Feature Article

Bioactive peptides extracted from hydrolyzed animal byproducts for dogs and cats

Ricardo S. Vasconcellos, Josiane A. Volpato, and Ingrid C. Silva

Department of Animal Science, State University of Maringá, Maringá, PR, Brazil

Key words: animal byproduct meals, functional food, hydrolyzed protein, pet food, rendering

Introduction

Worldwide, 350 million tons of meat are produced annually; 35% is chicken, 30% is pork, 20% is beef, and 15% is meat from other species. Milk and egg production are also important, estimated at 900 and 90 million tons, respectively (World Food and Agriculture—Statistical Yearbook 2022, 2022). Approximately 40% of the total animal live weight produced globally is inedible for human consumption, such as skin, blood and its elements, horns, hooves, viscera, bones, hair, and feathers. Thus, most of these inedible parts for humans are used to produce, via the rendering process, animal byproduct meal (ABPMs) for livestock and pet feeding (e.g., meat and bone meal, poultry byproduct meal, blood meal, animal plasma, and feather meal). About 9 million tons of ABPMs and fats are used annually to produce dry food for dogs and cats (Alexander et al., 2020).

Feeding animals with these ingredients optimizes the surplus of food for human consumption, which is considered adequate in the food recovery hierarchy. Therefore, rendering is important because it recycles slaughterhouse waste into nutritious natural ingredients to meet animal nutritional requirements.

Rendering yields ingredients with variation in quality, sometimes low nutrient bioavailability, and low added value. However, these ingredients usually contain essential amino acids in high concentrations and peptides with possible functionalities in animal health. Thus, the industry has increased the production and use of protein hydrolysates from these byproducts to find better alternatives for using ABPMs (He et al., 2013). More recently, the pet food industry has employed these ingredients, focusing on their nutritional and functional properties for dogs and cats. In addition to the greater bioavailability of amino acids, some benefits of protein hydrolysates are the release of bioactive peptides (BPs) that have antioxidant, antimicrobial, immunomodulatory, tissue repair, antihypertensive, and glycemic control effects (Zóia Miltenburg et al., 2021).

Many of these functional properties are still being studied in companion animals. The present review focuses on the productive aspects of BPs and functional proteins, their applications, and the health benefits already identified in dogs and cats.

Hydrolysis of animal protein

Protein hydrolysis is the process of breaking the peptide bonds between amino acids and proteins. Protein hydrolysates are classified according to their degree of hydrolysis and contain free AAs, small peptides, and large peptides. The proportions of each hydrolysate product depend on protein source, water quality, protease type, and microbe species. Proteins and peptides have different molecular weights; in general, polypeptides with a molecular weight of >8,000 Daltons (>72 AA residues) are referred to as proteins, and those with a molecular weight of <8,000 Daltons are peptides. In animal production, high-quality protein is not hydrolyzed. Only animal byproducts, such as brewer’s byproducts, plant ingredients containing anti-nutritional factors, and low-digestible proteins are hydrolyzed to produce peptides for animal feeding (Hou et al., 2017).

Fish, chicken, and beef meat byproducts are the most common animal material in protein hydrolysate production. Hydrolysis can alter the properties of proteins in three different ways: reducing the molecular weight, increasing the number of ionizable molecules, or exposing hydrophobic groups. These properties are considered in food formulation because they are

Implications

- Enzymatic hydrolysis produces bioactive peptides and hypoallergenic ingredients for pet food.
- Animal byproducts are the main sources of bioactive peptides and hydrolyzed proteins for dogs and cats.
- Prebiotic, antioxidant, anti-inflammatory, immunological, and antihypertensive effects of the bioactive peptides have been studied in dogs and cats.
- Hydrolyzed ingredients improve the palatability of extruded diets for dogs and cats.

Published by Oxford University Press on behalf of American Society of Animal Science 2024. This work is written by (a) US Government employee(s) and is in the public domain in the US.

https://doi.org/10.1093/af/vfae012
directly responsible for their functionality in food processing and the bioavailability of their components (He et al., 2013).

Chemical, enzymatic, and microbial methods can produce hydrolyzed peptides of animal proteins. Choosing a technique for protein hydrolysis depends on the raw material, and a combination of methods can be implemented. For example, feathers, bristles, horns, beaks, or wool proteins contain keratin and are mostly hydrolyzed via acid or alkaline treatment associated with microbial keratolytic enzymes. Other animal (casein, whey, gut, and meat) raw materials are usually subjected to enzymatic hydrolysis or microbial fermentation. Hydrolysis can last 4 to 48 h, depending on the material’s resistance and the method.

Chemical methods for protein hydrolysis include acid and alkaline hydrolysis treatments. In acid hydrolysis, raw materials are commonly treated with concentrated hydrochloric acid (HCl); however, sulfuric acid can be used as well. The most important factors that influence acid hydrolysis are the concentration and type of acid (hydrochloric acid or sulfuric acid), temperature (250 to 280 °F), pressure (32 to 45 psi), hydrolysis length (2 to 8 h), protein concentration and resistance of the raw material ingredient. These factors, individually and combined, affect the quality of the product. In the pet food industry, most acid-hydrolyzed proteins are used as flavor enhancers. Despite the low cost, acid hydrolysis may destroy some essential amino acids such as tryptophan, methionine, cystine, and cysteine. Furthermore, glutamine and asparagine are converted to glutamic and aspartic acids, respectively (Pasupuleti and Demain, 2010).

Protein alkaline hydrolysis requires agents such as Calcium, Sodium, or Potassium Hydroxide to be submitted to high temperatures (>100 °C; Dai et al., 2014). However, lower temperatures (27 to 55 °C) and shorter processing lengths (4 to 8 h) are often desirable to obtain peptides in the food industry (Pasupuleti and Demain, 2010). After alkaline hydrolysis, the product is dehydrated, pasteurized, or spray-dried. Alkaline hydrolysis is a common method for processing low-digestible proteins, such as feathers used in feather meal production and certain keratin material. The alkaline hydrolysis increases the digestibility and the content of free amino acids in the ingredient; however, it has a few disadvantages. Degradation of some amino acids like serine, threonine (Pasupuleti and Demain, 2010; Hou et al., 2017), cystine, methionine, and lysine has been observed (Papadopoulos et al., 1985; Kim et al., 2002).

In enzymatic hydrolysis, different proteolytic enzymes are used, the most common are papain, pepsin, protease complexes, pancreatin, trypsin, chymotrypsin, alkalases, thermolysin, and numerous other bacterial and fungal enzymes (Korhonen and Pihlanto, 2006). Depending on the purpose of hydrolysis, the enzymes (papain and pepsin, papain and actinase E, or trypsin alone) may be used alone or combined. Choosing the enzyme depends on the protein source, the degree of hydrolysis, and the other traits, such as bioactive properties, desired for the final product. Another type of hydrolysis is microbial, a type of enzymatic hydrolysis that occurs during a fermentative process. In the presence of substrate, microorganisms release proteases to break down complex nutrients in the medium and to take advantage of them in metabolism (Smid and Lacroix, 2013). The fermentative process can also produce new proteins, peptides, and free amino acids from microbial metabolism, which can improve the palatability, digestibility, and functionality of these ingredients (López-Pérez and Viniegra-González, 2016; Sandhu et al., 2017).

The industrial production process of protein hydrolysates

The overall procedure for hydrolysate production is described in Figure 1. The process can last 4 to 48 h, depending on the time and hydrolysis method.

Sources of animal protein hydrolysates

Hydrolysis improves the nutrient bioavailability and sensory attributes and releases BPs. Thus, producing protein hydrolysates from industrial byproducts is advantageous because it enhances these materials. For this reason, the production of hydrolysates from animal protein, including byproducts such as ruminant and pig leather, chicken feet, skin and intestine, liver, trachea, bones, blood, and plasma from different species has been widely studied to improve the usage of waste generated in meat production for human consumption. Thus, the bioactivity of these peptides as antioxidants, antihypertensives, antihyperglycemics, and anti-inflammatory agents has been studied.

Bioactive properties of animal protein hydrolysates

Dry food for dogs and cats contains protein derived from industrial byproducts of meat production for humans, which have been included in formulations up to 32% (Alexander et al., 2020). These ingredients are rich in essential and nonessential amino acids; however, most of these proteins are subjected to high temperatures during rendering, which impairs their digestibility and amino acid bioavailability. Animal protein meal industries have increased the production of hydrolyzed meals to improve the processing of these ingredients, especially via enzymatic hydrolysis, which yields peptides with bioactive properties.

Bioactive peptides.

BPs are small molecules (0.5 to 5 kDa) ranging from 3 to 50 amino acid residues linked together (tripeptides and oligopeptides) in different combinations and arrangements, with various biological roles (Hou et al., 2017). Endogenous peptides are produced by different glands and cells in the body. On the other hand, exogenous peptides can be provided via food, dietary additives, and medications (Lorenzo et al., 2018). The digestive process of BPs is facilitated by greater exposure to endogenous digestive enzymes.
Metabolism of BPs and their health benefits.

After food intake, proteins are first hydrolyzed by pepsin in the acidic environment of the stomach (pH 1.5 to 3). However, pepsin is an endopeptidase and does not break down protein into free amino acids. Therefore, products of pepsin reaction are partially hydrolyzed proteins and large peptides that will be further hydrolyzed by enteric (enterokinase) and pancreatic endo- (trypsin, chymotrypsin) and exo-peptidases (carboxypeptidases) in the intestine. After this step, free amino acids, small peptides (di- and tri-peptides), large peptides (oligopeptides), and, eventually, proteins that were not significantly hydrolyzed by pepsin and pancreatic enzymes (hair, feathers, and others resistant to endogenous enzymatic digestion) pass through the intestine. During the passage through the small intestine, they contact the brush border, and the membrane digestion phase begins. Peptidases in the brush border hydrolyze peptides into free amino acids, di- and tri-peptides, or oligopeptides. These molecules can then be absorbed via one of the four absorption pathways (Figure 2), hydrolyzed inside the enterocyte (intracellular digestion), or pass into the bloodstream, reaching target organs as peptides or free amino acids.

When ingested, peptides play roles in metabolism, interfering with inflammatory responses, blood pressure, weight control, and glycemic response. The activity of BPs depends on the electrostatic charge, chain size, amino acid sequences in the peptide chain, and molecular surface hydrophobicity. There are four pathways by which BPs are taken up: paracellular diffusion, transcellular passive diffusion, transcytosis, and carrier-mediated transport, which depend on chemical and physical properties (Amigo and Hernández-Ledesma, 2020).

Paracellular diffusion occurs by increasing the permeability of the junctions among enterocytes (tight junctions). In transcellular passive diffusion, the uptake depends on peptide hydrophobicity and charge neutrality. Transcytosis is a process of endocytosis by which peptides are absorbed by the cell with energy expenditure and subsequently processed inside the enterocyte. The last pathway, carrier-mediated transport, occurs via facilitated diffusion or secondary active transport. The PEPT1 pathway is the most common and is responsible for absorbing high amounts of the hydrolyzed peptides during digestion in mammals and birds (Miner-Williams et al., 2014).

Many BPs, such as the antihypertensive peptides extracted from pigs (RPR, KAPVA, and PTPVP), can resist the intestinal membrane protease action and reach their targets intact (Escudero et al., 2012); however, there are no estimates on the amount of BPs that escape gastrointestinal and enterocyte hydrolysis.

Due to the abundant expression and high capacity of the peptide carrier (PEPT1) in the small intestine, the rate of luminal absorption of peptides is higher than that of free amino acids (Vij et al., 2016). However, PEPT1 does not transport large peptides; its role is limited to di- and tri-peptides, regardless of the amino acid sequence. Before being transported to the bloodstream, BPs can influence the gut health. For instance, LKPT peptide stimulates the GLP-1 secretion in the ileum, by enteroendocrine cells (Thysgeur et al., 2020). As the GLP-1 influences central nervous system to decrease the permeability in the colon (Funayama et al., 2023), the consumption of BPs can favor the gut health in animals with chronic enteropathies as suggested by Meineri et al. (2022).
In addition to the intestinal health effects some animal BPs possess during digestion, other post-absorption effects have also been investigated. The scientific literature has reported studies performed to assess the effects of BPs as antioxidants (Hou et al., 2017), antihypertensive (Zóia Miltenburg et al., 2021), antimicrobial (Vidal et al., 2022), anti-inflammatory, and anxiolytics.

Immuno-logical activity.

Depending on their physicochemical characteristics, BPs may have both immunostimulatory and immunosuppressive activities. In humans, egg-derived peptides showed an immunostimulatory effect in chemotherapy patients (Mine and Kovacs-Nolan, 2006). In addition, peptides hydrolyzed from bovine sarcoplasmic proteins showed cytotoxic effects against breast cancer cells and inhibited gastric cell proliferation (Jang et al., 2008). Despite their relevance, these effects have not yet been studied in dogs and cats.

Protein hydrolysates have been extensively used in hypoallergenic diets for dogs and cats because they do not stimulate immune reactions due to their low molecular weight; thus, hypersensitivity symptoms are reduced (Olivry et al., 2017).

Osteoarthritis is a degenerative joint condition that affects humans and dogs as well. Dogs are mainly affected by joint dysfunctions and hydrolyzed collagen peptides have been extensively used to develop products for pet food that benefit joints; however, these effects are still to be proven. Schunck et al. (2017) observed improved chondrocyte function using hydrolyzed collagen in dogs. In this study, the authors observed an improvement in the biosynthesis of type II collagen and elastin compared to the control group. In another study (Ruff et al., 2016), hydrolysates from the eggshell and membrane improved the mobility of dogs with arthritis. On the other hand, chicken liver hydrolysate did not modify any immunological parameters in healthy dogs, especially those related to more intense and allergic inflammatory responses (Pinto et al., 2022). Many benefits are attributed to the hydrolyzed collagen, such as increasing bone strength and density, decreasing the extracellular matrix and the markers of joint degeneration, inhibiting inflammatory cytokines, and improving joint stability.

Antioxidant activity.

The antioxidant activity of BPs also depends on peptide chain, amino acid composition, and hydrophobicity. Most antioxidant peptides range from 4 to 16 amino acid residues with molecular weights ranging from 0.4 to 2 kDa (Zaky et al., 2022). Enzymes alter the antioxidant effects of BPs during hydrolysis. Saiga et al. (2003) observed greater antioxidant activity using the linolenic acid peroxidation system when peptides were obtained using actinase E compared to papain in the hydrolysis of porcine myofibrillar proteins. In another study, collagen from pig skin was hydrolyzed using three different enzymes to obtain antioxidant peptides (Li et al., 2007). However,
Antioxidant effects were not observed in a study with adult dogs (Pinto et al., 2023) fed diets formulated with hydrolyzed chicken liver as the main protein source (24%, 32%, and 40% crude protein). Like other antioxidant compounds, BPs quench or eliminate free radicals and synergize with other antioxidant compounds. The advantage is that they are also nutrients and act as antioxidant additives. Recently, Hu et al. (2020) assessed the effects of two plant hydrolysates (hydrolyzed corn gluten meal and hydrolyzed distiller’s dried grains with solubles using neutrases or alkalases) on the oxidation of corn and fish oils and reported a similar effect compared to the synthetic antioxidant butylated hydroxytoluene. This result brings new perspectives on using hydrolysates as antioxidants.

Antimicrobial activity.

Antimicrobial BPs (AMBPs) are classified according to their mechanism of action into short AMBPs (20 to 46 amino acid residues), basic AMBPs (rich in lysine or arginine), and amphipathic AMBPs that are commonly abundant in hydrophobic residues (leucine, isoleucine, valine, phenylalanine, and tyrosine; Haney and Hancock, 2013). The mechanism of action of AMBPs differs from that of conventional antibiotics. Like other BPs, AMBPs depend on the structure, composition, and amino acid sequencing to be effective on their targets, which can be Gram-positive or Gram-negative bacteria, fungi, or viruses (Reddy et al., 2004). However, AMBPs possess a higher bactericidal than bacteriostatic effect and play a potent immune modulator role (Kumar et al., 2020).

Antimicrobial resistance to antibiotics is a human and animal health issue; thus, AMBPs may be a viable alternative. Unlike conventional antibiotics, AMBPs bind to the membranes of microorganisms by electrostatic interactions without killing them until reaching the threshold level, hindering and reducing the development of resistance (Hollmann et al., 2018). Furthermore, hydrolyzed animal proteins can improve the intestinal health of pets. Diets containing hydrolyzed meat proteins have been effective in the long-term treatment of chronic enteropathies in dogs and can help regulate the gut microbiota.

Antihypertensive activity.

The antihypertensive peptides obtained from protein hydrolysates, especially from animal protein, are of great research interest. One of the most studied BPs in humans and animals is angiotensin-converting enzyme (ACE) inhibitors. Hypertension affects one in three adult people worldwide. More than 1.3 billion people take medications, and 4 out of 5 people are not treated. However, in animals, especially pets such as dogs and cats, cases of hypertension are rarely diagnosed, and ACE inhibitors are used for other purposes, largely for treating chronic kidney and heart diseases.

ACE accelerates the conversion of angiotensin I to angiotensin II, which promotes vasoconstriction and increases blood pressure. Hydrophobicity plays an important role in many functional and bioactive properties of food-derived peptides. C-terminal aromatic residues and N-terminal hydrophobic residues provide antihypertensive BPs with high activity in blocking the ACE function (Acquah et al., 2018). Antihypertensive drugs (ACE inhibitors) such as captopril, fosinopril, and ramipril have several side effects. As a result, alternatives, such as food-derived BPs ACE inhibitors to treat blood pressure, are commercially available (Hartmann and Meisel, 2007). Most studied in humans is the hydrolysis of milk protein, meat, fish, and egg to produce antihypertensive BPs. In companion animals, protein hydrolysates to inhibit ACE is recommended for patients suffering from heart disease and chronic renal failure in more advanced stages. Zóia Miltenburg et al. (2021) compared the effects of feeding cats two diets containing 25% conventional or enzymatically hydrolyzed poultry byproduct meal and observed that hydrolyzed meal reduced serum ACE activity by 11.2% (P < 0.10). These authors also assessed in vitro inhibitory effects on the ACE activity and observed 90% and 52% of ACE inhibition for the hydrolyzed and conventional meals, respectively. Changes in hydrolysis and peptide purification and improved peptide bioavailability could increase effectiveness in animals.

Glycemic control.

Type 2 diabetes mellitus (DM-2) is an obesity-related disease that affects approximately 9% of the human population and has a similar prevalence in pets. Among the therapeutic possibilities, dipeptidyl peptidase IV (DPP-IV) inhibitors, such as linagliptin, sitagliptin, and saxagliptin, have been used in cases refractory to metformin and other drugs, showing promising results. Recent studies have demonstrated that animal protein hydrolysates are a source of BPs with potent action to control blood pressure and glycemic response, with similar activity to those seen in ACE and DPP-IV inhibitors. Using an in vitro digestive system adapted for dogs, Thyssegeur et al. (2020) reported that tilapia protein hydrolysate has a potent DPP-IV inhibitory activity compared to the conventional (non-hydrolyzed) tilapia protein.

Although no in vivo study that assessed the DPP-IV inhibitory capacity of protein hydrolysates is found, Furrer et al. (2010) reported increased insulin secretion (about 20%) and reduced plasma glucagon secretion (36.7%) in young and healthy cats undergoing intravenous glucose tolerance and postprandial glycemic testing when administered DPP-IV inhibitor (NVP-DPP728, 0.5 mg/kg intravenously or 1 mg/kg subcutaneously). These peptides have two possible mechanisms of action: the first is related to the GLP-1 analog, and the second to their inhibitory effect on the DPP-IV. This result suggests that DPP-IV inhibitors benefit glycemic index in cats; therefore, protein hydrolysates could be used for this purpose. Figure 3 illustrates the main applications of protein hydrolysates containing BPs in dog and cat nutrition.
Palatability.

The strong bitter taste of protein hydrolysates has been reported to possibly limit intake in dogs and cats. However, the results of more recent palatability studies have shown otherwise. Pinto et al. (2023) and Zóia Miltenburg et al. (2021) reported that poultry byproduct hydrolysates were preferred over diets containing conventional poultry byproduct meal by dogs and cats, respectively.

Conclusion

The pet food industry has increased the production of enzymatically hydrolyzed ingredients, targeting digestibility and palatability improvements, as well as the ecological paw print. Furthermore, studies with hydrolyzed ingredients focus on assessing functionality, especially immune, antioxidant, blood pressure control, and prebiotic effects. However, scientific evidence on the beneficial effects of BPs on these functions is still limited. The increasing number of publications in recent years will contribute to developing new products, especially those targeting ill dogs and cats.

Acknowledgments

This manuscript was invited for submission by the American Society of Animal Science. The views expressed in this publication are those of the author(s) and do not necessarily reflect the views or policies of the American Society of Animal Science, the journal, or the publisher.

Conflict of interest statement

The authors report no real or perceived conflict of interest.

References


Downloaded from https://academic.oup.com/af/article/14/3/38/7696635 by guest on 25 June 2024

This page has been downloaded from our Open Access journal. It is available for personal use only. Commercial use is prohibited.
Antioxidant activity of peptides


About the Authors
Ricardo Souza Vasconcellos is an Associate Professor of the Animal Science Department at the State University of Maringá (UEM), in Southern Brazil. Graduated in Veterinary Medicine from the Federal University of Viçosa (UFV Brazil), he is PhD in Animal Nutrition, with emphasis on Dog and Cat Nutrition. He is a Professor and advisor in two Graduate Programs linked to the State University of Maringá. He also has worked in a Brazilian Pet Food Company, in the R&D department. The main research topics that he is working, are as follows: shelf life evaluation methods in Pet food; Quality of animal by product meals and animal fats as ingredient for Pet food; and Tools for the evaluation of Pet food sustainability. Corresponding author: ricardo.souza.vasconcellos@gmail.com
Josiane Aparecida Volpato is a PhD student in Animal Science, with an emphasis on Dog and Cat Nutrition at the State University of Maringá, in southern Brazil. She graduated in Animal Science from the State University of Mato Grosso do Sul (UEMS Brazil). She worked at a Brazilian Pet Food company, as Technical Manager. She works with feed formulation and food development for dogs and cats. The main research subjects are the following: quality of animal byproduct meals and animal fats as ingredients for pet food; Palatability of ingredients and foods for dogs and cats; Digestibility and In Vitro Fermentation; melanoidins extracted from the animal byproduct meals and their effects on the cat health.

Ingrid Caroline Silva is a PhD student in Animal Science at the State University of Maringá, with an emphasis on Dog and Cat Nutrition at the State University of Maringá, in southern Brazil. She is graduated in Animal Science from the State University of Ponta Grossa. Her research subjects are in sensory aspects of extruded cat foods with different ingredients; lipid oxidation; shelf life of food for dogs and cats; production of wet and dry Pet foods containing fresh or dried animal byproducts.