Effects of Milk-Derived Bioactive Peptides on Animal Intestinal Function and Mechanism of Action: Postprint

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Abstract

Milk-derived bioactive peptides (MDBPs) refer to relatively short, small-molecular-weight peptides present in milk that possess specific amino acid sequences and spatial structures, and participate in various metabolic pathways and physiological processes of the organism. Recent studies have demonstrated that MDBPs can influence nutrient absorption in the intestinal mucosa of animals, alleviate intestinal oxidative stress, improve the intestinal microecological environment, and enhance intestinal immunity. This review will focus on the effects of MDBPs on animal intestinal function and their underlying mechanisms of action.

Full Text

Effects and Mechanisms of Milk-Derived Bioactive Peptides on Animal Intestinal Function

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Abstract

Milk-derived bioactive peptides (MDBPs) are relatively short peptide fragments present in milk that participate in various metabolic pathways and physiolog-

ical processes, possessing specific amino acid sequences and spatial structures. Recent studies have found that MDBPs can influence nutrient absorption by the intestinal mucosa, alleviate intestinal oxidative stress, improve the intestinal microecological environment, and enhance intestinal immunity. This review summarizes the effects and mechanisms of MDBPs on animal intestinal function.

Keywords: milk-derived bioactive peptides; animal intestine; mechanism; oxidative stress; intestinal immunity

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Protein is an essential nutrient for maintaining life activities, and it is ultimately digested into amino acids or small peptides for absorption in the intestine. Studies have shown that small peptides can be absorbed intact with the assistance of intestinal transporters, characterized by rapid absorption rates, low carrier saturation, low energy consumption, and high efficiency. Moreover, supplying amino acids in peptide form can avoid the inhibitory effects caused by competition for binding sites among free amino acids, thereby promoting the absorption and utilization of protein feedstuffs[1]. Consequently, the nutritional functions and mechanisms of small peptides have attracted widespread attention in the fields of animal nutrition and medicine.

Bioactive peptides are relatively short molecular fragments (typically 2-9 amino acid sequences) with specific amino acid arrangements and spatial structures that possess various physicochemical properties and exert positive effects on multiple metabolic pathways and physiological processes. A common structural feature of bioactive peptides is the presence of hydrophobic amino acid residues, excluding combinations composed of proline, lysine, or arginine[2]. For young animals, maternal milk is a crucial source of nutrition and partial immune capacity. Milk-derived bioactive peptides (MDBPs) are low-molecular-weight peptides primarily prepared through protease hydrolysis of whey proteins and casein (CN) in milk or via microbial fermentation of dairy products[3]. Research has reported that MDBPs exhibit various physiological functions, including promoting intestinal absorption and utilization of mineral elements, regulating immunity, antithrombotic effects, antihypertensive activity, opioid-like activity, and antimicrobial properties[2-4].

Recent studies have demonstrated that MDBPs can promote nutrient absorption by intestinal epithelial cells, alleviate oxidative stress induced by weaning or

inflammatory bowel disease (IBD) in piglets, inhibit various harmful pathogenic bacteria to improve the intestinal microecological environment, and enhance the intestinal immune barrier function by promoting the proliferation and functional activity of macrophages and lymphocytes[1-5]. This review will focus on the effects and mechanisms of MDBPs on animal intestinal function.

1 Transport and Absorption of MDBPs in the Intestine

The intestine is the primary site for nutrient digestion and absorption, and its folded mucosa and villi greatly increase the contact area with digesta. Dietary proteins entering the intestine are hydrolyzed into small peptides and free amino acids. While amino acid transport is mediated by specific amino acid transporters, small peptide absorption primarily depends on high-capacity, low-affinity peptide transporters (PEPT) and their related peptide transporter receptors, which possess broad substrate tolerance and can transport structurally related compounds and drugs[5].

Intestinal absorption of small peptides primarily relies on the PEPT family (mainly PEPT1 and PEPT2)[6], and MDBPs are absorbed similarly to other small peptides. Vij et al.[7] demonstrated that Caco-2 cells can express PEPT1-like receptors involved in transporting the casein hydrolysate peptide VLPVPQK. PEPT1 is abundantly expressed in the duodenum, jejunum, and ileum of the small intestine, but is normally not expressed in the colon[7-8]. PEPT1 is expressed in highly differentiated intestinal epithelial cells (such as villus tips) but not in low-differentiated cells (such as crypts).

PEPT1 plays a major role in small peptide absorption. Daniel et al.[9] reported that PEPT1 mRNA and protein expression are regulated by miRNA-92b, which can inhibit pro-inflammatory responses in intestinal epithelial cells induced by bacterial-derived peptides by suppressing PEPT1 expression. STE20-related proline/alanine-rich kinase (SPAK) is a key regulator of renal tubular ion transport and blood pressure, and Warsi et al.[10] found that it can regulate the expression of PEPT1 and PEPT2 in the intestine. Additionally, the oxidative stress-related kinase oxidative-stress-responsive kinase 1 (OSR1) can downregulate PEPT1 and PEPT2 expression by reducing carrier protein abundance in the cell membrane, thereby affecting intestinal absorption and causing intestinal dysfunction[11]. The intact transport and absorption of small peptide fragments via carriers is fundamental for MDBPs to maintain their biological activity and exert physiological functions.

2.1 Effects on Intestinal Nutrient Absorption

Carbohydrates are the primary source of energy for animal organisms. Studies have shown that MDBPs can promote insulin secretion, accelerate glucose uptake into the liver, reduce postprandial blood glucose levels, and thereby promote peptide chain elongation and protein synthesis. The conserved amino acid sequence at positions 60-70 of -casein, Tyr-Pro-Phe-Pro-Gly-Pro-Ile-Pro-

Asn-Ser-Leu, exhibits opioid peptide activity and serves as the precursor of -casomorphins (-CMs). The enzymatic hydrolysis product -CM-7 has the amino acid sequence Tyr-Pro-Phe-Pro-Gly-Pro-Ile.

Research has shown that -CM-7 can downregulate the gene expression of intestinal glucose transporters (GLUT), reduce Na+-K+-ATPase activity, inhibit glucagon secretion while promoting insulin secretion, and increase cellular glucose consumption to lower blood glucose levels[12-13]. Zoghbi et al.[14] reported that -CM-7 can strongly stimulate intestinal mucin secretion through neural pathways and activation of opioid receptors. However, Zhang et al.[15] reported that -CM-7 can inhibit high glucose-induced epithelial-mesenchymal transition in proximal renal tubular cells by affecting the secretion of angiotensin II (Ang II) and transforming growth factor 1 (TGF- 1), rather than through opioid receptors. Additionally, studies have reported that -CM-5, with the amino acid sequence Tyr-Pro-Phe-Pro-Gly, can influence intestinal digestion and nutrient transport rates[16]. -CMs possess a conserved -Phe-Pro-Tyr-NH2 structure that confers resistance to enzymatic degradation, maintaining stable biological activity. These findings demonstrate that -CMs can affect glucose absorption by intestinal epithelial cells, nutrient transport, and promote digestive enzyme secretion and related gene expression.

Trace element absorption is crucial for protein synthesis, energy metabolism, and enzyme function in animal organisms. Caseinophosphopeptides (CPPs) exhibit strong mineral-binding capacity and can enhance intestinal absorption and bioavailability of trace elements such as calcium, iron, zinc, copper, and manganese. Most CPPs share common structural features, such as the -Ser(P)-Ser(P)-Ser(P)-Glu(E)-Glu(E)- sequence motif[17], which enables MDBPs to bind calcium, phosphorus, and other trace elements under intestinal pH conditions. Phosphopeptides possess clustered -Ser(P)-Ser(P)-Ser(P)-Glu(E)-Glu(E)- sequences with negatively charged side chains, particularly phosphate groups on these amino acids, providing binding sites for trace elements.

García-Nebot et al.[18] found that CPPs can enhance the bioavailability of iron and zinc in Caco-2 cells, and subsequently compared the iron-chelating capacity of CPPs derived from -CN and -CN hydrolysis, revealing that -CPPs exhibited stronger iron chelation than -CPPs at a concentration of 12.5 mol/L[19]. Boutrou et al.[20] reported that digestion of different -CN(1-25) chelates with intestinal brush border membrane vesicles showed that mineral-CPP binding could inhibit peptidase and phosphatase activities. LI Yao et al.[21] investigated the effects of small peptide-chelated trace elements on piglet development and found that small peptide chelation of multiple trace elements could improve trace element utilization and enhance piglet production performance. Supplying trace elements in the form of small peptide chelates in diets can significantly ameliorate symptoms of trace element deficiency in animals[22].

2.2 Alleviating Intestinal Oxidative Stress

The redox balance in the intestine is crucial for intestinal function and the survival of various intestinal microorganisms. Intestinal redox homeostasis is primarily maintained by the glutathione redox system (GSH/GSSG), the thioredoxin redox system (Trx/TrxSS), and the cysteine redox system (Cys/CySS). Reactive oxygen species (ROS) are normal byproducts of cellular metabolic activities, but excessive ROS can cause DNA damage, lipid peroxidation, protein structural damage, mitochondrial dysfunction, and induce apoptosis. Superoxide dismutase (SOD), glutathione peroxidase, and catalase (CAT) are involved in protecting cells from the destructive effects of ROS[23]. Animals subjected to weaning stress, inflammatory bowel disease, and other challenges are prone to intestinal oxidative stress, leading to intestinal dysfunction[24]. Weaning stress causes significant increases in malondialdehyde (MDA), nitric oxide (NO), and hydrogen peroxide (H2O2) concentrations, while superoxide dismutase activity decreases markedly, with downregulated expression of antioxidant-related genes and increased expression of ROS-producing genes[25]. IBD is caused by excessive release of ROS and free radicals from intestinal mucosal macrophages and neutrophils, which inhibits the activity of key enzymes maintaining redox homeostasis (such as superoxide dismutase)[26].

Studies have shown that enzymatic hydrolysates of whey protein and casein possess strong antioxidant activity, effectively scavenging ROS and free radicals and preventing lipid peroxidation[27-32]. Zhang et al.[27] suggested that the hydrophobicity of whey protein hydrolysates is responsible for their antioxidant capacity and resistance to oxidative damage. The hydrophobic residues at the termini of MDBPs readily bind to unsaturated fatty acids, thereby inhibiting lipid peroxidation[28]. Cheison et al.[29] found that the content of aromatic amino acids in whey protein hydrolysates positively correlates with antioxidant capacity, with higher content conferring stronger antioxidant activity. Casein hydrolysates can inhibit lipoxygenase activity, thereby preventing lipid peroxidation. Small peptides isolated from casein exhibit stronger scavenging abilities against diphenylpicrylhydrazyl (DPPH), hydrogen peroxide radicals, and superoxide anions compared to glutathione and carnosine[30]. Furthermore, -CM-7 and -CM-5 and other -CMs can significantly enhance the activity of antioxidant enzymes in intestinal mucosa and alleviate oxidative stress-induced damage to intestinal epithelial cells[3-4,12,31].

MDBPs typically exhibit stronger antioxidant activity when their composition is rich in histidine (His), tyrosine (Tyr), and cysteine (Cys), or when they contain phenylalanine (Phe), valine (Val), isoleucine (Ile), and leucine (Leu) at their termini[32]. MDBPs are of significant importance for alleviating intestinal oxidative stress in weaned piglets; however, the specific molecular mechanisms by which MDBPs relieve intestinal oxidative stress require further investigation.

2.3 Effects on Intestinal Microecological Environment

Microorganisms and mammals have co-evolved in a highly synergistic relationship, with the host immune system playing a crucial role in maintaining the homeostasis of resident microbial communities, effectively ensuring a mutually beneficial relationship while microorganisms promote the maturation of the host immune system[33]. Intestinal commensal flora can promote the development and maturation of the intestinal mucosal immune barrier, while the clearance of harmful microorganisms and pathogens depends on antimicrobial defensins secreted by Paneth cells (such as porcine -defensins[34]). Research has found that weaning can cause increased concentrations of harmful bacteria such as Escherichia coli and decreased concentrations of beneficial bacteria such as Lactobacillus in piglet intestines[35]. Imbalance in the intestinal microecological environment often leads to indigestion or diarrhea in pigs and induces IBD[36], with enterotoxigenic Escherichia coli (ETEC) proliferating extensively. Studies have shown that ETEC can affect the expression of tight junction proteins and their encoding genes, thereby causing damage to intestinal epithelial cells[37]. Further research indicates that ETEC can reduce the transmembrane resistance of intestinal epithelial IPEC-J2 cells and activate Caspase-3, triggering apoptosis[38-39].

MDBPs can effectively inhibit the activity of Gram-negative (G−) and Gram-positive (G+) bacteria, particularly demonstrating strong inhibitory capacity against foodborne pathogenic bacteria in the intestine. Studies have shown that antimicrobial peptides isolated from lactoferrin hydrolysates contain high levels of Cys and alanine (Ala), exhibiting significant inhibitory effects against Gram bacteria (including both G− and G+)[40]. In contrast, antimicrobial peptides isolated from casein hydrolysates contain high levels of proline (Pro) and show good inhibitory effects against Staphylococcus aureus and Escherichia coli[41]. Most lactoferrin-derived antimicrobial peptides consist of fewer than 50 amino acids and contain numerous hydrophobic and basic amino acids[42]. Peptide segments rich in Cys or with Ala at both termini can effectively enhance inhibitory capacity against pathogenic bacteria. Furthermore, the secondary structure of MDBPs also affects antimicrobial activity, with -sheet structures demonstrating stronger inhibitory effects against Gram bacteria (G− and G+) compared to -helical structures[43].

Yoon et al.[44] found that dietary supplementation with the synthetic antimicrobial peptide AMP-P5 could improve the growth performance of weaned piglets, significantly promote intestinal nutrient absorption, and reduce diarrhea rates. Jiang et al.[45] demonstrated that dietary supplementation with glycine (Gly)-glutamine (Gln) could alleviate growth inhibition and immune dysfunction in early-weaned piglets induced by Escherichia coli lipopolysaccharide (LPS).

The primary mechanism by which MDBPs inhibit microorganisms is the disruption of target cell membranes. Reddy et al.[46] found that antimicrobial peptides can form pore-like channels through a helical aggregation model, utilizing their

hydrophobic surfaces to interact with membrane phospholipid acyl side chains to create aqueous pores, which are then stabilized and enlarged by recruiting additional antimicrobial peptides, ultimately leading to leakage of intracellular contents. However, only antimicrobial peptides with appropriate size, optimal helical angle, and amphipathic properties can adopt the helical aggregation model; most antimicrobial peptides employ the toroidal pore model[47], where multiple peptides first aggregate parallel to the cell membrane and, upon reaching a threshold concentration, insert into the membrane to form ring-shaped pores, causing target cell disintegration as antimicrobial peptide concentration and pore number increase. Additionally, antimicrobial peptides can kill microorganisms by inhibiting DNA and RNA synthesis, suppressing protein and enzyme synthesis and activity, inhibiting Na+-K+-ATPase activity to affect energy supply, or by inhibiting microbial cell wall formation. The specific regulatory mechanisms of milk-derived antimicrobial peptides in improving the intestinal microbial environment remain unclear and require further investigation.

2.4 Effects on Intestinal Immune Function

The intestine is not only the site of nutrient digestion and absorption but also the largest immune organ in the body. When antigens enter the intestinal barrier, intestinal immune cells phagocytose them and trigger the host immune defense mechanisms. Lymphocytes are major components of the active immune system in animals and play an important role in the intestinal mucosal immune barrier. The coordination of T lymphocyte subset ratios is crucial for immune function in mammals, while the proliferation and differentiation of lymphocytes in the intestinal lamina propria affect intestinal immune tolerance and response.

MDBPs can regulate lymphocyte proliferation, promote macrophage phagocytosis, and downregulate the production of certain lymphokines, thereby modulating the development of the immune system in young animals[3]. Caseinoglycomacropeptide (CGMP), derived from -casein hydrolysis, can regulate T lymphocyte subset balance, maintain stable intestinal immune function, and modulate the intestinal microecological environment to indirectly enhance intestinal immunity[48-49]. Zhou et al.[50] reported that the QEPVL (Gln-Glu-Pro-Val-Leu) sequence from MDBPs can significantly enhance lymphocyte activity both in vitro and in vivo, increase lymphocyte proliferation rates, and elevate antimicrobial peptide concentrations. QEPVL can also inhibit LPS-induced inflammation by regulating nitric oxide release and the production of cytokines interleukin-4 (IL-4), interleukin-10 (IL-10), interferon- (IFN-), and tumor necrosis factor- (TNF-). Additionally, MDBPs derived from -casein hydrolysis can promote macrophage phagocytosis and improve related inflammatory responses[1]. Moreover, -CM-7 exhibits opioid peptide activity and can upregulate the expression of lymphocyte-related genes in the intestine, enhancing intestinal mucosal barrier function[3-4,14]. Many MDBPs with opioid and immunomodulatory activities can activate relevant signaling pathways and

receptors, enhance cytokine concentrations, activate immune-related enzymes, and thereby promote immune cell proliferation and differentiation, improving overall animal immunity[3-4].

In summary, MDBPs can promote intestinal nutrient absorption, alleviate oxidative stress induced by weaning or enteritis, inhibit harmful intestinal pathogens, improve the intestinal microecological environment, promote intestinal immune cell proliferation, and enhance macrophage phagocytosis. In-depth research on the mechanisms of MDBPs' effects on intestinal health is not only important for animal nutrition and production but also provides valuable reference for human intestinal health. Compared to chemical drugs, MDBPs are natural and side-effect-free, offering broad application prospects for treating human intestinal diseases and cardiovascular disorders. Currently, research on MDBPs requires further in-depth investigation, and future studies should integrate molecular biology with omics technologies and modern microscopy to analyze and explore the structure and detailed mechanisms of action of MDBPs.

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