EXPLORING THE IMPACT OF GLYCEROL MONOLAURATE ON GUT HEALTH: A REVIEW OF IN VIVO STUDIES

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ABSTRACT

Glycerol monolaurate (GML), a naturally occurring medium-chain monoglyceride, has attracted increasingly interest due to its special antivirus ability and potential benefits on gut health. This review focuses on animal research to explore how GML supplementation impacts intestinal morphology, gut microbiota composition, and immune function. Studies show that GML could enhance the structure of the villus, which may in turn lead to better nutrient absorption. GML has been shown to resemble properties of prebiotics due to its ability to regulate microbial diversity of the intestines for favorable microbiota such as *Barnesiella* or *Bacteroides* and to enhance production of short chain fatty acids. The results have shown that GML exhibits anti-inflammatory activities through its capacity to lower pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, increase anti-inflammatory cytokines like IL-10 and TGF-β1, and enhance expressions of tight junction proteins.

BIOGRAPHICAL SKETCH

Xuanni Chen was born in Shenzhen, China. She got her bachelor degree in Guangdong Technion-Israel Institute of Technology, Guangdong Province, China, majored in Biotechnology and Food Engineering. As an undergraduate student, she involved in the project of developing walnut peptide nanoparticles in the food chemistry lab. She began her study in Food Science focusing on gut health at Cornell University under the guidance of Dr. Elad Tako in 2023. Xuanni loves exploring dietary supplements and functional foods that can improve people's health. She wants to research how the foods we eat impact overall wellness and find new ways that nutritional products can lead to better health outcomes. Her goal is to develop practical dietary supplement and functional food solutions that make a positive difference.

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INTRODUCTION

Monoglycerides, dietary lipids, are amphiphilic or dual-atomic molecules possessing a glycerin head joined with a saturated fatty acid by an ester bond. Unlike the hydrophilic glycerol head and the hydrophobic fatty acid tail, monoglycerides interact well with water and fats. Ultimately, monoglycerides are excellent multifunctional molecules and having emulsification ability and potential bioactivity because of their dual natural tendency. Glycerol monolaurate (GML) or monolaurin ($C_{15}H_{30}O_4$), commonly obtained from human breast milk, coconut oil, and palm kernel oil, is a medium-chain monoglyceride. GML can be synthesized by lauric acid and glycerol reactions with the same chemical structure and function as cellular occurring ones (Khandara et al., 2024). GML is generally recognized as safe (GRAS) by the USA's FDA (Food and Drug Administration), implying its safety for human consumption in the aim of different food preparations.

In the form of monoglycerides, GML from coconut oil can be taken up by gut cells without the need to undergo any further hydrolyzation (Dayrit, 2015). While the exact mechanisms of GML absorption remain under investigation (X. Luo et al., 2022), research suggests the possibility of both direct absorption of intact GML and absorption of lauric acid, its hydrolysate, which may be further metabolized or potentially re-esterified to GML within the body (Dayrit, 2015). This flexibility in metabolic pathways and the unique ability of lauric acid to bypass the lymphatic system for direct transport to the liver constitute the potential for GML to exert diverse effects. A

study has found that GML can be detected from the human blood sample, demonstrating its presence throughout the systemic circulation and calling for further inquiry regarding its possible systemic roles (Barberis et al., 2021). These extraordinary properties make GML an object of opportunity regarding promoting gut health and overall physiological functions.

GML features multispecies antibacterial activity against prevalent bacteria, fungi, and protozoa in vitro (Jackman et al., 2020; Jiang et al., 2018; C. Luo et al., 2014). The possible mechanisms of GML to kill bacteria are a). attacking cellular membranes and interfering with cellular processes, particularly Gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes* affected (Schlievert & Peterson, 2012); b). the equal ability to interfere with signal transduction and transcription (Schlievert et al., 1992); and c). block the process of exotoxin synthesis and, thus, disable the bacteria (Schlievert et al., 1992). GML can similarly stimulate the effectiveness of other antimicrobial agents like EDTA when used in synergism (Schlievert & Peterson, 2012). Research has identified GML as a key contributor to the antimicrobial properties of human breast milk that make oral consumption potentially similarly effective (Schlievert et al., 2019).

The research on GML's effectiveness against coronaviruses, including SARS-CoV-2 (the virus that brought about COVID-19), still needs to be elucidated. On the bright side, this broad-spectrum antiviral property exhibited by GML against other enveloped viruses points to possible areas of exploration. Interference by GML with the lipid envelope that is vital in terms of viral entry and infectivity implicates coronaviruses due to them also possessing a lipid envelope layer

(Aldridge, 2020). This mechanism and GML's demonstrated dual ability to hinder viral replication and assembly in other viruses point to similar outcomes for coronaviruses (Welch et al., 2020). This potential extends beyond coronaviruses, encompassing the full spectrum of the enveloped viruses. Until now, GML has demonstrated efficacy against Herpes Simplex Virus (HSV), influenza viruses, Human Immunodeficiency Virus (HIV), measles virus, African Swine Fever Virus (ASFV), Yellow Fever Virus (YFV), and zika virus in both in vitro and in vivo studies (Thormar et al., 1987; Aldridge, 2020; Welch et al., 2020).

Most GML products in the current market propaganda themselves as immune support dietary supplements. Despite the absence of human data, GML exhibits a complex and concentration-dependent influence on the immune function in vitro. At low concentrations (around 0.1 μg/mL), GML acts as an immunostimulant, primarily targeting T lymphocytes. It stimulates the growth of T cells and subsequently improves host immunity through adaptive resistance mechanisms (Witcher et al., 1996). Nevertheless, GML's role extends beyond simply boosting immune activity. It demonstrates anti-inflammatory properties by modulating the production and expression of pro-inflammatory cytokines like IL-1α (Interleukin-1 alpha), IL-6 (Interleukin-6), IL-18 (Interleukin-18), TNF-α (Tumor Necrosis Factor-alpha), and IFN-γ (Interferon-gamma) in vitro (Witcher et al., 1996; Silva et al., 2018). This implies a delicate equilibrium whereby GML fine-tunes the immune response, promoting efficient defense against pathogens while preventing excessive inflammation and potential tissue damage. GML's interaction with human serum albumin appears to contribute to its regulatory effects on T cell signaling and cytokine production (M. S. Zhang & Houtman, 2016). The fine balance between immune stimulation and

anti-inflammatory cytokine production embodies the therapeutic potential of GML, which may serve as a protective agent in such conditions.

This review is the primary goal of assessing animal studies systematically and synthetically in search of novel insight into the effect of GML on gastrointestinal health. Although a mountain of information has been collected on GML antimicrobial properties and its suspected influence on the digestive system, there remains a significant gap in the literature concerning its comprehensive effects on gut health. In this review, we aspire to eliminate the void by assessing the efficiency, mode of action, and safety of GML, considering gut microbiota, intestinal barrier and factors of inflammation and immunity. This review is centered on introducing GML's medicinal potential, which should steer the next investigative studies in the new field of gut health.

MATERIALS AND METHODS

A search was conducted using PubMed, Scopus, and Science Direct databases to identify relevant studies published up to April 20, 2024. The following keywords are used for the searching: (glycerol monolaurate OR monolaurin) AND (intestinal morphology OR villus OR crypt depth OR gut microbiota OR microbiome OR microbial composition OR immune function OR cytokine OR inflammation OR tight junction protein OR short-chain fatty acid OR SCFA). The search was limited to articles published in English. A total of twelve papers were included in this review. Studies conducted in vivo on the impact of GML on gut health were used. Each paper included in this review was carefully examined by the authors.

RESULTS

Effects on Intestinal Health and Function

Several studies have evaluated the effects of GML on indicators of intestinal morphology like villus height and crypt depth. In juvenile pompano fish, dietary GML increased villus lengths and muscle thickness in the intestine (Lin et al., 2023). Similar improvements in villi morphology were observed across different species when supplemented with GML. Research on broiler chickens (Liu et al., 2020) and laying hens (Cui et al., 2023) demonstrated increased villus length and thickness in the intestine. GML supplementation yielded enhanced villus height-to-crypt depth ratio (V/C ratio) in both experiments. However, in piglets, jejunal villus length decreased after GML supplementation compared to the antibiotic control group. The ileum showed no difference in lymphocytes, goblet cells, villus height, or crypt depth between groups (Cui et al., 2020). Another study in mice (Mo et al., 2019) claimed that the overall morphology of duodenum and colon remained unchanged.

GML supplementation enhanced intestinal immunity in post-weaning piglets and broilers. GML supplementation alleviated the decrease induced by lipopolysaccharide in the expression of tight junction proteins (zonula occludens 1, occludin, and claudin-2) in broilers (Kong et al., 2022). For broilers having a basal diet, increase of zonula occludens 1 was also observed in the 600 mg/kg GML group (Kong et al., 2021). Same findings in the piglets' study and laying hens' study (Cui et al., 2020, 2023) indicated that GML can increase the expression of zonula occludens 1, claudin-1 and occluding protein in the jejunum. In mice supplemented with high does GML, no

significant difference was found in the mRNA expression of mucin 2, zonula occludens 1, occludin, claudin-1 and jam-1 in the ileal section (Mo et al., 2019). Higher levels of serum immunoglobulin A and immunoglobulin G were observed in the lipopolysaccharide plus GML group in broilers (Kong et al., 2022). Increased serum immunoglobulin A was also found in the ileum of piglets taking a low-protein diet when treated with GML (Cui et al., 2020). No impact on the D-Lactate (D-LACT) and Diamine Oxidase (DAO) concentration by GML supplementation in post-weaning piglets (Cui et al., 2020).

Multiple studies reveal alterations in microbial composition with GML supplementation. In zebrafish, 750 mg/kg GML supplementation exhibited a significant reduction in the phylum *Firmicutes*, driven by decreases in the families *Staphylococcaceae*, *Listeriaceae*, and *Carnobacteriaceae*. Increase in the phylum *Fusobacteria*, particularly the family *Fusobacteriaceae*, and the phylum *Proteobacteria*, particularly in the families *Vibrionaceae* and *Shewanellaceae* was also observed (Y. Wang et al., 2021). Feeding GML increased *Barnesiella*, *Bacteroides*, *Faecalibacterium*, *Odoribacter*, *Parabacteroides*, and CHKC001 cecal level of broilers (Kong et al., 2021). In laying hens, *Bacteroides* and *Alistipes* increased after 1000 mg/kg administration of GML (Cui et al., 2023). Similarly, in basal diet mice, GML supplementation at 400 and 800 mg/kg also increased the relative abundance of *Barnesiella*. However, a higher dose of 1600 mg/kg did not show the same effect (Mo et al., 2019). In high-fat diet mice, high dose GML elevated *Firmicutes* and *Verrucomicrobia* level while lowered *Bacteroidetes* and *Proteobacteria* level. There are also changes of microbiota at family, genes, and species level (Zhao et al., 2020). In basal diet mice, GML supplementation led to a decrease in the phylum

Tenericutes, specifically the family *Anaeroplasmataceae*. While lower doses of GML (400 and 800 mg/kg) increased the relative abundance of *Porphyromonadaceae* and decreased *Bacteroidaceae* and *Erysipelotrichaceae*, a higher dose (1600 mg/kg) significantly increased the *Proteobacteria* content, particularly *Sutterellaceae*, and decreased *Desulfovibrionaceae* (Mo et al., 2019). GML supplementation promoted the growth of *Lactobacillus*, *Barnesiella*, *Bacteroides*, *Coprobacter*, *Gordonibacter*, *Lachnoclostridium*, *Anaerostipes*, and *Pseudoflavonifractor* in LPS-challenged broilers, while reducing the abundance of *Parabacteroides*, *Marvinbryantia*, and *Phascolarctobacterium* (Kong et al., 2022). GML treatment significantly increased α-diversity in zebrafish and high-fat diet mice (Y. Wang et al., 2021; Zhao et al., 2020). No obvious difference was found for α-diversity in four study (basal diet mice, low-fat diet mice, LPS-induced broilers, and basal diet broilers) (Mo et al., 2019; Jiang et al., 2018; Kong et al., 2022; Liu et al., 2020). Decreased Simpson and Shannon indices was observed in broilers with high dose GML (Mo et al., 2019). The β-diversity was also altered in high-fat diet mice, basal diet mice, and broilers (Mo et al., 2019; Jiang et al., 2018; Liu et al., 2020). When broilers got doses of GML from moderate to high (450 mg/kg to 600 mg/kg), butyric acid, propionic acid, valeric acid, and all total short chain fatty acids (SCFAs) increased remarkably in vivo (Liu et al., 2020). Additionally, the concentrations of laying hens` propionic acid and acetic acid rose within ceca (Cui et al., 2023). GML supplementation decreased lipopolysaccharide concentration in mice on a high-fat diet (Zhao et al., 2020).

Anti-inflammatory and Immune Modulating Effects

GML demonstrates anti-inflammatory effects in some studies. In broilers, 300 mg/kg and 1200

mg/kg GML reduced serum proinflammatory cytokines like IL-1β (Interleukin-1 beta), IL-6, and TNF-α (Wang et al., 2020). Ileac TLR2 (Toll-Like Receptor 2), MyD88 (Myeloid Differentiation Primary Response 88), IL-1β and TNF-α was reduced in laying hens with GML treatment (Cui et al., 2023). The effect of GML on reducing TNF-α and IL-6 were also shown in mice on a high-fat diet (Zhao et al., 2020). No significant difference in IL-1β and IL-6, IFN-γ was observed in post-weaning piglets when taking 2 kg/T GML (Cui et al., 2020). Also, no significant difference was found in TNF-α, IL-6, IL-1β, IFN-γ, IL-12/p70, LPS (lipopolysaccharide) and LBP (LPS-binding protein) in mice taking high dose of GML (Mo et al., 2019). GML supplementation was observed to increase the production of IL-10 (Interleukin-10) and TGF-β1 in zebrafish (Y. Wang et al., 2021). High dose GML administration increased the circulating levels of TGF-β1 and IL-22 (Interleukin-22) in mice (Mo et al., 2019). IL-10 expression level increased while IL-8 expression level decreased in juvenile pompano Trachinotus ovatus when giving GML (Lin et al., 2023). Decreased expression of NF-κB (Nuclear factor kappa-light-chain-enhancer of activated B cells) was seen in the gut of LPS-induced broilers, basal diet broilers, and juvenile pompano Trachinotus ovatus (Kong et al., 2021, 2022; Lin et al., 2023). GML supplementation also elevated survival rates after challenge tests with *Vibrio parahaemolyticus* of juvenile pompano Trachinotus ovatus (Lin et al., 2023).

DISCUSSION

Promising Effects on Intestinal Health and Function

Nutrients are largely absorbed by intestinal villi. Lower crypt depth, longer villi, and thicker intestinal wall contribute to magnifying intestinal mucosal area, which improves digestion while helping to sustain intestines (Zeitz et al., 2015). Reviewed researches indicate that supplementing GML has several possible benefits for intestines in diverse animals. Improvements in villus morphology, observed in zebrafish, broilers, and laying hens, suggest enhanced nutrient absorption capacity due to increased surface area (Lin et al., 2023; Liu et al., 2020; Cui et al., 2023). However, the effect of GML on villus length in piglets is unclear. One study reported a decrease in jejunal villus length compared to the antibiotic control group (Cui et al., 2020). Yet, another (Mo et al., 2019) showed no significant changes. The single study on piglets found no significant changes in D-LACT and DAO levels (Cui et al., 2020). Since there is no study reported that GML lower the villi height compared to the basal diet control group, GML can be recognized as harmless to the gut morphology and would not impede normal gut function.

Further contributing to improved gut health, GML appears to play a crucial role in enhancing intestinal barrier function. The intestinal epithelium is the central coordinator of mucosal immunity that composed of a single layer of cells. It forms a dynamic interface that selectively permits the absorption of essential nutrients, ions, and water while simultaneously acting as a frontline defense against the intrusion of noxious luminal substances and invading pathogens (Allaire et al., 2018). The tight junctions are important to sustain the integrity of this barrier.

These complex networks of transmembrane and cytoplasmic proteins act like seals between epithelial cells (González-Mariscal et al., 2003). In this review, some researches have proved that in broilers, laying hens, and piglets the expression of tight junction proteins is upregulated by supplementing GML, like claudin, zonula occludens-1, and occludin (Kong et al., 2021, 2022; Cui et al., 2020, 2023). The strengthening of the intestinal barrier directly impacts gut health by reducing permeability. This, in turn, limits the passage of inflammatory triggers, which promotes homeostasis and overall well-being. In piglets and broilers, the elevated levels of IgA and IgG have shown that the humoral immunity of GML contributes to sustaining gut health (Kong et al., 2022; Cui et al., 2020). The immune defense of gastrointestinal tracts relies predominantly upon IgA; besides, it is the most common immunoglobulin in mucosal areas. IgA influences intestinal and extra-intestinal diseases mediated by commensal microbiota by regulating its colonization within gut (Takeuchi & Ohno, 2022). It acts as a first line of defense by neutralizing pathogens and preventing their attachment to the gut lining. IgA also contributes to preserve a symbiotic and colonic homeostasis-rich microbial ecosystem (Nakajima et al., 2018). IgG is more frequently linked to systemic immunity, but recent research has shown how important it is for gut health and host-microbiota interactions. It can help remove pathogens by binding to particular antigens on them. IgG can also support the memory of the immune system. This is critical for long-term defense against recurrent infections (Sterlin et al., 2020).

Modulating Gut Microbiota Composition

After GML supplementation, an intricate interaction between diet, dosage, and species is shown through the changes in gut floras. GML's antimicrobial attributes are potentially conducive to the

decrease in some bacterial populations (H. Zhang et al., 2007), probably because GML is capable of interfering with intracellular signaling and destroying the completeness of microbial cell membranes (Dayrit, 2015). In respect of zebrafish, *Firmicutes* decreases, especially *Carnobacteriaceae*, *Staphylococcaceae*, and *Listeriaceae*, possibly on account of GML's protection against Gram-positive bacteria (Mueller & Schlievert, 2015). Similarly, among mice, *Tenericutes* declines, particularly *Anaeroplasmataceae*, in conformity with GML's defense against this phylum (Mueller & Schlievert, 2015). Reversely, *Fusobacteria*, specifically *Fusobacteriaceae*, arose among zebrafish, potentially owing to their intrinsic resistance to GML or their capability of thriving in the altered microbiota environment. This is rational since *Fusobacteriaceae* consists of Gram-negative bacteria, and there is no direct evidence suggesting GML targeting this family both in vitro and in vivo. Various studies reveal growing beneficial bacteria, including *Barnesiella*, *Bacteroides*, *Alistipes*, and *Faecalibacterium* (Kong et al., 2022, 2021; Liu et al., 2020; Cui et al., 2023), which denotes a prospective prebiotic efficacy of GML stimulates these commensals to grow. These bacteria are known for their roles to produce short-chain fatty acid (SCFA), which contributes to gut health by providing energy for colonocytes, sustaining gut barrier integrity, and modulating immune responses (Leylabadlo et al., 2020; Parada Venegas et al., 2019; K. Wang et al., 2021). In broilers and laying hens, more SCFAs are generated in favor of this underlying prebiotic efficacy (Liu et al., 2020; Cui et al., 2023). The contrasting responses to GML dosage, such as the variable abundance of *Barnesiella* and the increase in *Proteobacteria* at higher doses, focuses on the demand for in-depth exploration of possible damage to gut homoeostasis and dose-dependent effects (Mo et al., 2019; Jiang et al., 2018), which is also apparent in diverse researches on the changing effects of alpha and beta diversity (Y. Wang et al., 2021; Zhao et al., 2020; Mo et al., 2019; Jiang et al., 2018; Kong et al.,

2022; Liu et al., 2020), where GML's effect seemingly depends upon the elements, like species, dosage, diet, and intestinal flora composition.

Enhancing Immune Function

Numerous researches indicate GML's potential of decreasing inflammation and modulating the immune response (Mo et al., 2019; Zhao et al., 2020; Kong et al., 2021, 2022; Y. Wang et al., 2021; Cui et al., 2023). It can reduce the proportion of TNF-α, IL-1β and other pro-inflammatory cytokines in mice and broilers, revealing the anti-inflammatory effect of GML (Wang et al., 2020; Zhao et al., 2020). Similarly, another example is that mice on a high-fat diet treated with GML experienced lower IL-6 and TNF-α levels, which proves the possibility of GML in addressing inflammation caused by diet disorders (Zhao et al., 2020). These cytokines, despite their significance for immune reactions, is still harmful when in the dysregulation state. IL-1β plays an important role in initiating and amplifying inflammation, and its overproduction might cause inflammatory bowel disease (IBD) and other gut-associated inflammations (Mao et al., 2018). Though IL-6 is important to a healthy immune reaction, its chronic growth in guts can aggravate IBD and even potentially play roles in the progression of colorectal cancer. As a pivotal severe inflammation mediator, TNF-α is also associated with IBD and capable of destroying the gut lining, leading to increased permeability and more problems (Mudter & Neurath, 2007). The reduction of ileac TLR2, MyD88, IL-1β, and TNF-α in laying hens after GML treatment is indicative of its function in attenuating the innate immune response, which is frequently overactive in inflammatory diseases of the gut (Cui et al., 2023). However, in post-weaning piglets and mice given high doses of GML, no significant changes were observed in the levels of

all various cytokines. Thus, a dose-dependent response or a possible ceiling effect hypothesis can be made, where higher doses do not equate to increased efficacy (Cui et al., 2020; Mo et al., 2019). GML also appears to promote an anti-inflammatory response by increasing the production of anti-inflammatory cytokines like IL-10 and TGF-β1 (Wang et al., 2021; Mo et al., 2019). IL-10 is closely associated with IBD, and mice and humans lacking IL-10 will exhibit severe intestinal inflammation (Ip et al., 2017). Like IL-10, TGF-β1 is also critical for preserving intestinal homeostasis due to its strong anti-inflammatory and immunosuppressive properties, supports the integrity of the epithelial barrier, and facilitates intestinal repair and regeneration—all of which are necessary for reducing inflammatory conditions of the gastrointestinal tract (Chen et al., 2023; Tie et al., 2022; Troncone et al., 2018). This further supports GML's potential to maintain intestinal health and reduce inflammation. The observed decrease in NF-κB expression within the gut further supports GML's anti-inflammatory profile. This was seen in LPS-induced broilers and juvenile pompano. NF-κB is a key regulator of inflammation. Thereby, suppressing its expression might cause to decrease cytokine storms and inflammatory signaling (Kircheis et al., 2020). The enhanced survival rates of juvenile pompano challenged with *Vibrio parahaemolyticus* after GML supplementation show its potential in supporting the gut's defense against pathogenic bacteria possibly through the modulation of the gut microbiota and immune system.

Limitations and Future Research Directions

Although it is shown GML potentially acts as a gut health modulator, some restrictions still emerge for in-depth exploration. Clinical trials are important to verify the effect of GML in

animal models. What`s more, it is significant to elucidate the molecular mechanisms of gut absorbing GML in follow-up researches. Besides, greater gut health approaches can be developed by exploring prospective synergic actions between GML and dietary supplements or feed additives. Overall, addressing the identified limitations and pursuing the proposed research directions are crucial steps towards fully realizing the potential of GML as a nutraceutical agent. This will contribute to promote gastrointestinal health and alleviating gut-related disorders in both animal and human populations.

CONCLUSION

This review from in vivo animal studies demonstrates the promising potential of glycerol monolaurate in modulating gut health. GML supplementation was associated with improved villus architecture and increased expression of tight junction proteins. This indicates a boost for the nutrient absorption capacity and intestinal barrier function. In the main time, GML appeared to foster the growth of beneficial gut bacteria like *Barnesiella*, *Bacteroides*, and *Faecalibacterium*, yet reducing potential pathogenic populations. These microbial shifts correlated with increased SCFAs production reflects a potential prebiotic effect. GML exhibited anti-inflammatory properties by downregulating pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, while upregulating anti-inflammatory mediators like IL-10 and TGF-β1. It is suggested that GML can alleviate excessive inflammation and promote gut homeostasis from its ability of immunomodulatory and decreasing NF-κB expression. These intestinal alterations, collectively, suggest potential mechanisms by which GML supplementation exerts beneficial effects on gut health. However, the findings were heterogeneous, reflecting variations in animal models, dosages, and treatment durations across studies. While this review provides promising results, further in vivo research is crucial. This will help establish optimal dosages and treatment regimens for both animals and humans.

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