

# TECHNICAL BULLETIN

Arm & Hammer Animal Nutrition



## CELMANAX™ supplementation modulates microbiome and enhances gut health, immune function, and productivity parameters of Tilapia.

### INTRODUCTION

CELMANAX™ is a multicomponent, all-natural feed supplement containing Refined Functional Carbohydrates™ (RFC™) that has Generally Recognized as Safe (GRAS) status as a feed ingredient. It is used across species to improve gut health and productivity.

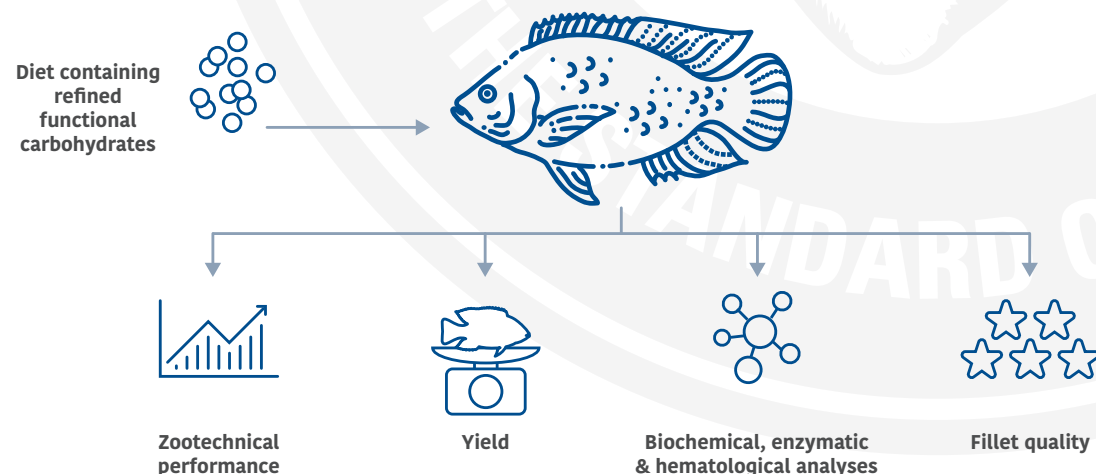
### STUDY OVERVIEW

A dose study<sup>1</sup> was conducted to determine the effect of dietary supplementation of CELMANAX on productive parameters, metabolic and immune biomarkers and gut health of tilapia. The 90-day study was conducted in tanks containing water with a salinity of 3g/L. Fish with starting weight  $20.73 \pm 0.39g$  (n=750) were assigned to 5 experimental treatments with 6 replicates per treatment, each with about 25 fish/replicate. Fish were fed commercial pelleted corn-soy diet.

#### Following treatments were fed:

- **0:** control diet
- **50:** Diet supplemented with CELMANAX SCP 50 g/MT
- **100:** Diet supplemented with CELMANAX SCP 100 g/MT
- **300:** Diet supplemented with CELMANAX SCP 300 g/MT
- **500:** Diet supplemented with CELMANAX SCP 500 g/MT

Growth performance was measured every two weeks, and at the end of the experiment, 30 fish from each treatment were weighed individually to measure weight gain and calculate feed conversion ratio. Thirty fish were euthanized and analyzed for intestinal morphology, carcass traits, biochemical and immune parameters, and intestinal microbiota composition. Data were analyzed using statistical software R (version 4.2.1, 2022), and treatment effects were considered significant at  $P \leq 0.05$  and showing a trend for  $0.05 < P < 0.10$ .



## RESULTS

### Growth Performance, yields and meat characteristics

CELMANAX supplementation increased the final weight ( $P<0.0001$ ), weight gain ( $P<0.0001$ ), and average daily gain (ADG) ( $P<0.0001$ ) of the tilapia in a linear and quadratic manner. There was no significant effect on feed intake (FI) ( $P=0.287$ ) and feed conversion ratio (FCR) ( $P=0.2268$ ) (Table 1 and Figure 1). CELMANAX supplementation at 100 g/t gave the best overall productive efficiency of the fish.

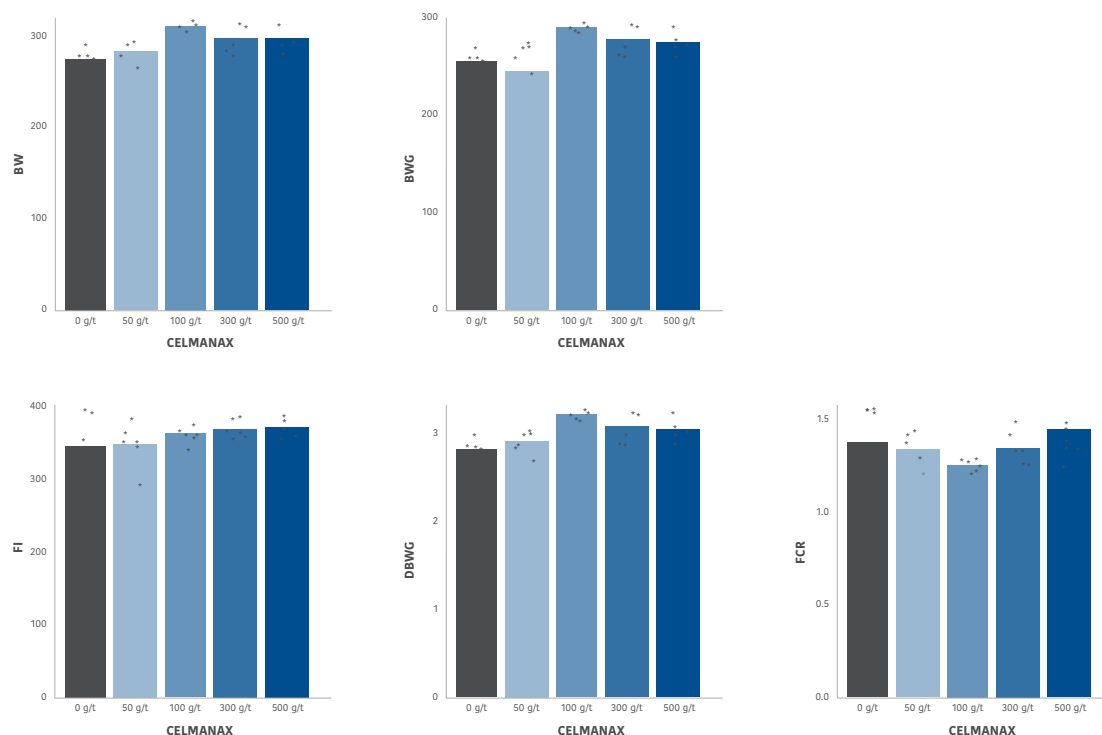
**Table 1.** Effect of increasing dose of CELMANAX supplementation on growth performance of Nile tilapia after 90 days.

CELMANAX, g/t	Initial Weight	d-90 Body Weight	Weight Gain	ADG	FI	FCR
0	20.63	273.33 <sup>c</sup>	252.70 <sup>c</sup>	2.81 <sup>c</sup>	344.16	1.36
50	20.73	281.09 <sup>bc</sup>	260.35 <sup>bc</sup>	2.89 <sup>bc</sup>	346.47	1.33
100	20.73	308.60 <sup>a</sup>	287.86 <sup>a</sup>	3.20 <sup>a</sup>	358.86	1.25
300	20.77	296.27 <sup>ab</sup>	275.50 <sup>ab</sup>	3.06 <sup>ab</sup>	367.23	1.34
500	20.80	293.29 <sup>ab</sup>	272.49 <sup>ab</sup>	3.03 <sup>ab</sup>	370.90	1.36
P-Value	0.97	<0.0001	0.0001	0.0001	0.29	0.23
SEM	0.17	4.58	4.61	0.05	10.41	0.04
C.V. (%)	2.04	3.86	4.18	4.18	7.13	7.1
Linear	0.55	0.027	0.03	0.03	0.44	0.42
Quadratic	0.82	0.001	0.001	0.001	0.47	0.21

Different letters in the columns indicate significant differences between treatment means.

**Figure 1.** Effect of increasing dose of CELMANAX supplementation on growth performance of Nile tilapia after 90 days.

■ 0g/t ■ 50g/t ■ 100g/t ■ 300g/t ■ 500g/t



A linear dose dependent response on body length and a quadratic increase in body width was observed with CELMANAX supplementation (Table 2). The relative weight of the fish spleen was significantly affected by the treatments ( $P=0.0062$ ), exhibiting a quadratic relationship with CELMANAX supplementation showing a decrease in spleen weight (Table 3). Increase in spleen size as noted in control treatment is an indicator of stress or inflammation. Fillet yield exhibited a significant quadratic effect and increased in response to all doses of CELMANAX supplementation, with the maximum yield achieved at 300 g/t dose. This will enable producers to adjust supplementation in accordance with their performance and profitability goals.

**Table 2.** Effect of increasing dose of CELMANAX on body length (BL), body height (BH), body width (BW) and head size (HS) of Nile Tilapia after 90 days.

CELMANAX, g/t	BL, cm/fish	BH, cm/fish	BW, cm/fish	HS, cm/fish
0	24.44	9.80	3.71 <sup>b</sup>	6.34
50	24.45	10.10	3.83 <sup>a</sup>	6.46
100	24.62	10.20	3.85 <sup>a</sup>	6.35
300	24.93	9.95	3.86 <sup>a</sup>	6.61
500	24.99	9.79	3.56 <sup>c</sup>	6.31
P-Value	0.19	0.24	0.04	0.13
SEM	0.21	0.15	0.08	0.09
C.V. (%)	3.32	5.94	8.19	5.42
Linear	0.02	0.31	0.08	0.98
Quadratic	0.53	0.16	0.01	0.03

*Different letters in the columns indicate significant differences between treatment means.*

**Table 3.** Effect of increasing dose of CELMANAX on the viscerosomatic index (IVS), hepatosomatic index (HSI), spleen index (SI), visceral fat index (VFT) and fillet with skin (FWS) and fillet without skin (Fillet) of Nile tilapia after 90 days of experimental period.

CELMANAX, g/t	IVS, %	HIS, %	SI, %	VFT, %	FWS, %	Fillet, %
0	7.48	1.91	0.42 <sup>a</sup>	1.11	33.83	24.71 <sup>b</sup>
50	7.96	2.29	0.30 <sup>ab</sup>	0.30	34.58	27.65 <sup>ab</sup>
100	7.85	2.26	0.28 <sup>b</sup>	0.28	36.83	29.02 <sup>a</sup>
300	7.37	2.13	0.28 <sup>b</sup>	0.30	35.46	30.01 <sup>a</sup>
500	8.29	2.07	0.29 <sup>b</sup>	0.33	37.33	28.19 <sup>ab</sup>
P-Value	0.084	0.33	0.006	0.04	0.063	0.003
SEM	0.25	0.19	0.029	0.22	0.96	0.88
C.V. (%)	12.64	25.43	36.79	187.85	10.48	12.29
Linear	0.19	0.87	0.039	0.14	0.29	0.034
Quadratic	0.16	0.25	0.027	0.07	0.72	0.003

*Different letters in the columns indicate significant differences between treatment means.*

**Table 4.** Effect of increasing dose of CELMANAX on meat texture, water holding capacity (WHC), weight loss by cooking (WLC), pH, luminosity (L), and factors a and b of meat Nile tilapia after 90 days.

CELMANAX, g/t	Texture, kgf/cm <sup>2</sup>	WHC, %	WLC, %	pH	L	a	b
0	0.39	66.40 <sup>ab</sup>	89.13	5.90	39.80	4.08	10.77
50	0.27	72.04 <sup>ab</sup>	88.61	5.91	40.28	5.59	10.43
100	0.75	72.46 <sup>ab</sup>	86.79	5.88	40.71	5.49	12.32
300	0.56	72.81 <sup>a</sup>	88.55	6.04	40.76	3.62	9.89
500	0.52	65.20 <sup>b</sup>	88.84	5.97	41.53	3.84	10.90
P-Value	0.29	0.017	0.07	0.18	0.77	0.05	0.09
SEM	0.15	1.63	0.53	0.05	0.97	0.48	0.56
C.V. (%)	3.32	4.05	1.04	1.36	4.12	18.39	8.85
Linear	0.52	0.17	0.57	0.07	0.25	0.04	0.53
Quadratic	0.28	0.003	0.11	0.28	0.92	0.75	0.73

*Different letters in the columns indicate significant differences between treatment means.*

A quadratic effect of CELMANAX supplementation was noted on meat water holding capacity with all doses except the highest dose showing an increase compared to control. Tilapia supplemented with CELMANAX at 300 g/t had the best water holding capacity leading to juicier fillets and reduced water loss during cooking (Table 4).

#### **Biomarkers of immune function and oxidative stress**

Biochemical, enzymatic and hematological results are summarized in table 5 and 6. Supplementation at 300 g/t resulted in the lowest cortisol response. Supplementation with CELMANAX led to a tendency in increase in lysozyme levels, and a significant reduction in malondialdehyde (MDA), an increase in catalase (CAT) levels, and a decrease in glutathione reductase (GR) (Table 5). This may suggest a synergistic effect of reduced oxidative stress and enhanced immune response in Tilapia supplemented with CELMANAX.

The hematological parameters of tilapia were notably affected by the supplementation of CELMANAX at 50 g and 100 g dose (Table 6). An increase in erythrocyte count, hematocrit levels, mean corpuscular volume, platelet and total leukocyte and its subtype count all indicate an improvement in overall health and immune status.

**Table 5.** Effect of increasing dose of CELMANAX on cortisol, glucose, lysozyme, malondialdehyde (MDA), catalase (CAT) and glutathione reductase (GL) in Nile Tilapia.

CELMANAX, g/t	Cortisol, mcg/dL	Glucose, mg/dL	Lysozyme, $\mu\text{m/L}$ of plasma	MDA, nM/g of protein	CAT, U/L	GR, U/L
0	3.50 <sup>ab</sup>	84.65 <sup>a</sup>	303.61 <sup>b</sup>	15.23 <sup>a</sup>	291.17 <sup>b</sup>	236.75 <sup>a</sup>
50	5.40 <sup>a</sup>	70.90 <sup>ab</sup>	370.94 <sup>a</sup>	12.12 <sup>b</sup>	362.39 <sup>a</sup>	161.68 <sup>b</sup>
100	4.26 <sup>ab</sup>	84.65 <sup>a</sup>	337.26 <sup>ab</sup>	11.77 <sup>b</sup>	326.78 <sup>a</sup>	153.25 <sup>b</sup>
300	2.87 <sup>b</sup>	52.80 <sup>c</sup>	350.08 <sup>ab</sup>	11.09 <sup>b</sup>	350.22 <sup>a</sup>	136.52 <sup>b</sup>
500	4.97 <sup>ab</sup>	67.00 <sup>bc</sup>	338.20 <sup>ab</sup>	11.69 <sup>b</sup>	317.92 <sup>a</sup>	124.02 <sup>b</sup>
P-Value	0.014	<0.001	0.027	0.006	0.04	0.0002
SEM	0.51	3.74	13.21	0.73	17.94	14.51
C.V. (%)	27.23	11.62	8.69	13.2	12.17	19.97
Linear	0.82	<0.001	0.54	0.015	0.83	<0.01
Quadratic	0.07	0.002	0.098	0.01	0.06	0.017

*Different letters in the columns indicate significant differences between treatment means.*

**Table 6.** Effect of increasing dose of CELMANAX on hematological parameters: erythrocytes (Ery, million/mm<sup>3</sup>), hematocrit (Hct, %), hemoglobin (Hb, g/dL), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin concentration (MCHC, g/dL), platelets.

CELMANAX, g/t	Ery, MM/mm <sup>3</sup>	Hct, %	Hb, g/dL	MCV, fL	MCHC, g/dL	Plt, $\mu\text{L}$	WBC/mm <sup>3</sup>	Neu, $\mu\text{L}$	Lym, $\mu\text{L}$	Mon, $\mu\text{L}$	Eos, $\mu\text{L}$
0	2.21	33.70 <sup>b</sup>	12.80 <sup>b</sup>	151 <sup>a</sup>	38.20	1360 <sup>c</sup>	19990 <sup>e</sup>	2798 <sup>e</sup>	15992 <sup>e</sup>	999 <sup>c</sup>	199 <sup>e</sup>
50	2.97	45.15 <sup>a</sup>	18.10 <sup>a</sup>	152 <sup>a</sup>	40.10	1460 <sup>a</sup>	59300 <sup>a</sup>	10674 <sup>a</sup>	47440 <sup>a</sup>	593 <sup>d</sup>	593 <sup>a</sup>
100	2.95	45.35 <sup>a</sup>	17.20 <sup>ab</sup>	154 <sup>a</sup>	39.00	1340 <sup>d</sup>	39580 <sup>b</sup>	7124 <sup>b</sup>	31664 <sup>b</sup>	395 <sup>e</sup>	395 <sup>b</sup>
300	2.23	30.94 <sup>b</sup>	12.90 <sup>b</sup>	139 <sup>c</sup>	41.80	1290 <sup>e</sup>	27570 <sup>d</sup>	3859 <sup>c</sup>	22056 <sup>d</sup>	1378 <sup>b</sup>	275 <sup>d</sup>
500	2.31	33.32 <sup>b</sup>	13.70 <sup>ab</sup>	144 <sup>b</sup>	41.10	1450 <sup>b</sup>	29640 <sup>c</sup>	3853 <sup>d</sup>	23712 <sup>c</sup>	1778 <sup>a</sup>	296 <sup>c</sup>
P-Value	0.05	<0.001	0.005	<0.001	0.18	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SEM	0.23	1.12	2.52	6.23	2.26	67.14	82.63	73.91	88.17	59.24	24.15
C.V. (%)	20.47	6.63	16.73	1.69	6.24	8.67	15.24	27.16	31.18	19.51	10.25
Linear	0.162	<0.001	0.082	<0.001	0.052	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Quadratic	0.51	0.27	0.52	0.002	0.268	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

*Different letters in the columns indicate significant differences between treatment means.*

## Intestinal morphology

A quadratic response of CELMANAX supplementation was seen on villus height, lamina propria and surface area with the 100 to 300 g/t dose promoting best morphofunctional optimizations of the intestinal epithelium, enhancing the integrity and absorptive efficiency of the intestinal villi in Nile tilapia (Table 7).

Liver histology demonstrated preserved tissue architecture across all treatments, while splenic sections exhibited improved organization of white pulp at 100–300 g/t, suggesting immunomodulatory benefits without inducing hyperactivity (data not shown).

**Table 7.** Effect of increasing dose of CELMANAX on intestinal histomorphometry.

CELMANAX, g/t	Villus Height, $\mu\text{m}$	Villus Width, $\mu\text{m}$	Height/Width	Lamina propria, $\mu\text{m}$	Intestinal Wall thickness, $\mu\text{m}$	Surface area, $\mu\text{m}$
0	172.49 <sup>b</sup>	34.53 <sup>c</sup>	7.06 <sup>b</sup>	5.09 <sup>ab</sup>	27.97 <sup>ab</sup>	0.82 <sup>bc</sup>
50	192.72 <sup>ab</sup>	50.72 <sup>a</sup>	9.45 <sup>a</sup>	3.86 <sup>c</sup>	23.42 <sup>c</sup>	1.10 <sup>a</sup>
100	206.15 <sup>a</sup>	43.13 <sup>b</sup>	8.91 <sup>a</sup>	4.85 <sup>bc</sup>	24.82 <sup>bc</sup>	0.86 <sup>bc</sup>
300	212.94 <sup>a</sup>	37.40 <sup>bc</sup>	8.20 <sup>ab</sup>	5.95 <sup>a</sup>	29.73 <sup>a</sup>	0.73 <sup>c</sup>
500	168.37 <sup>b</sup>	40.05 <sup>bc</sup>	7.95 <sup>ab</sup>	4.28 <sup>bc</sup>	23.67 <sup>c</sup>	0.96 <sup>ab</sup>
P-Value	0.0001	<0.001	0.003	<0.001	0.02	<0.001
SEM	7.76	1.49	0.44	0.27	1.61	0.05
C.V. (%)	18.21	16.14	23.51	24.90	27.83	24.15
Linear	0.41	0.10	0.58	0.73	0.74	0.58
Quadratic	<0.001	0.39	0.06	0.001	0.13	0.009

*Different letters in the columns indicate significant differences between treatment means, determined by Tukey's test ( $P < 0.05$ ), performed in addition to linear and quadratic regression analysis.*

## Intestinal Mucosal Microbiota

Multivariate analyses confirmed distinct clustering of microbiota profiles among the supplemented groups (Figure 2).

Figure 2. Biplot of Principal Component Analysis (PCA) of the Intestinal Microbiota in Tilapia Fed with CELMANAX.

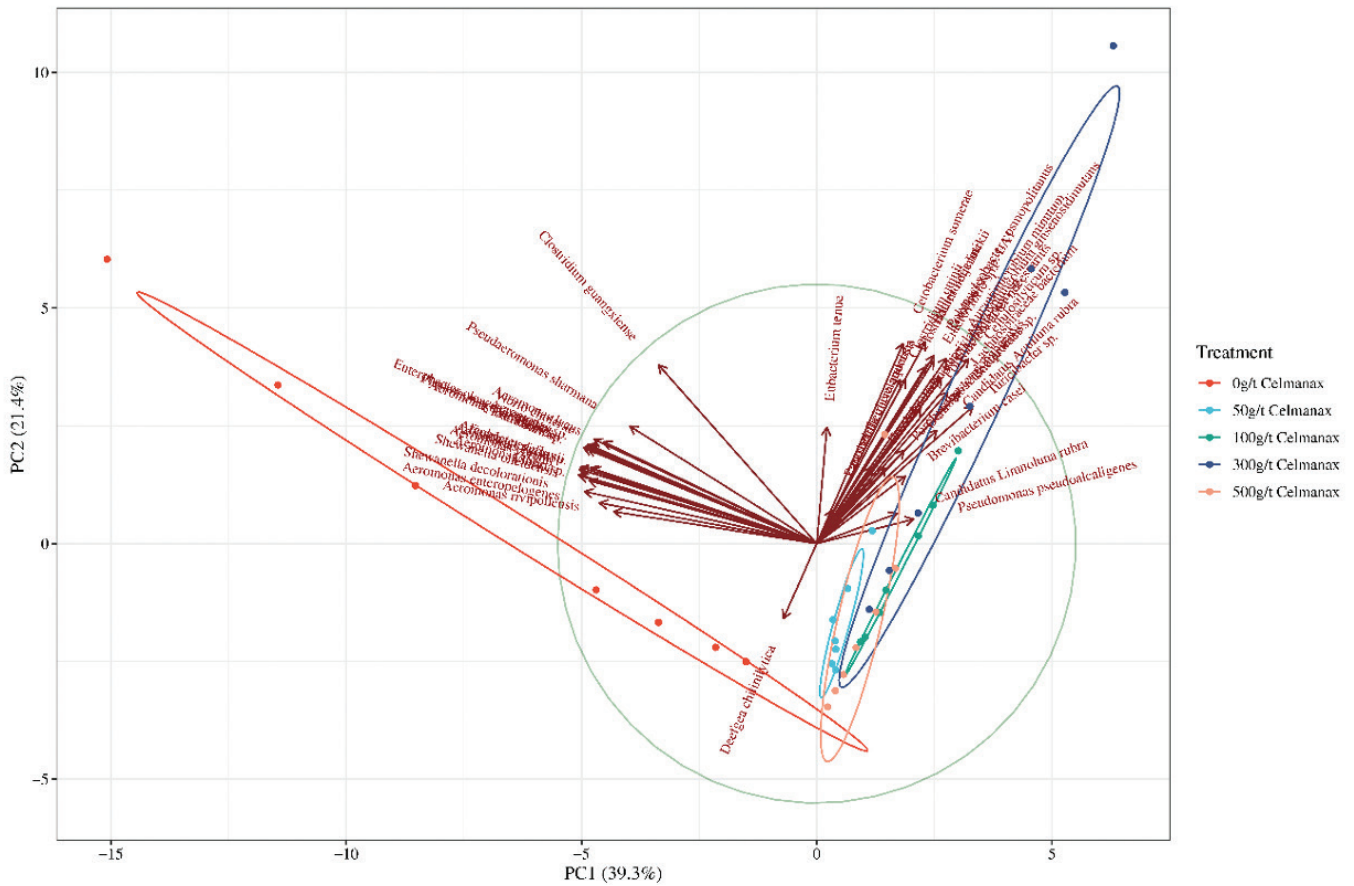
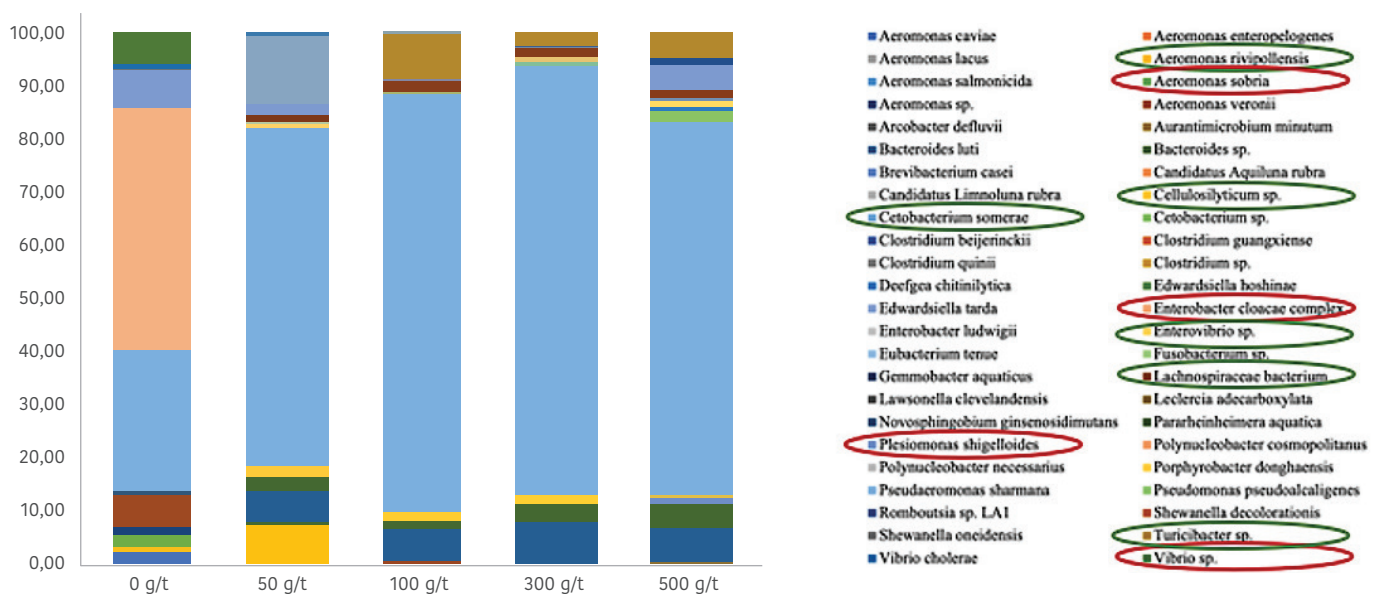


Figure 3. Relative abundance by treatments.



Additionally, CELMANAX modulated intestinal microbiota, promoting beneficial taxa such as *Cetobacterium somerae*, *Turicibacter sp.*, and *Aeromonas rivipollensis*, while concurrently reducing potential pathogens such as *Vibrio sp.* and *Plesiomonas shigelloides*.

*Cetobacterium somerae* has been implicated in enhancing host growth performance and modulating immune responses. Higher supplementation levels of CELMANAX demonstrated marked reductions *Aeromonas sobria*, *Vibrio sp.*, and *Plesiomonas shigelloides*, suggesting a potential antimicrobial or inhibitory effect of CELMANAX against these known pathogenic taxa.

## CONCLUSIONS

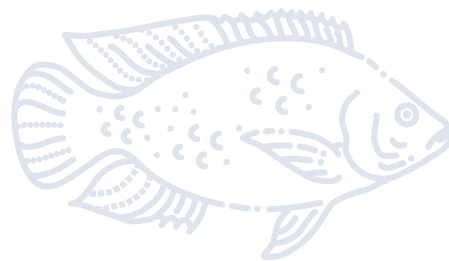
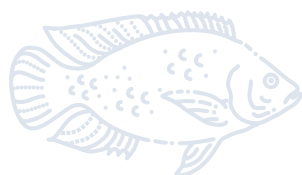
The supplementation of 50 g/t of RFCs was found to enhance the immune response in tilapia. Moreover, the 100 g/t dosage not only sustained this immunological advantage but also significantly improved growth performance and meat quality.

Multivariate analysis indicated that the 100 g/t and 300 g/t doses exhibited superior productive efficiency, while the 50 g/t and 100 g/t doses were more effective in bolstering immune resilience against prevalent farming stressors.

Intestinal morphological changes indicate an enhanced absorptive capacity and improved gastrointestinal functionality. Furthermore, a positive modulation of the intestinal microbiota composition was observed, characterized by an increased abundance of beneficial bacterial populations.

These findings support CELMANAX supplementation to be an effective feed additive to promote intestinal health, thereby contributing to enhanced nutritional precision and efficiency in contemporary aquaculture.

It is therefore concluded that supplementation at 100 g/t provides the optimal balance between immune support and productive performance. This dosage is recommended as a continuous strategy in contemporary tilapia farming, in accordance with the principles of precision nutrition.



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To learn more about CELMANAX, contact your ARM & HAMMER™ representative or visit [ahanimalnutrition.com](http://ahanimalnutrition.com).

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