Leiber

Leiber® Beta-S:

purified ß-glucans for aquaculture

Dr. Holger Kühlwein

Global KAM Aquaculture

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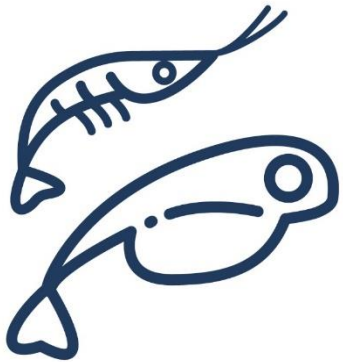
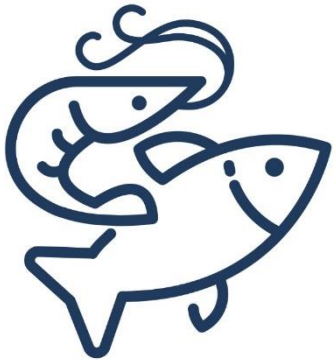
Overview

- Background information and product properties
- Functionalities – experimental results in fish
- Functionalities – experimental results in shrimp
- Excursion: human cancer research
- Conclusions and recommendations for use



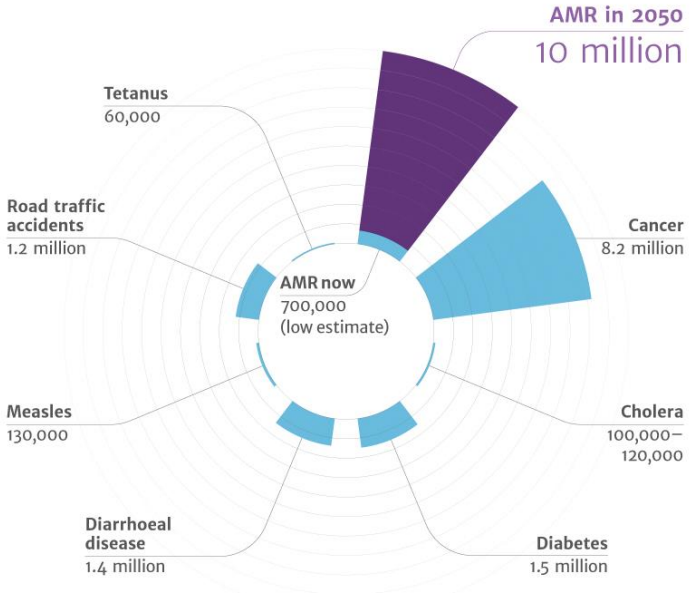


Leiber® βeta-S

Background information and product properties

Why so much interest in functional feeds?

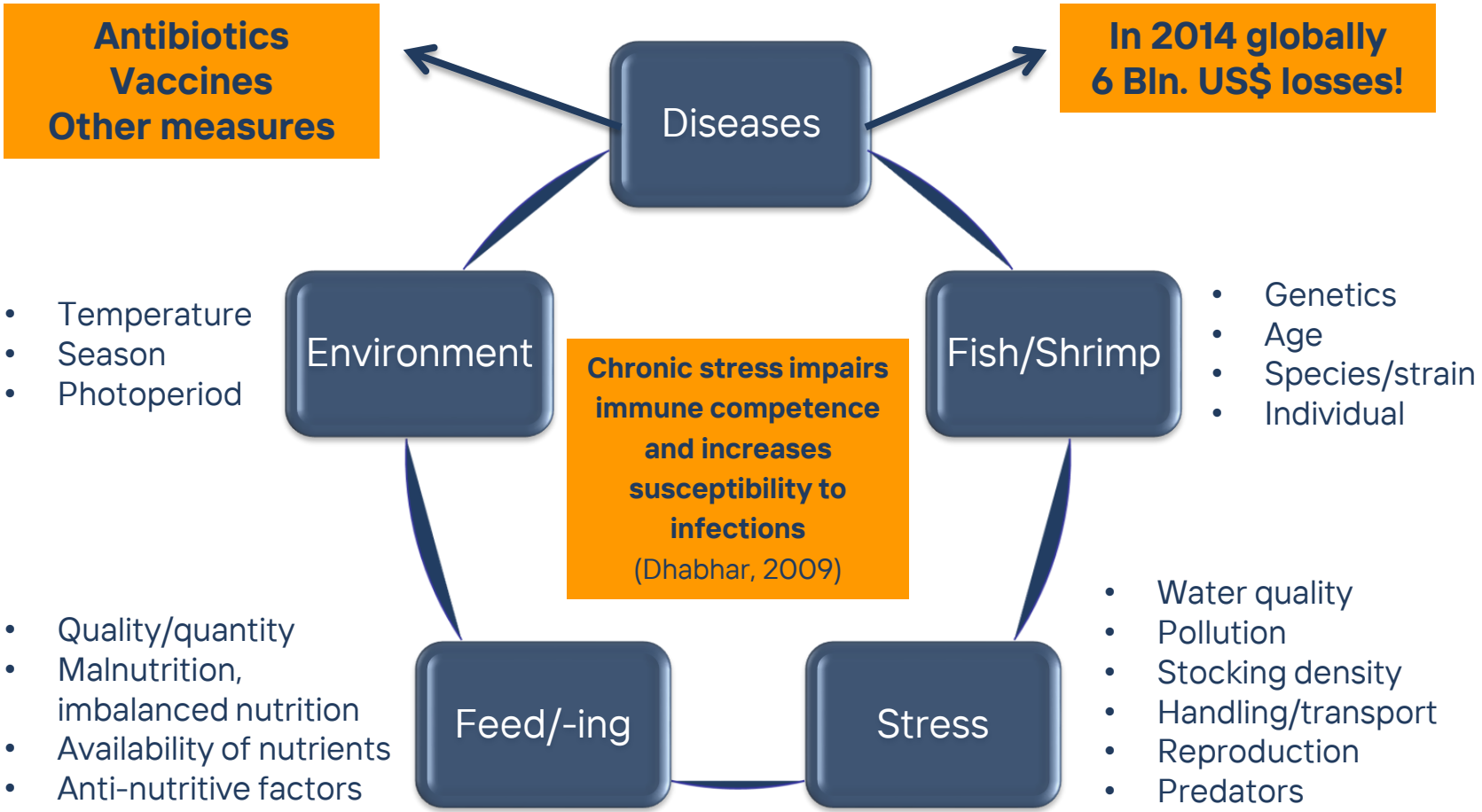
- Issues with disease pressure and compromised immunity in intensive aquaculture husbandry
- Related economic losses
- Antimicrobial resistance and ban of AGP's (pressure from media, consumers/retailers, authorities, etc.)
- Economic improvements of husbandry (weight gain, feed conversion, general survival rates, etc.)
- Animal welfare
- Food security
- Sustainability



"Deaths attributable to antimicrobial resistance every year compared to other major causes of death"

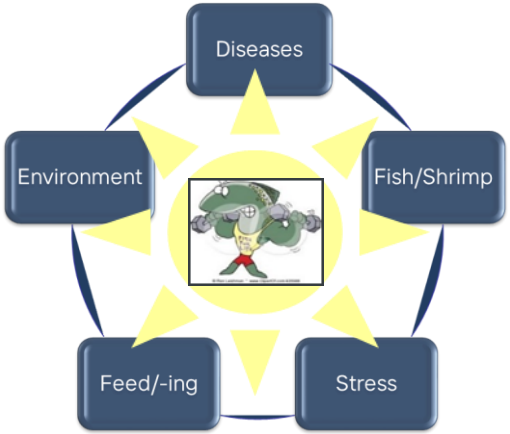
From: The Review on Antimicrobial Resistance 2014

Factors on immune competence and health status



„Prevention is better than cure“

- Maximize immune competence!
- Prophylactic immune activation with immune modulators
- Complementing and increasing the efficacy of vaccines



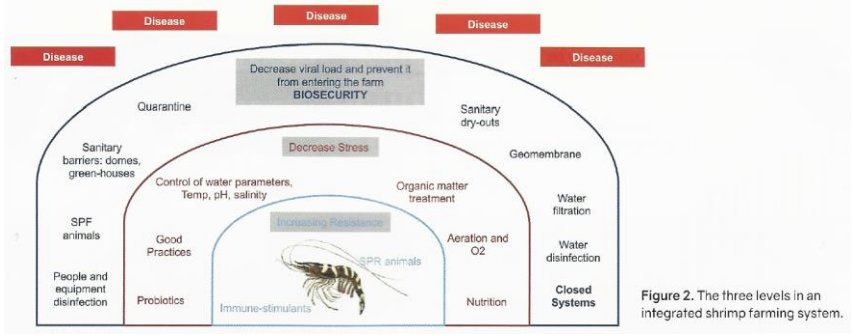


Figure 2. The three levels in an integrated shrimp farming system.

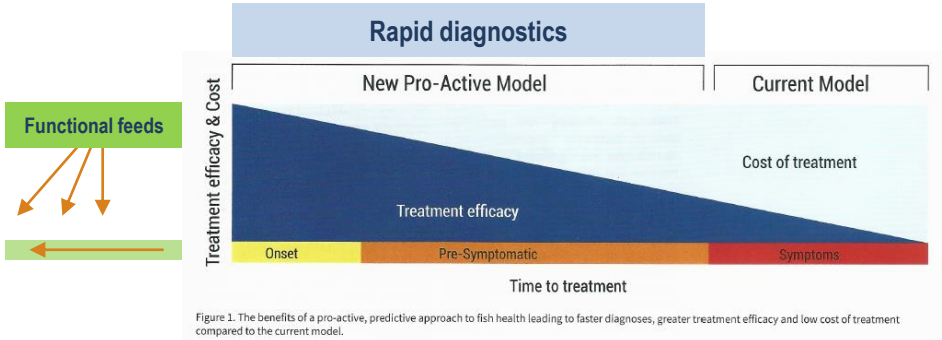


Figure 1. The benefits of a pro-active, predictive approach to fish health leading to faster diagnoses, greater treatment efficacy and low cost of treatment compared to the current model.

(Erasmus of Rotterdam)

What alternatives?

- Prebiotics
- Probiotics
- Plant extracts
- Animal by-products
- Others, e.g. organic acids, algae, …

Table 7: Type of prebiotics studied across different animal species

Substance / Agent	Number of articles	Porcine	Poultry	Bovine	Caprine & Ovine	Fish	Rabbits	Equine	Pets
Mannan oligosaccharide (MOS)	93	12	48	2	2	25	1	0	4
Glucan	62	9	19	2	1	31	0	0	0
Fructooligosaccharide (FOS)	45	4	13	0	1	15	0	2	10
Yeast cell wall (YCW)	43	6	22	1	1	10	0	0	3
Inulin	29	7	10	1	1	6	0	0	4
Chitooligosaccharide (COS)	12	6	4	1	0	1	0	0	0
Galactooligosaccharides (GOS)	10	2	2	0	0	3	0	1	2
Lipopolysaccharide (LPS)	6	3	0	1	0	2	0	0	0
Arabinoxylan oligosaccharides (AXOS)	5	1	0	0	0	3	1	0	0
Inactivated yeast-bacteria	5	3	0	0	1	1	0	0	0
Xylooligosaccharide (XOS)	5	2	2	0	0	1	0	0	0
Galactomanan	4	4	0	0	0	0	0	0	0
Probiotic in general terms	4	0	3	0	0	1	0	0	0
Transgalactooligosaccharide (TOS)	3	0	1	0	0	2	0	0	0
Galactoglucomannan oligosaccharide-arabinoxylan complex (GGMO-AX)	3	0	3	0	0	0	0	0	0
Levan	3	1	1	0	0	1	0	0	0
Polydextrose	3	2	0	0	0	0	0	0	1
Peptidoglycan	1	0	0	0	0	1	0	0	0
Chitin	1	0	0	0	0	1	0	0	0
Galacto-mannan-oligosaccharides (GMOS)	1	1	0	0	0	0	0	0	0
Acidic oligosaccharides (AOS)	1	0	0	0	0	0	0	1	0
Arabinogalactan	1	0	0	0	0	0	0	0	1
Phosphorylated mannans (MAN)	1	1	0	0	0	0	0	0	0
Arabinoxylan	1	0	1	0	0	0	0	0	0
Mannobiose	1	0	1	0	0	0	0	0	0

TOTAL	343

IRTA, 2015. Review of immune stimulator substances/agents that are susceptible of being used as feed additives: mode of action and identification of end-points for efficacy assessment. EFSA supporting publication 2015:EN-905. 266 pp.

Which parameters for assessment?

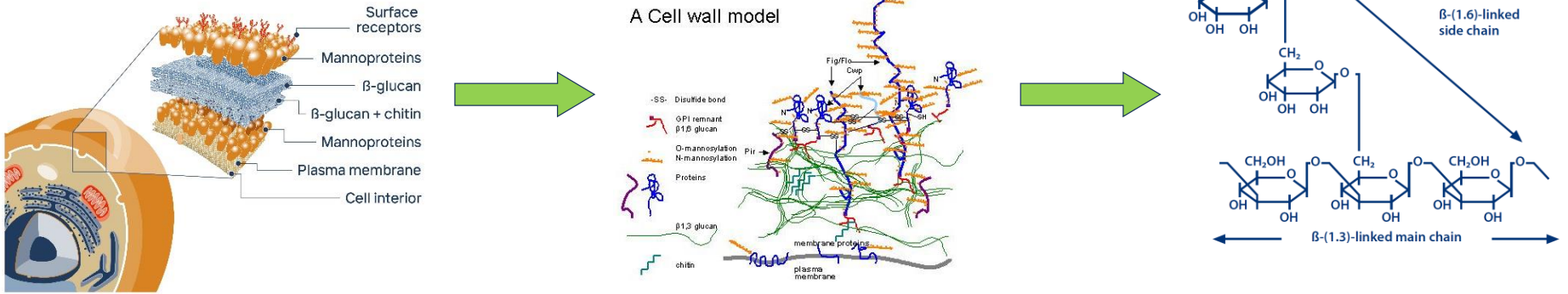
Table 90: Number of studies for each end-point when prebiotics are studied in fish (Freshwater fish)

FISH – PREBIOTIC (freshwater) (n=34)		Number of articles
Immunological parameters studied (fish)	Phagocytic activity	8
	Complement activity	6
	Leucocyte count	10
	Immunoglobulin quantification	8
	Haematocrit	8
	Respiratory (oxidative) burst	11
	Phosphatase activity	1
	Protease activity	0
	Phenoloxidase activity	1
	Lysozyme activity	12
	Peroxidase activity	2
	Bacterial activity	2
	Intestine morphology	6
	Other	31
Health status	Diarrhoea	0
	Mortality	13
	Morbility	0
	Performance	18
	Carcass traits	4
	Skeleton	0
	Other	1
	Not defined	12
Number of studies with challenge		14

IRTA, 2015. Review of immune stimulator substances/agents that are susceptible of being used as feed additives: mode of action and identification of end-points for efficacy assessment. EFSA supporting publication 2015:EN-905. 266 pp.

What are ß-glucans?

- Polysaccharide chains of β-1,3-linked D-glucose monomeres; linear, or branched with β-1,6- (or β-1,4-) linked side chains
- Isolated from a variety of sources, e.g. yeast cell walls, cereals, algae, bacteria, mushrooms
- Most important β-glucan with longest scientific track record from yeast *Saccharomyces cerevisiae*
- Immune modulating effects proven in aquatic organisms, livestock and pets as well as in humans



(Lipke *et al.*, 2001)

Impacts on efficacy

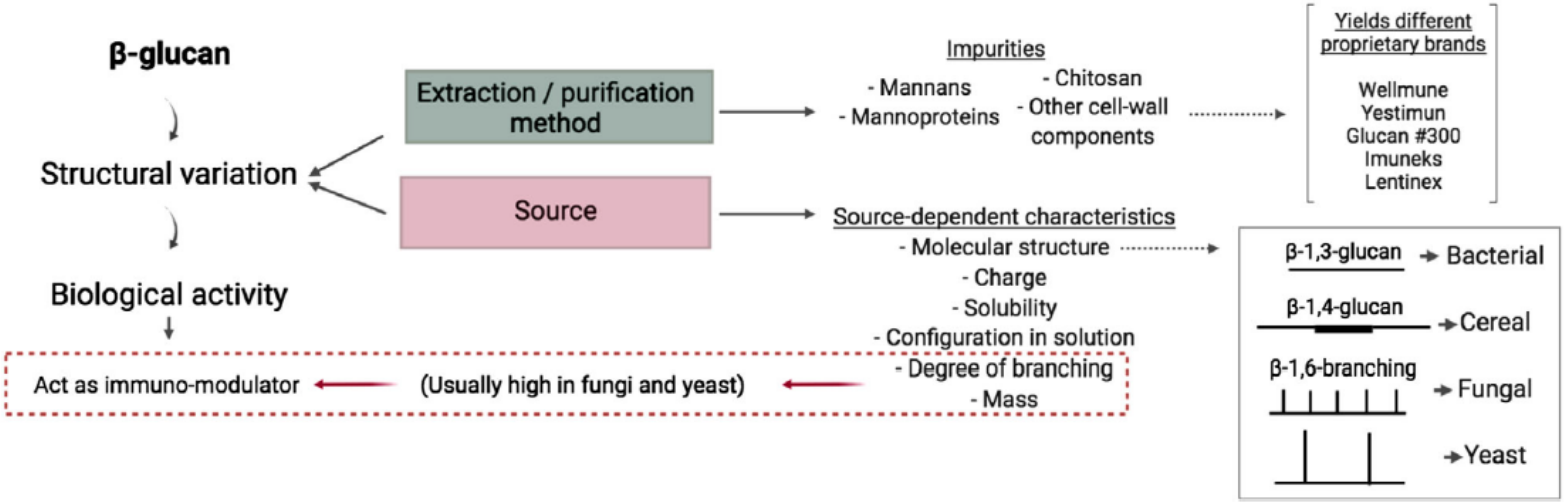
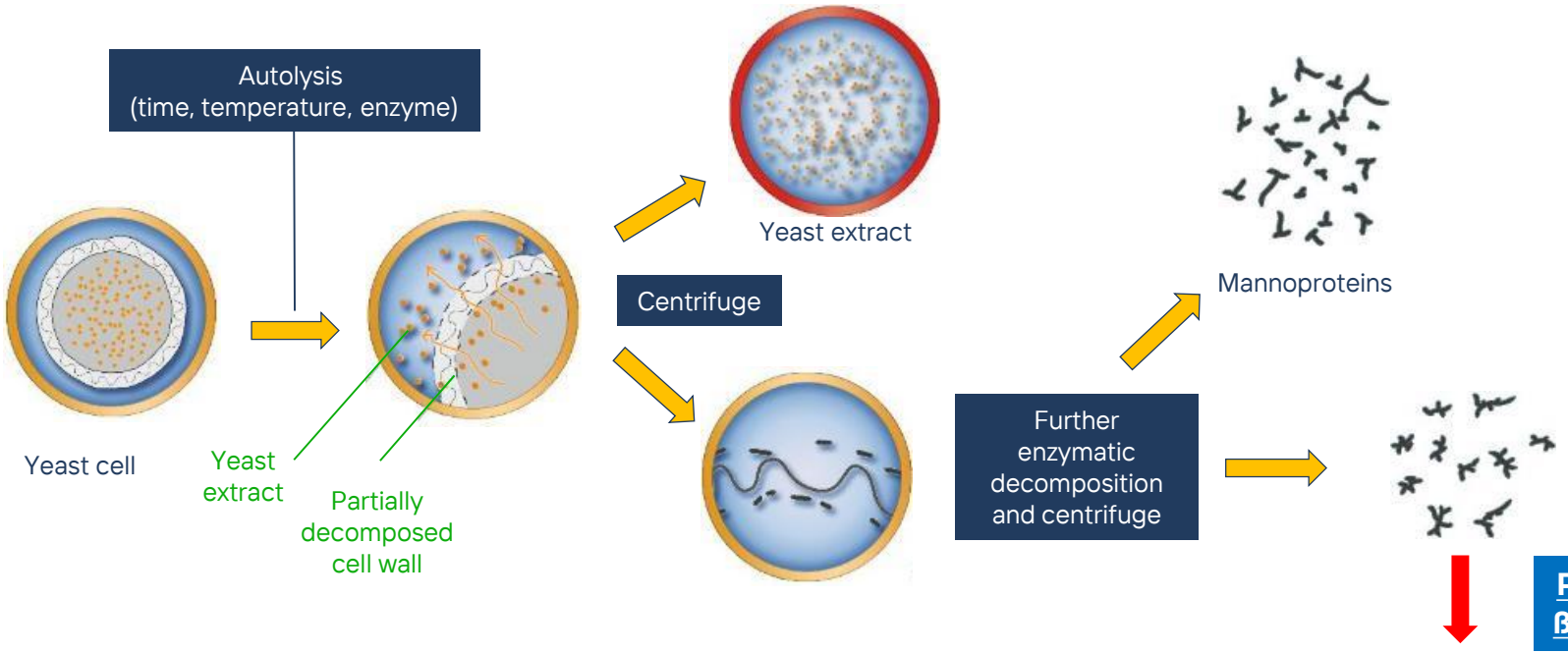


Figure 1. β-Glucan structure–activity relationship. Variability in β-glucan structure is due to differences in source and extraction and/or purification methods, which likely explains the divergent functionalities that exist among β-glucans. Depending on the source, differences arise such as the nature of molecular linkages and the degree of branching, together with variability in mass, charge, solubility, and configuration in solution (single helix, triple helix, or random coil), as well as in impurity levels and content. These variabilities will result in different interactions with the host. Bacterial β-glucans represent the most basic form of the polysaccharide with a linear β-1,3 structure; cereal β-glucans follow the same pattern with dominant β-1,4 stretches; fungal (e.g., mushroom) and yeast (i.e., single cell fungi) β-glucans have frequent β-1,3-D-glucose side chains at β-1,6 branching points that are short and spaced in fungal species (e.g., mushroom) and longer in yeast species. Further variations to these general structures are common. Only highly purified β-1,3-1,6-glucans with a high degree of branching along the β-1,3-glucan backbone and a high molecular weight are able to exert immunomodulatory properties.

(De Marco *et al.*, 2021)

Autolysis and extraction process



Leiber® Beta-S

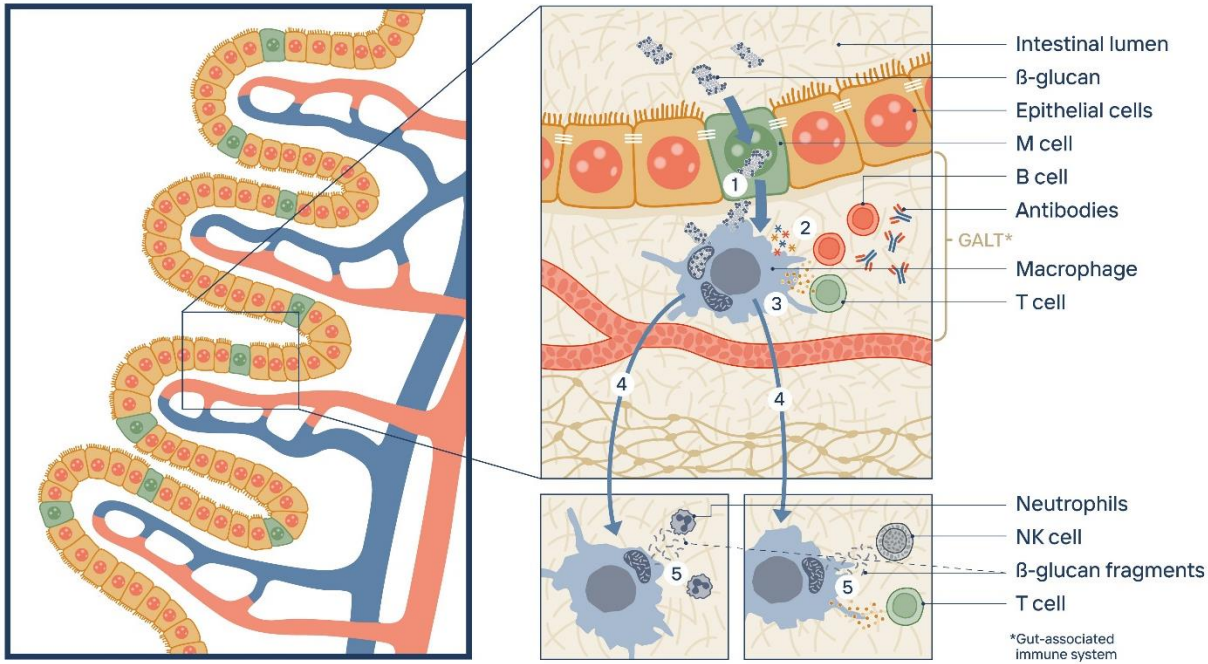
Leiber® Beta-S: product characteristics

- Highly purified, spray-dried ß-1,3-1,6-D-glucans from brewers' yeast *Saccharomyces cerevisiae*

	Leiber® Brewers' yeast	Leiber® Beta-S
Protein (%)	46	4
ß-1,3-1,6-glucans (%)	10 – 15	80
Mannan (%)	7 – 10	< 2
Ash (%)	8	3
Lysine (%)	3.2	0.2
Dosage (kg/to feed)	10 - 50	0.05 – 0.25

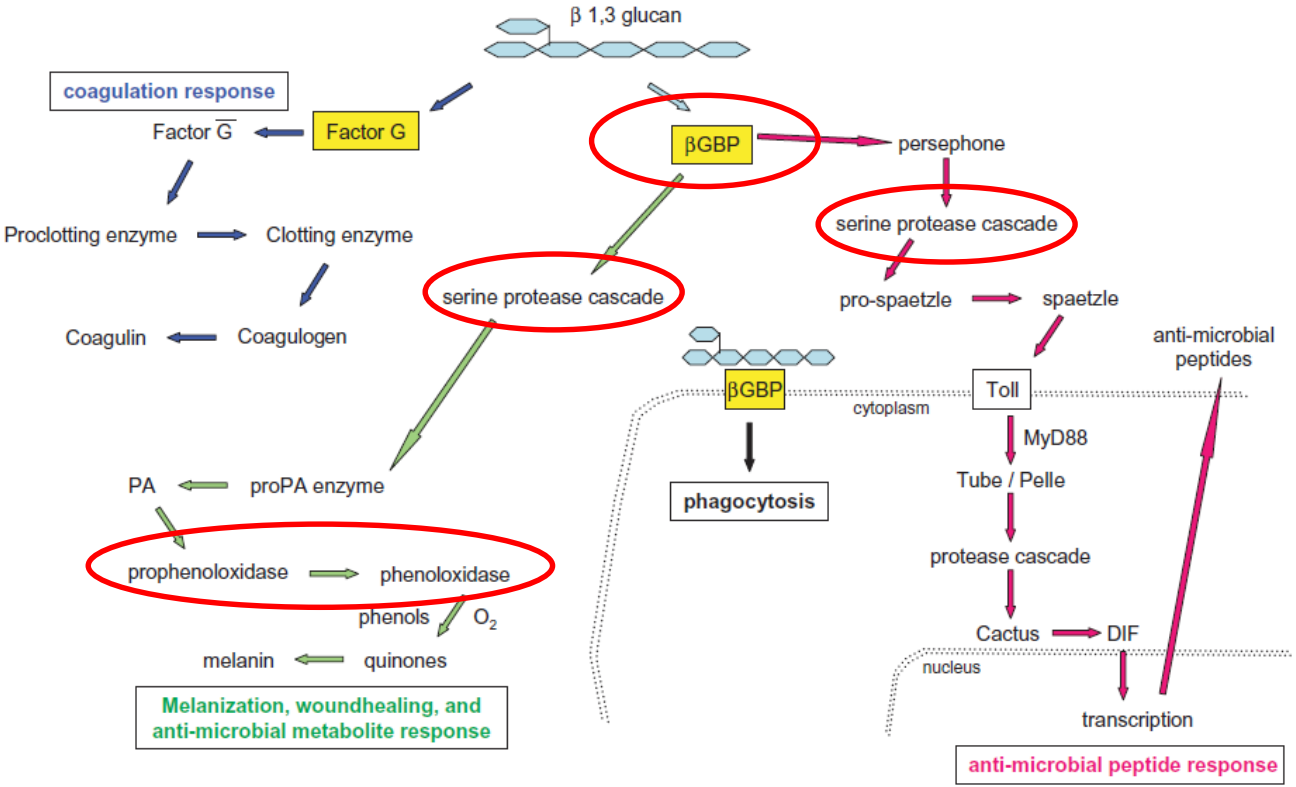
Mode of action of ß-glucans...in vertebrates

1. Uptake of ß-glucans from the gut lumen via M cells and phagocytosis by macrophages (or also via dendritic cells)
2. Activation of macrophages increases phagocytosis and the release of reactive oxygen and nitrogen radicals (oxidative burst), antimicrobial peptides and lysozyme → increased combat of pathogens
3. Release of cytokines and chemokines recruits more immune cells. Macrophages present antigenic structures to T and B cells, causing B cells to subsequently produce antibodies
4. Activated macrophages migrate to organs of the immune system where they break down the ß-glucans into smaller fragments [1]
5. In the organs of the immune system (bone marrow, spleen and lymph nodes), these ß-glucan fragments and cytokines are released, activating other immune cells such as granulocytes and NK and T cells



[1] Fragmentation 3-5 days after ingestion, release of fragments days 5-10, diminishing days 14-21

Mode of action of ß-glucans...in invertebrates

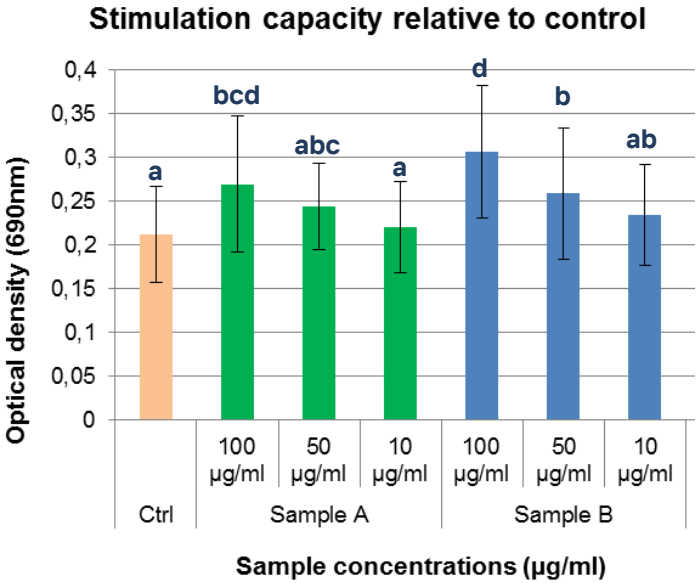


(Brown & Gordon, 2005)

Functionalities –
experimental results in fish

Leiber® Beta-S: benchmarking respiratory burst

- Smink & Wedzerai, Feed Innovation Services, 2017, Wageningen, NL
- In vitro trial with carp head kidney cells (macrophages and neutrophilic granulocytes)
- NBT-assay (Nitroblue tetrazolium) using triplicate samples
- Leiber® Beta-S is significantly better than the control and the main competitor!



Different superscripts indicate a significant difference ($p < 0.05$)

Pairwise comparison of respiratory burst activity

Product	NBT value	Δ
Control	0.212	
Sample A (Competitor)	0.244	+ 15 %
Sample B (Leiber® Beta-S)	0.266	+ 25 %
P-value:		
Sample (A vs B)	**0.039**	**+ 66 %**

Leiber® Beta-S: short-term feeding 4 weeks to carp & trout

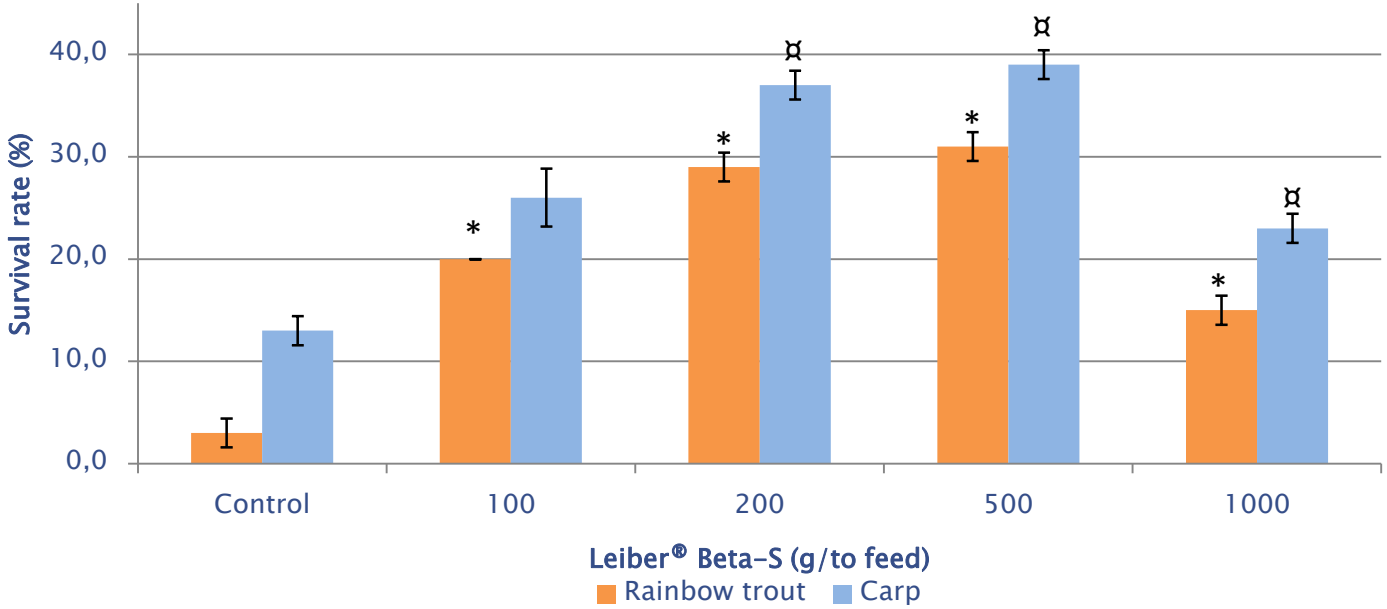
- Siwicki *et al.*, 2008
- Significant increase in non-specific cellular and humoral immunity ($n = 20$)
- ß-glucans are recognized by highly specific receptors on immune cells (phagocytes, e.g. macrophages)

	Control day 28	Beta-S day 0	Beta-S day 28	Difference %
Number of animals (n)	20	20	20	
Leiber® Beta-S (%)	0	0	0,02	
Rainbow trout:				
Phagocytes – oxidative burst	$0,35^{b}$	$0,33^{b}$	$0,52^{a}$	+ 49
Phagocytes – killing activity	$0,34^{b}$	$0,32^{b}$	$0,48^{a}$	+ 41
T-Lymphocyte activity	$0,42^{b}$	$0,44^{b}$	$0,63^{a}$	+ 50
B-Lymphocyte activity	$0,30^{b}$	$0,30^{b}$	$0,50^{a}$	+ 67
Lysozyme (mg/L)	$23,4^{b}$	$24,1^{b}$	$37,0^{a}$	+ 58
Total immunoglobulin (g/L)	$18,9^{b}$	$18,7^{b}$	$28,9^{a}$	+ 53
Carp:				
Phagocytes – oxidative burst	$0,31^{b}$	$0,30^{b}$	$0,52^{a}$	+ 68
Phagocytes – killing activity	$0,30^{b}$	$0,31^{b}$	$0,49^{a}$	+ 63
T-Lymphocyte activity	$0,40^{b}$	$0,41^{b}$	$0,58^{a}$	+ 45
B-Lymphocyte activity	$0,27^{b}$	$0,27^{b}$	$0,47^{a}$	+ 74
Lysozyme (mg/L)	$1,4^{b}$	$1,4^{b}$	$3,4^{a}$	+ 143
Total immunoglobulin (g/L)	$10,5^{b}$	$10,7^{b}$	$18,5^{a}$	+ 76

^{a,b} = *values with different superscripts in a row indicate a significant difference ($p < 0,05$)*

Leiber® Beta-S: short-term feeding 4 weeks to carp & trout

- Siwicki *et al.*, 2008
- Significant increase of survival rates following bacterial infections after 4 weeks of feeding
- Prophylactic boosting of the immune system aids the defence mechanisms



Effects of dietary Leiber® Beta-S on survival rates of rainbow trout & carp after a challenge with *Aeromonas salmonicida* and *Aeromonas hydrophila*, respectively, if the experimental diets were fed for 4 weeks prior to infection ($n = 50$)

Leiber® Beta-S: long-term feeding 6 months to trout

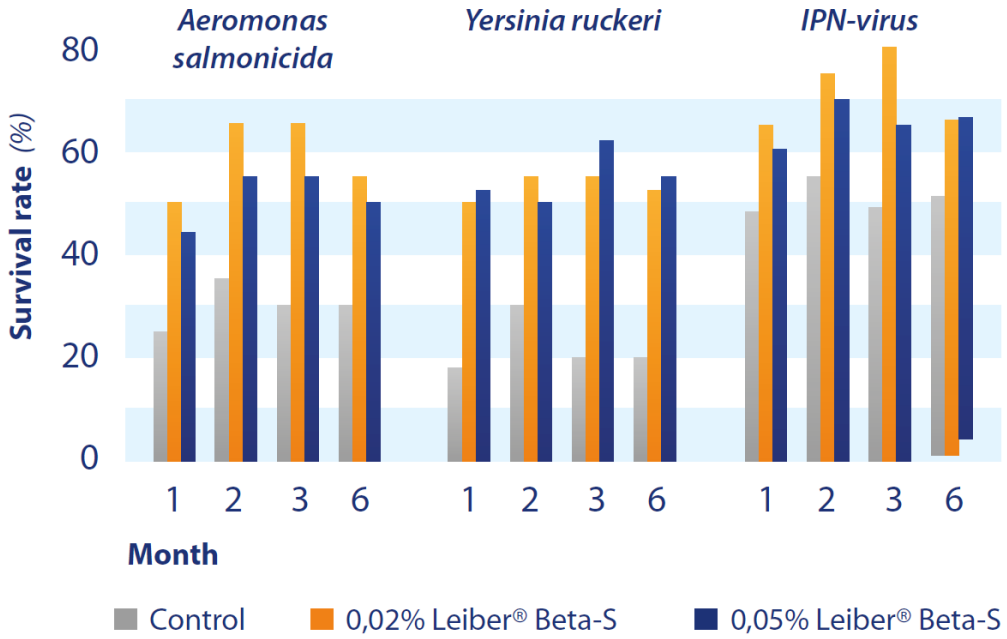
- Siwicki *et al.*, 2009
- Significant increase in non-specific cellular and humoral immunity ($n = 10$)
- This effect maintained even when long-term fed for up to 6 months (no depressive effect on immunocompetence)

	Control	Beta-S 0,02 %	Beta-S 0,05 %
Phagocytes - oxidative burst (OD 620 nm)			
after 1 month	0,34[b]	0,46[a]	0,47[a]
after 3 months	0,33[b]	0,49[a]	0,52[a]
after 6 months	0,32[b]	0,47[a]	0,42[a]
Phagocytes – killing activity (OD 620 nm)			
after 1 month	0,41[b]	0,50[a]	0,52[a]
after 3 months	0,39[b]	0,54[a]	0,53[a]
after 6 months	0,38[b]	0,51[a]	0,45[a]
T-lymphocytes activity (OD 620 nm)			
after 1 month	0,45[b]	0,55[a]	0,56[a]
after 3 months	0,45[b]	0,60[a]	0,58[a]
after 6 months	0,43[b]	0,57[a]	0,50[a]

	Control	Beta-S 0,02 %	Beta-S 0,05 %
B- lymphocytes activity (OD 620 nm)			
after 1 month	0,33[b]	0,42[a]	0,43[a]
after 3 months	0,34[b]	0,47[a]	0,45[a]
after 6 months	0,36[b]	0,45[a]	0,42[a]
Lysozyme (mg/L)			
after 1 month	35,5[b]	44,5[a]	43,0[a]
after 3 months	37,5[b]	46,5[a]	42,0[a]
after 6 months	36,5[b]	43,5[a]	41,0[a]
Total immunoglobuline (g/L)			
after 1 month	7,4[b]	8,4[a]	8,6[a]
after 3 months	7,5[b]	9,1[a]	8,9[a]
after 6 months	7,8[b]	8,9[a]	8,5[a]

Leiber® Beta-S: long-term feeding 6 months to trout

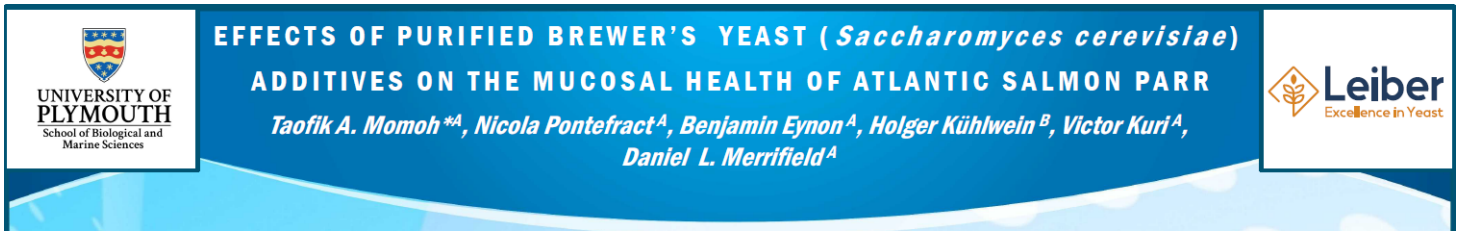
- Siwicki *et al.*, 2009
- Increased survival rates after bacterial and viral infections also after 6 months long-term feeding



Effects of dietary Leiber® Beta-S on survival rates of rainbow trout after a challenge with pathogens if the experimental diets were fed for 1, 2, 3 and 6 months prior to infection ($n = 40$)

Leiber® Beta-S: effects on mucosal health in salmon parr

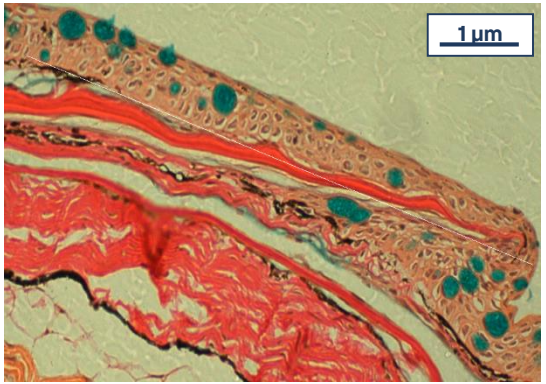
- Salmon parr (Ø IBW 21 g) fed for 4 weeks with: 0 %, 0.02% Beta-S and 0.2% Biolex® MB40
- Feeding rate 1.5 % of BW/day
- The basal diet had 48 % crude protein using 13 % soy bean meal, 15 % fishmeal and 8 % fish oil
- 5 fish per tank sampled from duplicate tanks (*n* = 20 fish/tank) at the end
- Slight improvements with Beta-S for growth and FCR
- Significant increases in goblet cells in gut and skin, and in microvilli length and density in distal gut
- Gene expression work ongoing (RNA extraction completed)

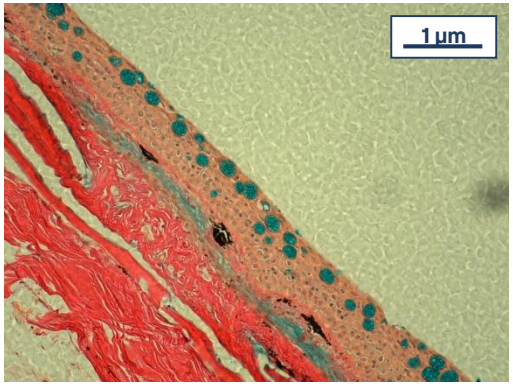


Poster presented at Aquaculture America conference in New Orleans 23rd – 26th February 2023 – best poster award

Leiber® Beta-S: effects on mucosal health in salmon parr

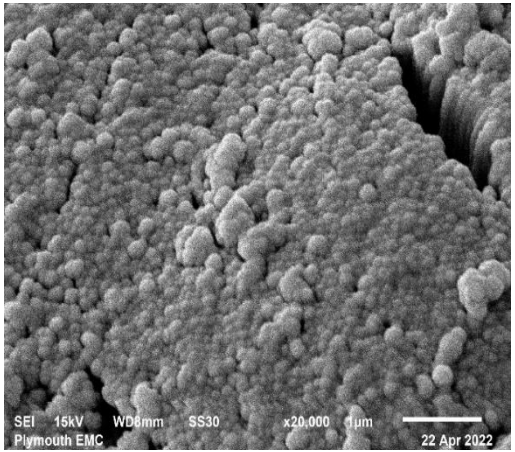
	Control	Beta-S	Biolex® MB40	*P*-Value
Goblet cell counts skin	22.7 ± 2.7[a]	33.8 ± 3.3[b]	27.0 ± 2.6[ab]	0.0459
Goblet cell counts distal gut	10.5 ± 0.1[a]	10.8 ± 1.4[a]	14.6 ± 1.3[b]	0.0422

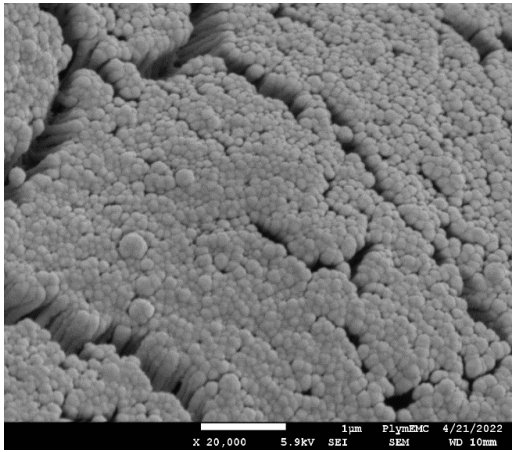


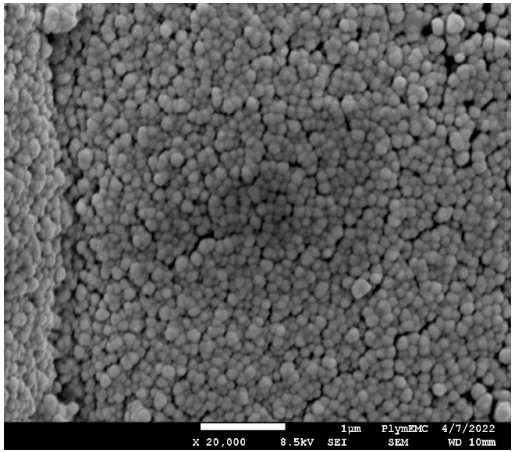


Leiber® Beta-S: effects on mucosal health in salmon parr

	Control	Beta-S	Biolex® MB40	*P*-Value
Microvilli density (per µm^2)	142.7 ± 4.7^a	191.5 ± 5.6^b	178.4 ± 10.4^b	0.0001

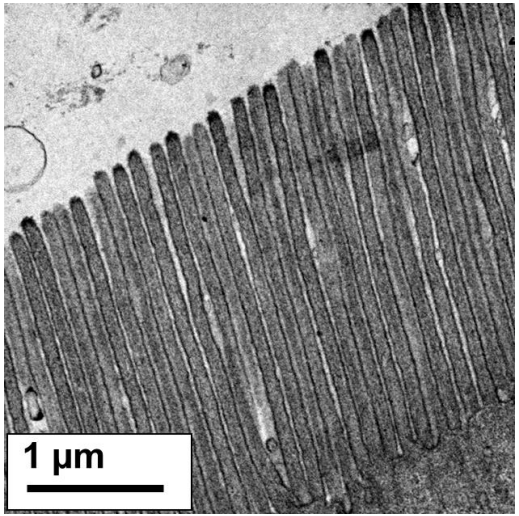


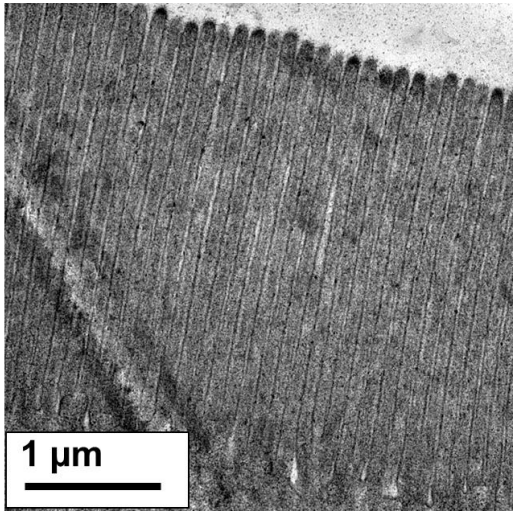


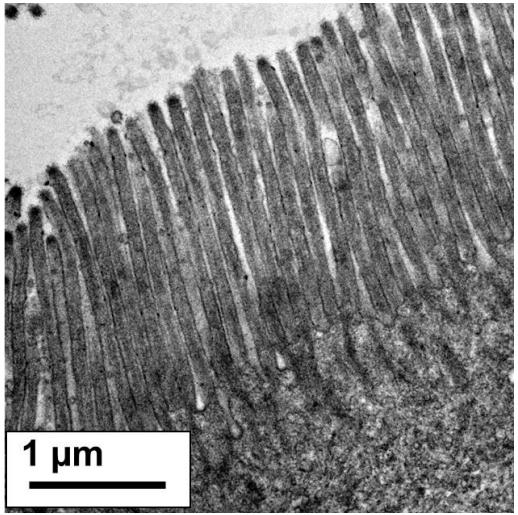


Leiber® Beta-S: effects on mucosal health in salmon parr

	Control	Beta-S	Biolex® MB40	P-Value
Microvilli length (µm)	1.58 ± 0.04[a]	1.86 ± 0.03[b]	1.46 ± 0.03[c]	<0.0001







Leiber® Beta-S: lasting immune effect in eel after withdrawal

- Siwicki *et al.* Centr Eur J Immunol 2015; 40 (1); 5-10)
- Significant increase in non-specific cellular and humoral immunity not only after 4 and 8 weeks of feeding, but also a further 8 weeks after stopping the feeding of Leiber® Beta-S (control feed only)!

Influence of β-glucan Leiber®Beta-S on selected innate immunity parameters of European eel (*Anguilla anguilla*) in an intensive farming system

ANDRZEJ K. SIWICKI[1], PATRYCJA SCHULZ[2], STANISŁAW ROBAK[3], KRZYSZTOF KAZUŃ[1], BARBARA KAZUŃ[1], EDWARD GŁĄBSKI[1], EWA SZCZUCIŃSKA[2]

[1]Department of Pathology and Immunology, Inland Fisheries Institute in Olsztyn, Poland
[2]Department of Microbiology and Clinical Immunology, University of Warmia and Mazury in Olsztyn, Poland
[3]Department of Ichthyology, Inland Fisheries Institute in Olsztyn, Poland

Abstract

Nutritional support plays an important role in promoting high cellular and humoral innate immunity activity and in preventing outbreaks of disease. The effects of β-glucan Leiber®Beta-S dietary supplementation on selected nonspecific immune parameters in juvenile European eel (Anguilla anguilla)

Leiber® Beta-S: lasting immune effect in eel after withdrawal

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Excellence in Yeast

Table 1. Immune parameters in eel after four and eight weeks of feeding with 0.02% Leiber®Beta-S (glucan-fed group) and in the control group (mean ± SD, *statistically significant $p < 0.05$)

Immune parameter	Weeks of fed with Leiber®Beta-S			
	4 weeks		8 weeks	
	control group	glucan-fed group	control group	glucan-fed group
metabolic activity of spleen phagocytes (RBA, OD 620 nm)	0.42 ±0.04	0.50 ±0.03*	0.43 ±0.03	0.59 ±0.04*
potential killing activity of spleen phagocytes (PKA, OD 620 nm)	0.38 ±0.03	0.48 ±0.04*	0.39 ±0.04	0.54 ±0.03*
pronephros lymphocytes ConA (LyP-ConA, OD 620 nm)	0.44 ±0.04	0.56 ±0.05*	0.43 ±0.03	0.59 ±0.05*
pronephros lymphocytes LPS (LyP-LPS, OD 620 nm)	0.30 ±0.03	0.41 ±0.04*	0.32 ±0.04	0.43 ±0.05*
lysozyme activity in serum (mg l^{-1})	9.3 ±0.8	13.5 ±0.7*	9.7 ±0.5	14.0 ±0.8*
Ig level in serum (g l^{-1})	10.5 ±1.0	12.5 ±0.9*	10.2 ±0.9	12.9 ±0.7*

Table 2. Immune parameters in eel after eight weeks of feeding with 0.02% Leiber®Beta-S and after an additional eight weeks in ponds; parameters were measured in both control and glucan-fed group

Immune parameter	Control	Glucan-fed group
metabolic activity of spleen phagocytes (RBA, OD 620 nm)	0.41 ±0.03	0.48 ±0.03*
potential killing activity of spleen phagocytes (PKA, OD 620 nm)	0.40 ±0.04	0.45 ±0.03*
pronephros lymphocytes ConA (LyP-ConA, OD 620 nm)	0.43 ±0.04	0.51 ±0.04
pronephros lymphocytes LPS (LyP-LPS, OD 620 nm)	0.31 ±0.03	0.40 ±0.05*
lysozyme activity in serum (mg l^{-1})	9.6 ±0.7	13.4 ±0.9*
Ig level in serum (g l^{-1})	10.9 ±1.1	12.4 ±0.8*

Leiber® Beta-S: the adjuvant effect

- Siwicki *et al.* Centr Eur J Immunol 2011; 36 (4): 212-214
- Pronounced adjuvant effect during vaccinations
- Enhanced levels of antibody-secreting cells (ASC) and specific antibodies in rainbow trout fed with Leiber® Beta-S supplemented diets for 4 weeks prior to vaccination

Influence of 1,3-1,6-β-D-glucan (Leiber® Beta-S) in diets on the effectiveness of anti-Enteric Redmouth Disease (AquaVac ERM) vaccine in rainbow trout (*Oncorhynchus mykiss*)

ANDRZEJ K. SIWICKI, KRZYSZTOF KAZUŃ, AGNIESZKA LEPA, BARBARA KAZUŃ

Department of Pathology and Immunology, Inland Fisheries Institute in Olsztyn, Poland

Abstract

This study was performed to investigate the influence of 1,3-1,6-β-D-glucan (Leiber® Beta-S), natural product, on the antibody secreting cells (ASC) and specific antibody levels after immunization of rainbow trout (Oncorhynchus mykiss) with anti-enteric redmouth disease vaccine (AquaVac ERM). Fish were fed

Leiber® Beta-S: the adjuvant effect

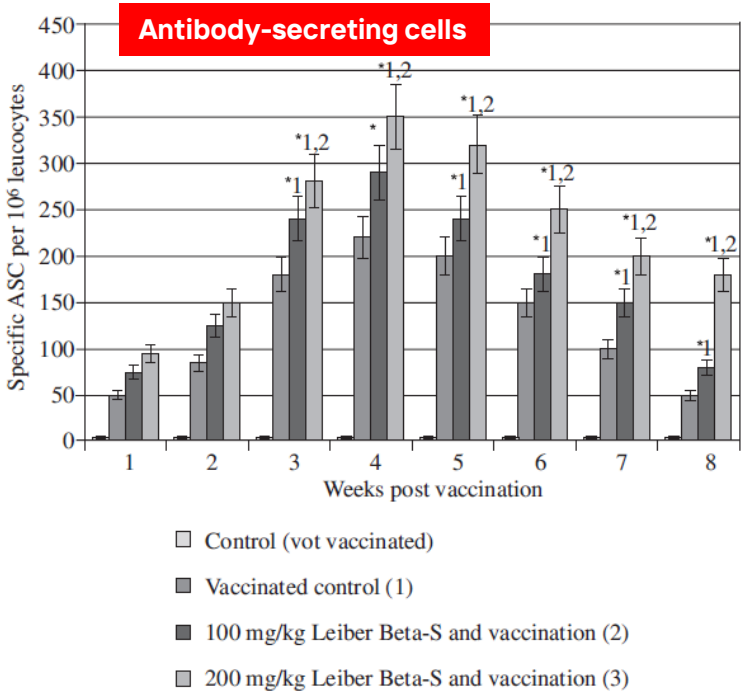


Fig. 1. Influence of Leiber® Beta-S on the specific ASC after vaccination against enteric redmouth disease in fingerling of rainbow trout (*Oncorhynchus mykiss*) (n = 10, mean ±SD; * – statistically significant differences, $P \leq 0.05$)

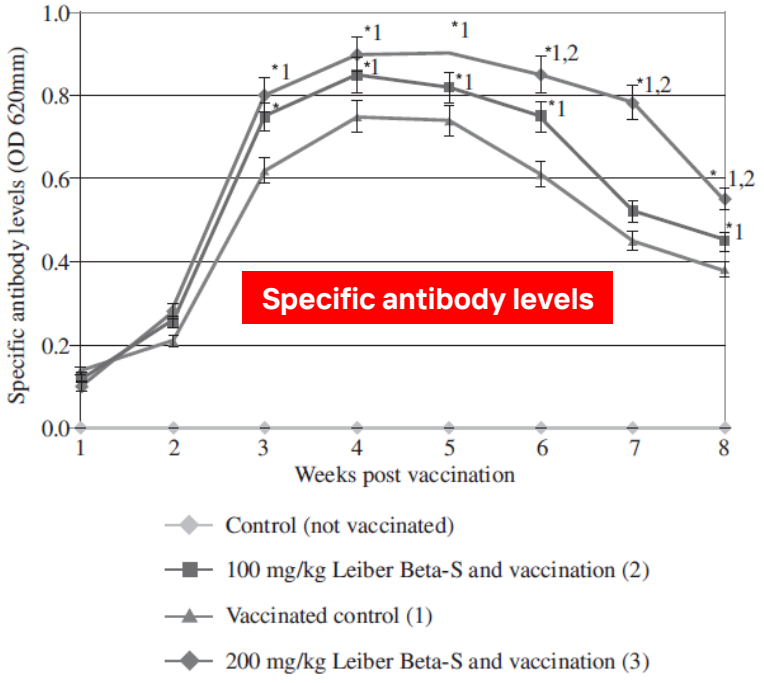


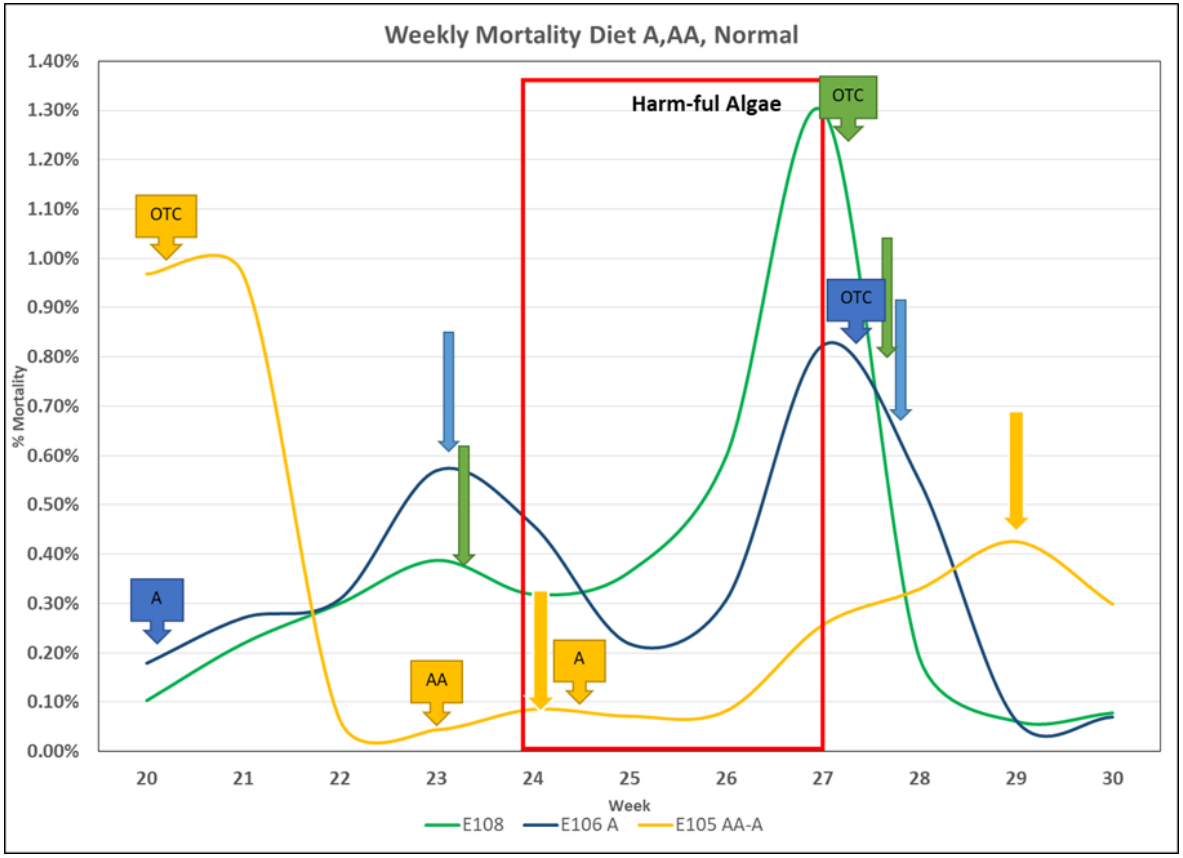
Fig. 2. Influence of Leiber® Beta-S on the specific antibody levels after vaccination against enteric redmouth disease in fingerling of rainbow trout (n = 10, mean ±SD; * – statistically significant differences, $P \leq 0.05$)

Leiber® Beta-S: 3rd party field trial Costa Rica 2018

- Spotted Rose Snapper, off-shore cages, production ~ 2000 to/y
- Stocking with ca. 3 g, grow-out to market size ~ 450 g
- Issues with *S. iniae* (no vaccination possible) & *Brooklynella hostilis* (Brooklynellosis/clownfish disease)

Leiber® Beta-S: 3rd party field trial Costa Rica 2018

- Red algae bloom ("Red Tide")



Treatments

- E105 AA: health premix with Beta-S
- E106 A: with organic acids
- E108: standard feed
- OTC: antibiotics
- Arrows: net changes

Leiber Yestimun®: immunity and gut microbiota in sole

- Carballo *et al.* Fish & Shellfish Immunol 2019; 92; 31-39
- Yestimun® is Leiber's food-grade, purified ß-glucan product (ß-1,3-1,6-glucan: min. 80% - max. 90%)
- Delivery of 1 mg/fish into the gut by oral intubation (reduce interference from feed components)

Fish and Shellfish Immunology 92 (2019) 31–39

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Yeast β-glucans and microalgal extracts modulate the immune response and gut microbiome in Senegalese sole (*Solea senegalensis*)

Carlos Carballo[a,f], Patricia I.S. Pinto[b], Ana Patricia Mateus[b,c], Concha Berbel[a], Cláudia C. Guerreiro[b], Juan F. Martinez-Blanch[d], Francisco M. Codoñer[d], Lalia Mantecon[e], Deborah M. Power[b,**], Manuel Manchado[a,*]

[a] IFAPA Centro El Toruño, Junta de Andalucía, Camino Tiro Pichón s/n, 11500 El Puerto de Santa María, Cádiz, Spain
[b] Centre of Marine Sciences (CCMAR), Universidade do Algarve, Campus de Gambelas, 8005-139, Faro, Portugal
[c] Escola Superior de Saúde da Universidade do Algarve, Campus de Gambelas, 8005-139, Faro, Portugal
[d] LifeSequencing, Parc Cientific Universidad De Valencia, Edif. 2, C/ Catedrático Agustín Escardino Benlloch, 9, 46980, Paterna, Spain
[e] Fitoplancton Marino, Dársena Comercial S/N (Muelle Pesquero), 11500, El Puerto de Santa María, Cádiz, Spain
[f] Universidad de Málaga, Departamento de Microbiologia, Campus de Teatinos, 29071, Malaga, Spain

Leiber Yestimun®: immunity and gut microbiota in sole

- Short-term changes gene expression in the intestine

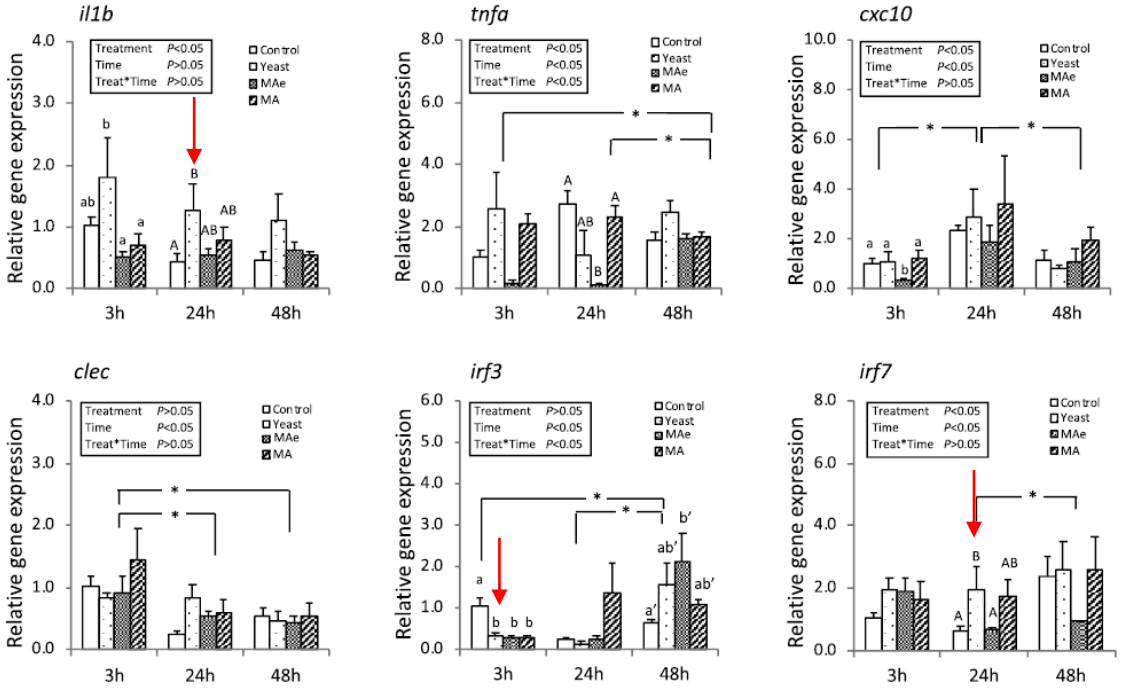


Fig. 2. Relative gene expression levels in the intestine at 3, 24, and 48 h after oral intubation. The four experimental groups are indicated: control, treated with insoluble yeast β-glucans (Yeast), microalgal extracts (MAe) and whole-cell microalgae (MA). Data are expressed as the mean fold change (mean ± SEM, n = 3) from the calibrator (control 3 h after oral intubation). A two-way ANOVA was used to determine statistical differences. Different letters indicate significant differences between oral intubation treatments for a specific time point. Asterisks denote significant differences between samples at different times post-intubation. Significant differences were set at $P < 0.05$ (Two-way ANOVA).

Leiber Yestimun®: immunity and gut microbiota in sole

- Short-term changes gene expression in the spleen after 24 h

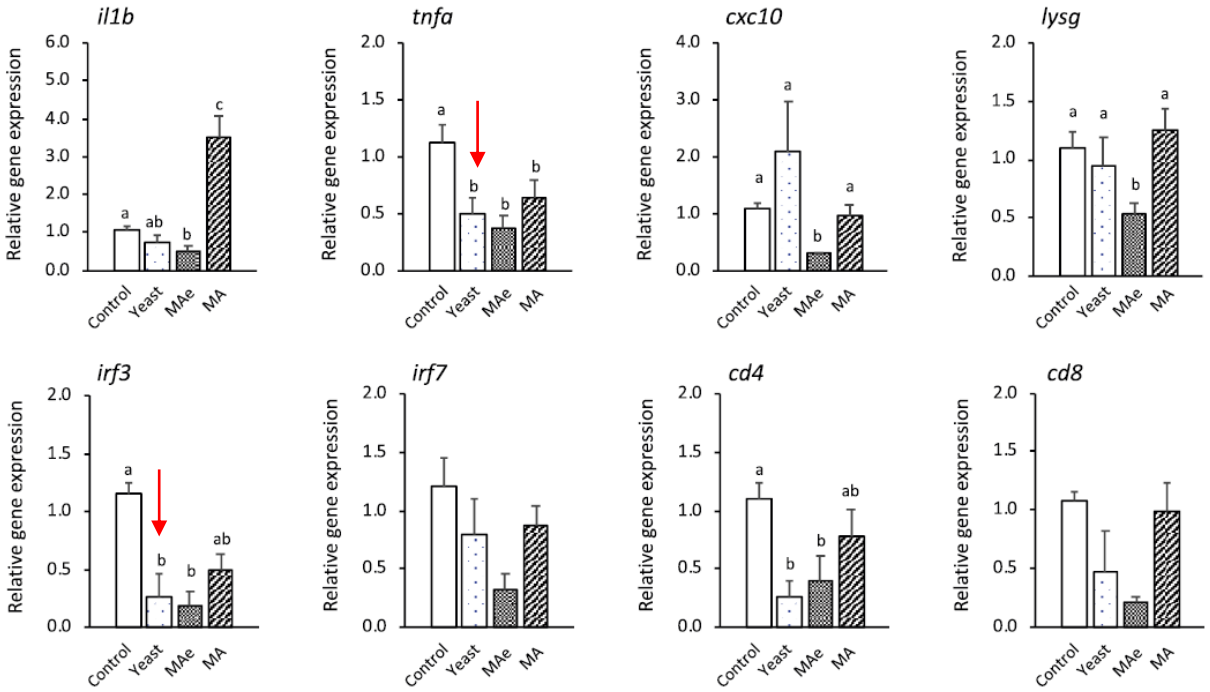


Fig. 3. Relative gene expression levels in the spleen 24 h after oral intubation. The four experimental groups are indicated: control, treated with insoluble yeast β-glucans (Yeast), microalgal extracts (MAe) and whole-cell microalgae (MA). Data are expressed as the mean fold change (mean ± SEM, n = 3) from the calibrator (control 3 h after oral intubation). Different letters indicate significant differences between oral intubation treatments. Significant differences were set at $P < 0.05$ (one-way ANOVA).

Leiber Yestimun®: immunity and gut microbiota in sole

- Long-term changes gene expression in gut and spleen after 7days

Table 1

Relative expression levels at 7 days after oral intubation. The fold-changes between the control (Ctrl) group with respect to yeast β-glucans (Yeast), microalgal polysaccharide-enriched extract (MAe) and whole-cell microalgae (MA) are indicated. A one-way ANOVA was carried out followed by Tukey's posthoc test (significance at $P < 0.05$ is indicated by "*"; ns, not significant). The "-" denotes that expression was not quantified.

Gene name	Gene description	Intestine						spleen					
		Ctrl *vs* Y		Ctrl *vs* MAe		Ctrl *vs* MA		Ctrl *vs* Y		Ctrl *vs* MAe		Ctrl *vs* MA	
il1b	Interleukin 1b	0.60 ± 0.11	ns	0.80 ± 0.17	ns	1.17 ± 0.27	ns	2.13 ± 1.05	ns	2.17 ± 0.69	ns	3.23 ± 0.54	*
tnfa	Tumor necrosis factor α	1.41 ± 0.11	ns	1.90 ± 0.56	ns	1.90 ± 0.41	ns	0.98 ± 0.35	ns	0.58 ± 0.09	ns	0.46 ± 0.19	ns
lysg	g-type lysozyme	1.17 ± 0.06	ns	1.11 ± 0.33	ns	1.18 ± 0.10	ns	1.74 ± 0.25	ns	1.84 ± 0.35	ns	1.90 ± 0.55	ns
cxc10	Chemokine cxc10	1.19 ± 0.11	ns	1.45 ± 0.91	ns	3.46 ± 1.20	ns	1.24 ± 0.21	ns	1.92 ± 0.59	ns	3.27 ± 1.00	ns
irf3	Interferon regulatory factor 3	1.07 ± 0.37	ns	1.34 ± 0.69	ns	2.88 ± 1.60	ns	1.01 ± 0.21	ns	0.60 ± 0.23	ns	0.60 ± 0.18	ns
irf7	Interferon regulatory factor 7	2.81 ± 0.80	*	4.36 ± 1.37	*	4.74 ± 1.67	*	1.02 ± 0.14	ns	0.93 ± 0.33	ns	1.10 ± 0.25	ns
hamp1	hepcidin	0.82 ± 0.06	ns	0.82 ± 0.09	ns	1.04 ± 0.24	ns	-		-		-	
clec	c-type lectin	1.85 ± 0.27	*	1.87 ± 0.39	*	2.97 ± 0.17	*	-		-		-	
cd4	cluster of differentiation 4	-		-		-		1.04 ± 0.33	ns	0.48 ± 0.16	ns	1.30 ± 0.35	ns
cd8a	cluster of differentiation 8a	-		-		-		0.91 ± 0.13	ns	1.65 ± 0.25	ns	0.85 ± 0.17	ns

Leiber Yestimun®: immunity and gut microbiota in sole

- Gut microbiome modulation after 7 days
- Treatments reduced microbiome species richness and bacterial diversity
- Reduction of genus Vibrio (control 93% vs. Yestimun 86%)

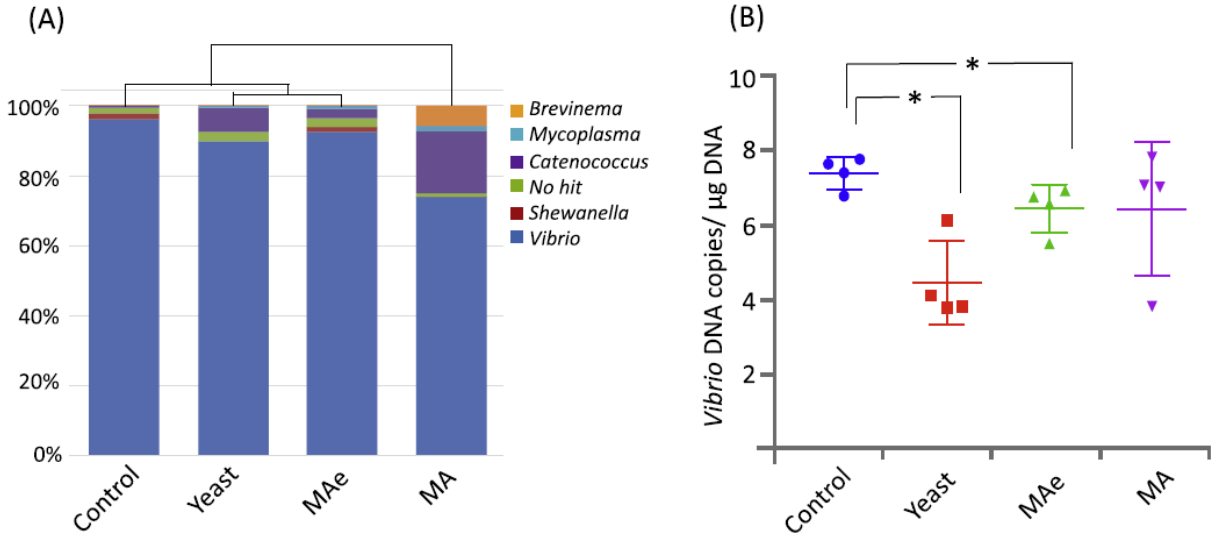


Fig. 4. A- Intestinal microbiome composition (in percentage) for the main bacterial genera, present at > 1% representation. The scheme represents the hierarchal clustering of the main bacterial genera in the gut of the four experimental groups: control, treated with insoluble yeast β-glucans (Yeast), microalgal extracts (MAe) and whole-cell microalgae (MA). B- Quantification of *Vibrio* DNA in the intestine of fish (n = 5) from each treatment group by qPCR with genera-specific primers and normalized using µg of DNA used in each PCR (bars represent the mean ± SEM). * indicate significant differences compared to the control with $P < 0.01$(Student's t-test).

Leiber Yestimun®: immunity and gut microbiota in sole

- What does it all mean?
  - Solubility is key for ß-glucan activity - particulate ß-glucans with major local effect on gut and microbiota
  - Upregulation of glucan receptor C-type lectin strong indicator for recognition by gut immune cells
  - Yestimun® triggered a fast and transient induction of IL1ß in the gut followed by a shift in microbiota (esp. significant decrease in *Vibrio* abundance)
  - *Vibrio* is a highly relevant for disease outbreaks in Senegalese sole
  - Opposing response of antiviral defense irf3 gene suggest a cross-talk between type 1 interferon and interleukin1 cytokine pathways to modulate inflammatory responses and bacterial populations

Functionalities –
experimental results in shrimp

Leiber® Beta-S: 90 – day feeding to whiteleg shrimp

- Boonanuntanasarn *et al.*, Aquaculture Nutrition 2015
- Improvements in growth, proximate composition and haemolymph when ß-glucan fed alone
- Improvements in intestinal microbiota and gut morphometry when fed combined with probiotics
- Shrimp fed for 90 days with ß-glucan alone or in combination with probiotics (*B. subtilis* and *P. acidilactici*)

Aquaculture Nutrition

Aquaculture Nutrition 2015 doi: 10.1111/anu.12302

Effects of dietary supplementation with β-glucan and synbiotics on growth, haemolymph chemistry, and intestinal microbiota and morphology in the Pacific white shrimp

S. BOONANUNTANASARN[1], U. WONGSASAK[1], T. PITAKSONG[1] & S. CHAIJAMRUS[2]

[1] *School of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand;* [2] *Department of Biology, Faculty of Science, Naresuan University, Muang, Phitsanulok, Thailand*

Leiber® Beta-S: 90 – day feeding to whiteleg shrimp

Table 2 Growth performance of *Litopenaeus vannamei* fed with experimental diets[1]

Parameters	Experimental diets			
	C	β-glu	β-glu+*Bs*	β-glu+*Pa*
Initial weight (g)	0.15 ± 0.02	0.15 ± 0.02	0.16 ± 0.02	0.16 ± 0.02
Final weight (g)	3.45 ± 0.36^{a}	5.30 ± 0.73^{b}	4.51 ± 0.41^{ab}	5.20 ± 0.38^{b}
Weight gain[2] (g)	3.29 ± 0.36^{a}	5.16 ± 0.74^{b}	4.36 ± 0.42^{ab}	5.04 ± 0.38^{b}
Total length (cm)	7.66 ± 0.26^{a}	9.14 ± 0.25^{b}	8.35 ± 0.48^{ab}	9.28 ± 0.08^{b}
FE[3]	0.37 ± 0.04	0.54 ± 0.08	0.45 ± 0.05	0.51 ± 0.04
Survival (%)	80.0 ± 5.7	80.0 ± 3.5	77.1 ± 7.3	85.7 ± 7.8

+ 53 %

[1] Values are means ± SD of five replicates. Means with a different superscript in each row differed significantly from each other ($P < 0.05$).

[2] Weight gain = final mean body weight − initial mean body weight.

[3] Feed efficiency (FE) = wet weight gain × dry feed fed^{-1}.

Leiber® Beta-S: 90 – day feeding to whiteleg shrimp

- Wongsasak *et al.*, Aquaculture, 2015
- Significant increases in gene expression of lipopolysaccharide and β-1,3-glucan-binding protein (LGBP) and superoxide dismutase activity (SOD) when ß-glucan fed alone

Aquaculture 436 (2015) 179–187

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Effects of dietary supplementation with β-glucan and synbiotics on immune gene expression and immune parameters under ammonia stress in Pacific white shrimp

Umaporn Wongsasak [a], Sirilux Chaijamrus [b], Suksan Kumkhong [a], Surintorn Boonanuntanasarn [a,*]

[a] School of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, 111 University Avenue, Tambon Suranaree, Muang, Nakhon Ratchasima 30000, Thailand

[b] Department of Biology, Faculty of Science, Naresuan University, Phitsanulok 65000, Thailand

Leiber® Beta-S: 90 – day feeding to whiteleg shrimp

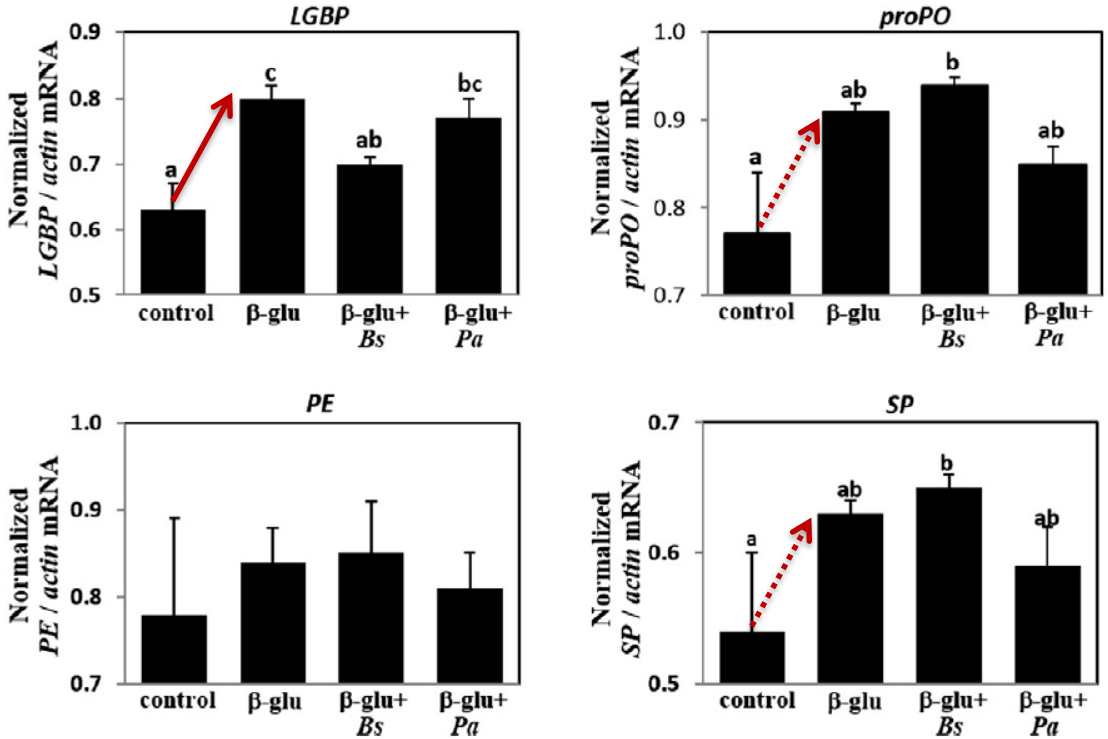


Fig. 2. Effects of dietary supplementation with β-glucan and probiotics on expression level of immune genes. Real-time RT-PCR was performed to examine the mRNA levels of lipopolysaccharide and β-1,3-glucan-binding protein (*LGBP*), prophenoloxidase (*proPO* peroxinectin) (*PE*), and serine protease (*SP*). The mRNA level of each gene was normalized to the β-actin mRNA level after log 10 transformation of each concentration. The values are means ± SE from at least five samples (RNA-pool of five shrimps) after duplicate PCR analysis. Values with different letters are significantly difference at $P < 0.05$.

Leiber® Beta-S: 90 – day feeding to whiteleg shrimp

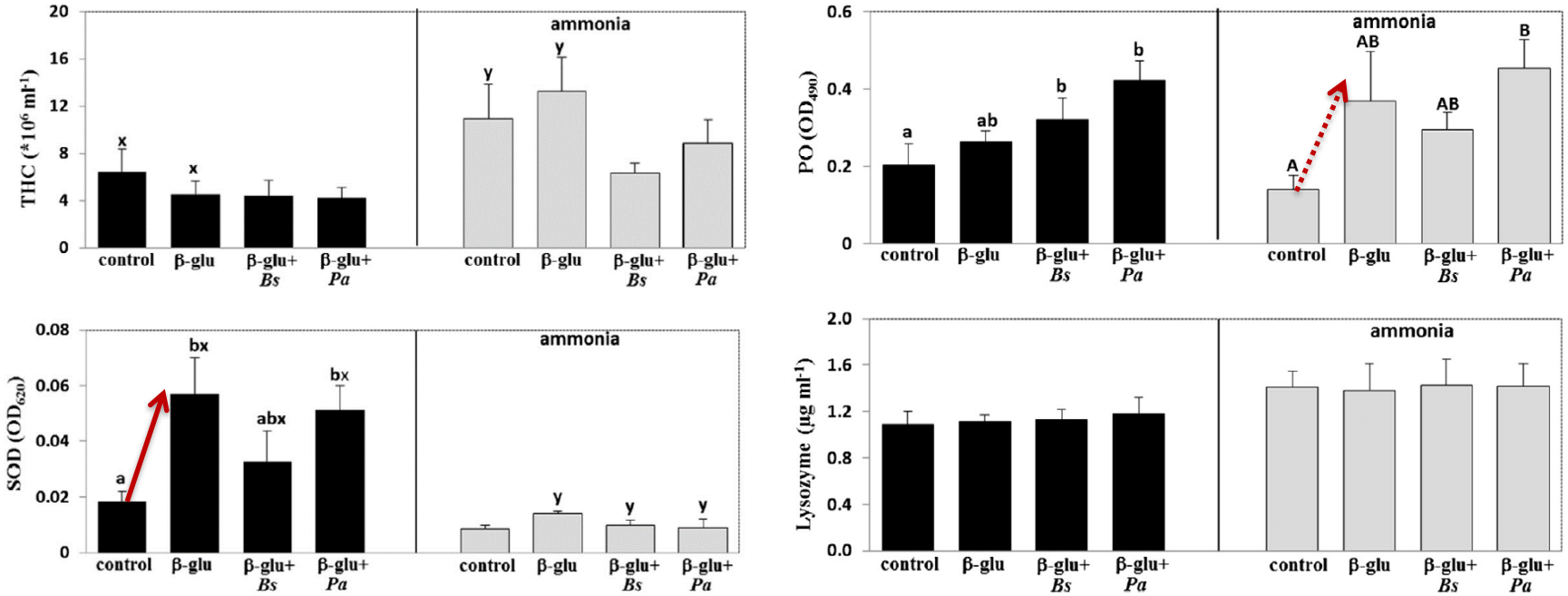


Fig. 3. Effects of dietary supplementation with β-glucan and probiotics on immune parameters in *L. vannamei*. Black and gray bars show the tested immune values, including total hemocyte count (THC), superoxide dismutase activity (SOD), phenoloxidase activity (PO), and lysozyme activity under normal and ammonia stress conditions, respectively. The different lower case letters denote significant differences in immune parameters among experimental diets at $P < 0.05$. The different capital letters denote significant differences in immune parameters among experimental diets at $P < 0.05$ under ammonia stress. The alteration of immune parameters between normal and ammonia stress within each experimental diet is indicated by x and y.

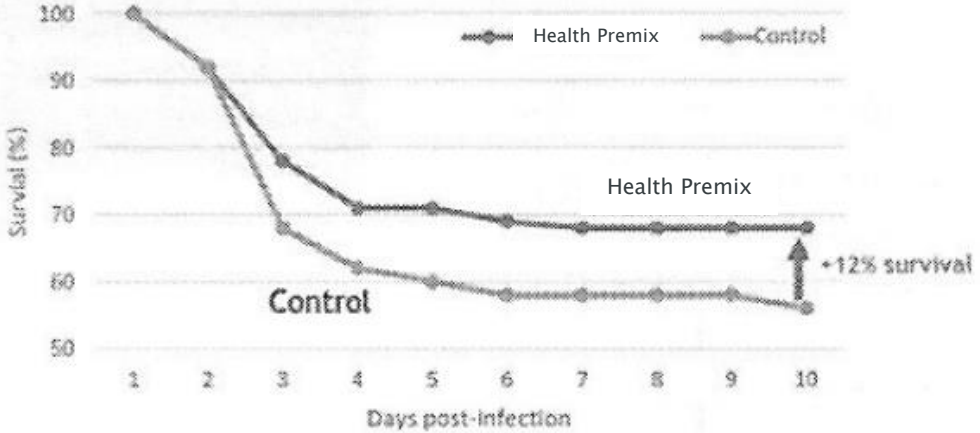
3rd party lab trial *L. vannamei* Ecuador 2017

- Trial at CENAIM, Ecuador (Centro Nacional de Acuicultura e Investigaciones Marinas)
- Phase 1:
  - 50 l tanks with 25 shrimp/tank (= 16 shrimp/m^2)
  - initial body weight 3.8 g → 32 days → feeding 2x per day ad libitum
- Phase 2:
  - 36 tanks each 40 l → 15 shrimp/tank (= 16 shrimp/m^2)
  - 10 days → feeding 2x per day ad libitum
  - WSSV-challenge in 18 tanks via WSSV-infected shrimp tissue (PCR +)

3rd party lab trial *L. vannamei* Ecuador 2017

- Beta-S @ 150 g/to in health premix with other health promoting components

Results



Treat.	Total n. of Haemocytes/ml		Granular Haemocytes/ml	
Health Premix	$3,8 \pm 0,8 \times 10^7$	a	$1,8 \pm 0,2 \times 10^7$	a
Control	$2,7 \pm 0,5 \times 10^7$	b	$1,2 \pm 0,2 \times 10^7$	b

- 6 g Final Weight

Source: CENAIM Ecuador 2017

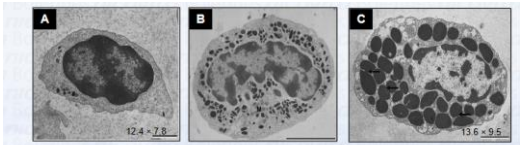
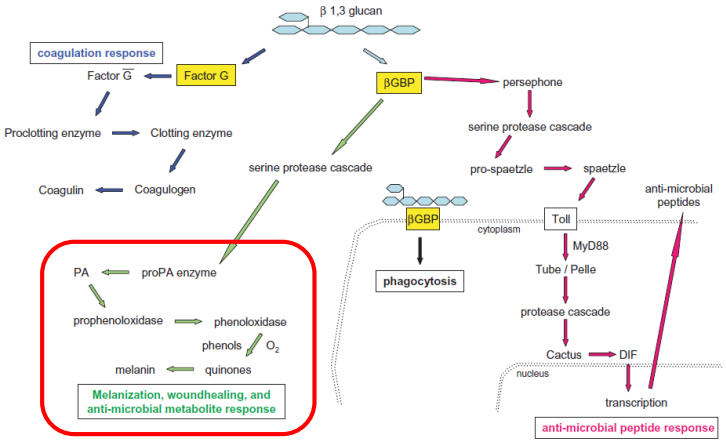


Figure 3. Classification of hemocytes: (A) Hyaline, (B) Semi-granular, (C) Granular (Modified photos from Giulianini *et al.*, 2007)

Table 1
Crayfish and crab haemocytes

Haemocyte type	Function in immunity
Hyaline cell	Phagocytosis[a]
Semigranular cell	Encapsulation[b]
	Phagocytosis (limited)[a]
	Storage and release of the proPO system[c]
	Cytotoxicity[d]
Granular cell	Storage and release of the proPO system[c]
	Cytotoxicity[d]

Johansson *et al.*, 2000



Excursion:

human cancer research

Leiber Yestimun®: anti-tumor chemotherapy

- Chae *et al.* Int J Biol Macromol 2019; 136; 1169-1175
- 2 weeks after tumor inoculation Yestimun® (daily 100 mg/kg) orally administrated to male BALB/c mice
- GEM (Gemcitabine) i.p. injected every third day starting at day 14 (a highly effective chemotherapy drug, but with adverse side effects - mainly myelosuppression – often hindering further treatment)
- Myelosuppression causes pancytopenia and immunosuppression in a dose-dependent manner

International Journal of Biological Macromolecules 136 (2019) 1169–1175

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

Yeast (1 → 3)-(1 → 6)-β-D-glucan alleviates immunosuppression in gemcitabine-treated mice

Jin Sung Chae [a,e,1], Hocheol Shin [b,1], Youngju Song [c], Hee Kang [d], Chang-Hwan Yeom [a], Sukchan Lee [b,*], Youn Seon Choi [e,*]

[a] Yeom Chang-Hwan Hospital, Seoul 06605, Republic of Korea
[b] Department of Integrative Biotechnology, Sungkyunkwan University, Suwon 16419, Republic of Korea
[c] Department of Biomedical Science and Technology, Graduate School, Kyung Hee University, Seoul 02447, Republic of Korea
[d] Humanitas College, Kyung Hee University, Yongin 17104, Republic of Korea
[e] Department of Family Medicine, Korea University Guro Hospital, Seoul 08308, Republic of Korea

Leiber Yestimun®: anti-tumor chemotherapy

- Significant reduction of tumor volume and weight through GEM

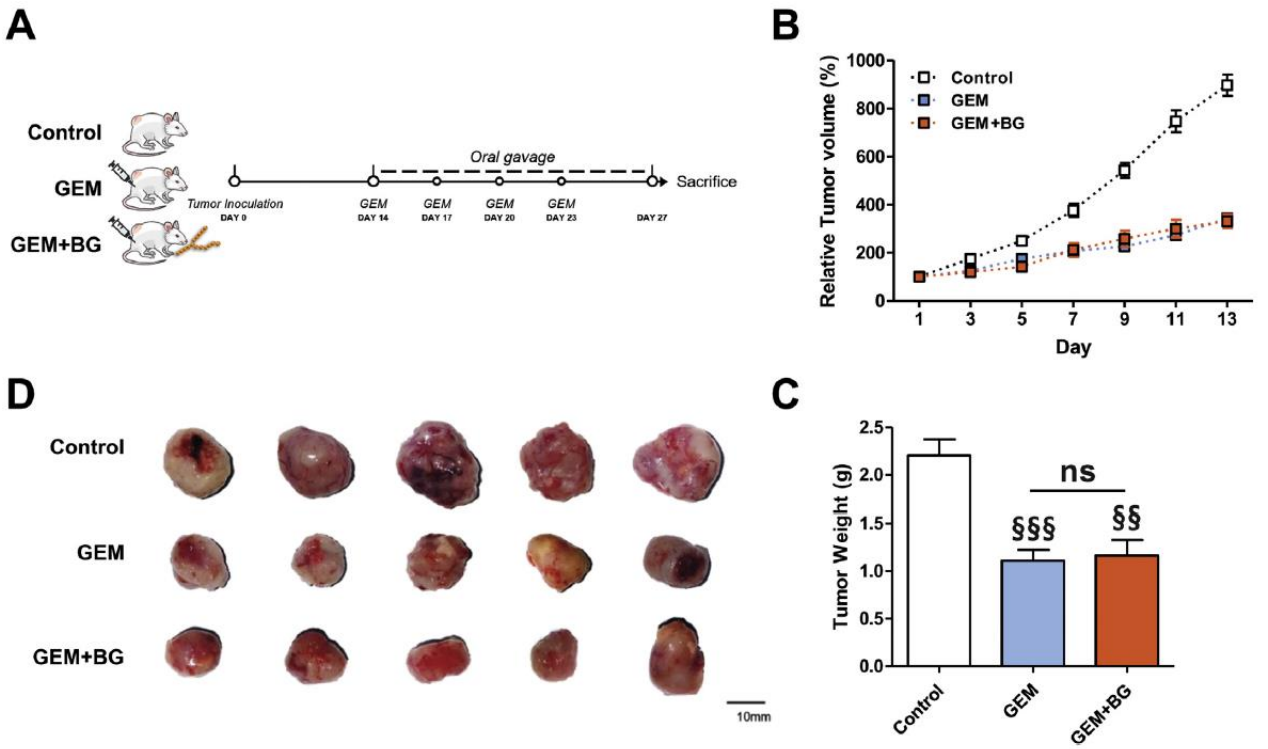
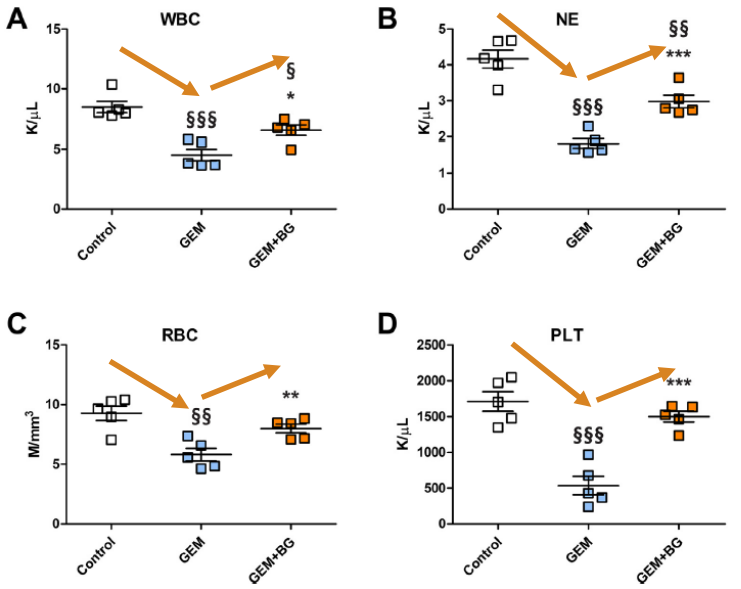


Fig. 1. Antitumor activity of gemcitabine in CT-26 bearing mice. (A) Experimental protocols used in this experiment. (B) Antitumor effect of gemcitabine on tumor growth. (C) Terminal tumor weight of CT-26-bearing mice. (D) Terminal tumor size of CT-26-bearing mice. Data are presented as mean ± SEM. §§$P < 0.01$, §§§$P < 0.001$ vs. control.

Leiber Yestimun®: anti-tumor chemotherapy

- Highly significant recovery from GEM-induced pancytopenia (total white blood cell, neutrophil, red blood cell and platelet levels in blood) through Yestimun® → alleviation of suppression of myeloid cells
- Highly significant increase in gene expression of major cytokines related to myelopoiesis (GM-CSF, G-CSF and M-CSF) through Yestimun® transferred to lymphoid tissue



In haematology, myelopoiesis in the broadest sense is the production of bone marrow and all cells that arise from it (all blood cells)

Yestimun® was able to mitigate immunosuppression in myeloid cells!

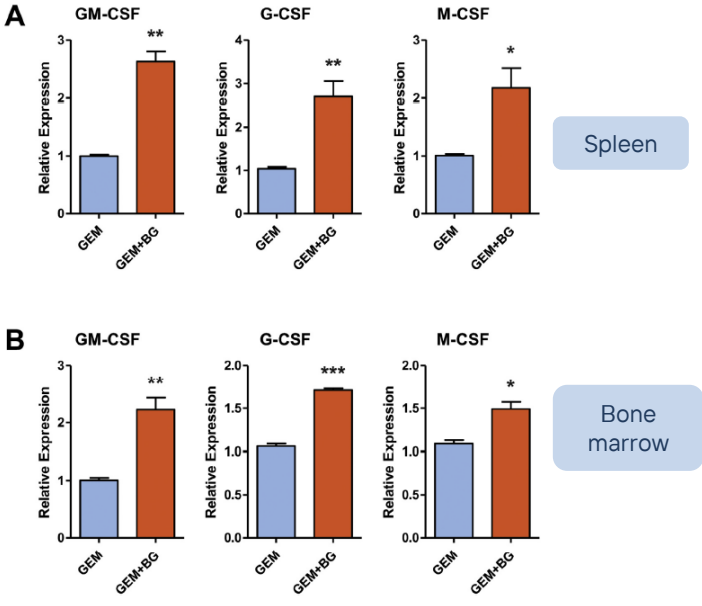


Fig. 2. Yeast beta-glucan alleviates the pancytopenia in GEM-treated mice. (A) White blood cell, (B) neutrophil, (C) red blood cell, and (D) platelet cell counts were determined. Data are presented as mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. gemcitabine alone; §$P < 0.05$, §§$P < 0.01$, §§§$P < 0.001$ vs. control.

Fig. 3. Hematopoietic cytokine mRNA expression in spleen and bone marrow. Quantitation of mRNA expression of GM-CSF, G-CSF, and M-CSF in spleen (A) and bone marrow (B). Data are presented as mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. gemcitabine alone.

Leiber Yestimun®: anti-tumor chemotherapy

- GEM reduced cytotoxicity of splenocytes by 43% whereas Yestimun® recovered that to 94% of controls
- GEM significantly reduced IFN-γ to 46% and IL-2 levels to 48%. Yestimun® restored that to 90% and 98%, respectively.

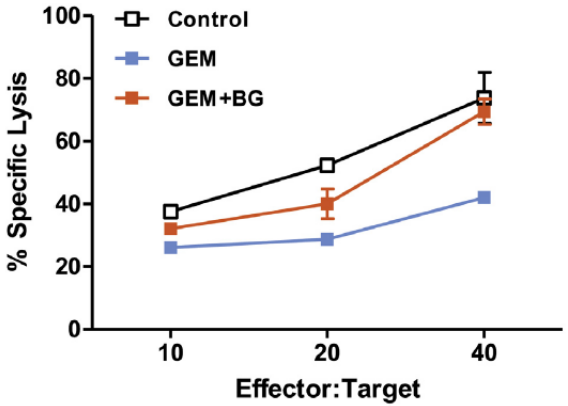


Fig. 4. Effect of yeast beta-glucan on the cytotoxicities of splenocytes from gemcitabine-treated mice. Measurement of splenocyte activity against the mouse lymphoma cell line YAC-1. Viability was examined at OD 450 nm using a microplate reader.

Yestimun® restored natural killer cell activity against YAC-1 tumor cells

IL-2 and IFN-γ are key cytokines in the immune response to tumors

Yestimun® was also able to mitigate immunosuppression in lymphoid cells!

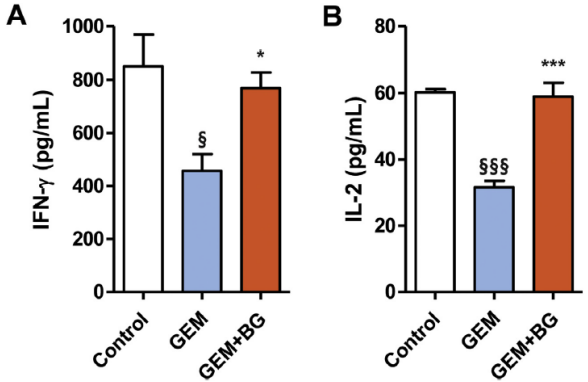


Fig. 5. Effect of yeast beta-glucan on IFN-γ and IL-2 cytokines secreted in CAC-treated splenocytes of gemcitabine-treated mice. Splenocytes were cultured with CAC. Cytokine levels of IFN-γ (A) and IL-2 (B) in the supernatant were measured by ELISA. Data are presented as mean ± SEM. *$P < 0.05$, ***$P < 0.001$ vs. gemcitabine alone; §$P < 0.05$, §§§$P < 0.001$ vs. control.

Leiber Yestimun®: anti-tumor chemotherapy

- GEM-treated mice showed bone marrow damage: hypocellularity, with a reduced myeloid/erythroid ratio, and sinusoidal alterations
- Yestimun® lead to a normalization of these damages!

Hypocellularity = abnormal decrease in the number of cells present

Myeloid/erythroid ratio = a decreased ratio may mean a depression of leukopoiesis or normoblastic hyperplasia depending on the overall cellularity of the bone marrow.

Sinusoidal alterations = pathological alterations of blood capillaries

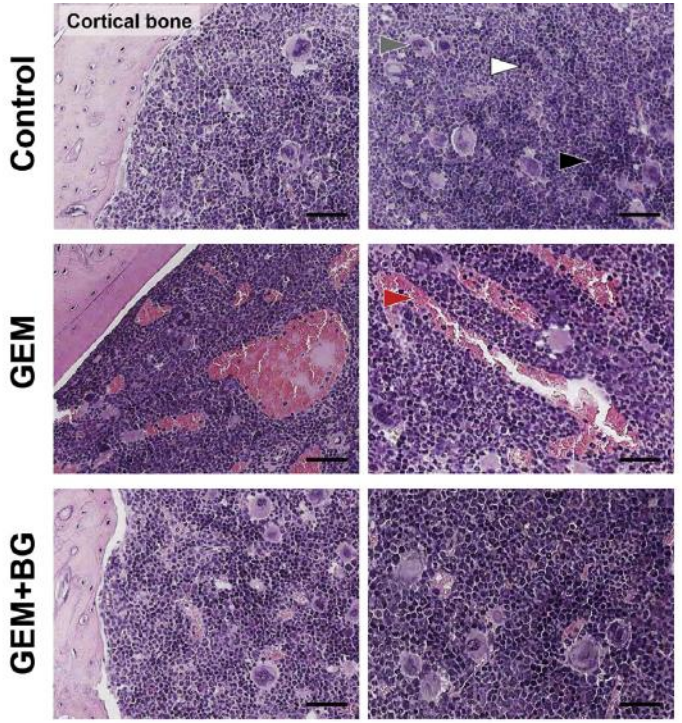


Fig. 6. Effect of yeast beta-glucan on bone marrow tissue damage induced by gemcitabine. Femurs were removed, and bone marrow was fixed, decalcified, and stained with H&E. Microscopic scale bar represents 50 μm (*black arrow*, erythroid cell; *grey arrow*, megakaryocyte; *white arrow*, myeloid cell; *red arrow*, sinusoid).

Leiber Yestimun®: anti-tumor chemotherapy

- What does it mean?
  - Orally administered Yestimun® effectively alleviated myelosuppression associated with gemcitabine-induced pancytopenia
  - Analysis of myelopoiesis-related cytokine expression demonstrated that Yestimun® up-regulated hematopoietic response in gemcitabine-treated mice
  - Yestimun® restored cytotoxicity of splenocytes against YAC-1 in gemcitabine-treated mice
  - Yestimun® displayed a positive effect on gemcitabine-damaged bone marrow tissue
  - In conclusion, Yestimun® has the potential to be used as adjuvant for alleviating chemotherapy-induced immunosuppression

Overall, this study demonstrates the high potency of dietary highly-purified ß-glucans to combat immunosuppression!!

Conclusions and recommendations for use

Conclusions

- Leiber® Beta-S improves immunocompetence (non-specific & specific immunity)
- Leiber® Beta-S thereby increases stress & disease resistance, and survival rates
- Leiber® Beta-S has a pronounced adjuvant effect in vaccinations
- Leiber® Beta-S improved growth performance and feed conversion is possible
- Leiber® Beta-S is overall a great tool to boost the immune system and to prophylactically prepare fish and shrimp against occurring diseases!

Recommended usage

- 50 – 250 g/to in feed (0.005 – 0.025%) → usually 200 g/to feed
- Before periods of higher stress (e.g. handling, transport, environmental changes) & pathogen pressure

  → prophylactic use

  → in order to prevent or reduce mortalities and other losses
- Limited immune capacity of larvae → support of immunity build-up and antibody production
- Prior to vaccinations → improving the effectiveness of vaccinations (adjuvant effect)
- Long-term administration possible (no immunodepression)

Product portfolio - Aquaculture

Immune competence	Gut health	Performance & Metabolism	Palatability
Challenge			
High pathogen pressure Challenges (handling, transport, vaccination,...) Environmental changes Limited immune capacity of larvae	Digestion Intestinal development Challenging diets Mycotoxin or pathogen contamination	Phases of high nutrient demand (e.g. larvae growth, broodstock) Intestinal development and gut integrity Performance Species with a short digestive tract	Diets with attractability and palatability issues Phases of high nucleotide demand Challenging diets negative "off-taste"
Product			
Leiber® Beta-S/Leiber® Beta-S Plus Highly purified ß-glucans Prophylactic use 4 weeks in advance Dosage Leiber® Beta-S: 200g/to Dosage Leiber® Beta-S Plus: 1kg/to	**Biolex® MB40** Yeast cell walls with high levels of MOS and ß-glucans Follow-up from Leiber® Beta-S use ideal to maintain high immune competence Dosage Biolex® MB40: 1-2kg/to	**CeFi® pro** Autolyzed brewers' yeast Peptides, amino acids and nucleotides Dosage CeFi® pro: 2-10kg/to	**Leiber NuTaste®** Range of brewers' yeast extracts Different taste profiles and functionalities Dosage Leiber NuTaste®: 1-5kg/to
Benefit			
Raising resistance Increased immune competence Higher stress and disease resistance As a result, higher survival rates Increases vaccination effect (adjuvant effect) Immune system build-up in larval and juvenile stages	**The inner protective shield** Helps maintain and develop gut health (eubiosis) Prebiotic effect on the gut microbiome Binding of certain pathogens and mycotoxins Improves gut morphology and barrier function Immunity support	**The All-rounder** High levels and bioavailabilty of nutrients and active ingredients Cell health support Stimulates the metabolism Positive effects on feed intake and performance Promotes intestinal integrity and immunity	**Natural. Pure. Delicious** Higher feed intake and less feed loss Naturally rich in free amino acids, especially free glutamic acid Concentrates of nucleotides and nutrients (proteins, peptides, amino acids, vitamins,...) Positive effects on growth and feed conversion Feed material in food quality

Thanks a lot for your attention!

Leiber
Excellence in Yeast

Contact details

Dr. Holger Kühlwein

Key Account Manager Aquaculture

Phone +49 173 5255293

E-Mail h.kuehlwein@leibergmbh.de

Website www.leibergmbh.de