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Antiviral Activity of Plant-Based Additives Against African Swine Fever Virus (ASFV) in Feed Ingredients

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ABSTRACT

Background: African swine fever (ASF) is one of the deadliest swine diseases with haemorrhagic symptoms and a high mortality rate. Plant-derived additives are potential antiviral agents against viruses due to their environmental and user-friendly properties.

Objectives: This study aims to evaluate the efficacy of plant-based additives (Phyto.A04 and Phyto.B) compared to an organic acid blend (OAB) in inactivating ASF virus (ASFV) in cell culture and feed.

Methods: ASFV-spiked feed was treated with individual or combined additives such as OAB, Phyto.A04 and Phyto.B. The viability of ASFV after treatment of ASFV-spiked feed with additives was then confirmed by both methods, real-time PCR and cell culture.

Results: The results of the in vitro test with cell cultures showed that all three additives (OAB, Phyto.A04 and Phyto.B) exerted a strong virucidal effect on ASFV in porcine alveolar macrophage cells. OAB at a concentration of 0.3% reduced the virus concentration from 4.48 $\log_{10}$ HAD_{50}/mL after 1 day of treatment (day 1) to 3.29 $\log_{10}$ HAD_{50}/mL after 3 days of treatment (day 3) and remained undetected after 7 days of treatment (day 7). In Phyto.A04 with 1%, the virus was only detectable on day 1 (3.53 $\log_{10}$ HAD_{50}/mL). Phyto.B with 0.01% and 0.05% both showed good efficacy in completely inhibiting virus presence on days 3 and 7.

Conclusions: All additives, OAB, Phyto.A04 and Phyto.B, were able to inactivate ASFV in a dose-dependent manner, as confirmed by cell culture and PCR methods. The combination of additives at different concentrations consistently improved the virucidal results.

1 | Introduction

African swine fever (ASF) is a highly contagious and fatal swine disease that causes severe haemorrhagic symptoms and is a major concern for livestock worldwide. The disease continues to be endemic in sub-Saharan Africa and other countries in Asia, including China, Vietnam, Thailand, Korea, Laos and Cambodia (Ge et al. 2018; Kim et al. 2020; Le et al. 2019; Mai et al. 2021; Matsumoto et al. 2020). The African swine fever virus (ASFV), a double-stranded DNA virus with a genome length of 170–193 kbp, is the causative agent of the disease (Dixon et al. 2013). The virus is classified into 24 genotypes and over eight serogroups

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based on the B646L gene (p72 protein) and EP402R gene (CD2v protein), respectively (Gallardo et al. 2009; Sanna et al. 2017).

In Vietnam, the first outbreak of ASF was reported in 2019 in Hung Yen province (Le et al. 2019). After 5 months of spreading, the Vietnamese government announced that 6 million pigs had been culled, and ASF was officially reported in every province (Mai et al. 2021). Non-commercial pig farms, such as households and backyards, hold a large proportion of Vietnamese pig producers. These farms are usually susceptible to swine diseases due to poor disease management, lack of veterinary knowledge, low biosecurity methods, swill feeding and so on. In Vietnam, both commercial and non-commercial pig farms use high-grade feed as a required standard to avoid contamination. However, feed ingredients are considered a risk factor for introducing and transmitting swine viral diseases (Niederwerder and Hesse 2018). Previous studies have shown that ASFV can survive on glass, rubber and metal for more than 7 days at 25°C, 3–5 days at 33°C, 1–2 days at 42°C and years in freeze conditions depending on different types of organs (Arzumanyan et al. 2021; Nuanualsuwan et al. 2022). This suggests concern about the contamination of pig feed as the average temperature of Vietnam is 25–33°C in the summer and below 20°C in the winter, depending on the different areas of Vietnam. The transmissibility of ASFV was considered possible through feed (Niederwerder et al. 2019). ASFV can infect pigs through contaminated natural plant-based feed, and the viability of the virus in the feed depends on the amount of virus present and the volume of feed consumed (Niederwerder et al. 2019). In Romania, for example, ASFV outbreaks on family farms between May and September 2019 were believed to have been caused by plant-based feeds transported from epidemic areas (Boklund et al. 2020). Another example comes from Latvia, where the outbreaks of ASF in pigs in 2014 were attributed to the use of ASF-contaminated grass and crops (Oļševskis et al. 2016). In Estonia, ASF outbreaks in commercial pigs in 2015 and 2017 were thought to have originated from ASFV-contaminated grain (Nurmoja et al. 2020). The use of dried blood contaminated with ASFV as a feed additive was also believed to be the cause of ASFV transmission in China (Wen et al. 2019; Zhai et al. 2019).

Therefore, mitigating the risk of ASF associated with feed products is a priority for affected and non-affected countries. Poultry, cattle and swine feed are commonly treated with chemical additives to minimise bacterial and viral pathogens such as porcine epidemic diarrhoea virus (PEDV), *Salmonella enterica*, avian influenza virus, porcine delta coronavirus and *Escherichia coli* (Amado et al. 2013; Cottingim et al. 2017; Toro, van Santen, and Breedlove 2016; Trudeau et al. 2016). Recent studies have also focused on feed treatment with chemical compounds to reduce the risk of food contamination with ASFV (Jackman et al. 2020; Niederwerder et al. 2021). However, chemical treatment usually causes environmental issues and is banned in other regions, such as the European Union [Regulation (EU) 2018/183] and Vietnam (Regulation QĐ 867/1998). Plant-based additives have been used as alternatives for their environmentally friendly origin and safety. This study aims to evaluate the efficacy of plant-based additives (Phyto.A04 and Phyto.B) compared to an organic acid blend (OAB) in inactivating ASFV in cell culture and feed ingredients.

2 | Materials and Methods

2.1 | Cells, Viruses, Feed Ingredients and Additives

Primary porcine alveolar macrophages (PAMs) used for the study were collected from the lungs of healthy pigs that were 8–10 weeks old, and the absence of Porcine circovirus type 2 (PCV2), Classical swine fever (CSF), Porcine reproductive and respiratory syndrome (PRRS), and ASF viruses was confirmed by real-time PCR (Median Diagnostics Inc., http://www.mediandiagnostics.com). This study was conducted in the biosafety facility of the Faculty of Veterinary Medicine, Vietnam National University of Agriculture (VNUA), Hanoi, Vietnam. All virus experiments were conducted in accordance with good experimental practice guidelines. In addition, the animal experiments were approved by the Committee on Animal Research and Ethics of the Faculty of Veterinary Medicine of VNUA with approval number CARE −2021/10 and an approval date of 4 December 2021. The PAM cells were cultured in a medium that included RPMI 1640 (Gibco), 10% foetal bovine serum (FBS), and 1% antibiotic. For cell culture, the cells were seeded onto tissue culture plastic plates at a density of approximately 4×10^5 cells/cm^2. Dead cells were removed by washing after 24 h, and the cells were then cultured at 37°C with 5% CO_2. The virus strain VNUA/HY/ASF1/Vietnam/2019, the causative strain belonging to p72 genotype II, was used for the study and originated from infected pigs during the first outbreak of ASF in Vietnam (Le et al. 2019). Commercial industrial pig feed (without antibiotics and formaldehyde), which contained cereal flour, meat, bone meal and minerals, was purchased from the American Feeds Company (http://goldcoin.com.vn/en). The feed was screened using real-time PCR to ensure an ASFV-negative status before use.

The additives used in this study, including OAB (powder form), Phyto.A04 (liquid form) and Phyto.B (powder form), were kindly provided by Dr. Eckel Animal Nutrition GmbH & Co. KG. OAB is a mixture of formic acid, lactic acid, calcium formate and calcium lactate as well as citric acid in a ratio of 8.2:1:1.7:11.9:2.5 and a pH value of 2. Phyto.A04 is a preparation of bioactive substances with hops. It contains a CO_2 extract from the flowers of *Humulus lupulus*, which contains hop-soft resin in a total concentration of 0.1% at a pH of 11. Phyto.B (pH = 5.04) is a preparation of bioactive substances containing anhydrous licorice extract (*Glycyrrhiza glabra*) from aqueous extraction.

2.2 | Detection of ASFV Genome

The genomic DNA of ASFV in the PAM cell culture and ASFV-spiked feeds were extracted using Qiagen DNeasy Blood & Tissue Kit (Qiagen). The presence of viral DNA was identified by a commercialised real-time PCR VDx ASFV qPCR Ver 2.1 (Cat. No. NS-ASF-31) kit targeting the p72 gene (Median Diagnostics Inc., http://www.mediandiagnostics.com).

2.3 | In Vitro Test of Plant-Based Additives Against ASFV in Cell Culture and Feed Ingredients

100 g of feed was prepared in each Ziploc bag and used for the study. All experiments were designed as described in Table 1. In

TABLE 1 | Experiment design for in vitro test of plant-based additives against ASFV (10^8HAD_{50}) in cell culture and feed.

Code	Name	Mixture
P1	Positive control	100 g feed + ASFV
N1	Negative control	100 g feed solely
C1	Control 1	100 g feed + 0.3% OAB + 1% Phyto.A04 + 0.05% Phyto.B
C2	Control 2	0.3% OAB + 1% Phyto.A04 + 0.05% Phyto.B + ASFV
T1	Treatment A	100 g feed + 0.3% OAB + ASFV
T2	Treatment B	100 g feed + 1% Phyto.A04 + ASFV
T3	Treatment C	100 g feed + 0.01% Phyto.B + ASFV
T4	Treatment C	100 g feed + 0.05% Phyto.B + ASFV
T5	Treatment A + B	100 g feed + 0.3% OAB + 1% Phyto.A04 + ASFV
T6	Treatment A + C	100 g feed + 0.3% OAB + 0.05% Phyto.B + ASFV
T7	Treatment B + C	100 g feed + 1% Phyto.A04 + 0.05% Phyto.B + ASFV
T8	Treatment B + C	100 g feed + 1% Phyto.A04 + 0.01% Phyto.B + ASFV
T9	Treatment A + B + C	100 g feed + 0.3% OAB + 1% Phyto.A04 + 0.01% Phyto.B + ASFV
T10	Treatment A + B + C	100 g feed + 0.3% OAB + 1% Phyto.A04 + 0.05% Phyto.B + ASFV

Note: 0.3% OAB corresponds to 0.3 g; 1% Phyto.A04 corresponds to 1 mL; 0.05% Phyto.B corresponds to 0.05 g; and 0.01% Phyto.B corresponds to 0.01 g.

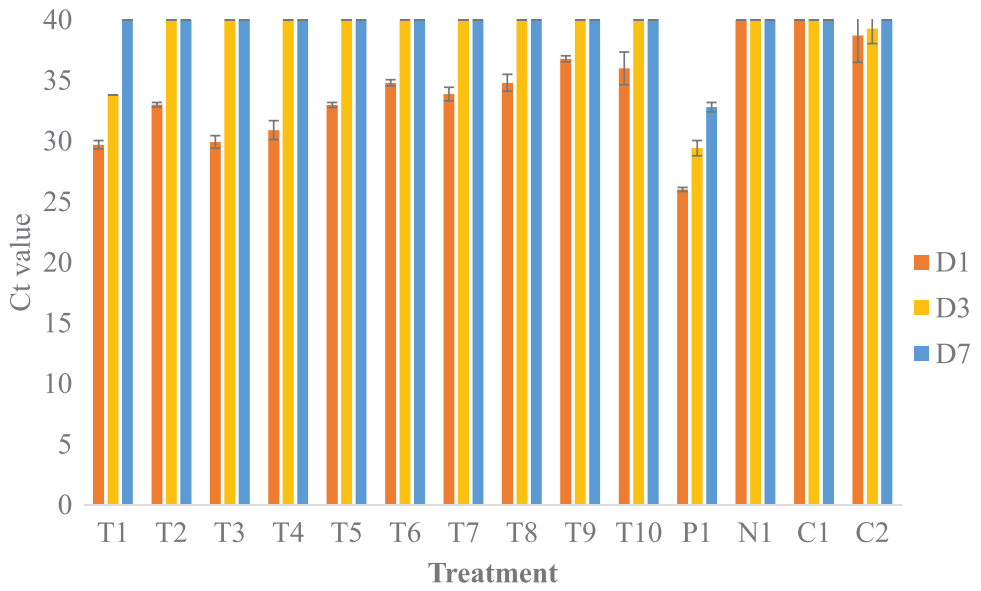

FIGURE 1 | Results of ASFV detection after treatment of ASFV-spiked feed with additives after 1 (D1), 3 (D3), and 7 (D7) days using real-time PCR (Median Diagnostics Inc., http://www.mediandiagnostics.com). Ct value ≥40: negative; Ct value <40: positive.

detail, for virus contamination of the feed, after the addition of additive products into the appropriate feed bags, 10 mL of ASFV (VNUA/HY-ASF1/Vietnam/2019) solution (10^7 HAD_{50}/mL) was added into each bag, followed by shaking for 10 minutes to mix well. All bags were then incubated at room temperature (approximately 25°C). After incubating for 1, 3 and 7 days, 50 mL of RPMI 1640 medium was added to each bag, mixed well, and the supernatant was collected by centrifuging at 4000 rpm for 10 min. The resulting supernatants, referred to as 'Treatment', were filtered using a 0.45 μm filter to remove impurities and limit bacteria. Serial 10-fold dilutions of the Treatment (coded as T1–T10 in Table 1 and Figures 1 and 2) were then prepared in RPMI medium for virus titration on PAM cell cultures. Each diluted Treatment was inoculated into triplicate wells of PAM cell plates (100 μL per well). In parallel, ASFV-infected commercial pig feed without additives (coded as P1), feed alone (coded as N1), feed plus with additives (0.3% OAB + 1% Phyto.A04 + 0.05% Phyto.B) without ASFV (coded as C1), ASFV-infected additives 0.3% OAB + 1% Phyto.A04 + 0.05% Phyto.B + ASFV without feed (coded as C2), ASFV-infected and -uninfected PAM cells were used as controls. Each experiment was repeated three times. The cell culture plates were incubated for 2 h at 37°C in a 5% CO_2 incubator. The Treatments were then replaced with 200 μL of cell culture media containing RPMI 1640 (Gibco), 10% foetal bovine serum (FBS) and 1% antibiotic. After 48 h of incubation, 20 μL of 1% porcine red blood cell in RPMI medium was added to

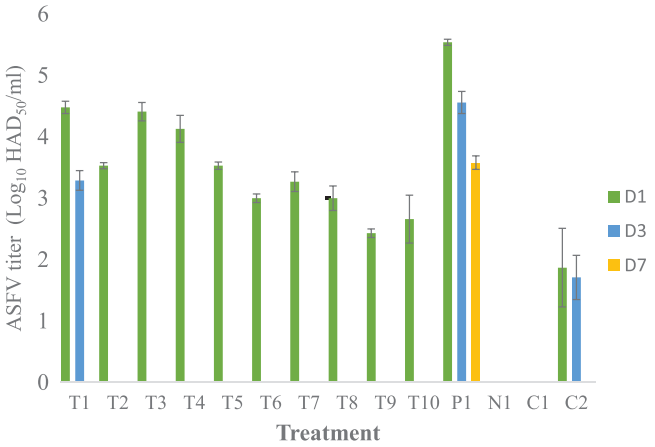

FIGURE 2 | Results of ASFV titration on PAM cells after treatment of ASFV-spiked feed with additives after 1 (D1), 3 (D3), and 7 (D7) days.

each well of PAM cells. The formation of hemadsorption (HAD) rosettes on ASFV-infected PAM cells was observed daily under an inverted microscope for 5 days. The titer ($\log_{10}$ HAD_{50}/mL) of the recovered virus was calculated using the Reed–Muench study (Reed and Muench 1938).

3 | Results

3.1 | Detection of ASFV in Feed Ingredients After Additive Treatments Using Real-Time PCR

The antiviral activity of plant-based additives OAB, Phyto.A04 and Phyto.B against ASFV was determined in their single (T1–T4) and combination (T5–T10) forms (Table 1). In this study, the viability of ASFV after treatment of ASFV-spiked feed with additives was confirmed by both methods, real-time PCR and cell culture. The results of ASFV detection by a real-time PCR (Median Diagnostics Inc., http://www.mediandiagnostics.com) showed that single treatments, including T1, T2, T3 and T4, could inhibit ASFV. Treatment T1 (0.3% OAB) produced the Ct value of 29.70 on day 1, then increased to 33.82 on day 3 and was negative on day 7. Treatment T2 showed a better result with detecting the virus on day 1 and negative on days 3 and 7. Different concentrations in Treatment T3 also produced similar results to Treatment T2. A higher concentration of Treatment T4 was also effective at reducing virus concentration (Figure 1).

In the dual combine groups, Treatments T5, T6, T7 and T8 only had a viral presence on day 1 with the Ct values of 32.98, 34.81, 33.88 and 34.81, respectively, and then were negative for ASFV at days 3 and 7. The effectiveness of virucidal additives was even better with the triple combination. The Ct values of the Treatments T9 and T10 were 36.79 and 36, respectively, on day 1 and then undetectable on days 3 and 7.

3.2 | Detection of ASFV After Additive Treatments by Cell Culture

In vitro trial results showed that all three additives (OAB, Phyto.A04 and Phyto.B) expressed a strong virucidal effect on ASFV in PAM cells. Treatment 1 with 0.3% concentration of OAB reduced the viral concentration from 4.48 $\log_{10}$ HAD_{50}/mL on day 1 to 3.29 $\log_{10}$ HAD_{50}/mL on day 3 and remained undetected on day 7. In Treatment 2 with 1% Phyto.A04, the virus was only detectable on day 1 (3.53 $\log_{10}$ HAD_{50}/mL). Treatments T3 (0.01% Phyto.B) and T4 (0.05% Phyto.B) both showed good effectiveness at completely inhibiting viral presence at days 3 and 7. With Treatments T2, T3 and T4, the presence of ASFV could not be detected on days 3 and 7. The positive control with only virus produced the highest $\log_{10}$ HAD_{50}/mL value of 5.54 on day 1, reduced to 4.56 $\log_{10}$ HAD_{50}/mL on day 3 and 3.58 $\log_{10}$ HAD_{50}/mL on day 7 (Figure 2).

Among the Treatments, Treatment 2 (1% Phyto.A04) was the most effective additive in reducing virus concentration. In the double combination groups, ASFV was detected in Treatments T5, T6, T7 and T8 only on the first day with HAD_{50} values of 3.53, 3.0, 3.27 and 3.0, respectively, while no ASFV was detected on the third and seventh day. The efficacy of the virucidal additives was even better with the triple combination. In Treatments T9 and T10, the virus was only detected on the first day with an HAD_{50} value of 2.43 and 2.66, respectively, and was then no longer detectable on the third and seventh day.

Comparison between the single and combined treatments of additives revealed that combining additives at different concentrations had better virucidal results. With all the combined treatments (T5–T10), Treatment T9 expressed the lowest viral load with 2.43 $\log_{10}$ HAD_{50}/mL on day 1 and went undetected on days 3 and 7. The results of the cytotoxicity test showed that the feed alone (coded as N1) and the feed plus with additives without ASFV (coded as C1) were safe for PAM cells (data not shown).

4 | Discussion

The ongoing ASF outbreak has had a major impact on swine production globally. Therefore, biosecurity efforts to limit the spread and transmission of ASFV are considered a top priority. It has been recognised that feed and feed ingredients can act as a vehicle for disease transmission and facilitate the spread of ASF, contributing to the transboundary spread of diseases such as ASF (Dee et al. 2018). According to a previous experimental study, the ASFV Georgia 2007 strain could survive for more than 30 days in transatlantic shipping conditions for 9 out of 12 tested feed ingredients, including organic soybean meal, conventional soybean meal, soy oilcake, pork sausage casings, choline, moist cat food, dry dog food, moist dog food and complete feed (Dee et al. 2018). Other ingredients, such as lysine, choline, vitamin D and complete swine feed in meal form, have also been shown to support wide-ranging pathogen stability (Stoian et al. 2020). A previous report has shown that complete feeds do not significantly reduce ASFV titers in ASFV-contaminated feeds during the incubation period (Dee et al. 2018). Our study also showed that complete feeds did not significantly reduce ASFV titers in ASFV-contaminated feeds during the 7-day incubation (P1 in Figures 1 and 2). Therefore, feed and feed ingredients are considered vectors for transboundary viral diseases and are also considered factors in transmitting ASF through the oral route. To avoid further transmission and reduce economic losses for owners, it is necessary to mitigate the risk of feed contamination

in herds. Although feed ingredients can be disinfected by physical and chemical means, their quality may be reduced. In this study, we proposed new and friendly plant-based additives that can easily be mixed with feed or feed ingredients to reduce viral contamination. The results showed that all additives exhibited good virucidal activity. The presence of ASFV in ASFV-spiked feed was reduced in all Treatments (Treatments 2–10) on day 1 and undetectable on days 3 and 7, as confirmed by both real-time PCR and cell culture methods, while Treatment 1 with 0.3% OAB showed negative results only on day 7 (Figures 1 and 2). Among the additives, the additive Phyto.A04 at a concentration of 1% showed the best virucidal effect, followed by Phyto.B at 0.05% and 0.01% and OAB at 0.3%. Phyto.A04, which was used in this study, is a preparation of bioactive substances with hops. It contains a CO_2 extract from the flowers of *H. lupulus*, which contains hop-soft resin in a total concentration of 0.1%. An earlier study has shown that soft resin compounds in hops are effective against gram-positive bacteria over a long period of time (Howard 1953). It is assumed that this effect is based on their surfactant-like properties (Behr and Vogel 2009). It cannot be ruled out that the described efficacy of these compounds against the outer membrane of gram-positive bacteria can also be observed in a similar way against enveloped viruses such as ASFV. Phyto.B, which was used in this study, is a preparation of bioactive substances containing anhydrous licorice extract *(G. glabra)* from aqueous extraction. Previous reports showed that the antiviral properties of licorice, likely due to the compound glycyrrhizin, are still under investigation (Huan et al. 2021). However, antiviral activity against enveloped viruses such as the HIV-1 virus has been demonstrated (Harada 2005). Of the additive combinations, Treatment 9 (0.3% OAB + 1% Phyto.A04 + 0.01% Phyto.B) showed the best virus reduction with a $\log_{10}$ HAD_{50}/mL value of 3.11, followed by Treatment 10 (0.3% OAB + 1% Phyto.A04 + 0.05% Phyto.B) with a $\log_{10}$ HAD_{50}/mL value of 2.88. The lowest efficacy of the additive combination was observed with Treatment 5 (0.3% OAB + 1% Phyto.A04). These results showed better antiviral activity of ASFV compared to previous reports tested with caprylic acid and formaldehyde additives at 0.03% (Jackman et al. 2020; Niederwerder et al. 2021). The results suggest that 1% Phyto.A04 alone or a combination of the additives OAB at 0.3% and Phyto.A04 at 1% and Phyto.B at 0.01% have the most impressive virus reduction ability.

The antiviral properties of various substances against ASFV have been extensively studied, focusing on the effects of pH on virus inactivation. It is well documented that both strongly acidic and strongly alkaline environments can inhibit ASFV activity. In particular, ASFV is sensitive to pH values below 3.9 and above 13, which can lead to the inactivation of the virus (Juszkiewicz et al. 2020; Plowright and Parker 1967). Although Phyto.A04 is an alkaline additive extracted from the flowers of *H. lupulus* with a pH of 11, and Phyto.B is an acidic additive containing anhydrous licorice extract *(G. glabra)* with a pH of 5.04, both showed strong anti-ASFV activity. Their pH values of 5.04 and 11 for Phyto.B and Phyto.A04, respectively, are far beyond the pH values of below 3.9 and above 13 that inactivate ASFV. Although we believe that the antiviral effect of these additives is due to specific bioactive compounds and not to their pH values, we recognise that this hypothesis is not directly supported by the pH data obtained under the experimental conditions. Further studies are needed to clarify the role of pH in the antiviral effect of these additives. Feed and feed ingredient biosecurity has become an important and widely recognised target to prevent viral swine diseases on farms. Biosecurity measures in feed and feed ingredients are essential to reduce the risk of ASFV at all stages of swine production, and implementing biosecurity procedures for feed can help to eradicate these risks (Reicks 2019; Stewart et al. 2020). In addition to biosecurity considerations for feed and feed ingredients, physical and chemical treatments of feed or ingredients can reduce the risk of ASFV contamination. For example, strict quarantine of feed and feed ingredients or storing them after importation from high-risk countries and regions is a strategy that allows the virus to degrade before these feed and feed ingredients are introduced into swine diets (Niederwerder et al. 2019). This study evaluated the efficacy of plant-based additives against ASFV in both cell culture and feed ingredients. Although commercial-grade feed is produced under strict control and regulations, there are still risks of contamination during transportation and delivery to pig producers' facilities. Additional treatment with safe and human-friendly additives could provide a better scenario in which contamination risks are further reduced. Some reports from the beginning of the ASF outbreaks in Vietnam revealed that ASFV could infect herds despite all applied biosecurity regulations. Contaminated feed ingredients may have been a cause of these outbreaks. This study may benefit commercial pig farms in avoiding ASF infection.

5 | Conclusions

In summary, this study showed that all additives, OAB, Phyto.A04 and Phyto.B, were able to inactivate ASFV, which was confirmed by cell culture and PCR methods. The PCR and cell culture results are the same and consistent. The efficacy of these additives provides a new approach to reducing the incidence of ASFV in feed ingredients using plant-based additives. However, the molecular interactions between the additives and ASFV and the effects of the additives on the health and production of pigs need to be further investigated.

Author Contributions

Conceptualisation: Niku Moussavi Biuki, Bernhard Eckel, Viktor P.L. Eckel and Van Phan Le. Formal analysis: Thi Ngoc Ha Lai and Thi Bich Ngoc Trinh. Funding acquisition: Van Phan Le and Thi Lan Nguyen. Investigation: Van Phan Le. Methodology: Van Phan Le. Project administration: Van Phan Le and Thi Lan Nguyen. Resources: Van Phan Le and Thi Lan Nguyen. Software: Thi Ngoc Ha Lai, Thi Bich Ngoc Trinh, Thi Tam Than and Nguyen Tuan Anh Mai. Supervision: Van Phan Le. Validation: Van Phan Le. Visualisation: Thi Ngoc Ha Lai and Thi Bich Ngoc Trinh. Writing—original draft: Thi Ngoc Ha Lai, Thi Bich Ngoc Trinh and Nguyen Tuan Anh Mai. Writing—review and editing: Van Phan Le.

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Ethics Statement

This study was conducted in the biosafety facility of the Faculty of Veterinary Medicine, Vietnam National University of Agriculture (VNUA),

Hanoi, Vietnam. All virus experiments were conducted in accordance with good experimental practice guidelines. In addition, the animal experiments were approved by the Committee on Animal Research and Ethics of the Faculty of Veterinary Medicine of VNUA with approval number CARE −2021/10 and an approval date of 4 December 2021.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data are provided by the corresponding author with reasonable requests.

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/vms3.70070.

References

Amado, I. R., J. A. Vázquez, P. Fucinos, J. Méndez, and L. Pastrana. 2013. "Optimization of Antimicrobial Combined Effect of Organic Acids and Temperature on Foodborne *Salmonella* and *Escherichia coli* in Cattle Feed by Response Surface Methodology." *Foodborne Pathogens and Disease* 10, no. 12: 1030–1036. https://doi.org/10.1089/fpd.2013.1559.

Arzumanyan, H., S. Hakobyan, H. Avagyan, R. Izmailyan, N. Nersisyan, and Z. Karalyan. 2021. "Possibility of Long-Term Survival of African Swine Fever Virus in Natural Conditions." *Veterinary World* 14, no. 4: 854–859. https://doi.org/10.14202/vetworld.2021.854-859.

Behr, J., and R. F. Vogel. 2009. "Mechanisms of Hop Inhibition: Hop Ionophores." *Journal of Agriculture and Food Chemistry* 57, no. 14: 6074–6081. https://doi.org/10.1021/jf900847y.

Boklund, A., S. Dhollander, T. Chesnoiu Vasile, et al. 2020. "Risk Factors for African Swine Fever Incursion in Romanian Domestic Farms During 2019." *Scientific Reports* 10, no. 1: 10215. https://doi.org/10.1038/s41598-020-66381-3.

Cottingim, K. M., H. Verma, P. E. Urriola, F. Sampedro, G. C. Shurson, and S. M. Goyal. 2017. "Feed Additives Decrease Survival of Delta Coronavirus in Nursery Pig Diets." *Porcine Health Management* 3: 5. https://doi.org/10.1186/s40813-016-0048-8.

Dee, S. A., F. V. Bauermann, M. C. Niederwerder, et al. 2018. "Survival of Viral Pathogens in Animal Feed Ingredients Under Transboundary Shipping Models." *PLoS ONE* 13, no. 3: e0194509. https://doi.org/10.1371/journal.pone.0194509.

Dixon, L. K., D. A. Chapman, C. L. Netherton, and C. Upton. 2013. "African Swine Fever Virus Replication and Genomics." *Virus Research* 173, no. 1: 3–14. https://doi.org/10.1016/j.virusres.2012.10.020.

Gallardo, C., D. M. Mwaengo, J. M. Macharia, et al. 2009. "Enhanced Discrimination of African Swine Fever Virus Isolates Through Nucleotide Sequencing of the p54, p72, and pB602L (CVR) Genes." *Virus Genes* 38, no. 1: 85–95. https://doi.org/10.1007/s11262-008-0293-2.

Ge, S., J. Li, X. Fan, et al. 2018. "Molecular Characterization of African Swine Fever Virus, China, 2018." *Emerging Infectious Diseases* 24, no. 11: 2131–2133. https://doi.org/10.3201/eid2411.181274.

Harada, S. 2005. "The Broad Anti-Viral Agent Glycyrrhizin Directly Modulates the Fluidity of Plasma Membrane and HIV-1 Envelope." *Biochemical Journal* 392, no. Pt 1: 191–199. https://doi.org/10.1042/bj20051069.

Howard, G. 1953. "Evaluation of Hops. I. A Review." *Journal of the Institute of Brewing* 59, no. 1: 36–52. https://doi.org/10.1002/j.2050-0416.1953.tb02710.x.

Huan, C., Y. Xu, W. Zhang, T. Guo, H. Pan, and S. Gao. 2021. "Research Progress on the Antiviral Activity of Glycyrrhizin and Its Derivatives in Liquorice." *Frontiers in Pharmacology* 12: 680674. https://doi.org/10.3389/fphar.2021.680674.

Jackman, J. A., A. Hakobyan, H. Zakaryan, and C. C. Elrod. 2020. "Inhibition of African Swine Fever Virus in Liquid and Feed by Medium-Chain Fatty Acids and Glycerol Monolaurate." *Journal of Animal Science and Biotechnology* 11, no. 1: 114. https://doi.org/10.1186/s40104-020-00517-3.

Juszkiewicz, M., M. Walczak, N. Mazur-Panasiuk, and G. Woźniakowski. 2020. "Effectiveness of Chemical Compounds Used Against African Swine Fever Virus in Commercial Available Disinfectants." *Pathogens* 9, no. 11: 878. https://doi.org/10.3390/pathogens9110878.

Kim, H. J., K. H. Cho, S. K. Lee, et al. 2020. "Outbreak of African Swine Fever in South Korea, 2019." *Transbound and Emerging Diseases* 67, no. 2: 473–475. https://doi.org/10.1111/tbed.13483.

Le, V. P., D. G. Jeong, S. W. Yoon, et al. 2019. "Outbreak of African Swine Fever, Vietnam, 2019." *Emerging Infectious Diseases* 25, no. 7: 1433–1435. https://doi.org/10.3201/eid2507.190303.

Mai, N. T. A., X. D. Vu, T. T. H. Nguyen, et al. 2021. "Molecular Profile of African Swine Fever Virus (ASFV) Circulating in Vietnam During 2019–2020 Outbreaks." *Archives of Virology* 166, no. 3: 885–890. https://doi.org/10.1007/s00705-020-04936-5.

Matsumoto, N., J. Siengsanan-Lamont, L. J. Gleeson, et al. 2020. "Evaluation of the Diagnostic Accuracy of an Affordable Rapid Diagnostic Test for African Swine Fever Antigen Detection in Lao People's Democratic Republic." *Journal of Virological Methods* 286: 113975. https://doi.org/10.1016/j.jviromet.2020.113975.

Niederwerder, M. C., S. Dee, D. G. Diel, et al. 2021. "Mitigating the Risk of African Swine Fever Virus in Feed With Anti-Viral Chemical Additives." *Transboundary and Emerging Diseases* 68, no. 2: 477–486. https://doi.org/10.1111/tbed.13699.

Niederwerder, M. C., and R. A. Hesse. 2018. "Swine Enteric Coronavirus Disease: A Review of 4 Years With Porcine Epidemic Diarrhoea Virus and Porcine Deltacoronavirus in the United States and Canada." *Transboundary and Emerging Diseases* 65, no. 3: 660–675. https://doi.org/10.1111/tbed.12823.

Niederwerder, M. C., A. M. M. Stoian, R. R. R. Rowland, et al. 2019. "Infectious Dose of African Swine Fever Virus When Consumed Naturally in Liquid or Feed." *Emerging Infectious Diseases* 25, no. 5: 891–897. https://doi.org/10.3201/eid2505.181495.

Nuanualsuwan, S., T. Songkasupa, P. Boonpornprasert, N. Suwankitwat, W. Lohlamoh, and C. Nuengjamnong. 2022. "Persistence of African Swine Fever Virus on Porous and Non-Porous Fomites at Environmental Temperatures." *Porcine Health Management* 8, no. 1: 34. https://doi.org/10.1186/s40813-022-00277-8.

Nurmoja, I., K. Mõtus, M. Kristian, et al. 2020. "Epidemiological Analysis of the 2015–2017 African Swine Fever Outbreaks in Estonia." *Preventative Veterinary Medicine* 181: 104556. https://doi.org/10.1016/j.prevetmed.2018.10.001.

Oļševskis, E., V. Guberti, M. Seržants, et al. 2016. "African Swine Fever Virus Introduction Into the EU in 2014: Experience of Latvia." *Research in Veterinary Science* 105: 28–30. https://doi.org/10.1016/j.rvsc.2016.01.006.

Plowright, W., and J. Parker. 1967. "The Stability of African Swine Fever Virus With Particular Reference to Heat and pH Inactivation." *Archives of Virology* 21, no. 3: 383–402. https://doi.org/10.1007/bf01241738.

Reed, L. J., and H. Muench. 1938. "A Simple Method of Estimating Fifty Per Cent Endpoints." *American Journal of Epidemiology* 27, no. 3: 493–497. https://doi.org/10.1093/oxfordjournals.aje.a118408.

Reicks, D. L. 2019. "Effective Biosecurity to Protect North American Studs and Clients From Emerging Infectious Disease." *Theriogenology* 137: 82–87. https://doi.org/10.1016/j.theriogenology.2019.05.041.

Sanna, G., S. Dei Giudici, D. Bacciu, et al. 2017. "Improved Strategy for Molecular Characterization of African Swine Fever Viruses From Sardinia, Based on Analysis of p30, CD2V and I73R/I329L Variable Regions." *Transboundary and Emerging Diseases* 64, no. 4: 1280–1286. https://doi.org/10.1111/tbed.12504.

Stewart, S. C., S. S. Dritz, J. C. Woodworth, C. Paulk, and C. K. Jones. 2020. "A Review of Strategies to Impact Swine Feed Biosecurity." *Animal Health Research Reviews* 21, no. 1: 61–68. https://doi.org/10.1017/s146625231900015x.

Stoian, A. M. M., V. Petrovan, L. A. Constance, et al. 2020. "Stability of Classical Swine Fever Virus and Pseudorabies Virus in Animal Feed Ingredients Exposed to Transpacific Shipping Conditions." *Transboundary and Emerging Diseases* 67, no. 4: 1623–1632. https://doi.org/10.1111/tbed.13498.

Toro, H., V. L. van Santen, and C. Breedlove. 2016. "Inactivation of Avian Influenza Virus in Nonpelleted Chicken Feed." *Avian Diseases* 60, no. 4: 846–849. https://doi.org/10.1637/11465-070616-ResNote.

Trudeau, M. P., H. Verma, F. Sampedro, et al. 2016. "Comparison of Thermal and Non-Thermal Processing of Swine Feed and the Use of Selected Feed Additives on Inactivation of Porcine Epidemic Diarrhea Virus (PEDV)." *PLoS ONE* 11, no. 6: e0158128. https://doi.org/10.1371/journal.pone.0158128.

Wen, X., X. He, X. Zhang, et al. 2019. "Genome Sequences Derived From Pig and Dried Blood Pig Feed Samples Provide Important Insights Into the Transmission of African Swine Fever Virus in China in 2018." *Emerging Microbes and Infections* 8, no. 1: 303–306. https://doi.org/10.1080/22221751.2019.1565915.

Zhai, S. L., W. K. Wei, M. F. Sun, D. H. Lv, and Z. H. Xu. 2019. "African Swine Fever Spread in China." *Veterinary Record* 184, no. 18: 559. https://doi.org/10.1136/vr.l1954.