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**EXPERIMENT TO FIND OUT PROBABLE  
SOURCE OF NITROFURAN  
METABOLITES IN FARMED PRAWN  
(GOLDA) IN BANGLADESH**

**Dhaka, Bangladesh  
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## **EXPERIMENT TO FIND OUT PROBABLE SOURCE OF NITROFURAN METABOLITES IN FARMED PRAWN (GOLDA)**

### **Summary**

As one of the activities included in the National Action Plan to combat antibiotic contamination in farmed prawn (*golda*), the USAID-funded PRICE project designed an experiment to identify the probable source of the nitrofurans metabolites that are leading to rejection of prawn exports to Europe. The experiment was implemented with the close and invaluable collaboration of the Bangladesh Fisheries Research Institute (BFRI) and the Department of Fisheries (DOF).

The experiment was conducted in BFRI ponds located in Cox's Bazar, using 12 different types of feed in 12 different but identical ponds. Significant presence of nitrofurans metabolites was found after three and a half months in *golda* fed with shrimp feed, poultry feed and – to a lesser degree - with fish feed, as well as in *golda* fed with prawn shells. On the contrary, no nitrofurans metabolites were detected in *golda* which received no supplemental feed or were fed with snail meat, poultry droppings, cow dung or fertilizer. After six months, *golda* nourished with supplemental feed and with prawn shell still showed presence of nitrofurans metabolites, although in smaller quantities.

The final stage of the experiment consisted in trying to determine if the nitrofurans metabolites would be eliminated by the live *golda* if the offending feed was eliminated from its diet. After having eliminated shrimp feed from their diet for five days, *golda* still exhibited the presence of nitrofurans metabolites, but these had disappeared after 10 days. The same occurred in the case of fish feed.

The results of this experiment – although they require corroboration – suggest two important conclusions of relevance to the shrimp industry:

1. Nitrofurans metabolite contamination probably originates in certain shrimp, poultry and fish feed.
2. It may be possible to eliminate nitrofurans metabolite contamination in live prawns by modifying their diet sufficient number of days before harvesting.

## **Detailed report**

As agreed under National Action Plan (NAP) to conduct an experiment to trace probable sources of nitrofurans in farmed prawn (golda), PRICE explored the probable sites where a joint experiment could be conducted jointly by USAID/PRICE and MoFL (DoF and BFRI). It was difficult to find identical sized, similar amount of water holding capacity based series of mini-ponds in a specific location that may be refilled and drained at will. Based on visit by COP and TLA of PRICE to Cox's Bazar, the mini-pond complex of Marine Fisheries and Technology Station (MFTS), BFRI was considered suitable.

Initially, discussion was made with Station Chief of MFTS, BFRI, Cox's Bazar whether MFTS can spare no less than 16 mini-ponds, at least for 6 months, without seriously hampering their routine activities. The CSO of MFTS, BFRI realizing that this is a joint program with MoFL and for national interest, agreed to provide 16 ponds for the experiment on condition that DG, BFRI agrees with the proposal. However, CSO mentioned that during rainy season it will be difficult to replace rearing animals to other ponds, as well as draining ponds and bottom treatments. TLA of PRICE then discussed with DG, BFRI who agreed/supported the initiative and jointly drafted a MoU that was ratified by MoFL by July 15, 2009. On July 21, 2009 the MoU was signed between PRICE and BFRI.

While the discussion with BFRI was in progress and official MoU signing was underway, DG, BFRI verbally instructed CSO of MFTS in Cox's Bazar to assist on the NAP on experiment initiative and accordingly, MFTS Cox's Bazar collected most of the aquatic organisms from the said 16 ponds and either replaced those to other ponds, stored, preserved or sold. The ponds were then drained out, left-over fish were killed by application of rotenone, and bottom debris was partially removed. All ponds were ready by the fourth week of July, 2009.

After signing of MoU by COP, PRICE and DG, BFRI on July 21, 2009, TLA of PRICE went to Cox's Bazar on 22<sup>nd</sup> July. He facilitated surrounding all ponds with fine-meshed synthetic nets, leveled water height similar in all ponds, marked all ponds with mini-sign boards based on different treatments, collected different inputs to be tested under farming, trained MFTS staff and hired personnel on the experimental methodologies.

On early morning, July 25, in presence of BFRI, DoF and PRICE representatives, pre-nursed golda PLs were stocked in experimental ponds. The stocking density was maintained at a rate of 5 PL/m<sup>2</sup> so that sufficient samples could be raised for testing ultimately by LC-MS/MS. Nine test trails with replications were conducted with different suspected ingredients: fish feed, shrimp/prawn feed, poultry feed, poultry droppings, snail meat, etc. All but two test groups had replications.

MFTS, BFRI at Cox's Bazar supervised the experiment and round the clock observation was ensured with the 6 month assignment of three staff to provide inputs/feeds/etc in specified time and look after farming management strictly under isolation on test-trial basis. All efforts were made to prevent cross-contamination among the test trails.

Adequate measures were taken to prevent submerge/flooding of ponds due to torrential rain or heavy downpour within a short time. Standby facilities were in place to discard water if it rose above a set level, or pumping water in, if it dropped below the required level.

A supplemental feed was manufactured incorporating powder of prawn shell. Prawn shell was collected from the Khulna region, especially from farmed origin. The shells were dried in sunlight and further dehydrated in an oven overnight at 60°C to make it fragile, and then milled with a high-speed blender to make it powder.

The prawn shell powder was fed to one group. Another group of prawn was fed with snail meat. The rest of the tests included no-supplemental feed and fertilization with liming only; no-supplemental feed but fertilization by NPK; no-supplemental feed but fertilization with cow dung; no-supplemental feed but fertilization with poultry droppings. Test feeds included fin fish feed, prawn/shrimp feed and poultry feed. All tests were with replication, except those with snail meat and prawn shell.

On 28 August, prawns in experimental ponds were observed by partial netting. Prawns under feeding by supplemental feeds showed relatively better growth compared to ones without supplemental feed, fertilized by cow dung or poultry droppings. Almost all ponds showed the presence of tiny tilapia, despite the repeated use of rotenone. The tilapia was removed manually, as much as possible.

In spite of the size variations, survival rates appeared excellent. Prawns were routinely observed during the daytime by using feed trays, and at night using torch-light. 24-hour presence by the side of the experimental ponds was ensured.

## **Nitrofurantoin Test on Farmed Golda**

- **First Metabolite Test on Farmed Prawn at FIQC**

At the end of three and a half months of the experiment the first sample was collected from the entire test rearing, to be sent to FIQC Dhaka Laboratory, DoF for nitrofurantoin test. On November 11, samples from all tests were collected, washed, weighed and quickly frozen with a code. Some natural golda were also collected earlier from distant farming zones and were frozen as well, to be used as a control group.

On November 12, 2009 frozen samples together with some natural golda in insulated boxes were airlifted to Dhaka and handed over to the officer in charge of the FIQC Laboratory. The laboratory was requested to conduct only nitrofurantoin metabolites analysis in the supplied samples.

The result of this first test is summarized in Table 1:

**Table 1. Test Result for Prawn Harvested after 3 ½ months**  
(Type of Product: Farmed and Natural Golda)

Sample code	PRICE Code	Source /treatment	Nitrofurans metabolites. ppb (Parts per billion)			
			AMOZ	AOZ	AHD	SEM
1	N-1	Natural -1	ND*	ND	ND	ND
2	N-2	Natural-2	ND	ND	ND	ND
3	SM	Snail Meat	ND	ND	ND	ND
4	SF	Shrimp Feed	ND	<b>0.28</b>	<b>3.40</b>	<b>7.83</b>
5	FF	Fish Feed	ND	ND	ND	<b>3.57</b>
6	PF	Poultry Feed	ND	<b>0.2</b>	<b>8.62</b>	ND
7	PD	Poultry dropping	ND	ND	ND	ND
8	CD	Cow Dung	ND	ND	ND	ND
9	NPK	Urea, TSP, Potash	ND	ND	ND	ND
10	NS	No supplement	ND	ND	ND	ND
11	FS	Feed with Prawn Shell-1	<b>0.32</b>	<b>0.25</b>	<b>2.08</b>	<b>5.01</b>
12	FS	Feed with Prawn Shell-2	ND	<b>0.33</b>	<b>2.37</b>	<b>4.79</b>

\*ND: Not detected

Source: FIQC Dhaka Laboratory

- **Second Metabolite Test on Farmed Prawn at FIQC**

At the end of 6 months, the experiment to detect probable source of nitrofurans metabolites was terminated and samples were collected from each test rearing for the determination of level of nitrofurans metabolites in raised prawn fed on diverse feeds or without feed supplements. The collected samples were then sent to FIQC Dhaka Laboratory, DoF for nitrofurans metabolite test.

On January 25, 2010 samples from all tests were collected, washed, weighed and quickly frozen with a code. Some natural golda were also collected earlier from distant sources and frozen as well, to be used as a control group.

On January 27 frozen samples in insulated boxes were transported to Dhaka and handed over to the officer in charge of the FIQC Laboratory. The laboratory was requested to conduct only nitrofurans metabolites analysis in the supplied samples.

The summary of this second group of tests is presented in Table 2:

**Table 2. Test Result for Prawn Harvested after 6 months**  
(Type of Product: Farmed and Natural Golda)

Sample code	PRICE Code	Source /treatment	Results on PPB			
			AMoz	AOZ	AHD	SEM
1	NS-1	No supplement	ND*	ND	ND	ND
2	NS-2	No supplement	ND	ND	ND	ND
3	SM-1	Snail Meat	ND	ND	ND	ND
4	SF-1	Shrimp Feed	<b>0.37</b>	ND	ND	<b>0.31</b>
5	SF-2	Shrimp Feed	<b>0.20</b>	ND	ND	<b>0.26</b>
6	PF-1	Poultry Feed	<b>0.26</b>	ND	ND	ND
7	FF-2	Fish Feed	ND	ND	ND	<b>0.25</b>
8	PF-1	Poultry Feed	<b>0.24</b>	ND	ND	ND
9	CD	Cow dung	ND	ND	ND	ND
10A	NPK	Fertilization	ND	ND	ND	ND
10B	NPK	Fertilization	ND	ND	ND	ND
11A	N	Natural-1	<b>0.29</b>	ND	ND	ND
11A	N	Natural-2	ND	ND	ND	ND
12	PD	Poultry droppings	ND	ND	ND	ND
13	PS	Prawn Shell-1	ND	<b>0.32</b>	ND	ND
14	PS	Prawn Shell-1	<b>0.51</b>	<b>0.33</b>	ND	ND

\*ND: Not detected

- **Removal of Metabolite from farmed prawn and its Test at FIQC**

At the later stage of the 6 month experiment, based on initial finding after 3 and half month rearing, it was decided to set up another study with contaminated live prawns to explore the possibility whether contaminated prawns can be subjected to a test by withdrawing feeds that caused accumulation of nitrofurantol metabolites.

It was decided to set up an experiment with three mini-ponds so that contaminated prawns from three tests that showed presence of nitrofurantol metabolites could be used to see whether it is possible to remove accumulated nitrofurantol metabolites from contaminated live prawn. The recovery test of metabolites was designed by withdrawing the feeds that led to the contamination.

Therefore, it was necessary to prepare some feed that was free from probable nitrofurantol parent drugs or metabolites. One feed was prepared using dry fish meal exclusively of marine sources.

Contaminated prawn fed on fish, shrimp and poultry feeds were pooled together and distributed among the three ponds. A few prawn survived in groups fed with lab made feed incorporated with shell powder of prawn and these prawns were pooled with shrimp feed group. These prawns were then fed with a laboratory made feed free from probable nitrofurantol drug or metabolites.

During the 6 month experiment no artificial aeration was used in any experimental ponds. However, in the recovery tests three paddle wheels were used in the three ponds to ensure that no accidental mortality occurs. Also, to control planktonic feeds in the test culture, plankton-feeding fin fish were used, together with contaminated prawns.

### Sampling from nitrofurans metabolites removal test

After setting the experiment in new ponds with contaminated prawns and withdrawing contaminated feeds and introducing laboratory feed free from nitrofurans parent drugs and metabolites, sampling was done at 5-day intervals.

Initial three batches of samples, i.e, 5, 10 and 15 day of withdrawal of contaminated feeds, were sent to FIQC laboratory in Dhaka. The result of the test is summarized in Table 3, shown below.

**Table 3. Nitrofurans Metabolite Contamination after Withdrawal of Contaminated Feed  
(Type of Product: Farmed and Natural Golda)**

Sample code	PRICE Code	Source /treatment	Results on PPB			
			AMAZ	AOZ	AHD	SEM
1	SF0	<b>Shrimp feed</b> 5 Day Withdrawal	<b>0.46</b>	ND*	ND	<b>0.26</b>
2	SF5	<b>Shrimp feed</b> 10 Day Withdrawal	ND	ND	ND	ND
3	SF10	<b>Shrimp feed</b> 15 Day Withdrawal	ND	ND	ND	ND
4	PF0	<b>Poultry Feed</b> 5 Day Withdrawal	<b>0.25</b>	ND	ND	ND
5	FF0	<b>Fish Feed +Prawn Shell</b> 5Day Withdrawal	<b>0.28</b>	ND	ND	ND
6	PF5	<b>Poultry Feed</b> 10 Day Withdrawal	ND	ND	ND	ND
7	PF10	<b>Poultry Feed</b> 15 Day Withdrawal	ND	ND	ND	ND
8	FF5	<b>Fish Feed +Prawn Shell</b> 10 Day Withdrawal	ND	ND	ND	ND
9	FF10	<b>Fish Feed +Prawn Shell</b> 15 Day Withdrawal	ND	ND	ND	ND
10	SF15	<b>Shrimp feed</b> 20 Day Withdrawal	ND	ND	ND	ND

11	PF15	<b>Poultry Feed</b> 20 Day Withdrawal	ND	ND	ND	ND
12	FF15	<b>Fish Feed Fish Feed</b> <b>+Prawn Shell</b> 20 Day Withdrawal	ND	ND	ND	ND

\*ND: Not detected

### **Inconsistencies in nitrofurans metabolites measurements**

After three & half month of rearing, presence of different forms of nitrofurans metabolites (AMOZ, AOZ, AHD and SEM) was found in prawns fed with supplemental feeds prepared in Lab with powdered prawn shell. The group fed with commercial shrimp feed showed the presence of AOZ, AHD and SEM. The prawn fed with fish feed showed only the presence of SEM. The group fed with poultry feed detected AOZ and AHD.

All other groups of prawn fed with natural feeds like snail meat or group without feeds or fertilization, groups raised by fertilization with lime and NPK, poultry droppings, or cow dung did not show presence of any form of nitrofurans metabolites.

It was decided to continue the experiment by further culturing the prawns with identical feeds, and samples were again tested after 6 months of rearing. The presence of nitrofurans metabolites were found in test animals fed on lab-made feed with shell powder (AMOZ and AOZ), fish feed (SEM), shrimp feed (AMOZ & SEM), and poultry feed (AMOZ).

At the end of 3 and half months rearing, no AMOZ was detected in groups fed on shrimp, fish or poultry feed, but at the end of 6 month culture the presence of AMOZ was detected in all these three groups, together with a sample of natural prawn collected through WFC. But no AOZ or AHD were traced in groups fed with shrimp and poultry feeds.

The levels of AHD and SEM after three & half month in the group with shrimp feed were high, but after 6 month AHD was not detected and SEM level was low. Similarly, the fish feed group showed high level of SEM after 3 month, but low level after 6 months. The poultry feed group showed high level of AHD after 3 months, but no traces of it after 6 months. The group fed with lab made feed + prawn shell powder showed no presence of AHD and SEM, though they showed high level of these after 3 month.

The recovery test is still continuing. We are collecting samples by 5 day intervals. First 4 batches of sample (5, 10, 15, 20 day of withdrawal of contaminated feeds) analysis indicated the presences of metabolites upto 5 day (AMOZ only) in all three groups. However, no samples showed any indication of metabolites presence in 10, 15 or 20 day after withdrawal of fish, shrimp and poultry feeds.

**Though the results received from LC-MS/MS testing on nitrofuran metabolites is inconsistent in two tests conducted in 3 and 6 month reared prawns in terms of the level & type of metabolites, it is significant that once the suspected feeds were withdrawn and the prawns were fed with clean feed no presence of nitrofuran metabolites was detected 10 days after withdrawal of the suspected feeds.**

### **Important Observation**

It is important to ensure that feed are free from parent drugs. However, in case prawns become contaminated with nitrofuran metabolites despite all precautions, it may be possible to remove metabolites with farming manipulation /withdrawal of contaminated feed.

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