SAO PAULO STATE UNIVERSITY - UNESP

JABOTICABAL CAMPUS

ANIMAL FIBER INCLUSION IN CATS DIETS: *IN VITRO* AND *IN VIVO* APPROACHES

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Veterinarian

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SAO PAULO STATE UNIVERSITY - UNESP

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POTENCIAL IMPACTO DESTA PESQUISA

O objetivo desta pesquisa foi explorar os diferentes aspectos da degradabilidade e fermentabilidade *in vitro* de diferentes ingredientes derivados de tecidos animais potencialmente pouco digestíveis, selecionar e comparar alguns deles como fonte de matéria orgânica fermentável para a formulação de dietas de manutenção para gatos adultos saudáveis e avaliar *in vivo* o impacto do consumo destes ingredientes sobre diferentes biomarcadores em potencial da funcionalidade intestinal. Estes resultados podem contribuir para o desenvolvimento metodológico de pesquisas e para discussões de aspectos nutricionais e de composição e função da microbiota intestinal da espécie que podem melhorar o uso sustentável de ingredientes na nutrição animal.

POTENTIAL IMPACT OF THIS RESEARCH

The objective of this research was to explore the different aspects of the *in vitro* degradability and fermentability of different ingredients derived from potentially poorly digestible animal tissues, to select and compare some of them as a source of fermentable organic matter for the formulation of maintenance diets for healthy adult cats, and to evaluate *in vivo* the impact of the consumption of these ingredients on different potential biomarkers of intestinal functionality. These results may contribute to the methodological development of research and to discussions of nutritional aspects and the composition and function of the intestinal microbiota of the species, which may improve the sustainable use of ingredients in animal nutrition.

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Câmpus de Jaboticabal

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: ANIMAL FIBER INCLUSION IN CATS DIETS: *IN VITRO* AND *IN VIVO* APPROACHES

AUTORA: ELOISE CRISTINA DE RAMOS
ORIENTADOR: AULUS CAVALIERI CARCIOFI

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Jaboticabal, 02 de julho de 2026.

AUTHOR'S CURRICULAR INFORMATION

Eloise Cristina de Ramos – was born on June 13th, 1993 in the city of Limeira, São Paulo. In 2011 enrolled in the Veterinarian Bachelor science program of Anhembi Morumbi University, in São Paulo. In 2013 proceed the course in the Faculty of Animal Science and Food Engineering, University of São Paulo - USP, Pirassununga, São Paulo, Brazil, graduating in 2018. During 18 months of the undergraduate years, participated in the Brazilian Scientific Mobility Program (BSMP) to United States of America at the University of Wisconsin River Falls and at Texas A&M University. Also, during the undergraduate period participated in scientific initiation program related dogs to primordial germ cells under Professor Dra. Daniele dos Santos Martins supervision and curricular internship in dogs and cats' clinical nutrition under Professor Dra Deise Carla Almeida Leite Dellova supervision. Between 2020 and 2022 concluded the Animal Sciences Masters program with Biochemistry and Animal Physiology specialty at the Faculty of Agricultural and Veterinary Sciences of "Júlio de Mesquita Filho" Sao Paulo State University, Jaboticabal Campus under Professor Dr. Aulus Cavalieri Carciofi supervision. Contributed as student representative of the Animal Science graduation program of Unesp during 2021-2022 (full member), 2023-2024 (alternate member) and also as student representative of the self-evaluation procedure of the graduation program (2021-2024). During 4 months the doctorate years, participated in the Sandwich Doctoral Program Abroad (PDSE) to the United States of America, at the Gastrointestinal Laboratory of Texas A&M University, College Station, TX, under Dr. Jan S. Suchodolski supervision.

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To the laboratory cats that lovely contributed to the trial – Arruda, Bartô, Batman, Bel, Charlote, Crepúsculo, Frida, Hortelã, Mental, Milk, Noturno, Pantera, Pisco, Pitica, Tico and Vampiro.

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SUMMARY

CERTIFICATE OF THE ANIMAL ETHICS COMMITTEE OF *IN VITRO* TRIAL

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

C E R T I F I C A D O

Certificamos que o projeto intitulado **"Fibra animal" – coprodutos de origem animal com potencial fermentativo em dietas para gatos – etapa in vitro",** protocolo nº 7626/2022, sob a responsabilidade do Prof. Dr. Aulus Cavalieri Carciofi, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 19 de outubro de 2022.

(We certify that the research project entitled **"Effect of animal-derived fermentable substrate ingredients in cats' diets - in vitro trial"**, protocol nº. 7626/2022, under the responsibility of Dr. Aulus Cavalieri Carciofi, which involves the production, maintenance and/or use of animals belonging to the Chordata phylum, vertebrate subfile (except man), for the purposes of scientific research (or teaching) - is in accordance with the precepts of Law no. 11,794, of October 8, 2008, in Decree 6,899, of July 15, 2009, and with the rules issued by the National Council for Animal Experimentation Control (CONCEA), and was approved by the Ethics Commission on Animal Use (CEUA), from the SCHOOL OF AGRICULTURAL AND VETERINARY SCIENCES, UNESP - JABOTICABAL – SP Campus, at October, 19, 2022 an ordinary meeting.)

Vigência do Projeto	18/11/2022 a 31/01/2023
Espécie / Linhagem	Felinos domésticos *(Felis catus)*
Nº de animais	6
Peso / Idade	3 a 6 kg / 1 a 6 anos
Sexo	Machos e Fêmeas
Procedência	Laboratório de Pesquisa em Nutrição e Doenças Nutricionais de Cães e Gatos "Prof. Dr. Flávio Prada".

Jaboticabal, 27 de fevereiro de 2026.

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CERTIFICATE OF THE ANIMAL ETHICS COMMITTEE OF *IN VIVO* TRIAL

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(We certify that the research project entitled **"Effect of animal-derived fermentable substrate ingredients in cats' diets - in vivo trial"**, protocol nº. 7625/2022, under the responsibility of Dr. Aulus Cavalieri Carciofi, which involves the production, maintenance and/or use of animals belonging to the Chordata phylum, vertebrate subfile (except man), for the purposes of scientific research (or teaching) - is in accordance with the precepts of Law no. 11,794, of October 8, 2008, in Decree 6,899, of July 15, 2009, and with the rules issued by the National Council for Animal Experimentation Control (CONCEA), and was approved by the Ethics Commission on Animal Use (CEUA), from the SCHOOL OF AGRICULTURAL AND VETERINARY SCIENCES, UNESP - JABOTICABAL - SP Campus, at October, 19, 2022 an ordinary meeting.)

Vigência do Projeto	01/02/2023 a 31/05/2024
Espécie / Linhagem	Felinos domésticos (*Felis catus*)
Nº de animais	36
Peso / Idade	3-6 kg / 1-6 anos
Sexo	Machos e Fêmeas
Origem	Laboratório de Pesquisa em Nutrição e Doenças Nutricionais de Cães e Gatos "Prof. Dr. Flávio Prada"

Jaboticabal, 30 de outubro de 2025

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INCLUSÃO DE FIBRA ANIMAL EM DIETAS PARA GATOS: ABORDAGENS *IN VITRO* E *IN VIVO*

RESUMO: Ao considerar os hábitos alimentares e a evolução dos gatos domésticos, porções indigeríveis de proteína com penas, pelos, e tendões – denominadas substratos fermentáveis de origem animal (ADFS) ou "fibra animal"– podem atuar como substratos orgânicos para a microbiota do cólon. Entretanto, a inclusão destes em dietas extrusadas para felinos continua inexplorada. Este estudo foi composto por ensaios *in vitro* e *in vivo* que avaliaram a degradabilidade, fermentabilidade e os efeitos fisiológicos de ADFS em comparação com fibras vegetais. No primeiro capítulo é realizada revisão da literatura a respeito, que dá suporte a possível participação dos ADFS como componentes que do ponto de vista evolutivo tiveram papel relevante no desenvolvimento e diferenciação da composição e atividade metabólica da microbiota intestinal de carnívoros. No capítulo dois é apresentado estudo experimental que incluiu métodos *in vitro* para avaliação de oito ingredientes proteicos com possível baixa digestibilidade ileal, quanto à degradabilidade *in vitro* de matéria orgânica (IVDOM) e fermentabilidade in vitro (IVF), utilizando inóculo fecal felino. A IVDOM foi determinada em quadruplicata, e a IVF foi avaliada nos resíduos insolúveis após 4, 8 e 24 horas de incubação anaeróbia. O inóculo fecal foi coletado de seis gatos adultos saudáveis alimentados com a mesma dieta de manutenção por pelo menos 21 dias. Os resíduos insolúveis provenientes da hidrólise de farinha de subprodutos de aves (HPMr) e de penas hidrolisadas ao final da hidrólise (FHr2) apresentaram os maiores valores de IVDOM, seguidos por penas cruas e farinha de penas (FM), enquanto o pelo de gato apresentou a menor degradabilidade ($P < 0,05$). Durante a fermentação, HPMr, FHr2 e FM produziram os maiores volumes de gás, enquanto HPMr e FHr2 apresentaram as maiores concentrações de ácidos graxos de cadeia curta ($P < 0,05$). Os demais substratos apresentaram fermentabilidade intermediária, embora todos tenham produzido respostas fermentativas superiores à fibra de cana-de-açúcar (controle negativo). Esses resultados indicam que vários coprodutos de origem animal podem atuar como substratos fermentáveis para a microbiota do intestino grosso dos felinos e sustentar a atividade metabólica microbiana. No capítulo três quatro dietas foram formuladas e extrusadas para conter 4% de matéria orgânica indigestível de origem vegetal—fibra de cana-de-açúcar (SF) e polpa de beterraba (BP)—e de origem animal—farinha de penas (FM) e resíduo sólido de proteína de aves hidrolisada (HPMr). A escolha dos resíduos de origem animal foi baseada nos resultados obtidos no estudo *in vitro*. Trinta e dois gatos (8 por dieta) foram alimentados durante 31 dias. A dieta FM apresentou maior digestibilidade aparente da matéria orgânica e da energia bruta em comparação com SF e BP ($P < 0,05$). O pH fecal foi mais alto e a amônia mais baixa em HPMr, enquanto os ácidos graxos de cadeia curta foram mais elevados em BP ($P < 0,05$). O colesterol e os fitoesteróis foram mais elevados nas fezes dos gatos alimentados com a dieta BP do que nas demais ($P < 0,05$). Efeitos semelhantes das dietas foram observados na diversidade e na composição geral do microbioma fecal dos gatos; no entanto, foram observadas maiores concentrações de ácidos biliares secundários e ácido litocólico, bem como maiores abundâncias de *Peptacetobacter hiranonis*, *Blautia* e *Oscillospiraceae* nos grupos ADFS ($P < 0,05$), apoiando a hipótese de que esses ingredientes podem atuar como uma fonte adequada de matéria orgânica fermentável para gatos

Palavras-chave: ácidos biliares, amônia, butirato; farinha de penas; fermentação de proteínas, *Peptacetobacter hiranonis*.

ANIMAL FIBER INCLUSION IN CATS DIETS: *IN VITRO* AND *IN VIVO* APPROACHES

ABSTRACT: Considering the feeding habits and evolutionary history of domestic cats, indigestible protein fractions such as feathers, hair, and tendons—referred to as animal-derived fermentable substrates (ADFS) or “animal fibre”—may act as organic substrates for the colonic microbiota. However, the inclusion of these ingredients in extruded feline diets remains unexplored. This study comprised both *in vitro* and *in vivo* trials that evaluated the degradability, fermentability, and physiological effects of ADFS in comparison with plant fibres. The first chapter presents a literature review supporting the potential role of ADFS as components that, from an evolutionary perspective, may have played a relevant role in the development and differentiation of the composition and metabolic activity of the intestinal microbiota of carnivores. In the chapter two, eight protein ingredients with low expected ileal digestibility were evaluated for their *in vitro* digestible organic matter disappearance (IVDOM) and *in vitro* fermentability (IVF) using feline faecal inoculum. IVDOM was determined in quadruplicate, and IVF was assessed in the insoluble residues after 4, 8, and 24 h of anaerobic incubation. Faecal inoculum was collected from six healthy adult cats fed the same maintenance diet for at least 21 days. The insoluble residues from poultry co-product meal hydrolysis (HPMr) and hydrolyzed feathers at the end of hydrolysis (FHr2) showed the highest IVDOM values, followed by raw feathers and feather meal (FM), whereas cat hair exhibited the lowest degradability ($P < 0.05$). During fermentation, HPMr, FHr2, and FM produced the greatest gas volumes, while HPMr and FHr2 higher concentrations of short-chain fatty acids ($P < 0.05$). The remaining substrates exhibited intermediate fermentability, although all produced greater fermentation responses than sugarcane fibre (negative control). These results indicate that several animal-derived co-products may act as fermentable substrates for the feline hindgut microbiota and support microbial metabolic activity. In chapter three, four diets were formulated and extruded to contain 4% indigestible organic matter from vegetable sources—sugarcane fibre (SF) and beet pulp (BP)—and animal sources—feather meal (FM) and solid residue of hydrolysed poultry protein (HPMr). The selection of animal-derived residues was based on the results obtained in the in vitro study. Thirty-two cats (8 per diet) were fed for 31 days. The FM diet showed higher apparent digestibility of organic matter and gross energy compared with SF and BP ($P < 0.05$). Faecal pH was higher and ammonia lower in HPMr, while short-chain fatty acids were higher in BP ($P < 0.05$). Cholesterol and phytosterols were higher in the faeces of cats fed the BP diet than in the others ($P < 0.05$). Similar diet effects were observed on faecal microbiome diversity and overall composition of cats; nevertheless, increased concentrations of secondary bile acids and lithocholic acid, as well as increased abundances of *Peptacetobacter hiranonis*, *Blautia*, and *Oscillospiraceae*, were observed in ADFS groups ($P < 0.05$), supporting the hypothesis that they may serve as a suitable source of fermentable organic matter for cats.

Keywords: ammonia, bile acids, butyrate, feather meal, protein fermentation, *Peptacetobacter hiranonis*.

CHAPTER 1 – GENERAL CONSIDERATIONS

1. INTRODUCTION

There is a global trend among dog and cat owners to regard their pets as family members. As pets continue to play an increasingly important role in human life, their health, particularly gastrointestinal functionality, has become a major concern for caregivers (Lee et al., 2024). In 2022, the cat's population in Brazil was of 33.6 million animals and the population growth from 2021 to 2022 was 6%, greater than all the average considered species (3.6%) (ABEMPET, 2024), reinforcing the need for nutritional solutions that are affordable for pet owners, beneficial to cats, and socially and environmentally responsible.

The nutritional composition of feline diets has a significant impact on gastrointestinal function. Dietary strategies can influence the intestinal microbiome, modulate mucosal immune responses, regulate genetic and epigenetic expression in intestinal tissues, enhance epithelial barrier function, and maintain physiological gut motility. Consequently, these effects contribute to the maintenance of overall health and well-being in cats (Kathrani, 2022; Butowski *et al*., 2022).

In commercial cat diets, particularly extruded products, animal co-products are the primary source of protein due to their favorable essential amino acid profile, high protein content, balanced proportions of protein, fat, and minerals, palatability, and widespread commercial availability (ABEMPET, 2024; Meeker & Meisinger 2015; Scarpim *et al*., 2024). Besides serving as a protein source, low-digestibility animal tissues such as keratin and collagen, when present in animal co-products, may undergo microbial fermentation in the colon. This process may result in effects similar to those of certain dietary fibres, including increased production of fermentation end products, reduced colonic pH, maintenance of gut motility, and modulation of faecal microbiota (Depauw *et al*., 2013). However, little research has investigated the effects of low-digestibility animal-derived protein sources on the feline intestinal environment. This may be partly due to the

extrapolation of concepts derived from human nutrition, in which protein fermentation is generally regarded as detrimental (Mukherjee *et* al., 2025). Nevertheless, such assumptions may not fully apply to cats. As obligate carnivores, cats evolved consuming whole prey, and indigestible animal tissues likely constituted the major source of organic substrates reaching the large intestine. Consequently, these substrates may have played an important role in shaping and sustaining the distinctive composition and metabolic activity of the feline gut microbiota (Morris, 2002; Lyu *et al.*, 2020; D'Hooghe *et al.,* 2024).

In this context, advances in scientific research require the continuous development of methods for hypothesis testing. Given the importance of dogs and cats to human society, alternative methodologies, such as *in vitro* models, have gained relevance as tools to reduce the use of animals in research while minimizing experimental and maintenance cost (Williams *et al.*, 2005; Harmon, 2007).

Taking this into consideration, this review aims to examine the relationship between feline feeding habits and gastrointestinal function, highlight the relevance of animal co-products as sources of indigestible and potentially fermentable organic matter in feline diets, and evaluate the use of different methodological approaches to investigate the potential implications of consuming low-digestibility animal tissues.

6. CONCLUSION

The ADFS evaluated, FM and HPMr, were well accepted by the cats and did not negatively affect total tract apparent digestibility or faecal characteristics, nor increased branched-chain fatty acids or ammonia in the faeces. These ingredients exhibited low fermentability, showing results similar to those observed for SC, a low-fermentable plant fibre source. However, it is possible that they were more extensively enzymatically digested and absorbed at the ileal level, reaching the colon for microbial fermentation to a lesser extent than the added vegetable fibre sources. At the inclusion level evaluated, plant- and animal-derived fermentable organic matter sources exerted similar effects on the faecal microbiome diversity and overall composition of cats. Nevertheless, some relevant effects with potential implications for feline health were observed, including increased concentrations of secondary bile acids and lithocholic acid, decreased ursodeoxycholic acid, and increased abundances of *Peptacetobacter hiranonis*, *Blautia*, and *Oscillospiraceae* compared with the vegetable fibre sources. In conjunction, these results support the hypothesis that proteinaceous, low-digestible material of animal origin may serve as a suitable source of fermentable organic matter for cats, a domesticated carnivore.

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DISCLOSURE STATEMENT

The authors Bruna C. Lopes, Jonathan L. Turck and Jan S. Suchodolski are employed by the Gastrointestinal Laboratory, which offers assays for intestinal function and microbiota analysis on a fee-for-service basis. None of the other authors declare any conflict of interest.

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7. REFERENCES

Adams, S., Che, D., Qin, G., Rui, H., Sello, C. T., & Hailong, J. (2018). Interactions of dietary fibre with nutritional components on gut microbial composition, function and health in monogastrics. *Current Protein and Peptide Science*, *19*(10), 1011-1023. https://doi.org/10.2174/1389203719666180508111843

AOAC. Official Methods of Analysis of AOAC International. (2019). 21st ed. Gaithersburg Maryland: *AOAC International. Official Methods*: 934.01; 942.05; 954.01; 991.43; 954.02.

Badri, D. V., Jackson, M. I., & Jewell, D. E. (2021). Dietary protein and carbohydrate levels affect the gut microbiota and clinical assessment in healthy adult cats. *The Journal of Nutrition*, *151*(12), 3637-3650. https://doi.org/10.1093/jn/nxab308

Blake, A.B.; Toresson, L.; Chen, C.-C.; Ishii, P.E.; Phillips, R.K.; Giaretta, P.R.; Cavasin, J.P.; Lidbury, J.A.; Suchodolski, J.S. (2025). Altered iso- and oxo-Faecal Bile Acid Concentrations in Dogs with Chronic Enteropathy. *Pets*, 2, 18. https://doi.org/10.3390/pets2020018

Barry, K. A., Middelbos, I. S., Vester Boler, B. M., Dowd, S. E., Suchodolski, J. S., Henrissat, B., et al. (2012). Effects of dietary fibre on the feline gastrointestinal metagenome. *Journal of Animal Science,* *90*(13), 4450–4463. https://doi.org/10.2527/jas.2011-4956

Butowski, C.F., Thomas, D. G., Young, W., Cave, N. J., McKenzie, C. M., Rosendale, D. I., et al. (2019). Addition of plant dietary fibre to a raw red meat high protein, high fat diet, alters the faecal bacteriome and organic acid profiles of the domestic cat (Felis catus). *PLoS ONE* 14(5): e0216072. https://doi.org/10.1371/journal.pone.0216072

Butowski, C. F., Thomas, D. G., Cave, N. J., Bermingham, E. N., Rosendale, D. I., Hea, S. Y,. Stoklosinski, H. M., & Young, W. (2022). In Vitro Assessment of Hydrolysed Collagen Fermentation Using Domestic Cat (*Felis catus*) Faecal Inocula. *Animals* 12, no. 4: 498. https://doi.org/10.3390/ani12040498

Callegaro, K., Brandelli, A., & Daroit, D. J. (2019). Beyond plucking: feathers bioprocessing into valuable protein hydrolysates. *Waste Management*, *95*, 399-415. https://doi.org/10.1016/j.wasman.2019.06.040

Carciofi, A. C., Takakura, F. S., De-Oliveira, L. D., Teshima, E., Jeremias, J. T., Brunetto, M. A., & Prada, F. (2008). Effects of six carbohydrate sources on dog diet digestibility and post-prandial glucose and insulin response*. *Journal of Animal Physiology and Animal Nutrition*, 92(3), 326–336. https ://doi.org/10.1111/j.1439-0396.2007.00794.x

Correa Lopes, B., Chen, C. C., Sung, C. H., Ishii, P. E., Medina, L. F. D. C., Gaschen, F. P., ... & Pilla, R. (2024). Correlation between Peptacetobacter hiranonis, the baiCD gene, and secondary bile acids in dogs. *Animals*, 14(2), 216. https://doi.org/10.3390/ani14020216

da Silva, C. B., dos Santos Neto, E. L., Bastiaens, L., Simons, Q., Spranghers, T., Deruytter, D., ... & Hesta, M. (2026). Is chitin an interesting fibre source for cats? Effects on palatability, digestibility, and health markers. *Journal of Insects as Food and Feed*, *1*(aop), 1-12. DOI:10.1163/23524588-bja10387.

Deb-Choudhury, S., Bermingham, E.N., Young, W., Barnett, M.P., Knowles, S.O., Harland, D., ... & Dyer, J.M. (2018) The effects of a wool hydrolysate on short-chain fatty acid production and faecal microbial composition in the domestic cat (*Felis catus). Food & Function,* 9(8), 4107-4121. https://doi.org/10.1039/C7FO02004J

de-Oliveira, L. D., de Carvalho Picinato, M. A., Kawauchi, I. M., Sakomura, N. K., & Carciofi, A. C. (2012). Digestibility for dogs and cats of meat and bone meal processed at two different temperature and pressure levels. *Journal of Animal Physiology and Animal Nutrition*, 96(6), 1136-1146. https://doi.org/10.1111/j.1439-0396.2011.01232.x

Depauw, S., Hesta, M., Whitehouse-Tedd, K., Vanhaecke, L., Verbrugghe, A., Janssens, G.P. (2013). Animal fibre: the forgotten nutrient in strict carnivores? First insights in the cheetah. *Journal of Animal Physiology and Animal Nutrition*. 97(1):146-54. doi: 10.1111/j.1439-0396.2011.01252.x. Epub 2011 Nov 10. PMID: 22074361.

D'Hooghe, S. M-T. J., Bosch, G., Sum, M., Cools, A. Hendriks, W. H., Becker, A. A. M. j.., & Janssens, G. P. J. (2024). How important is food structure when cats eat mice? *British Journal of Nutrition*, 131 (3), 369-383. doi:10.1017/S0007114523002039

FEDIAF. Nutritional Guidelines for complete and complementary pet food for cats and dogs The European Pet Food Industry. (2024). Bruxelas: The European Pet Food Industry Federation.

Félix, A. P., Souza, C. M. M., & de Oliveira, S. G. (2022). Biomarkers of gastrointestinal functionality in dogs: A systematic review and meta-analysis. *Animal Feed Science and Technology*, *283*, 115183. https://doi.org/10.1016/j.anifeedsci.2021.115183

Fischer, M. M., Kessler, A. M., De Sá, L. R. M., Vasconcellos, R. S., Filho, F. R., Nogueira, S. P., ... & Carciofi, A. C. (2012). Fibre fermentability effects on energy and macronutrient digestibility, faecal traits, postprandial metabolite responses, and colon histology of overweight cats. *Journal of Animal Science*, *90*(7), 2233-2245.https://doi.org/10.2527/jas.2011-4334

Giordano, M.V., Crisi, P.E., Gramenzi, A., Cattaneo, D., Corna, L., Sung, C-H., Tolbert, K. M., Steiner, J. M., Suchodolski, J. S. & Boari, A. (2024). Faecal microbiota and concentrations of long-chain fatty acids, sterols, and unconjugated bile acids in cats with chronic enteropathy. *Frontiers of Veterinary Sciences.* 11:1401592. doi: 10.3389/fvets.2024.1401592

Hervera, M., Baucells, M. D., Blanch, F., & Castrillo, C. (2007). Prediction of digestible energy content of extruded dog food by in vitro analyses. Journal of animal physiology and animal nutrition, 91(5-6), 205-209. https://doi.org/10.1111/j.1439-0396.2007.00693.x

Hewson-Hughes, A.K., Hewson-Hughes, V.L., Colyer, A., Miller, A.T., Hall, S.R., Raubenheimer, D., & Simpson, S.J. (2013) Consistent proportional macronutrient intake selected by adult domestic cats (*Felis catus*) despite variations in macronutrient and moisture content of foods offered. *Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology*, 183(4), 525–536. https://doi.org/10.1007/s00360-012-0727-y

Huss, M., Elger, T., Kunst, C., Loibl, J., Krautbauer, S., Liebisch, G., ... & Buechler, C. (2025). Faecal Arachidonic Acid: A Potential Biomarker for Inflammatory Bowel Disease Severity. *International Journal of Molecular Sciences*, *26*(9), 4034. https://doi.org/10.3390/ijms26094034

Kayser, E., Finet, S. E., & de Godoy, M. R. (2024). The role of carbohydrates in canine and feline nutrition. *Animal Frontiers*, 14(3), 28-37. https://doi.org/10.1093/af/vfae017

Kerr, K. R., Dowd, S. E., & Swanson, K. S. (2014). Faecal microbiota of domestic cats fed raw whole chicks v. an extruded chicken-based diet. Journal of nutritional science, 3, e22. https://doi.org/10.1017/jns.2014.21

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Ko, KS, Fascetti AJ. Dietary beet pulp decreases taurine status in dogs fed low protein diet. 2016. *Journal of Animal Science and Technology*. 58:29. doi:10.1186/s40781-016-0112-6

Laflamme, D. P. (1997) Development and validation of a body condition score system for cats: a clinical tool. *Feline Practice*., v. 25, n. 5/6, p. 13—18.

Lai, J., Zentek, J., & Grześkowiak, Ł. M. (2026). Dietary Fibre Regulation of Gut Microbiota and Bile Acid Metabolism in Animals: Implications for Animal Nutrition. *Veterinary Sciences*, *13*(2), 209. https://doi.org/10.3390/vetsci13020209

Lin, H., Peddada, S.D. (2020). Analysis of compositions of microbiomes with bias correction. *Nature Communications.* 11, 3514. https://doi.org/10.1038/s41467-020-17041-7

Loureiro, B. A., Sakomura, N. K., Vasconcellos, R. S., Sembenelli, G., Gomes, M. D. O. S., Monti, M., ... & Carciofi, A. C. (2017). Insoluble fibres, satiety and food intake in cats fed kibble diets. Journal of animal physiology and animal nutrition, 101(5), 824-834. https://doi.org/10.1111/jpn.12468

Martínez-Alvarez, O., Chamorro, S., Brenes, A. (2015). Protein hydrolysates from animal processing by-products as a source of bioactive molecules with interest in animal feeding: a review. *Food Research International*. 73, 204–212. https://doi.org/10.1016/j.foodres.2015.04.005.

Meeker, D. L., & Meisinger, J. L. (2015). Companion Animals Symposium: Rendered ingredients significantly influence sustainability, quality, and safety of pet food. *Journal of Animal Science*, 93(3), 835–847. https://doi.org/10.2527/jas.2014-8524

Moreno, A. A., Parker, V. J., Winston, J. A., & Rudinsky, A. J. (2022). Dietary fibre aids in the management of canine and feline gastrointestinal disease. *Journal of the American Veterinary Medical Association*, *260*(S3), S33-S45. https://doi.org/10.2460/javma.22.08.0351

Mukherjee, A., Farsi, D.N., Garcia-Gutierrez, E., Akan, E., Millan, J. A. S., Angelovski, L., Bintsis, T., Gérard, A., Güley, Z., Kabakcı, S., Kahala, M., Merabti, R., Pavli, F., Salvetti, E., Karagözlü, C., Bağlam, N., Hyseni, B., Bavaro, S., Papadimitriou, K., Doo, E-H., Chassard, C., Praćer, S., Vergères, G., Cotter, P. D., & Mojsova, S. (2025) Impact of fermented foods consumption on gastrointestinal wellbeing in healthy adults: a systematic

review and meta-analysis. *Frontiers in Nutrition*. 12:1668889. doi: 10.3389/fnut.2025.1668889

Namysl, S., Pelucchi, M., Herbinet, O., Frassoldati, A., Faravelli, T., & Battin-Leclerc, F. (2019). A first evaluation of butanoic and pentanoic acid oxidation kinetics. *Chemical Engineering Journal*, *373*, 973-984. https://doi.org/10.1016/j.cej.2019.05.090

Németh, K., Tóth, I., Lányi, K., Schilling-Tóth, B. M., Csorba, S., Žura Žaja, I., & Sterczer, Á. (2026). The Collaborative Collapse: Bile Acid Dysmetabolism as a Central Pathogenic Driver in Canine and Feline Multi-Systemic Disorders—From Mechanisms to Precision Therapeutics. *Veterinary Sciences*, 13(2), 182. https://doi.org/10.3390/vetsci13020182

Paßlack, N., Kohn, B., Doherr, M. G., & Zentek, J. (2018). Influence of protein concentration and quality in a canned diet on urine composition, apparent nutrient digestibility and energy supply in adult cats. *BMC Veterinary Research*, *14*(1), 225. https://doi.org/10.1186/s12917-018-1517-x

Pacheco, G.F.E., Pezzali, J.G., de Mello Kessler, A., Trevizan, L., 2016. Inclusion of exogenous enzymes to feathers during processing on the digestible energy content of feather meal for adult dogs. *Revista Brasileira de Zootecnia*. 45, 288–294. https://doi.org/10.1590/S1806-92902016000600002.

Pinna, C., Stefanelli, C., & Biagi, G. (2014). In vitro effect of dietary protein level and nondigestible oligosaccharides on feline faecal microbiota. *Journal of Animal Science*, *92*(12), 5593-5602. https://doi.org/10.2527/jas.2013-7459

Plantinga, E. A.; Bosch, G.; Hendriks, W. H. (2011) Estimation of the dietary nutrient profile of free-roaming feral cats: possible implications for nutrition of domestic cats. *The British Journal of Nutrition*, v. 106, S1, p.S35-S48. https://doi.org/10.1017/S0007114511002285

Pryce, J. D. (1969). A modification of Barker-Summerson method for determination of lactic acid. *Analyst*. 94:1151-1152. https://doi.org/10.1039/an9699401151

Rochus, K., Janssens, G. P., & Hesta, M. (2014). Dietary fibre and the importance of the gut microbiota in feline nutrition: a review. *Nutrition Research Reviews*, *27*(2), 295-307. https://doi.org/10.1017/S0954422414000213

Ruiz-Capillas, C., & Jiménez-Colmenero, F. (2004). Biogenic Amines in Meat and Meat Products. *Critical Reviews in Food Science and Nutrition*, 44(7–8), 489–599. https://doi.org/10.1080/10408690490489341

Rowe, J.C.;Winston, J.A. Collaborative Metabolism: Gut Microbes Play a Key Role in Canine and Feline Bile Acid Metabolism. *Veterinary Sciences*. 2024, 11, 94. https://doi.org/10.3390/vetsci11020094

Scarpim, L. B., De Ramos, E. C., Pacheco, L. G., Goloni, C., De Souza Theodoro, S., De Castro, T. S. A., & Carciofi, A. C. (2024). Hydrolysed poultry byproduct meal in extruded diets for cats. *Archives of Animal Nutrition*, 78(1),45–59. https://doi.org/10.1080/1745039X.2024.2312700

Silvio J, Harmon DL, Gross KL, McLeod KR. Influence of fibre fermentability on nutrient digestion in the dog. *Nutrition*. 2000;16:289–295. doi:10.1016/S0899- 9007(99)00298-1

Suchodolski, J. S. (2022). Analysis of the gut microbiome in dogs and cats. *Veterinary clinical pathology*, *50*, 6-17. https://doi.org/10.1111/vcp.13031

Sung, C.H.; Marsilio, S.; Chow, B.; Zornow, K.A.; Slovak, J.E.; Pilla, R.; Lidbury, J.A.; Steiner, J.M.; Park, S.Y.; Hong, M.P.; et al (2022). Dysbiosis index to evaluate the faecal microbiota in healthy cats and cats with chronic enteropathies. *Journal of Feline Medicine and Surgery*. 24, e1–e12. https://doi.org/10.1177/1098612X221077876

Sung, C.-H.; Pilla, R.;Marsilio, S.; Chow, B.; Zornow, K.A.; Slovak, J.E.; Lidbury, J.A.; Steiner, J.M.; Hill, S.L.; Suchodolski, J.S (2023). Faecal Concentrations of Long-Chain Fatty Acids, Sterols, and Unconjugated Bile Acids in Cats with Chronic Enteropathy. *Animals*, 13, 2753. https://doi.org/10.3390/ani13172753

Sung, C. H., Marsilio, S., Pilla, R., Wu, Y. A., Cavasin, J. P., Hong, M. P., & Suchodolski, J. S. (2024). Temporal variability of the dominant faecal microbiota in healthy adult cats. *Veterinary Sciences*, *11*(1), 31. https://doi.org/10.3390/vetsci11010031

Telles, N. J., Simon, B. T., Scallan, E. M., Gould, E. N., Papich, M. G., He, Y., ... & Katherine Tolbert, M. (2022). Evaluation of gastrointestinal transit times and pH in healthy cats using a continuous pH monitoring system. *Journal of Feline Medicine and Surgery*, *24*(10), 954-961. https://doi.org/10.1177/1098612X211062096

Vale, S. R., & Glória, M. B. (1997). Determination of biogenic amines in cheese. *Journal of AOAC International*, *80*(5), 1006–1012. https://doi.org/10.1093/jaoac/80.5.1006

Verbrugghe, A,, & Hesta, M, (2017) Cats and Carbohydrates: The Carnivore Fantasy? *Veterinary Sciences.* 4, no. 4: 55. https://doi.org/10.3390/vetsci4040055

Zhang, Y., Yang, R., & Zhao, W. (2014) Improving digestibility of feather meal by steam flash explosion. *Journal of Agricultural and Food Chemistry*, 62(13), 2745–2751. https://doi.org/10.1021/jf405498k

Yang, Y., Gao, W., Zhu, R., Tao, L., Chen, W., Zhu, X., ... & Zhang, X. (2025). Systematic identification of secondary bile acid production genes in global microbiome. *mSystems*. 10: e0081724. https://doi.org/10.1128/msystems.00817-24

Yao, C. K., Muir, J. G., & Gibson, P. R. (2016). Insights into colonic protein fermentation, its modulation and potential health implications. *Alimentary Pharmacology & Therapeutics,* 43(2), 181-196. https://doi.org/10.1111/apt.13456