



Animal Health

Leiber CeFi[®] Pro: autolyzed brewer's yeast for aquaculture Product properties and functionalities

June 2020



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Production, product properties and mode of action





Inner workings of a counterflow spray dryer



Autolyzed yeast from 100% brewer's yeast (Saccharomyces cerevisiae)

	Leiber [®] Brewers' Yeast unextracted	CeFi [®] Pro
Crude protein, CP (%)	46	50
Lysine (%)	3.2	3.6
Methionine (%)	0.6	0.8
Oils and fats (%)	2.7	3.0
Nucleic acid protein (% in CP)		12
Dosage (kg/to feed)	10 - 50	2 – 5



Modes of action

- CeFi[®] Pro consists of both the yeast cell wall and the cell contents
- The yeast cell contents are released by a gentle and effective autolysis ("perforating the cell wall")
 - * The cell contents consist of high levels of valuable nutrients incl. amino acids, nucleic acid protein, vitamins, etc.
 - Therefore, CeFi[®] Pro has a high attractability and palatability effect
 - The autolysis renders the cell contents remarkably faster and easier available (high bioavailability)
 - ◆ High native levels of natural RNA components, the nucleotides, nucleosides and the pyrimidine and purine bases
 → support and accelerate cell division, cell regeneration and cell repair)
 - Higher demand for nucleotides during high physiological stress (e.g. reproduction, high growth, immunological stress)
 - Certain cell types (e.g. lymphoid tissue & epithelial cells of the gut mucosa) can produce nucleotides only insufficiently
- In addition, the yeast cell walls result in the well-known gut health promoting effects
 - Prebiotic effect, pathogen/mycotoxin binding, immunity support, biofilm formation, improvements in gut morphology
- Bacteriostatic effect due to bioactive hops components only in brewer's yeast products!!



Benchmarking shrimp trial



Heizuo ^a, Cheng ^b and Kühlwein ^c, 2016

^a Chinese Academy of Fishery Sciences, ^b Vitamex Shanghai Ltd. Nuscience, ^c Leiber GmbH

- 2 weeks acclimatization in holding tanks
- ✤ 480 L. vannamei stocked in sixteen 800 L tanks
- 30 shrimp/tank (initial body weight 2.38 ± 0.24 g)
- 4 replicate tanks per treatment
- Isonitrogenous & isocaloric diets

42.4% crude protein, 6.4% crude fat, 13.2% crude ash

- Feeding 3 times daily for 8 weeks
- Autolyzed yeast supplemented to control diet: 0.2%

СО	COMP 1	COMP 2	CeFi [®] Pro
Control	2 kg/to feed	2 kg/to feed	2 kg/to feed
(no yeast)	Competitor 1	Competitor 2	Leiber [®] product



Fish meal Sovbean meal Wheat flour Peanut meal Squid visceral meal Meat & bone meal ■Ca (H2 PO4)2 · H20 Fish oil Soybean lecithin Vitamin premix Mineral premix Choline chloride ĭKCI Stay C Molt hormone



Growth performance and feed conversion week 8 (n = 4)



Final body weight (g)







Feed conversion ratio

7



Relative difference of yeast autolysate treatments vs. control

	COMP1	COMP2	CeFi Pro
Final body weight (g)	+ 6.7 %	+ 0.3 %	+ 8.2 %
Weight gain ratio (%)	+ 10.9 %	+ 1.6 %	+ 11.3 %
Specific growth rate (%)	+ 4.7 %	+ 0.6 %	+ 4.7 %
Feed conversion ratio	- 5.6 %	+ 0.6 %	- 6.9 %



Conclusions

- ✤ Leiber CeFi[®] Pro
- ... outperforms all other treatments regarding growth performance & feed conversion
- significantly increased growth performance & feed conversion vs. control and competitor 2
- ... numerically improved growth performance & feed conversion vs. competitor 1 (FCR improved by 1.32%)



Preliminary field trial data from shrimp hatcheries in Ecuador 2020



Original comments and explanations by distributor ChemTech S.A.:

- * The different hatcheries used different methods to top-coat CeFi® Pro onto the feed
- For survival and size (PL's/g) mean values of all tanks per treatment are listed
- At harvest, hatcheries usually deliver 10 20% of the ordered quantity to customers as free of charge bonus on top, i.e. the real survival is 10 - 20% more
- Same applies to shrimp nauplius suppliers
- Control tank feeds generally contain already certain top-coated ingredients (various immunostimulants, vitamins, etc.), which have been establishes and routinely used in the respective hatchery



DOBLE "	O" HATCHERY										
ZONE: LA	A DIABLICA (SA	LINAS)									
TANK	SRTART DATE	FINISH DATE	TONS	DENSITY/L	SOURSE	SURVIVAL	SIZE/G	# SIZE	DOSIS/KG	PRODUCT	OBSERVATIONS
1	10.11.2019	26.11.2019	15	133	TEXCUMAR	72%	250	2	10g	CEFI PRO	BETTER GROWTH
2	10.11.2019	26.11.2019	15	133	TEXCUMAR	72%	250	2	10g	CEFI PRO	LOW SIZE DISPERSION
3	10.11.2019	28.11.2019	15	133	TEXCUMAR	65%	300	4	10g	CONTROL	MORE DAYS OF CULTIVATION
4	10.11.2019	28.11.2020	15	133	TEXCUMAR	65%	300	4	10g	CONTROL	LESS SURVIVAL
OCEANL	AB HATCHERY										
ZONE: RI	O CHICO (SAN	TA ELENA)									
TANK	SRTART DATE	FINISH DATE	TONS	DENSITY/L	SOURSE	SURVIVAL	SIZE/G	# SIZE	DOSIS/KG	PRODUCT	OBSERVATIONS
1	06.02.2020	24.02.2020	10	125	SHRIMPLAB	80%	280	2	20g	CEFI PRO	BETTER GROWTH
2	06.02.2020	24.02.2020	10	125	SHRIMPLAB	80%	280	2	20g	CEFI PRO	LOW SIZE DISPERSION
3	06.02.2020	24.02.2020	10	125	SHRIMPLAB	80%	280	2	20g	CEFI PRO	LESS DAYS OF CULTIVATION
4	06.02.2020	24.02.2020	10	125	SHRIMPLAB	80%	280	2	20g	CEFI PRO	MORE SURVIVAL
5	06.02.2020	24.02.2020	10	125	SHRIMPLAB	80%	280	2	20g	CEFI PRO	BETTER GROWTH
6	06.02.2020	24.02.2020	10	125	SHRIMPLAB	80%	280	2	20g	CEFI PRO	LOW SIZE DISPERSION
7	06.02.2020	24.02.2020	10	125	SHRIMPLAB	80%	280	2	20g	CEFI PRO	PL 12 CULTIVATION STAGE
8	06.02.2020	24.02.2020	10	125	SHRIMPLAB	80%	280	2	20g	CEFI PRO	HEALTHIER
9	06.02.2020	24.02.2020	10	125	SHRIMPLAB	80%	280	2	20g	CEFI PRO	MORE LIPIDS
10	06.02.2020	24.02.2020	10	125	SHRIMPLAB	80%	280	2	20g	CEFI PRO	BETTER APPEARANCE
M1	06.02.2020	28.02.2020	10	125	SHRIMPLAB	78%	300	4	20g	CONTROL	PL 12 CULTIVATION STAGE
M2	06.02.2020	28.02.2020	10	125	SHRIMPLAB	78%	300	5	20g	CONTROL	MORE SIZE DISPERSION
M3	06.02.2020	28.02.2020	10	125	SHRIMPLAB	78%	300	4	20g	CONTROL	MORE DAYS OF CULTIVATION
M4	06.02.2020	28.02.2020	10	125	SHRIMPLAB	78%	300	5	20g	CONTROL	MORE SIZE DISPERSION
M5	06.02.2020	28.02.2020	10	125	SHRIMPLAB	78%	300	4	20g	CONTROL	LESS GROTH
M6	06.02.2020	28.02.2020	10	125	SHRIMPLAB	78%	300	5	20g	CONTROL	MORE SIZE DISPERSION
M7	06.02.2020	28.02.2020	10	125	SHRIMPLAB	78%	300	4	20g	CONTROL	LESS SURVIVAL



ROTTY S.	A. HATCHERY										
ZONE: M	AR BRAVO (SA	LINAS)									
TANK	SRTART DATE	FINISH DATE	TONS	DENSITY/L	SOURSE	SURVIVAL	SIZE/G	# SIZE	DOSIS/KG	PRODUCT	OBSERVATIONS
5	08.01.2020	26.01.2020	22	95	TEXCUMAR	82%	250	2	8g	CEFI PRO	BETTER GROWTH
6	08.01.2020	26.01.2020	22	95	TEXCUMAR	82%	250	2	8g	CEFI PRO	LOW SIZE DISPERSION
1	08.01.2020	28.02.2019	22	95	TEXCUMAR	75%	330	4	8g	CONTROL	PL 12 CULTIVATION STAGE
2	08.01.2020	28.02.2019	22	95	TEXCUMAR	75%	330	4	8g	CONTROL	LESS SURVIVAL
3	08.01.2020	29/2/2019	22	95	TEXCUMAR	75%	330	5	8g	CONTROL	MORE SIZE DISPERSION
4	08.01.2020	29/2/2019	22	95	TEXCUMAR	75%	330	4	8g	CONTROL	MORE DAYS OF CULTIVATION
PABLO C	ISNEROS HATC	HERY									
ZONE: SA	AN PABLO (SAI	NTA ELENA)									
TANK	SRTART DATE	FINISH DATE	TONS	DENSITY/L	SOURSE	SURVIVAL	SIZE/G	# SIZE	DOSIS/KG	PRODUCT	OBSERVATIONS
6	08.01.2020	26.01.2020	20	150	TEXCUMAR	85%	250	2	10g	CEFI PRO	BETTER GROWTH
7	08.01.2020	26.01.2020	20	150	TEXCUMAR	85%	250	2	10g	CEFI PRO	LOW SIZE DISPERSION
1	08.01.2020	28.02.2019	20	150	TEXCUMAR	78%	300	4	10g	CONTROL	PL 12 CULTIVATION STAGE
2	08.01.2020	28.02.2019	20	150	TEXCUMAR	78%	300	4	10g	CONTROL	LESS SURVIVAL
3	08.01.2020	29/2/2019	20	150	TEXCUMAR	78%	300	5	10g	CONTROL	MORE SIZE DISPERSION
4	08.01.2020	29/2/2019	20	150	TEXCUMAR	78%	300	4	10g	CONTROL	MORE DAYS OF CULTIVATION
5	08.01.2020	29/2/2019	20	150	TEXCUMAR	78%	300	4	10g	CONTROL	LESS GROTH
MBL HAT	TCHERY										
ZONE: LA	A DIABLICA (SA	LINAS)									
TANK	SRTART DATE	FINISH DATE	TONS	DENSITY/L	SOURSE	SURVIVAL	SIZE/G	# SIZE	DOSIS/KG	PRODUCT	OBSERVATIONS
8	08.03.2020	24.03.2020	15	120	TEXCUMAR	78%	220	2	8g	CEFI PRO	BETTER GROWTH
9	08.03.2020	24.03.2020	15	120	TEXCUMAR	79%	220	2	10g	CEFI PRO	MORE SURVIVAL
10	08.03.2020	26.03.2020	15	120	TEXCUMAR	75%	280	4	10g	CONTROL	MORE DAYS OF CULTIVATION



Conclusions

- CeFi Pro administered to shrimp larvae overall...
- ... increased growth performance and reduced the cultivation period for about 2-4 days
- … improved survival rates by 2-7%
- ... led to a more uniform size distribution (number of size classes reduced from about 4-5 down to 2)



Enrichment diets for turbot larvae



Miest & Arndt, 2014/15

GeoMAR, Germany

- Turbot larvae (Scophthalmus maximus) reared in twelve 75L-tanks filled with 30L FSW at 16 ± 1° C
- Stocking density 80 larvae/L
- Feeding daily with 3 prey items/mL (rotifers enriched for 3 hours with the treatments)
- From 13 dph larvae additionally fed enriched Artemia sp. nauplii (1 nauplius/mL)
- 4 replicate tanks per treatment (randomly distributed)
- ◆ 20 larvae sampled in the morning before feeding to analyze growth, RNA:DNA ratio and tryptic activity

СО	LOW	HIGH
Control	S.Presso +	S.Presso +
(S.presso, INVE, Belgium)	1 g/L CeFi [®] Pro	3 g/L CeFi [®] Pro



Survival rates

Survival was significantly increased due to administration of *Brachionus* fed the high dose of CeFi Pro!



Days post hatching



Final body weight

- No difference in dry weight of larvae at 12 dph, but significantly increased at 25 dph with CeFi Pro!
 CO = 6.25±0.64 mg vs. LOW = 9.08±1.19 mg (p = 0.058) vs. HIGH = 10.97±1.28 mg (p ≤ 0.01)
- No significant difference in larval length





RNA/DNA ratio

- ♦ 12 dph: significantly higher with LOW (2.26±0.05) compared to CO (2.00±0.08) and HIGH (2.10±0.09)
- ◆ 25 dph: significantly higher with LOW (2.45±0.13; p ≤0.01) and HIGH (2.63±0.10; p ≤0.001) compared to CO (1.96±0.09)





Tryptic activity

- Not affected at 12 dph
- At 25 dph: significantly higher with LOW (20.41±7.24 nmol/min) compared to CO (1.34±0.34 nmol/min) and HIGH (2.28±0.77 nmol/min) (both p ≤0.0001)





Conclusions

- CeFi Pro administered to turbot larvae via enriched live feeds...
- ✤ ... significantly increases survival up to 25 dph
- ✤ ... significantly improves growth at 25 dph
- significantly increases RNA/DNA-ratio up to 25 dph (marker for nutritional status, metabolism)
- significantly increases tryptic activity at 25 dph (marker for protein metabolism)



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ORIGINAL ARTICLE



Dietary supplementation of autolysed yeast enhances growth, liver functionality and intestinal morphology in African catfish

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Experimental design

- ✤ 240 African catfish (*Clarias gariepinus*) stocked in 12 rearing tanks
- 20 fish per tank (water volume 33 L)
- Initial body weight 22.5 ± 1.15 g per fish
- ✤ 3 replicate tanks per treatment, randomly allocated
- Feeding twice a day to apparent satiation for 7 weeks
- Weekly batch-weighing
- ♦ Water temperature 29.0 ± 0.29 °C, pH 6.85 ± 0.34, O₂ > 5 mg/L, ammonia 0.34 ± 0.1 mg/L
- Leiber CeFi[®] Pro (AY autolyzed yeast) supplementation:

СО	0.3 % AY	0.6 % AY	1.0 % AY
Control	3 kg/to feed	6 kg/to feed	10 kg/to feed
(no yeast)	Leiber CeFi [®] Pro	Leiber CeFi [®] Pro	Leiber CeFi [®] Pro

СО

9.33

6.55

4.39

Experimental design

Moisture

Crude Protein

Crude Lipid

Crude Ash

Crude Fibre

NfE

Ingredients of the basal diet and proximate analysis of test diets (% of dry weight) *

0.6 % AY

1.0 % AY

Leiber CeFi[®] Pro supplemented at the expense of shrimp meal *

0.3 % AY

Isonitrogenous and isolipdic diets, pellet size 2 mm *

	9.27 ± 0.12	9.53 ± 0.17	9.13 ± 0.21	9.33 ± 0.12
	39.2 ± 0.1	38.6 ± 0.0	38.9 ± 0.3	38.9 ± 0.1
	13.9 ± 0.1	13.3 ± 0.2	13.7 ± 0.0	13.6 ± 0.1
200	6.79 ± 0.12	6.96 ± 0.06	6.84 ± 0.13	6.55 ± 0.02
50	4.02 ± 0.11	4.94 ± 0.11	4.35 ± 0.15	4.39 ± 0.09
50	35.7 ± 0.4	35.6 ± 0.1	35.6 ± 0.5	36.1 ± 0.3







Growth performance, FCR and general survival after 7 weeks (n = 3)

	со	0.3 % AY	0.6 % AY	1.0 % AY
IBW (g)	22,0 ± 0,7	22,2 ± 0,9	23,5 ± 1,4	22,3 ± 0,5
FBW (g)	91,0 ± 1,9 ^a	106,1 ± 6,5 ^b	98,1 ± 8,2 ^{ab}	$98,0 \pm 8,2$ ^{ab}
SGR (%)	2,90 ± 0,03	3,19 ± 0,11	2,92 ± 0,12	3,01 ± 0,19
MGR (g/kg ^{0,8} /day)	14,5 ± 0,1 ª	$16,0 \pm 0,5$ ^b	$14,8 \pm 0,5$ ^{ab}	15,1 ± 0,9 ^{ab}
FCR	1,25 ± 0,12	1,08 ± 0,02	1,15 ± 0,10	1,14 ± 0,06
PER (%)	1,53 ± 0,15	1,82 ± 0,06	1,67 ± 0,18	1,70 ± 0,13
HSI	1,06 ± 0,11	1,12 ± 0,16	1,30 ± 0,12	1,18 ± 0,22
VSI	10,2 ± 1,2 ª	11,6 ± 1,4 ^{ab}	10,9 ± 1,7 ^{ab}	13,0 ± 0,5 ^b
Survival (%)	91,7 ± 4,7	98,3 ± 2,4	95,0 ± 0,0	86,7 ± 8,5

Values with different superscripts indicate significant differences (*P* < 0.05). IBW, initial mean body weight; FBW, final mean body weight; MWG, mean weight gain; PWG, percentage weight gain; SGR, specific growth rate; MGR, metabolic growth rate; FCR, feed conversion ratio; PER, protein efficient ratio; HSI, hepatosomatic index; VSI, viscerosomatic index.



Relative difference (in %) of Leiber CeFi® Pro treatments vs. control

	0.3 % AY	0.6 % AY	1.0 % AY
Final body weight (g)	+ 16,5	+ 7,8	+ 7,7
Specific growth rate (%)	+ 10,0	+ 0,7	+ 3,8
Metabolic growth rate (g/kg ^{0,8} /day)	+ 10,3	+ 2,1	+ 4,1
Feed conversion ratio	- 13,6	- 8,0	- 8,8
Protein efficiency ratio (%)	+ 19,0	+ 9,2	+ 11,1
Survival (%)	+ 7,3	+ 3,6	- 5,5

Haemato-biochemistry after 7 weeks (n = 6)



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			× /	
	0% AY	0.3% AY	0.6% AY	1.0% AY
Haematocrit (% PCV)	$\textbf{35.0} \pm \textbf{2.78}$	$\textbf{36.3} \pm \textbf{1.76}$	35.5 ± 1.00	37.3±1.04
Haemoglobin (g dL-1)	11.7 ± 0.98	12.3 ± 0.65	11.9 ± 0.36	12.5 ± 0.31
RBC (10 ¹² L ⁻¹)	$\textbf{2.50} \pm \textbf{0.45}$	$\textbf{2.55} \pm \textbf{0.13}$	$\textbf{2.40} \pm \textbf{0.18}$	$\textbf{2.86} \pm \textbf{0.43}$
WBC (10 ⁹ L ⁻¹)	143 ± 24.4	101 ± 64.8	$\textbf{208} \pm \textbf{82.2}$	113 ± 66.9
Neutrophil (%)	24.6 ± 6.45	23.2 ± 7.77	$\textbf{24.4} \pm \textbf{3.61}$	$\textbf{27.0} \pm \textbf{11.7}$
Lymphocytes (%)	$\textbf{72.2} \pm \textbf{6.43}$	$\textbf{68.3} \pm \textbf{8.13}$	71.5 ± 3.12	$\textbf{70.5} \pm \textbf{11.4}$
Basophil (%)	$\textbf{0.67} \pm \textbf{0.76}$	$\textbf{0.33} \pm \textbf{0.58}$	$\textbf{0.67} \pm \textbf{0.76}$	$\textbf{0.33} \pm \textbf{0.58}$
Eosinophil (%)	1.50 ± 1.32	$\textbf{2.00} \pm \textbf{1.50}$	$\textbf{2.00} \pm \textbf{1.73}$	1.00 ± 0.76
Monocytes (%)	$\textbf{3.50} \pm \textbf{1.80}$	$\textbf{2.17} \pm \textbf{1.76}$	$\textbf{3.83} \pm \textbf{0.76}$	$\textbf{2.83} \pm \textbf{0.29}$
MCV (fL)	144 ± 23.1	142 ± 0.40	149 ± 9.77	133 ± 16.3
MCH (pg)	48.4 ± 7.38	$\textbf{48.2} \pm \textbf{0.26}$	50.0 ± 3.58	44.8 ± 5.51
MCHC (g dL ⁻¹)	$\textbf{33.6} \pm \textbf{0.54}$	$\textbf{33.8} \pm \textbf{0.26}$	$\textbf{33.5} \pm \textbf{0.24}$	$\textbf{33.6} \pm \textbf{0.15}$
AST (IU L ⁻¹)	160 ± 19.7	174 ± 14.6	146 ± 19.6	185 ± 32.0
ALT (IU L ⁻¹)	24.9 ± 2.17^{a}	$15.7\pm3.9^{\:c}$	16.8 ± 1.53^{bc}	$\textbf{22.4} \pm \textbf{2.67}^{\text{ ab}}$
ALP (IU L ⁻¹)	60.0±8.40	62.7 ± 12.5	75.7 ± 15.2	69.8±11.6

Values with different superscripts indicate significant differences (P < 0.05). PCV, packed cells volume; RBC, red blood cells; WBC, leucocytes; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; AST, aspartate transaminase; ALT, alanine transaminase; alkaline phosphatase.



Gut histology after 7 weeks (n = 6)

	со	0.3 % AY	0.6 % AY	1.0 % AY
Goblet cells (per 100 µm)	$4,78 \pm 0,87$ ^a	5,75 ± 1,49 ^b	6,32 ± 1,28 ^c	6,62 ± 1,28 ^c
IELs (per 100 μm)	$42,0 \pm 7,3$ ^a	47,9 ± 7,9 ^b	53,7 ± 8,2 °	53,4 ± 9,6 °

Values with different superscripts indicate significant differences (P < 0.05). IELs, Intraepithelial leucocytes.





Conclusions

- ✤ ... significantly increased growth performance at 0.3% supplementation
 - ightarrow final body weight by 16.5 % and metabolic growth rate by 10.3 %
- * ... and numerically increased specific growth rate, FCR, protein efficiency ratio and general survival
- ... significantly decreased ALT levels in blood at 0.3 % and 0.6% supplementation
 - \rightarrow indicating a protective effect on membrane integrity of liver cells
- ... significantly increased mucus-producing gut goblet cells at all supplementation levels
 - \rightarrow indicating an enhanced intestinal barrier function
- … significantly increased gut IEL's at all supplementation levels
 - \rightarrow indicating stimulation of the gut-associated lymphoid tissue (GALT),
 - \rightarrow thereby protecting and preventing pathogen invasion



The bacteriostatic effect

Bacteriostatic effects



- Hop effects:
 - ✤ calming
 - antioxidative
 - adstringent
 - bacteriostatic



Hildegard von Bingen (12. Jahrhundert)

"Der Hopfen trockne die Eingeweide aus, mache traurig und betrübt. Aber durch seine Bitterkeit bewirke er immerhin, dass sich Getränke, denen er zugesetzt ist, lange hielten"





eiher



Bacteriostatic effects

- Inhibitor test results
 - Inhibition of Staphylococcus aureus
 - Bacteriostatic effects
 - Resistant antibiotics
- Conclusion
 - Mode of action against resistant pathogens







Sample concentration *4 0.2% in feed*



Conclusions and recommendations for use



Conclusions

- CeFi[®] Pro has high levels and high bioavailability of nutrients and nucleotides
- CeFi[®] Pro can increase growth performance and feed conversion
- CeFi[®] Pro promotes cell division, regeneration and repair through nucleotides
- CeFi[®] Pro also achieves gut-health promoting effects through both nucleotides & yeast cell walls
- CeFi[®] Pro has a strong bacteriostatic effect against highly antibiotic-resistant pathogens



Recommended usage

- ♦ 2 5 kg/to in feed (0.1 0.5%) \rightarrow usually 3 kg/to feed
- To ensure and maintain high feed intake and growth performance

 \rightarrow in diets with attractability and palatability issues

- \rightarrow where challenging diets are used (high levels of plant ingredients, mycotoxins, etc.)
- Ideal for species with a short digestive tract (e.g. shrimp) through high bioavailability of nutrients
- Use during phases of high nucleotide demand (rapid larvae growth, reproduction, immune challenges, …)

Leiber® Beta-S, Beta-S Plus, Biolex® MB40 and CeFi® pro

General recommendation guideline for conceptual use in fish and shrimp





For more information please contact:

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Thanks a lot for your attention!

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