Aquaculture 590 (2024) 741040

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Poultry manure fertilization of Egyptian aquaculture ponds brings more cons than pros

Oskar Nyberg[a,g,*], Andreas Novotny[b], Ashraf S. Sbaay[c], Ahmed M. Nasr-Allah[c], Diaa A.R. Al-Kenawy[d], Cristiano M. Rossignoli[e,f], Patrik J.G. Henriksson[g,h,i]

[a] Department of Ecology, Environment and Plant Sciences, Stockholm University, Svante Arrhenius väg 20A, 104 05 Stockholm, Sweden
[b] Institute for the Oceans and Fisheries, Faculty of Science, Vancouver Campus, The University of British Columbia, AERL, 2202 Main Mall, Vancouver, BC, Canada V6T 1Z4
[c] WorldFish, Abbassa, Egypt
[d] Central Lab for Aquaculture Research (CLAR), Agricultural Research Center (ARC), Abbassa, Sharkia 44662, Egypt
[e] Institute for Future Initiatives (IFI), The University of Tokyo, Japan
[f] WorldFish, Jalan Batu Maung, Batu Maung, Bayan Lepas 11960, Pulau Pinang, Malaysia
[g] Stockholm Resilience Centre, Stockholm University, Albanovägen 28, 106 91 Stockholm, Sweden
[h] Beijer Institute of Ecological Economics, The Royal Swedish Academy of Science, Lilla Frescativägen 4 A, Stockholm, Sweden
[i] Institute of Environmental Sciences (CML), Leiden University, Einsteinweg 2, Leiden 2333 CC, the Netherlands

ARTICLE INFO

Keywords:
Aquaculture
Chicken manure
Eutrophication
ARGs
Profitability

ABSTRACT

Aquaculture is a crucial sector for Egyptian food production, providing a cheap source of animal protein while securing income and employment for a substantial part Egypt's population. Nile tilapia (*Oreochromis niloticus*) is the most commonly produced fish, usually farmed in earthen ponds around the Northern Delta Lakes. A common practice among farms is to fertilize ponds with chicken manure (CM) in order to increase nutrient levels and promote phytoplankton, consumed by the fish. However, with reports of use of antibiotics in Egypt's poultry sector, and that CM contains residues of antibiotics, antibiotic resistant pathogens and antibiotic resistance genes (ARGs) are production benefits large enough to compensate a potential health hazard?

Using production data from 501 aquaculture farms and fish pond sediment from 28 ponds we evaluated potential benefits in yields and profitability for farms using CM for fertilization, and used qPCRs to screen sediments for three antibiotic resistance genes coding for resistance to the most commonly used antibiotics in the poultry sector. The analysis showed no significant benefits to fish yields or profitability in farms where CM was applied, but a risk of significantly increased nutrient loads. Meanwhile, we detected increased abundances of *tetA* and *tetW* resistance genes in fish pond sediment where CM was applied. With the risk of disseminating ARGs and causing eutrophication of local waterways, we recommend that Egyptian tilapia pond farmers refrain from using CM and adopt best management practices for increasing farm profitability in order to to reduce environmental and health hazards.

1. Introduction

Aquaculture shows promise as a more sustainable and nutritious animal-based food (Golden et al., 2021; Gephart et al., 2021). In order to reduce stress caused by food production on land, it is important to utilize resources and manage water resources when producing aquatic plants and animals are important (Zhang et al., 2022). Most of the 87.5 million tonnes produced in global aquaculture in 2021 came from Asia (88.7%), while Africa only accounted for 2.7%. Within Africa, Egypt accounted for about 70% of all aquaculture output, with 1.57 million tonnes produced in 2021 (FAO, 2022). The majority of Egyptian aquaculture takes place in the northern Nile River delta, around the northern delta lakes. Aquaculture production mainly cater domestic markets, with negligible exports. Kafr El-Sheikh is the most important governorate with largest fish production in the country (Rossignoli et al., 2023). Aquaculture operations also take place to the south of the Nile River delta in Fayoum

---

Abbreviations: CM, Chicken manure; AB, Antibiotics; ARGs, Antibiotic resistance genes; qPCR, Quantitative polymerase chain reaction.

* Corresponding author at: Stockholm Resilience Centre, Stockholm University, Albanovägen 28, 106 91 Stockholm, Sweden.
E-mail address: oskar.nyberg@su.se (O. Nyberg).

https://doi.org/10.1016/j.aquaculture.2024.741040
Received 4 December 2023; Received in revised form 7 April 2024; Accepted 3 May 2024

governorate, adjacent to desert oases using naturally ocurring ground water. Fish provide an inexpensive source of protein in Egypt, proving an affordable source of animal protein (FAO, 2022; FAO, 2023). Nile tilapia (*Oreochromis niloticus*) makes up 67% of Egyptian aquaculture production (Shaaban et al., 2021), mainly produced in earthen ponds and fed commercial extruded pellets. Tilapia are often co-cultivated with African catfish (*Clarias gariepinus*), thinlip mullet (*Chelon ramada*, also known as *Mugil capito)* and gray mullet (*Mugil cephalus)*. The majority of fish farming operations are small to medium scale private operations, implying aquaculture's important role for income and employment (Macfadyen et al., 2011). Yet sustainable strategies for maintaining high yields are lacking (Soliman and Yacout, 2016). Lately, disease has started to occur more frequently without adequate disease diagnostics present (Eissa et al., 2016; Soliman and Yacout, 2016; Nicholson et al., 2017).

Egyptian fish farmers depend on the commercial feeds, but pond fertilization is often applied to enhance natural food in ponds and reduce feed costs (Macfadyen et al., 2011; Dickson et al., 2016; El-Sayed, 2020; Rossignoli et al., 2023). Chicken manure (CM) is commonly used as fertilizer, as it is abundant, cheap, and readily available product that is rich in nitrogen. Egypt's poultry sector produces two million tonnes of CM yearly, of which between 3.5% and 10% are used in aquaculture (Hasan et al., 2007; Shatokhin et al., 2017). Previous studies document increased fish growth as a result of fertilization (Kang'ombe et al., 2006; El-Sayed, 2020, with optimal performance if fertilizer is added in the starting phase of the aquaculture production cycle (Diana et al., 1994; Green et al., 2002). Excess nutrients in CM not utilized by the fish are in part sedimented, but some of the nitrogen (ca 65%) and phosphorous (ca 40–45%) are emitted with pond effluents (Green and Boyd, 1995; Nhan et al., 2008). This results in eutrophication of local water resources.

Apart from potentially deteriorating water quality, there are other reasons to be cautious about the practice of fertilizing pond water with CM. Dahshan et al. (2015) describes severe misuse of antibiotics (ABs) in Egypt's poultry sector with unregulated sales, little governance, and lack of education among farmers. This, in turn, leads to high concentrations of AB residues and multi-resistant human pathogens remaining in chicken droppings. Subsequently, presence of AB resistant enteric pathogens, such as *Escherichia coli* and *Salmonella* sp., will increase in ponds fertilized with CM (Elsaidy et al., 2015). Residues of ABs have been found in fish tissues from aquaculture operations that have no history of using ABs (Mahmoud and Abdel-Mohsein, 2019), and in fish pond sediment post-harvest (Koeypudsa et al., 2005). These residues can accumulate due to poultry manure containing ABs used as a fertilizer in aquaculture operations (Koeypudsa et al., 2005). Ciprofloxacin and tetracycline are two ABs extensively used within Egyptian poultry farming that have been detected in high concentrations in poultry manure previously in Egypt (Dahshan et al., 2015). These ABs are deemed critically important as human medications (World Health Organization, 2019).

The use of untreated organic fertilizers has also been identified as a cause for mass mortalities in fish ponds in northern Egypt due to elevated nutrient levels, leading to ammonia toxicity, and increased prevalence of pathogens (Abu-Elala et al., 2016).

With previous studies identifying Egyptian CM application as a cross-sectoral vector for transmission of AB residues, pathogens, and antibiotic resistance genes (ARGs), there is a need to evaluate the sustainability of CM fertilization. CM fertilization might imply reduced biosecurity, by inefficient AB therapy to diseased fish, due to misuse of ABs in the poultry industry, thus creating a cross-sectoral management problem relating to ARG development. Downstream human health issues might potentially arise from resistant pathogens, as a consequence of ARG proliferation within food production systems (Marshall and Levy, 2011; Liu et al., 2016).

To critically assess the potential benefits and downsides of pond fertilization strategies and whether CM use in Egyptian pond aquaculture increases fish production and profitability, this study evaluates production data from 501 Egyptian tilapia pond farms collected across six of the most productive governorates in Egypt over three years (2017–2019). Fish production, eutrophication potential, and profitability are considered in relation to chicken manure addition. Secondly, we performed quantitative polymerase chain reaction (qPCR) on DNA extracted from fish pond sediments that were collected from 28 ponds and adjacent waterways in 2018, in order to assess the relative abundance of ARGs in farms where CM fertilization was present or absent. The analysis targeted three ARGs; *tetA*, *tetW,* and *qnrS*. We discuss the CM fertilization strategy trade-offs, cross-sectoral AB use problem, and review the feasibility of fertilization strategies in light of findings from analyzing interview data and results from qPCR analysis of pond sediment samples.

2. Materials and methods

2.1. Data collection

Semi-structured interviews on farming practices were conducted across northern Egypt together with aquaculture farmers or managers on three occasions; in 2017 as part of the WorldFish Aqualinc project (56 interviews); in 2018 by authors of the current study (46 interviews); and in 2019 as part of the a WorldFish 'FISH' baseline study for assessing productivity and profitability of the Egyptian tilapia aquaculture sector (399 interviews; Shikuku et al., 2020; Rossignoli et al., 2023). Interviews took place across the governorates (i.e., provinces) Kafr El-Sheikh, Beheira, Sharquia, Ismailia, Port Said, and Fayoum. Within the 2019 dataset, geographic detail were given down to markaz (i.e., district) level and was used to classify these interviews into the following markaz; Burullus, El Hamoul, El Ryad, Side Salm, and Metobas (Fig. 1). Data from the three surveys have varying degrees of detail, with only *O. niloticus* production recorded in the 2017 dataset. Interviews from 2018 and 2019 represent both monoculture systems (41% and 21%, respectively) and polyculture systems (59% and 78%, respectively) with two or three species co-cultivated with *M. cephalus* and *M. capito*. Four farms (1%) in the 2019 survey practice tilapia polyculture, together with mullet and *C. gariepinus*, as well as either Common Carp (*Cyprinus carpio*), or Grass Carp (*Ctenopharyngodon idella*).

During the data collection in 2018, interviews were carried out by trained field officers using questionnaires (see supplementary information (SI) 1). Interviews were undertaken in Arabic by WorldFish field officers and notes in Arabic were translated after the interviews. A WorldFish trainer representative from each governorate arranged the interviews and remained present during interviews. Since respondents were not always able to refer to written logs, curation of the dataset was deemed necessary, to safeguard against seemingly arbitrary responses or erroneous data entries. Some parameters (e.g., stocking densities, feed inputs or feed prices) were recorded as five to 25 times the average, hence data were omitted, based on the following conditions: 1) fish per m^3 exceeded two kg, and feed per m^3 exceeded five kg, 2) fish harvest exceeded the feed and manure input combined, and 3) price for feed exceeded the average price (i.e., 9.9 EGP Kg^{-1}) by 100%. Data were discarded if feed input or harvest data were missing (Table 1).

For details on methodology for the 2019 dataset, please see Shikuku et al. (2021). Because of the disproportionally large sample from Kafr El-Sheikh in the 2019 survey, this governorate was classified into districts (Markaz) to allow for better comparisons on a local scale. Due to the few interviews from Metobas markaz ($n = 2$), these were merged with Side Salm. Similarly, interviews from Port Said ($n = 4$) were merged with the adjacent governorate Sharkia (Table 1).

2.2. Assumptions in place for this survey

CM are usually delivered by trucks carrying about 7 m^3, and we assumed each cubic meter (m^3) of dried CM to weigh 600 kg (Griffiths and NSW Agriculture, 2003). Calculations of farming operations were

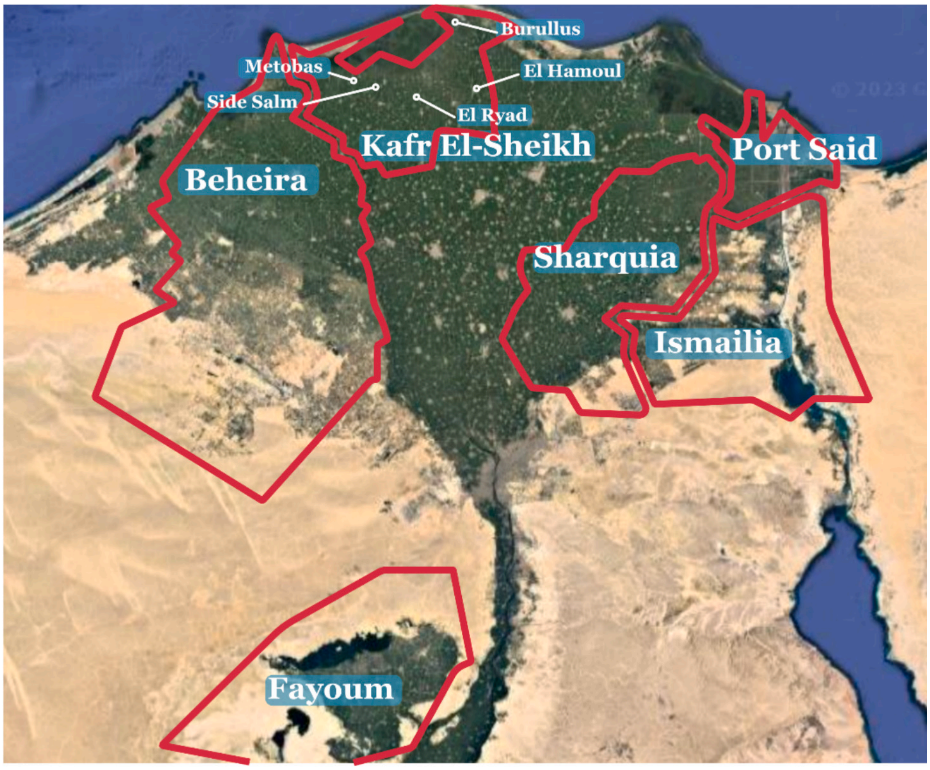

Fig. 1. Map showing the governorates (red outlines) and markaz (white circles) represented in the surveys for the current study. Source: Google Maps. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

extrapolated over eight months grow-out time, unless stated otherwise by farmers. Weekly use inventories were subsequently extrapolated over eight months growth cycle. If no pond depth was recorded, an average depth of 1.5 m was assumed. Some farmers stated they add manure to ponds continuously during grow-out, which was assumed to be continuous over the first two months during grow-out cycle.

Table 1
Interviews undertaken per governorate and markaz per mission. Data collection year 2017 = Interview data extracted from WorldFish Aqualinc LCA data collection in 2017. 2018 = interview data from the current authors' field survey in 2018. 2019 = FISH baseline study for assessing productivity and profitability of the Egyptian tilapia aquaculture sector. Markaz = District. Numbers within parentheses are the number of interviews remaining after data curation and data omissions.

Region	Data collection year			Total
	2017	2018	2019	
Beheira	9 (6)	–	–	9 (6)
Ismailia	9 (9)	–	–	9 (9)
Sharkia	19 (9)	6 (6)	–	25 (15)
Port Said	–	4 (2)	–	4 (2)*
Fayoum	–	11 (6)	–	11 (6)
Kafr El-Sheikh	19 (18)	25 (13)	*(Per Markaz)*	44 (31)
Burullus	–	–	37 (24)	37 (24)
El Hamoul	–	–	99 (74)	99 (74)
El Ryad	–	–	170 (128)	170 (128)
Metobas	–	–	2**	2**
Side Salm	–	–	91 (69)	91 (69)
	56 (42)	46 (27)	399 (297)	501 (366)

* Interviews from Port Said were merged with data from Sharkia due to few available data.

** Interviews from Metobas were merged with data from Side Salm due to few available data.

2.3. *Eutrophication potential*

A mass balance approach was used to calculate the accumulation of excess nutrients, considering total inputs (feed and fertilizers) and all outputs (fish). The N and P content in inputs and outputs were calculated according to parameters given in Table 2. N content in feeds is based on the stated feed protein content and converted using an average default factor of 5.6 (17.8%) reported by Mariotti et al. (2008). In the few cases where triple super phosphate ($n = 4$) or urea ($n = 1$) were recorded, the respective N and P contents were added nutrient inputs.

2.4. *Profitability*

Profitability was simplified as the sum of sales (EGP for all fish produced) minus the costs for feeds and fertilizers. Land rent, labor costs, and other operational costs were not included as they were deemed irrelevant for estimating the profitability of using chicken manure.

Table 2
Nitrogen (N) and Phosphorous (P) content for respective type of substance included in calculations of eutrophication potential.

Type of substance	N content	P content	Reference
Feed	% protein/ 5.6	0.8%	Mariotti et al. (2008), Bueno et al. (2019)
Chicken manure (dry, average)	3.40%	1.75%	El-Sayed (2013)
Triple super phosphate (TSP)	0%	20%	Boyd (2018)
Urea	45%	0%	Boyd (2018)
Fish	2.12%	0.75%	Boyd et al. (2007)

2.5. Pond sediment sampling

Sampling of tilapia pond sediments was conducted from May to June of 2018, in parallel to interviews situated at farm locations. Sediment samples from 28 ponds were collected from various regions across northern Egypt with the permission of each farmer (Table 3). A 2.5 m long polypropylene tube with a diameter of 5 cm and a rubber stopper as lid was used to collect a sediment core from the pond edge. With evenly spaced holes drilled in the side covered by tape, water could be drained without disturbing the top layer of sediment. The sediment core was transferred to a plastic container and a new 5 ml sterile syringe, with a cut-off tip, was used to extract a 5 ml "core" subsample from the center of the core (see Fig. S1 in SI2), which was transferred to sterile 10 ml polypropylene centrifuge tube and kept on ice until frozen in −18 °C. All equipment was sterilized with 95% ethanol prior to sampling.

2.6. DNA extraction and qPCR screening for ARGs

DNA was extracted from pond sediment, each 250 mg, using the DNeasy PowerSoil kit (Qiagen, Netherlands) and purified using Agencourt® AMPure® XP PCR Purification kit (Beckman Coulter, USA) following manufacturers' protocols. Extracts were quality tested for concentration and purity using NanoDrop 2000 photospectrometry (Thermo Scientific, Wilmington USA). Extracted DNA was subsequently frozen at −18 °C until qPCR work commenced (after roughly one year). Prior to qPCR assay, DNA concentrations in samples were analyzed using a Qubit® fluorometry (Qubit® dsDNA HS Assay Kit, Thermo Fisher) and subsequently diluted to a normalized concentration of 1250 ng/ml respectively. ARGs of interest were the tetA and tetW genes conferring resistance to tetracycline, and the qnrS gene conferring resistance to fluoroquinolone antibiotics. The 16S SSU rRNA gene (16S) was used as housekeeping gene for relative quantification of ARGs.

All qPCR reactions were performed using 20 μl total volume containing 10 μl LightCycler® 480 SYBR Green I Master Mix (Roche Life Science), 1 μl forward primer and 1 lL reverse primer (Table 4) at 10 pMol respectively, 2 μl DNA template (32–118 ng/μl) and 6 μl sterile DNA/RNA free water as diluent. qPCR reactions were performed as three technical replicates on a LightCycler® 480 system (Roche Diagnostics) with the following thermal cycling conditions: 95 °C for 5 min followed by 45 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 15 s and fluorescent acquisition. After the cycles, a melt curve analysis ran at 95 °C for 5 s, 60 °C for 60 s and increased to 97 °C at a rate of 2.2 °C per second with constant fluorescent acquisition. Cycle threshold values (Ct) were determined as the maximum of the second derivative of the amplification curve in the instrument software.

To be able to account for varying primer efficiencies, qPCR standard curves were employed for all genes. ARG sequences were downloaded from The Comprehensive Antibiotic Resistance Database (https://card.mcmaster.ca/, accessed 2021-05-05, SI2; Table S1) and synthesized by Eurofins genomics (eurofinsgenomics.eu/). Primer details are reported in Table 4. The target sequence for 16S (467 base pairs) was created with PCR from environmental samples using primers f331 and r797 (Hardwick et al., 2008). The product was purified using Agencourt® AMPure® XP PCR Purification kit (Beckman Coulter) according to manufacturer's instructions. ARG and 16S standards were adjusted to 1×10^{9} copies μl^{-1} and ten-fold serial dilutions (1×10^{8} to 1×10^{1} copies μl^{-1}) were used to create standard curves. Concentrations were verified using Qubit® fluorometry (Qubit® dsDNA HS Assay Kit, Thermo Fisher). The standard curves were run in triplicates and primer efficiencies (*P.Eff*) were calculated in the instrument software using Eq. 1.

$$\mathrm{P.Eff} = 10^{\frac{-1}{\mathrm{slope}\left(\mathrm{Ct}\sim log_{10}(\mathrm{CopyNr})\right)}} \tag{1}$$

Target samples were run in triplicates with all four primer sets in the same reaction. Two standard concentrations (1×10^{8} and 1×10^{4} for 16S, and 1×10^{5} and 1×10^{2} for target ARGs) were included in each qPCR run. Ct value <40 was regarded as positive, given that the Ct value for all three technical replicates had <2 Ct variation, and amplicons with a single melt peak at similar temperature as standards were considered valid during melt curve analysis. Relative abundance of ARGs was calculated as a ratio between target amplicon product and 16S product (Eq. 2) where *Eff* is the primer efficiency and *Ct* is the cycle threshold. Quantifying ARGs as relative measurement to the 16S rRNA gene allows for comparing sites with variable loads of organic matter and bacterial abundances to each other, since the 16S rRNA relates to the bacterial abundance in samples.

$$\text{Relative abundance} = \frac{\mathrm{Eff_{ARG}}^{-\mathrm{Ct_{ARG}}}}{\mathrm{Eff_{16S}}^{-\mathrm{Ct_{16S}}}} \tag{2}$$

2.7. Statistical analysis

When investigating the influence of CM fertilization on fish production, input and output parameters were normalized to stocking density (kg fish m^{-3}), to allow for production outcomes in farms of varying sizes to be compared. We subsetted production data per region and per CM application, and tested for normality using Shapiro-Wilks test for each subset and across the entire dataset. Rank sums for each production strategy per subset and across the country total were tested for differences using a Mann-Whitney test, using a two-sided wilcox.test in R. The influence of the amount of feed and CM applied to farms was tested using Spearman's correlation using the cor.test operation with method = "spearman" defined. Data curation, data management, statistical analyses, and visualizations of data were performed using R software, version 4.1.2 (R Core Team, 2021).

3. Results

After filtering the dataset to remove erroneous data entries, 135 (27%) of the 501 interviews were omitted. Data from the remaining 366 farms were used in the subsequent analysis (Table 1). Manure fertilization occurred on 104 (28%) of farms, at a mean input rate of 3.84 t ha^{-1} water surface and grow-out cycle, albeit with variability from 0.13 to 14.9 t ha^{-1} among farms (Table 5). Only five farms applied inorganic manures to ponds, with four adding TSP and one urea. The N, P, and costs for inorganic fertilizers were included in the calculations, but not treated to a separate analysis due to the few samples. The proportion of farms that apply CM are similar whether production is monoculture or polyculture, with slightly fewer in monoculture (24%) than in polyculture systems (28–33%).

3.1. Production outcomes related to CM fertilization

The number of interviews varied by region, from six interviews in Beheira and Fayoum governorates respectively, to 126 interviews in El Ryad, a district within the Kafr El-Sheikh governorate. To analyze how fish production was influenced by CM fertilization, the rank sums of the two categories were compared across the entire dataset. We found no significant differences ($p > 0.05$) in fish yields when comparing CM strategies across the entire dataset (W-value = 12,800, p-value = 0.367; Table 6). Meanwhile, fish yields varied greatly among and within

Table 3
Number of ponds per governorate sampled during May and June 2018. Use of chicken manure (CM) fertilizer recorded at sites where pond sediment was acquired.

Governorate	No CM use	CM used	Total
Fayoum	4	1	5
Kafr El-Sheikh	11	6	17
Sharqia	5	1	6
Total	20	8	28

Table 4
Details of the primer sequences and properties, and reference studies. P.Eff = Primer efficiency.

Target gene	Primer	Primer sequence	Documented annealing temp (°C)	Applied annealing temp (°C)	Size (bp)	Sensitivity (copies/μl)	Source	*Eff*
tetA	tetA-F2 tetA-R2	TCAATTTCCTGACGGGCTG GAAGCGAGCGGGTTGAGAG	55	60	96	10	Börjesson et al., 2009	1928
tetW	F R	GAGAGCCTGCTATATGCCAGC GGGCGTATCCACAATGTTAAC	60	60	168	Not reported	Wang et al., 2016	1956
qnrS	qnrSrtF11 qnrSrtR11	GACGTGCTAACTTGCGTGAT TGGCATTGTTGGAAACTTG	62	60	118	18	Marti and Balcázar, 2013	2,07
16S rRNA	F331 R797	TCCTACGGGAGGCAGCAGT GGACTACCAGGGTATCTAATCCTGTT	62	60	467		Hardwick et al., 2008	1938

Table 5
Manure application strategy and mean inputs per farming system. CM = Chicken manure. The minimum and maximum CM input reported within parentheses.

Species farmed in system	CM application			
	Number of farms		Mean CM application (t ha^{-1} per cycle)	Standard deviation CM application
	No	Yes		
Tilapia monoculture	93 (76%)	30 (24%)	4.76 (0.40–14.3)	4.00
Tilapia +1	95 (67%)	46 (33%)	2.93 (0.13–14.9)	3.68
Tilapia +2	73 (72%)	28 (28%)	4.34 (0.60–11.1)	2.71
Tilapia +3	1 (100%)	0	–	
Total	262 (72%)	104 (28%)	Total mean: 3.84	3.61

Table 6
Fish yields depending on CM fertilization strategy per region. For analysis, W-value and *p*-value are results from a Mann Whitney *U* Test of difference sum of ranks between groups. "n" represents the number of interviews from respective region and fertilization strategy. Standard deviation is presented in parentheses.

Governorate *Markaz*	CM application					
	No		Yes			
	n	Fish yields (kg m^3/)	n	Fish yields (kg m^3/)	W-value	*p*-value
Beheira	4	0.555 (0.029)	2	0.601 (0.048)	531.5	0.769
Fayoum	4	0.311 (0.137)	2	0.370 (0.150)	20	0.328
Ismailia	5	0.561 (0.146)	4	0.596 (0.185)	10	0.098
Kafr El-Sheikh	15	0.536 (0.285)	16	0.475 (0.181)	130	0.707
Burullus	19	0.613 (0.126)	5	0.774 (0.235)	522	0.527
El Hamoul	53	0.663 (0.182)	21	0.673 (0.207)	1032	0.188
El Ryad	104	0.597 (0.208)	24	0.638 (0.166)	26	0.135
Side Salm	46	0.595 (0.212)	25	0.610 (0.183)	9	0.905
Sharkia	12	0.458 (0.264)	5	0.587 (0.209)	10	0.125
Total	**262**	**0.596 (0.209)**	**104**	**0.612 (0.196)**	**12,800**	**0.367**

regions, despite having normalized production to kg fish per m^3 water. The mean fish yields appear slightly higher when CM is applied across all regions, with the exception of the Kafr El-Sheikh (Fig. 2), but no significant differences ($p > 0.05$) could be established between CM strategies in any governorate or markaz (Table 6).

Among semi-intensive Egyptian pond farms, there was a correlation between fish production and feed input (Fig. 3). Using a spearman rank correlation test between feed input and fish output, normalized to kg per m^3 water, this yielded a *rho* value of 0.89 (Fig. 3; S = 881,731, $p < 2.2$ e^{-16}).

Since the rank sums of the two categories of CM fertilization only compared production of fish between groups, regardless of the magnitude of CM inputs, we test the linear relationship between fish yield and manure addition (normalized to kg per m^3) using spearman correlation of ranks. The analysis showed a very weak linear relationship between manure addition and fish output (S = 247,226, *p*-value <0.001, *rho* = −0.319), visualized in Fig. 4. A negative *rho* indicates that with increased manure application per m^3, less fish is produced. However, the weak association between ranks, indicated by a *rho* close to zero, that the quantity of manure added to ponds will not alone sufficiently explain the fish output, as there are other co-dependent variables, such as feed addition.

3.2. Eutrophication impacts and profitability from CM fertilization

To evaluate how CM application influence potential eutrophication and profitability in Egyptian semi-intensive aquaculture, we compared rank sums of the two production strategies using Mann-Whitney tests. The data were not normally distributed despite normalizing nutrient emissions to kg per ton^1 fish produced and profitability calculated as 1000 EGP per ton fish. The analysis show that CM application causes significant increase in nutrient loss to the environment (W-value = 24,244 and *p*-value <2.2 e^{-16} for P-emissions, and W-value = 18,834 and p-value = 1.15 e^{-08} for N-emissions, Table 7). On average, farms that use CM as fertilizer emit 327% and 29% more phosphate and nitrogen, respectively (Fig. 5). Meanwhile, we were unable to detect a significant difference in profitability between the two groups (W-value = 14,596 and p-value = 0.287), despite excluding labor costs for fertilizer application.

3.3. Relative abundance of ARGs

We detected the *tetA* gene in 25 of the 28 pond sediment samples collected in 2018, as well as in a drainage canal sample (Fig. 6, SI2; Table S3). When comparing the sum of ranks between the two CM application strategies (yes/no), analysis showed a significant difference between the two groups (Table 8), and an increased abundance of tetracycline resistance genes *tetA* and *tetW* in ponds that applied CM compared to farms that did not. However, no linear relationship could be inferred between the amount of manure input and the relative abundance of the *tetA* and *tetW* genes, using spearman's correlation (S = 50.96, *p*-value = 0.85 for *tetA*, and S = 48.94, *p*-value = 0.79 for *tetW*). The highest abundance of ARGs was represented by *tetW* at site EG30, at a relative abundance of $1.03E^{-3}$, which is comparable to relative *tetW* abundances in wastewater lagoons from cattle feedlots with known prevalent use of tetracycline (Peak et al., 2007).

In the cases where the quinolone-resistance gene *qnrS* was detected,

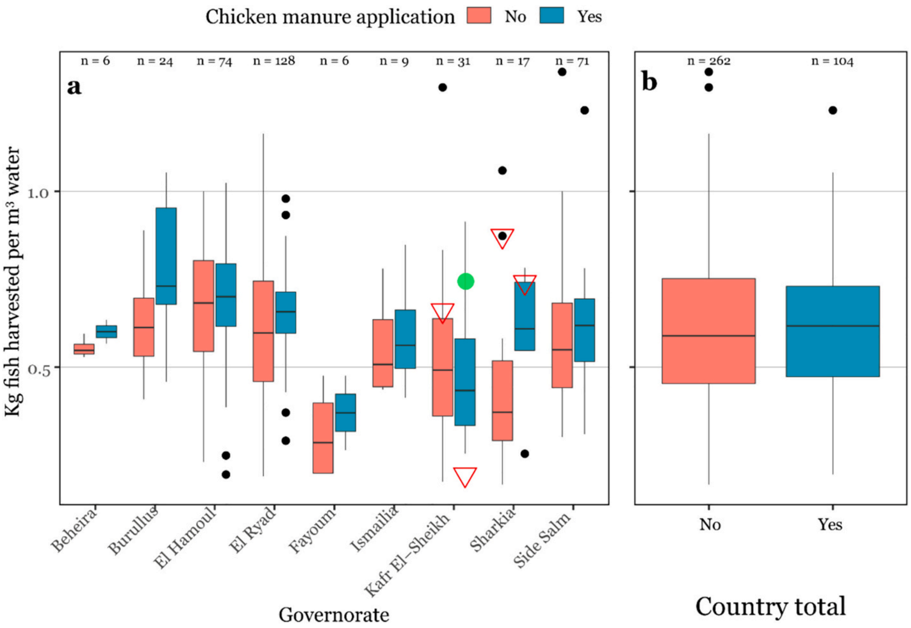

Fig. 2. Fish harvest reported as kg/m3 water per a) each governorate or markaz, and b) country total in Egyptian pond aquaculture related to chicken manure addition. Farms applying TSP are marked with a red triangle and the farm applying urea is marked with a green circle. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

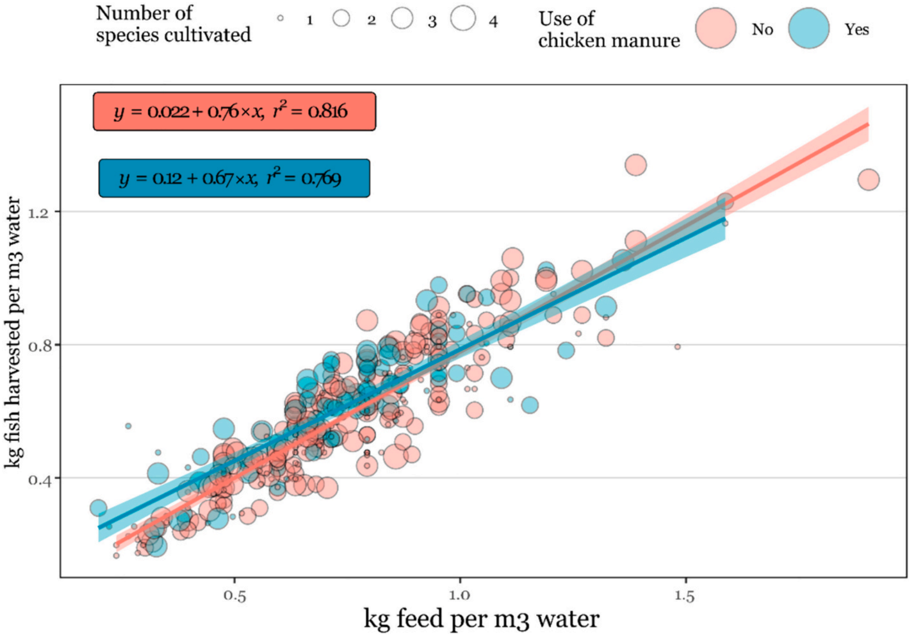

Fig. 3. Fish production correlated to feed input in Egyptian pond aquaculture (n = 366). Lines represent a fitted linear model for each CM application strategy. Equation and r^2 value for linear models are given in each respective box with colour corresponding to colour markings; red = no manure application, blue = manure is used. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the relative abundance was much lower than the *tet*-genes. The *qnrS* gene was detected in 40% of the farms that apply CM, but only in 13% of the farms that do not apply CM. The highest gene abundance was, surprisingly, detected in a sample from a farm not using manure (EG 3.1, see Fig. 6). Farms EG20, EG21, EG30, and EG31 all apply CM and the *qnrS* gene was detected at a ratio of 8,05E^{-7}, 1,03E^{-6}, 8,05E^{-7}, 8,59E^{-7} relative to the 16S rRNA gene, respectively. For EG25, the other site without CM application where *qnrS* was detected, the farmer experienced high mortalities at the time of sample collection.

4. Discussion

4.1. Eutrophication and profitability

Several studies support the concept of supplementing tilapia pond aquaculture systems with organic fertilizers, such as CM (Diana et al., 1994; Green et al., 2002; Kang'ombe et al., 2006; El Naggar et al., 2008; El-Sayed, 2020). However, when analyzing interview data from 366 pond tilapia aquaculture operations across the six main aquaculture producing governorates in Egypt and three different years, our results do

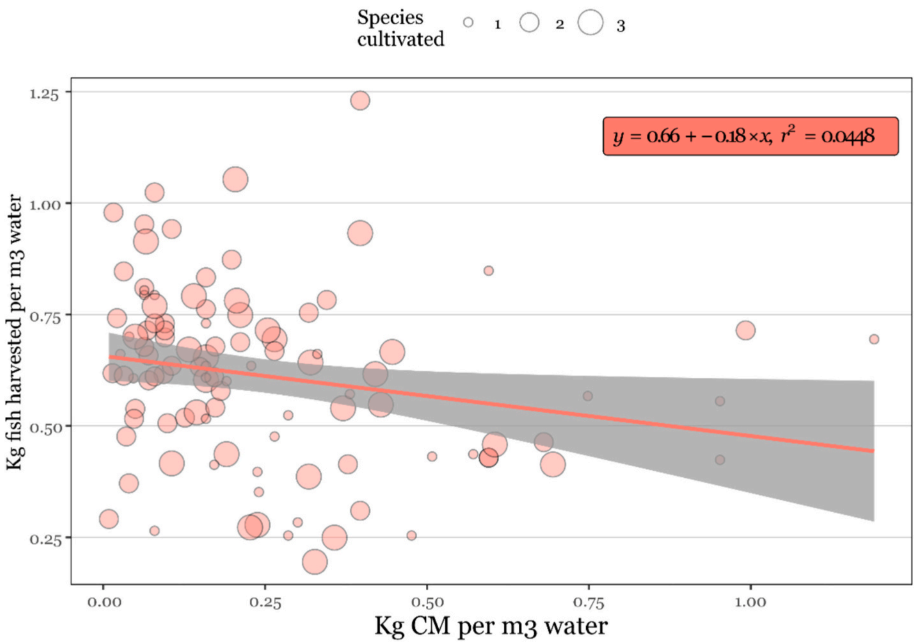

Fig. 4. Linear correlation between fish yields (kg per m^3) and manure inputs (kg per m^3) in ponds where CM is used.

Table 7
Outcomes from analysis of CM influence on N and P emissions and profitability per tonne fish respectively. W-value and *p*-value were results from a Mann Whitney *U* Test of difference sum of ranks between groups. "n" represents the number of interviews from respective region and fertilization strategy. Standard deviation is presented in parentheses. Statistical significance $p < 0.05$ is reported as an asterisk "*".

Outcome	CM application					
	No		Yes			
	n	mean	n	mean	W-value	p-value
Kg P per tonne fish	262	2.42 (1.60)	104	10.33 (8.90)	24,474	< 2.2E-16 *
Kg N per tonne fish	262	40.71 (11.01)	104	52.72 (19.64)	18,917	6.72E-09 *
Profitability 1000 EGP per ton fish	262	12.70 (5.40)	104	12.84 (4.84)	14,592	0.289

not indicate any significant increase in fish production, nor profitability, in Egyptian tilapia farms applying CM fertilization. The efficiency of CM fertilization depends on achieving optimal nutrient loading for each system, as well as other factors, such as soil composition, climate, and weather (Boyd, 2018; El-Sayed, 2020), and fish yields and profitability are also governed by other factors such as water quality (Kolding et al., 2008; Makori et al., 2017; Abd El-Hack et al., 2022), stocking densities (Rossignoli et al., 2023), feed quality, and feeding regimes (Abdel-Tawwab et al., 2010; Dickson et al., 2016). Pond fertilization using organic manures also contributes to stabilizing water quality as long as pH levels are maintained to buffer against ammonia toxicity (El-Sayed, 2013). However, excessive CM fertilization can deteriorate water quality and cause oxygen depletion which can reduced fish growth or increase mortality (Boyd and McNevin, 2015; Boyd, 2018), and nutrient rich pond effluents can influence biogeochemical processes in receiving freshwater systems and contribute to greenhouse gas emissions due to anoxia (Liikanen and Martikainen, 2003; Li et al., 2021). Based on mass balance calculations of inputs versus outputs, the use of CM correlated

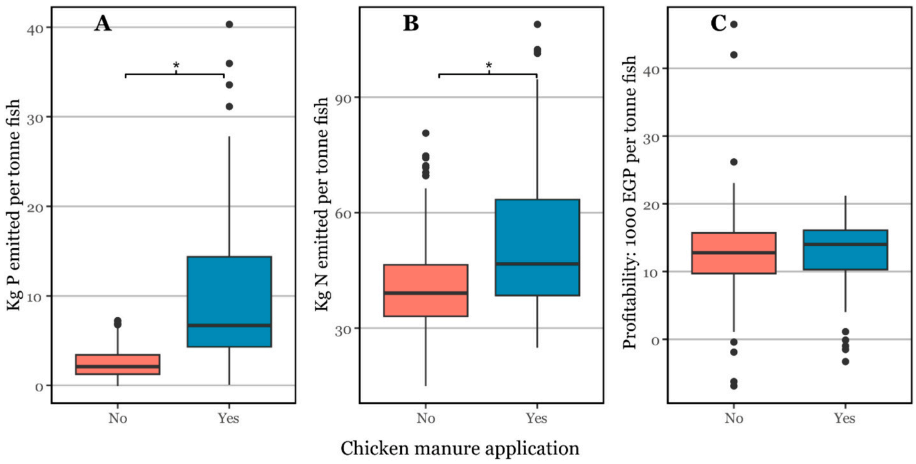

Fig. 5. CM application influence on; A) phosphate emissions to the aquatic environment; B) nitrogen emissions to the aquatic environment and; C) profitability per tonne fish. The median is indicated by a thick line, the box represents 25th and 75th percentiles, and the whiskers 1.5 times the interquartile range from the 25th and 75th percentiles respectively. Statistical significance between the two treatments ($p < 0.05$) is reported as an asterisk "*".

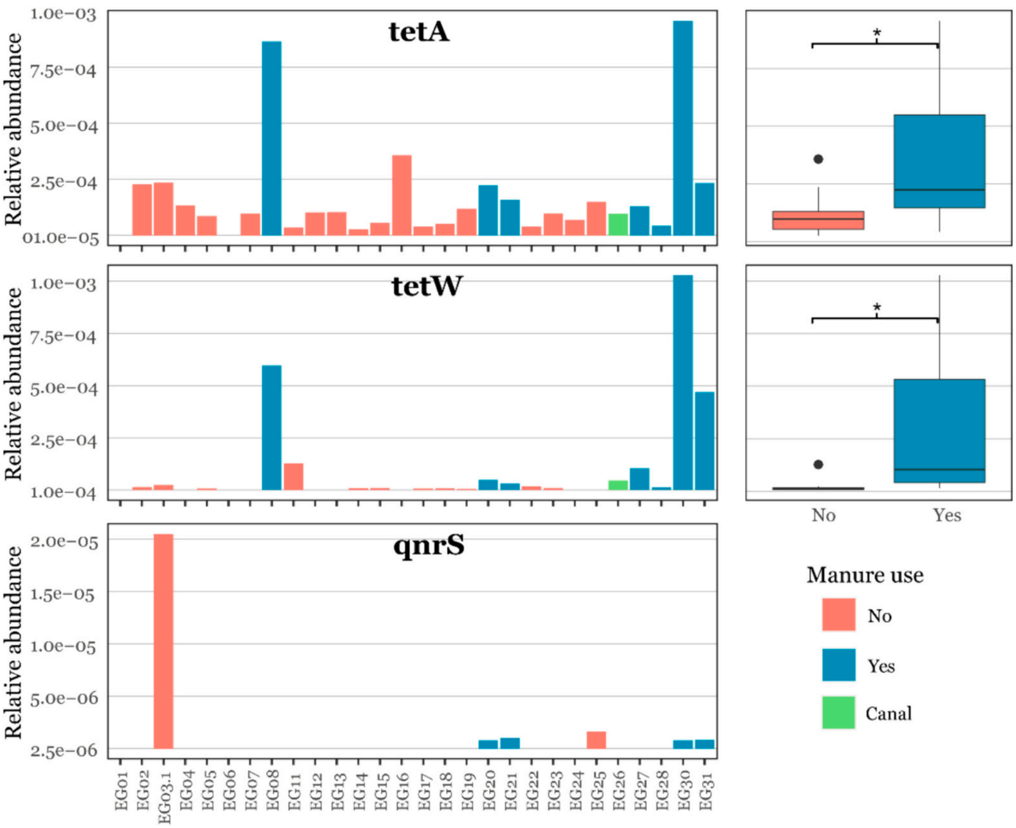

Fig. 6. Results from qPCR of ARGs showing relative abundance (of ARGs per 16S rRNA gene abundance) for genes tetA, tetW, and qnrS per site (left-side panels) and overall median relative abundance (right-side panels). The sample EG26 is taken from a drainage canal collecting water from multiple ponds, and is not included in the right-side panels. Right side panel for qnrS has too few data points to produce meaningful boxplots and is therefore not shown. Statistical significance between the two treatments ($p < 0.05$) is reported as an asterisk "*".

Table 8
Means and standard deviation of relative abundances of ARGs (ARG per 16S rRNA) between farms that do and do not apply CM. W-value and p-value are results from a Mann Whitney *U* Test of difference sum of ranks. Standard deviations are presented in parentheses. Statistical significance $p < 0.05$ is reported as an asterisk "*".

Gene	No		Yes			
	n	mean	n	mean	W-value	*p*-value
tetA	16	1.13E-04 (8.55E-05)	9	3.73E-04 (3.74E-04)	99	0.03 *
tetW	10	2.17E-05 (3.53E-05)	8	3.28E-04 (3.86E-04)	70	<0.01 *

with increased N and P emissions (29% and 327% respectively), which causes eutrophication in local waterways. These results rely an indication of potential eutrophication with only feed and fertilizer inputs correlated with fish yields and nutrient emissions, while any realized local effects will depend on fish pond soil biogeochemistry, effluent nutrient contents, and the recipient's capacity to buffer increased nutrient loads (Boyd and McNevin, 2015).

Water from the Nile river is prioritized for agricultural irrigation (Macfadyen et al., 2011; Wally, 2016), while fish farms are limited to downstream agricultural or municipal drainage canals. Farms may even be connected in series to the same channels resulting in re-use of water among aquaculture farms (Macfadyen et al., 2011). Agricultural drainage water is often rich in nutrients and may contain pesticides and heavy metal residues that can have negative effects to fish health in affected farms (Eltholth et al., 2015; Soliman and Yacout, 2016; Ali et al., 2020). In cases where drainage water is re-used, excessive CM fertilization brings a risk of exacerbating problems related to eutrophication, such as ammonia toxicity.

Best management practices (BMPs) in Egyptian tilapia aquaculture suggest optimal manure application rates of circa 3.7 t ha^{-1} per grow-out cycle (Dickson et al., 2016), which corresponds well to the national average of the current dataset (3.84 t ha^{-1} per cycle; Table 5), but CM fertilization rates in the current dataset range from 0.13 to 14.88 t ha^{-1} per cycle. A spearman correlation of ranks analysis shows a negative correlation between CM inputs and fish yields (Fig. 4), but the weak explanatory power of this test ($rho = -0.319$) could be explained by other influential factors over fish yields, such as feed input ($rho = 0.89$). BMPs should, however, consider our results and take into account the risks of eutrophication. Profitability might, in the meantime, need further considerations given the continuously increasing cost of aquaculture feeds in Egypt (El-Sayed et al., 2015).

4.2. *Antibiotic resistance genes related to CM fertilization*

Results from qPCR assays of tilapia pond sediment show that manure addition correlates with increased abundance of ARGs relative to 16S. Farms that apply CM show signatures of increased abundances of tetracycline resistance genes *tetA* and *tetW*, commonly detected in feces and manure (Abramova et al., 2023). The site with the highest relative abundance of *tet*-genes applied 7.63 t of CM per ha over the grow-out period (recorded as five tonnes weekly across the entire farm). In general, the *tetW* gene appears in higher relative abundances in farms where CM is applied, while the *tetA* gene is detected in most samples.

The *qnrS* gene is detected in relatively few farm sediments, of which two out of six sites surprisingly did not apply CM. The abundance of *qnrS* genes in pond sediments from site EG3.1 is high in comparison to the other five sites where it is detected (2.05×10^{-5} copies per 16S rRNA), yet comparing to the relative abundance of *tet* genes, this number is still in the lower range. The EG3.1 sample interestingly comes from one of the few farms with documented AB use, albeit use of oxytetracycline, not quinolone antibiotics (SI2, Table S2) and the relative abundance of *qnrS* genes is comparable to findings in fish farms in China with a history of both prophylactic and therapeutic administration of antibiotics which is recorded as 3.29E-04 to 7.20E-05 copies of *qnrS* per 16S (Lin et al., 2023). From these results, it seems as AB use, rather than manure application, in tilapia ponds correlate with increased abundance of quinolone resistance. The overall abundances of *tetA* and *tetW* genes we detected in Egyptian tilapia pond sediments are comparable to abundances in anthropogenically impacted environments, affected by manure pollution (Abramova et al., 2023).

While we detected increased abundance of ARGs as a consequence of CM fertilization in these tilapia production environments, it is hard to conclude what the subsequent effects might be. Food-borne pathogens carrying resistance genes have been attributed to cases where infections cause severe illness (Marshall and Levy, 2011), and the transfer of ARGs between different food animal sectors via manure should to be viewed as a potential health hazard. Aquaculture sediment may act as reservoirs for ARGs (Marti et al., 2014), and investigations into how ARGs present in farming environment contribute to health risks in food animal and human populations should be undertaken in consideration of a non-negligible link between animal production sectors according to "One Health" principals.

5. Conclusions

Based on interview data from 366 Egyptian tilapia pond farms over three years, use of CM does not contribute significantly to the production and profitability in Egyptian tilapia pond aquaculture. Instead, CM use correlates with increased ARG frequencies in tilapia pond sediment, but not above levels relative to other aquaculture operations globally. Fertilizing fish pond water with organic waste is in theory a sound practice, given that the organic waste does not contain pharmaceuticals or antibiotic resistant bacteria. However, effects of CM fertilization that are reflected in this dataset is eutrophication of the local water resources, and the underlying risk of ARG transfer from poultry farms to fish farms, which implies risks to both fish health and human health through reduced efficacy of antibiotic treatments. While there is large variation in CM application rates across Egyptian tilapia pond farms, we see no apparent economic benefits from CM application, but rather risks to fish, human, and environmental health. From this viewpoint we recommend that best management practices should be reviewed with regards to CM use. This could improve product quality, safety, reduce the impact of fish farming on the environment.

CRediT authorship contribution statement

Oskar Nyberg: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Andreas Novotny:** Writing – review & editing, Supervision, Resources, Methodology. **Ashraf S. Sbaay:** Writing – review & editing, Resources, Investigation. **Ahmed M. Nasr-Allah:** Writing – review & editing, Supervision, Resources, Methodology. **Diaa A.R. Al-Kenawy:** Writing – review & editing, Supervision, Resources, Methodology. **Cristiano M. Rossignoli:** Writing – review & editing, Methodology, Investigation, Data curation. **Patrik J.G. Henriksson:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

Funding support was provided by the through the Global Economic Dynamics and the Biosphere Program (GEDB), the Royal Swedish Academy of Sciences, Sweden. PJGH is funded by the FORMAS Inequality and the Biosphere Project (2020-00454), Ardevora Charitable Trust, IKEA Foundation, Gordon and Betty Moore Foundation (GBMF11613), and Walton Family Foundation (00104857), and the David and Lucile Packard Foundation (2022-73546). PJGH has undertaken this research as part of the CGIAR Research Initiative on Low-Emission Food Systems. This program is supported by contributors to the CGIAR Trust Fund.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2024.741040.

References

Abd El-Hack, M.E., El-Saadony, M.T., Nader, M.M., Salem, H.M., El-Tahan, A.M., Soliman, S.M., Khafaga, A.F., 2022. Effect of environmental factors on growth performance of Nile tilapia (*Oreochromis niloticus*). Int. J. Biometeorol. 66, 2183–2194.

Abdel-Tawwab, M., Ahmad, M.H., Khattab, Y.A.E., Shalaby, A.M.E., 2010. Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticus* (L.). Aquaculture 298, 267–274.

Abramova, A., Berendonk, T.U., Bengtsson-Palme, J., 2023. A global baseline for qPCR-determined antimicrobial resistance gene prevalence across environments. Environ. Int. 178, 108084.

Abu-Elala, N.M., Abd-Elsalam, R.M., Marouf, S., Abdelaziz, M., Moustafa, M., 2016. Eutrophication, Ammonia intoxication, and infectious diseases: interdisciplinary factors of mass mortalities in cultured Nile Tilapia. J. Aquat. Anim. Health 28, 187–198.

Ali, S.E., Jansen, M.D., Mohan, C.V., Delamare-Deboutteville, J., Charo-Karisa, H., 2020. Key risk factors, farming practices and economic losses associated with tilapia mortality in Egypt. Aquaculture 527, 735438.

Börjesson, S., Dienues, O., Jarnheimer, P.-Å., Olsen, B., Matussek, A., Lindgren, P.-E., 2009. Quantification of genes encoding resistance to aminoglycosides, β-lactams and tetracyclines in wastewater environments by real-time PCR. Int J Environ Health R 19, 219–230.

Boyd, C.E., 2018. Aquaculture pond fertilization. CABI Reviews 2018, 1–12.

Boyd, C.E., McNevin, A.A., 2015. Aquaculture, Resource Use, and the Environment. Wiley-Blackwell, Hoboken, NJ, USA.

Boyd, C.E., Tucker, C., Mcnevin, A., Bostick, K., Clay, J., 2007. Indicators of resource use efficiency and environmental performance in fish and crustacean aquaculture. Rev. Fish. Sci. 15, 327–360.

Bueno, G.W., Mattos, B.O.D., Neu, D.H., David, F.S., Feiden, A., Boscolo, W.R., 2019. Stability and phosphorus leaching of tilapia feed in water. Cienc. Rural 49, e20180429.

Dahshan, H., Abd-Elall, A.M.M., Megahed, A.M., Abd-El-Kader, M.A., Nabawy, E.E., 2015. Veterinary Antibiotic Resistance, Residues, and Ecological Risks in Environmental Samples Obtained from Poultry Farms. Egypt, Environmen Monit Assess, p. 187.

Diana, J.S., Lin, C.K., Jaiyen, K., 1994. Supplemental feeding of Tilapia in fertilized ponds. J. World Aquacult. Soc. 25, 497–506.

Dickson, M., Nasr-Allah, A., Kenawy, D.A.R., Kruijssen, F., 2016. Increasing fish farm profitability through aquaculture best management practice training in Egypt. Aquaculture 465, 172–178. https://doi.org/10.1016/j.aquaculture.2016.09.015.

Eissa, A.E., Moustafa, M., Abumhara, A., Hosni, M., 2016. Future prospects of biosecurity strategies in Egyptian fish farms. J. Fish. Aquat. Sci. 11, 100–107.

El Naggar, G., Ibrahim, N., Abou Zead, M.Y., 2008. Influence of fertilizers' types and stocking density on water quality and growth performance on Nile tilapia African catfish in polyculture system. 8th International Symposium on Tilapia in Aquaculture. WorldFish Center, Regional Center for Africa and West Asia, Abbassa, Abou-Hammad, Sharkia, Egypt.

Elsaidy, N., Abouelenien, F., Kirrella, G.A.K., 2015. Impact of using raw or fermented manure as fish feed on microbial quality of water and fish. Egypt. J. Aquat. Res. 41, 93–100.

El-Sayed, A.F.M., 2013. On-farm feed management practices for Nile tilapia (*Oreochromis niloticus*) in Egypt. In: Hasan, M.R., New, M.B. (Eds.), On-Farm Feeding and Feed Management in Aquaculture, FAO Fisheries and Aquaculture Technical Paper no, vol. 583. FAO, Rome, pp. 101–129.

El-Sayed, A.F.M, 2020. Tilapia Culture, second ed. Elsevier Inc.

El-Sayed, A.F.M., Dickson, M.W., El-Naggar, G.O., 2015. Value chain analysis of the aquaculture feed sector in Egypt. Aquaculture 437, 92–101.

Eltholth, M., Fornace, K., Grace, D., Rushton, J., Häsler, B., 2015. Characterisation of production, marketing and consumption patterns of farmed tilapia in the Nile Delta of Egypt. Food Policy 51, 131–143.

FAO, 2022. The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation, FAO.

FAO, 2023. Egypt. In: Salem, A.M., Saleh, M.A. (Eds.), Fisheries and Aquaculture Division [online]. Rome. [Cited October 13th 2023]. https://www.fao.org/fishery/en/countrysector/eg.

Gephart, J.A., Henriksson, P.J.G., Parker, R.W.R., Shepon, A., Gorospe, K.D., Bergman, K., Eshel, G., Golden, C.D., Halpern, B.S., Hornborg, S., Jonell, M., Metian, M., Mifflin, K., Newton, R., Tyedmers, P., Zhang, W., Ziegler, F., Troell, M., 2021. Environmental performance of blue foods. Nature 597, 360–365.

Golden, C.D., Koehn, J.Z., Shepon, A., Passarelli, S., Free, C.M., Viana, D.F., Matthey, H., Eurich, J.G., Gephart, J.A., Fluet-Chouinard, E., Nyboer, E.A., Lynch, A.J., Kjellevold, M., Bromage, S., Charlebois, P., Barange, M., Vannuccini, S., Cao, L., Kleisner, K.M., Rimm, E.B., Danaei, G., DeSisto, C., Kelahan, H., Fiorella, K.J., Little, D.C., Allison, E.H., Fanzo, J., Thilsted, S.H., 2021. Aquatic foods to nourish nations. Nature 598, 315–320.

Green, B.W., Boyd, C.E., 1995. Chemical budgets for organically fertilized fish ponds in the dry tropics. J. World Aquacult. Soc. 26, 284–296.

Green, B.W., El Nagdy, Z., Hebicha, H., 2002. Evaluation of Nile tilapia pond management strategies in Egypt. Aquac. Res. 33, 1037–1048.

Griffiths, N and NSW Agriculture, 2003. Poultry Litter: A Great Resource or an Environmental Hazard / Prepared by Neil Griffiths. NSW Agriculture, Paterson, N.S.W.

Hardwick, S.A., Stokes, H.W., Findlay, S., Taylor, M., Gillings, M.R., 2008. Quantification of class 1 integron abundance in natural environments using real-time quantitative PCR. FEMS Microbiol. Lett. 278, 207–212.

Hasan, M.R., Hecht, T., De Silva, S.S., Tacon, A.G.J., 2007. Study and analysis of feeds and fertilizers for sustainable aquaculture development (UNFAO fish tech pap no. 497).

Kang'ombe, J., Brown, J.A., Halfyard, L.C., 2006. Effect of using different types of organic animal manure on plankton abundance, and on growth and survival of *Tilapia rendalli* (Boulenger) in ponds. Aquac. Res. 37, 1360–1371.

Koeypudsa, W., Yakupitiyage, A., Tangtrongpiros, J., 2005. The fate of chlortetracycline residues in a simulated chicken-fish integrated farming systems. Aquac. Res. 36, 570–577.

Kolding, J., Haug, L., Stefansson, S., 2008. Effect of ambient oxygen on growth and reproduction in Nile tilapia (*Oreochromis niloticus*). Can. J. Fish. Aquat. Sci. 65, 1413–1424.

Li, Y., Shang, J., Zhang, C., Zhang, W., Niu, L., Wang, L., Zhang, H., 2021. The role of freshwater eutrophication in greenhouse gas emissions: a review. Sci. Total Environ. 768, 144582.

Liikanen, A., Martikainen, P.J., 2003. Effect of ammonium and oxygen on methane and nitrous oxide fluxes across sediment–water interface in a eutrophic lake. Chemosphere 52, 1287–1293.

Lin, X., Tan, A., Deng, Y., Liu, W., Zhao, F., Huang, Z., 2023. High occurrence of antibiotic resistance genes in intensive aquaculture of hybrid snakehead fish. Front. Mar. Sci. 9, 1088176.

Liu, Y.-Y., Wang, Y., Walsh, T.R., Yi, L.-X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X., Yu, L.-F., Gu, D., Ren, H., Chen, X., Lv, L., He, D., Zhou, H., Liang, Z., Liu, J.-H., Shen, J., 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect. Dis. 16, 161–168. https://doi.org/10.1016/S1473-3099(15)00424-7.

Macfadyen, G., Nasr Allah, A.M., Kenawy, D.A.R., Ahmed, M.F.M., Hebicha, H., Diab, A., et al., 2011. Value-Chain Analysis of Egyptian Aquaculture [project report 2011-54],. The WorldFish Center, Bayan Lepas, Penang.

Mahmoud, M.A.M., Abdel-Mohsein, H.S., 2019. Hysterical tetracycline in intensive poultry farms accountable for substantial gene resistance, health and ecological risk in Egypt- manure and fish. Environ. Pollut. 255, 113039.

Makori, A.J., Abuom, P.O., Kapiyo, R., Anyona, D.N., Dida, G.O., 2017. Effects of water physico-chemical parameters on tilapia (*Oreochromis niloticus*) growth in earthen ponds in Teso north Sub-County. Busia County. Fisheries and Aquatic Sciences 20. https://doi.org/10.1186/s41240-017-0075-7.

Mariotti, F., Tomé, D., Mirand, P.P., 2008. Converting nitrogen into protein—beyond 6.25 and Jones factors. Cri Rev Food Sci 48, 177–184.

Marshall, B.M., Levy, S.B., 2011. Food animals and antimicrobials: impacts on human health. Clin. Microbiol. Rev. 24 (4), 718–733. https://doi.org/10.1128/CMR.00002-11.

Marti, E., Balcázar, J.L., 2013. Real-time PCR assays for quantification of qnr genes in environmental water samples and chicken feces. Appl. Environ. Microbiol. 79, 1743–1745.

Marti, E., Variatza, E., Balcazar, J.L., 2014. The role of aquatic ecosystems as reservoirs of antibiotic resistance. Trends Microbiol. 22 (1), 36–41. https://doi.org/10.1016/j.tim.2013.11.001.

Nhan, D.K., Verdegem, M.C.J., Milstein, A., Verreth, J.A.V., 2008. Water and nutrient budgets of ponds in integrated agriculture-aquaculture systems in the Mekong Delta, Vietnam. Aquac. Res. 39, 1216–1228.

Nicholson, P., Fathi, M.A., Fischer, A., Mohan, C., Schieck, E., Mishra, N., et al., 2017. Detection of Tilapia Lake virus in Egyptian fish farms experiencing high mortalities in 2015. J. Fish Dis. 40, 1925–1928.

Peak, N., Knapp, C.W., Yang, R.K., Hanfelt, M.M., Smith, M.S., Aga, D.S., Graham, D.W., 2007. Abundance of six tetracycline resistance genes in wastewater lagoons at cattle feedlots with different antibiotic use strategies. Environ. Microbiol. 9, 143–151.

R Core Team, 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rossignoli, C.M., Manyise, T., Shikuku, K.M., Nasr-Allah, A.M., Dompreh, E.B., Henriksson, P.J.G., et al., 2023. Tilapia aquaculture systems in Egypt: characteristics, sustainability outcomes and entry points for sustainable aquatic food systems. Aquaculture 577, 739952.

Shaaban, N.A., Tawfik, S., El-Tarras, W., El-Sayed Ali, T., 2021. Potential health risk assessment of some bioaccumulated metals in Nile tilapia (*Oreochromis niloticus*) cultured in Kafr El-Shaikh farms, Egypt. Environ. Res. 200, 111358.

Shatokhin, Y., El Gammal, M., Prikhodko, D., 2017. Arab Republic of Egypt broiler poultry industry: investment challenges and opportunities (draft for discussion).

Shikuku, K.M., Tran, N., Henriksson, P., Nasr-Allah, A., Roem, A., Badr, A., Cheong, K.C., Sbaay, A., Calmet, M., Karisa, H., Rossignoli, C., 2021. Dataset for Baseline Integrated Assessment of Aquaculture Systems Performance in Egypt. https://doi.org/10.7910/DVN/VSQWEE.

Soliman, N.F., Yacout, Dalia M.M., 2016. Aquaculture in Egypt: status, constraints and potentials. Aquac. Int. 24, 1201–1227.

Wally, A., 2016. The state and development of aquaculture in Egypt. USDA FAS GAIN, pp. 1–14.

Wang, N., Guo, X., Yan, Z., Wang, W., Chen, B., Ge, F., Ye, B., 2016. A comprehensive analysis on spread and distribution characteristic of antibiotic resistance genes in livestock farms of southeastern China. PLoS One 11, e0156889.

World Health Organization, 2019. Critically Important Antimicrobials for Human Medicine, 6th Rev. World Health Organization, Geneva.

Zhang, W., Belton, B., Edwards, P., Henriksson, P.J.G., Little, D.C., Newton, R., Troell, M., 2022. Aquaculture will continue to depend more on land than sea. Nature 603, E2–E4.