Karomy *et al. J Pure Appl Microbiol,* **13**(**3**), 1543-1548 | September 2019
Article 5653 | https://doi.org/10.22207/JPAM.13.3.26
Print ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Effect of the Frequency of the Microsafe Spraying on the Production Performance and the Microbial Presence in Mouth and Vent of Broiler Chickens

Alfred S. Karomy[1]*, Qutaiba J. Gheni[1] and Saher S. George[2]

[1]Animal Production Department, Agriculture College, Basrah University, Basrah, Iraq. [2]Food Science Department, Agriculture College, Basrah University, Basrah, Iraq.

Abstract

The experiment aimed to study the effects of the number of microsafe detergent spraying periods on the microbial presence in mouth and vent of birds and their product characteristics. A total of 180 one-day old unsexual chickens (Ross-308) strain were used. The birds were randomly allocated to four treatments with 3 replicated of 15 birds each. The treatments were control (C) without microsafe spray and D3, D7 and D10 which were sprayed with microsafe detergent every 3, 7 and 10 days, respectively. Total bacteria count, coliform and *E.coli* bacteria at 14 and 28 days in both mouth and vent of birds were significantly (p <0.05) lower when microsafe detergent was sprayed at D3, D7 and D10 treatment as compared with control (C). *E. coli* O157H: 7 bacteria in mouth and vent of birds at 14 and 28 days, results were indicated high effectiveness of microsafe spraying period on the elimination of this type of bacteria. Body weight, weight gain, feed intake and feed conversation ratio at 28 days were significantly (p <0.05) higher in all number of microsafe spray periods treatments as compared with control.

Keywords: Microsafe, poultry, *E. coli, coliform*, productive characteristics**.**

***Correspondence:** Alfred_skh@yahoo.com

(Received: 06 May 2019; accepted: 03 June 2019)

Citation: Alfred S. Karomy, Qutaiba J. Gheni and Saher S. George, Effect of the Frequency of the Microsafe Spraying on the Production Performance and the Microbial Presence in Mouth and Vent of Broiler Chickens, *J Pure Appl Microbiol.*, 2019; **13**(**3**): 1543-1548. https://doi.org/10.22207/JPAM.13.3.26

INTRODUCTION

Poultry is one of the most important industries in the world. It participates in the production of 7.6 billion tons of poultry meat in a year (Allen and Fetterer, 2002). The development of this industry faces many determinants, including field conditions and new of modern breeding (Conway and McKenzie, 2007). Poultry diseases are one of the most challenges faced by breeders, which cause large economic losses due to a large number of mortality, low production and high economic cost of, vaccines and veterinary medicines (Simon, 2005).

Burbarelli *et al*., (2015) used both glutaraldehydes at 250 g/L and formaldehyde at 185 g / L at 0.5 % in water for sterilization of halls and breeding tools the result was that both were effective for killing microbes. Samaha *et al*., (2013) compared the use of 4 commercial sterilizers: ammonia hydroxide at 0.5% and 2.5% concentration, phenol concentrations (5, 10 and 20%) and Eco. Bio product with a concentration of 5, 10 and 20%, Results showed that phenol at 10% concentration for 24 hours had the best. Payne *et al*. (2005) evaluated using sterilizers for floor sterilization and hallway equipment, the treatments consisted of 4 different disinfectants, which included a phenolic compound, a quaternary ammonium compound, a nascent oxygen compound, a compound that contained potassium peroxymonosulfate and sodium chloride as the active ingredients, and a control. Each disinfectant was prepared according to the manufacturers' recommendations using distilled water. He noticed that the first three compounds led to a significant reduction in the incidence of salmonella and decrease in the number of air bacteria, the absence of unwanted odors and some of them cause sensitivity to the eyes and skin poison as well as could be used before and during rearing the birds in the farm.

Microsafe in terms of use during the presence of birds in the farm because it is non-toxic and without any side effects or allergic to the eyes and has no smell and the important thing is my potential Use it before and during the rearing chicks breeding because it consists of pure water oxidized by 99.97% (Super oxidized water). It is ready to be used without dilution or mixing with other non-toxic substances for humans and birds and has a significant effect on infection control and the spread of communicable diseases (Landa-Solis *et al*., 2005). Super-oxidized water (SOW) has been used as an effective sterilizer in hospitals (Schneider 2013). Sterilization of tools and medically equipment (Netson, 2000), as well as to reduce skin cancers (Sekiya *et al*., 1997). It was used as antimicrobial agent for salmonella (Rahman *et al.,* 2016). Microsafe is very effective in eliminating many viruses such as H5N1 and HIV HIV (Landa-Solis *et al*., 2005). Therefore, the present study aimed to demonstrate the effect of the number of periods spraying microsafe on the microbial presence in the mouth and the vent of the birds and their productive characteristics.

MATERIALS AND METHODS

The experiment was carried out in the poultry field of the Animal Production department for the period between 2/11/2017 to 29/11/2017. One hundred eighty one day old chickens distributed to 4 treatments with 3 replicates per treatment and 15 birds each. Chicks placed in floor cages, All administrative procedures were followed by sterilization, ventilation and suitable lighting, and fed on a ready-made feed containing 23% protein, 2850 Kcal energy / kg feed for starter and, 19% protein and 3170 Kcal / kg for finisher.

The treatments were as follows

1. C: control (without spraying Microsafe)

2- D3: Microsafe spraying every 3 days (9 times during the experiment)

3- D7: Microsafe spraying every 7 days (4 times during the experiment)

4- D10: Microsafe spraying every 10 days (2 times during the experiment)

The microsafe was sprayed at 15 mL per replicate and 1 m high from the birds' using a special pump to convert it to a very fine spray. We took measurements of the body weight, the rate of weight gain, amount of feed consumed, food conversion efficiency, total number of bacteria, *coliform* and *E. coli*. By a special sterilizer at age 14 and 28 days.

Technique of Petri film was also detected by the presence of *E. coli* O157H: 7 through the use of latex.

Method of Petri film to estimate total count bacteria and *E.Coli, Coliform*

The method of Petri film used for

counting bacterial number, which is characterized by high accuracy and showing shortening time as compared to conventional counting methods, the bacterial colonies of the red and blue developing on the segments of the Petri, some of them producing gas bubbles around their colonies has been fermented lactose sugar producing (Kanagawa *et al.,* 2018). It consists of a flat plate containing a circle divided into 20 small squares. One ml of dilution prepared for the implanting transferred by micropipette to the Petri film and left for a period to ensure equal spread. Then it incubated at a temperature of 37°C for 24 - 48 hours and then the colonies calculated (Blackburn and McCarthy 2000). The percentage of low total bacteria, *E. coli* and *Coliform* was calculated, according to Behrad *et al*. (2009). The differences between the averages were also tested using the least significant difference (L.S.D) using the SPSS (2013).

RESULTS AND DISCUSSION

Different spraying microsafe periods at treatments D3, D7 and D10 resulted showed a significant ($p<0.05$) decrease in the mouth and Vent total bacteria, coliform and *E.coli* bacteria at 14 days compare without microsafe spraying treatment (control C) table 1. These treatments were decreased (-52.15, -52.15 and -52.98%) respectively for total bacteria count compared with the control treatment. But *coliform* and *E. coli*, bacteria were decreased by -100% lower in mouth samples birds.

The vent samples at the same age were decreased for total bacteria count, (-12.93, -15.25, -13.51) %, coliform bacteria (-13.38, -17.91 and -14.37) % and for *E.coli* (-14.68, -20.63 and -15.87) % in treatments D3, D7 and D10 respectively.

Table 2 was illustrated the mouth and vent total bacteria, coliform and *E. coli* bacteria count at 28 day. It was indicated that the mouth bacteria count decreasing significantly ($p < 0.05$) in D3, D7 and D10 as compared with the control, it observed that the decrease percentage were (-33.64, -33.64 and -24.38)% for total bacteria, whereas the decrease in *coliform* (-15.38, -31.81 and -15.58)% while *E. coli* was -100% lower in treatments D3, D7 and D10 respectively.

Microsafe spraying treatments as compared with control for birds vents indicated the total count, *coliform* were decreased significantly ($p < 0.05$) to (-14.52, -14.68 and -29.04)% for total bacteria respectively while the decreased for the number of *Coliform* bacteria (-19.42 and -37.14 and -9.71)% respectively and the *E.Coli* bacteria was decreased by (-20.0, -40.0 and -7)% for the spray microsafe treatments respectively in relation with control.

It is clear appearance in table 1 and 2 that the effect of period's microsafe spraying, indicated the decrease in total count, *Coliform* and *E.Coli* bacteria in mouth and vent birds at 14 and 28 days, this result were shown to be agreement with Landa-Solis *et al*. (2005). When using this sterilizer in US hospitals, the results were 100% effective against *E. coli, Staphylococcus aureus, Salmonella*

Table 1. Effects of microsafe spraying on average on bacteria counts in mouth and Vent of birds in 14 days ± SE (CFU/g)

Treatments	Total Amount		Coliform		*E.coli*	
	Vent	Mouth	Vent	Mouth	Vent	Mouth
C	5.18 ± 0.01^a	4.18 ± 0.01^a	5.08 ± 0.02^a	4.00 ± 0.02^a	5.04 ± 0.01^a	3.70 ± 0.03^a
D3	4.51 ± 0.03^b	2.00 ± 0.02^b	4.40 ± 0.01^b	0.00 ± 0.00^b	4.30 ± 0.03^b	0.00 ± 0.00^b
D7	4.39 ± 0.02^b	2.00 ± 0.01^b	4.17 ± 0.02^b	0.00 ± 0.00^b	4.00 ± 0.01^b	0.00 ± 0.00^b
D10	4.48 ± 0.03^b	1.84 ± 0.02^b	4.35 ± 0.01^b	0.00 ± 0.00^b	4.24± 0.03^b	0.00 ± 0.00^b
			Average of decrease in bacteria numbers			
C	0	0	0	0	0	0
D3	-12.93	-52.15	-13.38	-100	-14.68	-100
D7	-15.25	-52.15	-17.91	-100	-20.63	-100
D10	-13.51	-55.98	-14.37	-100	-15.87	-100

Different letters between treatments in the same column mean significant $p<0.05$
C: control (without spray), **D3:** Spray Microsafe every 3 days, **D7:** Micro-sprayed every 7 days, **D10**: Micro-sprayed every 10 days

Table 2. Effects of micro save spraying on average on bacteria counts in mouth and Vent of birds in 28 days ± SE (CFU/g)

Treatments	Total count		Coliform		*E. coli*	
	Vent	Mouth	Vent	Mouth	Vent	Mouth
C	6.06 ± 0.04[a]	6.48 ± 0.02[a]	5.25 ± 0.04[a]	3.08 ± 0.01[a]	5.00 a± 0.02	2.84 ± 0.01[a]
D3	5.18 ± 0.04[b]	4.30 ± 0.02[b]	4.23 ± 0.02[b]	2.60 ± 0.01[b]	4.00 ± 0.01[b]	0.0± 0.00[b]
D7	5.17 ± 0.03[b]	4.30 ± 0.02[b]	3.30 ± 0.02[b]	2.10 ± 0.01[b]	3.00 ±0.02[c]	0.0± 0.00[b]
D10	4.30 ± 0.01[b]	4.90 ± 0.03[b]	4.74 ± 0.03[b]	2.60 ± 0.02[b]	4.65 ± 0.03[ab]	0.0 ± 0.00[b]
	Average of decrease in bacteria numbers					
C	0	0	0	0	0	0
D3	-14.52	-33.64	-19.42	-15.58	-20	-100
D7	-14.68	-33.64	-37.14	-31.81	-40	-100
D10	-29.04	-24.38	-9.71	-15.58	-7	-100

Different letters between treatments in the same column mean significant p<0.05

C: control (without spray), **D3**: Spray Microsafe every 3 days, **D7**: Micro-sprayed every 7 days, **D10**: Micro-sprayed every 10 days.

and other pathogenic bacteria. Microsafe have the ability to perform a wide spectrum action to eliminate pathogenic bacteria (Tanaka *et al*., 1996; Schneider 2013 and Nakano *et al*., 2015), as well as those agreed with Horiba *et al*. (1999) which showed a significant decrease ($p < 0.01$) in the number of pathogenic bacteria as a result of the use microsafe sterilization technique as compared with the solution of iodine in the feet of people suffer from foot ulcers due to diabetes, which reduced the time of healing wounds for these patients. The microsafe is 99.97% oxidative water reduces the time of sterilization, toxicity and reduce the infections (Netson, 2000). It has the ability to kill single-celled microbes such as bacteria, viruses, and other microorganisms. Its function is to analyze it into high concentrations of positive H+ and other negative charges OH-. These charges are unstable and the walls of the bacteria are single cell and viruses attract these ions because they have low concentrations of them, which make these ions to rush into the interior through their production of oxygen free radicals, Which are produced by the enzyme NADPH Oxidase These roots kill bacteria by penetration the walls of the cell and tearing and thus killing them with high efficiency (Horiba *et al*., 1999).

Table 3. Effects of microsafe spraying on present of *E coli* bacteria O157H:7 in mouth and Vent of birds in 14 & 28 days ± SE

Treatments	14 days		28 days	
	Vent	Mouth	Vent	mouth
C	+	+	+	+
D3	+	-	-	-
D7	+	-	-	-
D10	+	-	-	-

Table 4. Effect of microsafe spraying on the average live body weight and body weight gain (gm) of broiler chickens ± SE
Age (days)

Treatments	Weight gain			Body weight		
	7	14	28	7	14	28
C	164.5± 2.4	415.5± 5.2	1403± 18.2[b]	126.5± 2.4	250.5± 5.1	988± 17.8[b]
D3	165± 3.1	426.5 ± 6.1	1547± 20.3[a]	127.5± 3.1	261± 5.9	1121± 19.2[a]
D7	166± 2.5	430 ± 4.5	1525± 14.1[a]	127.5± 2.5	264.5± 4.3	1095.5± 14[ab]
D10	165.5 ±2.0	430.5 ± 4.3	1542.5 ± 17.8[a]	127.5 ± 2.0	265.5 ± 4.1	1112 ± 17.5[a]

Different letters between treatments in the same column mean significant p<0.05

C: control (without spray), **D3**: Spray Microsafe every 3 days, **D7**: Micro-sprayed every 7 days, **D10**: Micro-sprayed every 10 days.

Table 3 was indicated the effect of microsafe spraying on the presence of *E. coli* O157H: 7 bacteria in the mouth and Vent of the birds at the age of 14 and 28 days. It was showed high effectiveness of microsafe in the elimination of this type of bacteria, which are found naturally in bird feces but are not Active and became active with a decrease in bird immunity such as infection with viral diseases, Morita *et al*. (2000) refer to that the microsafe has a high effectiveness in reducing the effectiveness of viruses such as HIV-1, which affects humans by affecting the effectiveness of the surface protein hepatitis B virus.

Table 4 showed the effect of microsafe spraying during certain periods on body weight, weight gain rates at 7, 14 and 28 days. It's showed no significant differences during the 7 and 14 days' periods. while showed significant effects at 28 days, as compare with control but no significant change appears between treatments (D3, D7 and D10) the body weight and the weight gain in these treatments become (1547, 1525, and 1542.5) g and (1121, 1095 and 1112 g) for respectively compare with Control when the body weight became (1403) g and weight gain (988) g.

Table (5) referred to the effect of microsafe spraying during different periods on average feed intake and feed conversion efficiency. It's showed no significant differences during the period 7 and 14 days of the age of birds in all treatments. The control treatment was showed significantly higher ($p < 0.05$) in 28 days for feed intake which was 1922.5 g and Feed conversion coefficient 1.98 g feed/g body weight gain compare with spraying treatments where no different between them significantly and the amount of feed intake was (1836.6, 1868 and 1821 g) feed conversion coefficient (1.7 and 1.7 and 1.63) g/g for D3, D7 and D10 respectively. They were decreased significantly ($p < 0.05$) for these treatments as compared with control.

Table 5. Effect of microsafe spraying on the average feed intake and feed conversion (g) of broiler chickens ± SE

Treatments	Average feed intake			accumulation	Average feed conversion			accumullation
	7	14	28		7	14	28	
C	118.5 ± 4.5	368.5 ± 11.4	1922.5± 21.1^{a}	2409±12.3^{a}	0.90 ±0.01	1.39 ± 0.08	1.94 ± 0.09^{c}	1.76±0.063^{a}
D3	115.5 ± 6.6	350 ± 16.2	1836.5 ±24.2^{b}	2302±15.66^{b}	0.92 ± 0.02	1.39 ± 0.05	1.71 ± 0.08^{b}	1.53±0.052^{b}
D7	117 ± 3.9	353.5 ± 12.4	1868 ± 18.6^{b}	2338.5±11.63^{b}	0.91 ± 0.01	1.33 ± 0.04	1.73 ± 0.07^{b}	1.57±0.045^{b}
D10	116 ± 2.8	365.5 ± 8.2	1821 ± 15.9^{b}	2302.5±8.96^{b}	0.90 ± 0.01	1.37 ± 0.05	1.63 ± 0.06^{a}	1.53±0.042^{b}

Different letters between treatments in the same column mean significant p<0.05.
C control (without spray), **D3**: Spray Microsafe every 3 days, **D7**: Micro-sprayed every 7 days, **D10**: Micro-sprayed every 10 days.

Present of bacteria, neither harmful or benefit naturally presence in the digestive tract of birds. These have a clear effect on the health of birds thus affect the various functions such as feeding, immunity and physiology status of birds (Kohl, 2012). The microsafe spraying treatments was indicated that decrease numbers of harmful bacteria will help beneficial bacteria to grow and multiply and thus improve the productive performance of birds as body weight and weight gain with an improvement in the feed conversion coefficient of birds. Ahmed *et al*., (2015) showed that the (*Bacillus* spp.) and Lactobacillus spp., which represent significant beneficial bacteria not affected when using Clo2 in percentage (0, 0.05 and 0.1%) as a sterilizer, The effect was clear on *Salmonella* and *E. coli* bacteria, which had a positive effect on production performance For birds. Microsafe spraying has a positive effect through reducing odors emitted from bird feces as it plays a role in eliminating the bacteria that produce these odors when they degrade animal waste (Zhu, 2000).

We conclude from the above that it was possible to use microsafe sprayer during the breeding period of the broiler chickens for several times to reduce the harmful effects of the infectious microbes and improve the environmental conditions of breeding as it a component of water 99.97%, which reflects positively the production values.

ACKNOWLEDGEMENT

None.

CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

REFERENCES

1. Ahmed, S.T., Kim, G., Islam, Md.M., Mun, H.A.B.M. Bostami, A.B.M.R. and Yang, C. Effects of dietary chlorine dioxide on growth performance, intestinal and excreta microbiology, and odorous gas emissions from broiler excreta. *5 J. Appl. Poult. Res.*, 2015; **24**: 502–510. https://doi.org/10.3382/japr/pfv058
2. Allen, P.C. And Fetterer, R. H. Recent advances in biology and immunobiology of *Eimeria* species and in diagnosis and control of infection with these coccidian parasites of poultry. *Clin. Microbiol. Rev.*, 2002; **15**(1): 58–65. https://doi.org/10.1128/CMR.15.1.58-65.2002
3. Kanagawa, S., Ohshima, C., Takahashi, H., Burenqiqige, Kikuchi, M., Sato, F., & Kimura, B. Evaluation of Petrifilm Lactic Acid Bacteria Plates for Counting Lactic Acid Bacteria in Food. *J Food Protect*, 2018; **81**(6): 1030-1034. https://doi.org/10.4315/0362-028X.JFP-17-260
4. Behrad, S., Yusof , M.Y., Goh, K.L. and Baba, A.S. Mainpulation of Probiotics Fermentation of Yogurt by *Cinnamon and Licorice*: Effect on Yogurt Formation and Inhibition of *Helicobacter* Pylori Growth *In Vitro. International J Biolog, Biomolecul, Agricul, Food and Biotechnol Engg*, 2009; **3**(12): 563-567.
5. Blackburn, C.D.E. and McCarthy, J. D.. Modifications to methods for the enumeration and detection of injure *Escherichia coli* O157:H7 in foods. *International J Food Microbiol*, 2000; **55**: 285-290. https://doi.org/10.1016/S0168-1605(00)00205-1
6. Burbarelli, M.F.C., Merseguel, C.E.B., Ribeiro, P.A.P., Lelis, K.D., Polycarpo, G.V., CarToo, A.C.P., Bordin, R.A., Fernandes, A.M., Souza, R.L.M., Moro, M.E.G. and Albuquerque, R. The Effect of Two Different Cleaning and Disinfection Programs on Broiler Performance and Microbiological Status of Broiler Houses. *Brazilian J Poult Sci*, 2015; **17**(4): 575-580. https://doi.org/10.1590/1516-635X1704575-580
7. Conway, D.P. and McKenzie, M.E. Poultry coccidiosis diagnosis and testing procedures. 3rd ed. Iowa: Blackwell Publishing, 2007. https://doi.org/10.1002/9780470344620
8. Horiba, N., Hiratsuka, K., Onoe, T., Yoshida, T., Suzuki, K., Matsumoto, T. and Nakamura, H. Bactericidal effect of electrolyzed neutral water on bacteria isolated from infected root canals. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, 1999; **87**(1): 83-87. https://doi.org/10.1002/9780470344620
9. Kohl, K.D. Diversity and function of the avian gut microbiota. *J. Comp. Physiol. B.*, 2012; **182**: 591–602. https://doi.org/10.1007/s00360-012-0645-z
10. Landa-Solis, C., Gonzבlez-Espinosaa, D., Guzmב n-Sorianoa, B., Snyderb, M., Reyes-Terבn", G., Torresc, K. and Gutierrez, A.A. Microcyn: a novel super-oxidized water with neutral pH and disinfectant activity. *Journal of Hospital Infection*, 2005; **61**: 291-299. https://doi.org/10.1016/j.jhin.2005.04.021
11. Morita, C., Sano, K., Morimatsu, S., Kiura, H., Goto, T., Kohno, T., Hong, W.U., Miyoshi, H., Iwasawa, A., Nakamura, Y., Tagawa, M., Yokosuka, O., Saisho, H.
12. Nakano, T., Hayashi, H., Wu, H., Shimamoto, C., & Sano, K. Disinfection potential of electrolyzed strongly acidic water against Mycobacteria: Conditions of disinfection and recovery of disinfection potential by reelectrolysis. *Biomed Res*, 2015; **36**(2): 109-113. https://doi.org/10.2220/biomedres.36.109
13. Netson, D. Newer technologies for endoscope disinfection: electrotyzed acid water and disposable-component endoscope systems. *Gastrointest Endosc. Clin. N. Am.*, 2000; **10**: 319-328. https://doi.org/10.1016/S1052-5157(18)30134-X
14. Rahman, S.M.E., Khan, I. & Oh, D.H. Electrolyzed water as a novel sanitizer in the food industry: current trends and future perspectives. *Comprehensive Reviews in Food Science and Food Safety*, 2016; **15**(3): 471-490. https://doi.org/10.1111/1541-4337.12200
15. Payne, J.B., Kroger, E.C. and Watkins, S.E. Evaluation of Disinfectant Efficacy When Applied to the Floor of Poultry Grow-Out Facilities. *J. Appl. Poult. Res.*, 2005; **14**: 322–329. https://doi.org/10.1093/japr/14.2.322
16. Schneider, P.M. New technologies and trends in sterilization and disinfection. *American Journal of Infection Control*, 2013; **41**(5): S81-S86. https://doi.org/10.1016/j.ajic.2012.12.007
17. Samaha, H.A., Haggag, Y.N., Nossair, M. A. and Habib, H.M. Assessment Efficiency of Some Chemical Disinfectants Commonly Used Against Coccidia in poultry Farms. *Alexand J Veter Sci*, 2013; **39**: 82-90
18. Sekiya, S., Ohmori, K. and Harii, K. Treatment of infectious skin defects or ulcers with electrotyzed strong acid aqueous solution. *Artif. Organs*, 1997; **21**: 32-38. https://doi.org/10.1111/j.1525-1594.1997.tb00696.x
19. Simon M.S. Handbook on Poultry Diseases, 2nd Edition, American Soybean Association, 2005.
20 Spss. Statistical Package of Social Science. Ver. 22. Application Guide .Copy right by Spss Inc. USA., 2013.
21. Tanaka, H., Hirakata, Y., Kaku, M., Yoshida, R., Takemura, H., Mizukane, R., Ishida, K., Tomono, K., Koga, H., Kohno, S. and Kamihira, S. Antimicrobial activity of superoxidized water. *J. Hosp. Infect.*, 1996; **34**: 43-49. https://doi.org/10.1016/S0195-6701(96)90124-3
22. Zhu, J. A review of microbiology in swine manure odor control. Agric. *Ecosyst. Environ.*, 2000; **78**: 93–106. https://doi.org/10.1016/S0167-8809(99)00116-4