8-20-2000

Phototrophic Anaerobic Lagoons as Affected by Copper and Zinc in Swine Diets

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Gilley, John E.; Spare, Daniel P.; Koelsch, Richard K.; Schulte, Dennis D.; Miller, Phillip S.; and Parkhurst, Anne M., "Phototrophic Anaerobic Lagoons as Affected by Copper and Zinc in Swine Diets" (2000). *Biological Systems Engineering: Papers and Publications*. 10.
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PHOTOTROPHIC ANAEROBIC LAGOONS AS AFFECTED BY COPPER AND ZINC IN SWINE DIETS

J. E. Gilley, D. P. Spare, R. K. Koelsch, D. D. Schulte, P. S. Miller, A. M. Parkhurst

ABSTRACT. *Odor emissions from anaerobic lagoons containing large populations of phototrophic bacteria are usually minimal. This study was conducted to determine whether copper (123 ppm) and zinc (2,310 ppm) in diets fed to weanling pigs for therapeutic purposes affect phototrophic conditions within lagoons. Column reactors containing 47 L of swine lagoon sludge and supernatant were used to represent lagoons. The reactors were placed in an environmental chamber maintained at 24° C. Copper, zinc, and control manure were added to the reactors at a volatile solids loading rate of* $128\ g_{vs}\ m^{-3}\ da^{-1}$ *using a hydraulic retention time of 32.5 days. Bacteriochlorophyll a, copper, reduction-oxidation potential, salinity, sulfate, sulfide, and zinc were then measured for at least 99 days. Sulfide, total copper and total zinc were the only parameters to be significantly impacted. The copper and zinc concentrations in the sludge increased but that of supernatant in the individual reactors changed little during the study period. However, the addition of dietary copper significantly increased the concentrations of sulfides in the supernatant, creating a condition that appeared toxic to phototrophic bacteria. In contrast, a decrease in sulfide concentration resulted from the addition of dietary zinc, resulting in an environment that may have been favorable to phototrophic bacteria. Thus, to minimize potential odor concerns, zinc rather than copper may be the best choice as a dietary supplement for weanling pigs.*

Keywords. *Anaerobic bacteria, Lagoon effluent, Manure management practices, Odor control, Swine lagoon waste.*

Copper sulfate fed to weanling pigs at 100 to 250 ppm has stimulated growth (Kornegay et al., 1989). In addition, weanling pigs fed zinc oxide at 2,000 to 5,000 ppm have shown increased weight gain and reduced scouring (Hahn and Baker, 1993). Manure containing mineral supplements fed to swine for therapeutic purposes is often discharged into lagoons.

Anaerobic lagoons are one of the most widely selected alternatives for treating livestock waste because of their size, cost, and effectiveness (Barth, 1985). Odor, however, may be a problem in some anaerobic lagoons (Miner, 1981). Lagoons which are overloaded can result in odor emissions, even when the treatment facility is designed properly.

Phototrophic algae and bacteria that use light as a source of energy reduce odor (Cooper et al., 1965). Odor problems are usually minimal in lagoons that have large populations of purple sulfur bacteria (PSB) (Holt et al., 1994). Lagoons containing PSB usually turn pink, purple or red in the warmer summer months (Merrill et al., 1998). However, conditions required to maintain a healthy environment for PSB are not well understood. Copper and zinc in manure excreted by swine may influence the production of phototrophic bacteria.

LAGOON CHARACTERIZATION

Most species of PSB as well as other phototrophic species involve bacteriochlorophyll *a* (bchl *a*) in their photosynthetic activities. Thus, one of the methods frequently used to determine the presence of PSB in effluent involves the extraction of bchl *a* (Freedman et al., 1983). Bchl *a* serves as an indirect measure of PSB growth. Chen (1997) found in her investigation of eight swine lagoons that purple and non-purple lagoons had mean bchl *a* concentrations of 912 and 29 g L^{-1}. Bakke et al. (1999) measured bchl *a* concentrations in reactors fed at loading rates of 19, 64, and 256 $g_{vs}\ m^{-3}\ da^{-1}$ to be 127, 282, and 310 g L^{-1}, respectively, indicating that high loading rates do not necessarily prohibit the growth of PSB.

Reduction oxidation (redox) potential is used to determine the electrical potential between a standard hydrogen electrode and a reversible oxidation-reduction system (Barnes et al., 1985). In a study of swine waste lagoons, no odors were produced as long as the redox potentials of the surface were not less than –76 mV (Schulz and Barnes, 1990). Chen (1997) found that redox potential measured during the summer in purple swine lagoons was –288 mV compared to –332 mV in non-purple lagoons. Bakke et al. (1999) found that near the end of a 130-day experimental period, loading rates of 16 and

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Article was submitted for publication in March 2000; reviewed and approved for publication by the Structures & Environment Division of ASAE in September 2000.

This article is a contribution from USDA-Agricultural Research Service in cooperation with the Agricultural Research Division, University of Nebraska, Lincoln, and is published as Journal Series No. 12970.

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256 g_{vs} m^{-3} da^{-1} produced redox potentials of –73 and –7.5 mV, respectively.

Different species of PSB have varying tolerances for salinity. Some PSB persist in a relatively wide salinity range (Pfennig and Trüper, 1989). Several investigators have found that lagoons with salinity ranging from 5 to 8 dS m^{-1} perform satisfactory, while reductions in biological activity and increases in odor emission occur in lagoons with salinity above 10 dS m^{-1} (Fulhage, 1995). In her survey of purple and non-purple swine lagoons in southeast Nebraska, Chen (1997) found mean salinity levels of 2.5 and 6.1 dS m^{-1}, respectively, during the summer. Schulte and Koelsch (1998) found in an investigation of 37 lagoons in Minnesota, Nebraska, North Dakota, and South Dakota that when salinity levels exceeded 6 dS m^{-1}, concentrations of selected bacteria were generally low.

Pfennig (1978) described the sulfate-reducing bacteria (SRB) in anaerobic lagoons that contribute to the digestion of organic material. These anaerobic bacteria reduce sulfates to sulfides. Both PSB and SRB are found together in the same lagoon habitat, although SRB do not require light for their metabolism. Driven by energy from the sun, a sulfur cycle can be established using these two types of bacteria. A model of the sulfur cycle describing the movements of sulfides, sulfate and elemental sulfur in a phototrophic lagoon has been presented by van Lotringen and Gerrish (1978).

Sulfides are thought to originate from the breakdown of organic materials in the vicinity of the sludge, from sulfate reduction by SRB, and from dark period reduction of elemental sulfur by PSB (van Lotringen and Gerrish,1978). Sulfide can leave the cycle as hydrogen sulfide gas diffusing from the surface of the lagoon or as gas transported in bubbles rising from the sludge to the surface. Sulfates are introduced into the sulfur cycle in urine and are also produced by oxidation of sulfides by PSB. Sulfate is consumed through a chemical reduction process by the SRB, with sulfide being the end product. If there are no organisms available to consume the sulfate, or if the sulfate reducing activity occurs at a rate slower than the sulfate production by PSB, the sulfate concentration should increase.

Holm and Vennes (1970) discovered in their study of a phototrophic municipal lagoon that as PSB population increased to a peak, the sulfate concentration began to decline and sulfide concentration was reduced to a level that was undetectable. Lagoons for livestock waste usually use volatile solids loading rates much greater than municipal waste treatment facilities (Hart and Turner, 1965). Chen (1997) found no significant differences in sulfate concentrations between purple and non-purple swine lagoons, with values ranging from 20 to 35 mg L^{-1}. Sulfide concentrations in purple and non-purple lagoons were also similar, varying from 1 to 6 mg L^{-1}. For reactors containing swine manure at loading rates of 16, 64, and 256 g_{vs} m^{-3} da^{-1}, Bakke et al. (1999) measured sulfate concentrations of 62, 19, and 31 mg L^{-1}, respectively, and sulfide values of 2.0, 12.1, and 12.6 mg L^{-1}, respectively. The objective of this study was to determine the effects of copper and zinc in swine diets on the performance of phototrophic anaerobic lagoons.

MATERIALS AND METHODS

The nine column reactors used in this study to simulate anaerobic lagoons were fabricated from 20 cm diameter, 152 cm long PVC pipe having an end cap attached to the bottom end. A circular shim was glued below the end cap to allow the reactor to stand in an upright position. A port located 8 cm above the bottom of the reactor was used for extraction of sludge samples. Supernatant was removed from another port positioned 130 cm from the bottom of the reactor.

Sludge and supernatant were obtained as seed material from an anaerobic lagoon with a history of turning purple located near Grand Island, Nebraska. A boat was used so that material could be collected away from the edge of the lagoon. Approximately 6 L of sludge and 41 L of supernatant were added to each reactor. The initial composition of the supernatant and sludge which was added to the reactors is shown in table 1. The reactors were sealed at the top for transfer to an environmental chamber that was maintained at a temperature of 24°C. In the environmental chamber, a single 75 W halogen bulb was positioned 20 cm above each reactor. A timer switch was used to provide 12 h of continuous light to the reactors each day.

Swine feeding and manure collection were carried out on the University of Nebraska-Lincoln East Campus. A 3 × 3 Latin square design was used in the feeding operation. To generate and store the quantity of manure required in this experiment, three sets of pigs were fed three diets over three periods of time. Over the three periods, each set received all three diets. A set remained on one diet during a given period. Manure from a given set/period was added to an assigned reactor in the reactor operation stage. An experimental unit was considered to be the set/period/reactor, of which there were nine. By using a Latin square design, interactions occurring between swine set, swine diet, and feeding period could be minimized.

Manure was collected from swine fed a copper-sulfate diet (control plus 123 ppm copper), a zinc-oxide diet (control plus 2,310 ppm zinc) and a control diet. The copper-sulfate and zinc-oxide were fed to the animals in the form of an inorganic salt. The 12 barrows, with an average weight of 67 kg at the start of the feeding trial, were housed in individual stainless steel cages with unlimited access to feed and water. A stainless steel pan was positioned to catch all of the material falling through

Table 1. Characteristics of supernatant and sludge obtained from the lagoon

Parameter	Supernatant	Sludge
Bchl *a*, (µg L^{-1})	397	
Copper (mg kg_{TS}^{-1})		126
Copper (mg L^{-1})	0.20	
Redox (mV)	+6.0	
Total solids (mg L^{-1})	2,760	50,300
Zinc (mg kg_{TS}^{-1})		3,027
Zinc (mg L^{-1})	0.88	

the netting, and to divert the material to a plastic bucket. A walk-in refrigerator was used for storing the manure.

Manure from the control diet was first added to each of the nine reactors during a 97-day acclimation period. During the remainder of the study, manure from the copper, zinc, and control diets were added to the respective reactors. Sludge and supernatant characteristics are reported for the test period that began 20 days after copper and zinc were first introduced into the reactors. Thus, day 0 (shown in figures 1-9) corresponds with day 20 after Cu and Zn were initially placed in the reactors. An hydraulic retention time of 32.5 days and volatile solids loading rate of 128 g_{VS} m^{-3} da^{-1} were used during the test period. If the volume of manure and water to be added would bring the reactor volume above the 47 L maintenance limit, then an appropriate volume of supernatant was removed from the withdrawal port. The following supernatant characteristics, bchl *a* (Fry, 1988), redox potential, salinity, sulfate and sulfide (APHA, 1998), and copper and zinc (AOAC, 1990), were monitored to determine the performance of the reactors. The copper and zinc content of sludge were also measured.

The repeated measures analysis of variance (ANOVA) for a Latin square design was conducted using the General Linear Models Procedure (Littell et al., 1996). A probability level $p \leq 0.05$ was considered significant. Statistical tests were first performed to evaluate diet by time interactions. If a significant interaction was observed, then a generalization concerning the effect of diet on a particular reactor characteristic could not be made. Then the significance of diet on individual sampling days was examined. If no significant diet by time interaction was identified, then significant differences between diets as a whole were evaluated. If significant differences due to diet were determined, least significant differences (LSD) procedures were employed for mean separation at $p \leq 0.05$ (Steel and Torrie, 1980). The statistics used to determine significance of the data were applied to the entire test period. Further details concerning experimental and statistical procedures are reported by Spare (1999).

RESULTS AND DISCUSSION

COPPER AND ZINC CONTENT IN REACTORS

Manure from the swine fed a copper diet (123 ppm) contained copper concentrations of 878 mg kg_{TS}^{-1}, compared to 96 mg kg_{TS}^{-1} for the non-copper treatments. The daily input of copper into the copper treatment reactors was 7.8 mg, while the non-copper treatment reactors received 0.9 mg. The copper content of sludge was affected by diet as shown in figure 1. The addition of manure from the copper-diet caused a consistent increase in copper content of sludge. Copper content in sludge for the copper-treatment reactors increased from 385 mg kg_{TS}^{-1} on day 0 to 1208 mg kg_{TS}^{-1} on day 99, while copper content varied from 205 to 268 mg kg_{TS}^{-1} in the non-copper treatment reactors. ANOVA of copper content of sludge showed a significant diet by time interaction ($p = 0.0001$). The significance of diet on individual sampling days was then examined. With the exception of day 14, significant differences ($p \leq 0.05$) in copper content of sludge were found between the copper and non-copper diets.

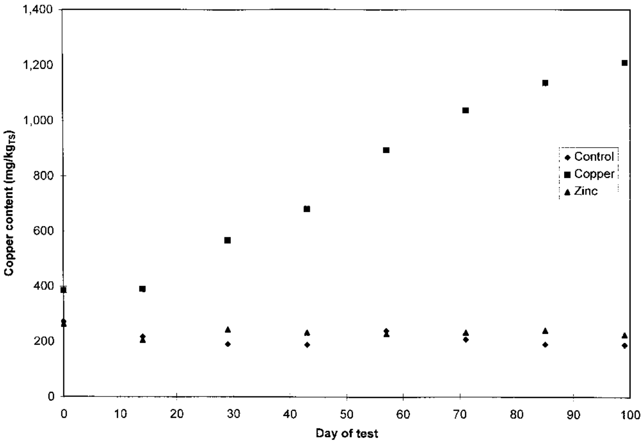

Figure 1–Copper content of sludge as affected by diet.

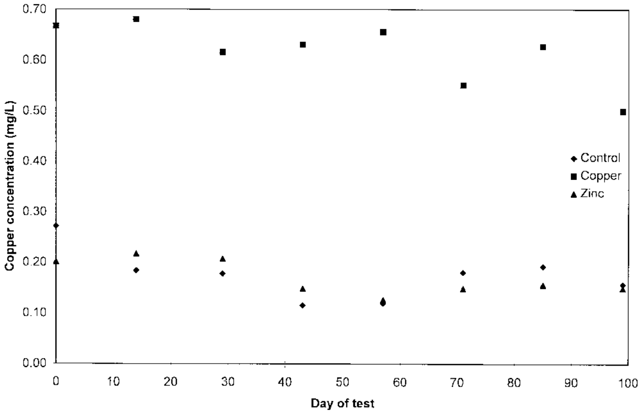

Figure 2–Copper concentration of supernatant as affected by diet.

Copper concentrations of supernatant were affected by diet as illustrated in figure 2. It is evident that copper concentrations in the copper treatment reactors varied little during the testing period. For the copper treatment reactors, copper concentrations ranged from 0.50 to 0.68 mg L^{-1}, while in the non-copper treatment reactors copper concentrations varied from 0.11 to 0.27 mg L^{-1}. ANOVA showed no significant ($p \leq 0.05$) diet by time interaction. However, copper concentrations were found to be significantly influenced by diet at the $p = 0.05$ level (table 2). It can be seen that copper concentrations of supernatant within the copper treatment reactors were significantly greater than the non-copper treatment reactors (table 3).

Swine fed a zinc diet (2,310 ppm) produced manure containing 12 800 mg kg_{TS}^{-1} of zinc, while manure from

Table 2. Analysis of variance showing the effects of swine diet on supernatant characteristics

Supernatant Characteristic	PR > F
Bchl *a*	0.63
Copper	0.05
Redox	0.09
Salinity	0.17
Sulfide	0.01
Zinc	0.02

Table 3. Tests for equality of copper, zinc, and sulfide concentration in supernatant as affected by diet

Constituent	Source	PR > F
Copper	Copper vs Control	0.03
Copper	Copper vs Zinc	0.03
Copper	Control vs Zinc	0.95
Zinc	Zinc vs Control	0.01
Zinc	Zinc vs Copper	0.01
Zinc	Control vs Copper	0.89
Sulfide	Control vs Copper	0.05
Sulfide	Control vs Zinc	0.02
Sulfide	Copper vs Zinc	0.01

the non-zinc treatments contained 810 mg kg_{TS}^{-1}. The daily input of zinc into the reactors from the zinc-manure and non-zinc-manure treatments was 114 mg and 8 mg, respectively. Zinc content of sludge was affected by diet as shown in figure 3. A consistent increase in zinc content resulted from the zinc diet. Zinc content in sludge for the zinc treatment reactors increased from 6635 mg kg_{TS}^{-1} on day 0 to 21 126 mg kg_{TS}^{-1} on day 99, while in the non-zinc treatment reactors, zinc content varied from 2899 to 3335 mg kg_{TS}^{-1}, respectively. A significant diet by time interaction (p = 0.0001) was found from ANOVA. For each of the individual sampling dates, significant differences ($p \leq 0.05$) in zinc content of sludge occurred between the zinc and non-zinc diets.

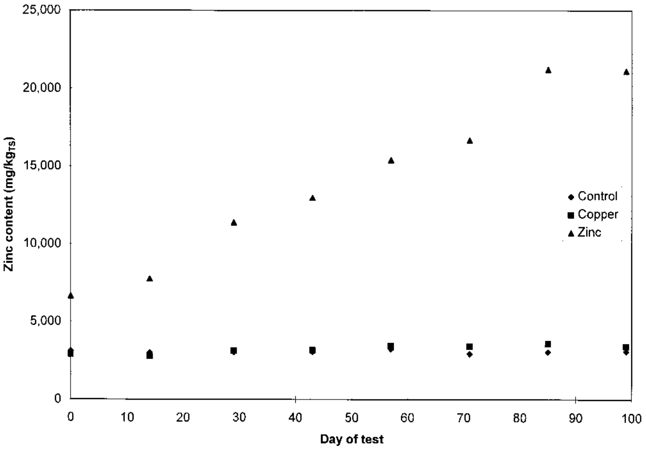

Figure 3–Zinc content of sludge as affected by diet.

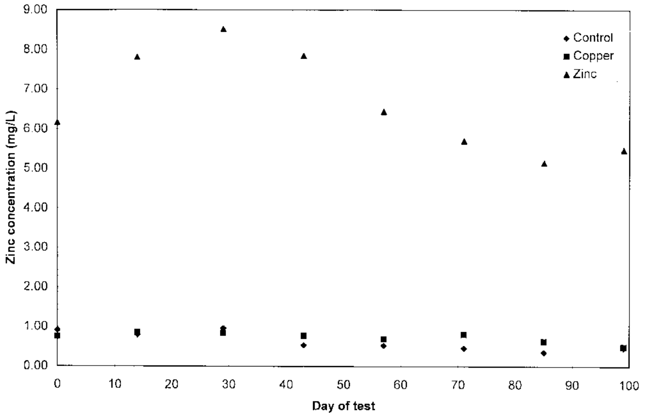

Figure 4–Zinc concentration of supernatant as affected by diet.

Zinc concentrations of supernatant were affected by diet as illustrated in figure 4. Zinc concentrations in the zinc-treatment reactors varied from 8.52 mg L^{-1} to a minimum of 5.14 mg L^{-1} near the end of the investigation, while in the non-zinc treatment reactors, zinc concentrations ranged between 0.36 and 0.96 mg L^{-1}. No significant ($p \leq 0.05$) diet by time interaction of zinc concentration of supernatant was indicated by ANOVA. However, diet was found to significantly influence zinc concentrations at the p = 0.02 level (table 2). Zinc concentrations of supernatant within the zinc-treatment reactors were significantly greater than the non-zinc treatment reactors (table 3).

MEASURED REACTOR PERFORMANCE

Bchl *a* concentrations of supernatant were affected by diet (fig. 5). Throughout the test period, bchl *a* concentrations generally followed the trend: zinc diet > control diet > copper diet. For the zinc diet, control diet, and copper diet reactors, bchl *a* concentrations varied from 705 to 1353, 698 to 986, and 424 to 898 mg L^{-1}, respectively. ANOVA showed no significant diet by time interaction for bchl *a* concentrations of supernatant. Although differences in bchl *a* concentrations were evident between diet treatments, the variations were not statistically significant (p = 0.63) because of the large differences in measurements obtained for the individual replicates. It is also evident from figure 5 that the bchl *a* concentrations in the zinc treatment reactors increased near the end of the experiment, and were substantially greater than the non-zinc treatment reactors. All three of the zinc treatment reactors were observed to have developed a pink color. In contrast, bchl *a* concentrations in the copper treatment reactors were consistently less.

Redox potential of supernatant was affected by diet as shown in figure 6. In general, redox potential of supernatant followed the trend: control diet > copper diet > zinc diet. For the control diet, copper diet, and zinc diet, redox potential ranged from –44 to –21, –46 to –23, and –48 to –28 mV, respectively. No significant diet by time interaction of redox potential was identified by ANOVA. In addition, differences in redox potential between diets were not statistically significant (p = 0.09) (table 2).

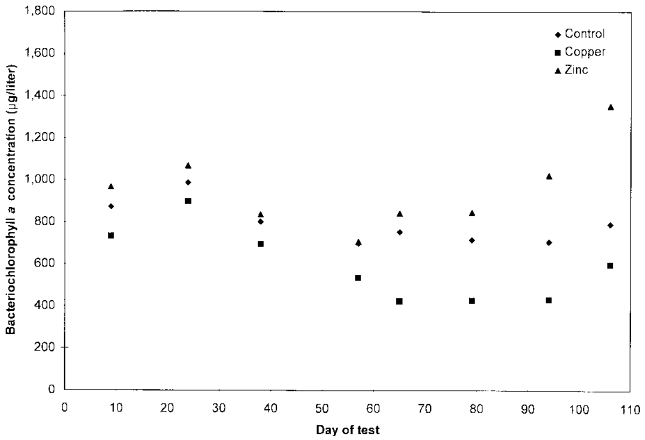

Figure 5–Bacteriochlorophyll *a* concentration of supernatant as affected by diet.

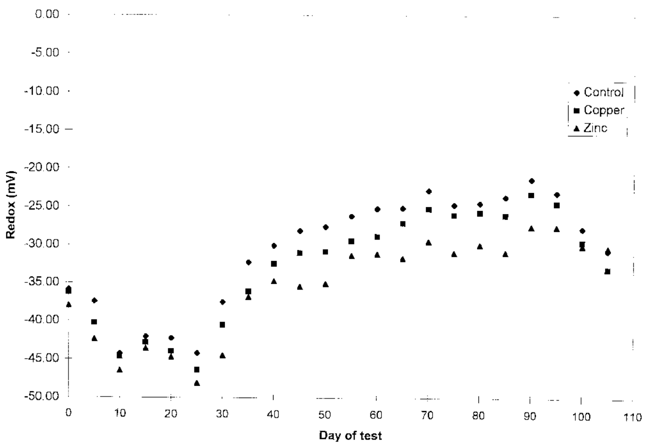

Figure 6–Redox potential of supernatant as affected by diet.

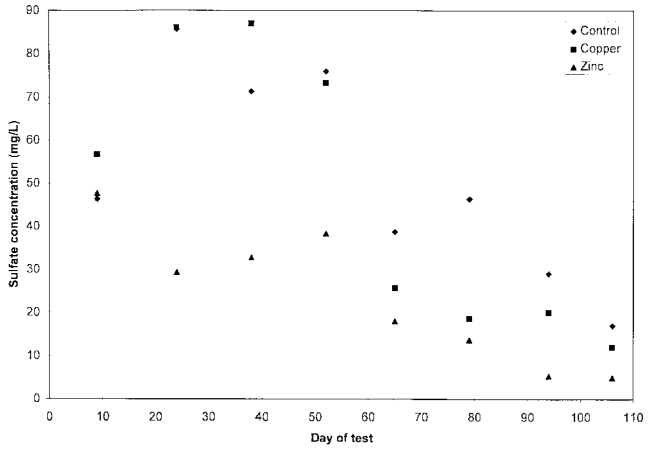

Figure 8–Sulfate concentration of supernatant as affected by diet.

During approximately the first third of the experiment, salinity levels were above the threshold of 6 dS m^{-1} identified by Schulte and Koelsch (1998) (fig. 7). As a result, the bacteria populations initially may have been stressed, requiring a longer time to reach an equilibrium condition. Throughout the test period, salinity followed the general trend: copper diet > control diet > zinc diet. For the copper diet, control diet, and zinc diet reactors, salinity varied from 5.8 to 10.2, 5.2 to 9.9, and 4.9 to 9.9 dS m^{-1}, respectively. Differences between the copper diet and non-copper diet treatments were most pronounced near the end of the test period. ANOVA showed no significant diet by time interaction for salinity of supernatant. The variations in salinity between diet treatments were not significantly different ($p = 0.17$) (table 2).

During the last five sampling dates, sulfate concentrations followed the trend: control diet > copper diet > zinc diet (fig. 8). For the control diet, copper diet, and zinc diet reactors, sulfate concentrations ranged from 17 to 86, 12 to 87, and 5 to 48 mg L^{-1}, respectively. ANOVA of sulfate concentrations of supernatant showed a significant diet by time interaction ($p = 0.0001$). However, with the exception of day 79, no significant differences in sulfate concentrations were found between the diet treatments for the individual sampling dates.

Sulfide concentrations followed the general trend: copper diet > control diet > zinc diet (fig. 9). For the copper diet, control diet and zinc diet reactors, sulfide concentrations varied from 34 to 73, 23 to 70, and 9 to 66 mg L^{-1}, respectively. ANOVA of sulfide concentrations of supernatant showed no significant ($p \le 0.05$) diet by time interaction. Sulfide concentrations were found to be significantly influenced by diet at the $p = 0.01$ level (table 2). Sulfide concentrations in the reactors representing each of the three manure sources were significantly different (table 3).

The solubilities for CuS and Cu_2S are 9×10^{-18} and 3×10^{-11} mg L^{-1}, respectively (Lawrence and McCarty, 1965). The result of adding manure containing dietary copper to the reactors would be expected to be a lower concentration of sulfides due to the copper ions complexing with available sulfides. However, at the end of the experiment, the copper treatment reactors contained higher concentrations of sulfides than the non-copper treatment reactors. Daily addition of copper to the copper treatment reactors was 7.8 mg. Sulfide concentrations in the copper treatment reactors near the end of the study were 40 mg L^{-1}. Approximately 1 mg of copper is required to complex with 0.5 mg of sulfide (Lawrence and McCarty, 1965). Thus, there may not have been sufficient quantities

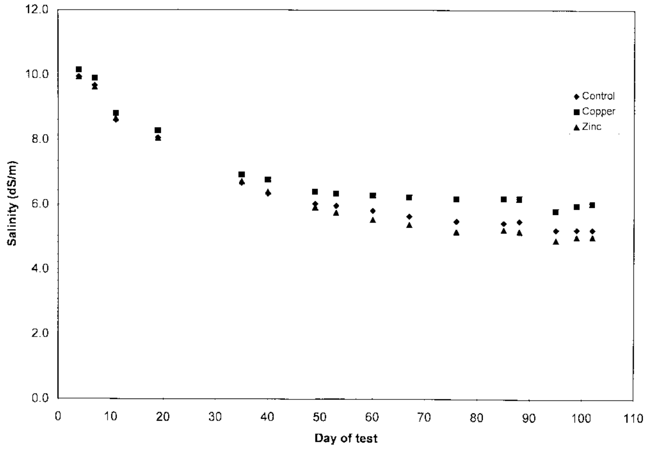

Figure 7–Salinity of supernatant as affected by diet.

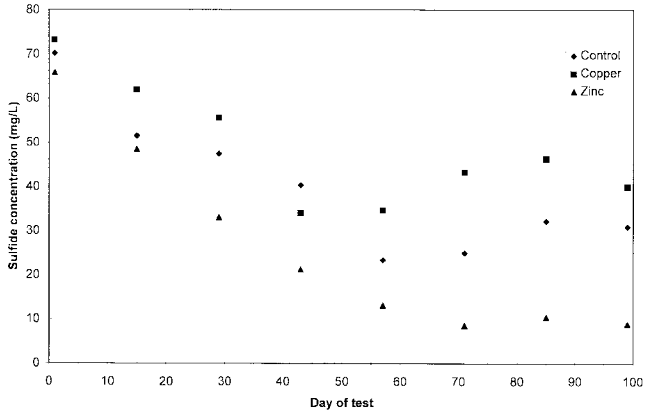

Figure 9–Sulfide concentration of supernatant as affected by diet.

of unbound copper available to fully complex with the sulfides.

Incomplete anaerobic digestion of organic materials may have increased sulfide levels in the reactors. The increased sulfide concentrations were thought to be caused by a smaller population of PSB. Levels of copper in the sludge approached 1200 mg kg_{TS}^{-1} near the end of the experiment. Thus, an environment toxic to methane-forming bacteria and PSB may have been created when copper was introduced into the reactors.

The solubility of ZnS is 3×10^{-7} mg L^{-1} while approximately 1 mg L^{-1} of Zn is required to complex with 0.5 mg of sulfide (Lawrence and McCarty, 1965). Zinc was added to the zinc treatment reactors at a rate of 114 mg d^{-1}. Sulfide concentrations in the zinc-treatment reactors were relatively stable at 9 mg L^{-1} near the end of the experiment, in contrast with the control treatments where 31 mg L^{-1} were observed. Thus, there may have been enough zinc introduced into the reactors to have caused substantial complexing with the sulfides.

Bchl *a* concentrations were always greater in the zinc treatment reactors, especially at the end of the study. Sulfate concentrations in the zinc treatment reactors were usually less than the non-zinc treatment reactors, and they steadily decreased near the end of the investigation. In addition, the supernatant color of the zinc treatment reactors was pink, in contrast with an absence of pink color in most of the other reactors. Thus, it appears that the addition of zinc may have created an environment that enhanced the production of phototrophic bacteria.

CONCLUSIONS

This study was conducted to determine the effects of copper and zinc in swine diets on the performance of phototrophic anaerobic lagoons. Significantly greater amounts of copper or zinc were present in the sludge and supernatant of the reactors containing manure generated from the copper or zinc diets. The copper or zinc content of sludge in the copper or zinc diet reactors consistently increased throughout the study period. In contrast, little change in the copper or zinc concentrations of supernatant occurred over time.

The bchl *a* concentrations in the zinc treatment reactors increased near the end of the experiment, and were substantially greater than the non-zinc treatment reactors. The zinc treatment reactors developed a pink color that is indicative of a healthy phototrophic bacteria population. In contrast, the bchl *a* concentrations in the copper treatment reactors were consistently less, and were considered marginal for healthy phototrophic conditions.

The concentration of sulfides in the reactors containing dietary copper increased significantly, creating a condition that appeared toxic to the bacteria in the reactors. In comparison, conditions favorable to phototrophic bacteria appeared to result from the addition of dietary zinc, which significantly decreased sulfide concentrations. Thus, to maintain healthy phototrophic conditions, zinc may be the best choice as a dietary supplement for swine.

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