



## NOTE

Avian Pathology

# Durability of alkaline agents' bactericidal efficacies in litter under field conditions

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**ABSTRACT.** Alkaline agents are well-known for their disinfection capacities against pathogens even at the presence of organic materials, but the durability of their bactericidal efficacies under field conditions is unknown. Therefore, within the present study, two alkaline agents, namely bioceramic (BCX) derived from chicken feces and food additive grade  $\text{Ca}(\text{OH})_2$  (FdCa(OH)<sub>2</sub>) derived from natural lime stone, were evaluated for the persistence of their bactericidal efficacies in litter, under simulated field conditions. BCX powder mixed at 50% concentration in litter or FdCa(OH)<sub>2</sub> powder at 20% concentration in litter lost their bactericidal efficacies at 3 days post exposure of chicks, and thereafter, both mentioned alkaline agents could not inactivate bacteria down to the acceptable level ( $\geq 3 \log_{10}$  CFU/ml reduction).

**KEY WORDS:** bioceramic, food additive grade calcium hydroxide, infection control/disinfection, livestock biosecurity, poultry production

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Meat consumption annually increases around the world, with a shift towards poultry consumption that highlights increasing role of the poultry industry [3]. However, the control of infectious diseases still remains a large problem for poultry productions. Enhancement of the biosecurity in livestock industry is the key point for successful farming, and for providing safe animal products, which can be facilitated through application of ideal biosecurity materials. Alkaline agents are well known for their strong bactericidal efficacies under field conditions, and most frequently are applied for the control and prevention of livestock's infectious diseases [1, 2, 11, 13, 18]. Furthermore, treatment of bedding materials and/or poultry wastes through application of lime is another practice to prevent environmental contamination and to enhance poultry performance [6, 12, 15]. Application of lime was suggested by Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan since highly pathogenic avian influenza (HPAI) outbreaks occurred in 2004, around the poultry houses, in order to enhance farms biosecurity [16]. Addition to them, application of lime is a common practice for disinfection of livestock farms, for the control of infectious diseases [4, 5].

Bioceramic (BCX) powder is a useful material with good disinfection capacities that is produced from chicken feces in Japan. Takehara *et al.* [16] demonstrated efficacies of BCX to inactivated avian influenza virus (AIV) for the first time; thereafter, Thammakarn *et al.* [17] reported its capacity to prevent fecal-oral transmission of infectious bursal disease virus (IBDV), alongside with its safety to chicks.

Food additive grade calcium hydroxide  $\text{FdCa}(\text{OH})_2$  powder is another novel product with strong bactericidal efficacy at the presence of organic materials. Hakim *et al.* [8] demonstrated its fast and strong bactericidal efficacy in feces. However, durability of its bactericidal efficacy under field condition remains unknown.

Despite wide application of alkaline agents at livestock farms, the durability of their bactericidal activity under field condition is unknown, and outbreaks of infectious diseases still persist as the most important challenges towards the livestock industry. Therefore, it is worthwhile to evaluate durability of alkaline agents, such as BCX [17] and  $\text{FdCa}(\text{OH})_2$  [8], for their persistent bactericidal capacities, under simulated field conditions.

BCX powder at pH 13 is derived from chicken feces through sintering process [17], and was kindly provided by NMG Environmental Development Co., Ltd. (Tokyo, Japan).  $\text{FdCa}(\text{OH})_2$  powder at pH 13 [8] was made of natural calcium carbonates from limestone through calcinations and grinding process, with the average diameter of the powder particle size at 10  $\mu\text{m}$ ; it was

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**Table 1.** Persistence of bactericidal efficacy of BCX and FdCa(OH)<sub>2</sub> powders in litter under the chicks exposure

Disinfectant	Bacteria	Days post exposure					
		0	1	2	3	4	5
BCX (50%)	<i>E. coli</i>	≥4.05 <sup>a)</sup> ± 0.00	≥4.05 ± 0.00	≥4.05 ± 0.00	3.08 ± 0.24	0.63 ± 0.33	Not tested
	<i>S. Infantis</i>	≥4.47 ± 0.00	≥4.47 ± 0.00	3.16 ± 0.01	2.99 ± 1.01	2.31 ± 0.52	Not tested
FdCa(OH) <sub>2</sub> (20%)	<i>E. coli</i>	≥3.96 ± 0.00	≥3.96 ± 0.00	≥3.96 ± 0.00	≥3.96 ± 0.00	1.24 ± 0.67	0.75 ± 0.00
	<i>S. Infantis</i>	≥4.47 ± 0.00	≥4.47 ± 0.00	4.17 ± 0.42	2.32 ± 0.14	3.03 ± 0.16	1.23 ± 0.33

BCX and FdCa(OH)<sub>2</sub> powders were mixed with litter and spread on the cages surfaces. Then, 6 chicks were kept per cage, and daily around 3 g samples were collected per cage and subjected to rifampicin resistant bacteria, and incubated for 6 hr exposure times in order to know whether still they kept their bactericidal efficacy. a) Data represent RF<sup>b)</sup> of two experiments as mean ± standard deviation. b) Reduction factor = log<sub>10</sub> (titer of control/ml) – log<sub>10</sub> (titer of treated samples/ml).

kindly provided by Fine Co., Ltd. (Tokyo, Japan). Litter (sawdust) was purchased from the local market, and used after being mixed with the disinfectant powders as bedding material in the experiments.

Bacterial suspension was prepared in accordance with the previously described method [7], but in order to make rifampicin resistant bacteria, 100 µl of log phase cultures of both *E. coli* and *S. Infantis* were employed through their surface plating on DHL agar containing different concentrations of rifampicin (from 0–100 µg/ml), individually, and followed by overnight incubation at 37°C. Colonies grown in the area containing higher concentration of rifampicin were picked up and sub-cultured on the DHL agar containing 100 µg/ml rifampicin. After overnight culture, around five colonies were picked up and subsequently cultured in the LB medium containing the same concentration of rifampicin. Log phase of bacteria from the solution culture was used for the experiments, and stocked in 10% skim milk at –80°C until used.

Bacterial inactivation was considered as reduction factor (RF), and was calculated using the equation below after conversion of bacterial titer to the log<sub>10</sub> CFU/ml:

$$RF = tpc - ta$$

Which thereon, *tpc* is the titer of bacteria from untreated sample in log<sub>10</sub> CFU/ml, while *ta* is the titer of recovered bacteria from treated samples. The inactivation rate was acceptable when the RF was greater than or equal to 3 [7, 10, 16, 18].

Bactericidal efficacy persistence test was designed to study duration of BCX and FdCa(OH)<sub>2</sub> powders' bactericidal efficacies in litter, under animal exposure. Animal work was conducted in strict accordance to the animal care guidelines of Tokyo University of Agriculture and Technology (Tokyo, Japan), with permit numbers of 26–45 and 27–20. Day-old commercial chicks, with no vaccination, here after called “conventional chicks”, were purchased from Kanto Co., Ltd. (Maebashi, Japan), and divided into groups of 6 chicks in rat cages (CLEA-0108-3, Clea Japan, Inc., Tokyo, Japan) containing 0, 50 and 20% w/w of BCX or FdCa(OH)<sub>2</sub> powders, respectively, in the total amount of 200 g in litter per cage, and kept inside the isolator (CL-5443, Clea Japan), while normal feed and water were provided. After proper mixing, 3 g of bedding materials containing BCX or FdCa(OH)<sub>2</sub> powders, and from 0% groups were sampled per cage, daily, and the collected samples were evaluated for bactericidal efficacy of the bedding materials, using rifampicin resistant *E. coli* and *S. Infantis* in accordance with previously described method [8]. Briefly, after measuring and adding of 0.5 g bedding material into 50 ml conical centrifuge tube, 100 µl of bacteria was inoculated and properly mixed. Then, samples were incubated at room temperature (25 ± 3°C) in a dark place. After 6 hr exposure times, 5 ml Tris-HCl was added to stop bactericidal capacity of bedding material and to harvest the remained bacteria through mixing, using vortex mixer for about 1 min. Serial tenfold dilution was prepared per sample and plated on DHL agar containing 100 µg/ml rifampicin to enumerate bacterial titer of the samples as previously described [7].

Data in the Table 1 present persistent bactericidal efficacies of BCX and FdCa(OH)<sub>2</sub> powders in the bedding materials within 6 hr of exposure time. Within 3 days post exposure of chicks, BCX and FdCa(OH)<sub>2</sub> powders lost their bactericidal efficacies in litter and could not reduce bacterial titer down to the acceptable level (RF ≥ 3). There was no reduction observed in the 0% group (data not shown).

Bedding materials in the poultry houses constitute significant environmental factors because of their affects not only to zoo-hygienic conditions of the farms, but also indirectly to the health and performance of poult. Nevertheless, applications of alkaline agents in the poultry houses are common practices for enhancement of the biosecurity, but still these practices are not effective, probably due to quack lose in the efficacy of alkaline agents under field conditions. Pathogens in the contaminated feces play a critical role in the transmission of infectious diseases from one to other animals, as well as in the contamination of surrounding environments [6]. Inactivation of pathogens present in the feces plays a fundamental role in the prevention of fecal-oral transmission of infectious diseases and in the enhancement of biosecurity at the livestock farms that can be possible through frequent application of disinfectants. Since BCX is recognized as feed for domestic animals (Livestock hygiene service center feed No. 538, MAFF), it is safe for the farm animals, while using it as mixed with the bedding materials. In addition to its excellent bactericidal and virucidal capacities, changing of the chicken feces to such valuable and environmental friendly material is another important point.

MAFF of Japan established a standard hazard analysis critical control point (HACCP) certification system for the livestock farms in 2009, in order to improve security for prevention of biological hazard contamination at the primary production site of the food chain, that is livestock farms [14], and this system drove farmers to enhance their farms biosecurity through application of

disinfectants to a good level, until to be recognized as HACCP certified farms by MAFF [9].

In conclusion, both BCX and  $\text{FdCa}(\text{OH})_2$  powders lost their efficacies to inactivate bacteria in the litter, which rejects their application as long term disinfectant materials, but they can be used for disinfection of chicken manure or other poultry wastes, just after harvesting from poultry farms, in order to prevent environment contamination. Both products are environmental friendly materials, with no risk to animal and human health, and can be used as biosecurity materials for the enhancement of biosecurity in the poultry productions. The findings of this study can also help farmers to make proper strategies for application of alkaline agents in their farms. Finally, the findings may help farmers to implement better strategies for controlling infections in their livestock farms.

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