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How important is food structure when cats eat mice?

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Abstract

Feeding whole prey to felids has shown to benefit their gastrointestinal health. Whether this effect is caused by the chemical or physical nature of whole prey is unknown. Fifteen domestic cats, as a model for strict carnivores, were either fed minced mice (MM) or whole mice (WM), to determine the effect of food structure on digestibility, mean urinary excretion time (MUET) of ¹⁵N, intestinal microbial activity and fermentation products. Faeces samples were collected after feeding all cats a commercially available extruded diet (EXT) for 10 d before feeding for 19 d the MM and WM diets with faeces and urine collected from day 11 to 15. Samples for microbiota composition and determination of MUET were obtained from day 16 to 19. The physical structure of the mice diet (minced or not) did not affect large intestinal fermentation as total SCFA and branched-chain fatty acid (BCFA), and most biogenic amine (BA) concentrations were not different ($P > 0.10$). When changing from EXT to the mice diets, the microbial community composition shifted from a carbolytic (*Prevotellaceae*) to proteolytic (*Fusobacteriaceae*) profile and led to a reduced faecal acetic to propionic acid ratio, SCFA, total BCFA ($P < 0.001$), NH_3 ($P = 0.04$), total BA ($P < 0.001$) and para-cresol ($P = 0.08$). The results of this study indicate that food structure within a whole-prey diet is less important than the overall diet type, with major shifts in microbiome and decrease in potentially harmful fermentation products when diet changes from extruded to mice. This urges for careful consideration of the consequences of prey-based diets for gut health in cats.

Keywords: Cats: Animal fibre: Physical structure: Faecal microbiota: Fermentation end product

The domestic cat (*Felis silvestris catus*), a strict carnivore, plays an important role in many people's lives around the globe^(1,2). The companionship cats provide is reciprocated by care including the provision of nutritious dry extruded or wet retorted foods, often throughout their lives. Nutritional science has led to detailed insights in feline digestive physiology and metabolism as well as the physicochemical properties of ingredients and foods that underlie the pet food industry's capacity to manufacture nutritious, high-quality and safe foods that support health and longevity of domestic cats⁽³⁾. This also includes nutritional concepts to promote gut health via prebiotic plant fibres that steer microbial populations and their fermentation products^(4,5). How we feed domestic cats, however, differs considerably from what cats consume in nature where they typically prey on small vertebrates including mice with little consumption of plant matter⁽⁶⁾. Though it is widely accepted that

the evolution on such a diet has led to an array of metabolic adaptations in cats⁽⁷⁾, the nutritional properties of nature-based diets as a food source for cats are largely understudied⁽⁸⁾.

The nutritional properties of dry extruded cat food differ considerably from animal prey such as mice. Dry extruded pet foods are mainly composed of finely ground dry ingredients from plant and to a lesser extent animal origin that are cooked and shaped into a kibble which is coated to enhance palatability. The extrusion process increases the digestibility of the food⁽⁹⁾ and lowers the amount of undigested fractions. Therefore, mainly plant fibres and undigested proteins reach the distal intestine where they are used by the microbial population⁽¹⁰⁾. The composition of these fractions largely shapes the large intestinal microbial composition as well as the fermentation products. The gastrointestinal microbiome modulates metabolic and immune responses and is of great significance for intestinal

Abbreviations: BA, biogenic amine; BCFA, branched-chain fatty acid; CP, crude protein; EXT, extruded diet; GE, gross energy; ME, metabolisable energy; MM, minced mice; MUET, mean urinary excretion time; OTU, operational taxonomic units; WM, whole mice.

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and systemic health^(4,11). It is able to generate various energy-containing metabolites from indigestible organic molecules such as SCFA^(12,13). Proteolytic bacteria break down undigested proteinaceous material which are used either in proteolytic fermentation or generation of microbial cell components⁽¹¹⁾. This also renders branched-chain fatty acids (BCFA) and as well as the potentially harmful NH_3 , various amines, phenols and indoles⁽¹⁴⁾. The presence of protein in the large intestine originates from undigested food, bacterial cells and endogenous losses⁽¹⁵⁾. When cheetahs (*Acinonyx jubatus*), which are also strict carnivores, were fed a whole prey diet, a higher acetic to propionic acid ratio (A/P) was observed compared with cheetahs fed supplemented beef⁽¹⁶⁾. This implied that the fermentation rate by intestinal microbiota was reduced as certain fractions such as hair, skin, cartilage and bone have a similar function as insoluble plant fibres^(16,17). The animal-derived plant fibre-like fractions have recently been termed 'animal fibre'⁽¹⁶⁾. The whole prey feeding also reduced BCFA, indole, phenol and serum indoxyl sulphate concentrations in the faeces of cheetahs⁽¹⁶⁾. To what extent this effect is caused by the chemical or the physical nature of whole prey is unclear.

That metabolic disorders can occur due to a lack of diet structure, for instance in ruminants, is well established⁽¹⁸⁾. But in carnivore species, the importance of food structure is largely unknown⁽¹⁹⁾. It was shown that the rate of gastric emptying in cats is affected by the physical nature of the food (wet *v* dry food)⁽²⁰⁾ and that adding 10% insoluble fibre to the diet delays gastric emptying and colonic filling time in dogs⁽²¹⁾. Also, the shape of the extruded kibble of commercial cat foods affects gastric emptying⁽²²⁾. When a cat eats a mouse, it is cut into relatively large pieces and swallowed without further size reduction as cats' cheek teeth have no grinding function⁽²³⁾. Ingested prey pieces may require further size reduction by digestive and physical actions of the stomach before they can pass the pylorus and be subjected to further digestion in the small intestine. Animal fibre including resistant proteins escape enzymatic digestion⁽¹⁶⁾. These fractions have different physico-chemical properties and, therefore, are also expected to differently impact the microbial populations and fermentation product profiles. Moreover, the physical structure of ingested food may change the grinding and digestive processes, including gastric emptying, digestion and fermentation.

We hypothesised that when cats are fed a more structure-rich diet, gastric emptying will be delayed, leading to an altered flow of substrate for the large intestinal microbiota. Therefore, the present study investigated the separated effect of food structure on digestive features, faecal microbiota and their fermentation products in cats by feeding minced mice (MM) and whole mice (WM), also comparing the results to the effect of the preceding extruded diet.

Experimental methods

Animals and experimental design

Experimental procedures were approved by the Central Animal Testing Committee, The Netherlands, and the Animal Welfare Body of Wageningen University (2017.W-0073.003). Fifteen

(seven males and eight females) neutered 1-year-old domestic shorthair cats with an initial body weight between 3.6 and 5.2 kg were utilised from the feline unit of the research facility Carus (Department of Animal Sciences, Wageningen University, Wageningen, The Netherlands). The body condition score ranged between 5 and 8 on a scale of 1 (anorexic) to 9 (obese)⁽²⁴⁾. The cats were group-housed but fed individually (08.00, 11.00, 14.00 and 17.00 h) to maintain stable body weight and were weighed twice weekly. During collection periods, cats were housed in metabolic cages (0.80 × 1.00 × 0.75 m) fitted with a litter tray, and each group had 2 × 1 h of socialisation and play per day in the hallway of the facility. The group rooms were temperature-controlled (20°C) and maintained on a 12:12 h light–dark cycle. Enrichment in group rooms included scratching posts, hammocks and toys. Cats had free access to water and were cared for daily by animal caretakers and the trial leader. The trial leader was aware of the group allocation during the conduct of the experiment, the outcome assessment and the data analysis.

The cats were offered one-quarter of a mouse at several time points before the actual start of the trial to test their acceptance. One cat did not accept the mouse and was, therefore, excluded from the study. Fifteen cats were assigned to two groups according to body weight and sex resulting in a mean body weight of 4.5 (SD 0.4) kg for the cats fed MM (*n* 8) and 4.4 (SD 0.7) kg for the cats fed WM (WM; *n* 7). All participating cats were first individually fed a commercial extruded dry cat food (EXT) for 16 d with faecal samples collected during the final 3 d for microbiota analysis and to create a baseline for faecal fermentative end-product concentrations (EXT preMM; EXT preWM). The study had a parallel design with one feeding period. After 10 d of feeding MM and WM, total faeces and urine were collected for 5 consecutive days (postMM; postWM). Afterwards, urine was collected on day 15 to determine baseline for ^{15}N values as on day 16, ^{15}N -glycine (50.0 mg/ml; 5.0 mg/kg BW)⁽²⁵⁾ was included in the diets to gain insight into gastric emptying with urine collected for 48 h post ^{15}N -glycine administration. Fresh faeces (within 15 min of defecation) for microbiota analyses were collected from day 16 to 19.

Diets

The extruded dry cat food was a commercial diet (Table 1). Specific pathogen-free mice, with an age range of 8 weeks to 12 months old, were obtained from Envigo (Envigo) and stored frozen until 30 min before feeding when they were thawed in a plastic bag in boiled water (water boiler) until they were defrosted. Mice were either minced in a blender or left whole receiving a midline incision to improve acceptability. The estimated metabolisable energy (ME) of mice was calculated using Atwater factors where $\text{ME (kJ/100 g DM)} = 16.7 \times \text{crude protein (CP)} + 35.6 \times \text{crude fat (EE)} + 16.7 \times \text{nitrogen-free extract (NFE)}$ ⁽²⁶⁾ with the latter determined as $100 - \text{moisture} - \text{CP} - \text{EE} - \text{crude fibre} - \text{crude ash}$ (all in % of DM). Food intake and leftovers were weighed every feeding time. The amount of food offered to individual cats was adjusted when the cat gained or lost more than 1% of body weight.

Table 1. Analysed chemical composition, amino acid composition and metabolisable energy content of the extruded dry cat food (EXT) and mice used in the experiment

Item	EXT*	Mice
Component (g/100 g DM)		
DM (g/100 g as is)	93.1	25.6
Crude protein	42.4	56.5
Acid hydrolysed fat	14.0	20.6
aNDF†	11.2	5.2
Crude ash	8.7	10.1
Crude fibre‡	2.2	0.6
Nitrogen-free extract	30.4	2.7
Amino acid composition (g/100 g AA)		
Lysine	5.4	6.4
Methionine	1.6	2.2
Cystine	1.4	2.4
Threonine	3.9	4.4
Tryptophan	1.0	1.0
Isoleucine	4.1	3.8
Arginine	7.0	6.8
Valine	5.1	4.9
Phenylalanine	4.4	4.1
Histidine	2.1	2.2
Leucine	7.3	7.7
Tyrosine	3.2	3.7
Alanine	6.1	6.2
Aspartic acid	10.8	10.6
Glutamic acid	15.7	14.1
Glycine	9.0	8.5
Proline	7.2	6.0
Serine	4.7	5.0
Metabolisable energy (kJ/100 g DM)		
Calculated‡	1751	1852
Analysed	n.a.§	1916

EXT, extruded dry cat food; aNDF, amylase-treated neutral detergent fibre; ME, metabolisable energy; NFE, nitrogen-free extract.

* Perfect fit™ adult 1+ rich in chicken (Mars Petcare Inc); ingredients: dried poultry protein (incl. 21 % chicken), wheat, greaves protein, soya protein, oils and fats (incl. 0.4 % sunflower oil), soya meal, rice (4 %), dried cereal protein, hydrolysed liver, minerals, cellulose, and dry algae.

† aNDF and crude fibre are techniques developed to quantify plant fibre, not animal fibre.

‡ The average of the values calculated by Atwater factors where ME (kJ/100 g DM) = 16.7 × % crude protein + 35.6 × % crude fat + 16.7 × % nitrogen-free extract. NFE was determined as 100 – % moisture – % crude protein – % crude fat – % crude fibre – % crude ash.

§ Not available.

Sample collection

The extruded dry cat food was subsampled, and sixteen defrosted mice were minced in a blender, freeze-dried (ScanVac CoolSafe™ freeze-dryer) and ground over a 1-mm mill (Retsch ZM 200). The cats were acclimated to modified plastic litter boxes consisting of two identical, close-fitted trays positioned to slightly sloping to one corner to ensure complete collection of faeces and urine. The top tray contained a series of small holes at the lowest point and was filled with about 200 g of polyethylene grains (diameter 2 to 4 mm). The bottom tray contained 5 ml of 2M HCl to acidify urine immediately after voiding. Specific gravity was determined by collecting a small amount of urine present in the top tray. The litter boxes were checked every 15 min, and urine was then collected and stored at –20°C. Afterwards, urine was freeze-dried for determining the gross energy (GE) and stable isotope analyses. For the latter, subsamples of approximately 1.0 mg were weighed on a

microbalance XP56 (Mettler-Toledo S.A.) into 8 × 5 mm tin capsules (Interchrom).

Fresh faecal pH was immediately determined after collection by sticking a pH probe directly in the core of the faeces at two different sites (within 15 min of defecation; pH meter WTW 340i, Geotech). The faecal samples were weighed and scored on a five-point visual scale where 1 is classified as ‘bullet like’ and 5 as ‘entire liquid stool’⁽²⁷⁾ and stored at –20°C. To subsample the faecal samples, they were placed overnight in a refrigerator (4°C). For NH₃, SCFA and biogenic amines (BA), subsamples of approximately 1.0 g were added to pre-weighed 15 ml of screw cap tubes (Sarstedt AG & Co. KG). One ml of 10 % TCA (for NH₃) or 1 ml of 0.1 M phosphoric acid (for SCFA) was added to the screw cap tubes. The contents of the tubes were vigorously mixed, weighed and stored at –20°C. For the determination of DM and the volatile organic compounds, approximately 1.0 g of subsample was added to a pre-weighed, 2-ml safe-lock tube (Eppendorf AG) and stored at –20°C (DM) or –80°C. Fresh faecal samples for microbiota analysis were immediately flash-frozen in liquid N₂ and stored at –80°C until DNA extraction.

Chemical analyses

Diets and faeces were analysed for DM and ash by drying to a constant weight at 103°C (EXT) or by lyophilisation (mice and faeces) and combustion at 550°C, respectively. Nitrogen was determined by the Kjeldahl method⁽²⁸⁾ with CP calculated as N × 6.25, and crude fat was determined by pre-hydrolysis according to the Soxhlet method⁽²⁹⁾. Crude fibre was analysed by acid-alkali digestion⁽³⁰⁾. A protease step (Megazyme; Subtilisin A from *Bacillus licheniformis*; 50 mg/ml) preceded the analyses of amylase-treated neutral detergent fibre (aNDF)⁽³¹⁾ because of samples having a high protein content. Both fibre analyses are developed to quantify plant fibre. Therefore, an underrepresentation of animal fibre is expected with these techniques⁽³²⁾. Analysis of amino acids of the diets (Table 1) was done by Feed & Food Quality (FFQ) with their in-house method based on EC 152/2009 (L54/23) for the oxidation and hydrolysis. The ultra-HPLC was according to the Waters ACCQ-tag method with pre-column derivatisation. Tryptophan was determined according to EC 152/2009 (L54/32). Dietary, faecal and urinary GE were determined using a Flash 2000 Elemental Analyzer (Thermo Fisher Scientific).

The δ¹⁵N isotope values were measured at the University of New Mexico Center for Stable Isotopes (Albuquerque, USA). The samples were combusted in a Costech 4010 elemental analyser (Costech) coupled to a Thermo Scientific Delta V mass spectrometer (Thermo Scientific). Reference material was atmospheric N₂ for N.

For the determination of NH₃ and SCFA, the subsamples were thawed, mixed and centrifuged at 14 000 rpm for 5 min at room temperature (Centrifuge Hereaus Megafuge 40R). Ammonia and SCFA concentrations were determined as described in Eertink *et al.*⁽³³⁾. BA were determined as described in Saarinen⁽³⁴⁾. In brief, sample material (1.0 g) was added in a 15-ml falcon tube and shaken on maximum speed for 15 min with 2 ml of extraction solution (2 % sulphosalicyl acid dehydrate in 0.1 M HCl) for the extruded diet (EXT) faecal samples and 5 ml for the



mice diet faecal samples. Afterwards, 0.5 ml of supernatant was transferred to 1.5 ml of Eppendorf cup together with 0.5 ml of internal standard solution (Heptylamine), mixed on a Vortex mixer, placed in an ice-bath for 15 min and centrifuged for 10 min (14 000 rpm; 18°C). The supernatant was pipetted for derivatisation with dansyl chloride. After derivatisation, 1.0 ml of supernatant was pipetted in vials. The vials were placed in the autosampler, and 30 µl was injected on the column (Agilent 5 TC-C18 250 × 4.6 mm) of the HPLC (Sykam S5200 analyser, Sykam GmbH). Detection took place by a fluorescent detector with an excitation at 250 nm and emission at 540 nm (RF-20A, SerCoLab). The faecal DM content was used to calculate SCFA, NH₃ and BA content in the original faeces. The volatile organic compounds phenol, x-cresol and indole were analysed as described in Vossen *et al.*⁽³⁵⁾. There were a few alterations made to this method, that is, 0.5 g of faecal sample was used, there was no internal standard added and the solid phase micro-extraction fibre was exposed to each sample for 40 min at 38.5°C. The compounds were identified by comparing the chromatograms with the National Institute of Standard and Technology Mass Spectral Library (version 2.0, 2005) and retention time matching with external standards (phenol, x-cresol and indole). The data were obtained by expressing the relative area of specific quantifications ions for phenol, x-cresol and indole (respectively *m/z* 94, 107 and 117). The detection limit was set at 1×10^4 . We report x-cresol as p-cresol.

Calculations

When urine is collected with modified cat litter boxes containing polyethylene grains, some inevitable losses of urine occurs. Therefore, we measured the difference in volume and weight between a known amount of urine added to the litter box and the amount that was recovered. This was repeated twelve times, and the following linear regression was derived to calculate the amount of voided urine:

$$y = 1 \cdot 1395x + 3 \cdot 8523$$

where *y* = urine (ml) voided and *x* = urine (ml) collected and $4.2 < x < 44.0$ ml.

The amino acids (AA) were calculated as a proportion of the sum of all AA (g/100 g AA) because of the large difference in DM% between EXT and mice.

GE (MJ/kg) was calculated after using the Flash 2000 Elemental Analyzer with the equation $0.3491 \times C$ (weight percentage, wt%) + $1.1783 \times H$ (wt%) + $0.1005 \times S$ (wt%) – $0.0151 \times N$ (wt%) – $0.1034 \times O$ (wt%) – $0.0211 \times \text{ash}$ (wt%)⁽³⁶⁾. The Flash 2000 Elemental Analyzer does not generate a value for O. Therefore, O + ash was calculated as $100 - H - N - S$. Then, an average was calculated when the outcome of this calculation was presumed as O and when it was presumed as ash.

Apparent total tract digestibility values (%) were calculated using the equation: $((\text{nutrient intake (g/d)} - \text{faecal nutrient output (g/d)}) / \text{nutrient intake (g/d)}) \times 100$. Dietary ME (kJ/kg) was calculated with data from individual cats utilising the equation $\text{ME} = ((\text{GE of food consumed (kJ/d)} - \text{GE of faeces$

collected (kJ/d)) - GE of urine collected (kJ/d)) / amount of food consumed (kg/d).

The MUET of $\delta^{15}\text{N}$ was calculated as an alteration on the calculation for the faecal mean retention time of Thielemans *et al.*⁽³⁷⁾:

$$\text{MUET (h)} = \sum t_i C_i \Delta t_i / \sum C_i \Delta t_i$$

where *t_i* is the average time between the collection time of sample *i* and that of sample *i*-1, *C_i* is the marker concentration in sample *i*, and Δt_i is the interval between *t_i* and *t_i*-1.

Histamine and pyrrolidine were not included in the calculation of total BA because several values were non-detectable when cats ate MM (histamine: *n* 2; pyrrolidine: *n* 3) and when cats ate WM (histamine: *n* 5; pyrrolidine *n* 1).

Molecular analysis

DNA extraction was performed with the Qiagen DNeasy®PowerLyzer® PowerSoil® Kit (ref 12855-100). The concentration of the DNA was measured using the Nanodrop ND-1000 spectrophotometer (Nanodrop Technologie). Ten µl genomic DNA extract was sent out to LGC genomics GmbH where the 16S rRNA gene V3-V4 hypervariable region was amplified as described in Van Landuyt *et al.*⁽³⁸⁾. The PCR mix included 1 µl of 10× diluted DNA extract, 15 pmol of both the forward primer 341F 5'-NNNNNNNNNTCCTACGGGNGG CWGCAG and reverse primer 785R 5'-NNNNNNNNNTGACT ACHVGGGTATCTAAKCC⁽³⁹⁾ in 20 µl volume of MyTaq buffer containing 1.5 units MyTaq DNA polymerase (Bioline), and 2 µl of BioStab II PCR Enhancer (Sigma-Aldrich). For each sample, the forward and reverse primers had the same unique 10-nt barcode sequence. PCR were carried out for 30 cycles using the following parameters: 2 min 96°C pre-denaturation; 96°C for 15 s, 50°C for 30 s and 70°C for 90 s.

DNA concentration of amplicons of interest were determined by gel electrophoresis. Next, about 20 ng of amplicon DNA of each sample was pooled for up to forty-eight samples carrying different barcodes. The amplicon pools were purified with one volume of AMPure XP beads (Agencourt) to remove primer dimer and other small mispriming products, followed by an additional purification on MinElute columns (Qiagen). Finally, about 100 ng of each purified amplicon pool DNA was used to construct Illumina libraries by means of adaptor ligation using the Ovation Rapid DR Multiplex System 1-96 (NuGEN). Illumina libraries were pooled, and size was selected by preparative gel electrophoresis. Sequencing was performed on an Illumina MiSeq using v3 Chemistry (Illumina).

Bioinformatics (16S rRNA gene profiling)

Read assembly and clean-up was largely derived from the MiSeq SOP described by the Schloss lab^(40,41). In brief, mothur (v.1.42.3) was used to assemble reads into contigs, perform alignment-based quality filtering (alignment to the mothur-reconstructed SILVA SEED alignment, v. 138.1), remove chimeras (vsearch v2.13.0), assign taxonomy using a naive Bayesian classifier⁽⁴²⁾ and SILVA NR v138.1 and cluster contigs into operational



taxonomic units (OTU) at 97% sequence similarity. All sequences that were classified as Eukaryota, Archaea, Chloroplasts or Mitochondria and those that could not be classified at all were removed. For each out, representative sequences were picked as the most abundant sequence within that OTU and singletons were removed prior to further data analyses.

Statistical analyses

Based on a power analysis, a sample size of n 8 cats per group was found to be sufficient (Settings: Power = 0.8; α = 0.05/2 (to correct for multiple testing); d = 1.75 (based on the study of Depauw *et al.*⁽¹⁶⁾); two-sample t test; conducted in R 4.0.3. using the pwr package). The power analysis was based on the significant difference in indole values after cheetahs had consumed two different diets in Depauw *et al.*⁽¹⁶⁾. The effect size d (i.e. difference between the means divided by the pooled standard deviation) was found to be 1.75. The outcome parameters can be grouped in apparent total tract digestibility, MUET, microbial composition and fermentation products, each with their unique statistical characteristics. All statistical analyses and graph visualisations were conducted in RStudio v3.6.3 and v.4.2.0 (faecal microbiota), using packages vegan v.2.6.2, microbiome v.1.18.0, phyloseq v.1.40.0 and ggplot2 v3.3.6. To compare the difference in food intake, faecal characteristics, apparent total tract digestibility and urine characteristics between WM and MM treatment, a linear regression was performed using lm function of the stats-package. To analyse the effect of both treatment (WM *v.* MM) and the difference between the baseline period (EXT preMM; EXT preWM) and the experimental period (postMM; postWM), a linear mixed model was used including both treatment and period and with cat as random factor using the lmer-function in the lmerTest-package⁽⁴³⁾.

Alpha diversity indices (Richness (observed OTU), Shannon, InvSimpson) were calculated on OTU after normalisation by scaling with ranked subsampling (SRS)⁽⁴⁴⁾ at 35 688 sequences per sample. Differences in α diversity measures were further assessed by Kruskal–Wallis and pairwise Wilcoxon tests with Benjamini–Hochberg standard false discovery rate (FDR-BH) correction for multiple testing and significance threshold at $P < 0.05$.

Beta diversity was assessed using Bray–Curtis dissimilarities and visualised using principal coordinate analysis plots. permutational multivariate analysis of variance (PERMANOVA) was used to identify significant differences between the four groups (EXT preMM, EXT preWM, postMM and postWM). All PERMANOVA analyses were run with VEGAN function adonis with 1000 permutations. Pairwise group comparisons were assessed with the function pairwise.adonis, and P-values were adjusted with the false discovery rate correction.

For taxonomic composition and differential abundance analyses, OTU assigned to phyla and family whose relative median abundance was $\leq 1\%$ were removed from further analyses. Differences in taxonomic profiles at phylum and family level between the four groups were analysed by Kruskal–Wallis and pairwise Wilcoxon tests with FDR-BH correction and significance threshold at $P_{\text{adj}} < 0.05$.

Linear discriminant analysis effect size (LEfSe) was used to estimate which microbiome attributes differ significantly between cats fed MM (postMM) *v.* WM (postWM). The Galaxy implementation of LEfSe with default options was used, including a threshold of 2.0 for the logarithmic linear discriminant analysis score for discriminate features. Differential abundance of OTU between EXT preMM and postMM, EXT preWM and WM, and postMM and postWM was determined with DESeq2 v.1.36.0. Original count data were used after filtering rows with fewer than five counts over the entire row and using the parametric Wald test in DESeq2 with α = 0.01.

Results

The average body weight loss of cats fed WM and MM was 1% throughout the study. One cat from the MM group and one cat from the WM group did not defecate during the first collection period (EXT preMM; EXT preWM). One cat from the MM group was less active for 1 day during diet adaptation and seemed generally unwell. This cat also had outlying results for NH_3 and agmatine and, therefore, all results from the fermentation end products were omitted from the analysis. No urine was collected from one cat who vomited after ^{15}N -glycine was added to the diet.

Diet, food intake, nutrient digestibility, faecal characteristics and mean urinary excretion time

The chemical composition of EXT and the mice is presented in Table 1. The ME content of mice was lower when calculated using the Atwater factors than analysed (Table 1). Mincing the mice had no effect on food intake, faecal output, faecal score, faecal pH, nor apparent macronutrient digestibility ($P > 0.10$) (Table 2). Faecal DM percentage was higher ($P = 0.01$) when feeding MM compared with WM (Table 3). Cats fed MM had a mean (SD) MUET of 17.8 (4.3) h, which was not different ($P = 0.33$) to those fed the WM 14.5 (7.2) h.

Mean (SD) DM intake of mice (WM + MM) was 28.1 (2.8) g/d which was lower ($P < 0.001$) than $\text{EXT}_{(\text{WM} + \text{MM})}$ of 45.5 (4.4) g/d and resulted in a lower ($P = 0.001$) mean (SD) faecal score of 1.5 (0.3) *v.* 2.2 (0.4), higher ($P < 0.001$) pH (6.96 (0.17) *v.* 5.92 (0.15)) and higher faecal DM % ($P < 0.001$) (Table 3).

Faecal microbial populations

A total of 1 611 224 reads from twenty-eight faecal samples (median (IQR): 54 157 (20 991)) passed quality filtering and were assigned into 1537 OTU. After normalisation, 1215 OTU were retained with the number of OTU varying between 191 and 385 per sample (median: 278).

Alpha diversity indices (Richness, Shannon diversity, InvSimpson) are shown in Fig. 1. There was a decrease in richness (Wilcoxon, EXT preMM *v.* postMM, $P_{\text{adj}} = 0.002$; EXT preWM *v.* postWM, $P_{\text{adj}} = 0.002$) when cats were fed mice, but an increased diversity based on InvSimpson (Wilcoxon, EXT preMM *v.* postMM, $P_{\text{adj}} = 0.002$; EXT preWM *v.* postWM, $P_{\text{adj}} = 0.02$) and Shannon diversity indices, though the latter was not significant (Wilcoxon, EXT preMM *v.* postMM,

Table 2. Food intake, faecal characteristics, apparent total tract macronutrient digestibility and urine characteristics of domestic cats fed minced mice (MM) or whole mice (WM)

Item	postMM (n 8)		postWM (n 7)		P*
	Mean	SD	Mean	SD	
Food intake, g DM/d	28	3	28	3	0.84
Faecal characteristics					
Output, g as-is/d	14	2	12	3	0.12
Output, g DM/d	7	1	7	2	0.99
Score	1.5	0.3	1.5	0.4	0.58
pH	6.94	0.20	7.00	0.15	0.75
Apparent total tract digestibility					
DM, %	74	3	75	6	0.92
Organic matter, %	82	3	83	4	0.51
Crude protein, %	83	3	85	3	0.38
Crude fat, %	92	1	93	2	0.33
Urine					
Volume, ml/d	43	11	43	5	0.91
Specific gravity	1.061	0.003	1.061	0.003	0.71

postMM, after eating minced mice; postWM, after eating whole mice.

* P-value indicating the effect of treatment in a linear regression model including minced mice *v* whole mice as fixed factor.

$P_{\text{adj}} = 0.05$; EXT preWM *v.* postWM, $P_{\text{adj}} = 0.15$). Beta diversity, as measured by Bray–Curtis dissimilarities, showed clustering between cats fed EXT and cats fed mice (ADONIS, $F = 24.25$, $P_{\text{adj}} < 0.001$) as well as a difference between cats fed MM *v* WM (ADONIS, $F = 5.30$, $P_{\text{adj}} = 0.002$) (Fig. 2).

At phylum level, the OTU were assigned to eleven phyla of which only five had a median relative abundance $> 1\%$ (Fig. 3(a)). The low abundant phyla were removed from further analyses. Firmicutes was the most abundant bacterial phylum in cat faeces when eating MM (median (IQR): 67.2 % (0.05)) or WM (55.4 % (0.15)), followed by Bacteroidota (postMM: 13.2 % (0.05); postWM: 16.4 % (0.10)), Fusobacteriota (postMM: 14.2 % (0.06); postWM: 11.0 % (0.06)), Actinobacteriota (postMM: 4.6 % (0.02); postWM: 8.0 % (0.02)) and Proteobacteria (postMM: 1.0 % (0.01); postWM: 1.5 % (0.01)), with a significant difference in relative abundance between postMM and postWM groups for Firmicutes (Wilcoxon, $P_{\text{adj}} < 0.001$), Bacteroidota (Wilcoxon, $P_{\text{adj}} = 0.01$) and Actinobacteriota (Wilcoxon, $P_{\text{adj}} = 0.001$). These differences were also seen with LEfSe analysis (online Supplementary Fig. 1).

When the food was changed from an extruded food to a mice diet, there was a significant decrease in relative abundance of Bacteroidota (Wilcoxon, $P_{\text{adj}} < 0.001$) and Proteobacteria (Wilcoxon, $P_{\text{adj}} < 0.001$), and a significant increase of Firmicutes (Wilcoxon, EXT preMM *v.* postMM, $P_{\text{adj}} = 0.005$; EXT preWM *v* postWM, $P_{\text{adj}} < 0.001$) and Fusobacteriota (Wilcoxon, EXT preMM *v.* postMM, $P_{\text{adj}} = 0.006$; EXT preWM *v.* postMM, $P_{\text{adj}} = 0.02$). The relative abundance of Actinobacteriota was significantly increased in cats changing from extruded to WM diet (Wilcoxon, $P_{\text{adj}} = 0.001$).

At family level, the OTU were assigned to sixty-seven families of which only seven had a median relative abundance $> 1\%$ (Fig. 3(b)). The predominant families in cats fed mice were *Fusobacteriaceae* (postMM: 14.2 % (0.06); postWM: 11.0 % (0.06)), *Peptostreptococcaceae* (postMM: 8.2 % (0.04); postWM: 10.3 % (0.09)) and *Lachnospiraceae* of which the latter was

significantly higher in relative abundance when cats were fed MM compared with WM (41.7 % (0.06) *v.* 35.7 % (0.16); $X^2 = 29$, $P < 0.001$). For the family *Succinivibrionaceae*, a small difference between the group fed MM (0.16 % (0.002)) *v.* WM (0.55 % (0.003); $X^2 = 4.10$, $P = 0.04$) was observed.

When switching from the EXT to the mice diets, there was a 41.2–51.5 % decrease ($P_{\text{adj}} < 0.001$) in relative abundance of *Prevotellaceae* (phylum: Bacteroidota) and a 5.4–5.1 % decrease ($P_{\text{adj}} < 0.001$) of *Succinivibrionaceae* (phylum: Proteobacteria). An increase of 10.7–13.9 % in relative abundance of *Fusobacteriaceae* was seen when switching to the mice diets (postMM, $P_{\text{adj}} = 0.006$; postWM, $P_{\text{adj}} = 0.02$) (Fig. 3(b)). For the most dominant family of the phylum Actinobacteriota, the *Coriobacteriaceae*, an increase was observed when cats were fed WM (EXT preWM: 1.9 % *v* postWM: 6.7 %) (Fig. 3(b)).

As determined by DESeq2 analysis, there were forty-five differentially abundant taxa (OTU) in faeces from cats fed MM compared with cats fed WM. The major group of taxa were classified as *Lachnospiraceae*. One OTU assigned to the genus *Clostridium* sensu stricto was 4.88 log₂-fold lower when cats were fed WM (Fig. 4).

For the cats changing from EXT to MM, 160 OTU showed a significantly different abundance in response to the dietary change; of these, fifty-three species increased and 107 decreased in abundance after feeding MM (Fig. 5(a)). For the cats changing from EXT to WM, 138 OTU showed a significantly different abundance with forty-eight species increasing and ninety OTU decreasing in abundance after feeding WM (Fig. 5(b)). The outcome of the dietary change is comparable between those fed MM *v* WM. Of species known to be involved in the production of SCFA, *Prevotella* group 9 showed a significant decrease (13.01–13.16 log₂-fold change) when changing from EXT to a mice diet. One OTU assigned to *Fusobacterium*, known to produce butyric acid and possibly phenol through protein fermentation^(45,46), increased significantly (5.10–5.28 log₂ fold change) when feeding mice. Changing from EXT to a mice diet generated an increase in abundance (3.41–6.54 log₂-fold change) of two OTU when fed MM and one OTU when fed WM assigned at genus level to *Clostridium* sensu stricto 1, another possible phenol producer⁽⁴⁵⁾.

Faecal fermentation concentrations

The ratio of acetic to propionic acid ($P = 0.09$) tended to be higher when mincing the mice (Table 3). Spermidine ($P = 0.02$) and spermine ($P = 0.006$) concentrations were greater in cats fed MM compared with WM. p-Cresol decreased when mincing the mice, but only numerically ($P = 0.18$) (Table 3). The concentrations of other volatile fatty acids, NH₃, BA, phenol and indole were also not different between MM and WM (Table 3).

The SCFA and BCFA concentrations, except for isovaleric acid, were significantly lower when cats were fed the mice compared with the extruded food (Table 3). Ratios of acetic to propionic acid were lower ($P < 0.001$) when cats were fed EXT compared with the mice diet (Table 3). The NH₃ ($P = 0.04$) and most BA concentrations were lower on the mice diets, except for a higher agmatine ($P < 0.001$) and tyramine concentration ($P < 0.001$) (Table 3). Phenol was not detectable when fed

Table 3. Faecal DM, ammonia, SCFA, and biogenic amine concentrations ($\mu\text{mol/g}$ of DM, unless defined differently) and volatile organic compounds (expressed as % AUC) of cats fed an extruded dry cat food (EXT; n 13) and minced mice (MM; n 7) or whole mice (WM; n 7)

Item	Minced mice				Whole mice				<i>P</i>		
	EXT preMM		postMM		EXT preWM		postWM		EXT v Mice	MM v WM*	Interaction†
	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
DM, %	38.7	1.6	52.4	3.6	39.1	2.7	59.6	3.9	< 0.001	0.81	0.01
Ammonia	118	25	96	17	111	23	87	7	0.04	0.50	0.91
Straight-chain fatty acids											
Acetic acid (A)	190.6	20.9	85.3	5.7	186.0	41.7	72.3	11.9	< 0.001	0.73	0.64
Propionic acid (P)	94.5	11.8	18.1	2.2	88.0	18.4	17.2	2.7	< 0.001	0.28	0.50
Butyric acid (B)	37.1	12.0	18.4	1.8	37.1	8.9	16.5	2.8	< 0.001	0.88	0.66
Total SCFA	321.6	36.0	121.7	7.5	311.1	61.0	106.0	15.6	< 0.001	0.60	0.85
A:P	2.0	0.1	4.8	0.6	2.1	0.1	4.2	0.7	< 0.001	0.72	0.09
A:B	5.6	1.5	4.7	0.6	5.2	1.4	4.4	0.6	0.19	0.55	0.89
BCFA											
Isobutyric acid	4.8	1.4	3.4	0.3	4.5	0.7	3.2	0.5	0.01	0.49	0.86
Valeric acid	18.1	4.7	4.5	1.7	16.7	1.3	4.7	2.6	< 0.001	0.38	0.48
Isovaleric acid	7.2	2.3	6.2	0.5	6.5	1.2	5.9	0.9	0.44	0.41	0.72
Total BCFA	30.1	8.4	14.1	2.3	27.7	4.3	13.8	2.4	< 0.001	0.40	0.59
Biogenic amines (BA)											
Agmatine (Ag)	2.50	0.26	6.69	1.13	2.33	0.48	6.24	0.78	< 0.001	0.61	0.62
Putrescine (Pu)	2.54	0.58	1.42	0.54	1.98	0.59	0.48	0.32	< 0.001	0.06	0.36
Pu:Ag	1.02	0.24	0.22	0.10	0.94	0.56	0.08	0.06	< 0.001	0.63	0.78
Cadaverine	13.84	2.81	0.60	0.22	12.41	1.30	0.19	0.06	< 0.001	0.12	0.41
Histamine	1.17	0.50	0.12	0.07	0.61	0.46	0.12	0.02	0.14	0.02	0.18
Tyramine	0.11	0.12	0.70	0.18	0.10	0.11	0.58	0.18	< 0.001	0.84	0.33
Spermidine	0.80	0.20	0.79	0.22	0.85	0.21	0.52	0.27	0.003	0.62	0.02
Spermine	0.02	0.002	0.02	0.01	0.02	0.002	0.01	0.004	< 0.001	0.74	0.006
Tryptamine	5.92	0.73	0.27	0.08	5.31	0.81	0.13	0.02	< 0.001	0.05	0.26
Pyrrolidine	0.19	0.05	0.08	0.05	0.17	0.03	0.06	0.01	< 0.001	0.33	0.92
Total BA‡	25.73	3.95	10.49	1.16	22.99	1.19	8.14	0.87	< 0.001	0.04	0.82
Volatile organic compounds											
Phenol	n.d.§	—	49.0	14.7	n.d.§	—	35.7	22.9	—	—	—
Para-cresol	112.2	80.9	13.7	3.9	71.6	42.2	23.2	8.8	0.08	0.13	0.18
Indole	114.5	72.0	107.6	54.2	98.9	42.1	110.9	35.7	0.69	0.61	0.65

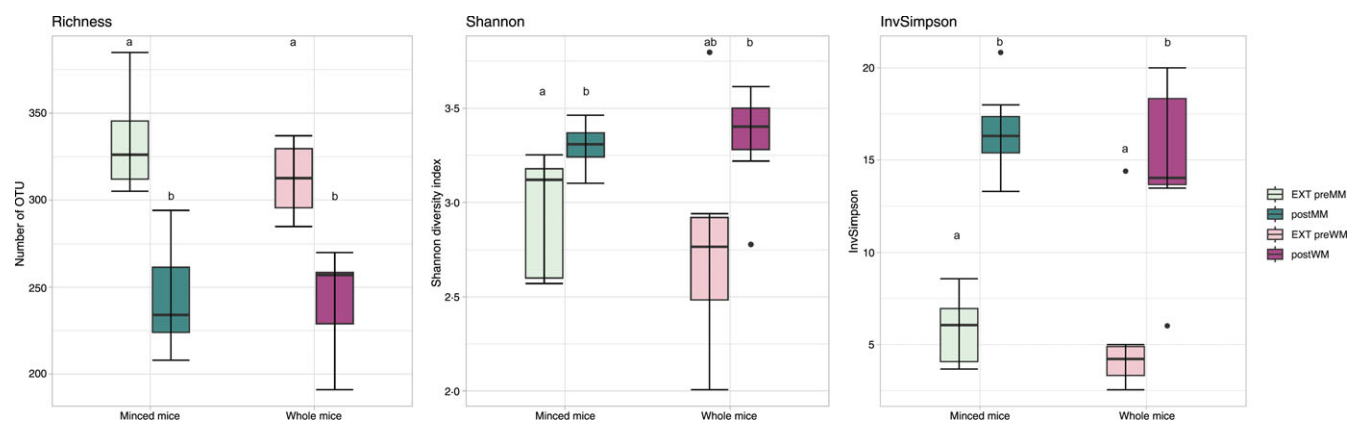
EXT preMM, extruded dry cat food before eating minced mice; postMM, after eating minced mice; EXT preWM, extruded dry cat food before eating whole mice; postWM, after eating whole mice; BCFA, branched-chain fatty acid; BA, biogenic amines.

* Comparison between the group minced mice (EXT preMM and postMM) and the group whole mice (EXT preWM and postWM).

† Interaction of group (minced mice v whole mice) and experimental time.

‡ Total biogenic amines = agmatine + putrescine + cadaverine + tyramine + spermidine + spermine + tryptamine.

§ Not detectable.

**Fig. 1.** Alpha diversity measures. Boxplot of microbial richness (number of observed OTU), Shannon diversity and InvSimpson from faecal samples of cats fed extruded food before changing to minced mice (EXT preMM; n 7) or whole mice (EXT preWM; n 6) and after feeding minced mice (postMM; n 8) or whole mice (postWM; n 7). The boxes denote interquartile ranges (IQR) with the median as a black line and whiskers extending up to the most extreme points within 1.5-fold IQR. Differences between groups with different letters are statistically significant as assessed by pairwise Wilcoxon tests with Benjamini–Hochberg false discovery rate correction for multiple testing and significance threshold at $P < 0.05$. OTU, operational taxonomic unit; EXT preMM, extruded dry cat food before eating minced mice; postMM, after eating minced mice; EXT preWM, extruded dry cat food before eating whole mice; postWM, after eating whole mice.

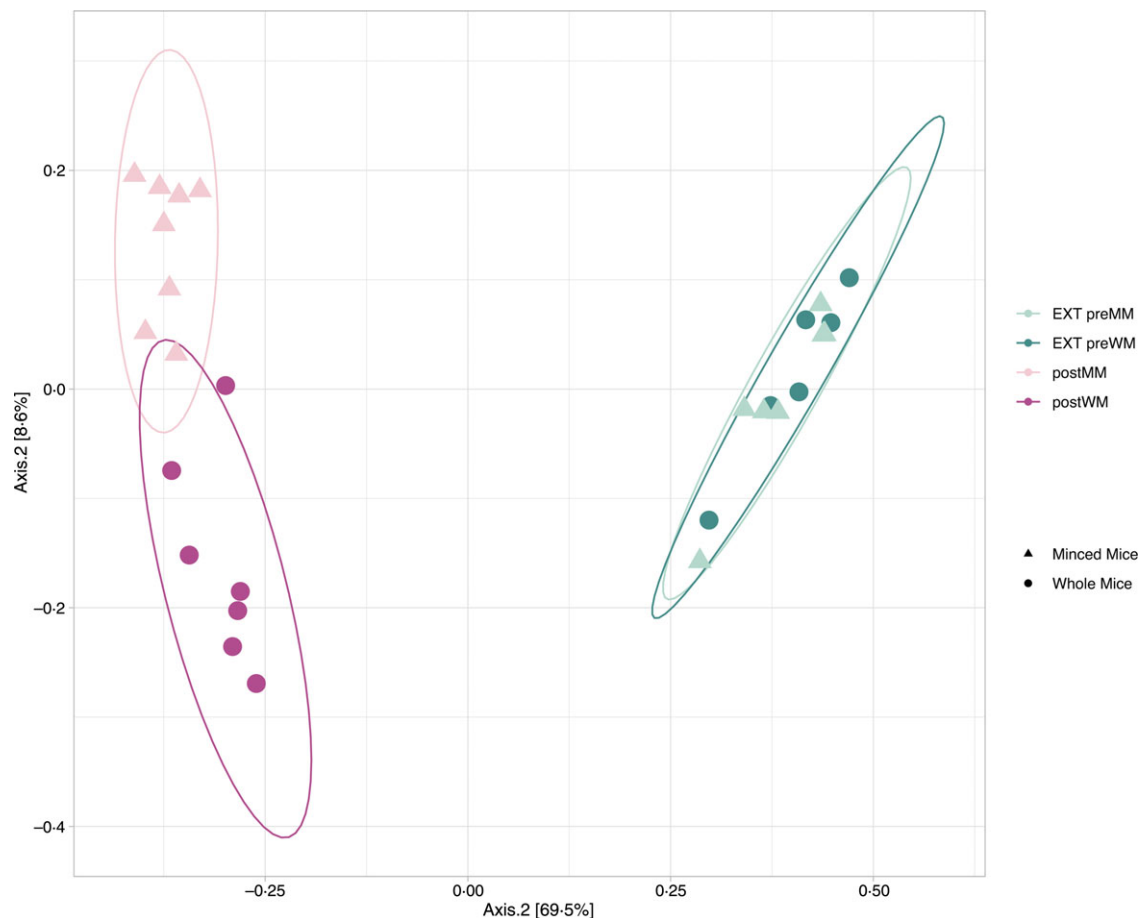


Fig. 2. Beta diversity. Principal coordinate analysis (PCoA) plot based on Bray–Curtis dissimilarities of microbial community structure in faecal samples of eight cats in the minced mice group (EXT preMM; postMM) and seven cats in the whole mice group (EXT preWM; postWM). Beta-diversity showed clustering between cats fed extruded diet and cats fed mice (ADONIS, $F = 24.25$, $P_{\text{adj}} < 0.001$) as well as a difference between cats fed minced mice v whole mice (ADONIS, $F = 5.30$, $P_{\text{adj}} = 0.002$). EXT preMM, extruded dry cat food before eating minced mice; postMM, after eating minced mice; EXT preWM, extruded dry cat food before eating whole mice; postWM, after eating whole mice.

EXT, whereas p-cresol was greater ($P = 0.08$) when cats were fed EXT. Indole concentrations were not different compared with the mice diets (Table 3).

Discussion

Feeding whole prey to felids has shown to benefit their gastrointestinal health^(16,47). Whether this effect was caused by the chemical or physical nature of whole prey is unknown. We observed comparable microbial profiles for MM and WM and mainly numerical differences in the fermentation end products, which suggests a limited protective effect of food structure against proteolytic fermentation. A mouse diet, however, appears to be protective compared with an EXT because of the observed large shift in faecal proteolytic fermentation end products and microbiota. Whether such fermentation profile is associated with an improved gut health and promotes longevity requires further study.

Animal prey contains little plant fibre, but it does contain animal fibre, that is, indigestible fractions from tissues such as hair, bone, cartilage and skin, that have comparable effects as plant fibre⁽¹⁶⁾, including impacting faeces consistency^(17,48). The

animal fibre in the mice was likely responsible for firm faeces observed in the present study. Apart from collagen, most animal fibres behave as insoluble fibres, hence mitigating fermentation⁽¹⁶⁾, similar to feeding straw to a ruminant. Mincing the mice seemed to reduce that effect, as reflected in the higher concentration of fermentation end products, albeit only significant for two of them. Smaller food particles provide a higher interaction surface between gut microbiota and their substrate but may also cause a shift in digestion site. Unfortunately, it was not possible to study ileal digestibility to provide that insight. In humans, it has been shown that less food structure increases digestibility, and therefore, less nutrients reach the hindgut to be fermented to produce SCFA⁽¹⁰⁾, whilst mincing the mice renders higher SCFA concentrations. The higher SCFA and BCFA concentrations when mincing the mice were accompanied by a higher NH_3 and higher BA concentrations, apart from histamine. Although the polyamines putrescine, spermidine and spermine are necessary for optimal growth and function of cells⁽⁴⁹⁾, high concentrations of BA are commonly considered as health-threatening⁽⁵⁰⁾. These higher concentrations can be explained by more proteolytic fermentation in the colon and suggests a health benefit of physical

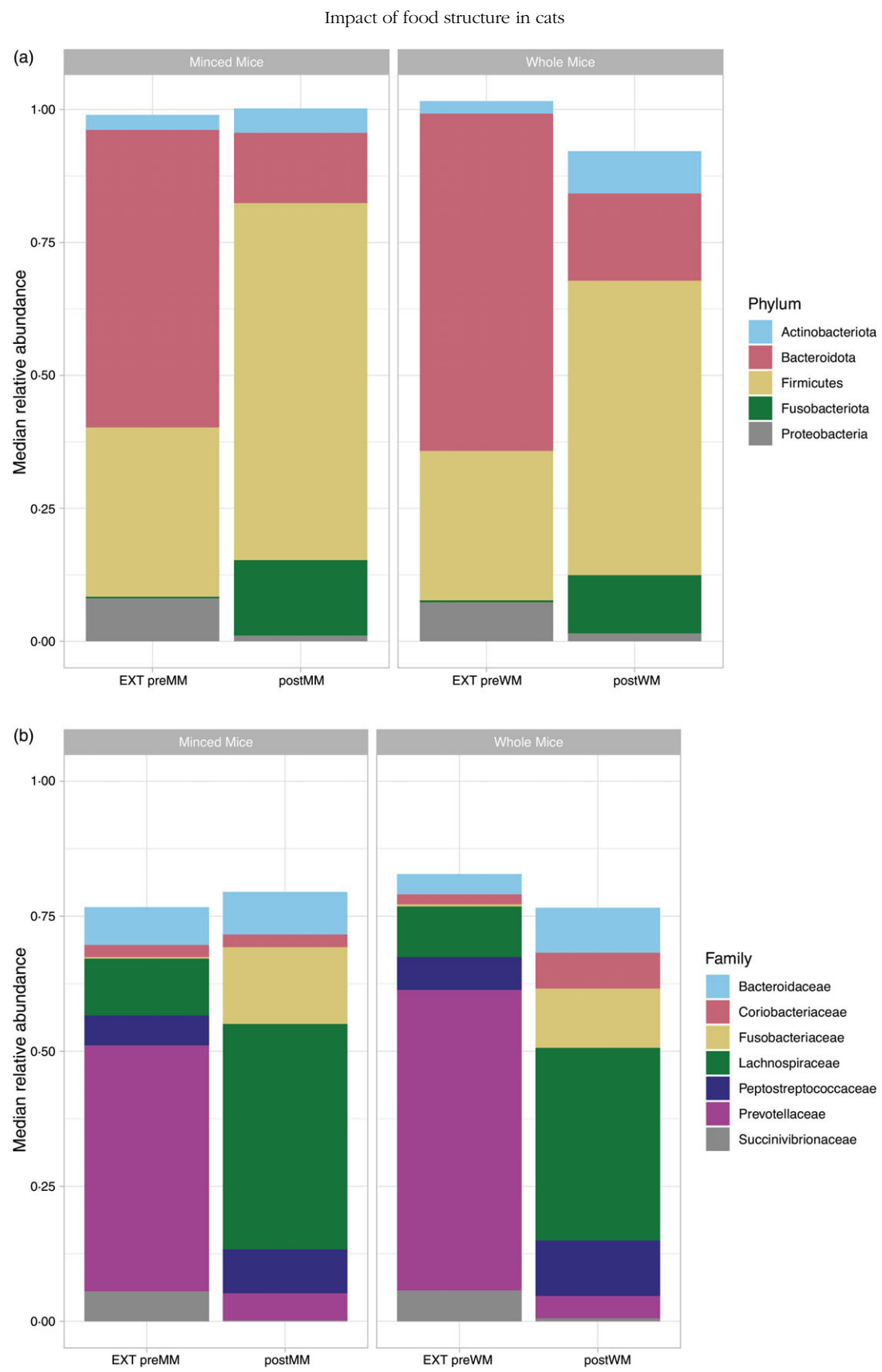


Fig. 3. Relative median abundance of phylotypes at phylum (a) and family (b) level in faecal samples collected from cats fed extruded food (EXT preMM; *n* 7) and after changing to minced mice (postMM; *n* 8) and from cats fed extruded food (EXT preWM; *n* 6) and after changing to whole mice (postWM; *n* 7). EXT preMM, extruded dry cat food before eating minced mice; postMM, after eating minced mice; EXT preWM, extruded dry cat food before eating whole mice; postWM, after eating whole mice.

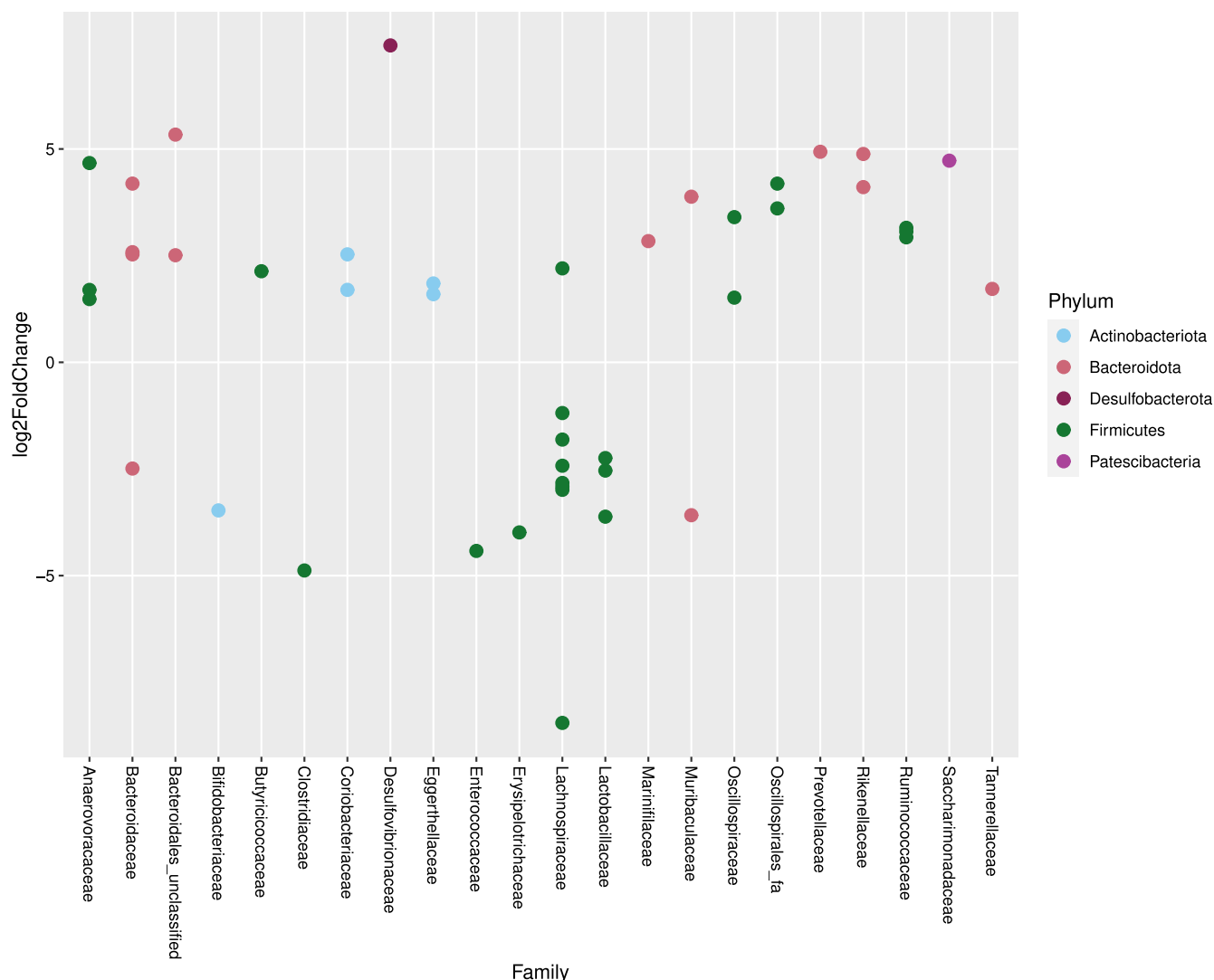


Fig. 4. Log2 fold change in abundance of taxa associated with cats fed minced mice or whole mice. A negative fold change implies a decrease in abundance when cats were changed to whole mice. Original count data were used after filtering rows with fewer than five counts over the entire row and using the parametric Wald test in DESeq2 with alpha = 0.01.

structure in the diet, at least for carnivores such as cats but possibly as a feature for more species, including humans. Mincing is the first step of processing and may exert a higher ratio of fermentable protein to insoluble fibre in the intestine. Despite the changes seen in the fermentation profile, the microbial profile was comparable between the MM and the WM diet.

Whole prey feeding was expected to take longer to be digested and metabolised compared with minced prey, but this was not supported by the apparent total tract digestibility data (Table 2) and the MUET calculated from the urinary marker excretion between MM and WM. Due to difficulties to adapt cats to the environment of climate respiration chambers⁽⁵¹⁾, the technique with stable isotopes and breath analysis⁽⁵²⁾ would have disturbed the results of the gastric emptying. Therefore, we alternatively developed the MUET technique which may not have been sensitive enough to detect a difference between the two mice diets. The higher variation of MUET in the WM may, however, point to a more heterogeneous gastric emptying which warrants for further investigation.

A remarkable finding in the present study was the large shift in fermentative end products and faecal microbiota when cats went from the extruded dry cat food to the mice diets. Straight-chain fatty acid, BCFA and total BA concentrations all decreased by feeding MM and WM. Although this shift was confounded by time, these differences can almost exclusively be attributed to the diets as the cats were maintained in a controlled environment and were their own control. The microbial profile and the fermentation end products in the cats on the EXT were similar for both groups (Fig. 2, Table 3). The values obtained for the EXT are highly comparable to those of other studies where healthy cats are also fed an extruded dry cat food^(5,53–55) and reflect normal colon values of cats fed extruded diets (online Supplementary Table 1).

The differences in SCFA abundance and profile between EXT and the mice diets can be considered functionally important. The high production of SCFA and isobutyric acid when EXT was fed relates to the abundance of *Prevotella* as the dominant genus, a known fermenter of complex carbohydrates⁽⁵⁶⁾. In humans,

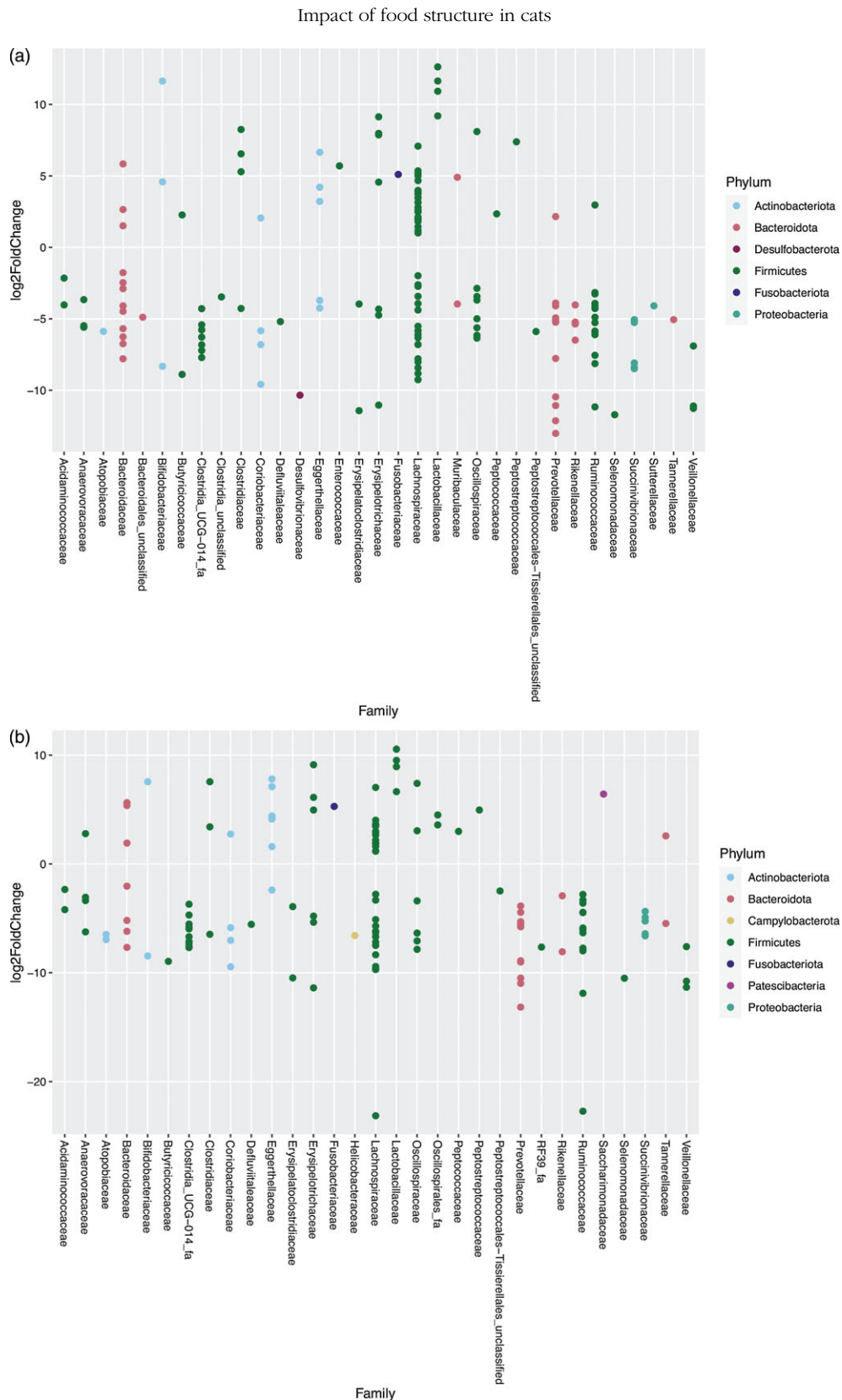


Fig. 5. Log2 fold change in abundance of taxa associated with changing the diet from an extruded dry cat food to minced mice (a) and an extruded diet to whole mice (b). A negative fold change implies a decrease in abundance when cats were changed to a mice diet. Original count data were used after filtering rows with fewer than five counts over the entire row and using the parametric Wald test in DESeq2 with $\alpha = 0.01$.

propionic acid production resulting from carbohydrate and protein fermentation is comparable, but total SCFA, acetic acid and butyric acid production is lower from protein fermentation⁽⁵⁷⁾. The butyryl-CoA:acetate CoA:transferase pathway dominates in omnivores and herbivores for butyric acid production. In carnivores, the butyrate kinase pathway dominates, which produces butyrate from protein⁽⁵⁸⁾. Therefore, the butyric acid production from protein fermentation is possibly not lower than that from carbohydrate fermentation. Because it was shown that proteolytic fermentation renders lower concentrations of SCFA than carbohydrate fermentation⁽⁵⁷⁾, with the possible exception of butyrate, this partly explains the greater concentration of SCFA in cats fed EXT compared with mice. Certain species of *Prevotella* have shown to produce succinate in mouse models⁽⁵⁹⁾ which could then be metabolised to propionic acid. The concentration of propionic acid was also greater when domestic cats were fed a kibble diet compared with when they were fed a raw diet in a study of Butowski *et al.*⁽⁵³⁾. Despite lower NH₃, the faecal pH increased when feeding mice, suggesting that the reduction in SCFA was the main cause of the pH increase.

The median relative abundance of *Fusobacterium spp.*, which has been implicated in proteolytic fermentation *in vitro*⁽⁶⁰⁾, increased when cats were fed the mice diets. It has been associated in humans with liver cirrhosis, appendicitis⁽⁶¹⁾, various oral cavity infections⁽⁶²⁾ and colorectal cancer, where putrescine and histidine pathways were most common⁽⁶³⁾. However, a study of Hooda *et al.*⁽⁶⁴⁾ showed that kittens stayed healthy, also the kittens with a higher abundance of *Fusobacterium* after being fed a high-protein, low-carbohydrate diet compared with when kittens were fed a moderate-protein, moderate-carbohydrate diet. The phylum Fusobacteriota has also been observed in healthy dogs⁽⁶⁵⁾. The higher faecal concentrations of putrescine and histamine when fed EXT can indicate that a higher abundance of *Fusobacterium* does not necessarily mean more proteolytic fermentation and a potential health risk. *Fusobacterium spp.* is one of the bacterial genera that produce butyric acid through catabolism of glutamate and lysine⁽⁶⁶⁾. Butyric acid has many beneficial functions⁽⁶⁷⁾, but these may be reduced when it is produced through protein fermentation: butyric acid uptake in the colonocytes is decreased because NH₃ generates inflammation and, therefore, butyric acid transporter expression is reduced⁽⁶⁸⁾. Colonocyte proliferation can also be decreased by p-cresol due to inhibition of cell respiration⁽⁶⁹⁾. The higher NH₃ and p-cresol concentrations in faeces from cats fed EXT implies that there was more proteolytic fermentation compared with the mice diets.

The higher total BA concentrations, except for agmatine and tyramine, confirm the higher proteolytic fermentation on the extruded diet. Arginine decarboxylase can convert arginine (highest in the mice diets) to agmatine with agmatinase able to further convert agmatine into putrescine. The putrescine: agmatine ratio can be an indicator for agmatinase and was, therefore, calculated. It clearly shows that the conversion by this enzyme was greater when cats were fed EXT than when they were fed the mice diets. This ratio was also numerically greater when cats were fed MM compared with cats fed WM. Because lipopolysaccharide induces a dose-dependent stimulation of

agmatinase whilst it decreases arginine decarboxylase⁽⁷⁰⁾, this finding could indicate that a finer structure and processing leads to increased inflammatory compounds.

Tyrosine is the precursor of p-cresol and tyramine. This amino acid was higher in the mice diets than in EXT but not of a magnitude that can explain the large increase in tyramine and especially not the drop in p-cresol. Certain strains that belong to *Coriobacteriaceae* can produce phenol and p-cresol, and certain strains of *Fusobacteriaceae* and *Clostridium sensu stricto 1* can produce phenol⁽⁴⁵⁾. The cats fed EXT had a greater median relative abundance of *Coriobacteriaceae* compared with cats fed WM, which supports the theory of more proteolytic fermentation. In contrast, there was a greater median relative abundance of *Fusobacteriaceae* in the mice diets and *Clostridium sensu stricto 1* in the MM diet, which is in line with the observed phenol concentrations.

Cadaverine is used by bacteria to protect themselves against acidic pH^(71,72) and is formed by direct decarboxylation of L-lysine. Despite the higher lysine concentration in the mice diets, the faecal concentration of cadaverine was greater when cats were fed EXT. In addition, BCFA, which were significantly higher when cats were fed EXT, are produced exclusively through the fermentation of branched-chain amino acids and therefore reliable markers of proteolytic fermentation⁽⁷³⁾. In humans, BCFA, NH₃, indoles and phenols have been associated with increased colon cancer⁽⁷⁴⁾ and ulcerative colitis⁽⁷⁵⁾. The mice clearly had a protective effect against the overall production of BA, suggesting a large effect of animal fibre as a chemical component, but the difference between MM and WM was mainly numerical, which suggest a limited protective effect of food structure against proteolytic fermentation.

The standardised amino acid digestibility of mice using the precision-fed cecectomised rooster assay⁽⁷⁶⁾ was, for most amino acids, higher than the apparent ileal amino acid digestibility of dogs fed an EXT diet^(77–79) (online Supplementary Table 2). The apparent ileal amino acid digestibility is strongly correlated with the apparent ileal N digestibility⁽⁸⁰⁾. Therefore, we may assume that part of the CP content of mice is more digestible than the CP content of EXT. Another part of the CP content of the mice diets is the poorly digestible animal fibre protein. This protein may act as poorly fermentable insoluble fibre, hence reducing fermentation, which is not present in the EXT diet that has a lower CP content than mice. In addition, the non-digested fractions of EXT may be more fermentable than that of mice.

As mentioned above, most animal fibre sources in prey, such as hair, skin and bone, seem to reduce fermentation, similar to insoluble plant fibres such as cellulose⁽⁸¹⁾. In addition, Depauw *et al.*⁽¹⁶⁾ showed in cheetahs that feeding whole rabbit resulted in higher A/P compared with when feeding supplemented beef, which is associated with slower fermentation rates due to dietary insoluble fibre⁽¹⁷⁾. The reduction in faecal concentrations of NH₃, SCFA and BCFA (except isovaleric acid) and the higher A/P when switching from an EXT to a mice diet agrees with that mechanism. This means that the fermentation rate by intestinal microbiota was slower because most animal fibres act as insoluble fibres^(16,17) or because of a lack of fermentable carbohydrates as an energy source for the microbiota. The





lower rate of protein fermentation seen in the study of Depauw *et al.*⁽¹⁶⁾ was possibly due to the physical barrier between bacteria and substrate.

Besides the differences in digestibility, there is also the difference in macronutrient composition of the EXT diet compared with the mice diets. Consequently, the fermentable substrates available for the gastrointestinal microbiota for fermentation is altered^(16,81). This will impact microbiota population as was also seen in a study of Kerr *et al.*⁽⁸²⁾. We acknowledge that the gut microbiota might not have been stable at the time of sampling, but it was already possible to demonstrate remarkable differences. Richness is typically perceived as a positive trait, and the decrease in richness when cats were fed mice could suggest that this diet is less optimal. However, carnivores have a lower microbial diversity than herbivores and omnivores, and the diversity also depends on the variety of the diet⁽⁸³⁾. Because the cats were only fed mice for a period of time, without added plant fibres, this can explain the decrease in richness. The difference in microbiota between the EXT and the mice diets cannot be attributed to any single factor or nutrient but only to the entire diet. Despite the mice diet having a similar macronutrient profile to the diet estimated for feral cats⁽⁶⁾, further research is necessary to investigate if variation in prey will influence intestinal fermentation. Additionally, the nutrient uptake from whole prey diets needs to be explored and to what extent animal fibre need to be included in a carnivore's diet to ensure the best health effect.

In conclusion, the comparison between cats consuming whole *v* MM demonstrated a lack of effect on digestibility and the MUET. The microbial profile was comparable between MM and WM, and there were mainly numerical differences in the fermentation end products that would indicate a protective effect of food structure against proteolytic fermentation. We postulate, from the marked reduction in proteolytic fermentation products in our study when shifting from an extruded to mice diet, that part of the CP of mice is more digestible than that of EXT and that the animal fibres in prey such as mice can protect against harmful protein fermentation. Another possible explanation is that there are insufficient fermentable carbohydrates that can be used as an energy source when consuming mice leading to an overall reduction in metabolic activity of the large intestinal microbiota. These findings can be used to optimise processing conditions in pet foods but may warrant further investigations in the health support function of food structure in all species, including humans.

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Supplementary material

For supplementary material/s referred to in this article, please visit <https://doi.org/10.1017/S0007114523002039>

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