A Comparison of the Effect of Anolyte and Effective Micro-organisms (Kyusei EM^{TM}) on the Faecal Bacterial Loads in the Water and on Fish Produced in Pig-cum-Fish Integrated Production Units

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Abstract. *The production potential of pig manure on fish growth and water quality in integrated pig-fish systems was investigated using effective micro-organisms (Kyusei EM^{TM}) with or without formulated pig feeds and Anolyte. Both Anolyte and EM effectively reduced faecal bacterial loads in pig manure. EM positively affected pig growth but this was obscured with the introduction from the second month of growth hormone and antibiotics in the pig diets. The application of manure from both treated and untreated pigs had a positive effect on fish yields, improving the feed conversion ratio of the fish to below 2. The EM-A containing manure, however, significantly improved the overall FCR producing a value of 1.4. The application of EM-A containing pig manure also had a marked effect on some faecal organism counts in the manure and in the water of the fish ponds, but also reduced the somatic coliphage numbers significantly. Faecal* Streptococci *and* E. coli *found in the kidneys, gills, spleen and liver of the* Mozambique tilapia *which were used as pond fish, may well have a medium to long term negative implication for the use of animal manures containing faecal bacteria. This aspect required serious attention in future research where agricultural waste products of this nature are used to stimulate fish pond production.*

Introduction

The incorporation of agricultural waste products into integrated aquaculture-agriculture food production systems, the advantages it has in reducing environmental pollution and the beneficial effect such waste products have on fish and crop production, had been practically demonstrated by research and development projects in South Africa and elsewhere (Prinsloo and Schoonbee, 1984 a,b,c; Prinsloo and Schoonbee, 1986: Prinsloo and Schoonbee, 1987; Prinsloo et al., 1999 a, b; Pullen and Shedeh, 1980; Edwards, 1991; Diallo, 1992). In recent years, innovative techniques which further positively affected the food production potential of integrated aquaculture-agriculture systems, were developed and applied in other fields of agriculture and environmental quality control (Higa, 1996; Higa, 1998; Sangakkara, 1998). One of these includes the EM Technology (Higa, 1996; Higa, 1998) whilst another, namely Anolyte (Hinze, personal communication) may play an important future role in combatting environmental pollution in intensive food production systems.

EM Technology is largely based on the rejuvenation of environmentally contaminated agricultural soils and the establishment of healthy soil conditions by the introduction of beneficial micro-organisms cultured, resulting in the replacement and/or elimination of potentially harmful micro-organisms and insects. At the same time the EM technology can also make a significant contribution towards the reduction and eventual total elimination of pesticides in previously cultivated soils (Parr et al., 1998).

By using animal manures in integrated aquaculture-agriculture systems, the problem of nutrient build-up as well as faecal contamination of fish pond water may affect fish production potential of water prior to its use in the irrigation of vegetable crops.

It was shown in the literature that fish produced in faecal bacterial contaminated water may be detrimentally affected by the occurrence of faecal *Streptococcus* belonging to the Lancefield Group D in tissues and organs of rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) (Boomker et al., 1979) but with no apparent affect on Mozambique tilapia *Oreochromis mossambicus* (Petersen, 1852) and banded tilapia sparmanii (A Smith, 1840). Hoshina et al. ((1958) suggested that these non-hemolytic enterococci may be a strain of *Streptococcus faecalis,* differing in its relation to its optimum pH and temperature requirements as well as the source from which it has been isolated. Barham et al. (1979) investigated the physiological affects of *Aeromonas* and *Streptococcus* infection on rainbow trout, suggest that Aeromonas infection may be of secondary nature following the occurrence of *Streptococcus* infection. Snieszko and Axelrod (1971) showed that *Aeromonas liquefaciens* may also cause infectious dropsy and naemorrhagic speticaemia in freshwater fish.

The present investigation deals with the following aspects of an integrated pig-fish production system. Different treatments, with and without the inclusion of EM and Anolyte technologies in the prodcution of pigs under controlled environmental conditions were applied. Manures from EM-A treated and untreated pigs, were then used as additional nutrients to promote *O. mossambicus's* growth in ponds. This was supplemented by using a formulated pelleted fish diet. Physical and chemical conditions of pond water were also investigated to evaluate nutrient enrichment of pond water by pig manure.

The occurrence of faecal bacteria, which included total Coliforms, faecal Coliforms, faecal *E. coli,* and faecal Streptococci (Group D), in pig manure of the four different experimental groups as well as the fish pond water, were determined. The possible affects of EM-A on specific faecal bacterial loads, are briefly considered, particularly so on Coliphages organisms in the fish pond aquatic environment. Streptococcil infections in kidneys, gills, livers and spleens of *O.mossambicus* pond fish and possible consequences threof on the fish health status, are considered.

Materials and Methods

Pigs used in the Investigation

A total of 40 mixed bred pigs (50 percent Duroc, 25 percent Landras, 25 percent Large White = F2) devided into four groups were obtained from the Mockford Farms, Pietersburg, The pigs were divided into 4 groups of 10 pigs each and housed in newly constructed disease free pig sties at a density of one pig/m^2. Unlimited water was supplied to each unit using drinking nipples. Pigs were fed *ad lib*, making use of commercially available pig feed troughs. Commercial diets were applied.

Feed Treatments

Different feed treatments were administered to four groups of pigs. Group 1 received 2.5 percent EM Bokashi (Kyan et al., 1999) mixed in with the food as well as Anolyte (a free radical oxidant) (Hinze, personal communication) which was sprayed into two of the pig house as a fine mist at a volume of 150 m/min/sprinkler, at intervals of 30 minutes for 8 hours per day. Two elevated mist sprinklers were installed at a height of 1.5 metres covering the entire surface area of the pig house where Anolyte was applied. Mist spray per time interval lasted 2 minutes.

Anolyte Technologies

The Anolyte technology was introduced into South Africa from Russia by Radical Waters, Co. ltd., Johannesburg, and consists of electrochemically activated NaC water produced which served as a sterilizing solution. pH of this solution was adjusted to 7.5 and diluted to 50 percent mixture with water before used in the mist spray programme. Although not utilized, a positively charged catolyte-anti-oxidant is also produced, suitable as a washing solution or vitamin enricher.

Preparation of EM Bokashi Used in Pig Food

The basic constituents (on a mass basis) included 70 percent wheat bran, 15 percent maize bran, 5 percent each of soya bean meal, fish meal and bone meal, respectively. The ingredients were properly mixed, using a concrete mixer. A 1:1:500 (on a volume basis) EM:molasses:water mixture was then sprayed on to the mixture to obtain an approximate moisture content of 35 percent. The mix was then transferred to a woven hessian bag and anaerobically sealed in a black plastic bag. A seven day period was allowed for obtaining the necessary state of fermentation of the material before being mixed in with the pig food.

Feeding Programme

Group 1 of the pigs received EM (2.5 percent) mixed in with the feed together with Anolyte spray. Group 2 received the standard rations without EM, but with the Anolyte spray. The Group 3 pigs, received EM only, mixed in with the feed whilst in the control group (Group 4) no EM or Anolyte applications were made. The feeding programme of all four groups of pigs were basically similar. During the first 30 days a formulated 18 percent protein pig weaner ration was provided. This was followed for the next 30 days with a super formulated 27 percent protein formulated diet. For the last 15 days of the pig feeding programme a 36 percent finisher formulated diet was provided. Pigs from groups 2 and 4 (Anolyte treatment and control) received growth hormone plus flavomycin as anti-biotic mixed in with the feed. The amount of food consumed by each group of pigs was carefully monitored on a daily basis.

Mass Determination of Pigs and Removal of Pig Manure

In order to assess the effects of the different treatments on pig growth, mass determination of individual pigs within each group were made every fortnight, The floors of the pig houses were thoroughly cleaned daily from pig waste and washed with water. Effluent water was discharged into gravel filters to remove any remaining solid wastes after which the wastewater was returned to the main reservoir for later use in fish ponds and for irrigation of different vegetable plots. Floors of pig houses receiving EM treated food, were also lightly sprayed with a 1:500 diluted solution of EM to reduce any odours arising from the pig manure.

Fish Production

Six ponds of 25 M^2 each provided with plastic canopies for temperature control, were used for stocking the fish. In each pond the tilapia *O. mossambicus* (Zululand strain) were stocked at a density of 40,000/ha. The mean individual mass of fish at stocking was 38.5 g. All Fish in each pond were individually weighed before stocking. Pig manure was applied to each pond for six days per week at 2.5 percent (dry mass of the total live biomass of the fish in the ponds. Mass adjustment of the pig manue dosage programme for the different ponds was made fortnightly, based on fish mass assessment using 20 –30 percent subsamples. Two sets of 3 ponds each were investigated. In the first three ponds, manure from EM-A treated pigs were used. The second group of ponds received manure from the untreated (control) pigs. In addition to pig manure, all fish ponds received a 30 percent protein formulated fish pellet daily for 6 days/week at 4 percent of the estimated fish biomass per pond. Due to the build up of algal growth in the ponds all ponds were aerated during the period of investigation. An Electror blower supplied air to the ponds. Water replacements started 30 days after the commencement of the fish feeding programme when approximately 20 percent of the volume of each pond was replaced once per week with water from the main reservoir. Final individaul mass determinations of the fish from each pond, including the wild spawn, were made to establish the total fish production over a period of 71 days for both sets of fish. A feed conversion ratio for the formulated feed was then calculated.

Water Chemistry

Chemical parameters of fish pond water were analysed according to Standard Methods (1995). Water temperature (c) was measured using s Thies hydro-thermograph. Dissolved oxygen concentrations ($mg.^{-1}$) were determined using an Oxy 92 oxygen meter. pH values were determined with a portable Hanna 8244 pH meter. The electrical conductivity ($S.cm^{-1}$) was recorded with a Hanna H1 8633 conductivity meter. Ammonia (NH_3-mg. $^{-1}$) nitrite (NO_2-mg^{-1}), nitrate (NO_3-mg^{-1}), orthophosphate (PO_4-mg^{-1}), as well as turbidity (NTU) were all determined using a Hach spectrophotometer. Magnesium ($MgCO_3$-mg^{-1}), calcium ($CaCO_3$-mg^{-1}), total hardness ($CaCO_3$-mg^{-1}) and alkalinity were titrimetically determined. Mean values, as well as ranges for each parameter, were determined and tabulated.

Bacteriological Analysis

Plaque Assay for Somatic Coliphages Using the Double Agar Layer Technique

The methodologies followed as assaying techniques employed in the evaluation of faecal bacterial loads in pig manure as well as in fish pond waer receiving treated and untreated pig manure, were conducted according to internationally accepted standards (Ketchum, 1942; Pelczar, 1916; Lenette et al., 1988; Grabow et al., 1995; SABS, 1990; Genthe and Du Preez, 1995).

Quantitative Assessments

Quantitative assessments of the various organisms were made which included total bacterial counts, total Coliforms, faecal Coliforms, faecal *Escherichia coli* (strain ATCC 25922), faecal *Streptococci* group D and somatic Coliphages.

Conventional plaque assays for somatic Coliphages were usually done on small volumes of water (1.0 m) based on techniques as describe by Adams (1959).

Inverted plates were incubated at 35-37 C for 24 h after which plaques were counted. Bacterial nutrient broth (Difco or equivalent) wereused as growth media. The respective compositions of phage bottom and phage top agar and the test procedure followed, are described in Grabow et al (1995).

Bacterial Sampling of Pig Manure

Fresh pig manure samples from each of the four treatments were collected at fortnightly intervals during the early morning for the necessary bacteriological analysis. Care was taken to do all collections under sterile conditions.

Collection and Treatment of Fish Pond Water for Bacterial Analysis

Water samples from fish ponds receiving manure from EM-A treated and untreated pigs respectively were sampled fortnightly and analysed for total bacterial counts, total Coliforms, faecal Coliforms, faecal *E coli*, faecal Streptococci and somatic Coliphages. Total Coliforms and somatic Coliphages were incubated at 37 C for 24h. Faecal Streptococci, faecal Coliforms and faecal *E. coli*, were incubated at 44 C for 24 h. Total bacterial counts were made after 48 h at 30 C. the investigation lasted for 9 weeks.

Bacterial Analysis of Selected Fish Organs

Target fish organs selected for analysis of faecal *Streptococci* group D and faecal *E.coli* included kidneys, gills, spleens and livers. To obtain sufficient material, three fish from each of the three different ponds, for each pig manure treatment, were randomly selected and the above mentioned organs were clinically removed under sterile conditions for bacterial analysis.

Table 1. A Comparison of the Stocking Densities and Initial Mean Individual and Total Biomass of the Pigs receiving different Treatments of EM Bokashi and Anolyte as well as the Final Individual and Total Biomass, Yields and Feed Conversion Ratio over a Period of 76 Days from 24 February – 11 May 1999 (Range Indicated in Brackets)

	Stocking Density and Initial Biomass			Final Number of Pigs	Mean Final Individual Mass (kg)	Mean Final Fish Biomass (kg)	Yields (kg)	Formulated Feed Dosage Quantitites (kg)	FCR
Treatment	**Mean Stocking Density /10m^2**	**Mean Initial Individual Mass (Kg)**	**Mean Initial Fish Biomass (kg)**						
EM-A	10	18.6 (15.5-22.6)	185.6	10	87.0 (77.0-94.0)	869.5	683.9	1928.0	2.82
A	10	18.6 (15.5-21.7)	185.6	10	88.4 (74.5-99.0)	884.0	698.4	1939.0	2.78
EM	10	17.7 (14.2-21.4)	177.3	10	83.1 (74.5-91.5)	831.0	653.7	1878.0	2.87
Control	10	17.8 (15.0-18.8)	177.9	10 (9)	84.7* (85.3) (75.0-94.5)	846.5* (767.5)	668.6* (589.6)	1906.0	2.85* (3.23)

* One mortality – weight of pig included in calculations

Results

Pig Production

Results on growth statistics of the pigs for the various feeding programmes are summarized in Table 1. Based on the mass increment, yields and feed quantities consumed by different groups of pigs, feed conversion ratios (FCR) suggest no significant difference in pig production between any of the pigs for the different treatments. In all cases, FCR ranged between a narrow 2.78 (Anolyte treatment) and 2.87 (EM-A treatment). One mortality occurred in the control group of pigs. It must be noted that prior to the inclusion of growth hormone which began 30 days after commencement of the investigation, both EM treated pigs had a slight weight advantage over the Anolyte and control groups.

Fish Production in Pig Manure Treated Ponds

Results of fish production in ponds receiving pig manure from EM-A treated and untreated pigs with formulated fish feeds over a period of 71 days are summarized in Tables 2 and 3. Fish mortalities in both sets of fish ponds were low and similar, ranging from 5.6 percent (control) to 6 percent (EM-A). Wild spawns took place in both sets of ponds and their respective contributions towards the yields were included in the final standing crops. Based on the initial estimated and final yields of the actual fish stocked in the two different treatments, the crop produced by the EM-A treated ponds exhibited a 4.0 percent better yield at the end of the investigation. Together with the fish mass contribution by the wild spawns in both sets of ponds, the EM-A ponds yielded an 18.5 percent higher total mass than those of the control ponds with yield of 4348 kg/ha as against 3688 kg/ha for the control ponds.

The respective feed conversion ratios of all fish produced as measured by the feed-crop ratio, amounted to 1.4 for EM-A treated compared to 1.7 the control ponds. The actual contribution of the pig manure cannot be calculated but must have played a significant role in the total yields obtained, judged by previous investigations by Prinsloo and Schoonbee (1984 a) and Prinsloo and Schoonbee (1986).

Table 2. **Results of the Mean Growth and Production of *O. mossambicus* receiving 30% protein Pelleted Fish Feed Supplemented with Manure from Pigs Treated with EM Bokashi and Anolyte over a Period of 71 days from 23 February – 3 May 1998**

Period	Days	Date	Mean and Extreme Temperatures	Stocking (S) and Final (F) Densities (fish/ha) Excluding Wild Spawns	Mean Individual Mass of Fish (g)	Estimated (E) and Final (F) Standing Crop (kg/ha)	Yield Successive Periods (kg/ha)	Production (kg/ha/d)	Calculated Pig Manure (dry matter) Quantities (kg/ha)	Formulated Feed Dosage Quantities (kg/ha)	Feed Conversion Ratio (FCR)
0	0	23/02/99	--	40 000(S)	38.6	1544.0	--	--	--	--	
1	9	24/02-4/3	25.4 (23.8-30.9)		42.7	1708.0(E)	164.0	18.2	104.3	500.3	
2	21	5/3-16/3	26.4 (21.0-31.1)		48.3	1932.0(E)	224.0	18.7	167.2	803.7	
3	34	17/3-29/3	25.0 (20.8-30.1)		62.4	2496.0(E)	564.0	43.4	176.7	926.4	
4	48	30/3-12/4	26.8 (21.0-31.0)		73.9	2956.0(E)	460.	32.9	270.4	1298.3	
5	62	13/4-26/4	24.9 (19.0-30.1)		82.6	3304.0(E)	348.0	24.9	320.2	1535.7	
6	71	27/4-3/5	20.9 (16.0-27.5)	37 733(F)	83.7	(2736.0*) 3155.5 5891.5(F)	4380.0**	--	220.2	1056.5	
								Total	1259.0	6120.9	1.4

* Wild spawn

** Total yield including wild spawn

Table 3. **Results of the Mean Growth and Production of *O. mossambicus* receiving 30% Protein Pelleted Fish Feed Supplemented with EM Bokashi and Anolyte over a Period of 71 days from 23 February – 3 May 1999**

Period	Days	Date	Mean and Extreme Temperatures	Stocking (S) and Final (F) Densities (fish/ha) Excluding Wild Spawns	Mean Individual Mass of Fish(g)	Estimated (E) and Final (F) Standing Crop (kg/ha)	Yield Successive Periods (kg/ha)	Production (kg/ha/d)	Calculated Pig Manure (dry matter) Quantities (kg/ha)	Formulated Feed Dosage Quantities (kg/ha)	Feed Conversion Ratio (FCR)
0	0	23/02/99	--	40 000(S)	38.5	1540.0	--	--	--	--	
1	9	04/03/99	25.4 (23.8-30.9)		46.6	1864.0(E)	324.0	36.0	115.2	492.8	
2	21	16/03/99	26.4 (21.0-31.1)		55.9	2236.0(E)	372.0	31.0	185.6	815.5	
3	34	29/03/99	25.0 (20.8-30.1)		60.3	2412.0(E)	548.0	42.2	242.2	1073.6	
4	48	12/04/99	26.8 (21.0-31.0)		71.3	2852.0(E)	440.0	31.4	281.4	1253.2	
5	62	26/04/99	24.9 (19.0-30.1)		77.6	3104.0(E)	252.00	18.0	332.7	1483.7	
6	71	03/05/99	20.9 (16.0-27.5)	37 867(F)	80.0	(2176*) 3032.0 5208.0(F)	3668.0**	--	362.6	993.1	
								Total	1519.7	6111.9	1.7

* Wild spawn
** Total yield including wild spawn

Water Quality Conditions in Fish Ponds

Results of the water quality conditions of fish ponds receiving EM-A and untreated pig manure are listed in Tables 4 and 5 respectively. The installation of plastic canopies over the fish ponds clearly elevated water temperatures which prevailed during all three months of the experimental period. At no time did the mean water temperatures decline below 20 C.

Values for dissolved oxygen were similar with mean values to be slightly higher during April and May in the EM-A treated pig manure ponds. In both sets of ponds the pH of the water was largely alkaline. Water used in both ponds had a similar conductivity and showed no undue build-up of dissolved salts over the period of investigation. Data for ammonia, nitrite and nitrate did not reflect any serious build-up of any of the three parameters showing that the nitrification process was effective in both pond systems. As a result of the concentration of soluble reactive phosphorous, phytoplankton activity can be expected in both types of fish ponds. The periodic addition of freshwater is reflected by the value obtained for turbidity in both types of ponds. Values for calcium, magnesium and total hardness corresponded in both cases with those of electrical conductivity and pH. This also applied to alkalinity.

Table 4. Water Quality Conditions of Fish Pond Water Receiving EM-A Treated Pig Manure During an Autumn Production Cycle (March-May 1999)

Analysis		Consecutive Months (March – May 1999)		
		March N=6	April N=6	May N=2
Temperature	$\bar{x}$	25.4	24.2	21.3
(°C)	range	(20.8-31.1)	(17.3-32.0)	(16.0-27.5)
Dissolved oxygen	$\bar{x}$	7.9	7.2	7.4
(mg/l^{-1})	range	(4.3-10.8)	(6.0-8.7)	(7.2-7.5)
pH	range	6.73-9.96	7.37-7.84	6.93-8.93
Conductivity	$\bar{x}$	201.8	238.8	201.5
(μ Scm^{-1})	range	(141.0-252.0)	(136.0-340.0)	(188.0-215.0)
Ammonia	$\bar{x}$	1.169	0.846	0.120
(NH_3mg/l^{-1})	range	(0.842-1.501)	(0.601-1.041)	(0.106-0.133)
Nitrite	$\bar{x}$	0.011	0.029	0.024
(NO_2mg/l^{-1})	range	(0.003-0.026)	(tr-0.087)	(0.021-0.027)
Nitrate	$\bar{x}$	3.15	2.83	3.05
(NO_3mg/l^{-1})	range	(tr-5.72)	(1.22-9.20)	(2.33-3.76)
Calcium hardness	$\bar{x}$	44.8	38.0	30.0
($CaCO_3mg/l^{-1}$)	range	(21.0-59.0)	(29.0-48.0)	(28.0-32.0)
Magnesium hardness	$\bar{x}$	20.0	17.2	30.0
($MgCO_3mg/l^{-1}$)	range	(17.0-23.0)	(7.0-30.0)	(13.0-47.0)
Total hardness	$\bar{x}$	64.2	55.2	55.0
($CaCO_3mg/l^{-1}$)	range	(39.0-82.0)	(32.0-71.0)	(45.0-55.0)
Alkalinity	$\bar{x}$	70.5	48.8	51.0
($CaCO_3mg/l^{-1}$)	range	(42.0-86.0)	(28.0-60.0)	(47.0-55.0)
Orthophosphate	$\bar{x}$	5.92	3.04	2.69
(PO_4mg/l^{-1})	range	(2.96-12.43)	(1.61-5.78)	(1.99-3.40)
Turbidity NTU		98.7	24.3	54.5

* Based on all ponds

Table 5. Water Quality Conditions of Fish Pond Water Receiving Untreated Pig Maure During an Autumn Production Cycle (March-May 1999)

Analysis		Consecutive Months (March – May 1999)		
		March N=6	**April N=6**	**May N=2**
Temperature	$\overline{x}$	25.4	24.3	21.3
(°C)*	range	(20.8-31.1)	(17.3-32.0)	(16.0-27.5)
Dissolved oxygen	$\overline{x}$	8.9	6.3	6.6
(mg/l^{-1})	range	(6.7-12.7)	(4.8-8.5)	(6.3-6.7)
pH	range	7.07-9.86	7.09-7.59	7.00-7.11
Conductivity	$\overline{x}$	232.7	234.5	220.3
(μ Scm^{-1})	range	(95.0-494.0)	(140.0-406.0)	(168.0-280.0)
Ammonia	$\overline{x}$	0.775	1.145	0.134
(NH_3mg/l^{-1})	range	(0.683-0.842)	(0.770-2.651)	(0.110-0.152)
Nitrite	$\overline{x}$	0.002	0.006	0.026
(NO_2mg/l^{-1})	range	(tr-0.006)	(0.001-0.012)	(0.019-0.036)
Nitrate	$\overline{x}$	2.64	3.38	5.5
(NO_3mg/l^{-1})	range	(tr-5.28)	(1.1.5.4)	(5.1-6.3)
Calcium hardness	$\overline{x}$	41.5	30.0	29.3
($CaCO_3mg/l^{-1}$)	range	(11.0-76.0)	(24.0-45.0)	(22.0-37.0)
Magnesium hardness	$\overline{x}$	20.2	25.8	25.3
($MgCO_3mg/l^{-1}$)	range	(9.0-40.0)	(0.0-43.0)	(15.0-43.0)
Total hardness	$\overline{x}$	61.7	55.8	48.0
($CaCO_3mg/l^{-1}$)	range	(27.0-116.0)	(28.0-72.0)	(42.0-49.0)
Alkalinity	$\overline{x}$	65.7	50.3	49.3
($CaCO_3mg/l^{-1}$)	range	(16.0-121.0)	(37.0-75.0)	(34.0-55.0)
Orthophosphate	$\overline{x}$	4.20	3.33	2.75
(PO_4mg/l^{-1})	range	(2.71-6.77)	(2.91-6.89)	(2.31-3.57)
Turbidity NTU		88.0	24.2	45.0
		(64.0-133.0)	(11.0-39.0)	(35.0-56.0)

* Based on all ponds

Bacterial Counts in Pig Manure

Total Coliforms

A comparison of the total Coliforms counts in the pig manure for the various combinations of EM and Anolyte treatments, as well as for the control feeding programme for four successive fortnightly periods, are listed in Table 6. In all cases, faeces from pigs receiving EM, in their feed and sprayed with Anolyte, were markedly lower in their Coliform counts. The addition of Anolyte alone does not appear to materially reduce the total Coliform counts where it was applied. In the case of Anolyte application alone, suggestions are, however, that this kind of treatment may well play a role in the reduction of faecal Coliform in the faeces of pigs (Table 6). The most variable results, however, occurred in the final data of the control experiments.

Faecal Coliforms

Results for the fourth fortnight period (21/04) (Table 6) are not available. Of all the results, the EM-Anolyte combination proved to be most effective in the reduction of faecal Coliform counts in pig manure, followed by that of the EM only treatment. Interestingly enough, the faecal Coliform counts in control pigs were on average lower that those of the Anolyte treated pigs which can largely be ascribed by the exceptionally high counts experienced during the first sampling period.

Faecal *E. coli*

A comparison of the results of faecal *E. coli* counts (Table 6) in pig manure of the various treatments suggest that despite variation in *E. coli* numbers, both the EM-Anolyte (separate and in combination) were effective in the reduction of this organism, compared to the mean values recorded for control pigs.

Faecal *Streptococci*

Faecal Streptococcai counts for the various tretments (Table 6) suggest the EM-combination with Anolyte to be the most effective in the reduction of the numbers of this organism, with EM to be the second most effective, followed by Anolyte. Control values for faecal Streptococci were clearly the highest.

Somatic Coliphages

It is important to note that the diets of the pigs containing EM-A were extremely effective in the marked reduction of somatic Coliphages followed by that of EM. Anolyte alone also showed some effect on the reduction of Coliphages with the control values clearly being the highest.

Bacterial Counts in Fish Pond Water Receiving EM-A Containing and Untreated (Control) Pig Manure

In each case, three ponds were sampled for bacterial analysis for various faecal organisms. Total bacterial counts in the fish ponds receiving manure from EM-A treated pigs were significantly lower than those from the control (untreated) pigs (Table 7). This also applied to total Coliforms and faecal Coliforms, and to a lesser extent in the case of faecal *E.coli*. There was a market effect on the reduction of faecal *Streptococci* in the water receiving manure from the EM-A treated pigs. As was the case for the somatic Coliphages in the manure of the EM-A treated pigs, the effect of this treatment clearly resulted in a significant reduction in the somatic Coliform organisms of the fish pond water.

Comparison of Faecal S*treptococci* and Faecal *E. coli* in the Kidneys, Gills, Spleen and Liver of Fish kept in Ponds Receiving Manures from EM-A Treated and Untreated Pigs

Results of faecal as well as Coliphage organisms were based on three replicates each for both pig manure and water samples, as well as from nine replicates of randomly selected fish analysed for faecal *Streptococci* and faecal *E coil* from the kidneys, gills, spleens and livers from ponds receiving manure from EM-A treated and untreated pigs (Table 7 and 8). These data clearly suggest that the manure from the EM-A treated pigs may have a marked bearing on the higher numbers of faecal *Streptococci* and faecal *E. coli* recorded from the different organs analysed.

Table 6. **Mean Values of Total Coliform, Faecal Coliform, Faecal *E.coli*-, Faecal Streptococci and Somatic Coliphage counts in 10 g of Pig Manure from Pigs Treated with EM and Anolyte, Anolyte, EM and No Treatment, Based on Composite Samples for each Analysis of Three Randomly Selected Individual Faecal Droppings from each of the Four Treatments. (Five Replicates were Analysed at Fortnightly Intervals over a Period of 71 days)**

Parameter	**Number of Samples N**	**Actual Count = Number x 10^x**		**Treatment of Pigs**			
				Mean Counts/ 10 g Manure			
				EM-Anolyte	**Anolyte**	**EM**	**Control (untreated)**
Total	5	x 10^4	$\bar{x}$	661.2	1 875.4	637.4	92 280.0
Coliforms			range	(16-1 500)	(380-6 000)	(27-1 200)	(1 100-450 000)
Faecal	5	x 10^3	$\bar{x}$	364.7	4 675.0	3 045.7	3 600.0
Coliforms			range	(200-1 000)	(1 000-10000)	(13-6 000)	(1 000-60 000)
Faecal	5	x 10^2	$\bar{x}$	26 342.0	25 480.0	13 303.6	56 600
E.coli			range	(310-97 000)	(1 000-49000)	(18-30 000)	(2 000-100 000)
Faecal	5	x 10^2	$\bar{x}$	271.4	1 946.6	1 290.2	1 2329.6
Streptococci			range	(9.800)	(3-6 100)	(11-4 400)	(18-38 000)
Somatic	5	x 1	$\bar{x}$	28.0	3 818	542.6	13 673.4
Coliphages			range	(3-110)	(0-19 000)	(1-2 600)	(10-63 000)

Table 7. **Mean Values of Total Bacterial, Total Coliform, Faecal Coliform, Faecal *E.coli*-, Faecal *Streptococci* and Somatic Coliphage Counts in 500 m*l* of Water from Fish Ponds Receiving Manure from EM-Anolyte Treated and Untreated Pigs, based on Composite for each Analysis of Three Samples from each Treatment. (Five Replicates were Analysed at Fortnightly Intervals over a Period of 71 days)**

Parameter	Number of Samples	Actual Count = Number x 10^x		Treatment of Fish Ponds: Mean Counts/ 500 m*l* water — EM-Anolyte Treated	Untreated
Total bacterial count	5	x 10^3	$\bar{x}$	7 308.3	95 285.3
			range	(436-20 698)	(30 620-221 434)
Total Coliforms	5	x 10^3	$\bar{x}$	105.7	2 283.7
			range	(46.0-199.0)	(0.021-14 000)
Faecal Coliforms	5	x 10^3	$\bar{x}$	161.9	957.2
			range	(0.3-2 160)	(0.005-3 600)
Faecal *E.coli*	5	x 1	$\bar{x}$	21.6	50.5
			range	(1-80)	(3-280)
Faecal *Streptococci*	5	x 10^3	$\bar{x}$	4.0	235.9
			range	(0.002-14)	(0.004-2 400)
Somatic Coliphages	5	x 1	$\bar{x}$	10.7	2 745.0
			range	(0-100)	(0-30 000)

Table 8. **Mean Values of Faecal Streptococci and Faecal *E. coli* Counts in the Kidneys, Gills, Spleens and Livers of *O. mossambicus* from Fish Ponds Receiving Manures from EM and Anolyte Treated and Untreated Pigs. Actual Counts are Based on Composite Samples for each Analysis of Three Samples per Pond from Each Treatment.**

Parameters	Actual Count = Number x 10^x	Fish organs analysed: Kidneys — EM-A Treated	Kidneys — Untreated	Gills — EM-A Treated	Gills — Untreated	Spleen — EM-A Treated	Spleen — Untreated	Liver — EM-A Treated	Liver — Untreated
Faecal Streptococci	x 10^3	302.0 (0-900)	1.4 (0.02-4.0)	713.5 (0.4-2 000)	0.015 (0-0.13)	436.3 (0.780)	0.011 (0-0.011)	133.7 (0.5-400)	17 333 3 (0-44 000)
Faecal *E.coli*	x 10^3	90 020.0 (0.15-270 000)	10 233.3 (1 900-21 000)	72 433.3 (300-216 000)	1 100.1 (0.16-3 100)	6.7 (0-20)	1 300.0 (0.005-2 600)	85.0 (0-250)	390 001.7 (5-630 000)

Discussion

Data obtained on the use of Anolyte as a disinfectant in pig production showed some promise but further investigations into its use as control agent for the reduction or eradication of faecal bacterial loads in the pig houses as well as in ponds used in aquaculture-agriculture systems need to be undertaken.

The incorporation of antibiotics and growth hormones, in particular, in pig feeds, obscured the positive results of EM on pig growth and may well conceal the stress reduction effects of these substances on the animals. The single mortality amongst the control group of pigs, may have been avoided if EM was incorporated in all pig rations which, cost-wise, may be a substance of choice in reduction in stress related diseases of these and other animals. There is no doubt that the treatment of large quantities of manure generated on pig farms with EM may assist is solving some of the problems of environmental pollution and at the same time may lead to the production of a good quality compost which can be used in integrated aquaculture-agriculture systems.

Data on fish production in ponds clearly demonstrate the potential advantages of pig manure as supplementary nutrient in yields in combination with pelleted fish feeds. This approach may therefore also be beneficial in the application of other agricultural and industrial wastes incorporated in integrated aquaculture-agriculture systems.

Despite the fact that significant quantities of pig manure were used to fertilize ponds, water quality conditions remained good for the entire duration of the fish production period. The effectiveness of both the EM and Anolyte to reduce the numbers of somatic Coliform organisms in fish ponds, poses a serious problem as this phenomenon is contrary to what may be required for the reduction of faecal organisms in fish ponds. The present investigation suggests that, contrary to te findings of Boomker et al. (1979), the Mozambique tilapia *O. mossambicus*, may also be susceptible to infection by faecal *Streptococci* and *E. coli* in faecal bacteria contaminated ponds. This may have a direct bearing on the health status of pond fish produced with the application of pig an other animal manures as nutrient in fish ponds. Further investigations on diseases caused by faecal bacteria in fish must therefore be pursued.

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