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Mixture of oxytetracycline and erythromycin antibiotics enhances nutritional quality of a commercial feed, effluent quality, and but not water quality in catfish culture, *Clarias gariepinus*

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Abstract
Nutrition is a critical driver of farmed fish's ability to grow and reproduce. The increased cost and scarcity of fish feed have motivated additional research into alternatives and additives. As a result, fish meals must be augmented with additives. Feed additives are eatable substances that are added to fish meals in modest quantities to improve growth and minimize mortality rates. The current study investigated the synergistic effects of the broad spectrum antibiotics oxytetracycline (OXY) and erythromycin (ERY) combined in a ratio of 1:1 and administered to African Catfish, *Clarias gariepinus* through feed fed at 0% OXY/ERY gkg^{1}, 1% OXY/ERY gkg^{1}, 2% OXY/ERY gkg^{1}, and 3% OXY/ERY gkg^{1} inclusion levels for 8 weeks. At the conclusion of the trial, feed samples, effluent samples, and water samples were analyzed on Catfish, *Clarias gariepinus*. The results showed that *C. gariepinus*, Catfish fed a 1% OXT/ERY diet performed much better in the proximate analysis than the control diet. Fish fed with 1% OXY/ERY showed significantly greater levels of calcium (87.9±1.02), percentage nitrogen (3.9±0.31), available phosphorus (3.3±0.7), and available potassium (95.7±4.66) in their effluent compared to other antibiotic inclusion levels and the control diet. Despite the potential for OXY/ERY applications in *C. gariepinus* Catfish cultivation, the results of this study show that fish fed with varied concentrations of OXY/ERY may have an impact on the quality of the cultivated water when compared to the control group. Taking all of the aforementioned into consideration, supplementing *C. gariepinus* feed with OXY/ERY could improve feed and effluent quality; however, its application may contribute to water quality deterioration, particularly at concentrations more than 1% and for extended periods of time.

Keywords: Oxytetracycline, erythromycin, antibiotics, catfish, feed quality, effluent quality

1. Introduction

Intensification of catfish culture has resulted to issues like poor growth, water quality, and disease outbreaks (Phuong & Oanh, 2010) [32]. In an attempt to address these challenges, farmers have soughted to the use of antibiotics as growth promoters, health boosters and the general management of fish welfare (Nkansa, 2017) [30]. Among the trusted medications is the use oxytetracycline and erythromycin antibiotics to salvage fish farms and handle all fish health issues in aquaculture (Hinchliffe *et al*., 2018) [18]. Mostly, these additives are added or supplemented to already formulated commercial feeds that is been purchased from the open market (Upadhayay & Vishwa, 2014) [42]. However, these formulated feeds normally come with its nutritional contents and quality (Tangendjaja, 2022) [41]. Effect of diet supplementation in fish cultured are mostly centered on its effect on growth, health and to increase resistance under pathological conditions (Mahmoud *et al*., 2017) [24]. Much attention has not been given to the effect and impact of the inclusion of these additives on the feed quality, effluent quality and water quality. Soils are frequently susceptible to continuous degradation once they are used for cultivation; however, inorganic fertilizers used to mitigate the effects of poor yield from degrading soil are facing a lot of criticism on both the health and environmental fronts (Brust, 2019) [9]. Soil fertility challenges necessitated long-term management of soil resources in order to increase fertility to levels suitable for vegetable production (Ahmad *et al*., 2021) [3]. Increasing call for ecologically friendly fish farming practices has focused on how waste generated from aquaculture systems can be potentially useful in enhancing crop production other than releasing it into the environment which can have detrimental effect on our surrounding aquatic ecosystem (Ahmad *et al*., 2021; Ojukwu, 2018) [3, 31]. Maintaining and managing aquaculture waste in most developing countries has become problem and public health menace (Abdul-Rahman *et al*., 2016; Van Tung *et al*., 2021) [2, 43]. Catfish culture in our fresh water systems have high concentrations of fish culture effluent (Abdul-Rahman *et al*., 2016) [2]. Organic fertilizer (Fishpond effluent) has been successfully used to improve soil fertility and increase the production of vegetables in the past (Chiquito-Contreras *et al*., 2022) [10], but there is no information on the use of catfish effluent fed with a mixture of oxytetracycline and erythromycin antibiotics. Quality catfish effluent is essential for long-term and continuous vegetable production. Despite the growing benefits of antibiotic application in catfish culture, the nutritional value from the waste generated needs to be evaluated. A lot of effort has been made to improve soil fertility and crop production by using a variety of soil improving chemicals, but none has looked at the combined effect of oxytetracycline and erythromycin antibiotics on catfish effluents. As a result, there is a need to research into different ways to help manage waste from antibiotic treated farms as an additional benefit and also reduce the consequences of its discharge into the environment. According to studies, aquaculture effluent contains a high level of organic matter, nitrogen, phosphorus, potassium, and micronutrients (Chiquito-Contreras *et al*., 2022; Abdul-Rahman *et al*., 2016) [10, 2]. The effluent can be used to fertilize the vegetables as a liquid organic fertilizer (Masciandaro *et al*., 2013) [26]. Inorganic nutrients in effluent are readily available to plants, whereas organic nutrients are available through microbial decomposition (Chiquito-Contreras *et al*., 2022) [10]. As a result, this study seeks to assess the effect of oxytetracycline and erythromycin antibiotic class mixture on the nutritional quality of the feed and effluent quality from catfish tanks, *Clarias gariepinus*.

2. Materials and Methods

2.1 Test substance and stock preparation

Oxytetracycline (OXY) and erythromycin (ERY) were acquired from a recognized pharmacy in Tamale Metropolis, and stock solution was made in a ratio of 1:1 by dissolving 75 mg/kg of OXY and ERY in a sufficient amount of distilled water.

2.2 Preparation of experimental diets

A 2mm commercial fish feed (Designated A) containing 45% protein, 11% fat, 20% fiber, 9.5% ash, and 1.3% phosphorus was acquired from the open market. To prepare the experimental feed, 1 kg each of the commercial feed was weighed into four separate bowls. To one of the measured feeds, 100 ml of distilled was added and mixed thoroughly, representing the control diet. The experimental diets were made by mixing 1 kg of commercial feed with various concentrations of the antibiotic mixture (1%, 2%, and 3%), which were then remolded to represent 1%, 2%, and 3% of the OXY/ERY gKg^1, respectively.

2.3 Experimental fish and design

A total of 300 Catfish without abdominal distension, ragged fins, or bleeding of a mean weight of 100±1.0 g were obtained from the Water Research Institute (Tamale, Ghana) for use in the experiment. The fish were divided into twelve groups and allowed to adapt in concrete circular tanks containing around 80 liters of water for a. During the acclimation period, fish were fed twice per day with the control food at 2% of their body mass. The feed was given in two equal portions at 8:30 a.m. and 4: 00 p.m. daily. The fish were then assigned to the appropriate groups (CT, 1% OXY/ERY gKg^1, 2% OXY/ERY gKg^1, and 3% OXY/ERY gKg^1) in triplicate, with each tank containing 25 fish. The amount of food given out was modified biweekly based on the individual body weight of each group's fish. To maintain water quality, approximately 30% was refreshed daily.

2.4 Data collection of feed quality analysis

Following the feed quality study, 20 grams of the control diet and the different concentrations of antibiotic-treated diet groups were sampled in triplicate into a cleaned bottle provided by the SARI laboratory technician at 8 weeks. All proximate composition studies adhered to the Association of Official Analytical Chemists' criteria. All analyses were performed in triplicate.

2.5 Data collection on effluent and water quality

Water samples from the control group and the various inclusion levels of the antibiotic mixture treated groups were collected after eight weeks and placed in a transparent glass bottle using a water tester. It was subsequently stored on ice and transported to the Scientific for Water Research agency in Tamale for investigation of a complete physicochemical water quality parameters, including effluent nutrient analysis. Throughout the 8 weeks feeding trial, water temperature, pH, conductivity, turbidity, total dissolve solids were tested. In addition, water samples were obtained from each tank and processed through filter papers for effluent analysis.

2.6 Data analysis

SPSS version 16.0 was used to compare differences ($p< 0.05$) in proximal indices, effluent parameters, and physicochemical water quality factors between the control and OXY/ERY groups. The treatment means were compared using a one-way ANOVA with the Duncan range test. Tables and conversations show data as means with standard error.

3. Results

3.1 Proximate analysis of the feed nutrient: Commercial diets supplemented with OXT/ERY gKg^1 antibiotic at several concentration levels had considerably higher levels of protein, ash, fat, fiber, and phosphorus than the control diets with a exception of the moisture. However, among the antibiotic-treated animals, 1% OXY/ERY inclusion level consistently and significantly raised all proximal parameters tested compared to the control. With the exception of the 3% OXY/ERY, which showed statistical similarity to the control for some of the indices tested. Overall, a declining trend was seen, with 1% OXY/ERY > 2% OXY/ERY > 3% OXY/ERY > the control diet ($p< 0.05$, Table 1).

Table 1: Proximate analysis of the control and the antibiotic experimental treated diet

Parameters	Control	1% OXY/ERY	2% XXY/ERY	3% OXY/ERY
Protein	37%±6.61d	51%±5.28a	46%±4.81b	43%±6.74c
Ash	17.42±3.13d	29.21±4.47a	26.48±5.14b	23.21±6.82c
Moisture	10.31±2.18d	8.62±1.87b	7.89±2.31b	6.98±1.91b
Fat	9%±2.03cd	17%±3.19a	13%±2.72b	11%±2.16c
Fibre	7.2±2.03cd	13%±2.77a	10%±1.38b	8.1%±1.53c
Phosphorus	0.8%±0.02cd	1.7%±0.04a	1.2%±0.02b	0.9%±0.01c

Where OXY=Oxytetracycline, ERY=Erythromycin. Means± SE (Duncan rang test, *n* = 3) with different superscript letters in the same column denote significant difference; OXY/ERY gKg[1]

3.2 Samples of effluent analysed after the experiment

Table 2 displays the effluent parameters after the experimental period in both the control and antibiotic-treated groups. It was observed that all the antibiotic supplemented groups at varying inclusion levels significantly recorded high levels of all the characteristics examined in comparison to the control. 1% OXY/ERY significantly raised calcium levels (87.9±1.02a), percentage nitrogen (3.9±0.31a), available phosphorus (3.3±0.7a), and available potassium (95.7±4.66a) compared to other antibiotic inclusion levels and the control fed group. It is worth noting that when antibiotic concentrations increased, there was a declining tendency. Overall, the oxytetracycline and erythromycin supplemented group showed significant increases in catfish effluent, with the highest mean values in all nutritional characteristics tested ($p < 0.05$, Table 2).

Table 2: Effluent nutrient analysis after the termination of the experiment

Treatment	Parameters of fish effluent analysis			
	Calcium	% N	P (mg kg^{-1})	K (mg kg^{-1})
CT	63.3±2.01d	0.37±004d	0.89±0.04d	68.4±3 41d
1% OXY/ERY	87.9±1.02a	3.9±0.31a	3.3±0.7a	95.7±4.66a
2% OXY/ERY	78.4±3.9.4b	2.7±0.61b	2.3 ± 0.5b	83.1±3.67b
3% OXY/ERY	69.8±4.32c	1.6±0.08c	1.7 ± 0.09c	76±3.44c

Source: (CSIR/SARI, 2024) Where N = nitrogen, P = phosphorus and K = potassium, Where OXY=Oxytetracycline, ERY =Erythromycin. Means± SE (Duncan rang test, *n* = 3) with different superscript letters in the same column denote significant difference; OXY/ERY gKg[1]

Note: Different superscript letters in the same column denote significant difference

3.3 Samples of water quality analysed after the experiment

Table 3 also includes data on water quality factors after week 8 of the trial period. The antibiotic class mixture treated group showed significantly higher water quality indices compared to the control group ($p < 0.05$). There was a considerable increase in antibiotic inclusion levels, particularly at 3% OXY/ERY, compared to the control group ($p < 0.05$, Table 3).

Table 3: Water quality analysis after the termination of the experiment

Treatment	Parameters of fish effluent analysis			
	Con.	Turk	pH	TDS
CT	990±7.01a	210±3.9a	7.35±1.04a	607±9.41a
1% OXY/ERY	1021±12.02b	267±8.31b	7.72±1.7b	635±10.66b
2% OXY/ERY	1032±14.9c	286±12.61c	7.93±1.5c	658±13.67c
3% OXY/ERY	1038±17.32c	298±10.08c	8.4±1.9c	664±14.44c

Source: (CSIR/SARI, 2024) Where N = nitrogen, P = phosphorus and K = potassium, Where OXY=Oxytetracycline, ERY=Erythromycin. Means± SE (Duncan rang test, *n* = 3) with different superscript letters in the same column denote significant difference; OXY/ERY gKg[1]

Note: Different superscript letters in the same column denote significant difference

4. Discussion

Antimicrobials, particularly antibiotics, have been utilized as fish feed additives to promote fish growth and health (Dawood *et al*., 2018) [13]. These additives are typically added to pre-formulated feeds and then remolded, or they are sprinkled directly on commercially prepared diets (Sutili *et al*., 2018) [40]. Antibiotic supplementation in catfish food is the sole element that has an influence on all assessed parameters when compared to the control. The concentration of nutrients in the effluent increases as production and feeding intensity increase (Kumar *et al*., 2010; Lucas-González *et al*., 2018) [22, 23]. Fish feed is the only nutrient source delivered into the fish growing system (Colt *et al*., 2021) [11]. Some of the feed delivered goes uneaten and becomes feed waste. The undigested portion of the eaten nutrients is expelled as particulate feces (Davis & Hardy, 2022; McClements & McClements, 2023) [12, 27], which primarily comprise organic carbon and phosphorus. The nutrients absorbed by fish are partially retained in their body mass (Davis & Hardy, 2022) [12]. The remainder is expelled as dissolved nutrients through the gills, primarily as ammonia, and in urine as phosphate and ammonium (Glencross *et al*., 2023; Enwereuzoh *et al*., 2021) [16, 15].

The data suggests that diets supplementing a commercial diet with a mixture of oxytetracycline and erythromycin antibiotics enhanced the proximate analysis of the commercial diet by significantly increasing the indices measured by increasing the total crude protein, ash, moisture, fibre, fat, and phosphorus due to the different compounds found in the antibiotics (Reid, 2007; Roy & Mráz, 2021; Najafzadeh Nobar & Safari Sinegani, 2022) [34, 36, 29]. In a diet supplemented with oxytetracycline and erythromycin antibiotics, it was shown that 1% OXY/ERY had the highest proximate nutritional content than those fed the control diet. It therefore suggests that supplementing the catfish diet with oxytetracycline and erythromycin antibiotics at a concentration of 1% OXY/ERY - 3% OXY/ERY has the ability to boost the nutritional content of the formulated feed. However, maintaining lower doses such as 1% OXY/ERY recorded the best results. It therefore implies that incorporating OXY/ERY in a commercial diet has the tendency to raise final body weight, weight gain, lower feed conversion ratio, and condition factor, boost nutrient digestibility, and maybe the health condition compared to animals given the control diet. The present results are consistent with those obtained by (Wade *et al*., 2018; Zaikina *et al*., 2022; Rohani *et al*., 2022) [44, 47, 35]. Fish fed diets supplemented with a mixture of oxytetracycline and erythromycin can improve feed utilization and digestibility parameters as signs of enhanced digestion due to the increase in levels of the proximate content of the commercial feed compared to fish fed a control diet. These findings could be explained by the findings of (Edwards *et al*., 2023; Alagbe, 2021) [14, 5], who discovered that incorporating probiotics in one's diet can aid to improve nutrient digestion efficiency. Increased or decreased enzyme activity alters the microbial metabolism, which can be accomplished by more efficient digestive processes or by fortifying digest compounds prior to digestion (Hortillosa *et al*., 2022; Jeyakumar & Lawrence, 2022) [20, 21].

Organic fertilizers are required to replenish soil nutrients and enhance plant growth (Ye *et al*., 2020; Shaji *et al*., 2021) [46, 39]. The application of fish pond effluent has greatly boosted soil nutrient levels and can also be utilized to grow crops (Wuang *et al*., 2016) [46]. It was shown in the current study that feeding the catfish diet with a mixture of oxytetracycline and erythromycin antibiotics can dramatically boost the levels of nutrient in the waste water through their enhanced feces. The data may indicate that incorporating a mixture of oxytetracycline and erythromycin antibiotics can potentially increase the nutrient enrichment of waste, which could be used to improve soil nutrient availability and support plant growth rather than discharging it into the environment, which could contribute to increased pollution of our aquatic ecosystems and the overall environmental health. Catfish effluent fed with a mixture of antibiotic-supplemented in a range of 1% OXYLERY- 3% OXY/ERY diet may improve soil conditions and stimulate rapid plant development compared to the control (Al-Wabel *et al*., 2024) [6]. This could be due to the high nutrient concentration released by the fish through their enhanced digestion and metabolism into the fish pond effluent (Santoyo *et al*., 2021; Al-Wabel *et al*., 2024) [38, 6]. Fish pond effluent contains nutrients necessary for photosynthesis and dry matter formation, as well as improvements in soil characteristics (Al-Wabel *et al*., 2024) [6]. According to Abdel-Wahed *et al*. (2018) [1], fish pond effluent contains exchangeable Ca, Mg, and K, which might raise soil pH when released. This could account for waste water nutrient enrichment in the catfish group treated with antibiotic. Furthermore, the fish group supplemented with a mixture of oxytetracycline and erythromycin pond effluent enhance the percentage of nitrogen, accessible phosphorus, and exchangeable potassium. As a result, supplementing catfish diets with antibiotic effluent as waste will no longer be a burden on environmental health, but may be effective in supporting soil conditions and possibly plant growth by potentially contributing to the growth and development of both micro and macronutrients required for plant and soil growth.

In the present investigation, the mean values of water physicochemical parameters or corresponding range values in different treatments were high but favorable for the farming of catfish fed with varying inclusion levels of antibiotic mixture (Bhatnagar & Devi, 2013; Hlaváč *et al*., 2014; Roy *et al*., 2022; Haraz *et al*., 2023) [8, 19, 36, 17]. At the end of the study, the differences in water quality indicators assessed might be connected to the harmful inorganic nitrogenous chemicals detected in the antibiotics supplied to the fish, as shown in table 3. The observed increase in conductivity, Ph, total dissolve solids, and turbidity in the different concentrations of the antibiotic inclusion level compared to the control group could be attributed to the increased protein digestion/assimilation efficacy by fish, thus increasing faecal nitrogen or ammonia entering the water (Qi *et al*., 2020; Akindele *et al*., 2022; Mansour *et al*., 2023) [33, 4, 25], supported by the lower activities of digestive enzymes and fish production in antibiotic supplemented groups during the experimental period and possibly insufficient ammonia-oxidizing bacteria populations. Total soluble solids levels during the study period represent bacterial biomass production in the antibiotic-treated group (Naiel *et al*., 2022; Asha *et al*., 2024) [28, 7]. As a result, the inclusion of a combination of antibiotics in the diet can be reduced by bacteria entering culture water via fish excreta or by antibiotics altering the microbial population, which may have resulted in water quality changes.

5. Conclusion

Based on the findings of this study, the use of antibiotics in fish culture in Ghana and around the world may be reconsidered. Catfish farming in Ghana is becoming more commercial and intensive. Antibiotics are commonly utilized on farms and have become an important component of effective commercial aquaculture production. The current study demonstrated that feeding a commercial food with a mixture of oxytetracycline and erythromycin antibiotics produced good results in proximate analysis, effluent nutrient enrichment, but reduces water quality in catfish farmed in circular tanks. Supplementing the catfish diet with oxytetracycline and erythromycin at concentrations ranging from 1% to 3% OXY/ERY considerably boosted the total proximate variables, effluent nutrient analysis, but not water quality, particularly at 3% OXY/ERY. With one health framework, waste from aquaculture farms, which has become a major contributor to resistance and pollution, may be decreased and transformed into more profitable channels by employing effluent to increase soil and crop production. As a result, all relevant stakeholders can work together to examine this from a larger perspective across all cultural systems.

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