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EDITED BY
Johan Osorio,
Virginia Tech, United States

REVIEWED BY
Tao Ran,
Lanzhou University, China
Fernanda Rosa,
Texas Tech University, United States

*CORRESPONDENCE
Joshua A. Jackman
jjackman@skku.edu
Charles C. Elrod
cce1@cornell.edu

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Antimicrobial monoglycerides for swine and poultry applications

Joshua A. Jackman[1]*, Theresia A. Lavergne[2] and Charles C. Elrod[2,3]*

[1]School of Chemical Engineering and Translational Nanobioscience Research Center, Sungkyunkwan University, Suwon, South Korea, [2]Natural Biologics Inc., Newfield, NY, United States, [3]Department of Animal Science, Cornell University, Ithaca, NY, United States

The development of natural, broadly acting antimicrobial solutions to combat viral and bacterial pathogens is a high priority for the livestock industry. Herein, we cover the latest progress in utilizing lipid-based monoglycerides as feed additives to address some of the biggest challenges in animal agriculture. The current industry needs for effective antimicrobial strategies are introduced before discussing why medium-chain monoglycerides are a promising solution due to attractive molecular features and biological functions. We then critically analyze recent application examples in which case monoglycerides demonstrated superior activity to prevent feed transmission of viruses in swine and to mitigate bacterial infections in poultry along with gut microbiome modulation capabilities. Future innovation strategies are also suggested to expand the range of application possibilities and to enable new monoglyceride delivery options.

KEYWORDS

monoglycerides, antimicrobial, antiviral, swine, poultry, pathogen, virus, bacteria

Introduction

Infectious diseases caused by viral and bacterial pathogens are a major challenge in the livestock industry, contributing to significant productivity and economic losses (Swayne, 2013; Vanderwaal and Deen, 2018; Shurson et al., 2022). Hence, there is widespread interest in developing strategies to prevent and treat pathogenic infections related to various commercially significant viruses and bacteria.

One widely used industry approach is the prophylactic use of antimicrobial compounds, often delivered in the form of feed or water supplements, to inhibit pathogens (Page and Gautier, 2012; Dittoe et al., 2018; Stewart et al., 2020; Silveira et al., 2021). Historically, the most widely used type of antimicrobial compounds has been antibiotics, which can specifically inhibit bacteria and can also enhance animal growth performance in some cases (Dibner and Richards, 2005). However, growing

attention to the rise of antibiotic-resistant bacteria (Nhung et al., 2017; Haulisah et al., 2021) has led to stricter regulations such as the Veterinary Feed Directive issued by the US Food and Drug Administration (FDA) and resulted in more judicious antibiotic use (Dillon and Jackson-Smith, 2021). Likewise, antiviral compounds such as the anti-influenza drug amantadine have been used as prophylactics to prevent viral outbreaks from spreading in livestock populations, but such widespread usage has been suggested to cause a rise in drug-resistant viral strains (Yuan et al., 2022). Additionally, more broadly acting, disinfecting compounds such as formaldehyde are used to inhibit both bacteria and viruses (Bleichert et al., 2014), but health concerns related to carcinogenicity (Swenberg et al., 2013; Andersen et al., 2019) have led to its ban for use in feedstuffs in certain jurisdictions (*e.g.*, in the European Union, Japan, and Korea) and reduced use in general (Śmiechowska et al., 2021).

As such, there is extensive interest in exploring the use of naturally occurring antimicrobial compounds that can work against a wide range of bacteria and viruses, are safe to use, and do not elicit pathogen resistance. Considering these points, one ideal pathogen target is the lipid membrane that surrounds bacterial cells and most viruses that pose a threat to the livestock industry (Yoon et al., 2020a). Recent findings from the human antiviral medicine field demonstrate the performance merits of targeting pathogen membranes in physiological environments (Jackman et al., 2018; Jackman, 2022) and thus support the potential for translating such strategies to animal agriculture, especially if economical antimicrobial compounds with acceptable regulatory profiles can be utilized.

Towards this goal, natural antimicrobial lipids such as medium-chain monoglycerides (MCMG) have emerged as a promising option to tackle the virus and bacteria challenges facing the swine and poultry industries and are covered herein. Particular focus is placed on critically analyzing how MCMG are being utilized to support agricultural biosecurity and animal health in the swine and poultry production sectors. Notably, MCMG have had wide use and established regulatory acceptance (*e.g.*, as a food substance, they are determined to be Generally Recognized as Safe by the US FDA, the European Commission, and the Food Chemicals Codex) as antimicrobial preservatives, lubricants, stabilizers, and emulsifiers in other aspects of food production (Luo et al., 2022), which reinforces their application potential in the present context. Ongoing research has explored how MCMG can be utilized for specific application needs such as virus mitigation in feed and supporting animal health during bacterial infection. Recent attention to the molecular-level properties of MCMG is also helping to determine structure-function relationships and to rationalize why certain MCMG are more potent than other ones.

Monoglyceride structure and functions

MCMG are derivatives of medium-chain fatty acids (MCFA), which are classified as saturated fatty acids with 6- to 12-carbon long, aliphatic chains. Structurally, MCMG are esterified adducts of an MCFA and a glycerol molecule and have nonionic headgroups, which enable pH-stable behavior that supports robust performance in aqueous environments (Figure 1A). By contrast, MCFA have a carboxylic acid headgroup that is typically negatively charged above pH 5 and its ionization state is sensitive to the pH environment (Valle-Gonzaílez et al., 2018). Owing to their distinct melting points, C_6 and C_8 monoglycerides are typically found in liquid form while C_{10} and C_{12} monoglycerides are typically in powder form. Of note, MCMG mixtures are often supplied in liquid form due to MCMG miscibility.

The main biologically active constructs of MCMG are micelles, which are self-assembled structures of individual molecules that form above a critical micelle concentration (CMC) and the CMC value varies depending on the particular compound or mixture thereof (Yoon et al., 2020b). In general, MCMG with longer hydrocarbon chains have lower CMC values than MCMG with shorter chains due to stronger hydrophobic interactions between chains that promote self-assembly (Yoon et al., 2017). The nonionic character of MCMG enables micelle formation at lower compound concentrations than MCFA, which have anionic character and hence intermolecular electrostatic repulsion between the headgroups that can hinder micelle formation at low concentrations. This biophysical feature helps to explain why MCMG are typically more potent than MCFA since MCMG form micelles at lower concentrations.

Mechanistically, MCMG can inhibit a wide range of membrane-enveloped viruses and bacteria by displaying membrane-disruptive properties, which often occur at and above the CMC (Figure 1B). Fatty acids and monoglycerides mainly exhibit membrane-disruptive properties in their micellar form above their corresponding CMC values (Yoon et al., 2015), which is therefore an important determinant of potency, while they are appreciably less active or inactive in monomeric form. In the case of bacteria, MCMG treatment can induce bacterial cell membrane disruption, which leads to abrogating cell growth or viability depending on the specific conditions (Bergsson et al., 1998; Bergsson et al., 2001). The mechanisms involved in this antibacterial activity can include increased membrane permeability and/or lysis and concomitant effects on membrane-related cellular functions such as hindered electron transport chains, oxidative phosphorylation processes, and enzyme activities (Yoon et al., 2018; Casillas-Vargas et al., 2021). Most of the latter effects are consequences of increased bacterial cell membrane permeability, *i.e.*, membrane leakage that disrupts ion and chemical gradients between the

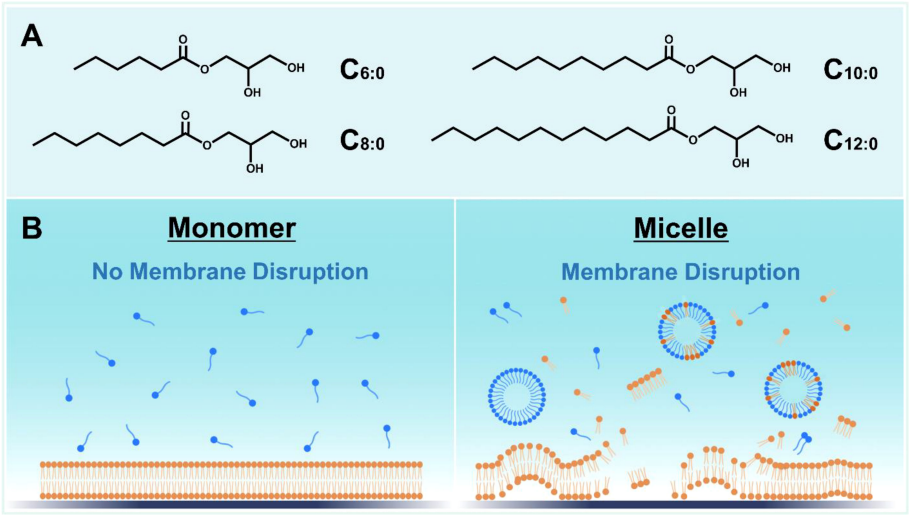

FIGURE 1
Overview of medium-chain monoglyceride (MCMG) structure and function. **(A)** Chemical structures of MCMG with saturated chains. **(B)** Schematic illustration depicting how MCMG in micellar form can disrupt phospholipid membranes such as those of bacteria and enveloped viruses while MCMG in monomeric form are largely inactive.

extracellular and intracellular spaces. MCMG can also inhibit membrane-enveloped viruses by damaging virus particles, which hinders infectivity and it is understood that membrane lysis is the main contributing factor to antiviral activity (Thormar et al., 1987; Thormar et al., 1994).

MCMG have been reported to inhibit a wide range of viral and bacterial pathogens that are important to swine and poultry production (Jackman et al., 2020a; Chen et al., 2021). Susceptible bacterial pathogens include *Escherichia coli*, *Streptococcus suis*, *Salmonella poona*, *Listeria monocytogenes*, and *Clostridium perfringens* (Wang and Johnson, 1992; Skřivanová et al., 2006; Wang et al., 2018), and susceptible viral pathogens include membrane-enveloped viruses such as avian influenza virus (AIV), infectious bronchitis virus, Newcastle disease virus, African swine fever virus (ASFV), porcine reproductive respiratory syndrome virus (PRRSV), and porcine epidemic diarrhea virus (PEDV) (Nur Ika, 2011; Jackman et al., 2020b; Lerner et al., 2020; Dee et al., 2021; Nefedova et al., 2021; Saleh et al., 2021). A common theme of these pathogens is that they are all coated with a lipid membrane envelope, which is the primary target for pathogen inhibition. Mechanistic studies have revealed that C_{10} and C_{12} monoglycerides have particularly high antimicrobial potency due to low CMC values, which allow them to be in the active micellar form at lower concentrations than shorter-chain monoglycerides (Kabara et al., 1972). Nevertheless, different bacteria and viruses have distinct lipid compositions and membrane properties so empirical testing is warranted to identify the best-performing MCMG or combination thereof for the target pathogen(s).

Compared to antibiotics, there are also two main advantages of MCMG for dealing with infectious diseases in livestock production: (1) MCMG exhibit broad-spectrum antimicrobial activity to inhibit viruses and bacteria, whereas antibiotics only work against bacteria (Churchward et al., 2018); and (2) there is anticipated to be a high barrier for pathogens to develop resistance to MCMG (Schlievert and Peterson, 2012), which supports the feasibility of using them across various applications such as mitigation rather than only in more specific therapeutic contexts. As described above, MCMG are also more potent than MCFA and have a more acceptable safety profile than other options like formaldehyde.

Bioavailability considerations

In terms of supply options, MCMG are widely found in various natural sources such as coconut oil and insect-derived oils (Dayrit, 2015; Borrelli et al., 2021; Dabbou et al., 2021). However, they are mainly present in triglyceride form, which typically do not exhibit antimicrobial activity and require enzymatic breakdown to yield the active MCMG form (Zentek et al., 2011). In addition to enzymatic breakdown of triglycerides, MCMG themselves can be broken down into free fatty acids and glycerol products by lipolytic enzymes. For example, certain bacteria such as *Staphylococcus aureus* can produce enzymes to hydrolyze MCMG (*i.e.*, glycerol monolaurate) and the resulting fatty acids can also exhibit similar biological activities by themselves (Ruzin and Novick, 2000) and in combination with MCMG (*i.e.*, in mixed micelles).

The gastrointestinal tract also contains lipolytic enzymes that can degrade MCMG, while it has also been discussed how MCMG can be absorbed in intact form and the hydrolytic propensity of MCMG can also vary depending on the specific stereochemistry of its molecular structure (Kabara, 2005). Indeed, MCMG are also found intact in complex biological matrices such as the milk of some mammals (Schlievert et al., 2019), and can alternatively be provided in highly purified form. The latter option is useful to incorporate a defined amount of MCMG into an aqueous or feed matrix, especially when antimicrobial activity is desired in the matrix itself. At present, feed delivery is the most widely used method for MCMG and we cover application examples of particular importance to dealing with microbial pathogens in swine and poultry production.

Application studies

Prevention of virus transmission in swine feed

Feed has been identified as a vector for pathogen transmission in swine production and there is ongoing exploration of chemical mitigants to stop feed transmission of viruses and bacteria (Niederwerder, 2021). One longstanding option has been formaldehyde, which can induce protein cross-linking to inhibit pathogens (Wilton et al., 2014; Dee et al., 2015). However, its use is being reduced or stopped in certain parts of the world due to recently enacted regulations as described above (Gosling et al., 2021). As such, there has been ongoing exploration of new classes of regulatory acceptable mitigants and one of the most promising options is membrane-disrupting MCFA (Baltić et al., 2017). In light of the typically greater biological potency of MCMG compared to MCFA and the demonstrated ability of MCFA to mitigate pathogens in feed, there have been several recent investigations exploring how MCMG might be useful to inhibit swine-related pathogens (Table 1).

In one study, the *in vitro* antiviral activity of GML monoglyceride against ASFV was compared to that of several individual MCFA in aqueous solution (Jackman et al., 2020b). It was observed that GML had the greatest antiviral potency and exhibited virucidal activity along with additional antiviral mechanisms. Practically, it was further demonstrated that GML could inhibit ASFV in a feed matrix, as indicated by a dose-dependent drop in viral infectivity, and the membrane-

TABLE 1 Recent examples of monoglycerides demonstrating *in vitro* and *in vivo* efficacy to inhibit swine- and poultry-related viral and bacterial pathogens.

Mitigant	Study type (pathogen)	Key result	Reference
Glycerol monolaurate (GML) or MCFA blend (51:29:7 C8:C10:C12)	*In vitro* solution and feed mitigation (ASFV)	-GML was more potent than individual MCFAs to inhibit ASFV in aqueous solution -GML inhibited infectious ASFV in feed by up to 88%, whereas MCFA blend was inactive -GML treatment had no effect on viral DNA in feed but impaired conformation of ASFV surface protein	Jackman et al., 2020b
Various commercial sources, including monoglycerides	*In vivo* feed mitigation with ice block challenge in pigs (PRRSV, PEDV, SVA)	-Monoglyceride-containing additives improved ADG and reduced mortality, as compared to positive control group -Also reduced clinical symptoms and reduced viral presence in rectal swabs, serum, and tonsils	Dee et al., 2021
Proprietary monoglyceride blend	*In vitro* and *in vivo* feed mitigation with ice block challenge in pigs (PEDV)	-Monoglyceride blend reduced PEDV in feed by 99% -Also prevented PEDV transmission to pigs and there were no clinical symptoms at much lower inclusion rate than MCFAs	Phillips et al., 2022
C3, C4, C5, C6, C8, C9, C10, and C12 monoglycerides	*In vitro* solution (various bacteria)	-GML was the most potent to inhibit Gram-positive *Staphylococcus aureus* and *Streptococcus suis* out of 34 mitigant candidates -C8/C10 monoglyceride blend additionally inhibited Gram-negative *Actinobacillus pleuropneumoniae*, *Salmonella* Typhimurium, and *Escherichia coli*	Neath et al., 2022
Proprietary blend of C4, C8, and C10 monoglycerides	*In vivo* challenge in broilers (*Eimeria* oocysts followed by *Clostridium perfringens)*	-Monoglyceride blend improved overall FCR -Monoglyceride blend reduced mortality associated with NE challenge	Gharib-Naseri et al., 2021
Proprietary blend of C4, C8, and C10 monoglycerides	*In vivo* challenge in broilers (*Eimeria* oocysts followed by *Clostridium perfringens)*	-Monoglyceride blend reduced mortality due to NE -Monoglyceride blend improved intestinal integrity and barrier function -Monoglyceride blend increased ability to digest more of the total energy of the feed	Kumar et al., 2021
Mixture of 1-monoglycerides (C1 to C7)	*In vivo* challenge in broilers (*Eimeria* spp., and *Clostridium perfringens* or *Salmonella typhimurium*)	-Mixture of organic acid 1-monoglycerides prevented acute necrotic enteritis -Mixture of organic acid 1-monoglycerides reduced colonization of *Salmonella typhimurium*	Tosi et al., 2017

disrupting mechanism also caused changes in the conformational properties of membrane-associated viral surface proteins. Specifically, the conformational stability of viral surface proteins depends on membrane integrity and membrane disruption therefore causes the loss of native protein conformation, impairing structure and potentially function as well (Salimi et al., 2020). Of note, the antiviral activity of GML did not cause a drop in viral nucleic acids found in the feed (*i.e.*, located in the core of ASFV particles), highlighting that GML can impair viral infectivity even when viral nucleic acids are still present. Together, these findings support that GML impaired viral infectivity by disrupting the lipid membrane envelope surrounding ASFV particles within the feed matrix, indicating that this MCMG can directly inhibit enveloped virus particles.

There has also been interest in developing swine models to evaluate the potential of MCMG-containing mitigant products to inhibit pathogen transmission. One popular model is based on an 'ice-block' challenge, whereby feed is inoculated with a frozen ice sample containing PRRSV, Senecavirus A (SVA), and PEDV that melts and disperses to contaminate the feed (Dee et al., 2021). Pigs are then fed the contaminated feed and biomarkers related to growth performance, clinical status, and virological infection signs are tracked over subsequent days as pigs respond to the pathogen exposure. The feed can be premixed with a mitigant candidate so that the effects of a mitigant on preventing disease transmission are evaluated, *i.e.*, it is envisioned that the mitigant inactivates the virus present within the feed matrix. Using this approach, several MCMG-containing mitigant products were shown to prevent disease transmission, as indicated by preventing infection-related mortality and reducing clinical symptoms. Sufficiently high inclusion rates of the MCMG-containing products in the feed also reduced viral presence in rectal swabs, serum, and tonsils, and also improved average daily gain (ADG).

Additional efforts have focused on understanding the range of effective inclusion rates of MCMG-containing products compared to that of formaldehyde-containing products, which is around 3.25 kg per ton in the latter case (Phillips et al., 2022). *In vitro* studies showed that 1.5 to 3.5 kg per ton inclusion rates of an MCMG-containing product inhibited PEDV infectivity in feed while further testing in the ice-block challenge model verified that these inclusion rates also effectively prevented disease transmission to pigs. On the other hand, an MCFA-containing product tested in parallel was only effective at an inclusion rate of 10 kg per ton. The higher effectiveness of the MCMG-containing product was rationalized by taking into account how MCMG is typically more potent than MCFA at a molecular level, and the data reinforced that MCMG is a potentially useful mitigant option to replace formaldehyde in light of similarly low effective inclusion rates.

While most recent MCMG mitigant studies have focused on preventing viral disease transmission in pigs, there is also potential to explore whether such mitigants can prevent bacterial disease transmission as well. For example, GML has been reported to most potently inhibit Gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus suis* out of over 30 mitigant candidates while a blend of 8- and 10-carbon long, saturated monoglycerides additionally inhibited Gram-negative bacteria such as *Actinobacillus pleuropneumoniae*, *Salmonella typhimurium*, and *Escherichia coli* (Neath et al., 2022). This knowledge can promote the development of improved MCMG-containing mitigant products that might be tailored for broad-spectrum antimicrobial activity or for more specific mitigation needs depending on the application context.

Mitigation of necrotic enteritis infection in poultry

Necrotic enteritis (NE) is a severe poultry disease that is caused by *Clostridium perfringens*, which is a Gram-positive bacteria (Timbermont et al., 2011). Necrotic enteritis is characterized by gross lesions in the jejunum and ileum of the small intestine as well as a sudden increase in mortality in two- to five-week-old broilers. Traditionally, NE was controlled by the use of antibiotics in feed. However, today, the decrease in use of antibiotics along with the increased use of coccidiosis (a predisposing factor to the disease) vaccines, has resulted in an increased incidence of NE (Adhikari et al., 2020). Thus, to combat the disease, the poultry industry is evaluating and utilizing non-antibiotic alternatives such as MCMG and related antimicrobial lipids that can inhibit the bacteria and curb the disease (Gomez-Osorio et al., 2021), as summarized in Table 1.

To evaluate the effectiveness of a short- and medium-chain monoglyceride blend to control NE, Gharib-Naseri et al. orally challenged broilers with *Eimeria* oocysts at nine days of age followed by inoculation with *Clostridium perfringens* at around 14-15 days of age (Gharib-Naseri et al., 2021). Broilers fed the monoglyceride blend had a numerically lower mortality rate than broilers that were not fed the blend. Also, from 0 to 35 days of age, challenged broilers fed the monoglyceride blend had a better feed conversion ratio (FCR). Therefore, the monoglyceride blend was able to alleviate some of the negative effects of NE. Additionally, in the trial, the monoglyceride blend was fed to broilers at two different inclusion rates (0.3% in starter and 0.2% in grower and finisher phases, or 0.3% in starter, 0.15% in grower, and 0.075% in finisher phases). Feed conversion was similar for challenged broilers fed either inclusion rate of the monoglyceride blend. However, the mortality rate tended to be lower for challenged broilers fed the higher level of the monoglyceride blend in the grower and finisher phases. Since all of the mortality occurred within the first four days following the challenge at 14 days of age, it is possible that the lower inclusion rate was not adequate. Thus, there is interest in

determining the optimal rate of inclusion and ensuring a sufficiently high level to control NE. Further analysis indicated that the monoglyceride blend improved cecal microbiome diversity, eliciting positive effects on healthy bacteria such as *Bacillus* species that are associated with growth performance and improved feed efficiency (Gharib-Naseri et al., 2021). These findings support that the monoglyceride blend may have enhanced immune function through microbiome modulation while interestingly the cecal level of *C. perfringens* was not affected.

In another study with a similar *Eimeria* spp. and *C. perfringens* challenge model, feeding broilers the monoglyceride blend only in the starter phase (0 to 10 days of age), at a rate of 0.5% of the diet, significantly reduced NE-related mortality compared to broilers that were not fed the monoglyceride blend (Kumar et al., 2021). In addition, feeding the monoglyceride blend to broilers in the starter phase increased the messenger RNA (mRNA) levels of jejunal genes related to tight junction protein (TJP1) and immunoglobulin G (IgG), which are important proteins that support gut integrity and immune health, respectively. During NE, the intestinal epithelium is damaged, resulting in a reduced immune response and reduced rate of nutrient absorption across the intestinal wall. Since challenged broilers fed the monoglyceride blend had upregulated TJP1 and IgG levels, this indicates that intestinal integrity and intestinal barrier function of broilers with NE disease can be improved by including the monoglyceride blend in the feed. Furthermore, this improved intestinal health resulted in increased rates of digestion and nutrient absorption. The latter was evidenced by the reported increase in digestion of the total energy in the diet of challenged broilers that were fed the monoglyceride blend. As described in the previous example, the cecal levels of *C. perfringens* in the treated group were not reduced compared to the control group, suggesting that the monoglyceride blend mainly exerted positive health effects by supporting immune functions, such as increased expression of jejunal tight junction and immunoglobulin genes associated with gut barrier functions, that may have counteracted the initial *Eimeria* spp. challenge prior to *C. perfringens* challenge (Kumar et al., 2021).

While alleviating the negative effects of NE is very important, means of preventing NE are also necessary for the poultry industry. Towards this goal, Tosi et al. reported that a specific mixture of short- and medium-chain monoglycerides, provided in feed at a rate of 0.5% from day 1 to 10 and at a rate of 0.025% from day 11 to 21, prevented NE (Tosi et al., 2017). Also, the specific mixture provided in the feed at a rate of 0.3% from day 1 to 34 reduced *Salmonella* colonization in broilers. Collectively, these data support the efficacy of MCMG within complex formulations to inhibit NE-related bacterial infections in poultry. Thus far, the mixtures used have been dry formulations and it would be desirable to further develop and test MCMG mixtures that can be supplied in liquid form.

Microbiome modulation

Another emerging application area for MCMG is microbiome modulation, which can support animal health and help to overcome viral and bacterial infections. Due to antimicrobial functions, MCMG have been shown to modulate the gut microbiome of swine and poultry by promoting the growth of healthy bacteria and helping to decrease the relative amounts of pathogenic bacteria. In the swine context, the addition of 1000 mg/kg GML to the daily diet of weaned piglets reduced the rate of diarrhea and led to marked alterations in gut microbiota (Li et al., 2022). Microbiome analysis of cecal contents indicated that GML promoted increased levels of healthy bacteria such as *Firmicutes*, *Lactobacillus*, and *Blautia* species while reducing the proportion of *Bacteroidota* and *Campilobacterota* species that are associated with various medical disorders.

There have also been numerous recent studies demonstrating that MCMG can modulate the gut microbiome in poultry as well. For example, a mixture of GML and C_{10} monoglyceride was added to the diet of laying hens at a dose of 300 mg/kg and caused a marked decrease in the cecal prevalence of the phylum *Proteobacteria*, which is associated with poor gut health, and increased levels of various healthy bacteria (Liu et al., 2020). Different doses (300-600 mg/kg) of the same MCMG mixture were incorporated into the diet of broiler chicks and increased the cecal prevalence of bacteria such as *Bifidobacteriaceae* and *Bacteroides*, the latter of which play important roles in gut metabolism and helps to protect against pathogenic microbes (Liu et al., 2022). Similarly, various doses (300-1200 mg/kg) of GML alone have also been added to the diets of broiler chicks and led to improved diversity of the cecal microbiome as well as increased levels of *Bacteroides (*Kong et al., 2021*)*. GML supplementation at a dose of 1200 mg/kg has also been shown to protect against immune stress and intestinal injury in liposaccharide-challenged broilers (Kong et al., 2022). Notably, these positive health effects were correlated with increased abundance of healthy gut bacteria involved in anti-inflammatory and antioxidant processes, and indicate that MCMG supplementation can modulate the gut microbiome to support animal health in addition to directly inhibiting pathogenic viruses and bacteria.

Future opportunities

The documented use of MCMG in recent research studies has demonstrated that they are effective in reducing viral infectivity in swine feed and in controlling bacterial-related infections in poultry. These application successes have been enabled by understanding how MCMG operate at a molecular level and rationalizing why they are typically more potent than

MCFA, which has translated into lower molar concentrations and inclusion rates. Moving forward, one of the areas of greatest opportunity lies in expanding the scope of MCMG-related studies to develop optimized mixtures in terms of not only antimicrobial efficacy but also in terms of controlling formulation properties, *i.e.*, powder or liquid supply, stability, and solubility. Current application uses have focused on incorporating MCMG into feed and developing water-miscible MCMG formulations for drinking water applications would also be advantageous, especially to rapidly respond to potential disease outbreaks. Such approaches might take advantage of recent innovations in lipid nanostructures and could also pave the way to developing aerosol formulations.

In terms of application scope, most studies have focused on preventing and treating viral infections while a renewed focus on mitigating disease transmission in livestock populations would be advantageous, especially to address some of the most pressing industry challenges. Since MCMG are broad-spectrum antimicrobial agents that target the lipid membrane surrounding bacteria and enveloped viruses, they also stand excellent potential to be readily deployed against evolving pathogen threats in the future and hence should be mainstays of the livestock industry for years to come. These findings also support that MCMG are active *in vivo*—an area that needs further exploration in the context of disease challenges in order to further understand how and where MCMG function. In the context of pathogen feed mitigation, it is also important to determine the extent to which MCMG inactivate pathogens in the feed matrix itself vs. in saliva upon ingestion. Altogether, there is excellent potential to continue exploring the use of MCMG to stop pathogenic viruses and bacteria while also building a more collective picture of how they modulate microbiome populations and of the interplay between these different functionalities to optimize practical utilization of MCMG in animal agriculture.

Author contributions

JJ and CE conceived and designed the study. JJ, TL, and CE performed the literature review. JJ, TL, and CE analyzed the data and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

TL and CE are employed by and JJ serves as a board member to the company Natural Biologics Inc.

Publisher's note

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