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EFFICACY OF PROBIOTICS TO CONTROL AMMONIA IN *OREOCHROMIS NILOTICUS* FISHPONDS IN VOLTA REGION, GHANA

Current intensification of aquaculture where the organic loads with toxic compounds like NH_3 and H_2S shoot up beyond the level where naturally occurring bacteria cannot decompose the wastes has necessitated the use of probiotics. ***Objective.*** *The objective was to use five different probiotics to treat ammonia and analyze the effects on water quality and changes in it in fishponds.* ***Methods.*** *Five different probiotic compositions were used, and the water quality was measured, mainly for ammonia concentration.* ***Results.*** *5 different ponds located in the village of Agortha, Volta Region, Ghana, were treated with 5 different products simultaneously for 3 months. The concentration of ammonia has come to zero in 2 ponds with pH equal to 8.* ***Conclusions.*** *The results revealed that probiotics addition was efficient in decreasing the ammonia concentration in fishponds.*

Keywords: *ammonia control, fishponds, probiotics.*

Aquaculture is considered an emerging food production sector all over the world. When artificial food is consumed with high-protein diets, merely 50—60% of protein is assimilated, and the rest of the protein reaches the environment either through feed wastage or fecal matter, which results in production of ammonia [1]. Ammonia is one of the critical parameters in aquaculture systems. It is toxic to fish and shrimp if it gets accumulated during the production process:

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high levels of ammonia affect the physiology of the fish and shrimp making them unable to assimilate energy from the feed consumed [2]. At extremely toxic levels of ammonia, fish and shrimps tend to become lethargic and go into a moribund state finally resulting in death. Inefficient management of ammonia in the aquatic ecosystem leads to poor growth, low survival, and high FCR (Feed Conversion Ratio), which ultimately leads to a high cost of production and simultaneous losses to the farmer [3, 4].

In fish ponds, the quality of water during the production period deteriorates mainly due to feed wastage, metabolic waste accumulation, decomposition of wasted feed, and other biotic compounds [2, 5]. Probiotics, which are usually beneficial bacteria, will uptake the organic matter directly or decompose it into harmless compounds, which ultimately increases the pond water quality [6—9]. In the past few years, probiotics have brought a revolutionary change in the water quality management of fishponds by improving water quality, inhibiting pathogens, and ultimately promoting the performance of fish in terms of growth and survival [4]. A wide range of probiotics with a variety of combinations has come into availability with a specific targeted application. In the current study, we have selected five different probiotics to decrease the level of ammonia and to determine their effect on water quality in fishponds.

Materials and methods. Fish and fishponds: Fishponds located in a farm at Agortha Village near Sogakope, Volta Region, Ghana, West Africa were chosen for the present study. The earthen ponds are designated as Ponds 1,2,3,4, and 5. In these five ponds, *Oreochromis niloticus* was stocked at a stocking density of 3 fish/m^2. The ideal pond size was 0.8 Ha, and the fish number stocked in each pond was 24000. The fish was provided with supplementary feed, which was a formulated floating fish feed, at a feeding rate of 1.8—2.5% based on their average body weight. Prior to the release of fish, all the ponds were treated with zeolites, lime, etc. to stabilize the pond environment. The particulars of various products and lime used in different ponds are given in Table 1.

The study was carried out during a culture period from December 15, 2020, to March 15, 2021. The extent (water spread area) of Ponds No 1, 2, 3, 4, and 5 was 0.52. 0.52, 0.52, 0.55, and 052 Ha, respectively. The depth of the ponds ranged between 0.8—1.2 m. All the ponds were treated with various probiotics for ammonia control.

Probiotics: Commercially available probiotics manufactured by three different companies were used which contain *Nitrosomonas* sp., *Rhodobacter* sp., *Nitrobacter* sp., *Rhodococcus* sp., *Bacillus subtilis* in Product A; *Nitrosomonas* sp., *Rhodobacter* sp., *Nitrobacter* sp., *Rhodococcus* sp. and *Pseudomonas denitrificans* in Product B; *Lactobacillus acidophilus, Bacilus licheniformis, Bacillus polymyxa, Bacillus megaterium, and Saccharomyces cerevisiae* in Product C; *Nitrosomonas* sp., *Rhodobacter* sp., *Nitrobacter* sp., *Rhodococcus* sp., and *Pseudomonas* sp. in Product D; *Nitrobacter* sp., *Nitrosomonas* sp., *Aspergillus* sp., and sulfur-consuming bacteria in Product E. We used these probiotics in the corresponding ponds at a dosage recommended by the manufacturer. All the probiotics were used as a single dose application since they were target-oriented toward the control of ammonia. The probiotic dosage was1 kg/Ha, and in most of the ponds, they were tested at a dose of 500 g/pond.

Water samples: Physiochemical parameters of water were studied on a daily basis until 5 days from the date of application. The physiochemical parameters such as temperature, TDS, ORP, Salinity, pH, EC, SG, DO, and ammonia were measured over a period of time. The first sample was collected before the application date, and later on water samples were collected on the 1st, 2nd, and 4th day according to the recommendation of the formulator of probiotics.

The correlation analysis for previous verification of normal distribution (Kolmogorov-

Table 1. **Mean of the results for parameters measured in experimental units of the present study (results are in triplicate)**

Parameters	Pond No-1 / SP06 — Product A				Pond No-2 / SP09 — Product B				Pond No-3 / SP16 — Product C				Pond No-4 / GP12 — Product D				Pond No-5 / SP12 — Product E			
	Day 0	Day 1	Day 2	Day 4	Day 0	Day 1	Day 2	Day 4	Day 0	Day 1	Day 2	Day 4	Day 0	Day 1	Day 2	Day 4	Day 0	Day 1	Day 2	Day 4
pH	9.2	9.2	8.8	8.9	8.8	8.8	8.2	8.5	6.1	6.1	6.3	6.2	6.2	6.2	6.5	6.4	7.6	7.8	7.5	7.5
ORP (mV)	130	186	190	190	170	170	190	186	287	289	268	275	273	268	280	296	209	220	226	216
NH_3 (PPM)	0.5	0.5	0.5	0	0.5	0.5	0.25	0	0.25	0.25	0.25	1	8	8	8	8	4	4	4	4
Temperature (°C)	29.3	29.3	29.2	29.3	29.4	29.3	29.2	29.3	28.7	28.9	29	28.8	29.2	29.3	29	29	29.1	29.3	29	29.1
EC (µS/cm)	2900	2800	2800	2760	2260	2200	2150	2150	1760	1700	1650	1650	1990	1900	1850	1850	3860	3850	3700	3600
TDS (PPM)	1450	1400	1400	1380	1130	1100	1075	1075	878	850	830	830	990	950	930	930	1930	1925	1850	1800
SG (kg/m)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
DO 3 Am (PPM)	2.9	2.6	3.8	4.1	2.1	1.9	2.8	3.2	1.6	1.6	1.9	1.9	1.4	1.3	1.9	2.1	3.1	3.3	3.4	3.6
DO 4 Pm (PPM)	11.4	9.6	8.8	8.8	9.9	6.8	7.5	7.9	5.6	5.5	5.6	6.1	3.6	3.7	4.2	4.4	4.5	4.8	4.6	4.7

Smirnov test) and variance homogeneity (Bartlet test [10]) as well as the software R [11] were applied for the obtained data analysis.

Results. Ammonia exists in either of two forms, un-ionized and uncharged form (NH_3), or as a positively charged ammonium hydroxide ion (NH_4+) when it dissolves in water [12]. The form that ammonia takes in water is highly dependent on the temperature and pH level of the water. The toxicity of ammonia is primarily attributed to the un-ionized form (NH_3). In general, high NH_3 concentration with more toxicity exists at high pH values. The relation between ammonia toxicity in relation to pH and temperature is shown in Table 1. The differences in ammonia concentrations observed on Day0 for all treatments, perhaps, would be to the marked environmental heterogeneity of available studied ponds.

During the short period of study, the variation in the water temperature was normal for tropical water and did not cross 32 °C in any of the ponds at any point of time, which can be seen in Table 1, and the amplitude of variation was low. There is a significant positive relationship between pH and DO (dissolved oxygen (r^2 =0.650; P < 0.050 at 3 AM and r^2 =0.810; P < 0.010 at 4 PM). It should be noticed that this higher value of pH is due to greater photosynthetic activity from abundant plankton in the pond water which also increases the DO of pond water during the daytime.

The oxidation-reduction potential (ORP) measures the ability of a water body to break down waste products such as contaminants and dead plants and animals. When ORP is high, it means that a lot of oxygen is present in the water. But here we got a negative relationship between ORP and DO levels recorded at 3 am (r^2 = —0.600; P < 0.050) and 4 pm (r^2 = —0.780; P < 0.010), which means that the higher ORP, the lower DO levels in the pond. A negative correlation is observed between pH and ORP in the ponds (r^2 = —0.950; P < 0.010), which means that the higher pH, the lower the ORP levels and vice versa. On a general trend in almost all trials, the ORP levels increased after the application of probiotics where the ammonia concentration came to zero, whereas in the other ponds, the ORP was either stable or only slightly increased with no reduction of ammonia concentration.

Electric conductivity (EC) and total dissolved solids (TDS) represent the mineral content in water and hence they exhibit a significant positive relationship in the ponds ($r^2 = 0.99$; $P < 0.01$). The higher values of conductivity were noticed in ponds with the higher salinity or more turbidity. The presence of highly soluble inorganic salts is responsible for the increase of conductivity in the water and also can be one of the reasons for high EC in some ponds. No significant correlation was found between pH and EC ($r^2 = 0.480$; $P > 0.050$). A negative correlation ($r^2 = —0.500$; $P > 0.050$) is observed between ORP and EC.

As for the whole trend of parameters, when the probiotic is applied to a pond, they are better in ponds with pH above 8.0, and their efficiency is less in low pH ponds. In ponds where we got good results for the reduction of ammonia, there was a significant drop in pH (>0.4) on the 2nd day and there was a significant increase in ORP (> 20 mV) except in product C. In addition, there was observed a significant drop in oxygen after the 1st day of application (0.2—0.3 ppm), where in the case of Product C, there is no change and in Product E, there is a slight increase in DO. Therefore, we can assume that the activation of probiotics, or the viable count of these products, is poor in water with low pH.

A negative correlation exists between ammonia and temperature ($r^2 = 0.050$; $P > 0.050$), which is almost insignificant. A positive correlation ($r^2 = 0.520$; $P < 0.050$) was found between ORP and ammonia and a negative correlation ($r^2 = —0.520$; $P < 0.050$) between pH and ammonia.

Discussion. Commercial aquaculture operations with high stocking densities of fish and feeding it with high protein content feeds gradually deteriorate the quality of water and bottom soil, which leads to the proliferation of pathogenic organisms like fungi, bacteria, and protozoans [13—15]. In addition, this creates stress on the fish and, ultimately, they will be subjected to diseases as well. In this study, the main studied parameter is the content of ammonia, which is very lethal to fish [2]. Ammonia can be observed in the ponds even from the beginning of culture, which can be due to the previous crop wastage if it was not dried or treated well. Ammonia in cultured ponds over a period of time keeps accumulating with the amount of feed used and from its wastage, fecal matter, dead fish, and decomposed organic matter [16]. However, if the natural microbes involved in the nitrogen cycle are present in the pond water, the accumulation is not high [8]. In another way, if the microbial colonies are fit enough to complete the nitrogen cycle, then we do not observe ammonia in the pond [17]. The results obtained in the present study denote an increase in water quality probably due to the addition of probiotics.

Usually, in fishponds, feed wastes and excreta increase the ammonia concentration, which is harmful to fish above 0.1 ppm, hence they are considered critical water parameters which are to be managed at the optimum level [18]. Oxidation of ammonia to nitrite by bacteria (*Nitrosomonas* sp.) and oxidation of nitrite to nitrate by other bacteria (*Nitrobacter*) are the principle stages of the nitrogen cycle, which means that the presence of these two bacteria is inevitable to convert and eliminate bacteria from the aquatic ecosystem [3, 19—23]. The use of probiotics for *O. niloticus* aquaculture and their efficient role in ammonia removal was observed using *B. subtilis* in [24], and *B. cereus, B. amyloliquefaciens*, and *Pseudomonas stutzeri* in *O. niloticus* aquaculture [19]. On this basis, the present study and literature antecedents support a conclusion that the addition of probiotics can reduce the ammonium concentration in fish ponds.

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ЕФЕКТИВНІСТЬ ЗАСТОСУВАННЯ ПРОБІОТИКІВ ДЛЯ КОНТРОЛЮ РІВНЯ АМІАКУ В РИБНИХ СТАВКАХ OREOCHROMIS NILOTICUS В РЕГІОНІ ВОЛЬТА, ГАНА

Інтенсифікація аквакультури, де органічне навантаження токсичними сполуками, такими як NH_3 і H_2S, перевищує рівень, при якому природні бактерії можуть розкладати відходи, призвела до необхідності застосування пробіотиків. **Мета** роботи полягала у використанні п›яти різних пробіотиків для зниження рівня аміаку та визначенні їх впливу на якість води у рибних ставках. **Методи.** У цьому дослідженні було використано п›ять різних композицій пробіотиків і виміряно якість води під їх впливом, головним чином, з точки зору концентрації аміаку. **Результати.** Для цього дослідження були оброблені різних ставків, розташованих у селі Агорта, регіон Вольта, Гана, п'ятьма різними продуктами одночасно протягом трьох місяців. Концентрація аміаку впала до нуля у двох ставках, pH в яких був вище 8. **Висновки.** Результати показали, що додавання пробіотиків є ефективним засобом для зниження концентрації аміаку в рибних ставках.

Ключові слова: *контроль рівня аміаку, рибні ставки, пробіотики.*