國立中興大學
National Chung Hsing University

&

NSP
矽牧公司
Si Moon

NSP for Life !

==========

(Friendly Derived Functions for Poultry Farming)

==========

Drug- and Antibiotic-free Poultry of Raising Chickens and Pigs

Li-Ker-Lin

Formulated with NSP (Natural Silicate Platelets)

NSP function is derived from the combined three properties,
(1) "physical capturing" for pathogenic bacteria, due to presence of high surface area (750 square-meter/gram) of NSP, rendering strong surface-adsorption force adhering with bacteria, organic toxins, and ammonia gas,
(2)"non-toxic" nature of NSP (evidenced by passing LD50 oral lethal dose test)—gaining free of drug-resistant that is often caused by chemical drugs and a concern of "food safety",
(3) "long-term stability"--being stable silicates (not organic chemical) and well dispersible in water, NSP behave to "friendly join the equilibrium of the Nature".

The above three properties collectively render the NSP multi-function and unique for substituting chemical drugs or antibiotics in poultry farming. The NSP formulated product, based on the field trails, enables to interrupt the growth of pathogenic bacteria, protozoan and viral infection. Further, NSP of adsorbing odorous gases and protecting the respiratory tract of poultry chickens have resulted the prevention of diseases and enhanced the health of the poultry. NSP can help the poultry farming for the issues of food safely and food security.

Papers Published

(Lin and Peng, ACS Applied Materials and Interfaces, 2 (6), **2010**, 1608)

ARTICLE

Evaluation on Cytotoxicity and Genotoxicity of the Exfoliated Silicate Nanoclay

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ABSTRACT The concern about toxicity for nanosilicate platelets (NSP) derived from natural montmorillonite clay is addressed. The NSP nanoclay was isolated from polyamine−salt exfoliation of the layered silicate clay into randomized individual plates, possessing multiple ionic charges on the surface of silicate plates with an average geometric dimension of ca. 80 × 80 × 1 nm^3. The material had been previously shown to be effective for antimicrobial and tendency for adhering onto the biomaterial surface based on the direct observation by using scanning electron microscope. The material safety on genotoxic effect was investigated by using three different test systems: the Comet assay test on Chinese Hamster Ovary (CHO) cells in vitro, micronucleus (MN) assay in vivo and the *Salmonella* gene mutation assay on strain TA98, TA100, TA102, TA1535 and TA1537. The Comet assay showed no DNA damage after 24 h of incubation with NSP of 1000 μg/mL. The MN test indicated no significant micronucleus induction in the CHO cells at the concentrations tested. With all five strains of *Salmonella typhimurium*, none of mutations was found. Furthermore, cytotoxicity of the same material was assayed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) release, showing a low cytotoxicity on CHO cells below 1000 μg/mL after 12 h incubation period and a dose-dependent effect after 24 h incubation. For feeding to rats, the acute oral toxicity was shown a low lethal dose (LD_{50}) or greater than 5700 mg/kg body weight for both male and female Sprague-Dawley rats. Overall, the study has demonstrated the safety of the NSP for potential uses in biomedical areas.

KEYWORDS: nanosilicate platelets • cytotoxicity • genotoxicity • nanomaterials • acute oral toxicity

NSP safety and non-toxicity test：

1. **mouse acute toxicity：= salt**
2. **cytotoxicity： < 1000 ppm**
3. **henotoxicity：None**
4. **human cell：promote growth and healing**

The safety of NSP with respect to cytotoxicity and genotoxicity has been evaluated.

1. A low lethal dose (**LD_{50} > 5,700 mg/kg or similar to NaCl**) was found.
2. Low cytotoxicity (1000 ppm on CHO cells) and none of genotoxicity were reported.

(Journal of Physical Chemistry C (2011) 115, 18770)

THE JOURNAL OF PHYSICAL CHEMISTRY C

ARTICLE

pubs.acs.org/JPCC

Inhibition of Bacterial Growth by the Exfoliated Clays and Observation of Physical Capturing Mechanism

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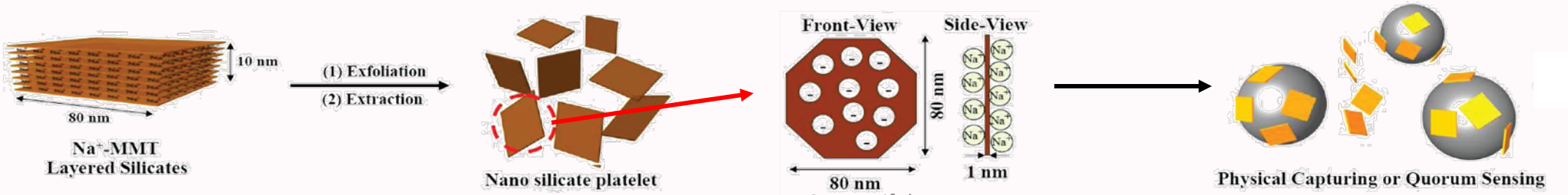
ABSTRACT: We observed unexpected antimicrobial behavior from the silicate platelets, prepared from the exfoliation of natural clays, for a broad spectrum of microorganisms. The antimicrobial properties are attributed to the unique structure of the exfoliated platelets, which possess an average dimension of 80 nm × 80 nm × 1 nm and polyvalent ionic charges (ca. 18 000 sodium ions/platelet) on the surface of ultrathin platelets. The thin shape and ionic character of the silicates enables them to physically adhere to microbe surfaces and be directly observed by scanning electron microscopy. The nanometer-sized thin silicates with the uniquely combined features of large surface areas and polyvalent charges have potential uses for biomedical treatments.

physical capturing or blocking "Quorum Sensing" of bacterial cross-talking signals --



NSP For Life!

NSP resists viral infection by physical action (animal experiment)
Including JEV, dengue and influenza A virus

(Hsu, Lin and Lin, Journal of Virology, 88, 2014, 4218)

JVI Journals.ASM.org

Surfactant-Modified Nanoclay Exhibits an Antiviral Activity with High Potency and Broad Spectrum

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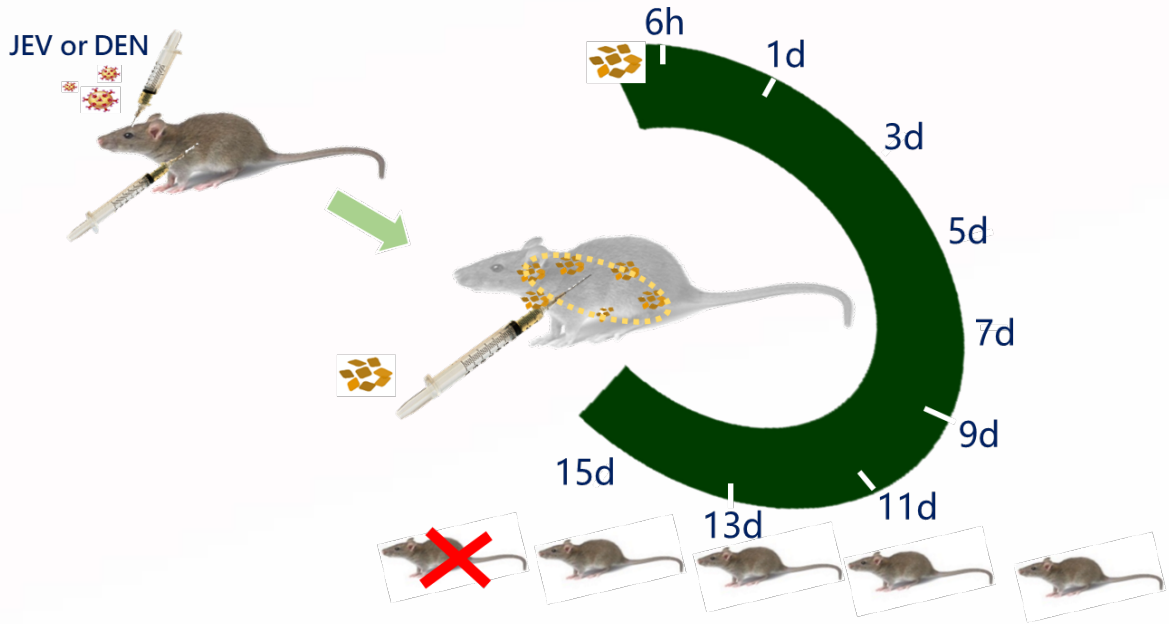
Institute of Biomedical Sciences[a] and Genomics Research Center,[c] Academia Sinica, Taipei, Taiwan; Institute of Polymer Science and Engineering, National Taiwan University, Taipei, Taiwan[b]

ABSTRACT

Nanomaterials have the characteristics associated with high surface-to-volume ratios and have been explored for their antiviral activity. Despite some success, cytotoxicity has been an issue in nanomaterial-based antiviral strategies. We previously developed a novel method to fully exfoliate montmorillonite clay to generate the most fundamental units of nanoscale silicate platelet (NSP). We further modified NSP by capping with various surfactants and found that the surfactant-modified NSP (NSQ) was less cytotoxic. In this study, we tested the antiviral potentials of a series of natural-clay-derived nanomaterials. Among the derivatives, NSP modified with anionic sodium dodecyl sulfate (NSQc), but not the pristine clay, unmodified NSP, a silver nanoparticle-NSP hybrid, NSP modified with cationic *n*-octadecanylamine hydrochloride salt, or NSP modified with nonionic Triton X-100, significantly suppressed the plaque-forming ability of Japanese encephalitis virus (JEV) at noncytotoxic concentrations. NSQc also blocked infection with dengue virus (DEN) and influenza A virus. Regarding the antiviral mechanism, NSQc interfered with viral binding through electrostatic interaction, since its antiviral activity can be neutralized by Polybrene, a cationic polymer. Furthermore, NSQc reduced the lethality of JEV and DEN infection in mouse challenge models. Thus, the surfactant-modified exfoliated nanoclay NSQc may be a novel nanomaterial with broad and potent antiviral activity.

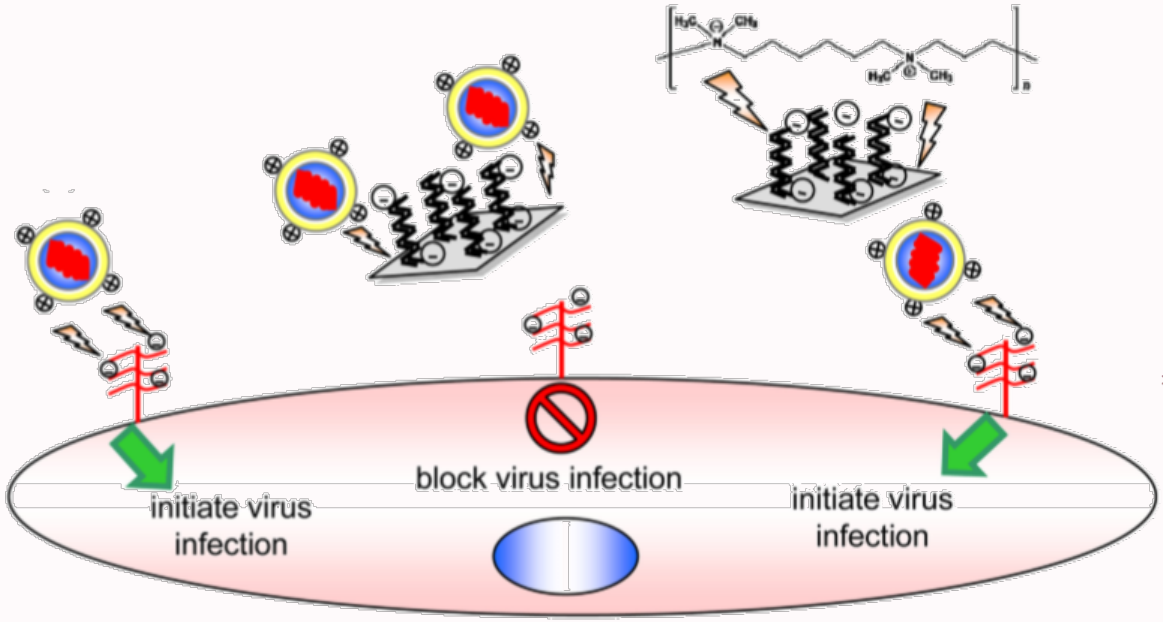


1. 注射NSP後, 病毒感染老鼠之存活率, 可從(0-20%)提昇至 (80-100%).
2. 解剖分析: NSP對肝臟安全性測試 ---無損害!

Animal experiment : NSP can reduce the fatality of the infected mice, before or after virus infection, the efficacy as high as 80-100% in reducing the death rate !
NSP has a high "therapeutic index", meaning the high safety and non-toxic nature.

NSP "physical shield effect" – neutralizing virus and adhering on host cell surface shielding off virus attack – anti-virus mechanism in (non-chemical) manner

(Surfactant- modified nanoclay exhibits an antiviral activity with high potency and broad spectrum, *Journal of Virology, 88, 4218-4228.)*



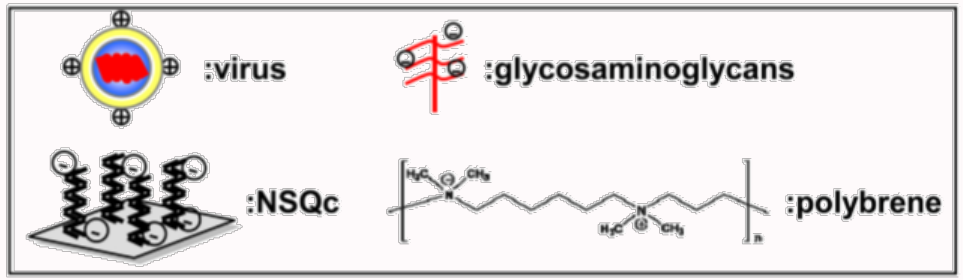
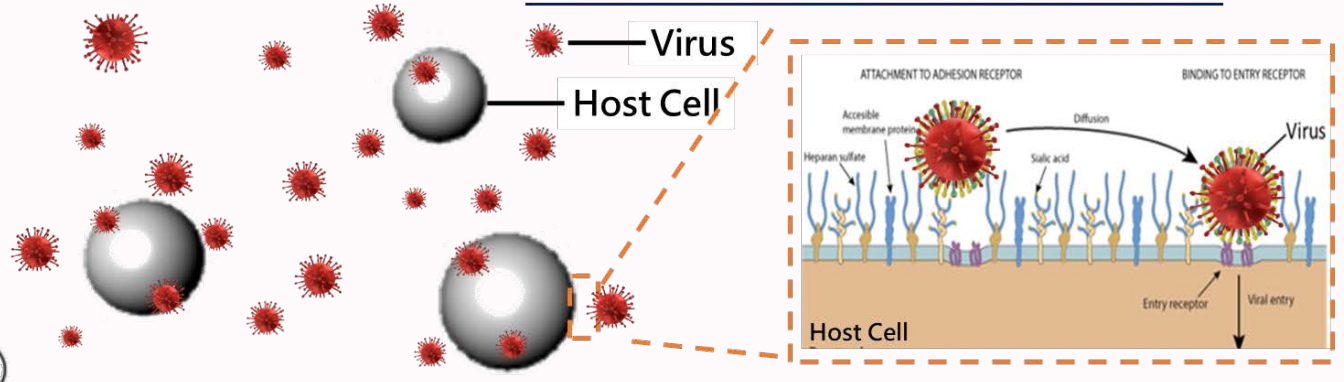
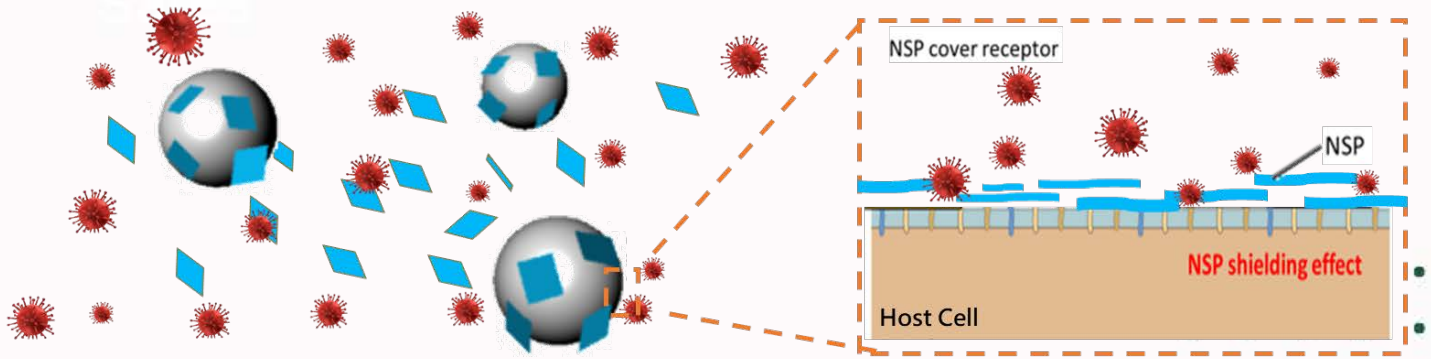


示意圖: 一般病毒入侵正常細胞之感染過程

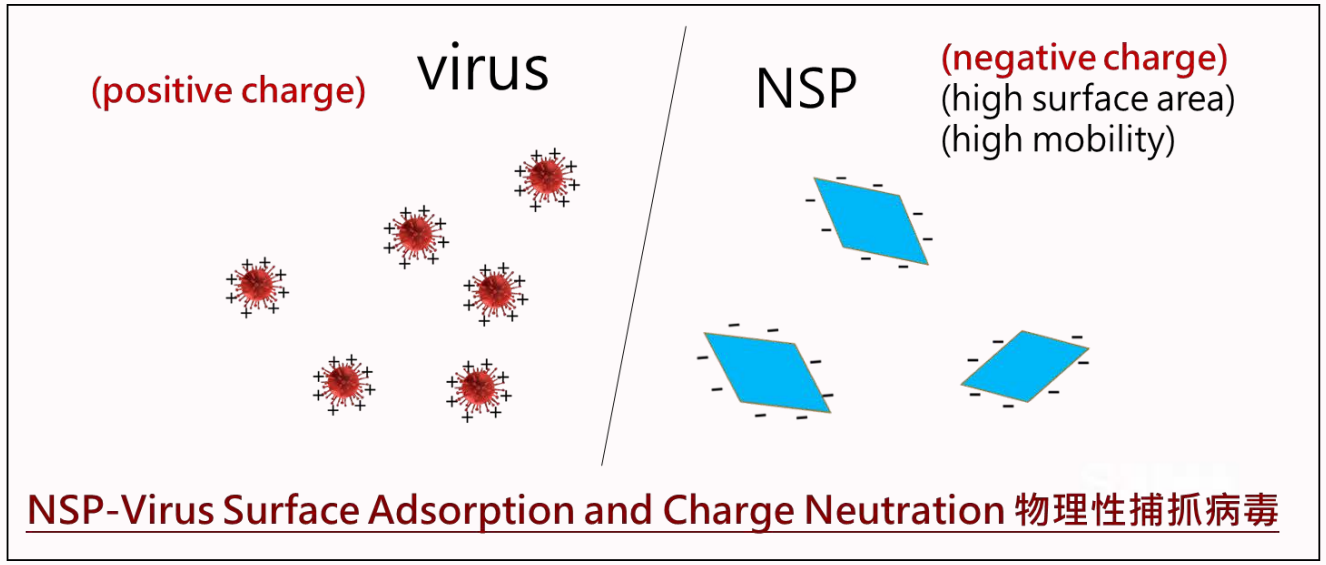


NSP"盾牌屏蔽"物理性捕捉病毒,阻斷病毒接觸細胞表面受體

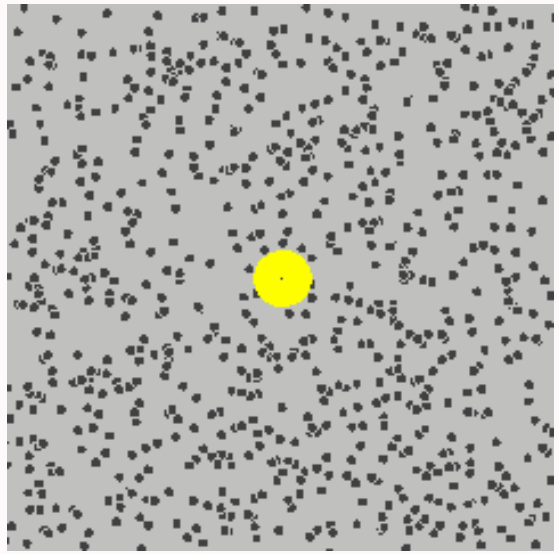


The basic principle of NSP anti-virus

1. NSP high surface area
2. Positive/Negative charge neutralization
3. NSP high mobility in water and in air
4. In vitro and in vivo (JEV, DEN and influenza A virus)



NSP-Virus Surface Adsorption and Charge Neutration 物理性捕抓病毒



JJ Lin, Yi-Ling Lin,et al. **2014.** Surfactant- modified nanoclay exhibits an antiviral activity with high potency and broad spectrum, Journal of Virology, 88, 4218; (Taiwan Patent I546080 (2013)*

JJ Lin et. al., 2011. Inhibition of Bacterial Growth by the Exfoliated Clays and Observation of Physical Capturing Mechanism, Journal of Physical Chemistry C, 115, 18770181.

Salmonella infection (chicken, pig feed/drinking water) avian influenza and bacterial infection

(International Journal of Nanomedicine, 7, **2012**, 2421-2423)

International Journal of Nanomedicine

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ORIGINAL RESEARCH

Efficacy and safety of nanohybrids comprising silver nanoparticles and silicate clay for controlling *Salmonella* infection

This article was published in the following Dove Press journal:
International Journal of Nanomedicine
11 May 2012
Number of times this article has been viewed

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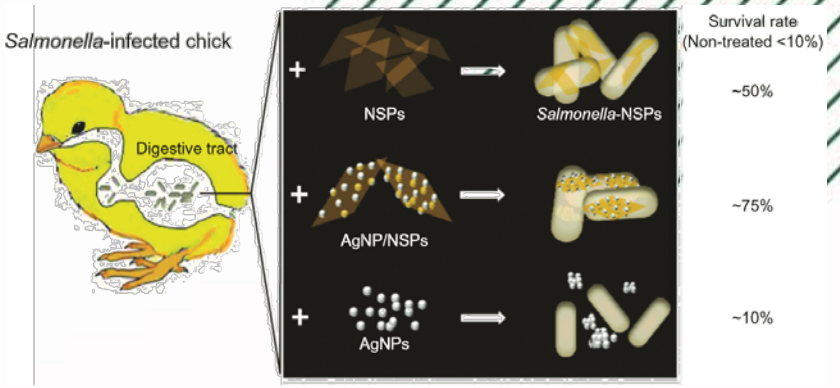
Abstract: Developing effective and safe drugs is imperative for replacing antibiotics and controlling multidrug-resistant microbes. Nanoscale silicate platelet (NSP) and its nanohybrid, silver nanoparticle/NSP (AgNP/NSP), have been developed, and the nanohybrids show a strong and general antibacterial activity in vitro. Here, their efficacy for protecting *Salmonella*-infected chicks from fatality and septicemia was evaluated. Both orally administrated NSP and AgNP/NSP, but not AgNPs alone, effectively reduced the systemic *Salmonella* infection and mortality. In addition, quantitative Ag analyses demonstrated that Ag deposition from AgNP/NSP in the intestines was less than that from conventional AgNPs, indicating that the presence of NSP for immobilizing AgNPs reduced Ag accumulation in tissue and improved the safety of AgNPs. These in vivo results illustrated that both NSP and AgNP/NSP nanohybrid represent potential agents for controlling enteric bacterial infections.

Keywords: silver, nanoparticle, biocompatibility, infection, cytotoxicity



- NSP and AgNP/NSP, but not AgNPs alone, were potential antimicrobial agents for controlling **enteric bacterial infection.**
- In contrast to the considerable Ag deposits, **the Ag accumulation in digestive tissues from AgNP/ NSP was significantly decreased**, showing improved **safety** over conventional AgNPs in animals.
- **Reduce the mortality rate of pullorum by 50%**
- **Improve the curative effect of antibiotics on pullorum by 70%**

NSP livestock feed added to remove toxins-FB1

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Evaluation of Efficacy and Toxicity of Exfoliated Silicate Nanoclays as a Feed Additive for Fumonisin Detoxification

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Supporting Information

ABSTRACT: The efficacy of nanosilicate clay platelets (NSCP), exfoliated silicates from natural montmorillonites, as a feed additive for ameliorating fumonisin B1 (FB1) toxicosis was evaluated. Toxicological mechanisms by NSCP were examined through proteomic and biochemical analyses. Dietary supplementation with NSCP at a low level of 40 mg/kg of feed improved growth performances in chickens with respect to FB1 toxicosis. Other issues of ameliorated symptoms including serum and/or hepatic aspartate aminotransferase activity, oxidative stress indicators, and sphinganine/sphingosine ratio, a hallmark of FB1 toxicosis, were considered. Chickens with NSCP inclusion alone at 1000 mg/kg of feed exhibited no changes in hepatic histology, oxidative status, and serum parameters and even had a higher feed intake. Proteomic analysis with liver tissues identified 45 distinct proteins differentially affected by FB1 and/or NSCP, in which proteins involved in thiol metabolism and redox regulation, glycolysis, carcinogenesis, and detoxification by glutathione *S*-transferase were promoted by FB1, whereas NSCP caused differential changes of protein abundances related to methionine/cysteine and choline/glycine interconversion for glutathione synthesis, redox regulation by peroxiredoxin, toxin/metabolite delivery by albumin, glycolysis, tricarboxylic acid cycle, adenosine triphosphate (ATP) synthesis, and chaperon escort for endoplasmic reticulum stress relief. Functional analyses confirmed the enhancement of hepatic metabolic processes for ATP and NAD(P)H production to meet the need for detoxification, antioxidative defense, and toxin/metabolite clearance by FB1 or NSCP ingestion. On the basis of the amelioration of FB1 toxicosis, global profile of hepatic protein expressions, and validated toxicological mechanisms, NSCP were concluded as a safe and effective agent for FB1 detoxification.

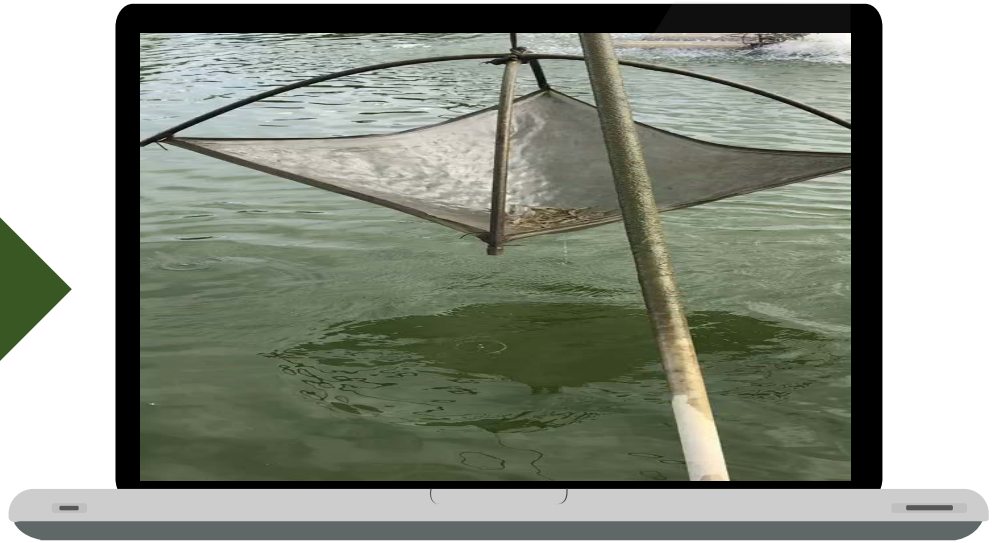
KEYWORDS: *nanosilicate platelets, fumonisin, detoxification, liver, proteomics*

- NSP(40 ppm): slow growth retardation in toxin-poisoned chickens (by 10%)
- Promote feeding effect (by 10.4%)
- In the study, dietary inclusion of NSP effectively improved the **growth performance and ameliorated FB1 toxicosis**, including oxidative stress, SA/SO ratio, and AST activity.
- Growth performances and toxicological analysis concluded NSP as a safe and effective agent for FB1 detoxification. Proteomic and function analyses suggested an enhancement of metabolic processes for ATP and NAD(P) H production for detoxification and antioxidative mechanisms, following toxin/metabolite clearance by NSP inclusion.

NSP treating the virus-infected shrimp

control

- ✓ Water: turbidity .
- ✓ Shrimp activity: less jumping, less color on shrimp shell



(video by clicking on picture)

NSP

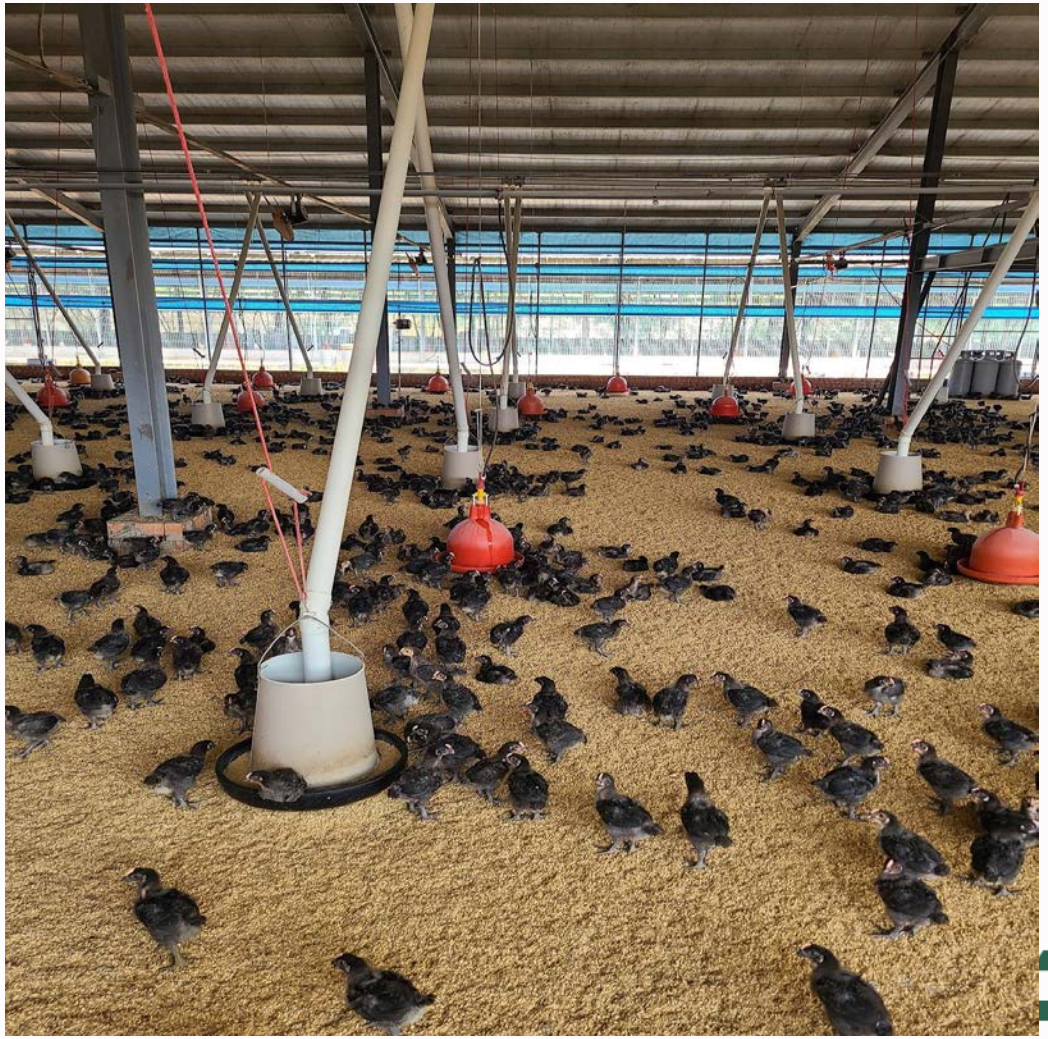
10 ppm
(calc. NSP weight / water in pond)

- ✓ Water: less turbidity
- ✓ Shrimp activity: gain back jumping activity after NSP

Day 2

(video by clicking on picture)

The disease of Coccidiosis (Chickens)—the cleaning and moisture of floor hygiene and odor of ammonia (NSP reducing the sporulation of coccidial oocysts in the life cycle)



已有農委會核准非屬飼料添加物函文

Livestock

NSP-formulated LIN-KER-LIN

固態

力克靈-S

Powder product
Registered in Taiwan Government

液態

力克靈

Liquid product Registered





NSP For Life!

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