



# THE USE OF BFT SYSTEM WITH PROBIOTIC TO LIMIT SHRIMP VIBRIOSIS

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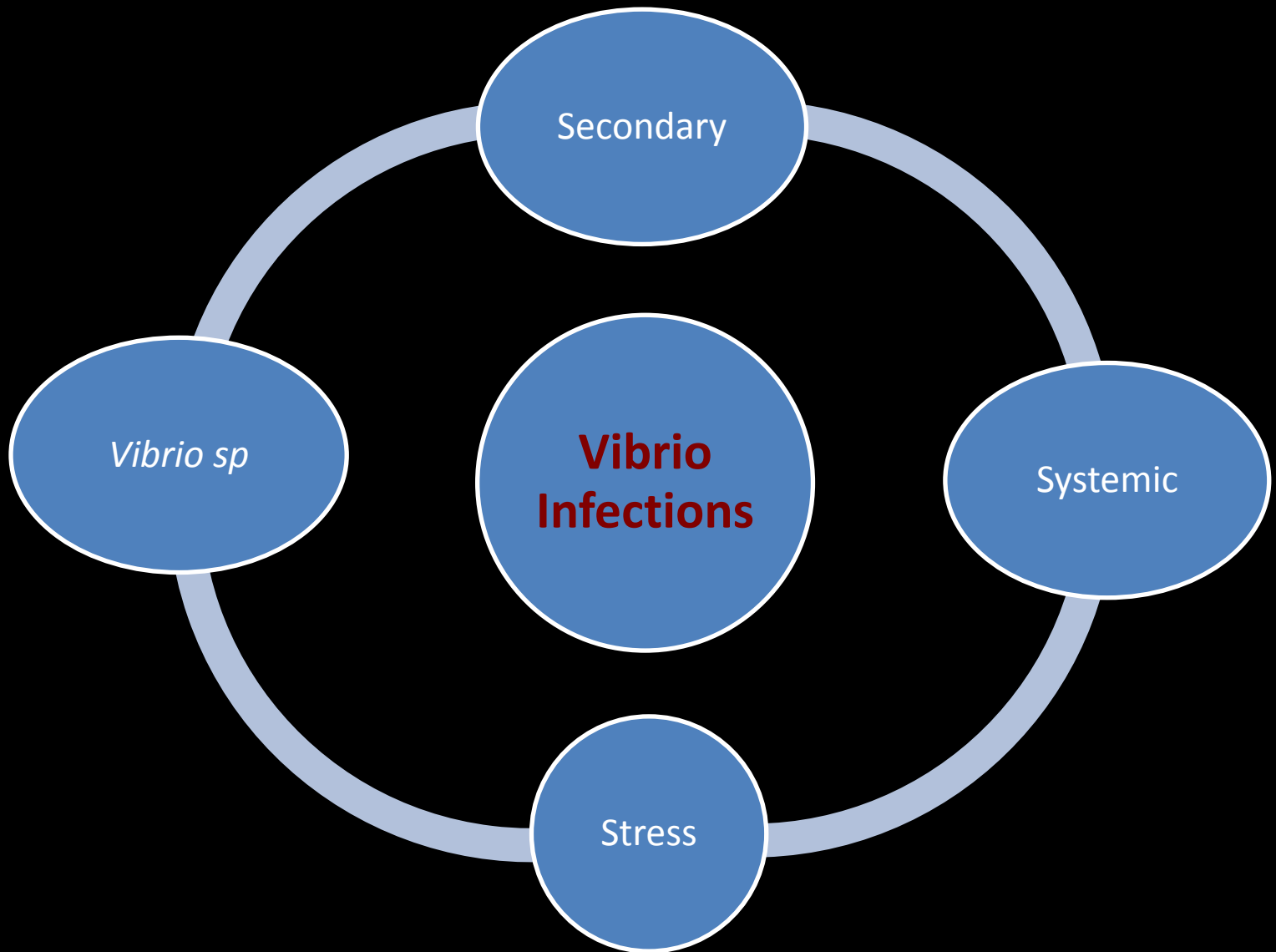
# MARINE STATION AQUACULTURE



Since 1985



Courtesy: Paulo Iribarrem



Secondary

*Vibrio sp*

**Vibrio Infections**

Systemic

Stress



➤ *Vibrio* affects growth or cause high mortalities during penaeid shrimp culture.



➤ On the other hand, probiotics may change microbial communities in aquatic culture environments



## OBJECTIVE

Analyze the effect of a probiotic in *Litopenaeus vannamei* cultured in a biofloc technology system contaminated with *Vibrio parahaemolyticus*.



# Raceway infected with *Vibrio* (1500 shrimps /m<sup>2</sup>)





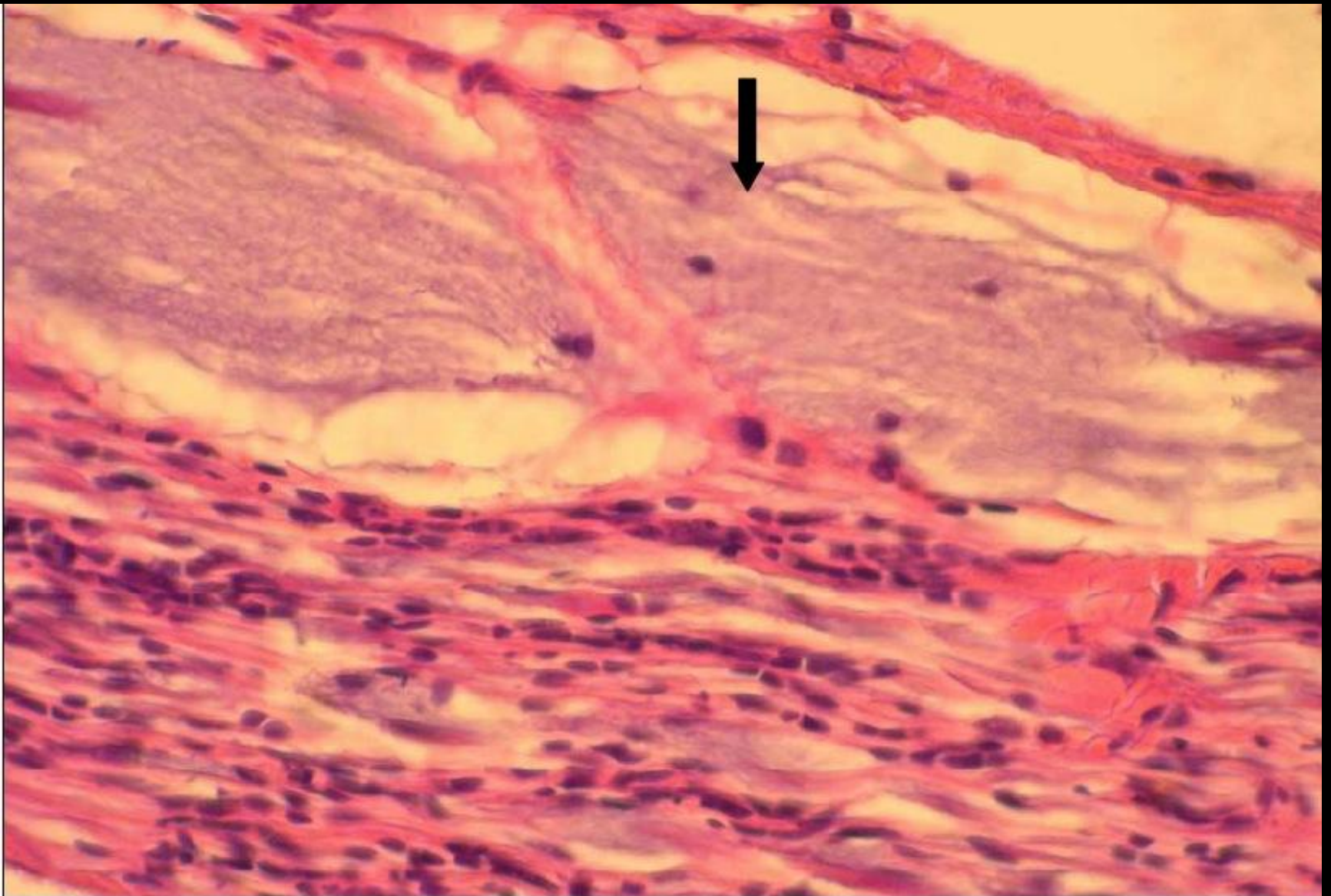


Figura 3: Cordón nervioso con colonias de bacterias azuladas pertenecientes a vobrios (flecha). H-E 20 X

**Necrosis was observed on the muscular fiber.  
These are colonies of *V. parahaemolyticus***

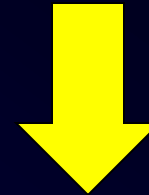
The experiment compared two treatments  
(three replicates)

CONTROL

(Biofloc without probiotic)



COMMERCIAL PROBIOTIC



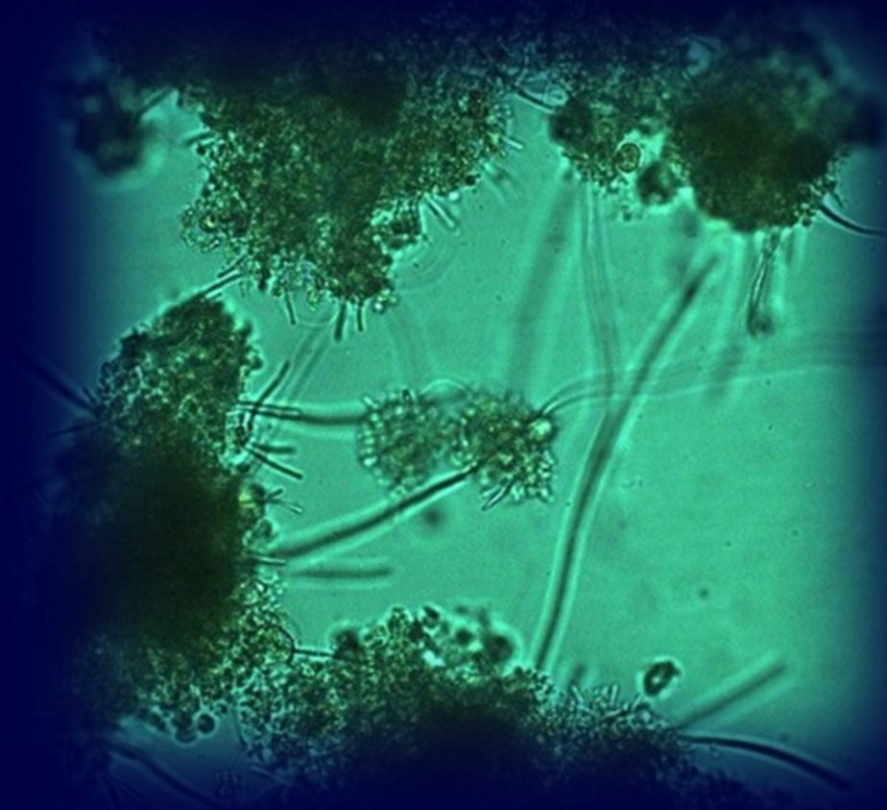
Multi-strain probiotic

➤ Added to the feed

- *Bacillus sp.*, *Enterococcus sp.* and  
*Lactobacillus sp*

➤ In the water

- *Bacillus sp.*, *Enterococcus sp.*,  
*Thiobacillus sp* and *Paracoccus sp.*





## Treatments were randomly assigned

- Greenhouse;
- Six tanks of 35 ton L lined raceways;
- 300 shrimps/m<sup>2</sup>
- Each tank was stocked with *Vibrio parahaemolyticus* infected juveniles





# Bioflocs

- Imhoff
- Secchi
- turbidity
- TSS
- Carbon source: molasses

## Water quality

- DO
- pH
- Alkalinity
- Nitrogen compounds

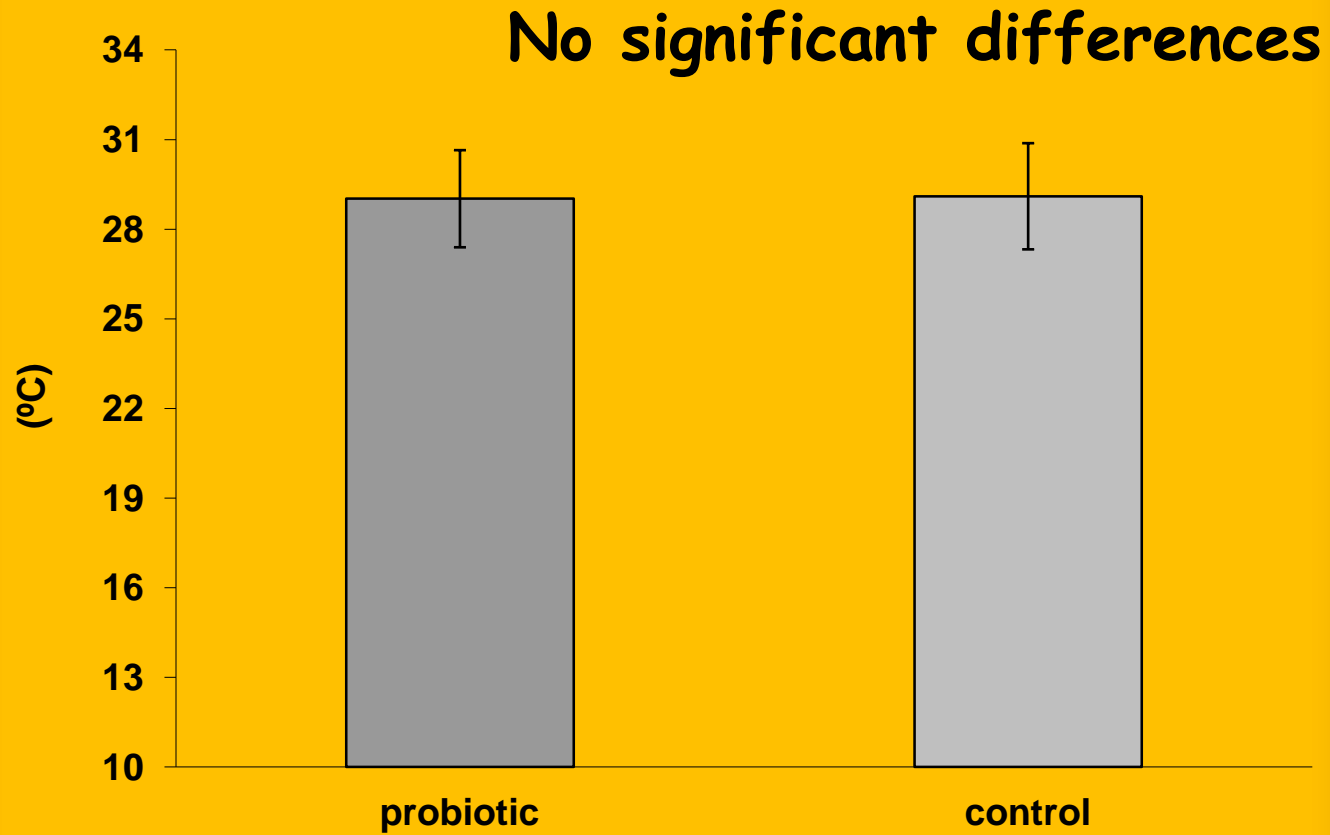


- Growth
- Weight gain
- FCR
- Survival



- Analysis of variance (ANOVA)
- Differences between means analyzed by Student's test ( $\alpha = 0.05$ ).
- The experiment lasted for 70 days

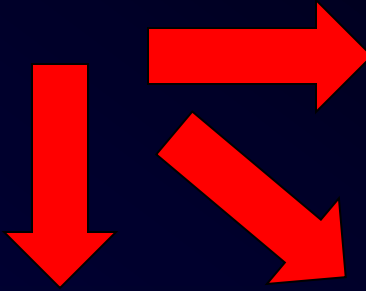
# Temperature



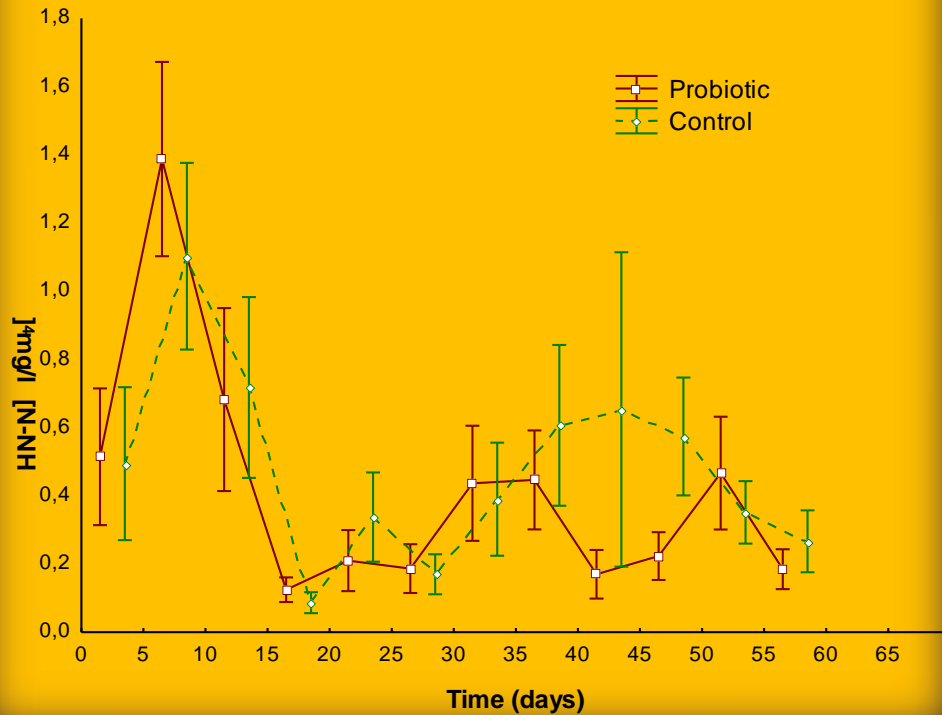


# ➤ Nitrogen compounds

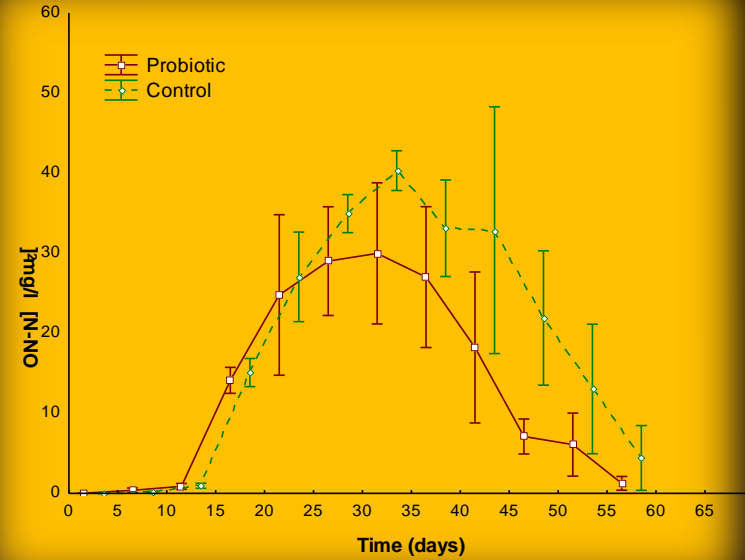
no significant differences



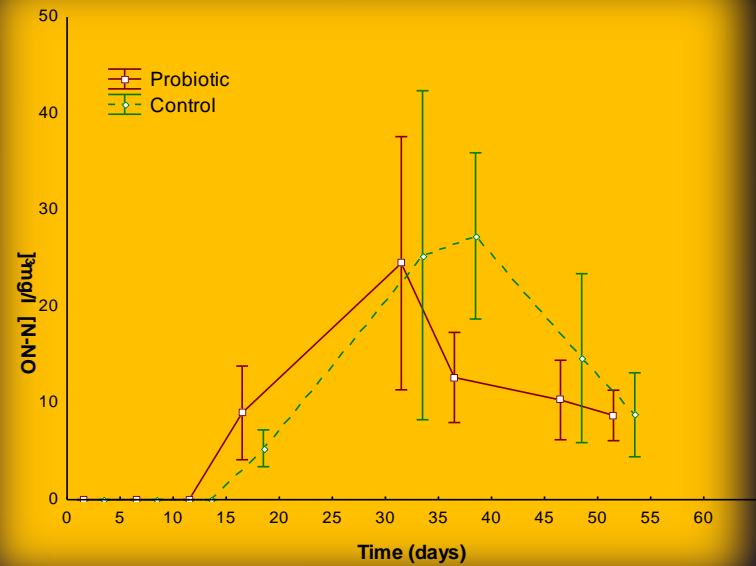
## Ammonia



## Nitrite



## Nitrate

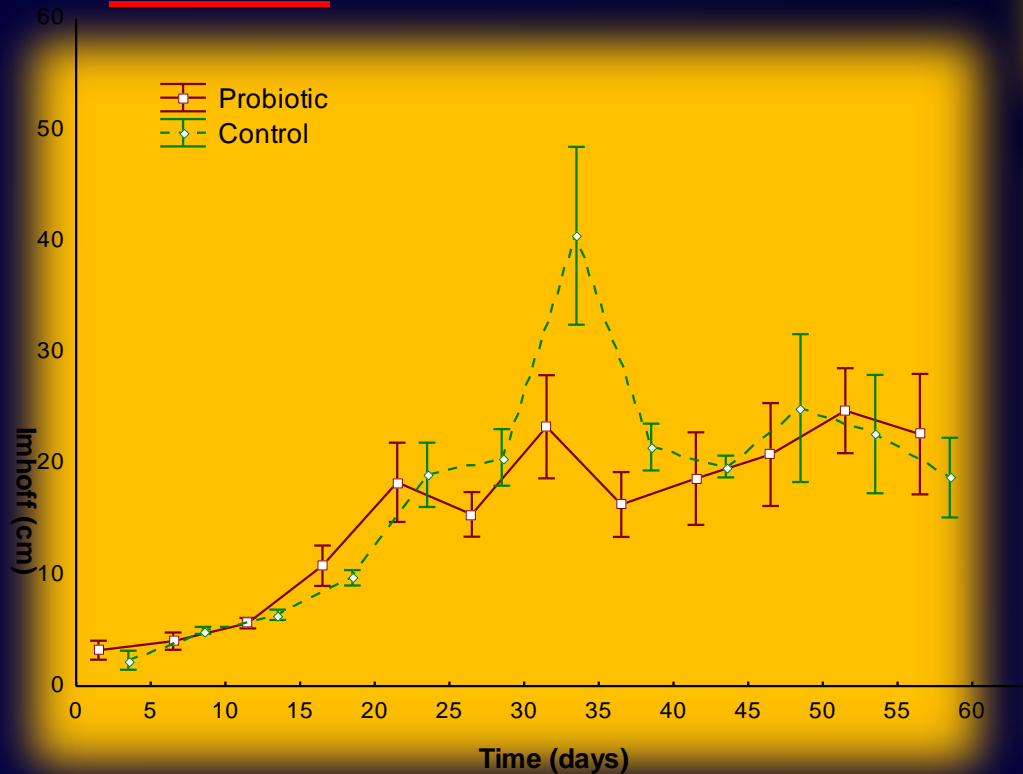


# Bioflocs Analysis

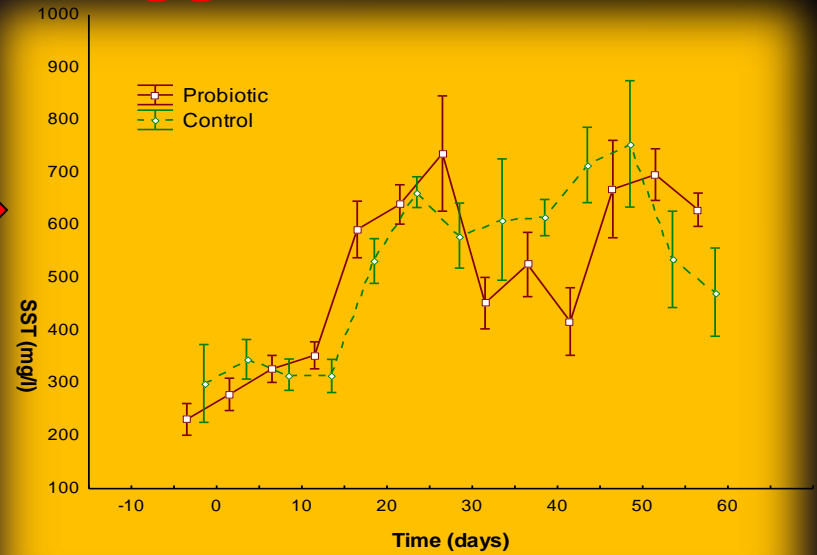
There were no significant differences



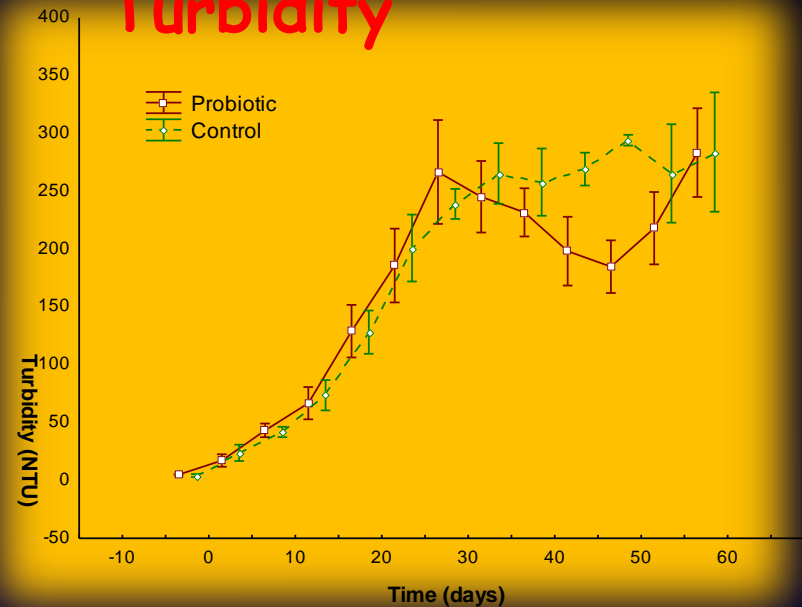
## Imhoff



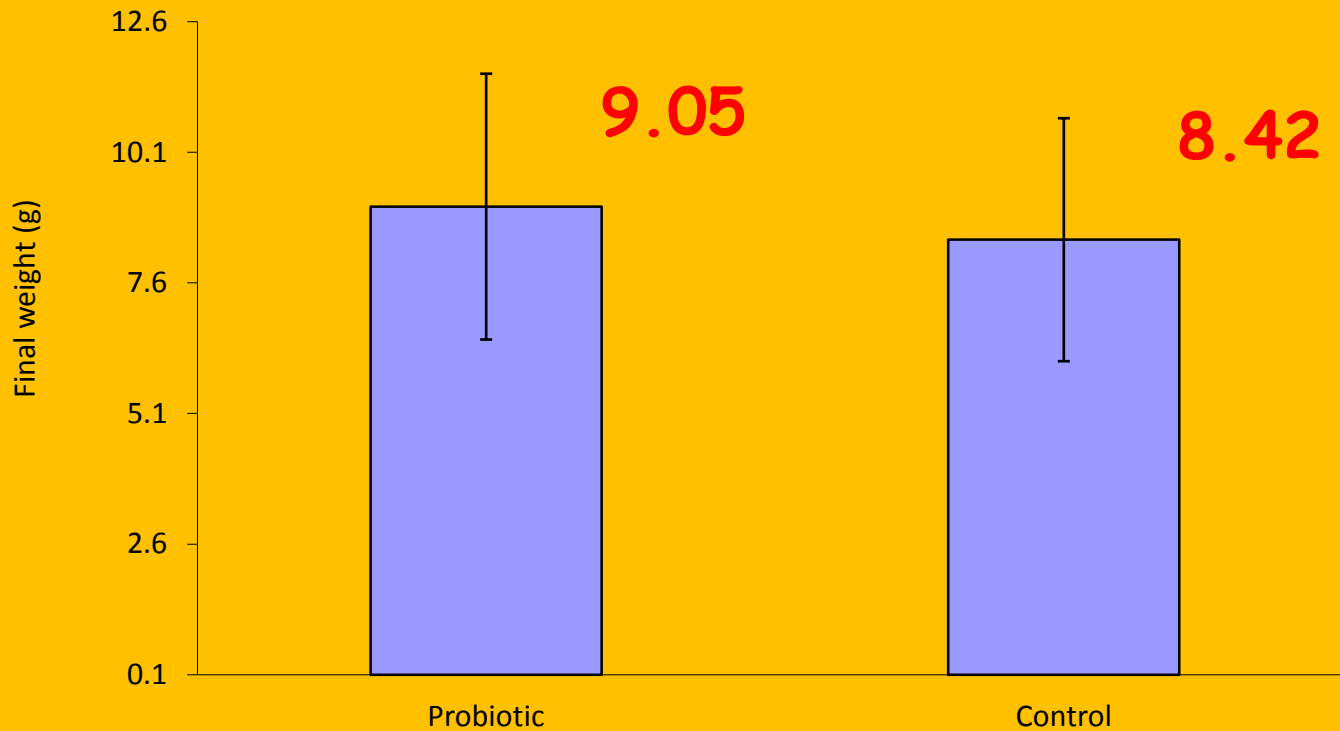
## TSS



## Turbidity



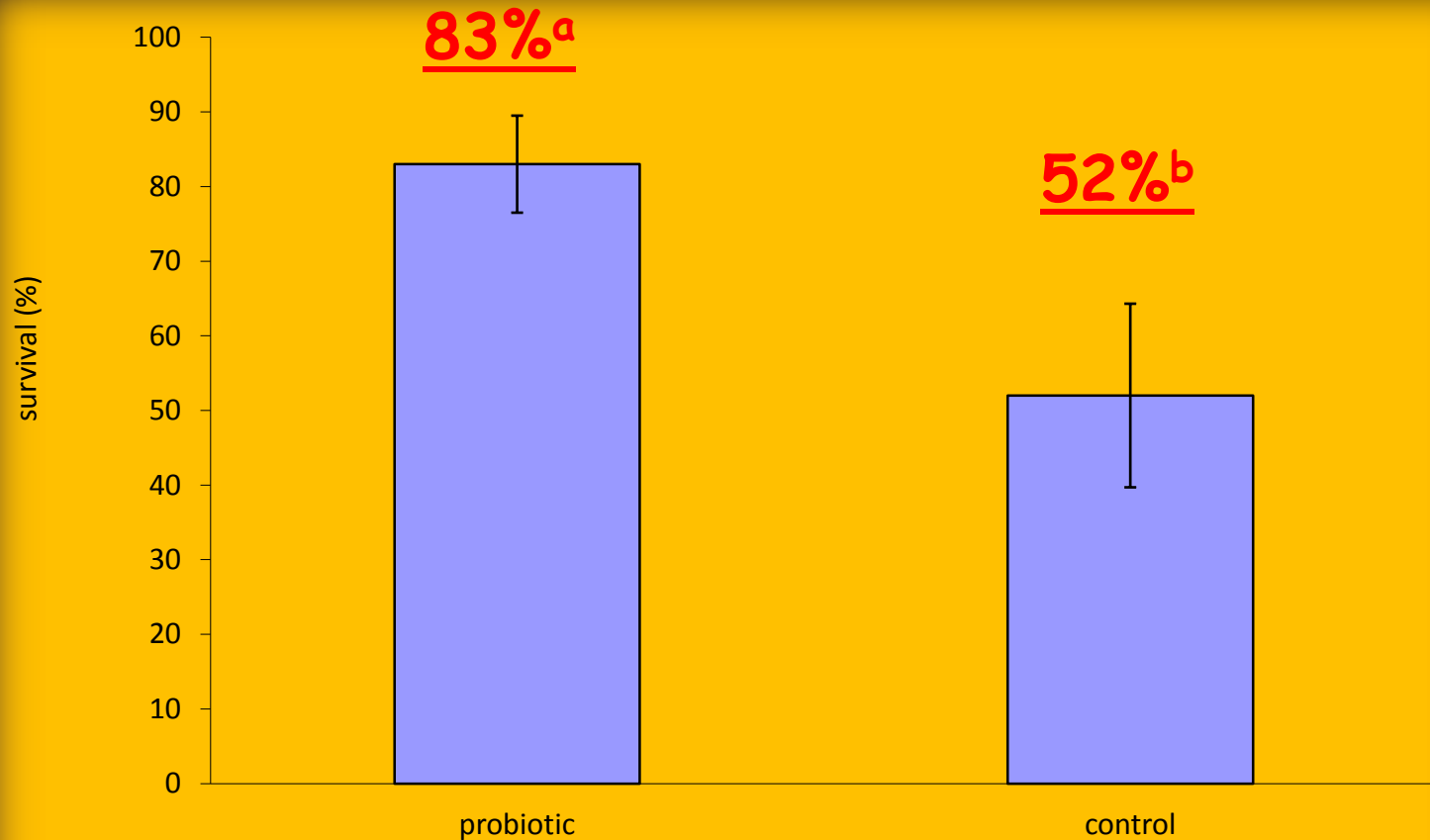
# Final weight



no significant differences



# Survival



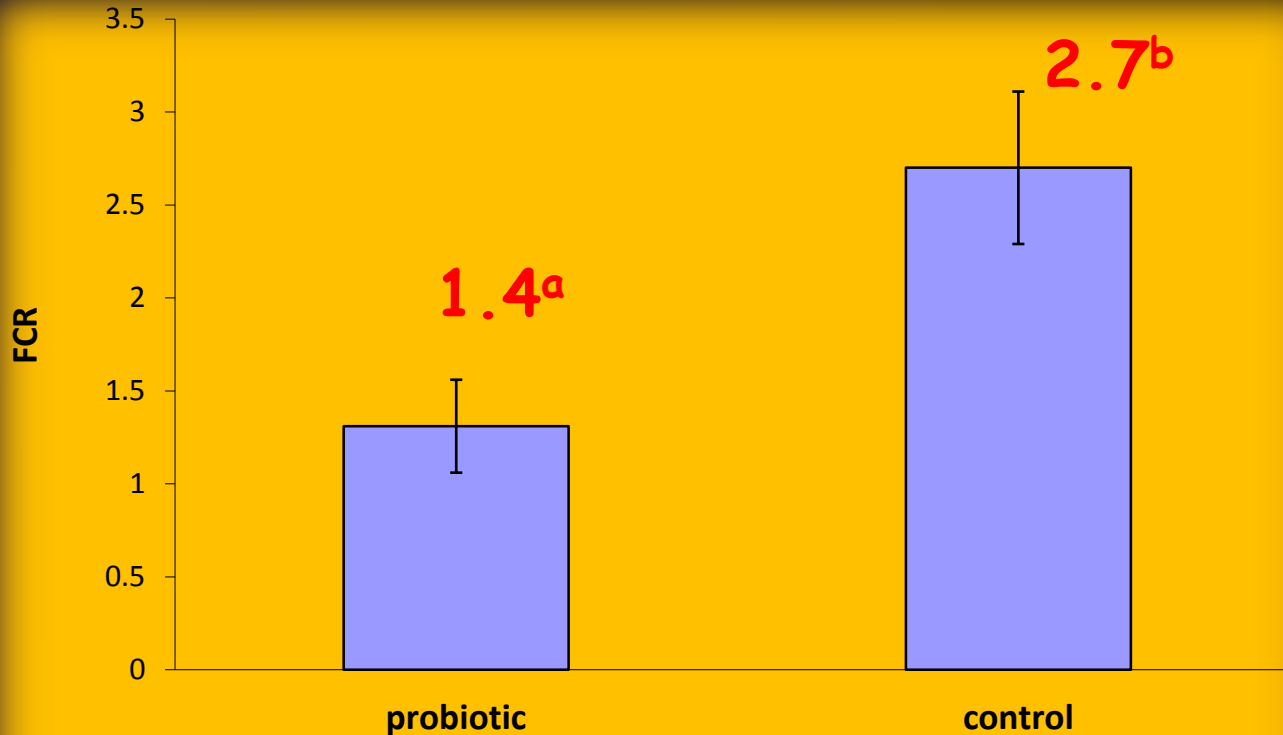
Survival was significantly higher in the probiotic treatment ( $P < 0.05$ ).

Final weight: 9.05 X 8.42

Survival: 83% X 52%

Even with a lower survival, which represents a lower density, shrimp treated with the probiotic had higher final weight

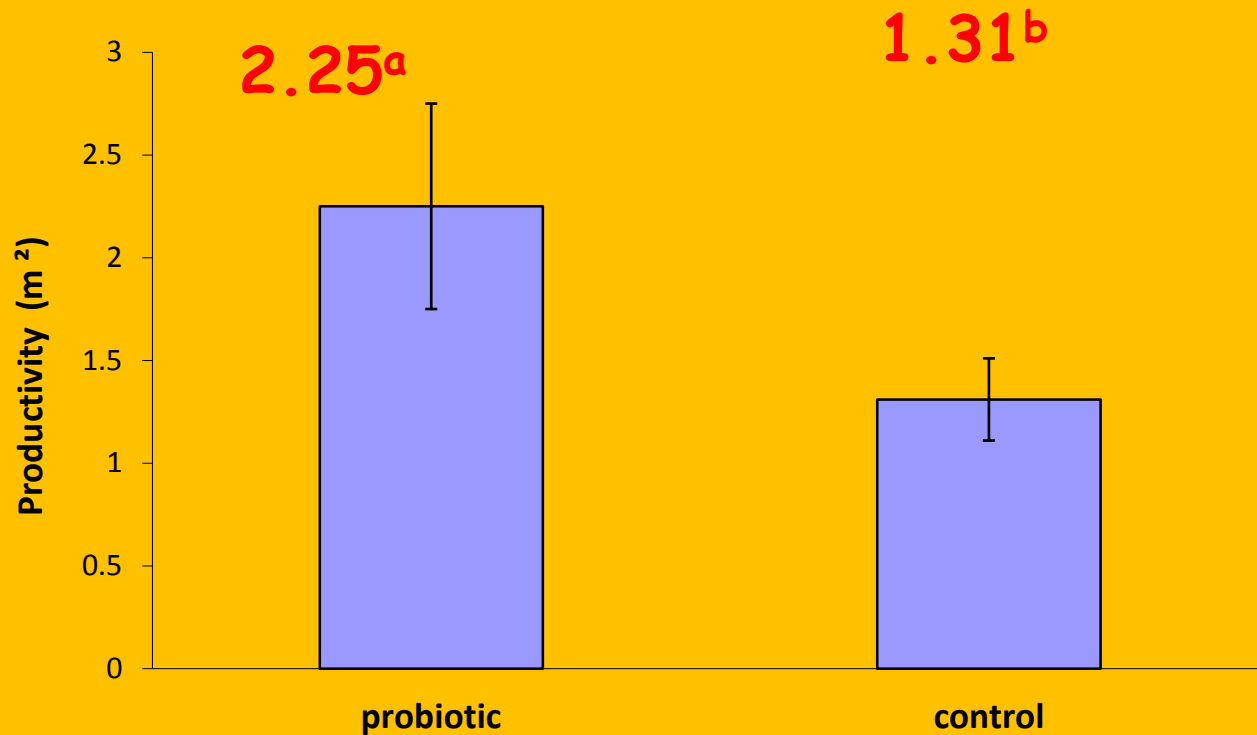
# FCR



The FCR was significantly lower in the probiotic treatment ( $P < 0.05$ ).



## Productivity (kg/m<sup>2</sup>)



Productivity was significantly higher in the probiotic treatment ( $P < 0.05$ ).

Results show that the commercial probiotic used here controlled the *Vibrio parahaemolyticus* in a biofloc culture system and improved the overall productivity of the system.

# Effect of probiotic in *Vibrio* infections in the culture of *Litopenaeus vannamei* (BFT system x Clear water)

Hostins et al, 2013



Biofloc +  
Probiotic  
BFT +P



Biofloc +  
BFT

12 tanks 400 L

*L. Vannamei* juveniles 13,9 g

300 shrimps / m<sup>2</sup>

30 days



Clear Water +  
Probiotic  
CW + P



Clear Water  
CW

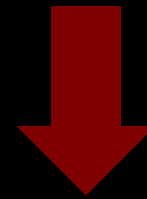


CONTROL

(Biofloc without probiotic)

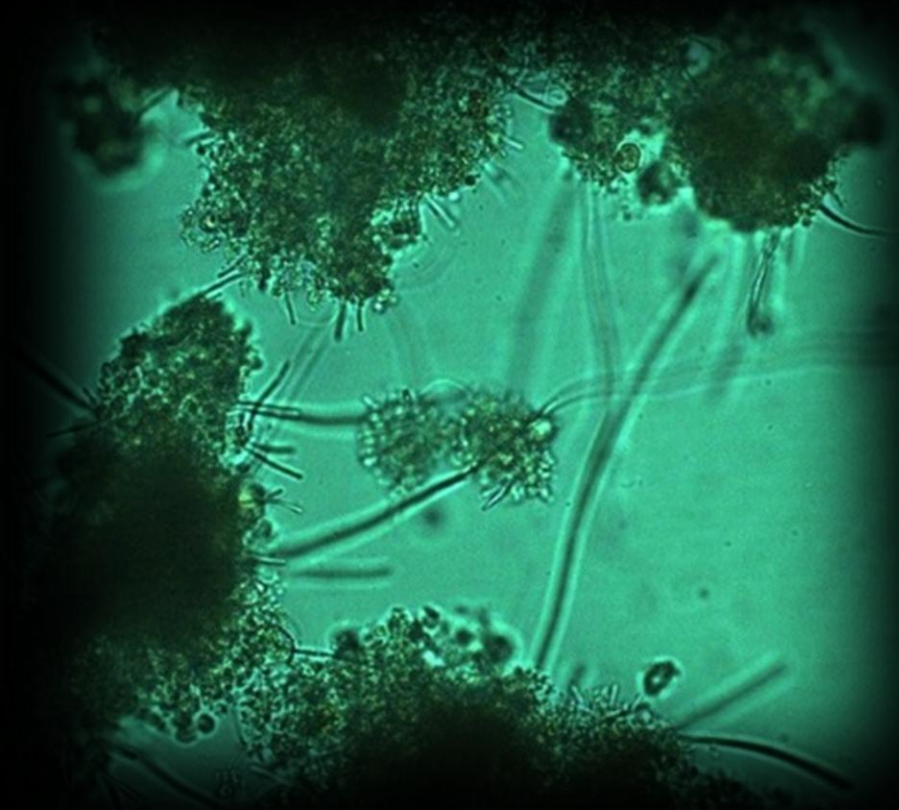


COMMERCIAL PROBIOTIC

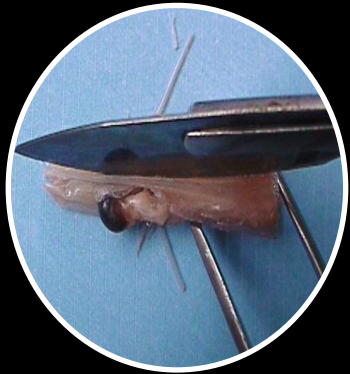


Multi-strain probiotic

- Added to the feed
- In the water



# Histopathological Analysis



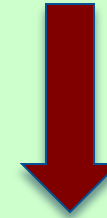
# Results

## Physical and chemical parameters

	BFT+P	BFT	CW + P	WC
T (°C)	26.08 ± 1.53	26.73 ± 1.35	25.94 ± 1.62	25.96 ± 1.39
DO (mg L <sup>-1</sup> )	6.01 ± 0.47	5.90 ± 0.41	6.17 ± 0.39	6.15 ± 0.34
pH	7.81 ± 0.28	7.87 ± 0.23	8.14 ± 0.15	8.14 ± 0.16
Salinity	32.4 ± 2.06	32.1 ± 2.03	31.2 ± 1.38	31.3 ± 1.35

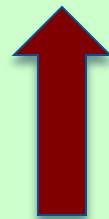
**No significant differences**

Water Exchange



Physical and chemical parameters

	BFT+P	BFT	CW + P	CW
N-AT (mg L <sup>-1</sup> )	<b>0.19 ± 0.64<sup>a</sup></b>	<b>0.13 ± 0.47<sup>a</sup></b>	<b>1.67 ± 1.05<sup>b</sup></b>	<b>1.69 ± 0.98<sup>b</sup></b>
N- NO <sub>2</sub> (mg L <sup>-1</sup> )	<b>2.28 ± 3.37<sup>a</sup></b>	<b>1.87 ± 3.27<sup>a</sup></b>	<b>0.10 ± 0.05<sup>b</sup></b>	<b>0.16 ± 0.09<sup>b</sup></b>
N- NO <sub>3</sub> (mg L <sup>-1</sup> )	<b>53.80 ± 20.5<sup>a</sup></b>	<b>54.00 ± 17.38<sup>a</sup></b>	<b>0.78 ± 1.42<sup>b</sup></b>	<b>0.50 ± 0.94<sup>b</sup></b>



Old Biofloc

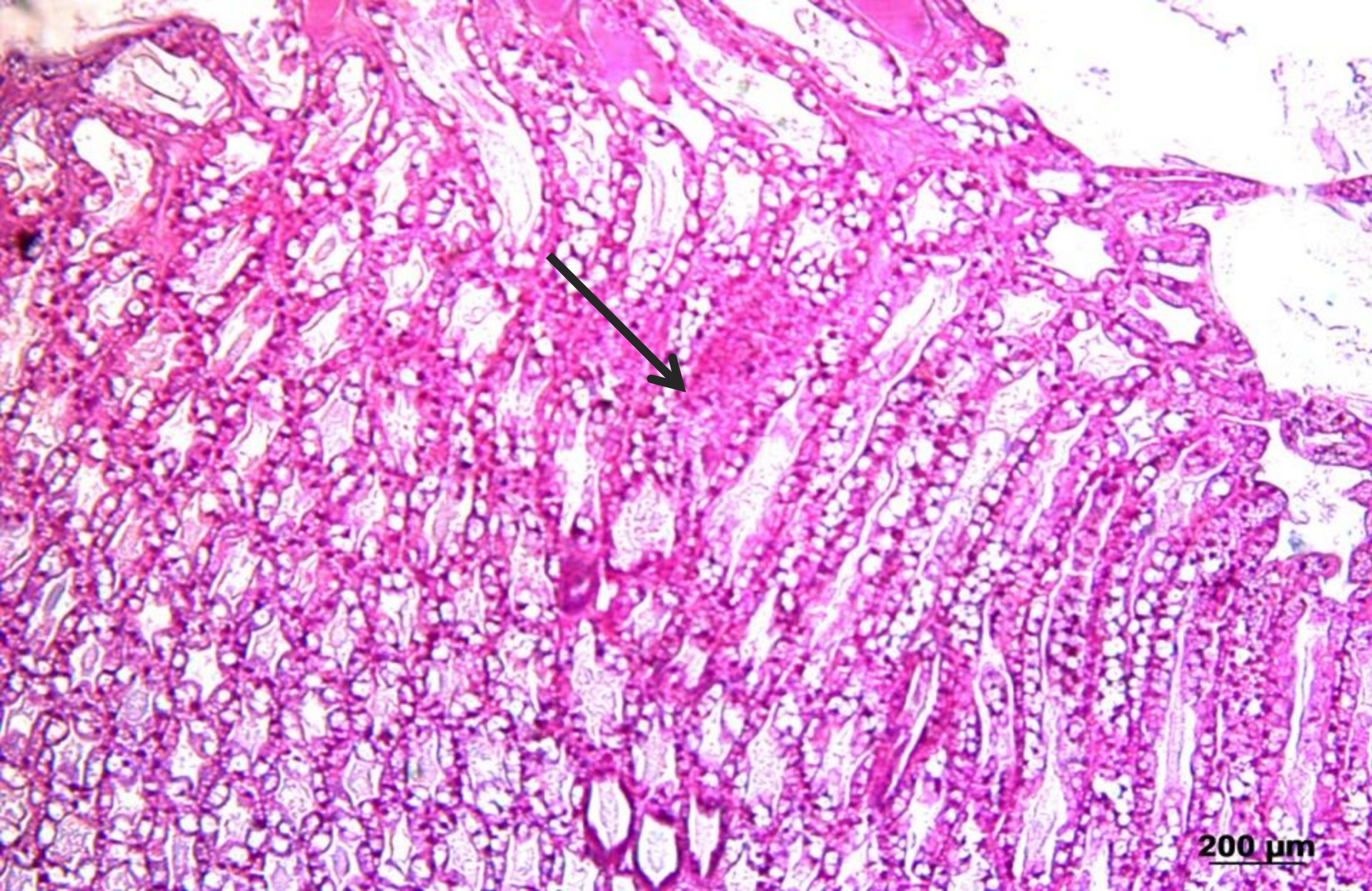
No significant differences

## zootechnical parameters

	BFT + P	BFT	CW + P	CW
Initial weight	<b>13.90 ± 3.60</b>	<b>13.90 ± 3.60</b>	<b>13.90 ± 3.60</b>	<b>13.90 ± 3.60</b>
Final weight (g)	<b>17.75 ± 3.83<sup>a</sup></b>	<b>16.17 ± 3.73<sup>b</sup></b>	<b>16.71±3.59<sup>b</sup></b>	<b>16.23±3.68<sup>b</sup></b>
Weight Gain (g)	<b>5.24±0.53<sup>a</sup></b>	<b>3.67±0.18<sup>bc</sup></b>	<b>4.35±0.97<sup>ab</sup></b>	<b>2.80±0.44<sup>c</sup></b>
Biomass (g)	<b>1947.9±89.2<sup>a</sup></b>	<b>1479.7±101.8<sup>b</sup></b>	<b>1827.4±140.4<sup>a</sup></b>	<b>1444.5±44.2<sup>b</sup></b>
Survival (%)	<b>88 ± 4.19<sup>a</sup></b>	<b>73 ± 4.27<sup>b</sup></b>	<b>86±2.2<sup>a</sup></b>	<b>75 ± 3.93<sup>b</sup></b>

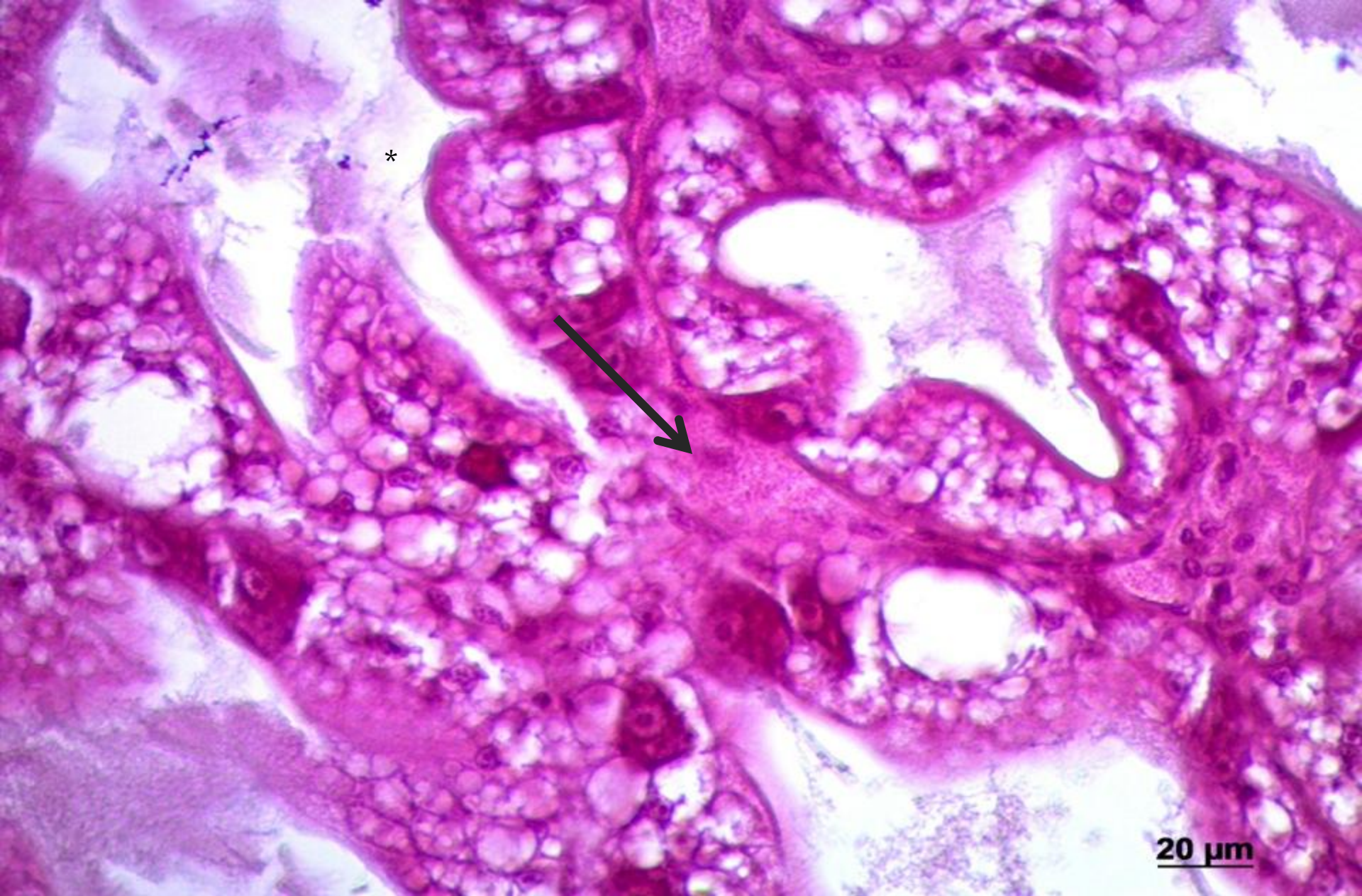
**Better results in the treatments with probiotic**





Initial sampling, Arrow indicates the hepatopancreas necrosis, evidence of vibrio infection (4x)

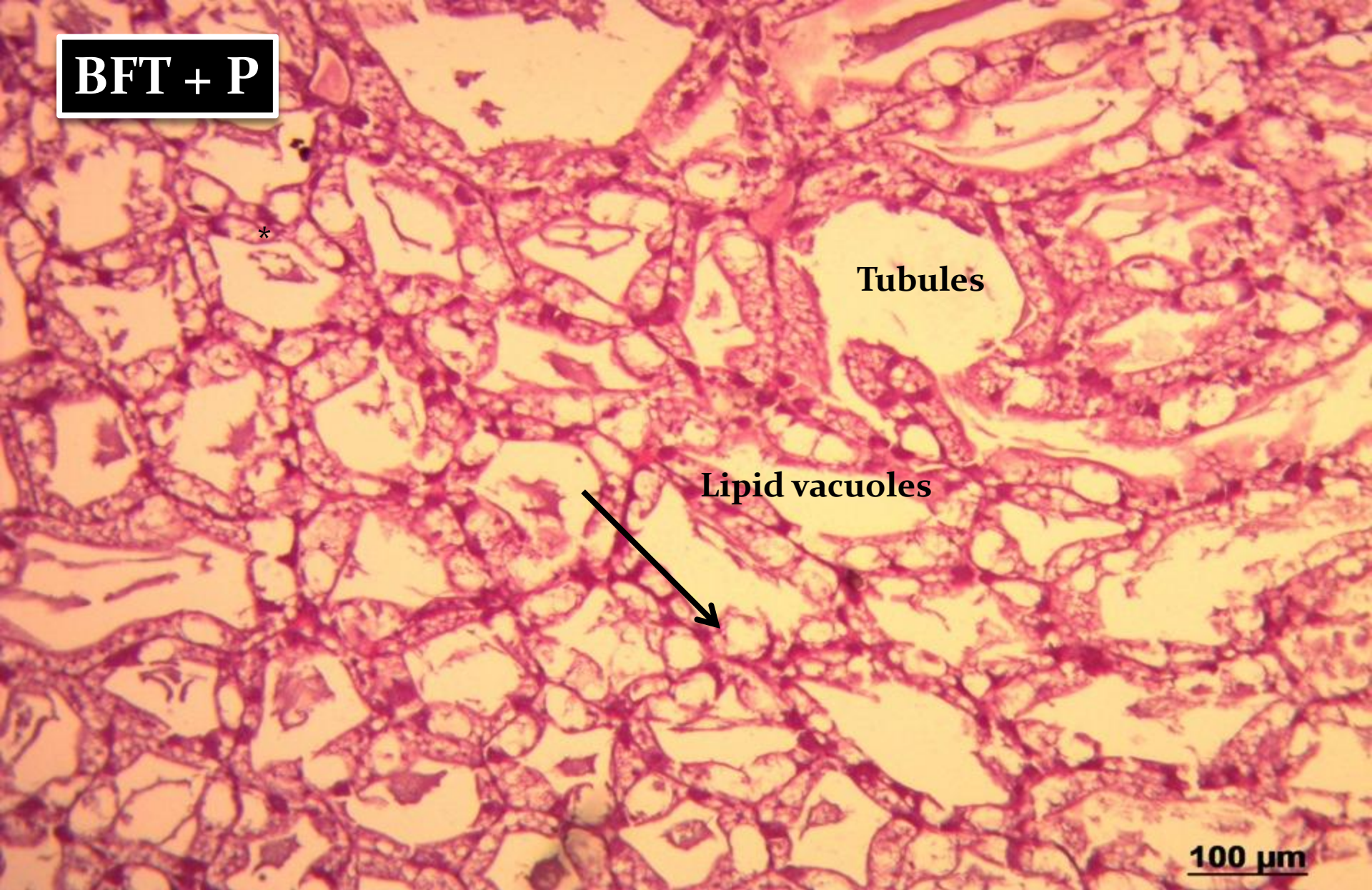




Arrow shows a colony of bacteria



**BFT + P**



**Tubules**

**Lipid vacuoles**

**100  $\mu$ m**

Final sample. Hepatopancreas healthy, with well formed tubules and lipid vacuoles (arrow) in good condition (10x).



Final sample

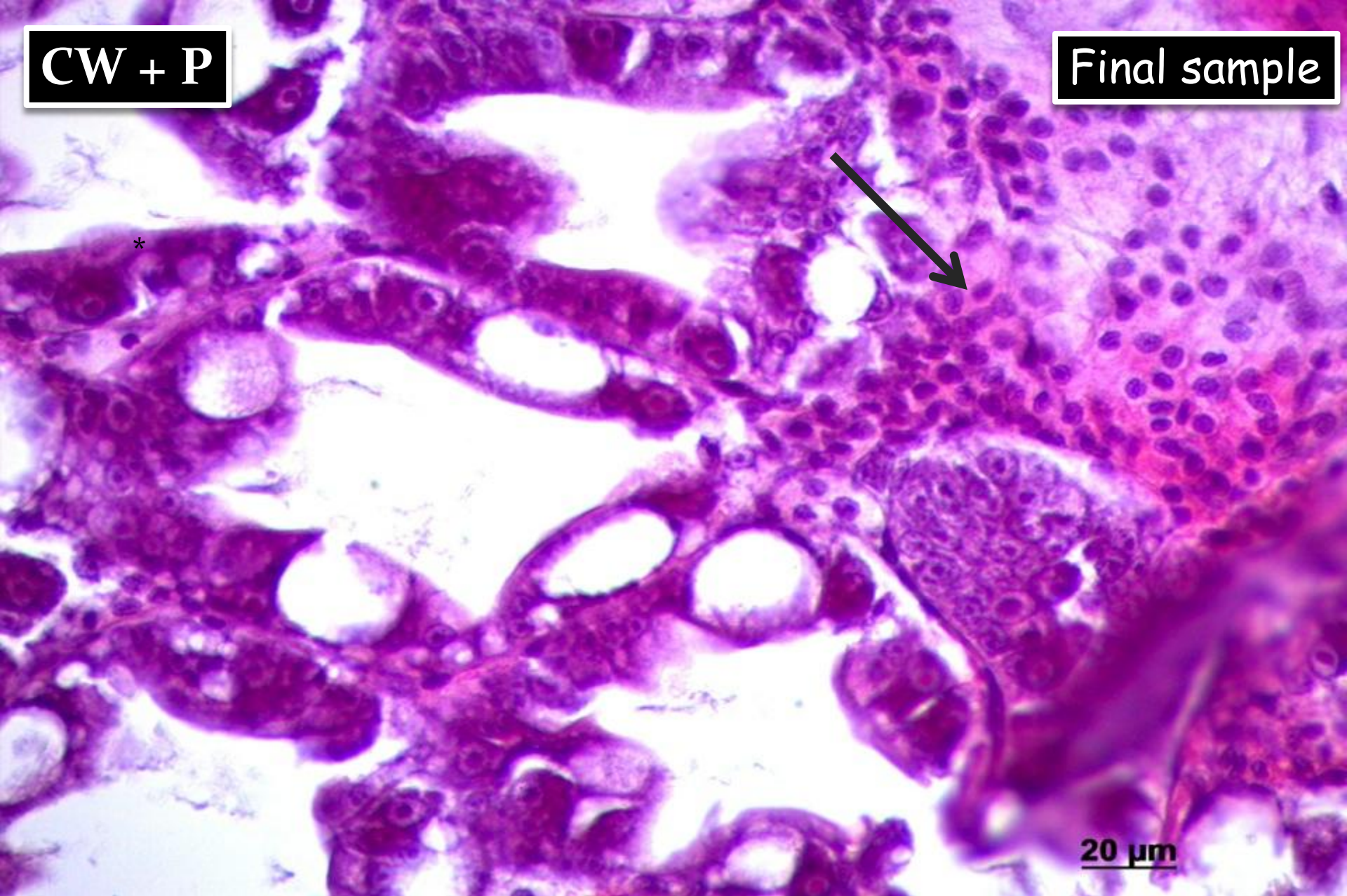


200  $\mu$ m



CW + P

Final sample

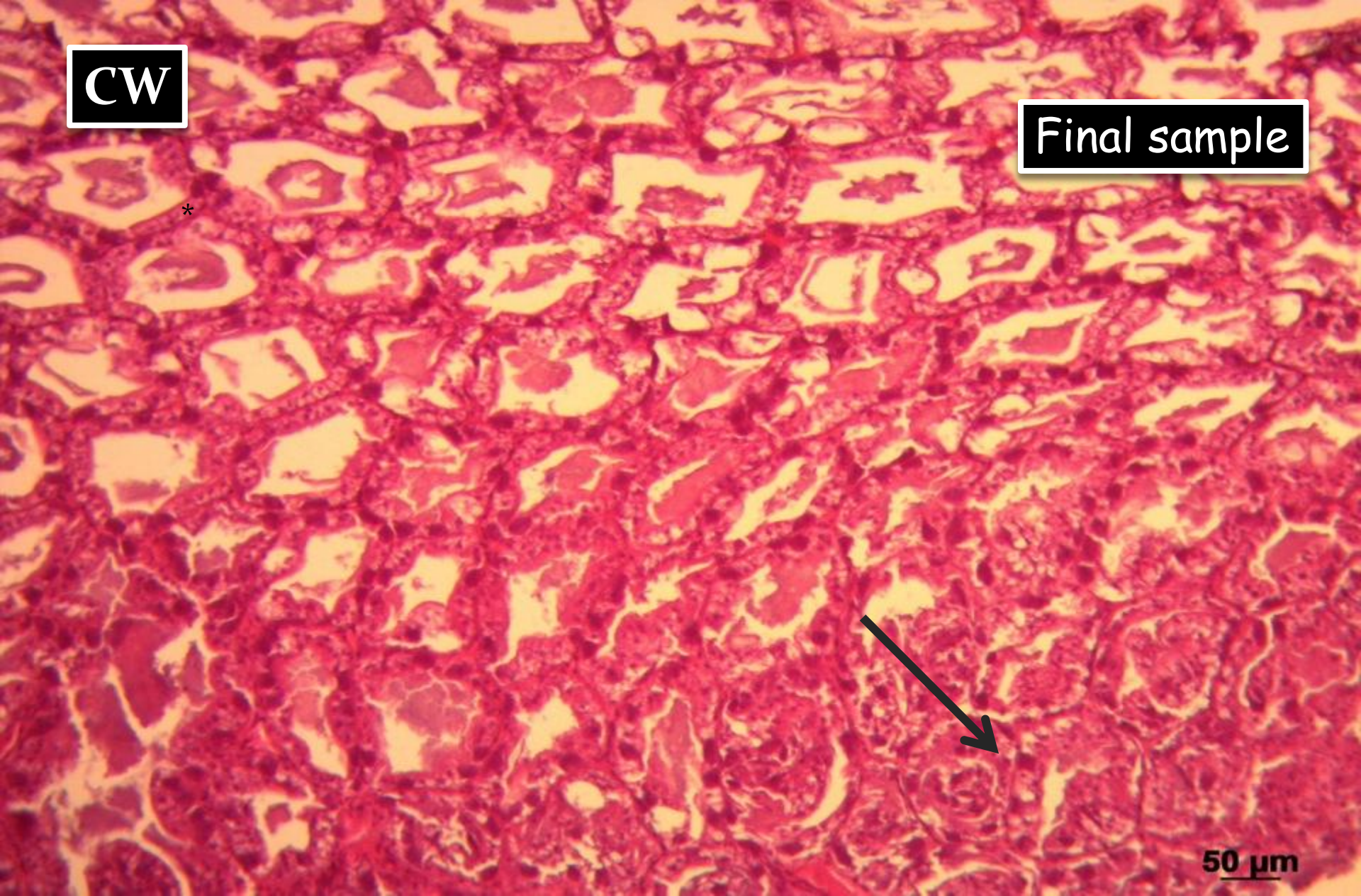


Region infiltrated hemocytes (arrow). This is indicative that there is a reaction of defense against infection. However necrosis is not observed (40x).



CW

Final sample



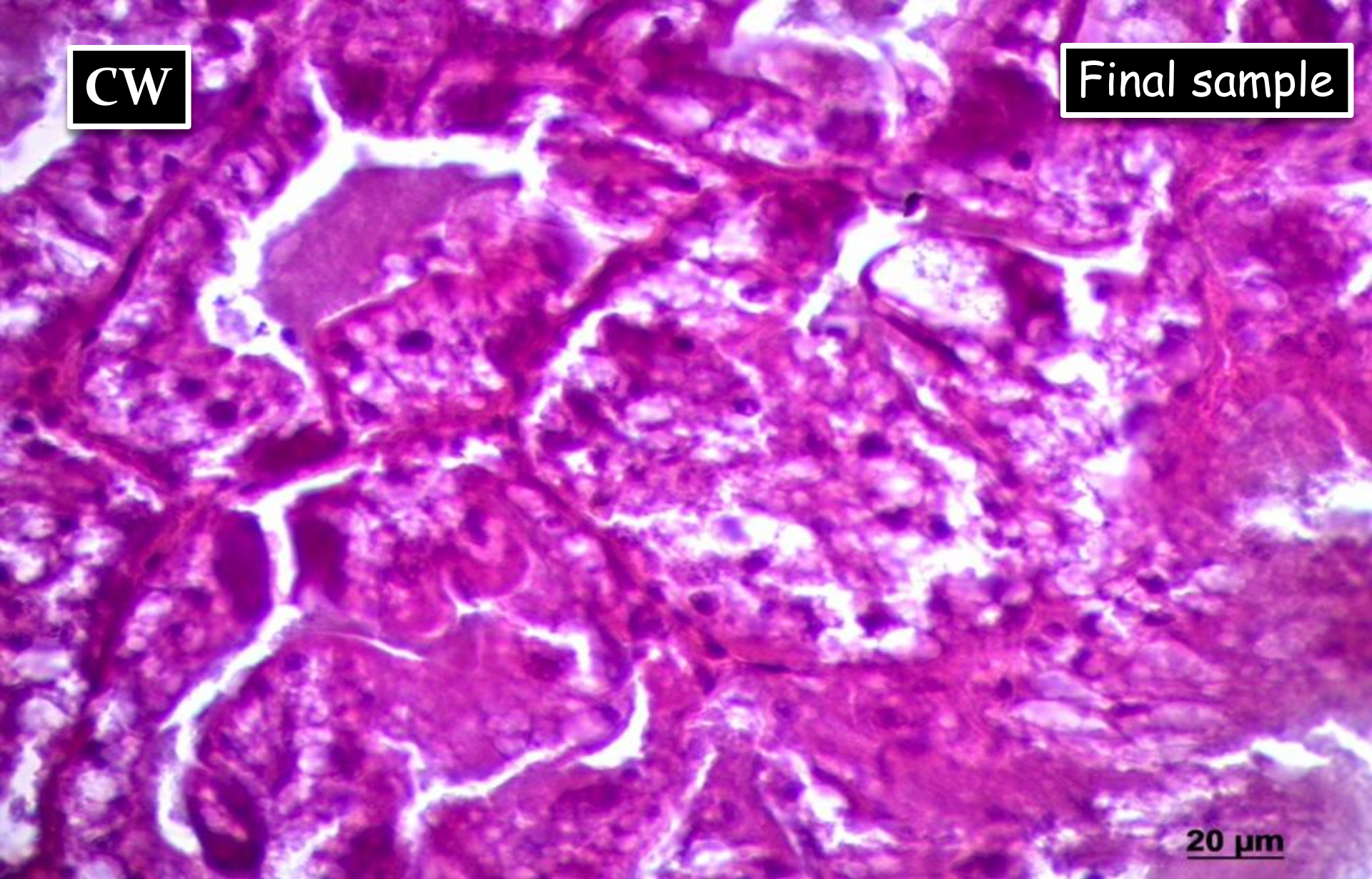
50  $\mu$ m

There lipids and observes a large amount of dead cells (arrow). The tubule wall is already collapsed. Indicates an advanced degree of infection (10x).



CW

Final sample



20 μm

**Same structure in larger increase. Tubules of the hepatopancreas collapsed and dead cells (40x).**

# CONCLUSIONS

- ✓ The use of probiotic contributes to the performance and survival of *L. Vannamei*
- ✓ Lesions caused by vibriosis in the hepatopancreas decreased
- ✓ The use of probiotic in BFT system provide the maintenance of the water quality, improved performance and increased disease resistance



# ACKNOWLEDGMENTS



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Thank you!