Stability and virucidal efficacies using powder and liquid forms of fresh charcoal ash and slaked lime against Newcastle disease virus and Avian influenza virus

Sakchai Ruenphet1, Darsaniya Punyadarsaniya1, Tippawan Jantafong1 and Kazuaki Takehara2

1. Department of Immunology and Virology, Faculty of Veterinary Medicine, Mahanakorn University of Technology, Thailand; 2. Department of Veterinary Medicine, Laboratory of Animal Health, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Japan.

Corresponding author: Sakchai Ruenphet, e-mail: rsakchai@hotmail.com
Co-authors: DP: darsaniya_pl@yahoo.de, TJ: jantafong1980@gmail.com, KT: takehara@cc.tuat.ac.jp
Received: 23-07-2018, Accepted: 14-11-2018, Published online: 02-01-2019

do: 10.14202/vetworld.2019.1-6
How to cite this article: Ruenphet S, Punyadarsaniya D, Jantafong T, Takehara K (2019) Stability and virucidal efficacies using powder and liquid forms of fresh charcoal ash and slaked lime against Newcastle disease virus and Avian influenza virus, Veterinary World, 12(1): 1-6.

Abstract

Aim: The present study was examined the virucidal activity comparison between fresh charcoal ash (FCA) and slaked lime (SL) against avian influenza virus (AIV) and Newcastle disease virus (NDV), using powder and liquid forms, either in the absence or presence of organic materials. In addition, both FCA and SL were evaluated for the persistence of virucidal activity in wet and dry conditions and stability of the solution.

Materials and Methods: Two hundred milligrams of FCA or SL powders were mixed with 100 µl of AIV or NDV in the absence of organic material or 33% of organic materials. In the same time, 400 µl of 1%, 5%, or 10% solution samples were mixed with 100 µl of each virus and then incubated at room temperature for an indicated time. After that, the mixed solution was stop activity of sample using 500 µl of 1M Tris-HCl pH 7.2. Each treatment was titrated onto Madin-Darby canine kidney cells or chicken embryo fibroblasts for AIV or NDV, respectively, for determining the efficacy of viral inactivation. In addition, the stability of powder under the wet-dry condition and solution stability under room temperature was examined.

Results: The results demonstrated that the FCA and SL in powder form could inactivate AIV and NDV even in the absence or presence of organic materials. In the liquid form, 5% and 10% of FCA could inactivate AIV and NDV either in the absence or presence of organic materials. Alongside, 1%, 5%, and 10% of SL could inactivate both viruses. 10% of FCA solution could inactivate virus at a shortest time when compared with other concentrations. In addition, the efficacy of wet-dry conditions of FCA was limited when compared with SL. On the other hand, it is demonstrated that the FCA solution was more stable and kept at room temperature longer than SL.

Conclusion: The FCA may, hence, be used as an alternative virucide, while applying it to prevent spreading of poultry disease on commercial chicken farms and also backyard chickens, especially in developing countries, including in rural areas of Thailand.

Keywords: alkaline agent, fresh charcoal ash, slaked lime, virucidal activity.

Introduction

Several viral diseases, especially avian influenza (AI) and Newcastle disease, have a strong negative impact on commercial chicken. Normally, the related viruses are shed from the respiratory and gastrointestinal systems of clinically or sub-clinically infected birds and circulate in the environment. Biosecurity on farms, such as cleaning and disinfection, is one of the best instruments to reduce the microbial load generally and the level of pathogens in particular, in poultry farms [1,2], especially the mentioned viruses. In general, several organic solvents, detergents, and disinfectants are applied for microorganism inactivation; however, their efficacy is decreased when contaminated with organic materials [2]. There were several trials to outline alternative materials for biosecurity enhancement, which are not affected by organic materials contamination. Several researchers adapted the alternative materials for biosecurity enhancement in spite of the presence of organic material contamination, using alkaline agents such as slaked lime (SL), bioceramic powder [3], scallop shell powder [3], calcinated eggshell [4], and nano-sized scallop shell powder [2].

Charcoal ash or wood ashes are waste products from restaurants and household after cooking, which have alkaline compounds such as calcite (calcium carbonate [CaCO₃]), lime (CaO), and portlandite calcium hydroxide (Ca(OH)₂) [5,6]. The alkalinity of charcoal ash is high, with pH from 9.3 to 13.5 [5].

The aims of the present study were to evaluate virucidal efficacies of fresh charcoal ash (FCA) against various chicken pathogens such as AIV and Newcastle disease virus (NDV), either in the absence
or presence of organic materials, to evaluate their stability under wet and dry conditions, and to appraise solution stability for biosecurity application in chicken farms.

Materials and Methods

Ethical approval

Ethical approval is not applicable to this study.

Sample preparation

The FCA powders prepared by burning wood charcoal and SL (Zapco\textregistered, Homeinter supply Co., Ltd., Nonthaburi, Thailand) were used for the present study. Both solution samples were prepared as 1%, 5%, and 10% dilutions using distilled water. A quantity of 1, 5, or 10 g of each powder was added to 100 ml of dW and centrifuged at 1750× g for 10 min. The resulting supernatants were used as 1%, 5%, or 10% solutions as described [4].

Viruses and cells

Low pathogenic AI virus, namely A/duck/Aomori/395/04 H7N1 [7], and virulent vNDV, namely NDV/chicken/Asean Country/2013 [8], were propagated in chicken embryonic eggs. After allantoic fluid harvesting, stock viruses were aliquoted and kept at –80°C for testing. Madin-Darby canine kidney (MDCK) cells and chicken embryo fibroblasts (CEF) were used for AIV and NDV titration, respectively.

Powder reaction

To determine virus inactivation, 200 mg of FCA or SL powders were mixed with 100 µl of AIV or NDV in the absence of organic material. In addition, for evaluating the presence of organic materials, 100 µl of each virus was mixed with 50 µl of fetal bovine serum (FBS), and then the mixture was added to 300 mg of each powder sample. After 3 min incubation at room temperature, the viruses were recovered with 900 µl or 850 µl of PBS, respectively, then centrifuged at 17,400× g for 3 min, and titrated onto MDCK cells or CEF for virus recovering [4].

Liquid reaction

About 400 mL of 1%, 5%, or 10% solution samples were mixed with 100 µl of each virus and then incubated at room temperature for an indicated time such as 5 s, 30 s, 1 min, 3 min, 5 min, 10 min, 30 min, 1 h, or 2 h. After that, the mixed solution pH was neutralized with 500 µl of 1M Tris-HCl pH 7.2. Each sample treatment was titrated onto MDCK cells or CEF for AIV or NDV, respectively. 500 µl of FBS was added to 10 ml of each sample concentration as 5% organic materials representation. To confirm the neutralizing efficacy of Tris-HCl, it was added to each solution sample before virus adding, namely at 0 s. Each treatment was tested in triplicates, and the titers were shown in mean with standard error (mean ± SE).

Virus titration

Each treated virus was diluted in 10-fold serial dilution using Eagle’s minimum essential medium (MEM, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) and inoculated onto MDCK or CEF cells containing equal volume of MEM with trypsin (Trypsin, Sigma, St. Louis, MO, U.S.A.) reaching final concentrations of 0.5 and 0 µg/ml, respectively. All of the inoculated cell plates were incubated at 37°C in 5% CO\textsubscript{2} incubator and observed for the cytopathic effect twice a day for 3 days. At the end of the incubation period, the hemagglutinin activity of the culture supernatant was tested using 0.5% chicken red blood cells. The 50% tissue culture infectious dose (TCID\textsubscript{50}/ml) was determined by Behrens and Kärber’s method [2].

Stability

The virucidal activity of FCA and SL stored under harsh conditions using wet-dry environmental transitions was also evaluated. A quantity of 3 g of FCA or SL powder in a 90-mm Petri dish was used for making suspensions in 10 mL with tap water, and the dish was kept at 37°C incubator for complete drying. The dried sample was collected and tested by powder reaction method. Resuspension and drying were repeated until virucidal efficacy ceased.

The stability of the solution sample was evaluated at 2, 4, 6, and 8 weeks post preparing and keeping at room temperature. Virus inactivation was determined within a 3-min incubation period.

Inactivation analysis

The reduction factor (RF) was used for determining virus inactivation. The RF is calculated using the following equation: RF = \frac{t_{0}}{t_{a}}; where t\textsubscript{0} is the titer converted into an index in log\textsubscript{10} of the positive control, and t\textsubscript{a} is the converted titer an index in log\textsubscript{10} of the recovered virus from the treated sample. Inactivation of the virus was considered effective when RF was ≥3 log\textsubscript{10} [2,9,10].

Statistical analysis

The SE is the standard deviation of virus titer distribution. SE was calculated by standard deviation using Microsoft Excel.

Results

The pH of 1%, 5%, and 10% FCA solutions was recorded by pH paper strip as 12.0, 12.5, and 13.0, respectively, while the overall concentration of SL was measured to be 12.5.

Table-1 shows that either FCA or SL powders could inactivate AIV and NDV both in the absence and presence of organic materials and reduced the virus titer to below the detection limit (2.5 log\textsubscript{10} TCID\textsubscript{50}/ml).

The AIV inactivation is shown in Table-1. At 5% and 10%, FCA without organic material contamination could inactivate AIV within 30 min and 30 s, respectively. Even in the presence of organic materials, inactivation occurred within 2 h and 1 min, respectively. However, all concentrations of SL could inactivate AIV in the absence and presence of organic materials within 10 min and 30 min, respectively (Table-2).

Table-3 shows NDV inactivation using both solution samples. In the absence of organic material
Table 1: The results are shown as Log_{10} TCID_{50}/ml (mean±SE) of avian influenza virus inactivating activity by means of reaction in the powder of fresh charcoal ash and slaked lime in the absence and presence organic materials.

| Conditions | Newcastle disease virus | Avian influenza virus |
|------------|-------------------------|-----------------------|
|            | Fresh charcoal ash      | Slaked lime            | Fresh charcoal ash | Slaked lime |
| Absence*   | Presence*               | Absence | Presence | Absence | Presence | Absence | Presence | Absence | Presence |
| t<sub>c</sub> | 8.00±0.43               | 7.75±0.25 | 8.42±0.38 | 6.75±0.25 | 7.00±0.25 | 6.67±0.29 |
| t<sub>d</sub> | <2.50±0.00              | <2.50±0.00 | <2.50±0.00 | <2.50±0.00 | <2.50±0.00 | <2.50±0.00 |
| RF*        | >5.50±0.43              | >5.25±0.25 | >5.29±0.38* | >4.25±0.25* | >4.50±0.25* | >4.17±0.29* |

*Absence of organic material. *Presence of organic material as 33%. *The titer converted into an index in log_{10} of treatment. *The titer converted into an index in log_{10} of the recovered AIV from the treated tube. *The reduction factor=t<sub>c</sub>-t<sub>d</sub>. *Inactivation regarded effective when RF was ≥3

Table 2: The results are shown as Log_{10} TCID_{50}/ml (mean±SE) of Avian influenza virus inactivating activity by means of reaction in 1%, 5%, and 10% fresh charcoal ash and slaked lime solution in the absence or presence organic materials.

| Conditions | Fresh charcoal ash | Slaked lime |
|------------|-------------------|------------|
|            | Absence organic materials | Presence organic materials | Absence organic materials | Presence organic materials | Absence organic materials | Presence organic materials |
| t<sub>c</sub> | 7.25±0.50          | 7.42±0.88  | 7.92±0.52 | 7.58±0.52 | 7.58±0.52 | 7.58±0.52 |
| 0 s<sup>b</sup> | 7.00±0.25          | 7.42±0.88  | 7.58±0.63 | 7.17±0.38 | 7.33±0.72 | 7.75±0.75 |
| 5 s<sup>c</sup> | NT                | 6.33±0.72  | NT        | NT        | NT        | NT        |
| 30 s<sup>d</sup> | NT                | 3.42±0.88* | NT        | NT        | NT        | NT        |
| 1 min<sup>e</sup> | NT                | 3.50±0.87* | NT        | NT        | NT        | NT        |
| 5 min<sup>f</sup> | NT                | NT        | NT        | NT        | NT        | NT        |
| 10 min<sup>g</sup> | NT                | NT        | NT        | NT        | NT        | NT        |
| 30 min<sup>h</sup> | 6.67±0.29          | 6.57±0.72  | 6.25±0.90 | 5.33±0.63 | 5.58±0.29 | 6.83±0.80 |
| 1 h<sup>i</sup> | 6.75±0.25          | NT        | 6.47±0.29 | 3.92±1.01 | 3.92±1.13 | 6.08±0.38 |
| 2 h<sup>j</sup> | 6.92±0.29          | NT        | 6.92±0.25 | 3.67±0.63* | NT        | NT        |

*Fetal bovine serum was added to fresh charcoal ash and slaked lime solution as 5% organic materials of total volume. *Concentration of fresh charcoal ash and slaked lime solution (%w/v). *The titer converted into an index in log_{10} of virus control. *Added 1M Tris-HCl before virus. *The titer converted into an index in log_{10} of the recovered virus after indicated time of treatment such as 5 s, 30 s, 1 min, 5 min, 10 min, 30 min, 1 h, 2 h, 3 h, and 6 h. NT: Not tested. *Inactivation effective when RF was ≥3
Table-3: The results are shown as log_{10} TCID₅₀/ml (mean±SE) of Newcastle disease virus inactivating activity by the mean of reaction in 1%, 5%, and 10% fresh charcoal ash and slaked lime solution in the absence or presence of organic materials.

| Conditions | Absence of organic materials | Presence of organic materials |
|------------|------------------------------|------------------------------|
| Slaked lime | 1%                           | 5%                           | 10%                          |
| Fresh charcoal ash | 1%                           | 5%                           | 10%                          |

### Discussion

The FCA or wood ash is the residue powder left after the combustion of wood, such as burning wood in a home fireplace or an industrial powder plant. The largest component of charcoal ash (about 25%) is CaCO₃, <10% is potash, and <1% phosphate [5,6,11]. However, there are trace elements of iron, manganese, zinc, copper, and some heavy metals [11]. All of these are, primarily, in the form of oxides [12]. In addition, Demeyer et al. [5] and Tarun et al. [6] described the FCA as strong alkaline that contains main inorganic materials such as calcium oxide and Ca(OH)₂. In water, calcium oxide changes to Ca(OH)₂. The SL is an inorganic compound, practically containing >70% Ca(OH)₂. In general, Ca(OH)₂ is a strong alkaline substance. Most of the enteropathogens are unable to survive in this high alkaline environment [14]. In general, SL is widely used as a disinfectant and involves problems associated with its use, such as corrosion and human irritation. The disinfection mechanism of SL is thought to act due to its high pH [15]. The present study showed virucidal ability of SL which affected to viruses in powder form and solution form.

In general, several disinfectants such as chlorine and quaternary ammonium compounds could not inactivate bacteria and viruses when contaminated with organic materials. However, the FCA and
Table-4: The RF (log_{10} TCID_{50}/ml) of fresh charcoal ash and slaked lime applied for inactivating Newcastle disease virus and Avian influenza virus under wet and dry conditions at consecutive re-suspension times with a 3-min incubation period.

| Number of times resuspended | Avian influenza virus | Newcastle disease virus |
|-----------------------------|-----------------------|-------------------------|
|                             | Fresh charcoal ash    | Slaked lime              | Fresh charcoal ash    | Slaked lime |
| 1                           | >5.00*                | >4.75*                  | >5.75*                | >5.00*      |
| 2                           | 0.50                  | >4.75*                  | 5.25*                 | >5.50*      |
| 3                           | 0.50                  | >4.75*                  | 0.00                  | >5.50*      |
| 4                           | 0.50                  | >4.75*                  | 0.50                  | >5.50*      |
| 5                           | 0.50                  | >4.75*                  | NT                    | >5.50*      |
| 6                           | NT                    | >5.00*                  | NT                    | >5.50*      |
| 7                           | NT                    | >5.00*                  | NT                    | >5.50*      |
| 8                           | NT                    | >5.00*                  | NT                    | 3.25*       |
| 9                           | NT                    | >5.00*                  | NT                    | 3.75*       |
| 10                          | NT                    | >5.00*                  | NT                    | 2.50        |
| 11                          | NT                    | >5.00*                  | NT                    | 1.00        |
| 12                          | NT                    | >4.75*                  | NT                    | 0.00        |
| 13                          | NT                    | 2.50                    | NT                    | NT          |
| 14                          | NT                    | 2.25                    | NT                    | NT          |
| 15                          | NT                    | 1.75                    | NT                    | NT          |

Inactivation regarded effective when RF was ≥3, RF=Reduction factor

Table-5: The RF (log_{10} TCID_{50}/ml) of fresh charcoal ash and slaked lime solution applied for inactivating Newcastle disease virus and Avian influenza virus after being kept at room temperature with a 3-min incubation period.

| Time point (week) | Concentration (%) | Avian influenza virus | Newcastle disease virus |
|-------------------|-------------------|-----------------------|-------------------------|
|                   |                   | Fresh charcoal ash    | Slaked lime              | Fresh charcoal ash | Slaked lime |
| 2                 | 1                 | 0.50                  | 0.50                    | 0.50                | 0.50        |
|                   | 5                 | 0.50                  | 2.50                    | 1.75                | 0.50        |
|                   | 10                | 3.5*                  | 3.75*                   | >5.50*              | 0.50        |
| 4                 | 10                | 3.50*                 | 3.25*                   | >4.25*              | NT          |
| 6                 | 10                | 3.25*                 | NT                      | >5.25*              | NT          |
| 8                 | 10                | 3.25*                 | NT                      | >5.0*               | NT          |

Inactivation regarded effective when RF was ≥3, RF=Reduction factor

SL could inactivate AIV and NDV even in the presence of organic materials in the present study. These findings are compatible with results obtained by several researchers which tested alkaline agents such as food additive Ca(OH)₂ (pH 12.5) and SL (pH12.5); that showed virucidal effect toward NDV [16]; calcinated eggshell powder (pH12.7), that showed virucidal ability against NDV, AIV and infectious bursal disease virus [4,17]; as well as scallop shell powder (pH 13.0) and SL that could inactivate AIV [3]. Lorcharoenrungrong et al. [18] reported that FCA could inactivate AIV, *Escherichia coli*, and *Salmonella* infantis in the presence of organic material and those findings are related to the present study. Finally, not only alkaline agent that could inactivate viruses even in the presence of organic material but also acidic agents were pointed at by Sonthipet *et al.* [8], who described the bactericidal and virucidal efficacies of potassium monopersulfate (pH 2.04); this acidic agent also inactivated AIV on virus-spiked clothes.

In addition, the wet-dry conditions and the stability of the solution sample were illustrated and lasted long enough to inactivate AIV and NDV under both conditions. The efficacy of wet-dry conditions of FCA is limited when compared with SL. This result indicated that the stability of FCA powder is not steady, especially in the rainy season, when FCA powder may be washed or soaked by rain, thereupon being affected similarly to the wet conditions in the present study. On the other hand, it was demonstrated that the FCA solution was more stable and kept at room temperature longer than SL. These stability results may be applied, suggesting an alternative disinfectant agent, especially for biosecurity enhancement on and around chicken farms.

**Conclusion**

Both powder and solution forms of FCA and SL could inactivate AIV and NDV under various concentrations, organic material presence, and during exposure or contact timing. Thereby, FCA might be used as an alternative material, while applying it to prevent spreading of poultry disease on commercial chicken farms and also backyard chickens, especially in developing countries, including in rural areas of Thailand.

**Authors’ Contributions**

KT supervised the present study. SR designed and coordinated the study. TJ, SR, DP and KT performed the experiment. SR analyzed the data and wrote the manuscript. The final manuscript has been read and developed in consultation with all authors.
Acknowledgments

The authors thank Dr. Dany Shoham, Bar Ilan University, Israel, for the grammatical review of the manuscript. This work was supported in part by a grant number 60-VET-DVM-4.1-014 in aid from Veterinary Medicine Faculty, Mahanakorn University of Technology (MUT), Thailand, and Faculty of Agriculture, Tokyo University of Agriculture and Technology.

Competing Interests

The authors declare that they have no competing interests.

Publisher’s Note

Veterinary World remains neutral with regard to jurisdictional claims in published institutional affiliation.

References

1. Gehan, Z.M., Anwer, W., Amer, H.M., EL-Sabagh, I.M., Rezk, A. and Badawy, E.M. (2009) In vitro efficacy comparisons of disinfectants used in the commercial poultry farms. Int. J. Poult. Sci., 8(3): 237-241.
2. Thammakarn, C., Satoh, K., Suguro, A., Hakim, H., Ruenphet, S. and Takehara, K. (2014) Inactivation of avian influenza virus, Newcastle disease virus and goose parvovirus using solution of nano-sized scallop shell powder. J. Vet. Med. Sci., 76(9): 1277-1280.
3. Thammakarn, C., Tsujimura, M., Satoh, K., Hasegawa, T., Tamura, M., Kawamura, A., Ishida, Y., Suguro, A., Hakim, H., Ruenphet, S. and Takehara, K. (2015a) Efficacy of scallop shell powders and slaked lime for inactivating avian influenza virus under harsh conditions. Arch. Virol., 160(7): 2577-2581.
4. Ota, M., Toyofuku, C., Thammakarn, C., Sangsiriratanakul, N., Yamada, M., Nakajima, K., Kitazawa, M., Hakim, H., Alam, S., Shoham, D. and Takehara, K. (2016) Calcinated egg shell as a candidate of biosecurity enhancement material. J. Vet. Med. Sci., 78(5): 831-836.
5. Demeyer, A., Voundi, N.J.C. and Verloo, M.G. (2001) Characteristics of wood ash and influence on soil properties and nutrient uptake: An overview. Bioresour. Technol., 77(3): 287-293.
6. Tarun, R., Kraus, R.N. and Kumar, R. (2003) A new source of pozzolanic material. Constr. Int., 25(12): 55-62.
7. Jahangir, A., Ruenphet, S., Shoham, D., Okamura, M., Nakamura, M. and Takehara, K. (2010) Haemagglutinin and neuraminidase characterization of low pathogenic H5 and H7 avian influenza viruses isolated from Northern pintails (Anas acuta) in Japan, with special reference to genomic and biogeographical aspects. Virus Genes, 40(1): 94-105.
8. Sonthipet, S., Ruenphet, S. and Takehara K. (2018) Bactericidal and virucidal efficacies of potassium monopersulfate and its application for inactivating avian influenza virus on virus-spiked clothes. J. Vet. Med. Sci., 80(4): 568-573.
9. Lombardi, M.E., Ladman, B.S., Alphín, R.L. and Benson, E.R. (2008) Inactivation 323 of avian influenza virus using common detergents and chemicals. Avian Dis., 52(1): 118-123.
10. Takehara, K., Yamazaki, K., Miyazaki, M., Yamada, Y., Ruenphet, S., Jahangir, A., Shoham, D., Okumura, M. and Nakamura, M. (2010) Inactivation of avian influenza virus H1N1 by photocatalyst under visible light irradiation. Virus Res., 151(1): 102-103.
11. Lerner, B.R. (2000) Wood Ash in the Garden. Purdue University, Department of Horticulture and Landscape Architecture, West Lafayette, IN, USA.
12. Misra, M.K., Ragland, K.W. and Baker, A.J. (1993) Wood ash composition as a function of furnace temperature. Biomass Bioenergy, 4(2): 103-116.
13. Siqueira, J.F. and Lopes, H.P. (1999) Mechanisms of antimicrobial activity of calcium hydroxide: A critical review. Int. Endod. J., 32(5): 361-369.
14. Aiello, S. (1998) The Merck Veterinary Manual. 8th ed. Merck & Co, Whitehouse Station, NJ, USA.
15. Takehara, K., Chinen, O., Jahangir, A., Miyoshi, Y., Ueno, Y., Ueda, S., Takada, Y., Ruenphet, S., Mutoh, K., Okamura, M. and Nakamura, M. (2009) Ceramic powder made from chicken feces: Anti-viral effects against avian influenza viruses. Avian Dis., 53(1): 34-38.
16. Paditporn, K., Ruenphet, S. and Takehara, K. (2016) Comparison of Virucidal Effects for the Newcastle Disease Virus between Slaked Lime and Food Additive Calcium Hydroxide. In: Proceedings of the 9th MUT Veterinary Annual Conference 2016, Mahanakorn University of Technology, Bangkok, Thailand. p13-18.
17. Thammakarn, C., Ishida, Y., Suguro, A., Hakim, H., Alam, S., Shoham, D. and Takehara, K. (2016) Calcinated egg shell as a candidate of biosecurity enhancement material. J. Vet. Med. Sci., 78(5): 831-836.
18. Lorcharoenrungroj, K., Takehara, K. and Ruenphet, S. (2016) Studies on Fresh Charcoal Ash to Inactivate Avian Influenza Virus, Escherichia coli and Salmonella Infants for Bioscience Enhancement On Chicken Farms. In: Proceedings of the 9th MUT Veterinary Annual Conference 2016, Mahanakorn University of Technology, Bangkok, Thailand. p5-12.

**********