end of weight loss in dogs on the HPHF diet (median 27% range 10–36%) than

	Energy was restricted for weight loss			HPMF (median 33% range 16–47%), P = 0.005
Blees et al. (2019)	HP Hight fat HCMP HCLP	36 beagles	4 weeks per diet	The HP diet resulted in lowest leptin peak concentrations (p = .004) and lower total leptin release from baseline to 9 hours post feeding (AUC p = .01), compared with HCLP diet. Baseline serum adiponectin concentrations were lower for the high fat diet (p = .018) and HCMP (p < .001) compared with HCLP. Lower adiponectin release with HP (p = .039), High fat (p = .05) and HCMP diet (p < .001), when compared to the HCLP diet. When compared to female dogs, male dogs had higher adiponectin baseline concentrations (p = .041) (AUC including baseline 3-, 6- and 9-hours post feed p = .023).
HP = high protein; HPHF= high protein high fibre; HPMF = high protein medium fibre; TDF= total dietary fibre; LBM = lean body mass; HF = high fibre; HCMP = high carbohydrate medium protein; HCLP = high carbohydrate low protein; AUC = area under the curve				

Fat and oils

Dietary fat is an essential part of a dog’s diet, providing more than double the energy (Kcals) per gram, compared with carbohydrates.[17] It is therefore crucial that dog food contain an adequate amount of fat, with FEDIAF recommending that dog food contain a minimum of 5.5% fat per 100g of dry matter.[17] Fats can be broadly separated into saturated and unsaturated fats. In humans, saturated fats are considered unhealthy as high consumption of these fats has been linked to high cholesterol and heart disease, however this unlikely in dogs. This is because dogs can consume considerable amounts of saturated fat and still have more high-density lipoprotein (HDL – “good” cholesterol), than low-density lipoprotein (LDL – “bad” cholesterol).[22] Saturated fats can be viewed as facilitative as they increase the palatability of food, provide energy, and aid in the absorption of important fat-soluble vitamins including vitamin A, E and D. Polyunsaturated fats on the other hand, are classed as functional fats as they have influence on many important biological functions including cell membrane structure, growth, development, and inflammation.[22,23,24]

Polyunsaturated fatty acids (PUFA) including omega 3 (n-3) and omega 6 (n-6) must be provided in a dog's diet as they do not synthesize them on their own. Linoleic acid (LA) and arachidonic acid (AA) are n- 6 fatty acids; while LA is essential for dogs AA is not as they can produce this from LA in the body (Lenox, 2016).[24] LA plays a key role in the maintenance of the cutaneous water barrier function, which is vital for healthy skin and coat. Essential n-3 fatty acids include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which can be derived from alpha linoleic acid (ALA) in the body if necessary. EPA and DHA are the main component of fish oil, (and the lesser-known, beef heart). These fatty acids are important for neurological development and modulating inflammatory responses and immune function (Bauer, 2008; Kaur, 2020).[22,23]

PUFA and inflammation

Both n-3 and n-6 have influence on inflammation responses, though the interaction between omega 3 and 6 and their exact role in the inflammatory response is complex and still not fully understood. When metabolised, n-3 and n-6 generate eicosanoids (signalling molecules), including, prostaglandins (PG) and leukotrienes. N-6 produces more of PGE2 and leukotrienes b4 which are considered pro inflammatory. N-3 on the other hand, produces more of PGE3 and leukotrienes b5 which are considered anti-inflammatory. This is due to their attenuation effect on inflammatory cytokines, as shown through attenuated DHT skin responses in dogs consuming fish oil supplements containing high amounts of n-3 (Wander et al. 1997; Hall et al 2003).[25,26] However, more recent research has highlighted that the mechanisms involved in inflammation are far more complex and n-3 and n-6 can have different effects on different parts of the immune system. For example, EPA has been found to reduce neutrophil action whereas DHA stimulates neutrophil phagocytic activity.[27]

It is important to note that inflammation is a normal and necessary response of the immune system to pathogenic stimuli. Inflammation becomes an issue in chronic disease such as cancer, osteoarthritis, and autoimmune diseases where inflammation is constant. It is not ideal to dampen inflammatory reactions in healthy dogs, thus it is important not to overdo omega 3 supplementation.[28] Furthermore, n-3 fatty acids are long-chain fatty acids with more than two double bonds. When these fatty acids are incorporated into cell membranes, they enhance membrane permeability, thereby increasing the vulnerability of cells to infiltration by free radicals and lipid peroxidation. As Wander et al. (1997) found, high intake of omega 3 at a ratio of 1.4:1 (n-6: n-3), significantly increased lipid peroxidation and reduced vitamin E in 20 healthy beagles. To counteract this, antioxidants like vitamin E and selenium have been found essential when consuming omega 3 fatty acids to protect cells from oxidative damage. However, depending on the n6: n3 ratio, extra supplementation with antioxidants may not be necessary. For example, Leblanc et al. (2005) found that a dose of 1.75 g/kg of dietary EPA and 2.2 g/kg of dietary DHA (on a dry matter basis), with an n-6:n-3 fatty acid ratio of 3.4:1 did not increase lipid peroxidation in their sample of dogs, with or without added vitamin E.[29]

PUFA and Osteoarthritis

Several studies reviewed here focused on n-3 supplementation and its influence on osteoarthritis (OA) (see table 2). Indeed, most studies showed omega 3 supplementation was beneficial. For example, significant decreases in the amount of carprofen used to treat OA was found in dogs when taking an n-3 supplement.[30] In another study, decreased lymphocyte proliferation was found in the n-3 supplemented group, which is beneficial for dogs with chronic inflammatory diseases.[28] Studies using gait analysis e.g., plate velocity force (PVF), to test the severity of OA showed no difference between fish oil groups and controls. However, when comparing within groups scores to baseline, the treated groups showed significant improvements whereas the control groups did not, indicating modest improvements in OA in treated groups.[31,32,33]

Supplementation of EPA and DHA also appears to exert positive effects on the ageing process. For example, aged dogs fed a diet enriched with antioxidants, EPA, and DHA had significantly longer telomere length. This is because telomere length in the control group decreased over time, while in the treated group telomere length stayed the same. This only occurred in old, not young, dogs. This finding also correlated with improvement in joint shoulder mobility in the test group.[34] Across all studies, improvements in owner satisfaction were most notable in treated groups compared with control groups. Owners' ratings of their dog's condition were shown to improve, [30,31,35] as well as improved clinical assessments depicting less severe OA,[30,36,37] following fish oil supplementation.

Table 2. Studies investigating PUFA supplementation on osteoarthritis

Reference	**Dog breed and sample size**	**Intervention Groups diets**	**Level/dose of fatty acids**	**Duration**	**Findings**
Leblanc et al. (2007)	15 healthy Walker Hound-cross	Control; Fish oil; fish oil plus vitamin E	1.75g/kg EPA and 2.2g/kg DHA	12 weeks	Significant decrease in lymphocyte proliferation in fish oil only group
Fritsch et al. (2010a)	109 dogs with OA symptoms breed not specified	Control; Commercial diet enriched with omega 3 fatty acids	3.5% omega 3 fatty acids	12 weeks	Significant decrease in carprofen dose for test group compared with control (P = 0.025)
Fritsch et al. (2010b)	177 dogs with OA symptoms. Breed not specified	Graded levels of omega 3 fatty acids	Level of EPA and DHA = 0.8% control diet A; 2% diet B; and 2.9% Diet C	90 days	Dose dependent increase in serum EPA and DHA. Improvements in clinical signs of OA, specifically weight bearing (P = .04), and lameness (P = .03), in dogs on diet C compared to dogs on diet A
Roush et al. (2010)	167 dogs with over 20 breeds including mixed-breed dogs, Labrador Retrievers, German Shepherd Dogs and Rottweilers.	Control and test group with approx. 31-fold increase in n-3 and 34 fold increase in n-6 compared to control.	Test meal 3.47% omega 3 fatty acids and 2.46% omega 6 fatty acids	6 months	Owner scores depicted a significant improvement in rising from a resting position (P = 0.033) and play (P = 0.011) at 0-6 weeks, and improvements in ability to walk at 6-12 weeks (P = 0.024) and 12-24 weeks (P = 0.003).

Hielm-Björkman et al. (2012)	71 dogs from many different breeds including mixed breed dogs, Collies, German Shepherd Dogs, Golden Retrievers, Labradors, and Rottweilers	Commercial food with placebo fish oil (control); Commercial food supplemented with fish oil (test)	Fish oil contained 450mg EPA, 100mg DHA and 27mg ETA per ml.	16 weeks	Significant improvement in owner satisfaction for test group compared to control (p= 0.029). significant improvement within test group for PVF (p= 0.021), HCPI (p= <0.001), NSAID use (p= 0.017), QOL (p= 0.045), mobility (p= 0.021), daily chores (p= 0.011), skin and hair (p= 0.025) compared to baseline values.
Moreau et al. (2013)	30 dogs diagnosed with OA breed not specified.	Commercial diet (control) commercial diet enriched with omega 3 (test)	Control diet omega 6/omega 3 ratio = 13:6 test diet ratio = 1:3 containing 1.08% EPA and DHA.	13 weeks	No difference between groups for PVF but within group improvement in omega 3 diet group at weeks 7 and weeks 13 compared to baseline (p < 0.001).
Rialland et al. (2013)	23 dogs including Labradors, golden retrievers, mixed breeds etc.	Green lipped muscle enriched diet day 30-90 (test) similar diet to test without EPA, DHA, Glucosamine and Chondroitin day 0-30(control)	0.45% EPA 0.25% DHA (test)	30 days	Within groups design. No significant difference in PVF on control diet (days 0-30). PVF was significantly higher on day 90 compared with day 0 P = 0.0004 and day 30 P = 0.003) following test diet. Significant improvement in owner scores for OA in test diet group
Manfredi et al. (2018)	42 Labrador retrievers	Commercial chicken based kibble (control) Commercial fish based kibble mixed with nutraceutical-supplement tablets containing hydrolysed fish	omega-3/6 ratio of 1:1	From approximately 60.04 ± 0.13 days old until 12 months old.	Radiographic analysis did not show difference in prevalence of hip and elbow dysplasia between groups. Based on blind clinical assessment the control diet was associated with a more severe OA at 12

		and vegetable protein, glucosamine, chondroitin sulphate, chitosamine, Boswellia serrata, devil's claw and green-lipped mussel			months of age (P = 0.038) compared with control.
Lorke et al. (2020)	74 shepherd dogs	Diet for adult dogs (control); food formulated for elderly dogs enriched with antioxidants, mitochondrial cofactors and omega-3 fatty (test)	Control – 0.22% omega 3 fatty acids test- 1.01% omega 3 fatty acids	6 months	Minimum telomere length for old dogs on test diet was significantly longer after 6 months compared with control (p = .026). Improved shoulder joint mobility shown through significant difference in minimum (p=.025) and maximum (p= .023) shoulder joint angles, and shoulder joint range of motion (p=.009) for test dogs compared with control at 6 months.
OA = Osteoarthritis; PVF = plate velocity force; HCPI = Helsinki Chronic Pain Index; NSAID = Non-steroidal anti-inflammatory drugs; QOL = quality of life					

PUFA and dermatitis

Research has also focused on the effects of PUFA supplementation on atopic dermatitis (AD) (See table 2.1 for breakdown of these studies). Collectively, the studies examined in this review revealed that dogs receiving PUFA supplementation experienced improvements in dermatitis symptoms, as well as enhancements in skin and coat quality. These improvements were assessed by clinicians and owners utilising a range of dermatitis severity rating scales such as the Canine Atopic Dermatitis Extent and Severity Index (CADESI).[38,39,40,41,42] Some studies showed dogs receiving PUFA supplements had increased overall lipid content in the stratum corneum[43] an increase in cholesteryl esters on the hair shaft,[40] increased plasma levels of alpha-linolenic acid (α – LLA) and EPA,[38] and rapid inclusion of n-3 fatty acids in the erythrocyte membrane.[44,42] Though only Combarros et al. (2020) noted a correlation between increases in plasma fatty acids and improvements in dermatitis symptoms. From the studies reviewed here, maximum improvement in atopic dermatitis is seen by at least 8 weeks, thus 2 - 3 months of PUFA supplementation is needed before any marked improvement in skin and coat quality.

Table 2.1 Studies investing PUFA on dermatitis

Reference	Dog breed and sample size	Intervention description	Level/ratio/dose of omega fatty acids	Duration	Findings
Mueller et al. (2005)	30 dogs with AD, breed not specified	Dietary supplements included: flaxseed oil (group 1) A commercial omega 3 supplement (group 2); Placebo supplement containing mineral oil (group 3)	Group 1 - 114 mg of α-LLA/kg and 34 mg of LA/kg Group 2 - EPA; 250 mg/capsule and DHA; 166 mg/capsule Group 3 - Mineral oil 200 mg/kg	10 weeks	No difference in cutaneous concentration of fatty acids or eicosanoids between groups; Group 2 had > 50% improvement in dermatitis symptoms seen in 5 dogs, as scored by clinicians and 4 dogs, as scored by owners. > 50% improvement seen in 4 dogs in group 1 and 1 dog in group 3 as rated by clinicians and owners. No correlation between clinical signs of atopic dermatitis and inclusion of n3 and n3 fatty acids in skin and plasma.
Glos et al. (2008)	43 dogs with non-seasonal AD. Breed included but not limited to : German shepherd, Labrador retriever, West Highland white terrier, Boxer, schnauzer, bearded collie and Husky.	Diet A: fish, hydrolysed chicken and rice. Diet B: fish and potato. Diet C: rice and hydrolysed chicken and soy. The control diet (D^d) commercially available diet.	Diet A: (Total PUFA %) 28.9; n-3 (% of fatty acids) 5.1; n-6 (% of fatty acids) 24.1 Diet B: (Total PUFA %) 16.9; n-3 (% of fatty acids) 4.6; n-6 (% of fatty acids) 12.6 Diet C: (Total PUFA %) 23.1; n-3 (% of fatty acids) 3.6; n-6 (% of fatty acids) 19.8 Diet D^d: (Total PUFA %) 24.3; n-3 (% of fatty acids) 1.6; n-6 (% of fatty acids) 2.9	8 Weeks	Diet B: significant improvements in CADESI (p=0.043) and Puritis scores (P= 0.012) Diet A: improved puritis (p=0.019) Diet D: improved CADESI (P=0.037) *Dogs who did not respond to elimination diet:* Significant improvements in Puritis for diets A P= 0.007) and B (P = 0.0137). Coat more lustrous on diet A due to high LA content.
Kirby et al. (2009)	24 dogs including hound-type mixed breeds and	Acclimation diet followed by increased fat diets containing graded levels of essential fatty acids	Diet A (3.1 g LA/1000 kcal, 0.42 g ALA/1000 kcal) Diet B (9.3 g LA/1000 kcal, 0.42 g ALA/1000	12 weeks	Skin hair and coat condition score significant (p<0.05) effects seen at week 12 – improvements in overall coat quality in diet B compared with diet A which is highest in

	Beagle dogs.		kcal); Diet C, 9.3 g LA/1000 kcal, 3.3 g ALA/1000 kcal) Total dietary fat in all diets was approximately 13% (as-is).		saturated fat. Improvements in scaliness and softness in Diet C group compared with A. Increase in cholesterol esters on hair shaft.
Popa et al. (2011)	5 dogs including 2 Labradors, 1 Coton, 1 West Highland white terrier and 1 Golden Retriever	One daily dose of Megaderm®/EFA-Z®	Containing omega-6 (linoleic acid 350 mg/ml, gamma linolenic acid 45 mg/ml) and omega-3 (eicosapentaenoic acid 25 mg/ml, docosahexaenoic acid 28 mg/ml), mixed 5:1 (v/v), and contains vitamin E 3.8 UI/mL	8 weeks	Following supplementation, protein bound lipids and free SC lipids showed significant increases in Cholesterol ($p=<0.05$ and <0.01 respectively), fatty acids ($p=<0.01$) and ceramides ($p=<0.01$) compared with before supplementation.
Stoeckel et al. (2011)	30 Beagle dogs	Fish oil (FO) group: fed 250g of special diet enriched with fish oil. Additive group (ADD): 300g of control diet with fish oil concentrate additive. Control Group: 300g commercial dry food	Enriched diet: EPA 1.66g/kg DHA 1.1g/kg; total; LA 35.64g/kg; total n6:n3 ratio 7.24:1. Control diet: No EPA or DHA; LA 19.69g/kg; total n6:n3 ratio: 6.61:1 Fish oil Additive: 14% EPA and 48% DHA. Rtio 3.75:1	12 weeks	Significant increases in n3 in the erythrocyte membrane for FO and ADD group ($p<0.01$); with corresponding decreases in n6 compared with control group from week 2 for FO (<0.01) and from week 8 for ADD. Despite differing ratios between FO and ADD group FA content of EM was similar at 12 weeks.
Müller et al. (2015)	34 dogs completed study. 21 different pure breeds and 5 mixed breeds were enrolled.	Test Group: preparation of plant oil and fish oil containing omega-3- and omega-6 polyunsaturated fatty acids Control Group: placebo (paraffin	3ml Test supplement contained contained approx. 360 mg alpha-linolenic acid (ALA), 410 mg eicosapentaenoic acid (EPA), 249 mg docosahexaenoic acid (DHA) and	12 weeks	Clinical evaluation used CADESI to decide medication dose every 4 weeks. At completion, the treated group needed a significantly lower dose of medication than the control group ($p=0.009$) indicating Fish oil supplement was successful in reducing medication for atopic dermatitis.

		oil for medical purposes) daily	the omega-6 fatty acids linoleic acid (LA) and docosapentaenoic acid (DPA) in minor fractions ratio 3.75:1 (n3:n6)		
Combarros et al. (2020)	24 dogs, breeds included: Bruno du Jura, Bleu de Gascogne and Griffon	All dogs consumed the same dry dog food however group A received a placebo capsule (group A, placebo) containing only microcrystalline cellulose. Group B received the test capsule containing fish oil and Vitamin E.	746.5 mg of fish oil including 230 mg of *n*-3 (with 160 mg of EPA and 100 mg of DHA in a triglyceride form) and 4.5 mg of vitamin E.	90 days	Dermatological clinical assessment scores showed significant improvement in Group B at 60 days compared with baseline, whereas Group A stayed the same. Group B fatty acid concentrations in the erythrocyte membrane showed significant increases in EPA from day 30 compared to baseline ($p<0.0005$), and DHA from day 30 compared to baseline ($p<0.0005$) and day 60 compared to day 30 ($p<0.05$).
AD = atopic dermatitis; α-LLA = alpha-linolenic acid; LA = linolenic acid; CADESI = canine atopic dermatitis; SC = stratum corneum; EM = erythrocyte membrane					

Carbohydrates

While there is no minimum carbohydrate requirement for dogs, (except in pregnant and lactating bitches),[17] dogs require glucose, as glucose is the main source of energy for cells. Dogs can obtain glucose from meat protein via gluconeogenic pathways, however, glucose from carbohydrates provides a more efficient source of energy. Thus, the addition of some carbohydrates can be beneficial, and allow for protein to be utilised for other important biological functions. Certain carbohydrates can also provide extra micronutrients and can have beneficial fermentative effects in the colon.[45]

Starch digestibility

Dogs are facultative carnivores, meaning they thrive on a meat diet, but are also capable of digesting some plant material. Unlike true omnivores, dogs possess carnivorous characteristics including pointy back teeth, a short simple digestive tract, taurine-conjugated bile acids, and lack of salivary amylase.[46] Over 12 thousand years of dog domestication and exposure to a human diet, dogs have developed certain genes involved in starch metabolism.[46] Namely AMY2B, MGAM and SLC5A1, which code for pancreatic amylase, maltase-glucoamylase and SGLT1 respectively. Due to lack of salivary amylase, the digestion of carbohydrates in dogs begins in the small intestine. Firstly, starch is cleaved by amylase to produce maltose, maltose is then hydrolysed by maltase-glucoamylase to produce glucose, which is then transported across the mucosal brush borer by SGLT1 for absorption.[47] Depending on the needs of the dog, glucose can be used for adenosine triphosphate (ATP) production, stored in the liver as glycogen, or stored in adipose tissue.[45]

There is a significant degree of variation in the number of copies of these genes found in dogs. For instance, Axelsson et al. (2013) observed that grey wolves (n=55) possess only 2 copies of AMYB2, whereas their sample of 136 dogs exhibited a range of 4-30 copies of AMY2B, indicating substantial diversity in copy numbers.[47] Subsequent research using improved Droplet Digital PCR (ddPCR) method, found AMY2B copies ranged from 2 to 21 in their sample of 266 dogs.[48] Additionally, Axelsson et al. (2013) found that 2 West Highland white terriers and a Chinese crested dog had no copies of MGAM. The number of copies of these genes, directly influences the efficiency of starch digestion and this is dependent on breed and individual characteristics.[48] Therefore, certain dogs may experience greater difficulty in digesting starch than others.

To ensure the bioavailability of nutrients in plant material such as vegetables, cereals, legumes, or grains, cooking is a necessary step to increase starch gelatinisation and improve digestibility, as dogs cannot digest raw starch, particularly legumes.[49,50] Cooking also reduces antinutrients present in many cereals, grains, and veg. These include but are not limited to phytic acid, phenols and tannins, which affect digestion and reduce the metabolism of important nutrients, particularly crude protein.[51] Reducing antinutrients to acceptable levels can be achieved through cooking, fermentation or soaking.[52]

To improve digestibility of carbohydrates in mildly processed foods, i.e., foods not processed by high temperature and high pressure, extra preparation of plant material, including soaking can help reduce antinutrient content, as it causes water soluble antinutrients to leach out into the water as shown in both legumes and sorghum.[53] While certain minerals can also leach out in the process, for example zinc and iron, it also greatly improves the bioavailability of these nutrients, as well as protein.[54]

Starch source also has a bearing on the digestibility of nutrients. Fortes et al. (2010) found, higher digestibility of macronutrients from cereals including high oil maize, broken rice, sorghum, and millet than from cereal by products including wheat bran, maize germ, and rice bran.[55] Additionally, particle size of certain starch sources has been shown to play a role in digestion. For example, Bazzoli et al. (2015) found that maize and sorghum at a fine particle size (360µm and 314µm respectively), significantly increased digestion of crude protein, dry matter, fat, and ash compared with coarse particle sizes of 619µm for maize and 594µm for sorghum. Additionally, at a fine particle size, sorghum produced the highest total short chain fatty acids (SCFA) compared with rice and maize[56] (see table 3 for a summary of these studies).

Table 3. Studies investigating the digestibility of different starches

Reference	Diet/intervention	Breed	Duration	Findings
Fortes et al. (2010)	Reference meal containing maize; poultry by-product meal; corn gluten meal, poultry fat; dried hydrolysed bovine liver; dicalcium phosphate; calcium carbonate; vitamin	8 cross-breed dogs	10 days per diet	TTAD showed wheat bran diet produced more faeces (g/dog/day) with a higher DM content than other diets (P<0.05) Cereals had higher TTAD of nutrients than cereal by-products (P<0.05). CP digestibility highest in High oil maize, sorghum and millet

	and mineral premix; and sodium chloride. Test : 700g reference diet with 300g of added test food, either: broken rice, high oil maize, millet, sorghum low in tannin (<0.06 g/kg), maize germ, rice bran and wheat bran.			diets. Lowest GE digestibility for maize germ, wheat bran and rice bran (P<0.05). High oil maize had the highest ME content, whereas wheat bran had the lowest (P<0.05).
Bazolli et al. 2015	Kibble diet containing 3 different starch sources, either Maize, Sorghum or broken rice at 3 different particle sizes, either fine, medium or coarse.	54 Beagles	10 days	For the rice-based diets, the raw material particle size did not change the digestibility of DM, fat, CP, or starch (P > 0.05). For maize and sorghum-based diets, TTAD was dependent on the particle size, the sorghum diet with smallest MGD had greater nutrient digestibility than the other diets (r2 < 0.72; P < 0.01). For dogs fed the maize-based diets, less starch gelatinization resulted in reduced digestibility of DM, CP, fat, and starch (data not shown; P < 0.05). For sorghum, a linear response was observed for digestibility of DM, CP, fat, and starch and starch gelatinisation (r2 < 0.76; P < 0.01). Rice digestibility was not affected by starch gelatinisation. Higher concentrations of total SCFA were observed for dogs fed sorghum-based diets (P < 0.05). Dogs fed sorghum-based foods with high MGD exhibited reductions in acetate and propionate concentrations and an increase in butyrate concentration (P < 0.05).

TTAD = total tract apparent digestibility; DM = dry matter; CP = crude protein; GE = gross energy; ME = metabolise energy; MGD = mean genomic diameter

It is important that complex carbohydrates are used in dog food, to ensure slow digestibility and promotion of satiety and glycaemic control. Dogs that have a glucose intolerance due to obesity and diabetes, or indeed to avoid glucose intolerance all together, it is necessary to provide dietary starch sources which delay and lengthen insulinemic and glycaemic responses. Several studies reviewed here investigated the effects of different starch sources on glycaemic and insulinemic parameters in dogs. Carciofi et al. (2008) found lentils, peas and sorghum provided greater glycaemic and insulinemic control than diets containing brewers rice, corn, and cassava flour, with sorghum producing the lowest immediate post-prandial glucose response, and lentils providing the lowest insulin response.[57] Adolphe et al. (2015), compared diets containing either yellow field peas or rice in their sample of obese dogs, and found that the pea diet produced lower serum insulin, though there was no difference in serum glucose levels; these results indicated a clear diet effect as no weight loss occurred during the study.[58] Teixeira et al. (2018), in their sample of diabetic dogs, found that a diet containing peas and barley produced more stable plasma glucose concentrations than maize.[59] De Godoy et al. (2014) found attenuated glycaemic and insulinemic responses in adult dogs consuming soluble corn fibres, which is a low digestible carbohydrate.[60] It appears that carbohydrates which produce the best glycaemic and insulinemic control are those which resist digestion in the small intestine but are fermented in the colon.

Table 3.1 studies investigating starch source on glycaemic and insulinemic parameters

Reference	**Diet/intervention**	**Dog breed**	**Duration**	**Findings**
Carciofi et al. (2008)	6 Kibble diets made with either corn, brewer's rice, sorghum, peas, lentils, or cassava flour as its exclusive starch source. Balanced to contain similar percentages [dry matter (DM) basis] of starch, fat, calcium and phosphorus as similar as possible. With TDF differing with starch source	36 mixed breed dogs (21 females, 15 males)	10 days	Incremental glucose response higher on lentil than on pea, cassava flour and corn diets ($p < 0.05$). Maximum glucose concentration occurred within the first hour for cassava flour, corn, brewer's rice and peas - significantly earlier than sorghum diet ($p < 0.05$). Immediate post-prandial response (AUC ≤ 30 min) was the greatest for brewer's rice, intermediate for corn, lentils, peas and cassava flour, and lowest for sorghum ($p < 0.05$). Regarding later responses, the AUC ≥ 30 min was greater for sorghum, lentil and pea diets than for brewer's rice, cassava flour and corn ($p < 0.05$). Post-prandial insulin was highest in dogs that had consumed corn-based diet and lowest for lentil-based diet ($p < 0.05$).

DeGodoy et al. (2014)	Soluble corn fibre (SCF), a low digestible carbohydrate (LDC) obtained by isolating an oligosaccharide-rich component from partially hydrolysed corn syrup. 2 solutions (25g SCF) mixed with water : G1-SCF and G2-SCF produced using different SCF production methods. G2-SCF has higher concentration of LDC than G1-SCF. Control: Maltodextrin (Malt)	5 purpose-bred female hound dogs	1 day on each SCF solution following 4 days of rest in between each dose.	Blood glucose concentration intermediate peak at 30 min for G1-SFA and 60mins for G2-SCF. Glucose AUC for Malt was greater (P < 0.05) than the AUC values for G1-SCF. Maltodextrin had a greater insulin AUC (P < 0.05) and RIR value than for G1-SCF. Maltodextrin had the greatest glucose AUC when compared with G2-SCF (P < 0.05). Area under the curve for insulin was greatest for Malt when compared with G2-SCF(P <0.05). G2-SCF generally resulted in lower responses than the G1-SCF.
Adolphe et al. (2015)	Diets were formulated to contain equal amounts of digestible energy, protein, starch, and fat based on data from previous digestibility trials, but each diet contained only one carbohydrate source (yellow field peas or white rice)	7 Beagles	12 weeks per diet with 8 week washout period	No significant difference in the peak serum glucose after feeding either the pea or rice diet in this normal weight sample of dogs. At the end of the 12-week feeding trial, fasting serum glucose and insulin did not differ in dogs on the pea or rice diets. The oral glucose challenge resulted in lower values for both peak serum insulin (p = 0.05) and serum insulin AUC (p = 0.05) in dogs fed the pea diet compared to the rice diet.
Teixeira et al. (2018)	Two extruded dry diets, based on peas and barley (PB) or maize (Mi). the Mi diet was processed with less restrictive grinding and extruder dimensions compared with the PB diet to decrease the gelatinisation index of the starch in the Mi diet.	15 Diabetic dogs receiving insulin: Mixed breeds, Labrador retrievers, poodle, cocker, Schnauzer, Pug, Dachshund	60 days	Results from continuous glucose monitoring system: Significant differences between PB and Mi diets for maximum glycaemia (P=0·01), difference between minimum and maximum glycaemia (P=0·03), mean time in hypoglycaemia (P<0·01) and mean time in hyperglycaemia (P<0·01) – indicating PB diet resulted in lower blood glucose levels.

TDF = total dietary fibre; AUC = area under the curve; LDC = low digestible carbohydrate

Grain free vs grain inclusive foods

Grain-free dog foods are increasing in popularity, mainly due to unsubstantiated marketing claims that grain free foods are healthier and 'hypoallergenic'.[61,62] 'Grain-free' appears to be more of a marketing term rather than a correct description of the food contents, as many 'grain-free' dog foods actually do contain grains such as green beans, amaranth, lupins and linseeds. Thus, "cereal free" is the scientifically correct term for these foods.[63] A survey by Baton et al. (2021), found that dog owners that believed their dog had a food allergy were 4 times more likely to feed 'grain-free' foods, compared with owners who did not suspect a food allergy. Conversely, owners who had their dog's allergies diagnosed by a veterinarian were significantly less likely to opt for no grain food.[62] This is because the most commonly diagnosed food allergies in dogs are chicken, wheat, dairy, eggs and beef, with allergies to grains such as corn and rice being the least diagnosed.[62] Grains are often thought of as a cheap filler i.e., provide bulk with no nutritional value. This however is not the case, as grains contain an abundance of nutrients, and heat processing makes them highly digestible, thus the notion these are cheap fillers is incorrect.[14] The digestibility of nutrients tends to be similar for grain free and grain-based dog food, though digestibility studies have shown dogs on grain-based diets displayed higher digestibility of dry matter, organic matter, and starch.[64,65,57] Additionally, Pezzali and Aldrich (2019), found that ancient grains had similar digestibility to grain-free diets with less processing required.[66] Crude fibre digestibility is less clear, with some studies finding higher digestibility of total dietary fibre (TDF) in grain-based food,[64,67] whereas others found that grain free foods displayed higher digestion of TDF.[65,66] Crude fibre digestibility seems to be dependent on the source and preparation of the carbohydrate used, rather than if it is a cereal grain or not, with peas lentils and sorghum diets showing higher digestibility of CF than corn and cassava flour.[57]

Table 3.2 Studies investigating cereal free and cereal inclusive diets on digestion of nutrients

Reference	Diet/intervention	Dog breed and sample	Duration	Findings
Carciofi et al. (2008)	6 Kibble diets made with either corn, brewer's rice, sorghum, peas, lentils, or cassava flour as its exclusive starch source. Balanced to contain similar percentages [dry matter (DM) basis] of starch, fat, calcium and phosphorus as similar as possible.	36 mixed breed dogs (21 females, 15 males)	10 days	Higher protein digestibility for sorghum-based diets than lentil-based diets but lower fat digestibility than dogs fed the cassava flour diet ($p < 0.05$). Higher DM, OM and GE in brewer's rice and cassava flour-based diets than in other diets ($p < 0.05$). Protein digestibility was higher in brewer's rice than sorghum, corn, pea and lentil diets ($p < 0.05$). Fat digestibility was greater for cassava flour and pea diets than other treatments (p <

	With TDF differing with starch source Grain inclusive = brewer's rice, sorghum, and cassava diets Grain free = Lentil and pea diets			0.05). Brewer's rice and cassava flour diets presenting the greatest digestibility, and pea and lentil diets the least (p < 0.05). Digestibility of TDF in pea, lentil and sorghum diets was higher than in corn and cassava flour treatments (p < 0.05),
Pezzali et al. (2019)	Kibble diets: Ancient grain diet (AG) with spelt, millet, and sorghum, and a grain-free diet (GF) which had potato, peas, and tapioca starch	12 Beagle dogs	14 days per diet – crossover	Total dietary fibre digestibility was 32% greater for dogs fed the GF when compared to those fed AG. Starch digestibility was statistically lower for dogs fed GF (P < 0.05). No differences were observed for DM, OM, CP, CF
Pezzali et al. (2020)	Grain based (GB) diet contained sorghum, spelt and millet; the grain free diet (GF) was formulated with peas, potatoes, and tapioca starch. Experimental diets were formulated to exceed the requirements of CP and sulphur amino acids for adult dogs (NRC, 2006), and were taurine supplemented.	12 Beagles	4 weeks	No differences were observed (P > 0.05) in ATTD of CP and crude fat between treatment. ATTD of organic matter was slightly higher (P < 0.05) for dogs fed GB
Kahraman and Inal (2021)	21 different commercial dry dog foods (7 lamb-grain, 7 chicken-grain, and 7 grain-free)	12 Golden retrievers	13 days	DM, OM and CF digestibility differed significantly between the three groups (grain-chicken meat, grain-lamb meat, grain-free) with higher values for the lamb-grain foods. The authors note that the higher Ether Extract in Grain free foods could have caused reduced digestibility of CF
Kahraman et al. (2022)	Grain based (GB) diets contained starches including corn gluten meal,	Twelve adult Golden Retriever dogs	15 days	crude fibre (CF) digestibility of grain-inclusive food was statistically higher (P<0.05).

	barely, corn, corn starch, rice. Grain free (GF) diets starches included potato flour, carrot flour and pea flour			Dry matter (DM), organic matter (OM), ether extract (EE) and crude protein (CP) digestibility coefficients of grain-inclusive and grain-free foods were not significantly different (P>0.05).
TDF = total dietary fibre; ATTD = apparent total tract digestibility DM = dry matter; OM = organic matter; CP = crude protein; CF = crude fibre; GF = grain free; AG = ancient grain				

The main issue with grain free dog food that has come to light in recent years, is its possible link to dilated cardiomyopathy (DCM) in dogs. In 2017 the Food and Drug Agency (FDA) received 524 reports of dogs with DCM who also had a history of eating grain-free food. In these reports, 91% of dogs diagnosed with DCM were consuming grain free foods with 93% containing peas or lentils. In most of these cases the dogs diagnosed with DCM were breeds not predisposed to DCM.[68] What's more, DCM was reversed in many of these dogs when switching to a grain-based diet, often supplemented with taurine and/or L-carnitine. This reaffirms the notion that these cases of DCM were related to diet. These reports were substantial enough for the FDA to issue a warning letter in 2018, reporting on the possible link between grain free diets and DCM and ordered an investigation into this.[61]

This is a complex issue and investigations are still ongoing. While there appears to be an association between some grain-free dog foods and DCM, the cause is not clear, and multiple biological compounds could be involved. Possible theories for a grain-free diet association with DCM include impaired taurine metabolism, nutrient deficiencies, decreased bioavailability of amino acids, or nutrient interactions.[7] Taurine is not required in a dog's diet if their food contains adequate bioavailable protein and sulphur containing amino acids. Grain-free diets however often contain foods which either have reduced taurine content or cause reduced synthesis of taurine. Quilliam et al. (2021) notes that the higher amylose and fibre content of the grain-free diet means that less nutrients are digested, including sulphur containing amino acids, leading to the production of less taurine.[69] Kaplan et al. (2018) note that dogs with DCM all consumed grain-free diets containing either alternative meat sources or diets rich in legumes, which could have explained the low blood taurine in some of these dogs.[70] Taurine status in dogs is not always affected by grain free (GF) feeding. For example, Pezzali et al. (2020) and Adin et al. (2021)[71] found no difference in taurine status between GF and grain-inclusive fed dogs, however bile acid losses in Pezzali et al. (2020)[65] were higher in GF fed dogs which could have indicated intestinal loses of taurine. Additionally, Adin et al. (2021) found that high-sensitivity cardiac troponin I (hs-cTnI), a marker for cardiac injury, was significantly higher in GF fed dogs compared with the grain inclusive fed dogs (p <.001), however they could not prove that these were clinically relevant findings for cardiac issues in their studies.[65,71]

Fibre

Most carbohydrates are a source of dietary fibre with common sources in dog foods including rice hulls, soybean hulls, beet pulp, bran, peanut hulls, and pectin.[72] Fibre is not considered an essential nutrient for dogs. Dogs are not able to get any energy from fibre therefore it is often used in weight management diets for dogs, to lower the calorie density of the food. The addition of fibre to their diet can however exert health benefits on the gastrointestinal tract, through promoting the growth of good bacteria and regulating bowl movements.[72,45] All fibres resist digestion in the small intestine and are either fermented quickly (fermentable fibre) or poorly (non-fermentable fibre) by microbiota in the large intestine.[73] Fibre can either be soluble meaning it disperses in water, or insoluble which does not. Although not a rule, soluble fibre including guar gum and fructo-oligosaccharides tends to be more highly fermentable than insoluble fibre such as cellulose.[74,73]

Studies reviewed here report the benefits of high-fibre foods during weight loss (see table 4 for studies). Low-fermentable fibres (LFF), such as cellulose, may improve the sensation of fullness by increasing bulk in the stomach and intestines. While high-fermentable fibres (HFF), such as beet pulp, may increase fullness through the production of SCFA which mediate the secretion of gastrointestinal satiety related hormones, which have been shown to be more effective at increasing satiety than a diet containing only LFF.[75] Additionally, the inclusion of HFF to a dog's diet improves glycaemic and insulinemic responses.[76,77] High fibre diets have been shown to improve body condition during weight loss, with dogs losing more fat and retaining more lean body mass compared with the low fibre group.[78] Increasing dietary fibre can also decrease the digestibility of nutrients including protein and fat.[18,74,79] So caution should be taken when deciding on fibre content, to ensure the reduction in bioavailability of these nutrients is not considerable enough to cause deficiencies.

Table 4. Studies investigating high fibre diets on weight management

Reference	**Diet/intervention**	**Dog breed**	**Duration**	**Findings**
Jewell et al. (2006)	Low fibre group contained 2% CF the High fibre groups containing 22% CF	12 intact female beagles	6 months total feeding time	At the end of the feeding period, average body weight was higher for dogs in the low-fibre groups (16.2 kg) than for those in the high-fibre groups (14.0 kg) ($P < .05$). Dogs fed low-fibre foods had an average final body fat percentage of 38.7% versus 31.1% for dogs fed the high-fibre foods ($P <0.05$).
Respondek et al. (2008)	Control: Dry dog food containing 24% protein, 32% fat, and 44% carbohydrates Test: Control food plus 1% short-chain fructooligosaccharide (scFOS)	8 obese Beagles	6 weeks with 3 weeks wash out	The rate of glucose infusion during the clamp was higher (P, 0.05) in obese dogs supplemented with scFOS than in those fed the control diet. HOMA-IR decreased ($P < 0.05$) in dogs supplemented with scFOS compared with the obese dogs fed the control diet.
Bosch et al. (2009)	Low-fermentable fibre (LFF) diet containing 8·5% cellulose or a high-fermentable fibre (HFF) diet containing 8·5% sugarbeet pulp	16 healthy beagles	13 days	The dogs fed the HFF diet tended to show a lower voluntary food intake (VFI) compared with the

	and 2% inulin			dogs fed the LFF diet (P¼0·058, Fig. 1)
Deng et al. (2013)	low-fibre (LF) diet containing 1% Solka-Floc (powdered cellulose fibre) and 1% soya hulls; a LFF diet containing 5% Solka-Floc and 3% soya hulls; a HFF diet containing 5% pectin (HM (high-methoxyl) Pectin; TIC Gums) and 3% scFOS	6 female hounds	7 days per diet	In contrast to dogs fed the HFF diet that had a sharp decrease in blood glucose from 60 to 90 min, dogs fed the LF and LFF diets maintained high glucose levels throughout the 90 min which did not return to baseline by 180 mins. Dogs fed the HFF diet had lower (P = 0·049) postprandial glucose IAUC$_{0-180\ \mathrm{min}}$ when compared with dogs fed the LF and LFF diets. IAUC$_{0-180\ \mathrm{min}}$ values for insulin and active GLP-1 were not different among the dietary treatments.
HOMA-IR = homeostasis model of insulin resistance; GLP-1 = glucagon-like peptide-1; LF = low fibre; CF = c rude fibre; HF = high fibre; HFF = high fermentable fibre; LFF = low fermentable fibre				

Studies investigating fibre particularly highlighted the difference in fibre needs and tolerances between small and large dog breeds. Large breeds appear to be less tolerant of high levels of fermentable fibre and resistant starch which can causes loose stools in these breeds.[73,80] Resistant starch, like fermentable fibre, resists digestion in the small intestine and is fermented in the large intestine. Goudez et al. (2011) found that adding graded levels of resistant starch from either maize or potatoes to a dog's diet did not affect stool quality in their sample of small dogs, with faecal scores always optimal in miniature schnauzers, or mostly optimal for miniature poodles and miniature schnauzers, 92.1% and 85% of the time respectively. In large dog breeds however, there was a linear relationship between level of RS and unacceptable faecal scores, with unacceptable scores reaching 89.1% when resistant potato starch was at 11.4%. The authors also note that one giant schnauzer had to be withdrawn from the study after consuming the diet containing 7.4% RS from maize, due to colitis symptoms.[80] Possible reasons for this sensitivity in large breeds could be explained by higher fermentative activity in the colon, which in part can be explained by the proportionally larger size of the colon, increased total transit time, and increased intestinal permeability, compared with small dogs. [73,80]

Probiotics

Probiotics are live microorganisms, (typically bacteria though sometimes yeasts), which can exert health benefits if consumed in adequate amounts. The gut microbiome of healthy mammals is made up of trillions of bacteria with high biodiversity, as well as archaea, viruses, and eukaryotic organisms, collectively known as microbiota. Microbiota influences several biological functions crucial to the maintenance of overall health, including digestion, immune function, and the brain-gut axis.[81,82] Key mediators involved in these processes are short chain fatty acids (SCFA), namely acetate, propionate, and butyrate, which are produced when gut microbiota ferment undigested starches and proteins.[81] The gut microbiome can be maintained or improved with the use of probiotics, though for probiotics to be effective they must survive digestion, reach the large intestine, then colonise and proliferate.[83]

Safety of probiotic supplementation

The studies reviewed here investigated many probiotics including but not limited to enterococcus faecium, Lactobacillus acidophilus, Lactobacillus fermentum, Bacillus subtilis, Bifidobacterium anamalis and Bacillus coagulans. The inclusion of probiotics in dogs' diets across studies reviewed here produced no adverse events. Probiotics appear safe for dogs, with no effect on general health including body weight and body condition score (BCS)[84,85] and may improve BCS.[86] Testing the safety and tolerability of Bifidobacterium anamalis for 12 weeks showed a daily dose of 50 billion colony forming units (CFU) was safe and tolerable in puppies, with all haematology parameters within healthy ranges. [85]

Effects on the microbiome

Probiotic supplementation appears to have positive effects on the composition of the intestinal microbial population in dogs, with studies reporting increases in bacteria such as Lactobacillus[87,88,84,89,86,90,83] and Bifidobacterium,[88] with reductions in bacteria such as clostridium,[84,90] staph, ecoli and enterococcus. [84,87,88] (See table 5 for doses used). A healthy microbiome has a balanced number of bacterial species, however, if a certain species of bacteria dominates the composition of the microbiome, this can lead to pathogenic strains emerging. One condition which can disrupt this balance is obesity. Microbiota mediates the brain gut axis by stimulating afferent vagal neurons (neurons which send information from the gut to the brain stem). This helps regulate the movement of food through the gastrointestinal tract and feeding behaviours,[91] however, this is disrupted in obesity. Probiotic supplementation has been correlated to improvements in BCS which Marelli et al. (2020) suggest is due to improvements in the composition of the gut microbiome, increasing its effectiveness in mediating neurons signalling hormones such as serotonin.[86] The microbiota also regulates fat storage. During obesity microbiota may be more effective in fermenting undigested foods and producing increased amounts of SCFA, thus increasing energy extraction from a diet (and subsequent fat storage), which has been proven in mice.[87] Marciňáková et al. (2006) therefore suggest improvements in dog's BCS following probiotic supplementation could be due to a shift in the balance equilibrium of the microbiota, which is less effective at fermenting food residues, to manage weight during obesity.

Table 5. Studies investigating probiotics effects on the microbiome

Reference	Probiotic dose/CFU	Dog breed sample	Duration	Findings
Baillon et al. (2003)	Probiotics incorporated into the food matrix at the time of manufacture to give a theoretical final concentration of 7.1 X 106 CFU/g; actual daily probiotic intake for each dog ranged from 1.97 X 109 CFU to 3.53 X 109 CFU, as measured via daily feed intake.	15 adult dogs	3 weeks of pro biotic supplementation	The number of clostridia and the percentage of clostridia in the total bacterial population decreased significantly during consumption of the probiotic-supplemented food. Exact matches to

				the probiotic strain were found, confirming that the probiotic organism was successfully isolated after passage through the canine gastrointestinal tract. Consistent with a change in the colonic microflora toward a healthier balance of microorganisms.
Marciňáková et al. (2006)	Enterococcus faecium strain EE3 109 colony-forming units (CFU) per mL, differed from 2 to 3 mL; up to 10 kg – 2 mL according to body mass of dogs	11 healthy dogs of different breeds (2 German shepherd, 2 Belgian shepherd, 1 Doberman, 1 dachshund, 1 Weimar setter and 4 crossbreeds)	Daily dose for 1 weeks	Average concentration of EE3 strain in the faeces of healthy dogs were 4.85 ± 2.43 log CFU/g. 3 weeks after cessation of the EE3 strain administration, the concentration increased, reaching 5.94 ± 1.61 log CFU/g at 2 months. The LAB concentration increased from the initial 5.31 ± 1.36 to 6.15 ± 1.57 log CFU/g after 1 week, and to 6.62 ± 1.39 log CFU/g after 3 months. Consumption of the EE3 strain was associated with a decrease of staphylococci. Moreover, significant decrease of Pseudomonas-like bacteria (p<0.001) was found 7 d after administration
Ren et al . (2011)	Candida utilis biomass was 8.69 lg colony-forming units (CFU)/ml, and Lactobacillus biomass was 9.61 lg CFU/ml. probiotics were enriched with selenium or zinc	20 “indigenous” dogs.	35 days	Lactobacillus increased extremely markedly (P<0.01), and E. coli decreased extremely significantly (P<0.01) in comparison with those of the control group. On day 30 Lactobacillus and

				Bifidobacterium were both extremely significant (P<0.01). decreasing amounts of E.coli, Staphylococcus, and Enterococcus were extremely significant (P<0.01).
González-Ortiz et al . (2013)	B. amyloliquefaciens CECT 5940 and E. faecium CECT 4515, each strain 5 ·x 108 CFU/g. daily dosage of 1 x 108 CFU of each probiotic strain	16 Beagle dogs	Day 7- day 45 supplementation period	B. amyloliquefaciens CECT 5940 colonies were only recovered in supplemented group. Due to the intestinal tract ubiquity of Enterococcus spp., it was not possible to distinguish between supplemented enterococci and common microbiota. Pathogenic clostridia counts were significantly reduced in probiotic group before finishing the probiotic supplementation (5.64 vs. 2.94 ± 0.53 log CFU/g faeces; p < 0.001). Total mesophilic aerobic bacteria increased only in supplemented group (9.86 vs. 10.35 ± 0.352; p = 0.407)
Strompfová et al. (2013)	Control group (C, n = 12) received placebo The probiotic group (LF, n = 12) received a fresh culture of Lactobacillus fermentum CCM 7421 (0.1 mL/kg of body mass, 108 CFU/mL The synbiotic group (LF+I, n = 12) received the probiotic (at same dose as the LF group) plus inulin	36 Adult dogs: German Shepherd (12), Belgian Shepherd (5), Slovenský kopov (3), Bayerische Gebirgsschweisshund (3), German Boxer (3), Rottweiler (2), Rhodesian Ridgeback (1), Weimaraner (1), Springer Spaniel (1), Dachshund (1), Fox Terrier (1), and cross-breed (3)	7 weeks (2 week treatment period)	The probiotic strain CCM 7421 was successfully isolated after passage through the canine GIT, and it was detected at concentrations of 104 to 107 CFU/g during the supplementation phase in both LF and LF+I groups persisting 35 days after cessation of treatment, at a range of 102–105 CFU/g. Clostridial numbers were significantly lower in the probiotic

				group at the end of dosing period (day 14, p < 0.05)
White et al. (2017)	Standard IBD therapy (ST) plus a multistrain probiotic. Lactobacillus plantarum DSM 24730, Streptococcus thermophilus DSM 24731, Bifidobacterium breve DSM 24732, Lactobacillus paracasei DSM 24733, Lactobacillus delbrueckii subsp. bulgaricus DSM 24734, Lactobacillus acidophilus DSM 24735, Bifidobacterium longum DSM 24736, and Bifidobacterium infantis DSM 24737 Dose: 112–225 x 10$_9$ CFU/10 kg or 450 billion bacteria daily for 10-20kg dog	34 dogs with IBD	8 weeks	Results based on 14 dogs in ST+probiotic group and 12 dogs in ST+placebo group. Only the total number of Eub338-positive bacteria were increased in dogs randomized to receive ST+probiotic (P < 0.02). ST+probiotic group showed increased total numbers of Lactobacillus spp. vs. dogs treated with ST alone (P < 0.001). ST therapy preferentially increased mucosal Bifidobacteria spp. (P < 0.05) while ST+probiotic therapy preferentially increased mucosal Lactobacillus spp. (P < 0.001).
Marelli et al. 2020	The LACTO group received food supplemented with L acidophilus CECT 4529 to a final concentration of 5.0 x 109 cfu/kg of food. Control – same diet with placebo supplement	40 healthy boxer dogs	35 days	Lactobacillus significantly (P≤0.01) higher in the LACTO group (5.64±0.26) than in the control group (4.50±0.22).
CFU = colony forming units; GIT = gastrointestinal tract; LAB = lactic acid bacteria; IBD = irritable bowel disease				

Probiotics and stool Quality

The studies which tested the supplementation of probiotics on outcomes related to stool quality were positive. These studies report improved stool quality,[92] decreased incidences of diarrhoea,[93,94] shorter time to resolution of diarrhoea,[95,96] and more normal stools,[97,98,92] following supplementation of probiotics in dogs experiencing gastrointestinal issues. The probiotics used in these studies varied in type, strain, and dose. (See table 5.1 for a breakdown of these).

Table 5.1. Probiotic strains and doses used in studies reporting improved stool quality

Reference	**Probiotic strains and dose**	**Other supplements/prebiotics used**
Gagné et al., (2013)	Enterococcus faecium SF68 (56.7 mg/g; 5.67 × 10^8 CFU/g), Bacillus coagulans (2.5 mg/g; 3.75 × 10^7 CFU/g), Lactobacillus acidophilus (14.4 mg/g; 7.2 × 10^8 CFU/g)	prebiotic, b vitamins and magnesium
Rose et al., (2017)	Enterococcus faecium strain NCIMB 10415 4b1707, at a dose of 2x 9 10^9 CFU	used a prebiotic FOS
Kelley et al., (2009)	B. animalis strain AHC7 at a dose of 2 × 10^{10} CFU	-
Herstad et al., (2010)	Blend of probiotics containing 4.2 billion live strains of Lactobacillus farciminis, Pediococcus acidilactici, Bacillus subtilis, Bacillus licheniformis and thermo-stabilised Lactobacillus acidophilus MA 64/4E in a 1 ml dose.	-
Fenimore et al., (2017)	Enterococcus faecium strain SF68 at 5 × 108 CFU per dose once daily	-
Nybroe et al. (2022)	Enterococcus faecium NCIMB 10415 at a dose of 109 CFU/kg of kibble	-
Kahraman et al. (2023)	2g B. subtilis (5× 108 CFU/g, BS1) and 4g B. subtilis (5 × 108 CFU/g, BS2)	-

Probiotics and nutrient digestibility

Certain probiotics have been shown to increase nutrient digestibility from food. For example, Acuff et al. (2021) found that B. coagulans GBI-30 at a concentration of 1.5 × 10^{10} CFU/g at a dose of 1.3 × 10^9 CFU consumed daily, improves digestibility of dry matter organic matter and gross energy, as well as displaying a trend for increased crude protein digestibility.[99] Kahraman et al. (2023) found the addition of B subtilis at a dose of 2g B. subtilis (5× 108 CFU/g, BS1) and 4g B. subtilis (5 × 108 CFU/g, BS2) to low quality low digestibility feed, improved the digestibility of Organic matter (OM), Dry matter (DM), Crude protein (CP) and crude fibre (CF).[92] One study reviewed here found their probiotics group supplemented with E. faecium SF68 at a dose of 5 x 10^8 CFU/g per day, displayed significant reduction in cobalamin resulting in moderate hypocobalaminemia, thought by the authors to be caused by higher consumption of cobalamin by microbiota.[100] Cobalamin (or vitamin B12) is an important vitamin, vital for many metabolic processes including the production of red blood cells, DNA and RNA, thus further studies may be required to ensure the safety of SF68. Nybroe et al. (2022), investigating E. Faecium *NCIMB 10415* at a dose of 3.8 – 5.0 x 10^9 CFU/kg CFU/kg also found slight reductions in cobalamin, however, this was not attributable to E. Faecium in their study.[98]

Vitamins, minerals & Bioavailability

Essential vitamins and mineral dogs require from their diet are outlined in table 6.

Table 6				
Vitamins	Vitamin A	Vitamin D	Vitamin E	Vitamin B1 (Thiamine)
	Vitamin B2 (Riboflavin)	Vitamin B3 (Niacin)	Vitamin B5 (Pantothe-nic acid)	Vitamin B6 (Pyridoxine)
	Vitamin B7 (Biotin)	Vitamin B9 (Folic Acid)	Vitamin B12 (Cyanocobalamin)	Vitamin K
Minerals	Calcium	Phosphorus	Potassium	Sodium
	Chloride	Copper	Iron	Magnesium
	Iodine	Manganese	Zinc	Selenium

Vitamins and minerals are only required in tiny amounts, but they have major roles within the body. Table 6.1 and 6.2 displays essential minerals and vitamins required by dogs, along with their functions and food sources.

Table 6.1. Essential dietary minerals, their role in the body and whole food sources.

Mineral	**Roles in the body**	**Whole food sources**
Calcium (Ca)	Ca is a constituent of bones and teeth. It mediates nerve and muscle function and is involved in blood coagulation. Ca is also required for the activation of several enzymes including Adenosine triphosphatase (ATPase) and has influence on membrane permeability.	Dairy products especially cheese, eggs, eggshells, oily fish such as sardines or sprats
Phosphorus (P)	P is a component of bones, teeth, and nucleic acids. It is present in every cell in the body and is involved in cellular energy exchange through phosphorylation, which is vital for the synthesis of adenosine triphosphate (ATP).	Meat, both muscle and organ meats, especially goose, duck, quail, rabbit, all fish, bones and eggs.
Sodium (Na)	Na acts as a buffer in the acid-base balance. It is also involved in muscle excitability, nerve impulses and the metabolism of monosaccharides, pyrimidines, bile salts and amino acids.	Table and sea salt, yeast extract, clams and other shellfish.
Potassium (K)	K is a component of the sodium potassium pump which regulates cell	Lean meat such as chicken, goose, rabbit, potatoes, nuts.

	potential. K interacts with hydrogen to maintain the acid-base balance. K is also involved in nerve impulse function, cardiac muscle contraction and is needed for glycogenesis.	
Magnesium (Mg)	Mg is necessary for oxidative phosphorylation and is a cofactor for several enzymes involved in carbohydrate metabolism and cellular energy homeostasis. It is also a constituent of bones and teeth.	Meat, organ meat especially liver, brewer's yeast, lentils, hemp seeds, soya beans.
Copper (Cu)	Cu is a constituent of several enzymes e.g., catalyse, lactase and ascorbic acid oxidase. Cu is also needed for the growth and formation of bone and myelin sheaths.	All types of liver, other organ meats, shellfish and other seafoods, hemp seeds.
Iodine (I)	Iodine is a component of thyroid hormones thyroxine and mono-, di- and tri-iodothyronine, and is mainly stored in the thyroid as thyroglobulin. Thus, is involved in process related to body weight, temperature, metabolism and cardiac function.	Kombu kelp and other sea vegetables, haddock, cod, shellfish, and yoghurt.
Iron (Fe)	Fe is a component of haemoglobin, myoglobin, succinate dehydrogenase and cytochromes. Thus, Fe is involved in oxygen transportation, oxidative phosphorylation, electron transfer and cell apoptosis. Fe is also involved in the regulation of neurotransmitters and myelination of spinal cord and brain.	Red meat, organ meats such as spleen, heart, liver, kidney, fish, hemp seeds, egg yolk, nuts, root vegetables.
Zinc (Zn)	Zinc functions as a cofactor of many enzymes involved in macronutrient metabolism and cell replication. Involved in tissue repair and epithelial cell division preventing parakeratosis.	Red meat, liver, other organ meats, oysters, crabmeat.
Manganese (Mn)	Mn acts as a cofactor of enzymes involved in nutrient metabolism, cartilage function, connective tissue biosynthesis, urea formation and the formation of bone matrixes.	Rice, soya, hemp seeds, tofu, nuts.
Chloride (Cl)	Cl makes up over 60% of anions in extracellular fluid. It is an important regulator of osmotic pressure and the acid-base balance. Cl is also the main anion in gastric juice and is involved in the production of acid in the stomach.	Table and sea salt, yeast extract, clams and other shellfish, drinking water.
Selenium (Se)	Se is a constituent of enzymes including glutathione peroxidase, involved in the cleavage of hydrogen peroxide and thioredoxin reductase which are involved	Crab, lobster, other shellfish, all kidneys, rabbit, turkey, egg yolks

	in sensing and regulating cellular redox. Thus, Se protects cells against oxidative damage from free radicals, particularly through destroying hydrogen peroxide. It also enhances the activity of the alpha ketoglutarate oxidase system involved in protein synthesis and inhibiting protein degradation in muscle tissue.	

Table 6.2. Essential dietary vitamins, their role in the body and whole food sources.

Vitamin	**Roles in the body**	**Whole food sources**
Vitamin A	Major role in maintaining epithelial tissue including keratinisation. Also involved in vision, growth, immune function, foetal development, cellular differentiation, and transmembrane protein transfer	All animal livers, cod liver oil, oily fish, turkey giblets,
Vitamin D	Regulates absorption, utilisation, and metabolism of calcium and phosphorus; stimulates osteoblasts; regulates muscle excitation; influences the activity of immune cells and has influence on gene expression and cellular signalling.	Cod liver oil, sardines and other small fish, eggs, milk
Vitamin E	Defence against oxidative damage; supports the production and activity of immune cells; aids in the production and maintenance of myelin and has influence on gene expression and cellular signalling.	Shellfish, most oils, sunflower seeds, nuts.
B1 (Thiamine)	An essential coenzyme involved in energy and carbohydrate metabolism; regulates appetite and digestion; has a role in maintaining cellular function, growth and division; involved in the production and maintenance of myelin and neurotransmitters.	Beef and lamb hearts, all pork meat, potatoes, peas, some flours, nuts
B2 (Riboflavin)	Component of enzymes involved in energy metabolism. Involved in defence against oxidative damage; necessary for the growth and maintenance of body tissues; maintenance of eye health; involved in the proper functioning of the nervous system.	All animal livers, duck and goose meat, turkey giblets, oily fish, eggs and milk
B3 (Niacin)	Required for DNA synthesis and repair; component of coenzymes	All meats but especially poultry and rabbit meat, all fish especially tuna, salmon halibut,

	involved in energy metabolism; plays a critical role in oxidative phosphorylation; involved in the synthesis of ceramides and some neurotransmitters.	
B5 (Pantothenic acid)	Involved in energy metabolism and the utilisation of nutrients and synthesis of phospholipids required for proper functioning of the nervous system	All meats but especially liver, kidneys, shellfish.
B6 (Pyridoxin)	Required for glucose generation; red blood cell function; niacin synthesis; nervous system function; immune response; hormone regulation and gene activation.	All lean meats, especially game birds, turkey, rabbit.
B7 (Biotin)	Involved in the production of keratin; acts as a coenzyme involved in metabolism of macronutrients, aids in the synthesis of neurotransmitters; maintenance of blood sugar levels; supports the proper functioning of the immune system.	Most animal livers, lamb and pork kidneys, , white fish such as plaice or basa, nuts and seeds
B9 (Folic Acid)	Plays a role in amino acid and nucleotide metabolism, red blood cell production and mitochondrial protein synthesis.	Most animal livers, shell fish, oily fish, venison,
B12 (Cyanocobalamin)	Functions as a coenzyme of methionine synthase and methylmalonyl-CoA mutase necessary for amino acid synthesis and metabolism. Also involved in red blood cell formation, DNA synthesis, metabolism and nervous system function.	Most animal livers, shell fish, oily fish, venison
Vitamin K	Activation of clotting factors, bone proteins, and other proteins.	Most leafy plant foods such as kale, spinach, cabbage, nettle, watercress,
Info taken from Soetan et al. (2010)[101] ;Tomar et al. 2022[102]; Feuer, D. (2006)[103]		

Nutrient Bioavailability

While food sources contain lots of essential nutrients, the bioavailability of these is key for proper utilisation of these nutrients in the body. Bioavailability refers to the proportion of a nutrient that is absorbed and reaches the bloodstream.[104] There are several factors which can affect the bioavailability of nutrients in food. For example, a low concentration of minerals in the raw ingredient will result in less minerals available for absorption.

A review by Soetan et al. (2010) identified that for plant-based foods, the location they are grown has a large impact on the amount of minerals the plant can absorb from the soil. This is because soil characteristics including PH, mineral concentration and composition differs depending on location.[101] Additionally, in a review by Neyestani et al. (2021), the authors concluded that high temperature cooking, including boiling, steaming, and frying causes high loss of minerals particularly for plant-based foods.[105]

Even if a plant is rich in minerals, plant-based foods contain anti-nutritional factors such as phytic acid and oxalic acid which can bind to nutrients creating complexes that are difficult to digest, this particularly affects Mg, P, Ca and Zn absorption.[101,105] Furthermore, the compartment in which nutrients are stored within the plant and the form of these nutrients, also has bearing on its bioavailability. For example, green leafy vegetables such as spinach are rich in vitamin K1 and pro vitamins such as beta-carotene. However, only between 5-10% of beta-carotene and less than 5% of vitamin k1 is available for absorption as these nutrients are stored in chloroplasts which are hard to break down. Carrots are also rich in beta carotene, however in crystallised form, which is not easy to absorb, whereas papayas contain beta carotene as lipid droplets which are more bioavailable.[106] For fruits and vegetables, the conversion efficiency of beta carotene is low with an estimated 12ug needed to form 1g of vitamin A.[106]

The bioavailability of nutrients from plants can be increased through processes which soften the vegetal matrix.[107] This can be achieved through thermal processing, and while this might degrade nutrient concentration, the bioaccessibility of these nutrients increases. Particle size can also play a role in some foods, for example, the small particle size achieved through liquifying spinach[108] and pulping carrots[109] increased the bioavailability of beta carotene compared with the whole food. Cooking the carrots after pulping further increased serum beta carotene from 21% to 27%.[109]

In comparison to plants, meat contains a higher bioavailability of many micronutrients. For example, vitamin A and vitamin B12 are only present in meat. Meat also contains heme iron which is considerably more bioavailable than non-heme iron found in plants.[110] In addition, folate, and zinc from meat, (particularly from red meat), are more bioavailable than plant sources. Offal contains the most nutrients with liver being particularly high in several micronutrients including vitamin B12, B1, B2, B6, vitamin A, magnesium, iron, and selenium.[110] Losses of certain nutrients are seen in meat following thermal processing. For example, boiling chicken breast at 90 degrees Celsius reduced iron content by 80% compared with raw chicken breast.[111] This is due to the combination of high thermal processing and the fact that Fe is water soluble. To avoid the risk of nutrient deficiency in dogs, FEDIAF recommend higher minimum micronutrient levels compared with the Nutritional Research Council (NRC) recommendations. This is in order to compensate for the possibility of low bioavailability of nutrients in commercial dog foods.[17,104]

Nutrients in commercial dog food

Although it is possible to get the necessary vitamins and minerals from whole foods, the vast majority of dog foods in the UK add synthetic vitamins and minerals. These can either be in organic form - i.e., bound to carbon or chelated to an amino acid, or in inorganic form which are compounds not bound to carbon. Research reviewed here has shown higher bioavailability of organic mineral supplements compared with inorganic supplements.[104,112,114] Commercial dog food often includes these nutritional additives because they use poor quality ingredients with low nutrient content and utilise high processing methods which degrades nutrients to such an extent that additional supplementation is required to comply with minimum guidelines. Synthetic additives, particularly inorganic ones are also used as they are cheaper than including large amounts of high-quality ingredients. Using tested and approved mineral additives also negates the need to test for these nutrients in the food as the synthetic additive should provide the necessary quantity. However, studies analysing the nutrient content of commercial pet foods containing nutritional additives found broad non-compliance with recommended nutrient minimums, legal maximums, and nutrient imbalances, as well as poor nutrient absorption.[114] (See table 6.3 for breakdown of these studies).

Table 6.3. Studies investigating the nutritional content of commercial pet foods.		
Reference	**Foods analysed**	**Findings**
Kastenmayer et al. (2002)[114]	Absorption of minerals in commercial dog food containing 20.1% protein, 11.1% fat, 1.5% fibre, 3.49 kcal/g metabolizable energy plus inorganic mineral additives per 250g food: Ca 4300mg Fe 54.5mg Cu 3.63mg Zn 37.0mg – much higher than AAFO recommendations.	Apart from copper, mineral absorption was low: Ca 10.1 ± 1.1 % Fe 8.8 ± 2.1 % Cu 23.1 ± 2.0 % Zn 11.5 ± 1.4 %
Davies et al. (2017)[1]	Analysed 177 “complete” popular cat and dog foods sold across the UK, 64 of which were for dogs. Pet foods covered 48 different brands and included veterinary/ therapeutic diets.	94% of wet pet foods and 61% dry pet foods did not fully comply with FEDIAF nutritional guidelines, with at least one essential mineral present below the minimum, or beyond the maximum recommended levels. 29% of wet dog foods and 20% of dry dog foods had clear mineral imbalances e.g., calcium phosphorus ratio. To ensure results were not due to a fault with the batch of food tested, a subset of wet foods from different batches were analysed which again showed non-compliance in line with initial results.
Pereira et al. (2018)[2]	Mineral analysis of 26 complete dry dog foods sold in Portugal and available in other European countries. Included Adult foods (20) and Puppy foods (6) from low, medium, and premium quality ranges. Food obtained from supermarkets, veterinary clinics and specialist stores.	Additives contributed 40.8-55.1% of total essential minerals present in food. < 25% of foods exceeded legal limit for Cu. > 50% of foods exceed legal limit for Zn. 50% of food above legal limit for Se with 25% below minimum NRC requirements. All foods over supplied macro elements with 50% supplying > 400% Ca; > 350% P; >750% Na; >200% Mg; > 150% K. 50% of foods oversupplied trace elements by > 600% Fe, >3000% Cu, >1500% Mn and > 450% Zn
Kazimierska et al. (2020)[63]	Mineral analysis of 30 complete dry extruded dog foods representing typical	1 out of 30 did not comply with minimum for Ca. Other essential minerals were well

	European dog food brands. Selected cereal-free and cereal inclusive foods to compare.	above the minimum but under maximum limits. Significant deviations from recommended ratios (between 30-98% of minimum ratio) for P:Zn (25/30 foods); also below minimum ratio for K:Mg (22/30) K:Ca (22/30) and Ca:Mg(13/30). 8/30 foods had inappropriate Ca:P ratio.
Dodd et al. (2021)[115]	Full nutrient analysis of 26 plant-based foods sold in Canada, 18 of which dog foods, 5 can foods and 3 classes as cat and dog foods.	4 of these foods complied fully with AAFCO recommendations and only 1 complied with FEDIAF. All 8 wet dog foods were low in 1 or more amino acids (methionine +cystine 8/8; methionine + cystine and methionine 3/8; 1/8 was same as previous plus low in lysine and tryptophan). 2 dog foods were below recommended levels for Na, 1 below for Na, Cl & Ca with 1 dry and 1 wet food providing an inverse Ca:P ratio and 1 dry food exceeding Ca:P ratio. 12 dog foods were low in vitamins.
Kępińska-Pacelik et al. (2023)[116]	41 dry dog foods sold in Poland analysed for mineral content and compared against FEDIAF and AAFCO mineral level recommendations	68% of foods were below the minimum level for Ca. 6 foods exceeded the maximum for P. 1 food had below minimum FEDIAF level for K. 14 did not meet FEDIAF or AAFCO min recommended level for Zn with 2 foods exceeding legal limit. 2 foods did not meet minimum for Mn.3 foods below min for Cu, with 1 food exceeding max Cu level. All foods below min ratio for Ca to P, highest of which was 0.68:1 (Ca:P). Only 22% met min Cu:Zn ratio. No foods met min Fe:Cu ratio.
AAFCO = Association of American Feed Control Officials		

Pereira et al. (2018) note that in the foods they tested, between 40- 55% of essential mineral were from synthetic mineral additives. The use of additives led to an oversupply for nearly all macro and trace elements in the foods they analysed. The authors discuss issues with over supplementation, such as the link between excess calcium and impairments in parathyroid gland activity and bone abnormalities.[2] While it was previously thought that excess calcium would simply be excreted from the body, a review conducted in 2015 found a distinct linear relationship between Ca intake and serum Ca levels, which puts into question the safety of current guidance on Ca supplementation.[117] Additionally, excessive sodium can lead to hypertension, and accumulation of Cu, due to excess intake, can lead to chronic hepatitis and cirrhosis.[2] Furthermore, excess iron relies heavily on a mechanism that regulates iron to work effectively, failure of this can lead to toxicity. Finally, while dogs appear to tolerate selenium up to 28 times above the minimum, Se below the minimum has been shown to reduce hair growth and thyroid hormones in puppies.[2] Pereira et al. (2018) conclude that using more bioavailable forms of these mineral (i.e. organic chelated versions), would lessen the need for excessive amounts. We note that using no additives and providing all minerals from whole food ingredients would also provide the same result. Kastenmayer et al. (2002) suggest that the low absorption of minerals in their study was due to the high elemental load lowering the digestibility of these minerals, as well as the use of inorganic additives. So, it would seem over supplementation of minerals is just as harmful as under supplementation, and also very common in dog foods containing nutritional additives. Collectively, these studies show the need for testing products before the addition of supplements and testing of final products, which is clearly lacking in many commercial brands.

It is not only individual mineral deviations from recommended levels that is an issue, but also how these interact with other nutrients creating nutrient imbalances. Sustained nutrient imbalances can deteriorate the health of pets, particularly a cause for concern if these foods are fed for long periods of time as Davies et al. (2017) note.

Nutrient interactions and 'food synergy'

It is vital to know how vitamins and minerals interact to ensure they are effectively utilised by the body (see table 6.3 and 6.4 for some important vitamin & mineral interactions). Vitamins and minerals can either enhance the absorption of other vitamins and minerals (agonist) or inhibit the absorption (antagonist). If antagonist nutrients are provided in excessive amounts this will reduce the absorption of certain nutrients. Having the right balance is therefore key here. While FEDIAF minimum recommendations provide the correct ratio, they only clearly point out recommendations for the Ca:P ratio, thus if nutrients deviate from the minimum FEDIAF recommendations, related nutrients must be adjusted accordingly.

Table 6.3 Mineral interactions with other minerals and vitamins.

	Minerals Interactions												**Vitamin Interactions**											
	Ca	P	Na	Cl	Fe	Mg	Mn	I	K	Zn	Cu	Se	A	E	D	K	B1	B2	B3	B5	B6	B7	B9	B12
Ca			🔴			🔴🟢			🔴	🔴			🔴🟢	🔴	🟢	🟢	🔴		🔴		🔴			
P	🔴🟢		🟢		🔴🟢	🔴🟢	🔴			🔴🟢	🔴				🔴🟢									
Na	🔴🟢				🟢	🔴🟢			🔴	🔴	🟢	🟢	🔴		🟢			🔴	🔴		🟢			
Cl																								
Fe	🔴	🔴	🟢				🔴🟢			🔴	🔴	🟢		🔴				🟢					🟢	🟢
Mg	🔴🟢	🔴	🔴		🔴🟢	🟢	🔴			🟢	🔴			🔴	🔴🟢		🟢				🟢		🔴	🔴
Mn	🔴	🔴			🔴🟢					🟢	🔴🟢			🔴		🟢	🟢							🔴
I																								
K	🔴🟢		🔴🟢		🟢	🟢	🟢			🟢	🔴				🔴		🔴				🟢			🔴
Zn	🔴	🔴🟢			🔴	🟢	🟢				🔴		🟢	🔴🟢	🔴🟢		🔴				🟢			🔴
Cu	🟢		🟢		🔴🟢				🔴	🔴🟢		🟢	🔴					🟢	🔴	🔴	🔴🟢		🟢	
Se	🟢		🟢		🟢		🟢		🟢	🟢	🟢		🔴	🟢				🔴	🟢					

🔴 = Antagonist 🟢 = Agonist 🔴🟢 = correct proportions of these nutrients are needed to work in synergy.

Table 6.4 Vitamin interactions with minerals and other vitamins

	Mineral interactions												Vitamin interactions											
	Ca	P	Na	Cl	Fe	Mg	Mn	I	K	Zn	Cu	Se	A	E	D	K	B1	B2	B3	B5	B6	B7	B9	B12
A	🔴🟢	🟢	🔴			🟢	🟢		🟢	🟢	🔴	🔴🟢		🔴🟢	🔴		🟢	🟢	🟢		🟢			🟢
E	🔴🟢	🟢	🟢		🔴🟢	🔴	🔴🟢		🟢	🔴🟢		🟢	🟢		🟢	🟢	🟢	🟢	🟢	🟢	🟢		🟢	🟢
D	🔴🟢	🔴🟢	🟢			🔴🟢	🟢		🔴	🔴	🟢	🟢	🔴	🟢		🟢		🟢	🟢					🟢
K	🔴						🟢						🔴🟢	🔴🟢					🟢		🟢			
B1		🟢	🟢		🟢	🟢	🟢		🔴🟢	🔴🟢	🔴🟢	🟢	🟢	🟢				🔴🟢	🟢	🟢	🔴🟢			🔴🟢
B2	🔴	🟢	🔴		🔴🟢	🟢	🔴		🟢	🟢	🔴🟢	🔴	🟢					🔴🟢	🟢	🟢	🟢		🟢	🟢
B3	🔴	🟢	🔴🟢		🟢	🟢	🟢		🟢	🟢	🔴🟢	🟢	🔴🟢	🟢			🟢	🟢		🟢	🟢		🟢	🟢
B5		🟢	🟢						🟢	🟢	🔴		🟢	🟢			🟢	🟢	🟢		🟢		🟢	🟢
B6	🔴	🟢	🟢		🔴🟢	🟢	🟢		🟢	🟢	🔴🟢	🟢					🔴🟢							
B7																								
B9					🟢	🟢				🟢	🔴🟢							🟢	🟢	🟢			🟢	🟢
B12	🟢		🟢		🟢	🔴			🔴	🔴	🟢	🟢		🟢	🟢		🔴🟢		🟢	🟢	🟢		🟢	

🔴 = Antagonist 🟢 = Agonist 🔴🟢 = correct proportions of these nutrients are needed to work in synergy.

One of the most widely known mineral interactions are calcium (Ca) and phosphorus (P), with Ca acting as both an agonist or an antagonist for P as excess Ca reduces P absorption, however, at the correct ratio of between 1:1 and 2:1 these minerals work in synergy. There are also many more interaction which must be considered. For example, vitamins A and E metabolism and bioavailability are dependent on zinc status;[101] copper interacts with iron, calcium, and phosphorus; copper can inhibit absorption of iron; zinc can inhibit the absorption of copper, and magnesium is needed for absorption of calcium.[102] Recommended ratios for some minerals are listed in table 6.5 (taken from Kepinska-Pacelik et al. (2023), based on FEDIAF 100g DM table).

Table 6.5 important mineral ratios	
Mineral	Ratio as per FEDIAF guidleines
Ca:P	1:1 – 2:1
Na:K	0.20:1
Ca:Mg	1:0.14
Cu:Zn	1:10.00
Fe:Cu	1:0.20

Interactions between nutrients, absorption of nutrients, and their bioavailability underpins the theory of 'food synergy', a concept described by Jacobs et al. (2009) which postulates that natural foods are better for humans and animals, than nutrient interpretations of these foods (i.e. nutritional supplements). Supplements have of course been beneficial in extreme deficiencies such as folic acid in pregnancy and calcium and vitamin D in osteoporosis prone groups. However, clinical trials have found that isolated compound versions of many of the nutrients found in food, (vitamin and mineral supplements), had no effects on health parameters.[118] Nutrients in food on the other hand are inherently coordinated. For example, nuts have high levels of unsaturated fats which are offset by the high level of antioxidant compounds also found in nuts. Additionally, there is much we don't know about the food matrix. Indeed, many of the effects we see may be the result of unidentified components in food. Thus, it is more beneficial, when possible, to obtain nutrients from foods rather than supplements.[118]

Discussion

This review of nutritional interventions in dogs has highlighted several important factors to consider when formulating dog food. Firstly, animal protein is superior to plant-based protein, as it contains all essential amino acids. Combining complimentary plant-based proteins as well as adding nutritional supplements can however provide adequate nutrition. Nevertheless, using animal protein in a dog food recipe would be advantageous to avoid the necessity for synthetic nutritional additives. Avoiding high pressure, high temperature cooking is important to ensure bioavailability of absorbed amino acids. Instead, gentle cooking increases digestibility of meat and bioavailability of the nutrients, with sous vide cooking standing out as a good method to reduce protein aggregation, as well as retaining water soluble nutrients in the bag. High protein diets are also advantageous for weight management and controlling glycaemic and insulinemic responses.

With regards to fats and oils, these are the main source of energy for dogs, thus are an essential part of their diet. PUFA are necessary for many biological processes, and studies here have also found they can provide some improvements to osteoarthritis and dermatitis in dogs. An optimal amount and ratio of n-6 to n-3 however is still elusive, as studies are using different amounts and ratios and seeing similar results. For atopic dermatitis, Muller et al. (2005) note that mechanisms other than PUFA supplementation may be involved due to the lack of correlation between PUFA intake, skin and plasma concentrations of PUFA, and clinical signs. DHT skin response tests have proven that high consumption of n-3 attenuates the inflammatory response, as well as reducing circulating vitamin E. Thus, high doses of n-3 should be restricted to dogs with chronic inflammatory diseases, and should also be supplemented with vitamin E.

The addition of carbohydrates to a dog's diet is not essential for all dogs, however it can provide an extra source of energy, allowing protein to be utilised for other important biological processes. How well dogs can digest protein depends on breed and can vary from dog to dog. While some dogs follow the wolf in the number of gene copies essential for starch digestion, others possess a higher number of copies, resulting in a greater efficiency when it comes to digesting starches. All dogs lack salivary enzymes responsible for breaking down starch, so are not as efficient as digesting starch as true omnivores. This review also highlighted that cooking grinding and soaking of plant-based foods reduces antinutrients and improves digestibility of other important nutrients. Additionally, if carbohydrates are added to dog food, it is important to use complex carbohydrates to maintain good glycaemic and insulinemic control. A major factor to arise from studies investigating carbohydrate sources in dog food was the effects of grain free feeding. In particular, this review highlighted that grain free foods are not inherently healthier than grain-based foods and that allergies to grains are rare in dogs. Additionally, studies revealed that dogs on grain-based diets digested more dry matter, organic matter and starch from their food, than dogs on grain free diets. This review also highlighted that the link between dilated cardiomyopathy and grain free foods, particularly those containing peas and lentils is apparent, but not fully understood. It is likely though that these diets either contained too much fibre, too little protein, or both, which can disrupt taurine synthesis.

While not essential for dogs, fibre is beneficial for maintaining gut health and regulating bowel movements. This review highlights that high fibre diets can be successfully used for weight management in dogs, as it lowers the caloric density of the food while increasing satiety. High fibre diets however should not be combined with low protein feed. Another important point to consider is the fermentability of different fibre sources and its effect on dogs. Given the clear differences in colonic fermentative activity between small and large breed dogs, it appears they could benefit from different ratios of fermentable to non-fermentable fibre. Limiting the amount of fermentable fibre for large breeds and increasing non-fermentable fibre, should improve stool quality. In small dogs on the other hand, increasing fermentable fibre and reducing non-fermentable fibre should increase colonic fermentative activity and reduce the chance of constipation.

Probiotic studies reviewed here show that the addition of probiotics to a dog’s diet can maintain and improve the composition of gut microbiota. This in turn can have positive effects on stool quality, body condition and improve the digestibility of nutrients in food. While the studies reviewed here used varying doses of probiotics, at least 1 billion CFU per daily dose is required to reap the benefits of probiotics.

This review underscores the importance of formulating dog foods with careful consideration of nutrient interactions, rather than simply meeting minimum nutrient requirements. Many existing dog foods rely on synthetic nutritional supplements to ensure adequate micronutrient content, often without assessing nutrient levels in the food before supplementation. Unfortunately, this approach can result in excessive amounts of certain nutrients, which can be toxic and interfere with the absorption and efficacy of other vital nutrients. The risks surrounding the use of synthetic nutrient additives are real. In 2019, the FDA recalled a range of Hill's prescription and Science canned dog foods due to reports of vitamin D toxicity, tragically resulting in the loss of some beloved dogs.[119] Similarly, in March of this year, Nestlé Purina recalled their Pro Plan Veterinary Diets after discovering dangerously high levels of vitamin D in their products.[120]

Finally, this review highlighted the importance of providing dogs with foods that contain bioavailable forms of essential macro and micronutrients. Just because a food contains certain vitamins and minerals does not mean that once consumed, they can be absorbed and utilised by the body. Vegetables and plant-based foods in particular contain many nutrients that are difficult to obtain. Preparing these foods properly is key if they are to be used in dog food. Meat and fruits on the other hand, contain nutrients that are highly bioavailable to the animal, making them readily absorbed and utilised. Furthermore, studies have revealed that inorganic synthetic nutrients demonstrate lower bioavailability compared to organic nutrient supplements. Nevertheless, research has also emphasized that even organic nutrient supplements cannot fully substitute the nutritional benefits offered by real food sources. This is because there is still a great deal we have yet to discover about the food matrix. Obtaining nutrients from real food sources not only provides a more balanced nutrient profile, but also allows any unknown mechanisms that facilitate nutrient absorption and utilisation to function effectively.

Conclusion

This systematic review highlights the importance of developing dog foods with a comprehensive understanding of nutrient needs and interactions. Animal protein, real food sources, and bioavailable nutrients are preferred over high carbohydrates and synthetic additives. The Proper dog food recipe will use these principles to enhance canine health and prevent potential risks associated with unbalanced diets.