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# Raw Meat-Based Diets: Current Evidence Regarding Benefits and Risks

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## Introduction

Dogs and cats evolved eating prey-based diets as their primary (dogs) or sole source (cats) of food. It is only within the past 100 to 150 years that commercial pet food products have been made and marketed for dogs and cats.<sup>1</sup> In a survey conducted in 2011,<sup>2</sup> 10.8% of 791 pet owners from 44 U.S. states and six countries fed a commercial or home-prepared raw meat-based diet (RMBD) as a major component of their pet's diet and 32.9% fed a home-prepared or commercial RMBD as some component of their pet's diet. Those that support RMBD are very passionate about their benefits, claiming improved pet health that includes improved immune function and decreased incidence of many chronic diseases, such as obesity, diabetes mellitus, allergies, feline urological syndrome, arthritis, and cancer.<sup>3-5</sup> Yet, no topic in veterinary nutrition has been more emotionally charged over the past 10 to 20 years, mainly due to the limited data from high-quality studies evaluating these diets. The purpose of this manuscript is to discuss the current evidence regarding the risks and benefits of these diets.

## Definition of RMBDs

Raw meat-based diets are those that include portions of uncooked domesticated or wild-caught food animal species and that are fed to pet dogs and cats in the home environment. These uncooked portions include skeletal muscle, bone and internal organs from mammals, fish and poultry. Raw-meat diets can be divided into two main categories: commercial or home-prepared.

The commercial RMBDs are fresh, frozen or freeze-dried diets intended to be nutritionally complete and balanced. These diets are created from recipes developed by companies marketing their specific brand of pet food. In addition to the fresh, frozen and freeze-dried commercial diets is a premix that includes vitamins and minerals intended to have a raw-meat protein source added by the pet owner to become a complete diet.

Home-prepared RMBDs include a variety of highly publicized feeding regimens, such as BARF<sup>3</sup> (biologically appropriate raw food), the Volhard<sup>4</sup> and the Ultimate Diet.<sup>5</sup> Additional RMBDs have been developed by veterinarians, breeders and owners.

## Glossary of Abbreviations

**AAFCO:** Association of American Feed Control Officials

**BARF:** Biologically Appropriate Raw Food

**DT:** Definitive Type

**FDA:** Food and Drug Administration

**RMBD:** Raw Meat-Based Diet

Some of the home-prepared RMBDs are based on a rotation of ingredients with the belief that this rotation will provide over a period of time necessary amino acids, fatty acids, vitamins, and minerals.

## Risks

Three primary risk factors have been reported with the consumption of raw diets by pets. These include overall nutritional adequacy, health concerns such as consumption of raw bones leading to dental fractures or gastrointestinal trauma, and consumption of pathogenic bacteria, viruses and protozoa not killed in the normal cooking process. The most frequently cited concern of these in the veterinary published literature has been food safety.

## Safety - Pets

The primary safety concern related to RMBDs is the risk of contamination with pathogens.<sup>6</sup> Raw meat, whether sold for human consumption or included in dry extruded or moist canned pet foods, can be contaminated with a variety of pathogens including *Salmonella* spp., *Escherichia coli* and *Campylobacter* spp. While care is used during processing, meat from food animals can acquire bacterial contamination from the hide, feathers or viscera during slaughter, evisceration, or processing and packing.<sup>7-10</sup> Because freezing does not destroy all these pathogens, both home-prepared and commercial RMBDs are at risk for being contaminated.

Several reports<sup>7-9</sup> have been published on the presence of *Salmonella* spp. and other pathogens in commercial and home-prepared RMBDs. Prevalence rates for contamination with *Salmonella* spp. in commercial RMBDs ranged from 20 to 48%.<sup>7,9,10</sup> Recently, a *Salmonella* spp. prevalence rate of 21% for 16 commercial RMBD samples was reported.<sup>10</sup>

Exotic cats fed raw meat diets have a high prevalence of shedding fecal *Salmonella* spp. For example, *Salmonella* spp. was isolated in 94% of fecal samples from a zoo and private big cat collection.<sup>11</sup> All exotic cats were clinically healthy and were being fed a raw horsemeat and chicken diet. Prevalence of fecal isolation of *Salmonella* spp. in apparent clinically healthy domestic cats

ranges from 0 to 18%.<sup>12-15</sup> As these values are based on one fecal culture, they probably underestimate prevalence due to the difficulty in isolating and culturing *Salmonella* spp. and due to its intermittent shedding in the host. Suspected clinical cases of salmonellosis require three negative cultures before ruling out the disease.<sup>12</sup> Susceptibility and severity of infection depend on multiple factors, including virulence of the pathogen strain, infectious dose and host resistance.<sup>14</sup> Host resistance factors include age, immunocompetence, stress, administration of glucocorticoids, and presence of chronic disease. Although cats may carry *Salmonella* spp. in their digestive tract without associated morbidity, there have been reports of morbidity and mortality in cats<sup>14,16,17</sup> and dogs<sup>18-21</sup> secondary to consumption of RMBDs.

Home-prepared RMBDs were evaluated in one study<sup>8</sup> in which eight of 10 home-prepared raw chicken-based diets fed to pet dogs had positive results when cultured for *Salmonella* spp. In addition, there are numerous reports<sup>14,22-24</sup> of racing Greyhounds, sled dogs, guard dogs, and cats with *Salmonella* infections attributable to consumption of contaminated raw meat, including reports of dogs and cats that died from *Salmonella*-related sepsis. It is not surprising to find high rates of contamination with *Salmonella* spp. in home-prepared diets because high rates of contamination with *Salmonella* spp. can be found in raw meats sold for human consumption. Rates of contamination differ among studies<sup>25-29</sup> but range from 21 to 44% of chicken samples purchased from retail locations throughout North America.

Contamination of RMBDs with other bacteria and pathogens has also been examined. *Escherichia coli* are part of the normal commensal gastrointestinal microbiome of mammals, yet certain strains of *E. coli* are known pathogens to both humans and animals. Verocytotoxic strains, including *E. coli* O:157:H7, are considered the most virulent causing hemorrhagic diarrhea. The overall prevalence of pathogenic bacterial contamination in raw meat and poultry sold for human consumption varies greatly, depending on the contaminant, the species of animal used to produce the raw ingredient, the amount of processing of the raw ingredient, i.e., the number of times the ingredient has been handled and the facility in which it is processed. Overall prevalence of *Campylobacter jejuni* in poultry ranges from 29 to 74%.<sup>25,26,29</sup> In human-grade raw beef products, the prevalence of pathogenic *E. coli* O157:H7 ranges from 0 to 28%.<sup>30,31</sup> Cattle are known to harbor large numbers of *E. coli* through fecal contamination, with as many as one in four animals at slaughter shedding *E. coli* O157:H7 in their feces.<sup>32</sup> The same fecal strains of *E. coli* have been recovered throughout the production-processing continuum and in the raw beef products.<sup>33</sup>

Pathogen contamination is not unique to unprocessed pet foods. Commercial pet foods have been subject to numerous recalls for *Salmonella* spp.<sup>34</sup> As an example, a pet food recall from a single manufacturing plant was linked to 29 human patients identified with *Salmonella enterica* serovar Schwarzengrund infections between 2006 and 2008.<sup>35</sup> Of 28 recalls and safety alerts because of confirmed or potential contamination of com-

mercially available pet foods with *Salmonella* spp. in 2011 and 2012, 17 were for dry extruded pet foods, one was for a RMBD, and 11 were for raw or insufficiently processed treats, especially raw pig ears.<sup>34</sup> Both pathogenic and chemical contamination in commercial processed pet foods has led to significant morbidity and mortality in pets with the most notorious being the 2007 recall secondary to melamin/cyanuric acid.<sup>36</sup> In North America, up to 39,000 dogs and cats may have developed kidney failure as a result of eating these contaminated commercial pet foods.

Other problems associated with commercial pet food recalls include vitamin D excess,<sup>37,38</sup> thiamine deficiency secondary to irritation<sup>39,40</sup> and mycotoxins.<sup>41,42</sup> There have also been warnings concerning a Fanconi-like renal syndrome in dogs after ingestion of chicken jerky treats manufactured in China.<sup>43</sup> In response to the melamine/cyanuric acid tragedy, the Food and Drug Administration (FDA) Amendments Act of 2007 was passed to strengthen the food recall process. It requires manufacturers to submit a report to the FDA no later than 24 hours after determining there is a reasonable probability that the use of or exposure to the food will cause serious adverse health consequences or the death of animals (or humans).<sup>44,45</sup>

Proponents of RMBDs argue that the gastrointestinal tracts of healthy pets can survive any raw meat product due to an abundance of gastrointestinal flora<sup>46</sup> and shorter intestinal length.<sup>5</sup> Although the gastrointestinal tracts of dogs and cats are shorter in comparison with that of humans, there is no evidence that a shorter gastrointestinal tract prevents infection with pathogens.

Parasite contamination is another safety concern when feeding RMBDs to dogs and cats. Cats are the definitive host for *Toxoplasma gondii*. *Toxoplasma* bradyzoites encyst in tissue, particularly muscle. Cats ingesting tissue cysts can go on to develop systemic infections. Kittens are particularly sensitive with transplacental exposure or ingestion through lactation resulting in significant morbidity and mortality.<sup>47</sup> In one study,<sup>48</sup> overall seroprevalence of antibodies to *Toxoplasma gondii* were 53% in cats fed raw meat diets compared to 23% in cats fed commercial heat-processed diets. Feeding a raw meat is a known risk factor for *Neospora caninum* in dogs.<sup>49</sup> Affected dogs are generally less than 6 months old and predominantly have signs of ascending hind limb paralysis.

### Safety – Owners

The potential impact on human health when feeding RMBDs to pets is another risk factor. As stated previously, raw meats are frequently contaminated with microorganisms including *E. coli*, *Salmonella* spp., *Campylobacter* spp., and *Listeria* spp.<sup>25-29,41,50-53</sup> Raw meats can also carry parasites such as *Toxoplasma gondii*, and *Trichinella* spp.<sup>54,54,55</sup> In addition to the diet itself being a source of pathogens for humans, other sources of contamination include food utensils, feeding bowls and areas of possible fecal contamination. The same pathogens isolated from raw diets have been found in dog's feces<sup>8</sup> and, subsequently, in owners/family members who have become ill.<sup>56,57</sup> The populations at greatest

risk for contracting illness in households feeding raw diets are the very young (infants and children), the elderly, pregnant women, and those who are immunocompromised.

Most human cases of salmonellosis are due to exposure to contaminated foodstuffs, but cases of human salmonellosis due to direct or indirect contact with animals have been reported.<sup>56–58</sup> As stated previously, *Salmonella* organisms can frequently live as a transient member of the intestinal microflora without causing illness; thus, a human or pet can be a carrier. Direct contact with infected or carrier animals or their feces is a risk factor for salmonellosis in humans.<sup>6,53,56,57,59</sup> Several studies<sup>8,60–62</sup> have found that dogs eating RMBDs are at risk for shedding *Salmonella* organisms in their feces. Results of these studies<sup>8,60–62</sup> indicate that between 3 to 50% of dogs fed RMBDs shed *Salmonella* in their feces. In one study,<sup>62</sup> when a single meal of a contaminated commercial RMBD was fed, seven of 16 dogs shed *Salmonella* spp. in their feces for up to seven days.

Fecal shedding of *Salmonella* spp. in cats can last from three to six weeks, and in some cases up to 14 weeks, after clinical illness.<sup>12,63</sup> In cats with salmonellosis, large numbers of bacteria are present in the mouth and their coat can be highly contaminated secondary to their grooming habits.<sup>64</sup> Most domestic cats spend a large amount of time in close proximity to their owners with ample potential for direct or indirect exposure to zoonotic organisms. Of particular concern is the increasing incidence of an antibiotic-resistant strain, *Salmonella* serovar Typhimurium definitive type (DT)104. This strain has become an important food safety concern because of its increased incidence in both humans and animals and its ability to cause serious disease with resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides, and tetracycline. Several studies<sup>58,63,65,66</sup> have identified domestic cats as carriers of multiresistant *Salmonella* Typhimurium DT 104 along with farm animals, dogs and birds.

Other pathogens also are of concern for humans exposed to pets shedding bacteria. *E. coli* O157:H7 transmission from an asymptomatic dog to humans has been documented.<sup>67</sup> *Toxoplasma gondii* infection is another concern to humans, particularly pregnant women. Although most cases of human toxoplasmosis are secondary to consuming undercooked meat or food that has been cross-contaminated from raw meat, toxoplasmosis can be passed from cats to humans through exposure to oocysts in the cats' feces. Cats with a newly acquired infection will shed the oocysts for about three weeks following infection. Thus, cleaning litter boxes during this timeframe can result in cross-contamination. If infection occurs to the fetus during pregnancy, abortion, premature birth or permanent neurological impairments can occur.<sup>68,69</sup>

### *Other Health Concerns*

In addition to the previously mentioned health problems, RMBDs that contain bones (i.e., the BARF diet) can potentially result in fractured teeth and gastrointestinal injury. Bones can cause obstruction or perforation of the esophagus, stomach,

small intestine, or colon. Bone foreign bodies were present in 30 to 80% of dogs and cats with esophageal foreign bodies.<sup>70–72</sup> Those who promote the feeding of raw bones claim that there are fewer problems with raw bones than with cooked bones<sup>73</sup>; however, to current knowledge, the frequency of obstruction or perforation with raw versus cooked bones has not been evaluated. Research is needed to better understand the frequency of these complications.

Another potential adverse health effect associated with RMBDs was identified in a recent report.<sup>74</sup> Authors of that report<sup>74</sup> identified and described 12 dogs with elevations in serum thyroxine concentration (six of which had clinical signs of hyperthyroidism) caused by eating an RMBD. All dogs had thyroxine concentrations within the reference range after the diet was changed.

### *Nutritional Adequacy*

A U.S. study<sup>75</sup> in 2001 revealed that all the home-prepared and commercial RMBDs tested (three home-prepared and two commercial RMBDs) had multiple nutritional imbalances, some of which could have important adverse effects on the health of the animals. Examples included a calcium-to-phosphorus ratio of 0.20, vitamin A and E concentrations below the minimum detectable value, and a vitamin D concentration nearly twice the Association of American Feed Control Officials (AAFCO) maximum amount.<sup>75</sup> Authors of a case report<sup>76</sup> of a growing dog fed an RMBD (a commercial premix plus raw ground beef prepared in accordance with instructions on the package label) reported that the nutritionally unbalanced diet resulted in vitamin D-dependent rickets type I and nutritional secondary hyperparathyroidism.

In a recent study<sup>77</sup> in Europe, investigators calculated amounts of 12 nutrients (e.g., calcium, phosphorus and vitamin A) for 95 homemade RMBDs being fed to dogs, as reported by the owners. In that study,<sup>77</sup> 57 diets (60%) had major nutritional imbalances. Therefore, there is concern that both commercial and homemade RMBDs may have important nutrient deficiencies and excesses. Investigators in three studies<sup>78–80</sup> evaluated the nutritional balance of commonly available home-prepared diet recipes. In the two studies<sup>78,79</sup> on animals with medical conditions, 94 recipes were evaluated, and none had adequate concentrations of all essential nutrients. In one of these studies,<sup>80</sup> investigators evaluated 200 recipes for healthy dogs and found that 190 (95%) recipes had at least one essential nutrient below AAFCO minimums and 167 (84%) recipes had multiple deficiencies.

### **Benefits**

As stated previously, numerous benefits have been ascribed to feeding pets RMBDs compared to feeding heat-processed foods. Reported benefits include improved skin and coat quality, improved digestibility and decreased incidence of many medical conditions.<sup>3–5,81</sup> To the author's knowledge, these claims have been based on anecdotal evidence rather than scientific study.

A founding premise regarding RMBDs is that these are the

optimal diets for health and wellness based on the theory that dogs and cats evolved over millions of years on a natural raw diet and logically this is their ideal food source. Advocates claim that processed foods are not what dogs and cats were programmed to eat during the long process of evolution and that foods similar to those eaten by the dog's/cat's wild ancestors are more biologically appropriate.<sup>3</sup> Cats are obligate carnivores with a strong predatory instinct.<sup>82</sup> Their natural diet in the wild includes a range of small prey species, such as mammals, reptiles, birds, and insects. Conversely, dogs have adapted to eating an omnivorous diet and can consume a variety of plant and animal products to meet their essential nutrient requirements.

Compared to cats, dogs have undergone an incredible variety of selection pressures resulting in large phenotypic differences from their ancestors. A recent study<sup>83</sup> found that there were 36 regions of the genome that differ between dogs and wolves, 10 of which play a critical role in starch digestion and fat metabolism. The authors of that study<sup>83</sup> concluded that these genetic differences in the genome between dogs and wolves have contributed to the ability of dogs to digest starch and fat and constituted a crucial step in the early domestication of the dog. Although carbohydrate digestibility in cats is nearly 100% for simple sugars and starches,<sup>84</sup> cats have limited ability to handle high concentrations of carbohydrate in their diet compared with other species secondary to having lower levels of pancreatic and intestinal amylase compared to dogs<sup>85</sup> and minimal glucokinase activity, an important glycolytic enzyme during high-carbohydrate loads.<sup>86</sup> Due to these metabolic adaptations, feeding high-carbohydrate commercial diets has been theorized to cause a variety of disorders in cats including obesity and diabetes.<sup>87</sup>

A frequently cited benefit to feeding raw food diets is that active digestive enzymes remain intact and thereby improve digestibility and bioavailability of foodstuffs.<sup>3,5</sup> Arguments against these claims are that protein enzymes are denatured and inactivated in the stomach secondary to hydrochloric acid and pepsin secretion,<sup>88</sup> and all the enzymes dogs and cats need for digestion are already produced in the gastrointestinal tract unless they have underlying exocrine pancreatic insufficiency.<sup>88,89</sup> The extent of enzyme degradation in the stomach is not completely quantified, but treatment for exocrine pancreatic insufficiency with raw pancreas is a common practice. In a study<sup>90</sup> comparing raw porcine pancreas to a commercial pancreatic enzyme supplement, the raw porcine pancreas had the highest level (39.1%) of supplemental lipase recovery in jejunally cannulated dogs compared to the commercial pancreatic supplement (26.2%). Dietary amylase and protease activity levels were still present in the jejunum from the raw pancreas extract and higher than enzyme levels in a dog with subclinical exocrine pancreatic insufficiency.

Several scientific studies<sup>91-93</sup> have documented improved digestibility in RMBDs compared to heat-processed diets. Digestibility of RMBDs versus dry extruded diets has been examined in both exotic and domesticated cats.<sup>91-93</sup> One study<sup>91</sup>

compared a raw meat diet with a dry kibble diet in sand cats and found the raw meat diet to have 10% higher digestibility in dry matter and energy and 15% higher digestibility in crude protein compared to the kibble extruded diet. A more recent study<sup>92</sup> looked at feeding the domestic cat's wild ancestor, *Felis lybica*, a commercial raw meat versus an extruded high-protein kibble diet. Crude protein digestibility in the raw diet was 8% higher compared to the extruded diet. In research done by the author,<sup>94</sup> significantly higher digestibility of dry matter (7 to 10%), organic matter (5 to 8%), protein (6 to 10%), and energy (3 to 6%) was seen in RMBDs compared to a canned heat-processed diet in both kittens and adult cats.

Proteins and amino acids undergo substantial physical changes during processing associated with the manufacture of pet foods. Processing conditions, which primarily involve application of heat but can also include pressure and water content, can have variable effects on protein digestibility and amino-acid bioavailability. The effects depend on the ingredients, temperature and type of processing (i.e., canning, extrusion used in the production of most commercial dry pet foods, and freezing or freeze-drying that would be performed with commercial RMBDs). In addition, food proteins can react with other food components, such as sugars, fats, oxidizing agents, acids, alkalies, polyphenols, and food additives. Heat processing during the manufacture of dry extruded or moist pet foods typically results in the denaturing of proteins and loss of secondary and tertiary protein structure. Processing can increase bioavailability of proteins through collagen breakdown and increased exposure to digestive enzymes, but it also can negatively affect amino acids through proteolysis, protein crosslinking, amino-acid racemization, protein-polyphenol reactions, oxidative reactions, and browning or Maillard reactions.<sup>95</sup> The Maillard reaction accounts for the most important losses of amino-acid bioavailability.<sup>96</sup>

Although conventional heat processing can have negative effects on animal tissue proteins, heat processing improves the bioavailability of some plant proteins secondary to denaturing of antinutritional factors. For example, legumes contain trypsin and chymotrypsin inhibitors that impair protein digestion and reduce protein bioavailability.<sup>97</sup> Heat processing denatures these inhibitors and, therefore, increases protein bioavailability.

Improved digestibility results in less digesta in the colon with less fecal matter. Decreased fecal output has been found in a study<sup>92</sup> of feral cats and in experiments conducted by this author.<sup>94</sup> Decreased fecal output is perceived as a benefit by some owners. Although nondigestible carbohydrates in the form of fiber are beneficial to the host,<sup>98</sup> undigested dietary protein results in increased amounts of colonic compounds, such as ammonia, phenols, indoles, and amines, which can play a role in diseases like colorectal, stomach and pancreatic cancers.<sup>99-101</sup> The author is not aware of any reported studies on the potential harmful effects of undigested dietary protein on colonic health in dogs or cats.

Heterocyclic amines are compounds formed when muscle

meat is cooked to a high temperature. Exposure to high concentrations (e.g., milligram/gram of food) of these compounds has been associated with cancer in research animals.<sup>102</sup> Concentrations found in both pet and human foods are much lower (nanograms/gram of food), but these concentrations may still have mutagenic activity.<sup>103</sup> The cumulative effects of these compounds on genomic instability and increased sensitivity to tumor promotion in pets and humans require investigation.

Another frequently cited benefit when feeding RMBDs is an improvement in immune function. In experiments conducted by this author,<sup>94</sup> domestic cats fed an RMBD for 10 weeks had a significant increase in lymphocyte and immunoglobulin production, though there were no significant changes over the same study period for cats fed a cooked commercial moist diet. In those experiments,<sup>94</sup> it was also found that cats fed the RMBD were fecal shedders of *Salmonella* spp. Exposure to higher microbial loads, including pathogens and microbial degradation products, changes in intestinal microflora, or nutritional differences in the diets may have stimulated the immune response detected for cats fed the RMBD. In the same study,<sup>94</sup> there were no significant differences found in herpes-specific titers after vaccination or parameters of innate immune response (oxidative burst or phagocytosis) between RMBD feeders or those fed the commercial canned heat-processed diet. Although this study evaluated serum immunoglobulin and innate immune response, further studies are needed to examine cell-mediated immunity (T-cell) response, secretory IgA levels and potential microbiome changes in pets fed RMBDs compared to heat-processed extruded or canned pet foods.

The thermal processing of proteins is important in food allergy because heat treatment can alter their allergenic sites. Antibodies recognize and interact with distinctive shapes called epitopes on the protein. In their native state, proteins are folded into compact tertiary structures determined by their amino-acid composition. The overall 3-D structure results in certain epitopes being buried within while others are exposed on the outside. Application of heat results in protein tertiary structure breaking down, exposing previously hidden epitopes on primary and secondary protein structures. Thus, heating can increase or decrease protein allergenicity, depending on the protein and the animal involved. In a study<sup>104</sup> looking at the effect of heating on immunogenicity of canned proteins in cats, a heated protein (casein) induced a salivary IgA response not seen in the raw product. Further studies looking at the effect of commercial heat treatment on immunogenicity of proteins in pet foods is warranted.

In another study<sup>94</sup> conducted by this author, an AAFCO growth feeding trial was conducted comparing two RMBDs (commercial frozen and premix added to raw meat) to a commercial heat-processed canned diet. Both raw diets had similar growth performance compared to a canned cat food and both passed an AAFCO growth trial. There were no significant differences in average daily gain or body tissue accrual/composition among the three diet treatments. Kittens fed the RMBDs

did have lower albumin and higher globulin levels compared to the control group, but this was not clinically significant as albumin levels were still within normal reference ranges.

## Conclusion

It is difficult to make overall recommendations regarding feeding raw diets to pets due to the current lack of good data and scientific studies. The infectious disease potential to both the pet and owners has been well-documented. Owners who elect to feed a commercial or home-prepared RMBD should be counseled on the risks to themselves and their pets. Since most home-prepared diets are deficient in one or more essential nutrients, a board-certified veterinary nutritionist should review these diets to ensure that they are balanced.

The use of intensive farming practices for meat production has increased dramatically over the past 50 to 100 years.<sup>105</sup> Raising livestock in confinement at high stocking density increases the risk of pathogen contamination in domestic animals intended for slaughter.<sup>30,33</sup> Raw meat obtained from these sources is quite different compared to intact prey fed upon by nondomesticated canids and felids. Further scientific studies examining the effects of long-term feeding RMBDs on microbiome, immunogenicity, immune function, gastrointestinal health, and disease are warranted.

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