



Study Report

A study on the impact of different feeding management strategies using Bioful Aqua on the mortality in rainbow trout, *Oncorhynchus mykiss*, after challenge with *Aeromonas salmonicida*.

Simuna Ivax, Bioful, Fisheries Information Centre

August 2020



Contents

Evaluation on the impact of different feeding management strategies using Bioful Aqua on the mortality in rainbow trout, *Oncorhynchus mykiss*, after challenge with *Aeromonas salmonicida*.

Study Report (August 2020)	1
Contents	2
1. Introduction to the AquaBioTech Group	3
2. Aim of the study	4
3. Materials and Methods	5
3.1. Feeding phase	5
3.2. <i>Aeromonas salmonicida</i> challenge	6
3.3. Statistical analysis	8
4. Results and Discussion	9
4.1. Weights, SGR, FCR and mortalities	9
4.2. <i>Aeromonas salmonicida</i> challenges	13

1. Introduction to the AquaBioTech Group

The **AquaBioTech Group** (**AquaBioTech Limited** - C22950) is an international aquaculture, fisheries and marine consultancy and engineering company with its headquarters based in Malta and offices in five other countries, although our work is truly global with clients and projects in over fifty five countries. Each department within the **AquaBioTech Group** has a specialist field of work, although all are in some way related to aquatic sciences, engineering and technology. The **AquaBioTech Group** is primarily involved in aquaculture, fisheries, environmental and other marine / oceanographic projects.

Individual staff at the Research and Development (R&D) facility, **ABT Innovia**, within the **AquaBioTech Group**, have extensive experience in Europe, the Mediterranean, Africa and Middle East aquaculture, bringing a range of skills and experiences to the table. As well as laboratory based R&D, the company has undertaken projects in many other regions such as Scandinavia, CIS countries, South America, Asia, Japan, Australasia and the United States of America. Our involvement in projects includes feasibility assessments for fish and shrimp farms; project design, construction, implementation & operational management; construction & management; implementation of sea based trials; feasibility assessments and market research studies for aquaculture products, fish or shrimp farms.

2. Aim of the study

To evaluate the potential impact of different feeding management strategies using Bioful Aqua on the mortality in rainbow trout, *Oncorhynchus mykiss*, after challenge with *Aeromonas salmonicida*.

3. Materials and Methods

The trial was structured into two phases, a feeding phase during which the fish were fed their respective diets, and a challenge phase during which the fish were exposed to *Aeromonas salmonicida* at different doses.

3.1. Feeding phase

3.1.1 Feeding phase: experimental system

The experimental system used in this experiment was housed in its own dedicated room, with limited and strictly controlled access to staff. The experiment was carried out in a recirculating aquaculture systems (RAS) with 9 500L culture tanks, drum filter, protein skimmer, UV and ozone sterilisation and biofilter. The average water temperature during the experiment was 15.7° C and oxygen content >7.5 ppm.

3.1.2 Experimental fish

Rainbow trout (*Onchorhynchus mykiss*), of about 7 g were used at the start of this trial. Fish were netted from the holding tanks, graded, and randomly allocated to the trial tanks to achieve a start number of 100 fish per tank. A backup batch of fish (feeding phase started 1 week later) were used following the same experimental condition to ensure a second main challenge could be carried out.

At the start of the feeding phase of the experiment, all the fish were weighed in bulk. Weights of all fish in bulk per tank were recorded after 10 days and at the end of the feeding phase that lasted for 21 days prior to challenge.

Water quality parameters were recorded daily for the duration of the study.

3.1.3 Feeds and feeding

During the feeding phase of the experiment, fish were fed just below satiation by hand three times a day using experimental feeds of 1.5mm followed by 2mm size pellets, according to

fish size. The experimental design for the two batches were based on a standard feed or the standard feed coated with Bioful Aqua as follows:

Batch 1 (4 tanks of 100 fish each)

- CTRL: standard feed only (Alltech Coppens, The Netherlands);
- 3WPT 0.5%: standard feed coated with Bioful Aqua at 0.5% inclusion;
- 3WPT 0.25%: coated with Bioful Aqua at 0.25% inclusion;
- 3WPT 0.5% + bath treatment: coated with Bioful Aqua at 0.5% inclusion, followed by a bath treatment 2 days prior to challenge at 1% for 15 minutes.

Batch 2 (3 tanks of 100 fish):

- CTRL: standard feed only (Alltech Coppens);
- 3WPT 0.5%: coated with Bioful Aqua at 0.5% inclusion;
- 3WPT 0.25%: coated with Bioful Aqua at 0.25% inclusion;

The feeds with the different inclusion levels of the product were prepared as follows:

- Non-chlorinated clean water at room temperature and pH adjusted with citric acid to 6.0 - 6.4 was used.
- Bioful preparation shaken and added to prepared water at the required inclusion rate. The solution was held at room temperature.
- After waiting for at least 3 hours, a sprayer was used to spray the prepared solution onto the feed and feed thoroughly mixed. All the tools that were in contact with the Bioful solution were cleaned completely before making a dilution and application.

The control (non-treated) feed was sprayed as above, except that instead of adding Bioful, an equivalent volume of pH 6.0-6.4 water was added to the final mix.

3.2. *Aeromonas salmonicida* challenge

3.2.1 Experimental system

The experimental system used in these challenges was housed in its own dedicated room, with strictly controlled and limited access to challenge staff. The experiments were carried out in tanks of 40L forming part of a RAS with UV, ozone sterilisation and biofilter.

3.2.2 Challenge procedure

After the conclusion of the feeding phase, 90 fish from each tank were moved to a challenge bay and inoculated with the pathogen. The two batches were subjected to a different experimental procedure as followed:

- Batch 1: 90 fish from each tank were split in three replicate tanks of 30 fish each and inoculated with *Aeromonas salmonicida* by intraperitoneal injection (IP) 0.05 ml at 8×10^6 CFU/fish;
- Batch 2: 90 fish from each tank were split in 3 replicates tanks of 30 fish each and inoculated with *Aeromonas salmonicida* by intraperitoneal injection (IP) 0.05 ml at two different doses: 15 fish with 8×10^6 CFU/fish (tank 3 and tank 4 had only 10 and 9 fish respectively); 15 fish with 8×10^5 CFU/fish; all fish were colour tagged. After the inoculation, 330 ml of Bioful Aqua were added to each tank; the following day an additional quantity of 100 ml of Bioful Aqua was added to each tank.

Fish were offered their respective diets during the challenges.

Mortalities were followed daily during the challenge period.

3.3. Statistical analysis

Growth and feed performances were determined using the following equations:

Specific growth rate, SGR (% body weight gain per day) = $(\ln W_t - \ln W_0)/\text{days} \times 100$

Where W_t = weight at end of trial in g and W_0 is weight at the beginning of the trial in g.

Feed Conversion Ratio, FCR = Total feed intake (g)/body weight gain (g).

Results of the final main challenge mortalities were analysed using ANOVA. A significance level of $p < 0.05$ was used.

4. Results and Discussion

4.1. Weights, SGR, FCR and mortalities

The results of the average weights of batch 1 and 2, SGR, FCR, feed intake and survival are presented in Table 1.

The overall growth of Batch 1 followed a similar trend among all treatments with a slightly higher growth recorded in fish fed 3WPT 0.25%. The SGR showed a higher value in fish fed 3WPT 0.25%, while the other three treatments had similar values.

The FCR was better with 3WPT 0.25% with a value lower than the other treatments while the FCRs of fish fed CTRL and 3WPT 0.5% were close to each other.

Regarding Batch 2, the overall growth was also better with 3WPT 0.25%; this treatment also showing a higher SGR and a lower FCR than the other treatments.

The results of feed intake and survival rate during the growth phase were quite similar.

Across the two batches, fish fed 3WPT 0.25% had a 6.5% better SGR and a 7.5% better FCR than the fish in the CTRL tanks. No statistics could be done on the results of the two batches; however, if the SGR and FCR results of 3WPT 0.25% are indexed against the results obtained by fish fed Ctrl for the two batches, significant values of 0.044 and 0.028 respectively are obtained.

The results are presented graphically in figures 1 to 6.

Table 1. Results of growth, SGR, FCR, feed intake and survival of the different treatments in batch 1 and 2.

Fish batch	Batch 1				Batch 2		
Treatment	CTRL	3WPT 0.25%	3WPT 0.5%	3WPT 0.5% + BT	CTRL	3WPT 0.25%	3WPT 0.5%
Initial Aw (g)	7.22	7.18	7.22	7.20	6.64	6.60	6.64
Final Aw (g)	13.34	13.90	13.41	13.44	11.40	11.64	11.18
SGR (%/day)	2.92	3.15	2.94	2.97	2.57	2.70	2.48
Feed intake (g)	424	425	435	430	384	384	384
FCR	0.69	0.63	0.70	0.69	0.81	0.76	0.85
Survival %	100.00	100.00	99.00	100.00	100.00	100.00	100.00

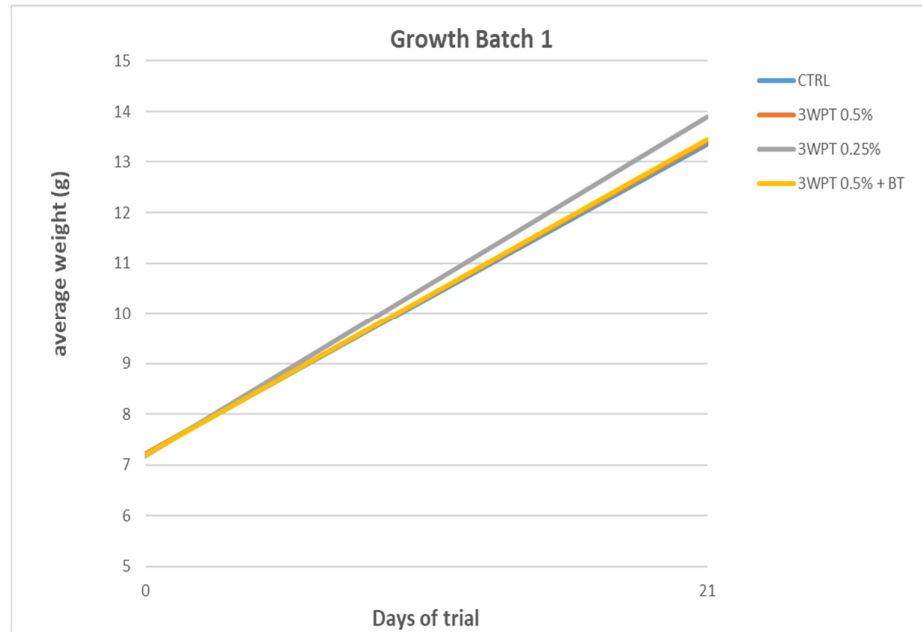


Figure 1. Fish growth of batch 1 during the growth phase

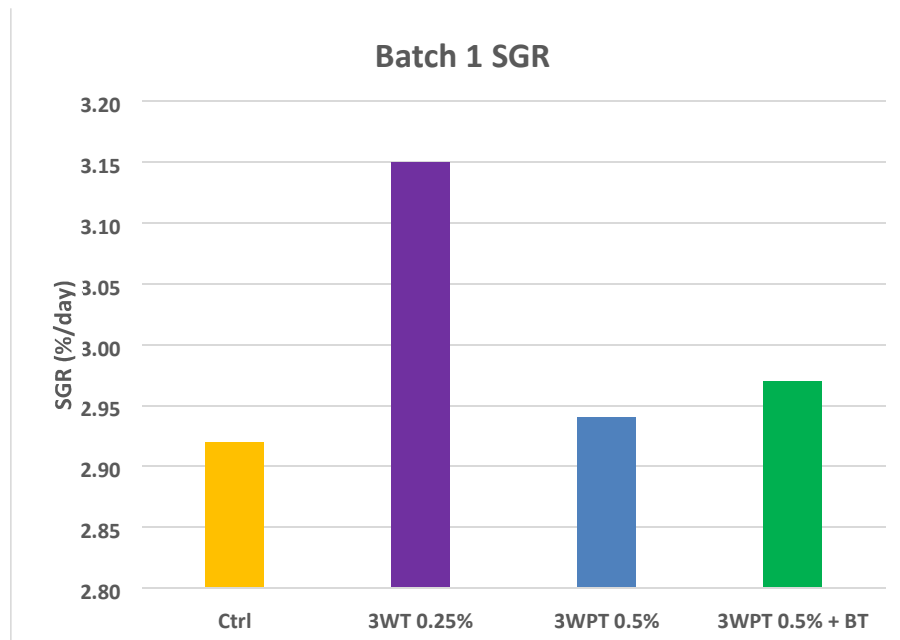


Figure 2. SGR (%/day) of batch 1 during the growth phase.

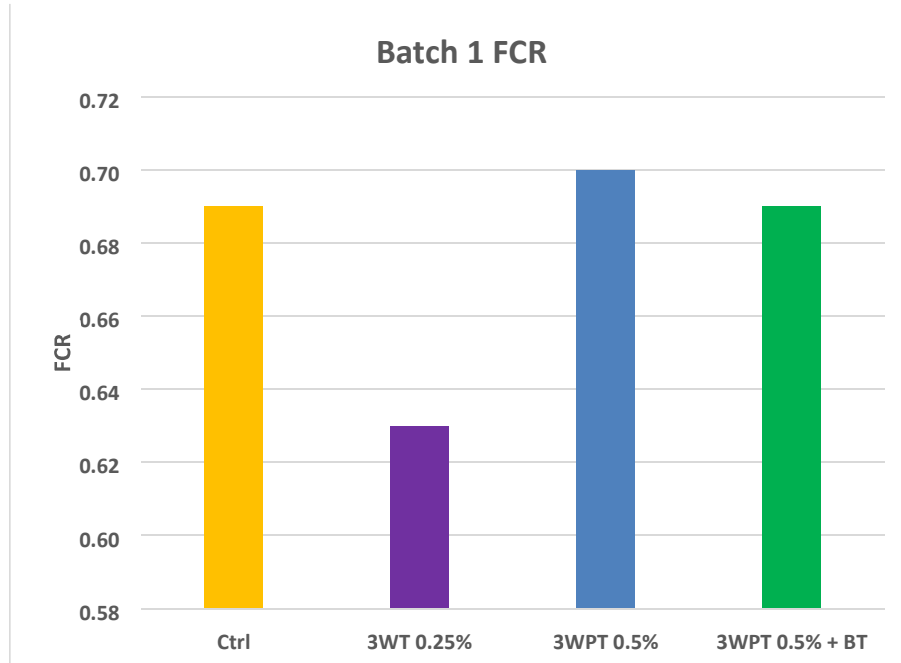


Figure 3. FCR of batch 1 during the growth phase.

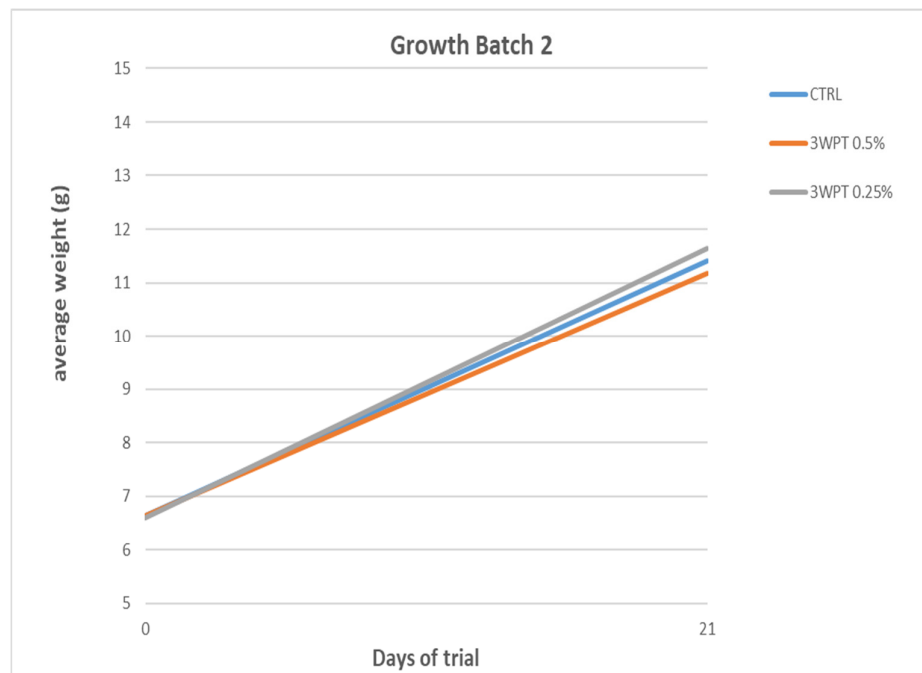


Figure 4. Fish growth of batch 2 during the growth phase

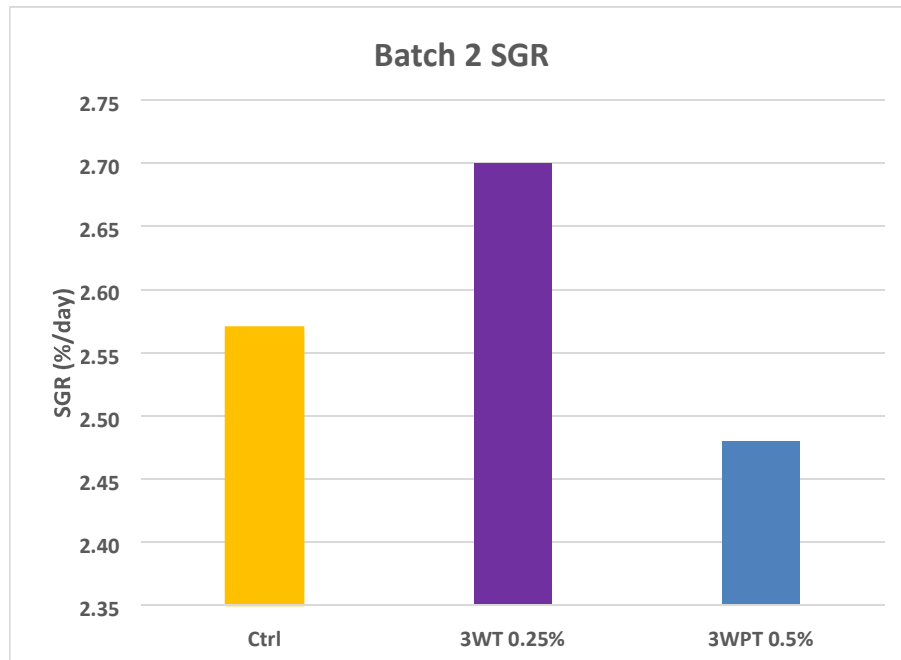


Figure 5. SGR (%/day) of batch 2 during the growth phase.

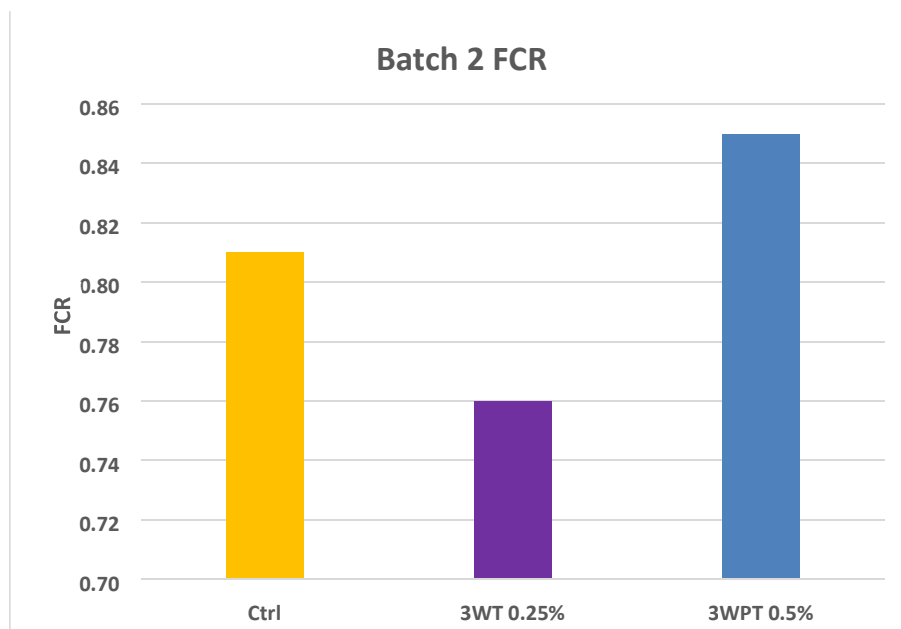


Figure 6. FCR of batch 2 during the growth phase.

4.2. *Aeromonas salmonicida* challenges

The results of the mortality data at the end of the two main challenges are reproduced in Table 2 and 3 respectively and graphically showed in figures 7, 8 and 9 respectively (day by day mortalities are shown in Appendix 5).

Table 2. Results of final accumulated mortalities of Challenge Batch 1 with *Aeromonas salmonicida*. Results are presented as means with standard deviation in brackets.

Dose: 8x10 ⁶ CFU/fish	Batch 1				ANOVA
Treatment	CTRL	3WPT 0.25%	3WPT 0.5%	3WPT 0.5% + BT	P value
% Accumulated Mortalities	90.00 (5.77)	94.44 (1.92)	93.33 (8.82)	92.22 (1.92)	0.780

Table 3. Results of final accumulated mortalities of Challenge Batch 2 with *Aeromonas salmonicida* at two different doses. Results are presented as means with standard deviation in brackets.

Dose: 8x10 ⁶ CFU/fish	Batch 2			ANOVA
Treatment	CTRL	3WPT 0.25%	3WPT 0.5%	P value
% Accumulated Mortalities	73.33 (11.55)	85.19 (4.63)	68.89 (7.70)	0.126

Dose: 8x10 ⁵ CFU/fish	Batch 2			ANOVA
Treatment	CTRL	3WPT 0.25%	3WPT 0.5%	P value
% Accumulated Mortalities	11.11 (10.18)	6.67 (0.00)	13.33 (6.67)	0.533

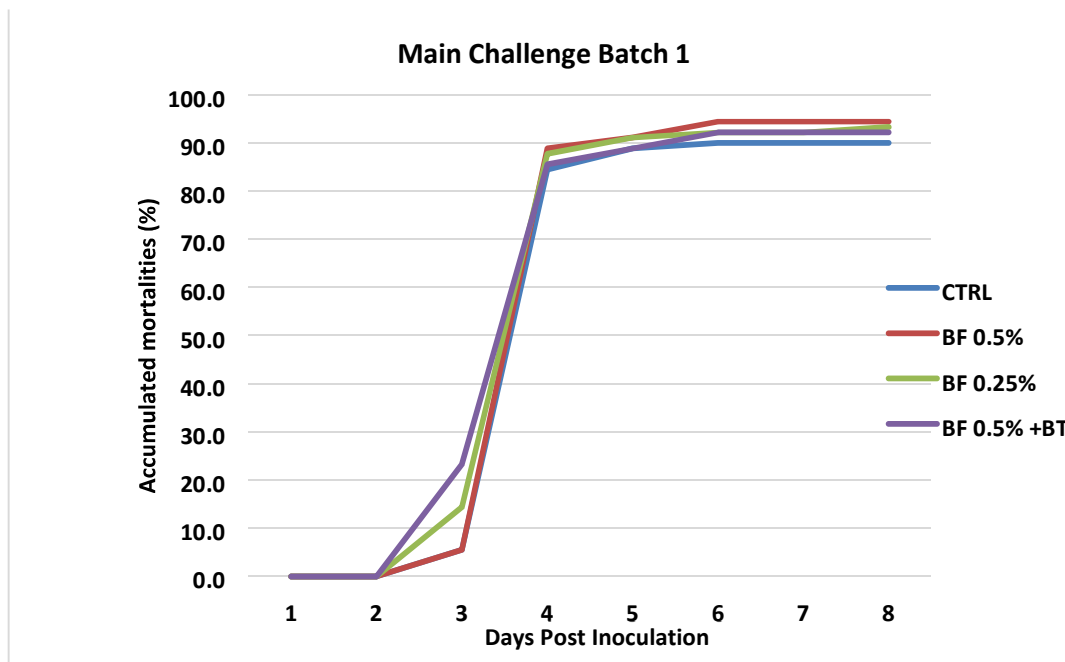


Figure 7. Accumulative mortalities of fish fed the different treatments during the first main challenge with *Aeromonas salmonicida* at dose of 8×10^6 cfu/fish.

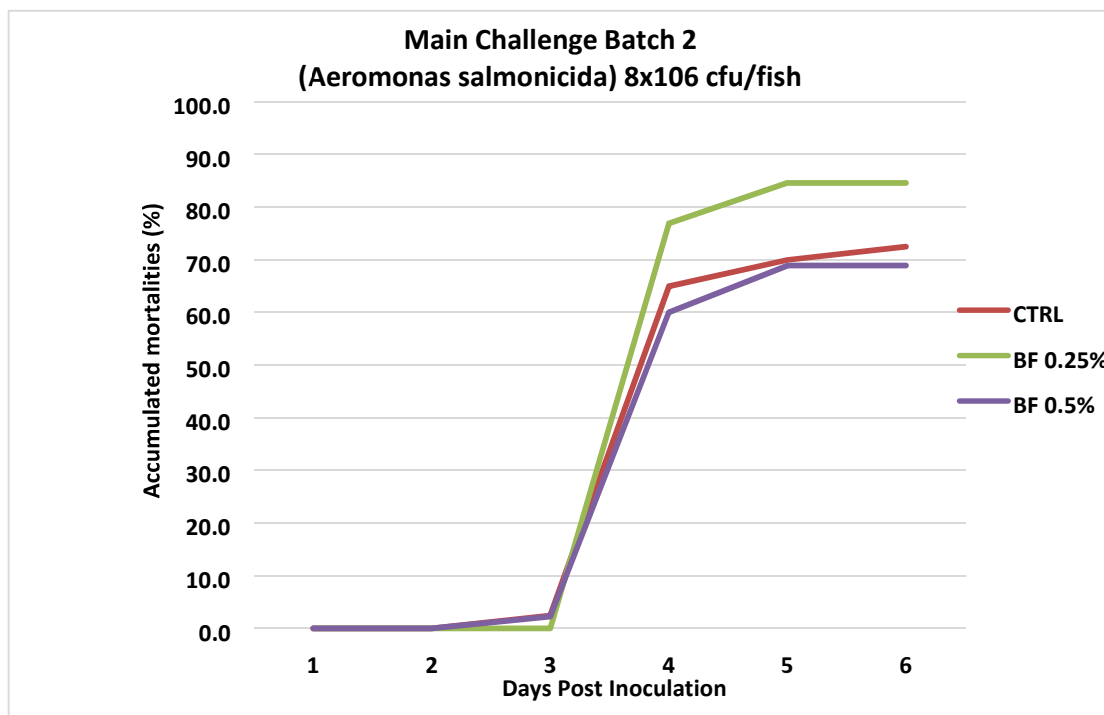


Figure 8. Accumulative mortalities of fish fed the different treatments during the second main challenge with *Aeromonas salmonicida* at dose of 8×10^6 cfu/fish.

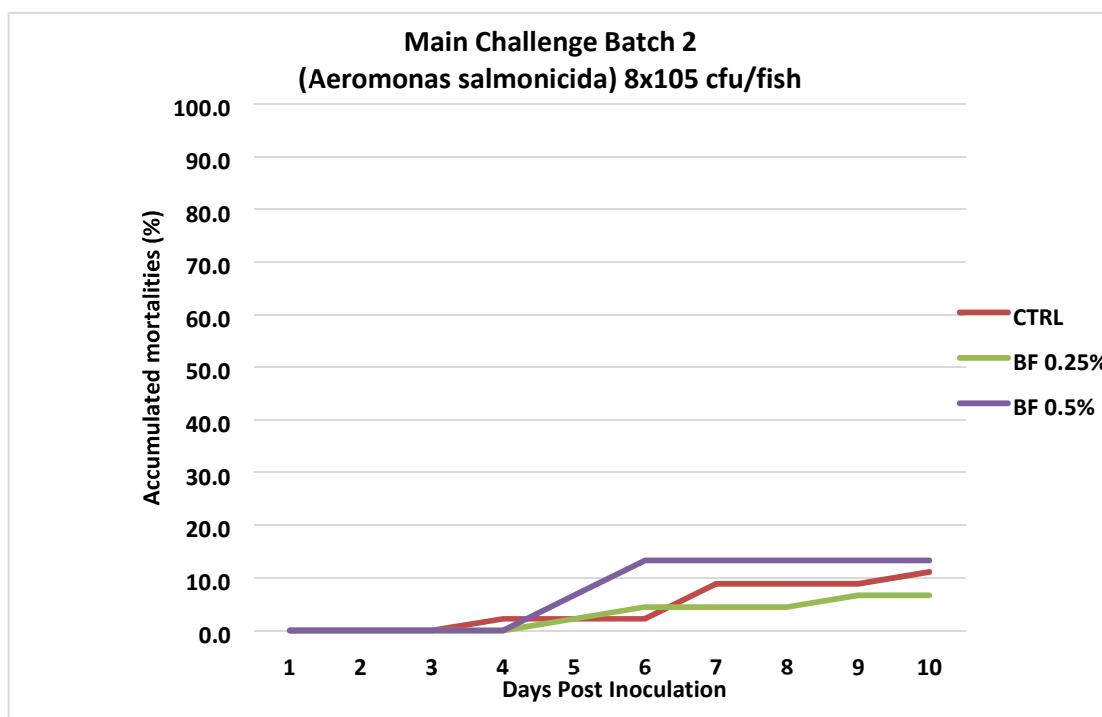


Figure 9. Accumulative mortalities of fish fed the different treatments during the second main challenge with *Aeromonas salmonicida* at dose of 8×10^5 cfu/fish.

The statistical analysis showed no significant differences among the treatments in all main challenges. Overall, the highest accumulated mortality in Challenge Batch 1 and Challenge Batch 2 (*Aeromonas salmonicida* at 8×10^6 cfu/fish) was observed in the diet with 0.25% of dietary inclusion of Bioful Aqua product. In the Challenge Batch 2 using the lower dilution, fish fed Bioful at 0.25% had the lowest mortality.

In conclusion, there is no statistical evidence of the effect of different doses Bioful Aqua product on the mortality in rainbow trout after an infection with *Aeromonas salmonicida*.

Appendix 1

Results of final mortalities in each tank during the two main challenges.

Challenge Batch 1	<i>Aeromonas salmonicida</i> (8x10 ⁶ cfu/fish)											
	CTRL			BF 0.25%			BF 0.5%			BF 0.5% + BT		
Tank	T1	T2	T3	T7	T8	T9	T4	T5	T6	T10	T11	T12
Day 0	0	0	0	0	0	0	0	0	0	0	0	0
Day 1	0	0	0	0	0	0	0	0	0	0	0	0
Day 2	2	2	1	3	5	5	2	3	0	5	6	10
Day 3	26	21	24	20	25	21	25	25	25	20	20	16
Day 4	0	2	2	2	0	1	1	0	1	2	1	0
Day 5	0	0	1	0	0	1	0	1	2	1	1	1
Day 6	0	0	0	0	0	0	0	0	0	0	0	0
Day 7	0	0	0	0	0	1	0	0	0	0	0	0
Total dead	28	25	28	28	29	28	25	30	29	28	28	27
% mortality	93.33	83.33	93.33	93.33	96.67	93.33	83.33	100.00	96.67	93.33	93.33	90.00

Challenge Batch 2	<i>Aeromonas salmonicida</i> (8x10 ⁶ cfu/fish)								
	CTRL			BF 0.25%			BF 0.5%		
Tank	T1	T2	T3	T4	T5	T6	T8	T9	T13
Day 0	0	0	0	0	0	0	0	0	0
Day 1	0	0	0	0	0	0	0	0	0
Day 2	0	1	0	0	0	0	0	0	1
Day 3	8	10	7	8	10	12	8	10	8
Day 4	1	0	1	0	2	1	1	1	2
Day 5	0	1	0	0	0	0	0	0	0
Day 6	0	0	0	0	0	0	0	0	0
Day 7	0	0	0	0	0	0	0	0	0
Day 8	0	0	0	0	0	0	0	0	0
Day 9	0	0	0	0	0	0	0	0	0
Total dead	9	12	8 ¹	8 ²	12	13	9	11	11
% mortality	60.00	80.00	80.00	88.89	80.00	86.67	60.00	73.33	73.33

¹ 10 fish/² 9 fish

Challenge Batch 2	<i>Aeromonas salmonicida</i> (8x10 ⁵ cfu/fish)								
	CTRL			BF 0.25%			BF 0.5%		
Tank	T1	T2	T3	T4	T5	T6	T8	T9	T13
Day 0	0	0	0	0	0	0	0	0	0
Day 1	0	0	0	0	0	0	0	0	0
Day 2	0	0	0	0	0	0	0	0	0
Day 3	0	1	0	0	0	0	0	0	0
Day 4	0	0	0	0	0	1	2	0	1
Day 5	0	0	0	1	0	0	1	1	1
Day 6	0	1	2	0	0	0	0	0	0
Day 7	0	0	0	0	0	0	0	0	0
Day 8	0	0	0	0	1	0	0	0	0
Day 9	0	1	0	0	0	0	0	0	0
Total dead	0	3	2	1	1	1	3	1	2
% mortality	0.00	20.00	13.33	6.67	6.67	6.67	20.00	6.67	13.33

Appendix 2

Picture 1. 500L RAS system used during the feeding phase.



Picture 2. Rainbow trout in the tanks during the feeding phase.

