

EFFECTS OF MANURE FERTILIZER ON OFF-FLAVOR SUBSTANCES IN WATER AND SEDIMENT FROM TILAPIA PONDS

NIWOOTI WHANGCHAI^{A*}, WITTAYA TAWONG^A, SUPRANEE WIGRAIBOON^A, TOMOAKI ITAYAMA^B, TAKASHI KUWABARAC AND NORIO IWAMI^D

^A Faculty of Fisheries Technology and Aquatic resources, Maejo University, Chiang Mai, 50290 Thailand

^B National Institute for Environmental Studies, 16-2 Onogawa, Tukuba, Ibaraki, 305-8506, Japan

^C Department of Biological Environment, Faculty of Bioresource Sciences, Akita Prefecture, University, 010-0195, Japan

^D Department of environmental systems, Meisei University, 2-1-1 Hodokubo, Hino, Tokyo, 191-8506, Japan

*Corresponding author; tel.: 66 53 873470 Fax: 66 53 873470 ext 130, niwoot@mju.ac.th

Abstract

The aim of this study was to determine the effects of dry chicken manure fertilizer (DCF) on the accumulation of off-flavor compounds (geosmin, GSM and 2-methylisoborneol, MIB) in water and sediment of tilapia ponds. Different doses of DCF application at the rate of 0, 187.5, 375.0, 562.5, 750.0, 937.5 kg/ha/week were designed for treatments 1-6 respectively. Water quality parameters, Biovolume of cyanobacteria in water and actinomycetes in sediment were also investigated. Off-flavor compounds (GSM and MIB) in both water and sediment were examined using SPME and GC-MS. The result showed that GSM in sediment, ammonia-nitrogen and phosphate-phosphorus in water increased when the doses of DCF increased. No correlation between water MIB and the doses of DCF was observed. GSM in sediment was highly correlated with the number of actinomycetes in sediment while there was no correlation of actinomycetes with MIB in sediment. The levels of GSM were higher than MIB in both sources. This study implied that GSM and MIB in sediment played the role in the off-flavors problem in fish. Therefore, the management of the sediment in old fish ponds should be concerned.

Key words: red tilapia, off-flavor, GSM, MIB (2-methylisoborneol), actinomycetes, sediment

INTRODUCTION

Since aquaculture is a major export industry in Thailand, high product quality and food safety are the major goals to ensure global competitiveness. Although there is a great diversity among culture systems, pond culture is the most commonly used system. Most tilapia culture is in ponds supplemented with organic or inorganic fertilizers. Under these conditions, natural food organisms supply substantial amounts

of nutrients required by fish (Lovell, 1989). Presently, most tilapia pond culture is dependent on green water establishment with fertilizer application, which, in turn relies on natural food community.

It was reported that nuisance odor, especially musty odor in water sources used for various purposes, such as agriculture, the water supply, food industry and fisheries, is the worldwide problem (Suguira *et al.*, 2004). In aquaculture activities, the problem facing the production was the accumulation of off-flavor in fish flesh caused by the presence of trace organic compounds in water and soil. The most troublesome odors are GSM (GSM) and 2-methylisoborneol (MIB) associated with cyanobacteria (Izaguirre *et al.*, 1982) and actinomycetes (Whangchai *et al.*, 2008) which were difficult to remove from the water (Montiel, 1983). GSM in river water affected rainbow trout farms in UK (Roberson *et al.*, 2006). In wild and farmed freshwater fish in Asia, the earthy-musty taint was reported (Yamprayoon and Noomhorm, 2000). The threshold level of GSM and MIB was 1.5 µg/ kg. (Robertson and Lawton, 2003)

In our previous study, tilapia cultured with green water was contaminated with off flavor (Whangchai *et al.*, 2008). This study aimed to determine the levels of GSM and MIB in both water and sediment of red tilapia ponds which applied different dry chicken manure levels. The target of this study is to improve culture system for better fish quality

MATERIALS AND METHODS

Experimental site and design and chicken manure application.

The study was carried out using six treatments with three replications. Treatment 1 was no fertilization; Treatments 2-6 were applied with dry chicken manure at the rate of 187.5, 375.0, 562.5, 750.0 and 937.5 kg /ha/week. Dry chicken manure containing 1.5 % total nitrogen and 1.7 % total phosphorus was applied weekly. Water depth was 0.80 m. in average. Water was added weekly to replenish evaporative loss, seepage, and percolation.

Assessment of GSM and MIB

Water and sediment samples were analyzed GSM and MIB using GC/MS SPME (Casey *et al.*, 2004). Ten milliliters of water or 5 g. of dry sediment mixed with 10 ml. distilled water was placed in a glass container, then 1.9 g of NaCl and 5 ml. of methanol were added. The vial was sealed with a crimp cap-fitted with a veton septum (Supelco).

The sample was then heated to 65 °C and exposed to the SPME fiber for a 12. minute absorption period while undergoing vigorous agitation. The autosampler was equipped with a 1 cm long divinylbenzene/carboxen/polydimethylsiloxane SPME fiber (Supelco). The fiber was withdrawn from the sample and desorbed at 270 °C for

5 minutes in the injection port of an HP 6890 gas chromatograph equipped with a 5973 mass selective detector (Agilent Technologies, Palo Alto, CA). For qualitative analysis, the oven was at 60 °C for 1 minute, then the temperature was programmed at 15 °C/min to 220 °C and maintained for 8 minutes.

Analysis of water quality parameters

The data was deigned to collect every 2 weeks. Physical and biological parameters were measured including ammonia, dissolved oxygen, nitrate, nitrite, orthophosphate, pH and temperature according to standard methods (APHA, 1989). Chlorophyll *a* was measured in the laboratory following Saijo (1975). Biovolume of cyanobacteria was carried out as described by Rott (1981). The actinomycetes in ponds sediment was also investigated following standard methods (APHA, 1989).

Calculation and statistical analysis

Data were analyzed using SPSS (version 11.5) software package to assess the level of significance at the 5% level. Analysis of variance (ANOVA) was performed with the parameters. Duncan's multiple-range test for variables was used for comparison of the treatments.

RESULTS

Water quality parameters

The water characteristics for all treatments were presented in Table 1. Chlorophyll *a*, ammonia- nitrogen and phosphate-phosphorus in water increased when the doses of DCF increased. Temperature was in the range of 29.5±0.5 - 30.2±0.5 °C.

Table 1. Water qualities (mean ± se) in experiment treatments.

Parameters	Chicken manure application (kg/ha/week)					
	0	187.5	375.0	562.5	750.0	937.5
Temperature (°C)	30.0±0.4	30.1±0.5	30.2±0.4	30.2±0.5	29.9±0.5	29.5±0.5
pH	7.05±0.08	7.97±0.18	7.71±0.21	7.54±0.19	8.19±0.19	7.75±0.06
Dissolved oxygen (mgL ⁻¹)	4.79±0.50	5.93±0.94	5.70±1.17	6.14±1.53	7.51±1.15	8.11±0.79
Chlorophyll <i>a</i> (µg L ⁻¹)	28.27±5.26	128.06±17.86	145.40±12.29	175.19±18.26	275.36±87.14	249.60±63.80
Ammonia-nitrogen (mgL ⁻¹)	0.22±0.04	0.39±0.02	0.36±0.02	0.65±0.01	0.51±0.02	0.89±0.32
Nitrite-nitrogen (mgL ⁻¹)	0.009±0.002	0.035±0.024	0.007±0.001	0.013±0.001	0.014±0.001	0.014±0.001
Nitrate-nitrogen (mgL ⁻¹)	0.10±0.06	0.14±0.05	0.22±0.128	0.41±0.16	0.37±0.20	0.22±0.02
Phosphate-phosphorus (mgL ⁻¹)	0.27±0.08	0.46±0.06	0.48±0.05	0.57±0.04	0.81±0.13	0.83±0.12

*Values in the same row, not sharing superscript letters are significantly different ($p < 0.05$)

The accumulation of GSM and MIB in water

Figure 1. presented the accumulation of GSM and MIB in the water. The range of GSM and MIB in ponds water were 13.8-22.0 ng/l and 0-17.2 ng/l, respectively. The highest concentration of GSM was in treatment with chicken manure of 562.5 kg/ha/week (22.0 ng/l) and the highest concentration of MIB was in treatment with chicken manure of 937.5 kg/ha/week (17.2 ng/l). Both water GSM and MIB were not directly dependent on fertilizer application.

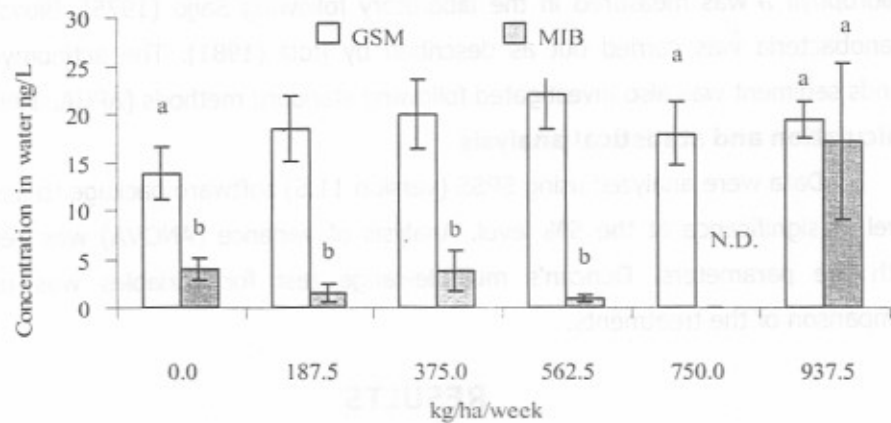


Figure 1. Accumulation of GSM and MIB in tilapia ponds water

The accumulation of GSM and MIB in sediment

The concentrations of GSM and MIB in tilapia ponds sediment were shown in figure 2. GSM accumulated into the sediment was higher than MIB. GSM concentration in sediment increased when increasing of DCM. The range of

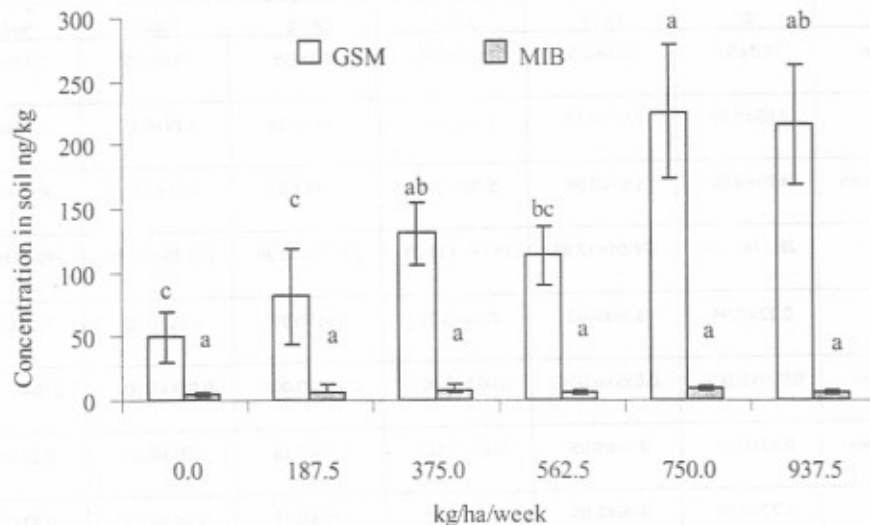


Figure 2. Accumulation of GSM and MIB in sediment of tilapia pond

Thereby, there was a declining concentration of GSM at high fertilizer loading (937.5 kg/ha/week, GSM 216.7 ng/kg). However, 2-methylisoborneol was highly concentrated into the water (17.2 ng/kg) if the fertilizer inputs were high but lower at soil bottom.

Biovolume of cyanobacteria in ponds water and Actinomycetes in sediment of

Biovolume of cyanobacteria in ponds water and Actinomycetes in sediment were investigated. The result biovolume of cyanobacteria was the highest in the treatment with 187.5 kg/ha/week (Fig. 3). The number of actinomycetes in pond sediment (Fig 4) tended to increase with the increasing of chicken manure. The highest number of actinomycetes was observed in treatment with highest dose of chicken manure.

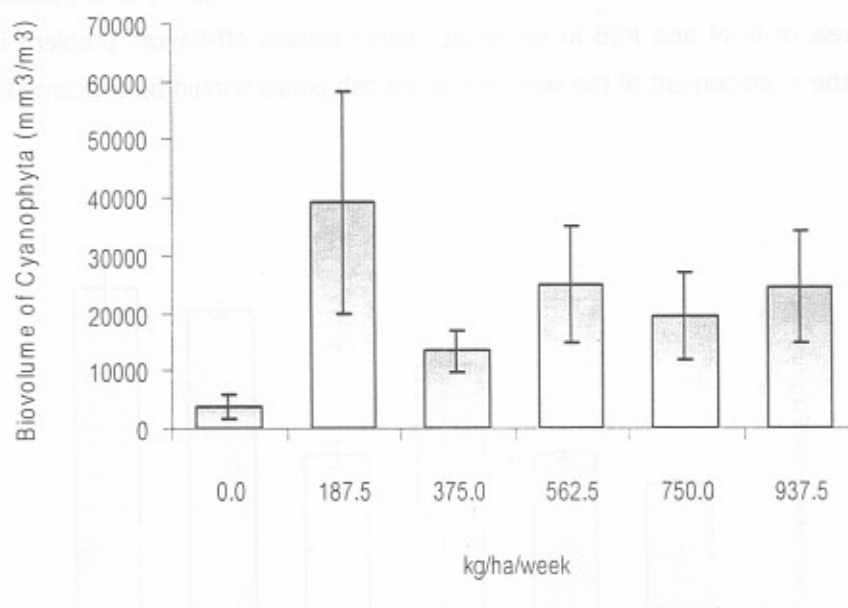


Figure 3. Biovolume of cyanobacteria (mm^3/m^3) in red tilapia cultured in tilapia ponds after culture

DISCUSSION

Most tilapia culture is in the ponds added with organic or inorganic fertilizers (Lim, 1989). Organic manure promotes natural food webs that improve tilapia production (Diana and Lin, 1994). Based on the results chlorophyll a increased with increasing DCM. This study also revealed that GSM in sediment increased when increasing of DCM. It is reported that GSM and MIB are produced by several species of cyanobacteria, including *Oscillatoria sp.*, *Lyngbya sp.*, *Phormidium sp.*, and *Anabaena sp.*, as well as some genera of *Streptomyces*, *Nocardia* and *Micromonospora* in aquatic

environments (Gerber, 1967; Tsuchiya *et al.*, 1978; Sugiura *et al.*, 1983). In previous study, we found that GSM in fish flesh was strongly correlated with both chicken manure loading and number of actinomycetes in ponds sediments. In this study, GSM, and MIB were found in both water and sediment. GSM in sediment was highly correlated with the number of actinomycetes in sediment while there was no correlation with MIB in sediment. Excess fertilizer accumulated in the pond sediments led to the proliferation of actinomycetes (Whangchai *et al.* 2008). Juttner and Watson (2007) reported that *Streptomyces* could produce GSM 52 ng/mg (dry weight) and some benthic cyanobacteria such as *Oscillatoria spp*, *Phormidium spp*, also produced GSM. MIB was produced by planktonic cyanobacteria such as *Planktotrix spp.* and some benthic cyanobacteria such as *Oscillatoria spp*, *Phormidium spp*, (Juttner and Watson, 2007). This study found that both GSM and MIB in sediment were very high in sediment compared to that in water, It is possible that high levels of GSM and MIB in sediment mainly caused off-flavors problem in fish. Hence, the management of the sediment in old fish ponds should be concerned.

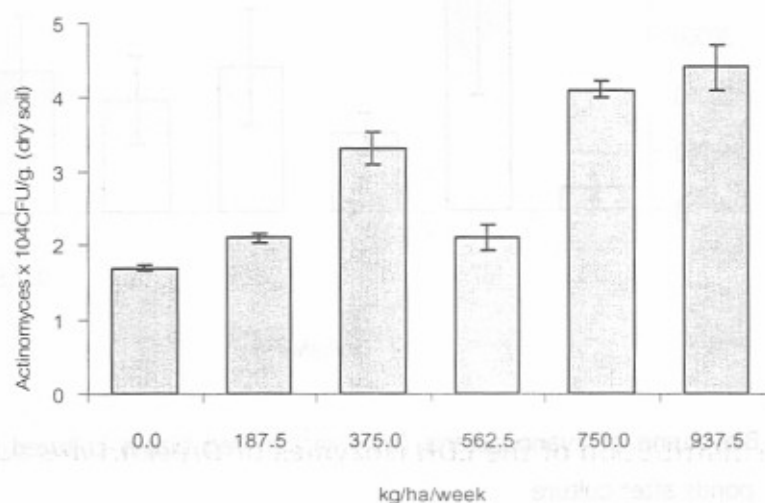


Figure 4. Actinomycetes in red tilapia ponds bottom soil which applied different dose of chicken manure.

Acknowledgments

This research was by funded by National Research Council of Thailand (NRCT).

REFERENCES

1. APHA (American Public Health Association). 1989. Standard Method for the Examination of Water and Wastewater. 15th ed. Washington DC: American Public Health Association. 1134 p.
2. Casey, C. G., W. L. Steven and V. Z. Paul. 2004. Instrumental versus sensory detection of off-flavors in farm-raised channel catfish. *Aquaculture* 236(๑-๔): 309-319.
3. Diana, J. S. and C. K. Lin. 1994. Supplemental feeding of tilapia in fertilized ponds. *Journal of the World Aquaculture Society* 25:497-506.
4. Gerber, N. N. 1967. GSM an earthy-smelling substance isolated from actinomycetes. *Biotechnol. Bioeng.* 9, 321-327.
5. Izaguirre, G., C. J. Hwang, S. W. Krasner and J. Micheal. 1982. Geosmin and 2-methylisoborneol from cyanobacteria in three water supply system. *Applied and Environmental Microbiology* 43(3): 708-714.
6. Juttner, F. and S. B. Watson. 2007. Biochemical and Ecological Control of Geosmin and 2-Methylisoborneol in Source Waters. *Applied and Environmental Microbiology*, 73(14), 4395-4406.
7. Lim, C., 1989. Practical Feeding-Tilapia. In: Lovell, T. (Ed.), *Nutrition and Feeding of Fish*. Van Nostrand Reinhold, New York, NY, pp. 163-183.
8. Lovell, T. 1989. *Nutrition and feeding of fish*. Van Nostrand Reinhold. New York. 260 p.
9. Montiel, A. J. 1983. Municipal drinking water treatment procedures for taste and odor abatement a review. *Wat. Sci. Technol.* 15 (1983), pp. 279-289.
10. Roberson, R. F. and L. A. Lawton. 2003. Off-Flavor problems an a potential solution within the U.K. trout industry. In: Rimando, A. M. Schrader K.K. (Eds.), *Off-Flavors in aquaculture*. ACS Symposium Series, vol. 848. Oxford Univ. Press. Washington, pp. 55-68.
11. Roberson, R. F., A. Hammond, K. Jauncey, M. C. M. Beveridge and L. A. Lawton. 2006. An investigation into the occurrence of geosmin responsible for earthy-musty taints in UK farmed rainbow trout (*Onchorhynchus mykiss*). *Aquaculture* 259:1-4, 153-163.
12. Rott, E. 1981. Some results from phytoplankton counting intercalibrations. *Schweiz.Z.Hydrol.* 43 (1): 34-62
13. Saijo Y. 1975. A method for determination of chlorophyll. *Jap. J. Limnol.* 36(3) 103-109.
14. Sugiura, N., O. Yagi and R. Sudo. 1983. Effect of various environmental factors in musty odor production by *Streptomyces*. *Jap. J. Water Poll. Res.* 6: 77-86.

15. Sugiura, N., M. Utsumi, B. Wei, N. Iwami, K. Okano, Y. Kawauchi and T. Maekawa. 2004. Assessment for the complicated occurrence of nuisance odours from phytoplankton and environmental factors in a eutrophic lake. *Research and Management*. 9: 195-201.
16. Tsuchiya, Y., A. Matsumoto and T. Okamoto. 1978. Volatile metabolites produced by *Actinomyces* isolated from Lake Tairo at Miyakejima. *Jpn. J. Pharmacol.* 98: 454-550.
17. Whangchai N., K. Kannika, S. Deejing, T. Itayama, N. Iwami, T. Kuwabara and Y. Peerapornpisal. 2008. Growth performance and accumulation of off flavor in red tilapia, *Oreochromis niloticus* x *Oreochromis mosambicus*, culture by green water system using chicken manure. *Asian Environmental Research*. (1) : 8-16.
18. Yamprayoon J. and A. Noomhorm. 2000. Noomhorm, Geosmin and off-flavour in Nile tilapia (*Oreochromis niloticus*), *J. Aquat. Food Prod. Technol.* 9 (2000), pp. 29-41.