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# Application of GML to ASFv

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## Abstract

The ongoing African swine fever virus (ASFv) epidemic has had a major impact on pig production globally and biosecurity efforts to curb ASFv infectivity and transmission are a high priority. It has been recently identified that feed and feed ingredients, along with drinking water, can serve as transmission vehicles and might facilitate transboundary spread of ASFv. Thus, it is important to test the antiviral activity of regulatory compatible, antiviral feed additives that might inhibit ASFv infectivity in feed. One promising group of feed additive candidates includes medium-chain fatty acids (MCFA) and monoglyceride derivatives, which are known to disrupt the lipid membrane surrounding certain enveloped viruses and bacteria.

## Method

1. Reagents: caprylic acid(8C), capric acid(10C), lauric acid(12C), glycerol monolaurate, an MCFA blend containing caprylic, capric, and lauric acids (51:29:7 ratio) plus silica carrier, GML

2. Virus: ASFv BA71V strain. The viral titer was measured by the Spearman-Kärber endpoint method and reported as log 50% tissue culture infective dose (TCID50) per mL (log TCID50/mL)

3. Feed sample preparation: A powdered mixture of MCFA or GML was used as the active ingredient and suspended in Eagle's minimum essential medium(EMEM). Two grams of commercial swine feed were mixed with the active ingredient by vortexing at inclusion rates of 0.25%, 0.50%, 1.0%, or 2.0% (wt/wt).

4. Method: Then, each feed sample was incubated with 100 μL of EMEM containing ASFv at $10^6$ TCID50 dosage for 30 min or 24 h at room temperature. After incubation, 20 mL of fresh EMEM was added to each feed sample and centrifuged at 3600×g for 40 min (4℃). The supernatant was collected, and aliquots were analyzed in virus recovery, PCR, and ELISA tests. Positive control (virus-only) samples were incubated with the same amount of virus without active ingredients.

## Results and discussion

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## 1. Antiviral activity in aqueous solution

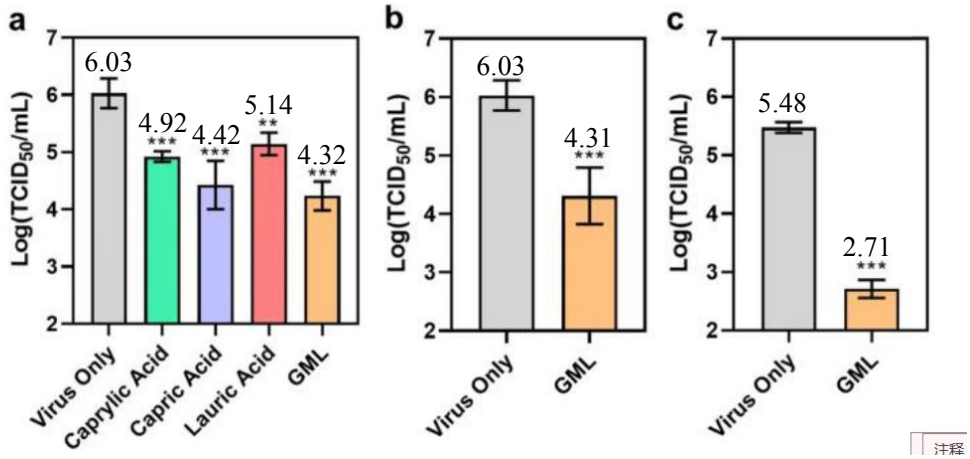


*Fig. a comparison of 8C, 10C, 12C and GML in solution to reduce virus infectivity (5mmol/L)*
*Figure B GML in solution reduces virus infectivity (250μmol/L)*
*Figure C GML in solution reduces virus infectivity (250 μmol/L at 37℃ for 1 h)*

The virus-only control had a mean viral titer of 6.03 log TCID50/mL. Treatment with 5mmol/L caprylic acid or capric acid caused significant decreases in mean viral titer down to 4.92 and 4.42 log TCID50/mL, respectively. **These changes correspond to greater than 92 and 97% reductions in viral infectivity**. Likewise, treatment with lauric acid or GML caused significant decreases in mean viral titer down to 5.14 and 4.32 log TCID50/mL, respectively. **These changes correspond to greater than 87 and 98% reductions in viral infectivity.** Thus, all tested MCFA and GML at 5 mmol/L compound concentrations exhibited virucidal activity and significantly reduced ASFv infectivity compared to the virus-only control.

Treatment with 250μmol/L GML caused significant decreases in mean viral titer down to 4.31, **which is equal to 98% reductions in viral infectivity.**

Treatment with 250μmol/L GML (kept at 37℃ for 1h) caused significant decreases in mean viral titer from 5.73 to 2.71, **which was equivalent to a 99.8% decrease in virus infectivity.**

## 2. Antiviral activity in feed

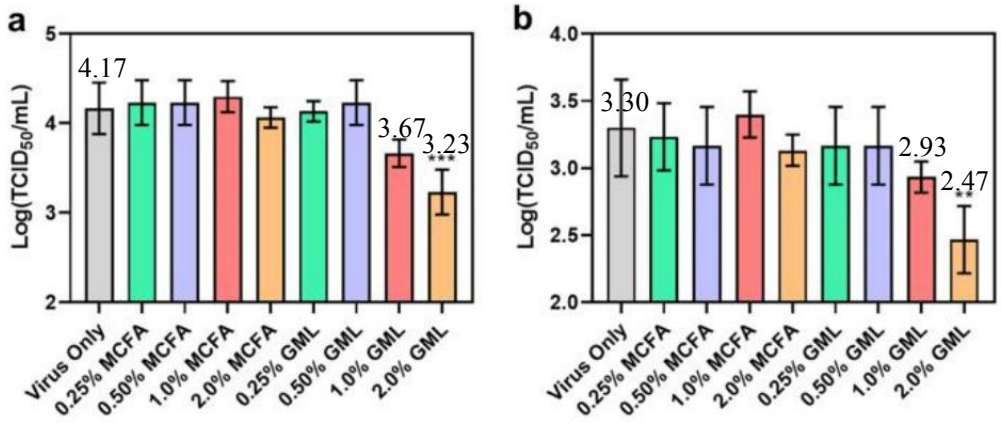


*Fig. a comparison of reducing virus infectivity by mixing MCFA(8C:10C:12C=51:29:7, silica carrier) and GML in feed (mixing for 30min)*
*Fig. b comparison of reducing virus infectivity by mixing MCFA(8C:10C:12C=51:29:7, silica carrier) and GML in feed (mixing for 24h)*

The additives(GML and MCFA) were incorporated into the feed at inclusion rates of 0.25%, 0.50%, 1.0%, and 2.0% and then the feed samples were spiked with ASFv inoculums. The viral titer in the feed was measured after a 30-min or 24-h incubation period.

After 30-min incubation, the virus-only control had a mean viral titer of 4.17 log TCID50/mL, while the viral titers of all MCFA-treated feed samples were similar, indicating that the MCFA blend did not reduce viral infectivity. The GML-treated feed samples exhibited dose-dependent reductions in viral infectivity at 1.0% and higher inclusion rates. The mean viral titers of 0.25% and 0.50% GMLtreated feed samples were similar to the virus-only control, while the mean viral titer of the 1.0% GML-treated feed sample tended to decrease to 3.67 log TCID50/mL, **which corresponds to a greater than 68% reduction in viral infectivity.** A significant decrease in viral infectivity occurred in the 2.0% GML-treated feed sample and the mean viral titer dropped to 3.23 log TCID50/mL, **which corresponds to a greater than 88% reduction in viral infectivity**. These data show that GML can quickly reduce viral infectivity in ASFv-contaminated feed whereas the MCFA blend was inactive.

After 24-h incubation, the mean viral titer of the virus-only control had decreased to 3.30 log TCID50/mL. The 1.0% GML dose tended to reduce the mean viral titer to 2.93 log TCID50/mL, **which corresponds to a 57% reduction in viral infectivity compared to the 24-h virus-only**

**control**. Moreover, the 2.0% GML dose significantly reduced the mean viral titer to 2.47 log TCID50/mL, **which corresponds to an 85% reduction in viral infectivity compared to the 24-h virus-only control**.

Moreover, compared to the 30-min virus control, the 2.0% GML additive reduced viral infectivity by 98% within 24 h, which proves that GML additive reduces viral infectivity in ASFv-contaminated feed samples.

## 3. Effect on virus genetic material

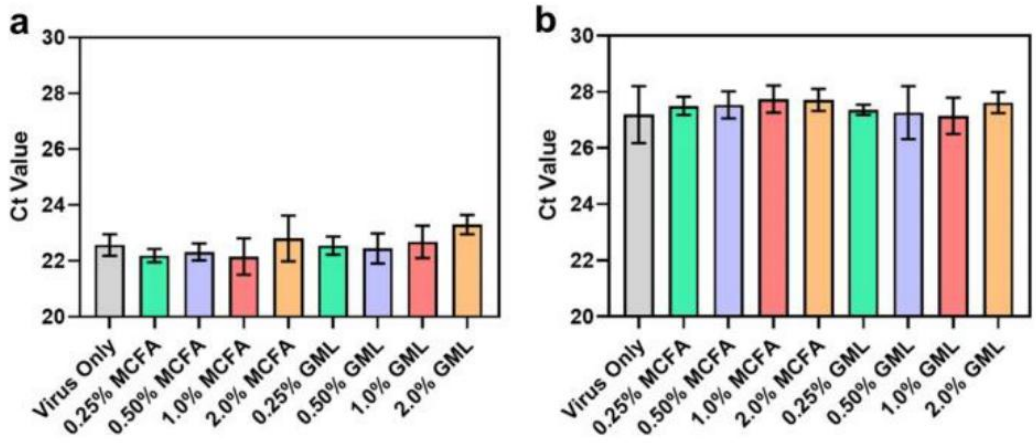


*Fig. A Comparison of the effects of MCFA(8C:10C:12C=51:29:7, silica carrier) and GML on virus genetic material in feed (mixing for 30min)*
*Fig. B Comparison of the effects of MCFA(8C:10C:12C=51:29:7, silica carrier) and GML on viral genetic material (mixed for 24 hours)*

The experiments were conducted because virus genetic material is often used as a diagnostic marker for virus contamination in feed and we measured the cycle threshold (Ct) value, which is inversely proportional to the amount of intact viral DNA present within a feed sample. A higher Ct value indicates less intact genetic material and vice versa.

After 30-min and 24-h incubation, the virus-only control and all MCFA-treated and all GML-treated feed samples had Ct values in the similar range, respectively. This result indicated that the MCFA and 0.25%, 0.50%, and 1.0% GML-treatments did not affect virus genetic material.

This finding indicates that the virus genetic material modestly degraded in all test groups due to the storage conditions, while the MCFA and GML additives did not further affect the integrity of virus genetic material. Together with the viral titer measurements, these data support that **GML**

**abrogates ASFv infectivity in feed through a virucidal mechanism that impairs virus particle structure but does not directly damage virus genetic material**.

### 4. Effect on antibody recognition of viral antigens

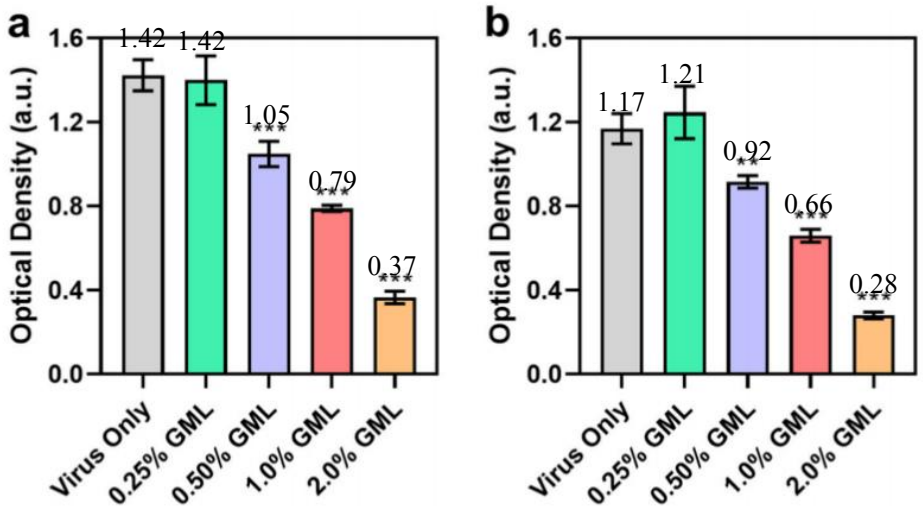


*Fig. A Effect of GML in feed on antibody recognition of p72 protein (mixing for 30min)*
*Figure B Effect of GML in feed on antibody recognition of p72 protein (mixed for 24h)*

The p72 protein is the major structural component of the viral capsid and also one of the key viral antigens. Therefore, we also conducted ELISA experiments on the ASFv-contaminated feed samples to determine if GML treatment affects antibody recognition of the antigenic p72 protein. A larger optical absorbance density (OD) signal indicates the presence of more structurally intact p72 protein and vice versa.

These data support that GML treatment caused decreases in the amount of structurally intact p72 protein. p72 proteins is known to interact with the inner lipid membrane in order to stabilize the capsid network. GML-mediated membrane disruption likely affects the outer and/or inner lipid membranes of ASFv particles, and such effects would in turn destabilize the capsid network, including inducing conformational changes in p72 protein structure.

## Conclusion

ASFv is genetically distinct from other swine viral pathogens and is the only member of the Asfarviridae family. In general, ASFv particles have a five-layer structure that consists of an inner

nucleoid containing virus genetic material, a core shell consisting of proteins, an inner lipid membrane coating, an icosahedral protein capsid, and an outer lipid membrane coating (“envelope”). A particularly unique feature of ASFv particles is that they have a complex structure that includes two distinct layers of lipid bilayer coating as opposed to the more conventional one layer in most other membraneenveloped viruses such as PEDv.

MCFA and GML can reduce the infectivity of ASFv in feed environments. Mechanistic studies further revealed that the antiviral activity of GML infeed caused conformational changes in viral membraneassociated ASFv p72 structural protein but did not affect viral genetic material, which is consistent with the known membrane-disruptive effects of GML. Continued development of suitable formulations to maximize the antiviral performance and industrial utility of MCFA and GML for water and feed delivery could help these additives become important tools in combating the infection and spread of swine viral pathogens, including ASFv.