EFFECT OF CLINOPTILOLITE ADDITION ON BACTERIAL COUNTS IN PIG SLURRY

Jasna Hrenović[1], Alenka Tofant[2], Mario Ostović[2], Dinka Milić[3]
[1]University of Zagreb, Faculty of Science, Rooseveltov trg 6, HR-10000 Zagreb, Croatia
[2]University of Zagreb, Faculty of Veterinary Medicine, Heinzelova 55, HR-10000 Zagreb, Croatia
[3]Dubravica Pig-breeding farm, Ltd., Pavla Štoosa 109, HR-10295 Dubravica, Croatia
E-mail: jasnah@biol.pmf.hr

ABSTRACT

The aim of this study was to determine the effect of natural zeolite (commercial preparation with 80% of clinoptilolite) on counts of different physiological groups of bacteria i.e. ***sulfide-producing, faecal and total coliform, mesophilic aerobic and anaerobic, and*** thermophilic bacteria in pig slurry. Zeolite was spread daily directly over partially slatted floor in a dose of 0.4 kg/1 m^2 during pig fattening period. The results demonstrated that the counts of all investigated groups of bacteria were lower after addition of zeolite when compared to control slurry.

Keywords: additive, natural zeolite, pig slurry, bacteria

INTRODUCTION

High capacity pig feedlots with slatted floor technology generate large volumes of slurry with a high concentration of organic matter, nutrients, trace elements, and variety of microorganisms, including pathogenic ones [1]. Microorganisms may persist in the slurry for a long time depending on storage condition, type of the slurry, the storage temperature and pathogen type. They will be inactivated after exposure to the environment but may survive long enough to be of public and/or animal health concern [2]. Survival of microorganisms is enhanced by the reduction of temperature and increased of solid content. Survival is the greatest at temperatures below 10°C and in slurries containing more than 5% solids [3].

Therefore, the need for and necessity of slurry hygienization in addition to its mechanical and biologic treatment have been increasingly emphasized. Processing strategies focus on minimization of environmental pollution and prevention of infection spreading.

Many scientific papers report about the possibilities of use the natural zeolites in the removal of chemical pollutants, i.e. binding of ammonium ions or trapping gaseous ammonia, from waste water including animal slurry; however the data about influence of the zeolites on bacterial counts in the slurry are rare and were carried out *in vitro* conditions [4].

In the present study the effect of the addition of natural zeolite, commercial preparation "ZeoClean", to fattening pigs' slurry was assessed from bacteriological point of view.

EXPERIMENTAL

The study was carried out at the Dubravica Pig-breeding farm during winter-spring period. It included two equal fattening units with partially slatted floor, each with 400 pigs on an average. The fattening period lasted 130 days. One unit served as a control unit. In the second unit the commercial preparation "ZeoClean", natural zeolite containing 80% of clinoptilolite, was daily directly spread over the partially slatted floor in the dose of 0.4 kg/1 m^2, according to the manufacturer's instructions.

Slurry samples were collected on 7 occasions from the channel under the slatted floor in sterile bottles and analysed within 4h. For bacteria determination supernatant was used after dry solid were settled down. The number of sulfide-producing bacteria (SPB) was determined in liquid sulfate-thiosulfate medium after incubation at 35°C for 48h [5]. The number of fecal coliforms (FC) and total coliforms (TC) was determined in MacConkey broth after incubation at 44.5°C for 24h and 35°C for 48h, respectively. The numbers of heterotrophic aerobic and anaerobic mesophilic bacteria, as well as thermophilic bacteria were determined on Nutrient agar plates. After inoculation of samples, plates for determination of aerobic heterotrophic bacteria were directly incubated at 35°C for 72h. Plates for determination of anaerobic heterotrophic bacteria were placed into Anaerocult A (Merck) and incubated at 35°C for 72h. Plates for determination of thermophilic bacteria were incubated at 55°C for 24h. The number of bacteria from liquid media was determined as the most probable number (MPN) using the standard evaluation tables, while the number of bacteria grown on solid media was determined as colony-forming units (CFU) per one L of slurry [6]. Statistical analysis was performed using the Statistica 8.0 (Statsoft Inc., 2008) software using the Student's T test.

RESULTS AND DISCUSSION

Pig slurry naturally contains an excess of 10^{10} bacteria *per* ml, some of them are considered to be potentially pathogenic [3]. Environmental and health problems related to the slurry microbiological composition include emanation of malodour, detrimental gases and spread of infections, so imperative of manure treatment is to imply additional procedures in order to reduce microbiological count. The zeolites have been increasingly used in various areas such as industry, agriculture, environmental protection, and even medicine. Natural zeolites are a family of minerals of volcanic origin that are made of crystalline aluminosilicates with excellent ion exchanging properties [7].

In this study the effect of natural zeolite, the slurry additive, on counts of different physiological groups of bacteria in the fattening pigs' slurry was investigated. The SPB are common inhabitants of pig caecum and are excreted with faeces in the slurry, where they are responsible for the characteristic malodour [8], which originates from hydrogen sulfide, one of the most potent malodours emitted from anaerobic pig slurry channels [9]. The FC and TC bacteria inhabit the pig intestine and are excreted with faeces in the slurry. Low temperatures generally help the growth and survival of enteric bacteria i.e. faecal coliforms which are considered to be pathogen. Other heterotrophic mesophilic (aerobic and anaerobic) and thermophilic bacteria are also present in the pig intestinal tract and excreted with faeces in the slurry. Results of the study are presented in Table 1 and Figs. 1-6.

Table 1. Numbers of bacteria in control and experimental samples of pig slurry expressed as mean±SD and *P* values for statistical difference. n=7 *per* measurement in each group.

Bacteria	Control	Zeoclean	*P* value
Sulfide-producing bacteria (MPN/L)	$1.61\pm4.14 \times 10^{10}$	$1.55\pm3.46 \times 10^{9}$	0.371
Faecal coliforms (MPN/L)	$1.18\pm1.74 \times 10^{8}$	$4.00\pm8.44 \times 10^{6}$	0.108
Total coliforms (MPN/L)	$6.71\pm9.25 \times 10^{8}$	$2.37\pm3.26 \times 10^{7}$	0.089
Aerobic mesophiles (CFU/L)	$9.62\pm5.57 \times 10^{10}$	$3.26\pm3.81 \times 10^{10}$	0.028
Anaerobic mesophiles (CFU/L)	$1.01\pm1.00 \times 10^{11}$	$4.36\pm7.07 \times 10^{10}$	0.241
Thermophiles (CFU/L)	$1.60\pm1.35 \times 10^{7}$	$4.49\pm4.93 \times 10^{6}$	0.056

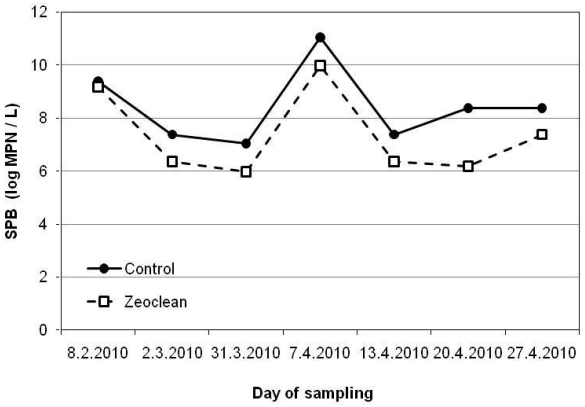

Figure 1. Counts of sulphide-producing bacteria (SPB) in control and experimental samples of pig slurry.

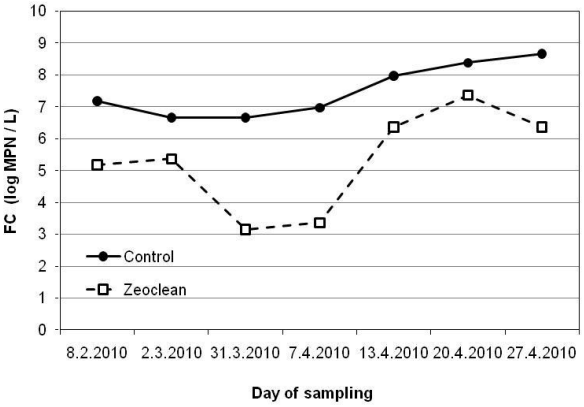

Figure 2**.** Counts of faecal coliforms (FC) in control and experimental samples of pig slurry.

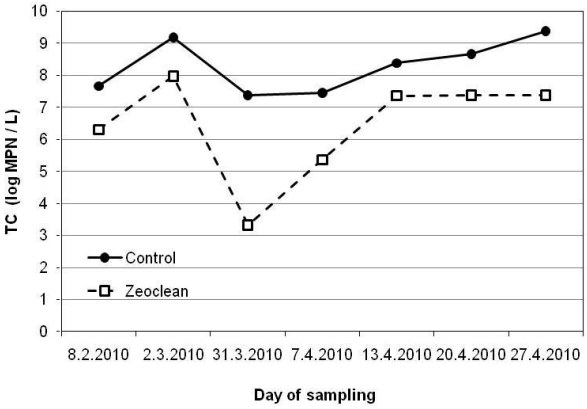

Figure 3. Counts of total coliforms (TC) in control and experimental samples of pig slurry.

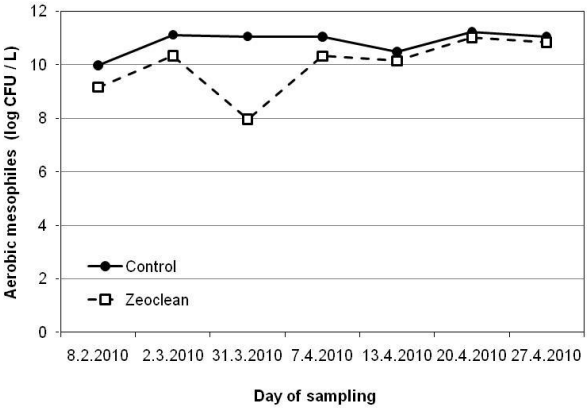

Figure 4. Counts of aerobic mesophilic bacteria in control and experimental samples of pig slurry.

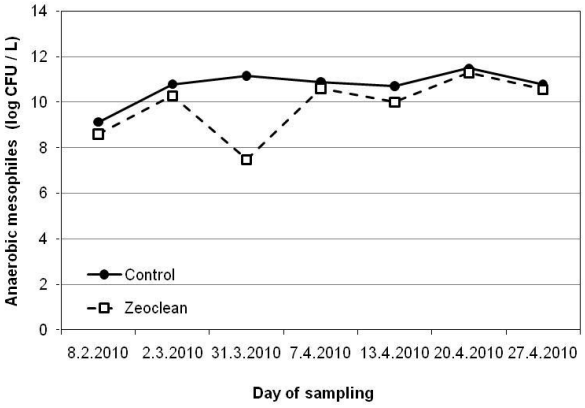

Figure 5. Counts of anaerobic mesophilic bacteria in control and experimental samples of pig slurry.

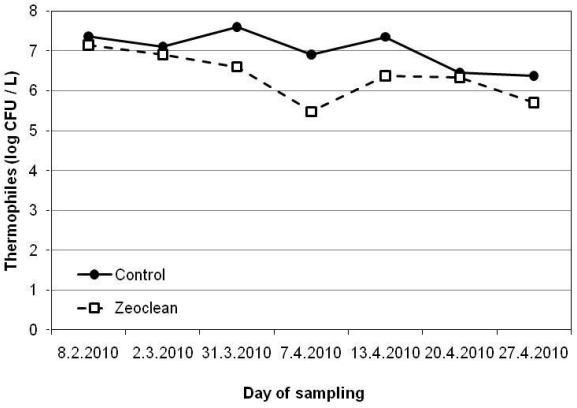

Figure 6. Counts of thermophilic bacteria in control and experimental samples of pig slurry.

According to the results, the addition of natural zeolite used as the slurry additive resulted in lower counts of all investigated groups of bacteria in the experimental when compared to the control slurry (Table 1). When assuming the results collected during whole study, the statistically significant (P<0.05) difference between control and experimental slurry was observed only for aerobic mesophilic bacteria. However, the single results collected on the same date (Figs. 1-6) showed lower counts of all physiological groups of bacteria in the experimental when compared to the control slurry. The highest difference between the control and experimental slurry can be seen for FC, which are considered as the most serious potentially pathogens among the investigated physiological groups of bacteria. The lower numbers of bacteria in the experimental when compared to the control slurry are explained by the process of spontaneous adsorption and immobilization of bacteria on the particles of natural zeolite [10].

CONCLUSION

The use of the "ZeoClean" as slurry additive resulted in reduced counts of different physiological groups of bacteria in the fattening pigs' slurry.

ACKNOWLEDGEMENT

Supported by the Ministry of Science, Education and Sports of the Republic of Croatia (grant nos. 119-1191155-1203 and 053-0531854-1865).

REFERENCES

[1] J. Venglovsky, N. Sasakova, M. Vargova, Z. Pacajova, I. Placha, M. Petrovsky and D. Harichova, *Bioresour. Technol*., 2005, **96**, 181-189.

[2] J. Venglovsky, J. Martinez and I. Placha, *Livest. Sci*., 2006, **102**, 197-203.

[3] D. Strauch, *Rev. sci. tech Off. int. Epiz*., 1991, **10**, 813-846.

[4] Z. Pacajova, J. Venglovsky, N. Sasakova, M. Petrovsky, M. Vučemilo and A. Tofant, *Proc. 4th Symp. D&D*, 131-136.

[5] B. Stilinović and J. Hrenović, *Folia Microbiol.*, 2004, **49,** 513-518.

[6] *APHA, AWWA, WEF: Standard Methods for the Examination of Water and Wastewater,* 21th ed. APHA, AWWA, WEF, New York, 2005.

[7] L. Ghaemnia, M. Bojarpour, Kh. Mirzadeh, M. Chaji and M. Eslami, *J. Anim. Vet. Adv.*, 2010, **9**, 779-781.

[8] T. Arakawa, Y. Ishikawa and K. Ushida, *J. Nutr. Sci. Vitaminol.*, 2000, **46,** 193-198.

[9] K.L. Cook, T.R. Whitehead, C. Spence and M.A. Cotta, *Anaerobe*, 2008, **14**, 172-180.

[10] J. Hrenović, T. Ivanković and D. Tibljaš, *J. Hazard. Mat.*, 2009, **166**, 1377-1382.