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PR1ME
Animal Health

Leiber Biolex® MB40: prebiotic brewer's yeast cell walls for aquaculture
Product properties and functionalities

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Prebiotic brewer’s yeast cell walls

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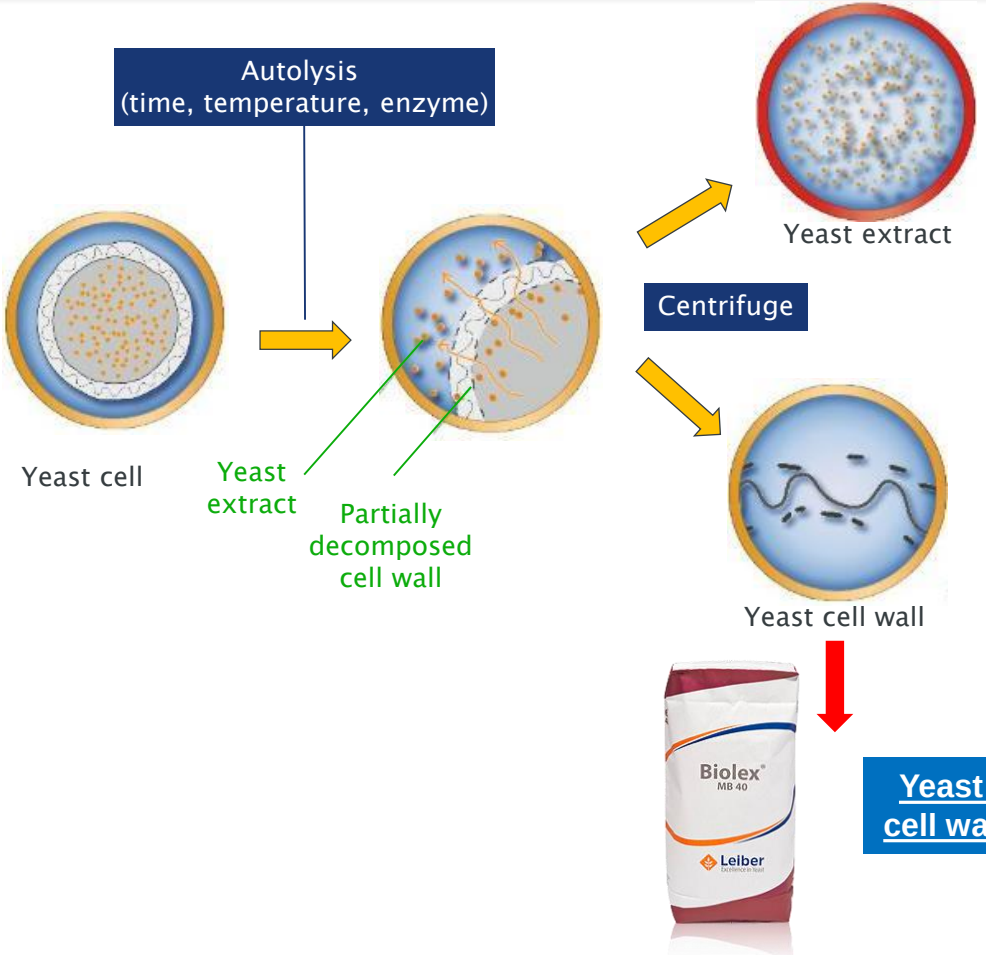
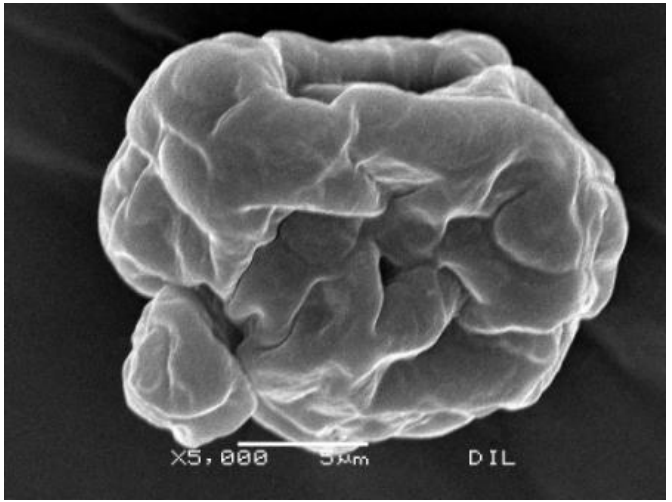
Production, product properties and overview “mode of action”

Prebiotic brewer's yeast cell walls





Prebiotic brewer's yeast cell walls

	Leiber® Brewers' Yeast unextracted	Biolex® MB40
Protein (%)	46	25
ß-Glucan (%)	10 - 15	25
Mannan (%)	7 - 10	20-25
Oils and fats (%)	2.7	7.5
Ash (%)	8	4
Dosage (kg/to feed)	10 - 50	0.5 – 2

Prebiotic brewer’s yeast cell walls

Modes of action

- Bacteriostatic effect due to bioactive hops components – only in brewer’s yeast products!!
- Prebiotic effect on beneficial microorganisms
- Binding of certain pathogen and their toxins as well as certain mycotoxins
- Activation of immunity (ß-glucans in the yeast cell wall activate immune cells)
- Formation of a biofilm on the gut mucosa (barrier function)
- Improvements in gut morphology

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The bacteriostatic effect

Prebiotic brewer’s yeast cell walls

Bacteriostatic effects

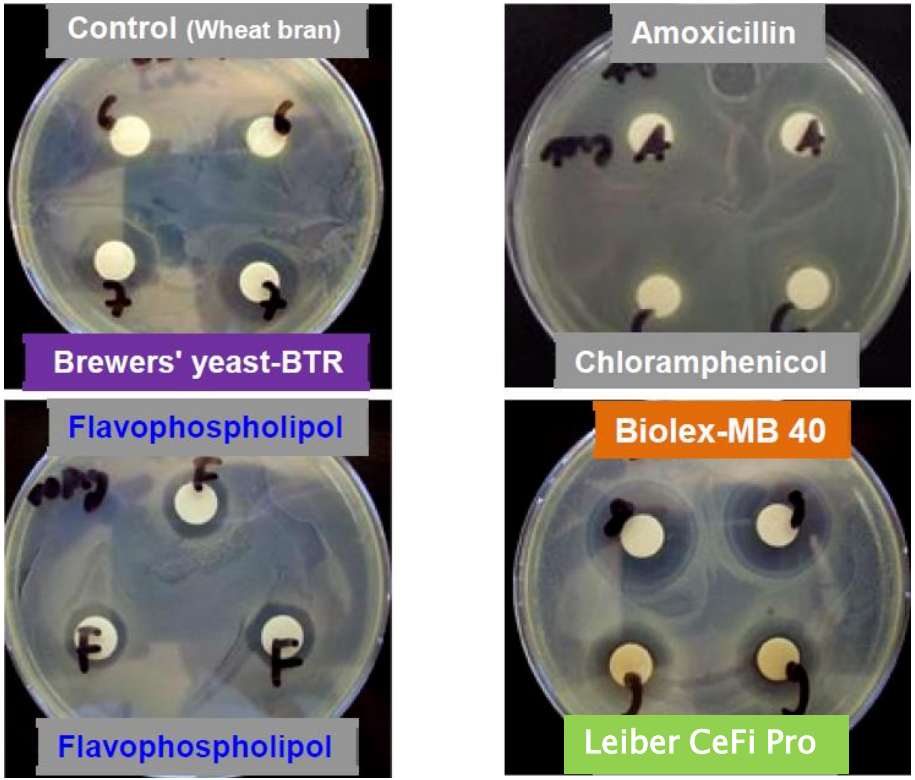
- Hops – only in brewer‘s yeast → through hop ingredients (α-acids, ß-acids, polyphenols – flavonoids)
- Hop effects:
  - calming
  - antioxidative
  - adstringent
  - bacteriostatic

Prebiotic brewer's yeast cell walls

Bacteriostatic effects

- Inhibitor test results
  - Inhibition of *Staphylococcus aureus*
  - Bacteriostatic effects
  - Resistant antibiotics

- Conclusion
  - Mode of action against resistant pathogens



Sample concentration ≙ *0.2% in feed*

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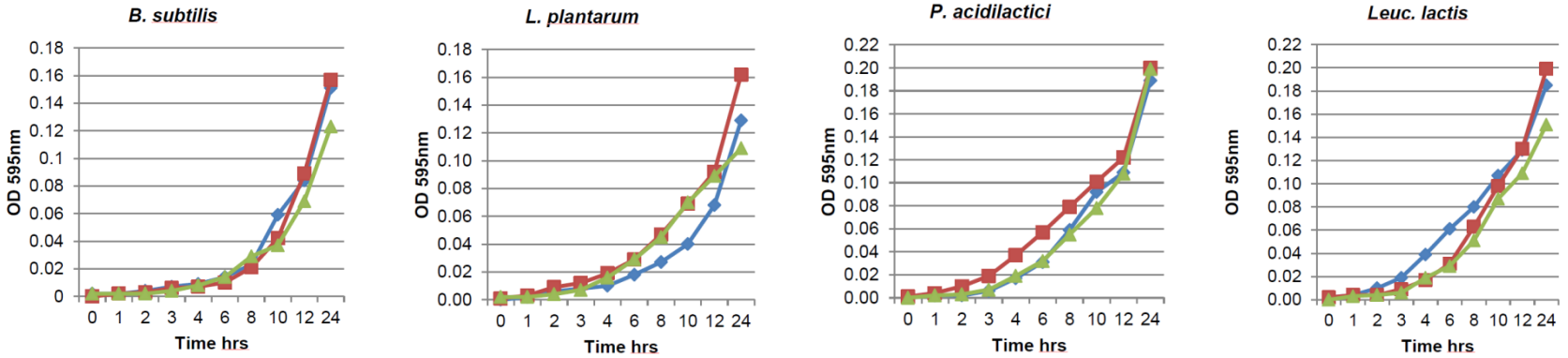
The prebiotic effect

Prebiotic brewer's yeast cell walls

The prebiotic effect – utilization by probiotic bacteria

- Peggs and Merrifield, Plymouth University (2014) – *in vitro*



Bacillus subtilis, *Lactobacillus plantarum*, *Pediococcus acidilactici* and *Leuconostoc lactis* in DMEM minimal media with Biolex® MB40 (5% w/v) as the only carbon source. Each strain was tested in triplicates.

Literature examples of beneficial effects of the tested probiotics in fish/shrimp			
Bacillus subtilis	*Lactobacillus plantarum*	*Pediococcus acidilactici*	*Leuconostoc lactis*
Rainbow trout (Brunt *et al*., 2007) Olive flounder (Cha *et al*., 2013)	Tilapia (Ren *et al*., 2013) Rainbow trout (Perez-Sanchez *et al*., 2011)	Tilapia (Standen *et al*., 2013) Blue shrimp (Castex *et al*., 2013)	Black Porgy (Zhang *et al*., 2013)

Prebiotic brewer's yeast cell walls: *in vivo* experiment

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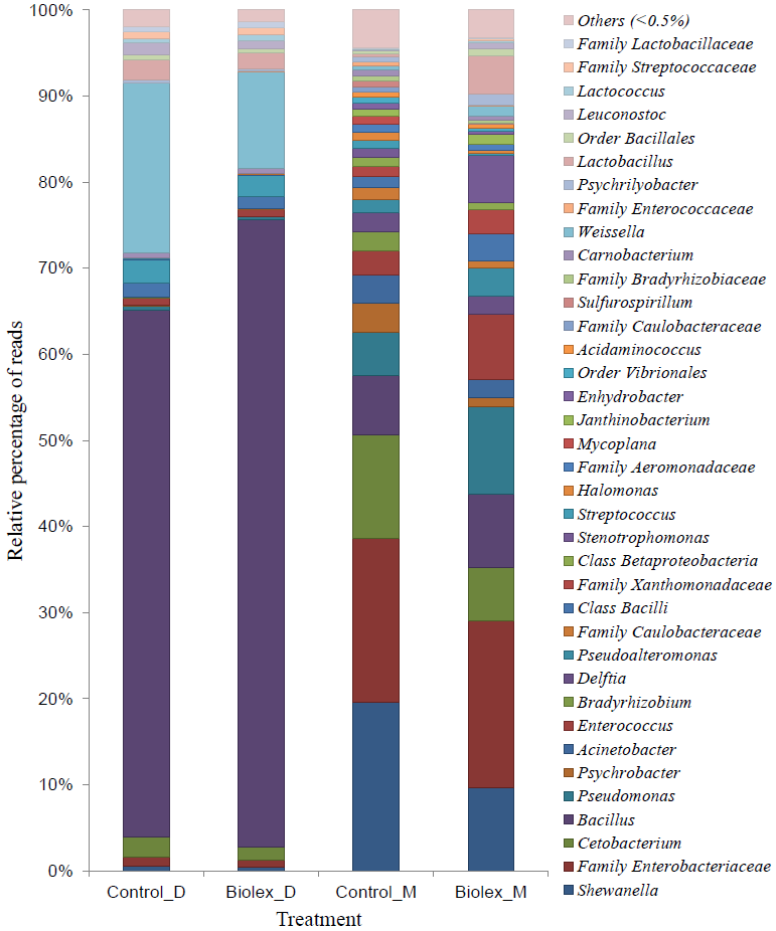
Effect of dietary Biolex® MB40 on the gut microbiome of rainbow trout

- Peggs and Merrifield, Plymouth University (2015)
- Rainbow trout with initial body weight of 128.1 ± 0.6 g in triplicate tanks
- Feeding for 28 days at 2% of body weight 2 isonitrogenous (43% protein) and isolipidic (20% lipid) diets
- Aseptical sampling of mucosa and digesta → high-throughput sequencing (Ion Torrent)

	Control	Biolex® MB40
Fishmeal (Herring meal LT94)	28.00	28.00
HP 100, soy protein concentrate, 54% protein	10.00	10.00
SPC 60, soy protein concentrate, 60% protein	13.03	13.03
Glutalys	10.74	10.74
Vital wheat gluten	5.03	5.03
Fish oil	7.63	7.63
Sunflower oil	9.00	9.00
Corn starch	15.07	14.87
Mineral/vitamin premix	1.00	1.00
CMC binder	0.50	0.50
Biolex® MB40	-	0.20

Prebiotic brewer's yeast cell walls: *in vivo* experiment



The relative proportion (%) of reads from the intestinal digesta (D) and mucosa (M) by treatment, assigned at the genus level >0.5%.

Prebiotic brewer's yeast cell walls: *in vivo* experiment

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Effect of dietary Biolex® MB40 on the gut microbiome of rainbow trout

Abundance (%) of important OTUs in digesta and mucosa samples

Taxon	Control	Biolex® MB40	P-value	Evaluation
Digesta				
Staphylococcus	0.4 ± 0.0[a]	0.2 ± 0.1[b]	< 0.01	*Pathogenic potential*
Bacillus	61.2 ± 14.2	72.9 ± 9.9		Probiotic potential (see *in vitro* trial)
Mucosa				
Shewanella	19.6 ± 4.3[a]	9.6 ± 7.7[b]	< 0.02	*Pathogenic potential*
Psychrobacter	3.5 ± 1.7[a]	1.1 ± 0.8[b]	< 0.02	*Pathogenic potential*
Weissella	0.5 ± 0.4[a]	1.2 ± 0.6[b]	< 0.05	Probiotic potential
Bacillus	6.9 ± 2.9	8.5 ± 5.2		Probiotic potential (see *in vitro* trial)
Lactobacillus	0.4 ± 0.6	4.4 ± 6.9		Probiotic potential (see *in vitro* trial)
Leuconostoc	0.1 ± 0.1	0.6 ± 0.7		Probiotic potential (see *in vitro* trial)

Prebiotic brewer’s yeast cell walls

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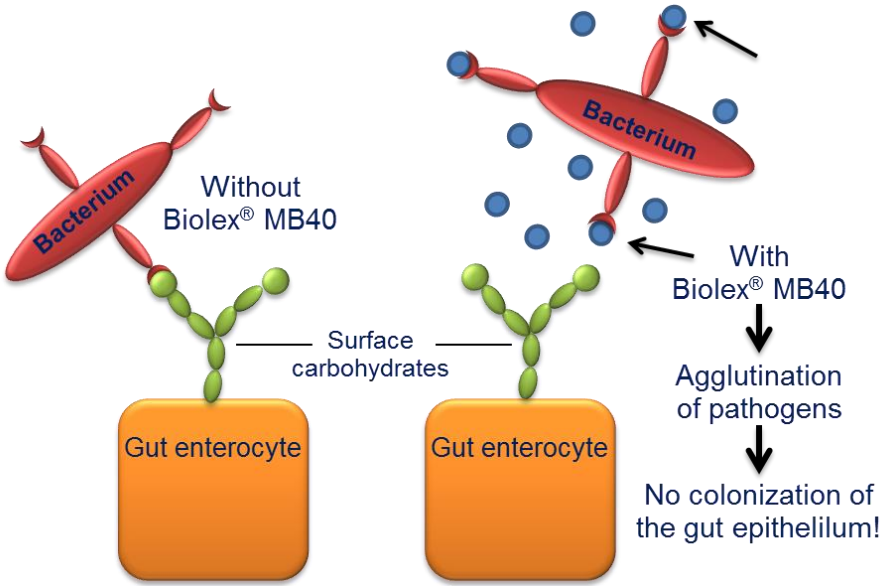
Pathogen and mycotoxin binding

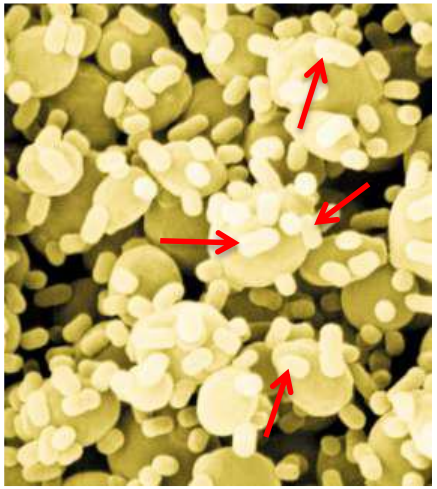
Prebiotic brewer's yeast cell walls

Binding of certain pathogens and their toxins

- Mannose binding lectins of yeast cell wall recognize carbohydrate patterns on surface of many pathogens
- High binding strength and deactivation of pathogens and toxins in the gut lumen
- Pathogens are inhibited from colonizing and attaching to the gut epithelium

> Excretion with the faeces!!





Scanning electron microscopy showing the agglutination of bacteria (arrows) Gedek, 2001

Prebiotic brewer's yeast cell walls

Binding of certain mycotoxins

- Mycotoxin binding through high concentration of MOS and surface receptors (fibrills) in the yeast cell wall
- Support of the mycotoxin degrading microbiota
- Mycotoxin binding Zearalenon - Experiment Fruhauf *et al*., 2012
  - Binding of ZON in vitro (buffer pH 3, buffer pH 6,5 and gastric juice)
  - Test material: 32 toxin binders (2 on clay basis as controls,10 MOS/ß-glucan products and 20 mixtures)
  - Aim of the study: binding effect depending on the ash content and on the MOS/ß-glucan content

Prebiotic brewer's yeast cell walls

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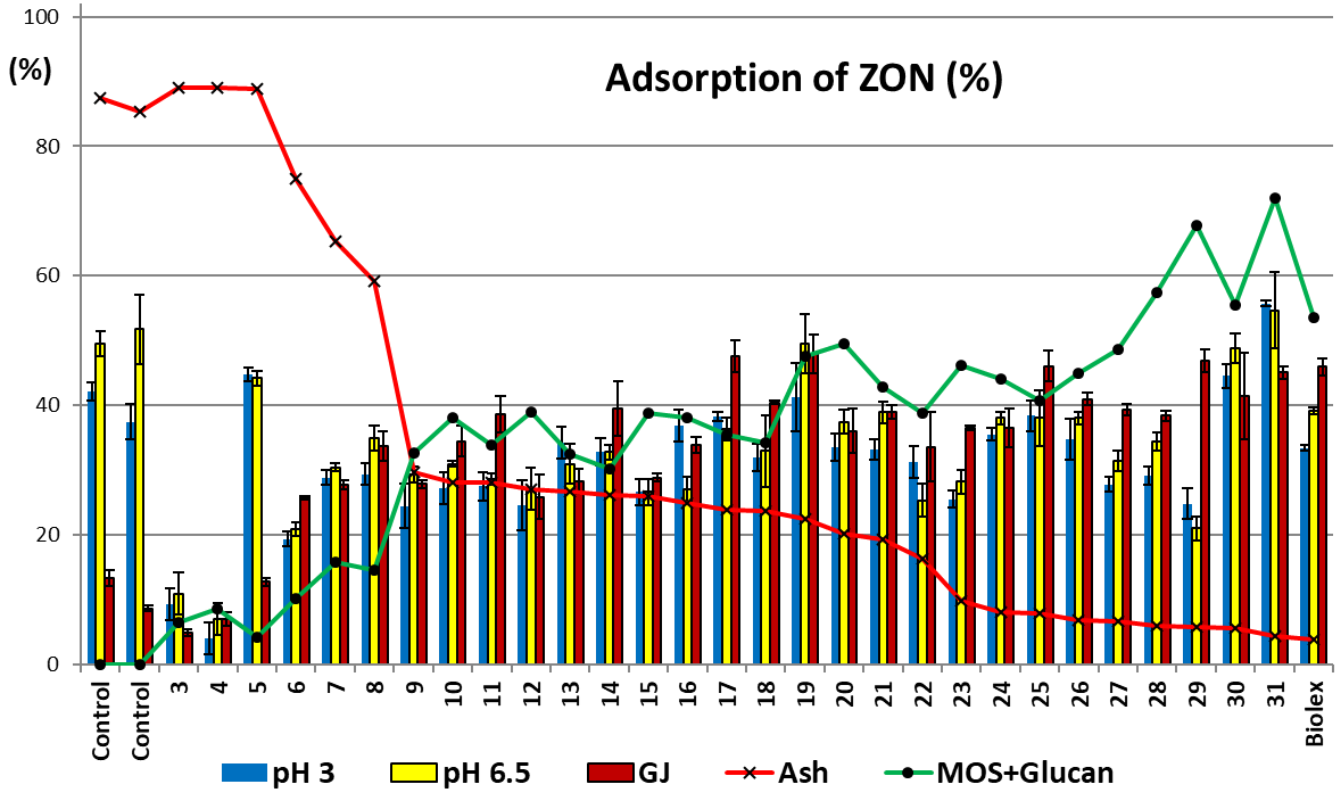
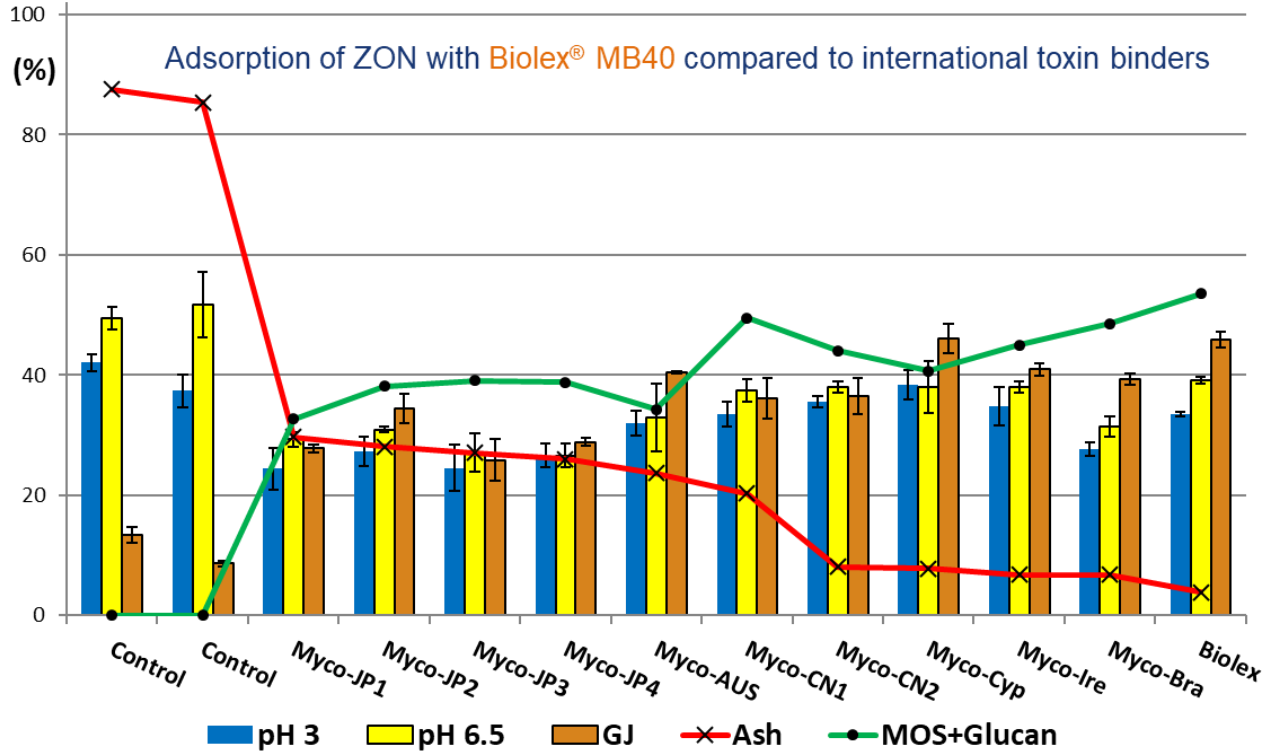
Binding of certain mycotoxins



Prebiotic brewer's yeast cell walls

Binding of certain mycotoxins

Results in comparison with registered Mycotoxinbinders!!



Prebiotic brewer’s yeast cell walls

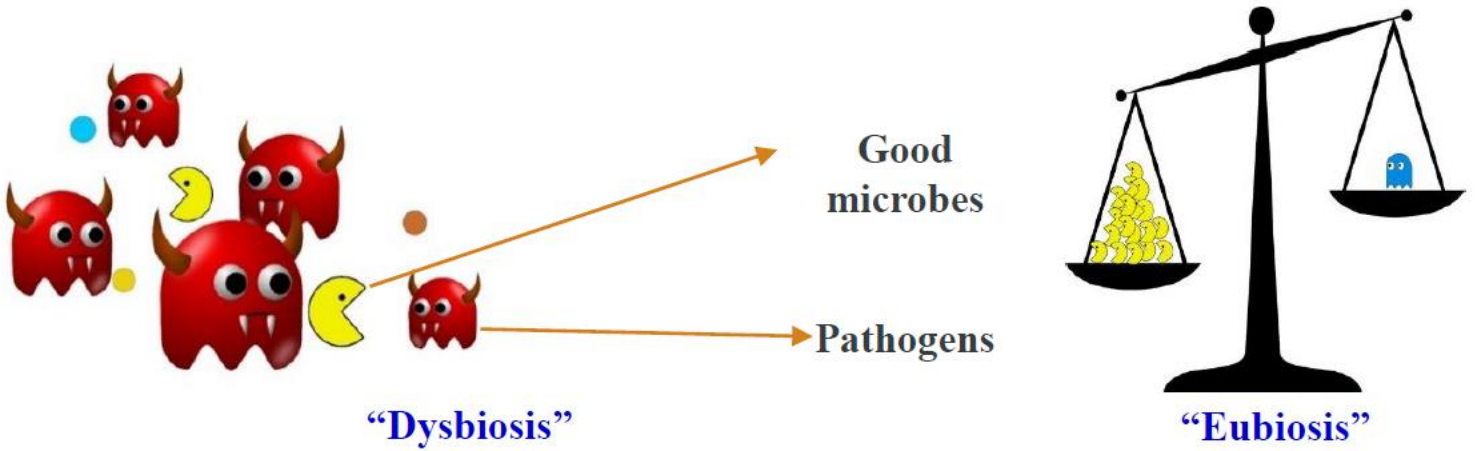
Binding of certain mycotoxins

- No binding effect with bentonite on ZON
- Limited effect with “mixed” products (bentonite/MOS)
- The higher the mannan and ß-glucan content, the higher the adsorption
- Biolex® MB40 shows the best binding effect in gastric juice
- Conclusion: Biolex® MB40 is most efficient in binding zearalenone!

Prebiotic brewer’s yeast cell walls

Effects on the gut microbiota in summary

- Prebiotic effect: brewer‘s yeast cell wall as fermentable substrate for the intestinal microbiota
- Bacteriostatic effect from hops components
- Result together with the pathogen and mycotoxin binding:
- Indirect reduction of harmful microbiota and support of a physiological microbial environment in the gut



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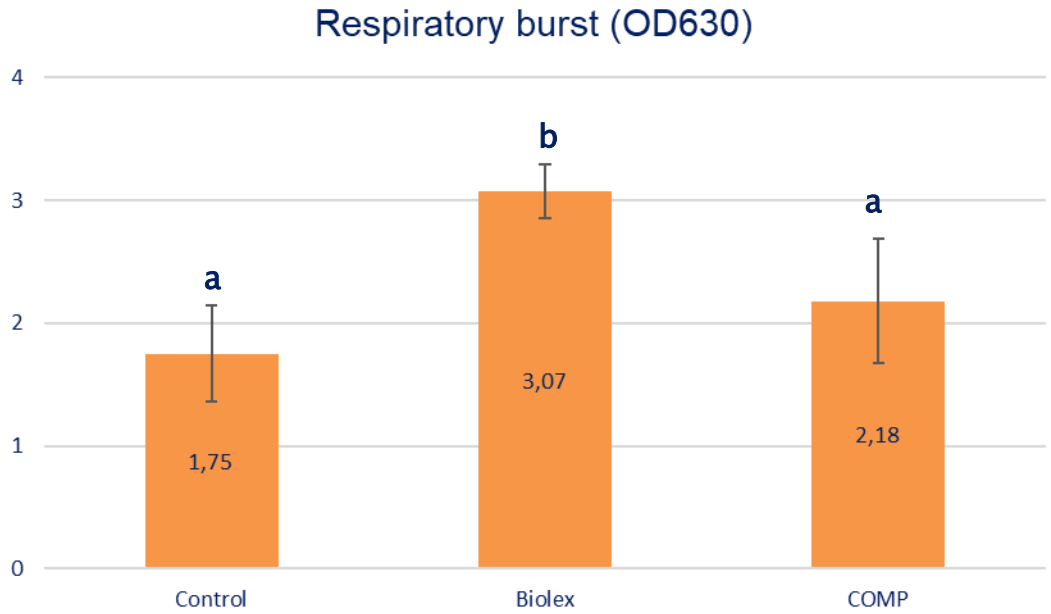
Activation of immunity

Prebiotic brewer's yeast cell walls

Activation of immunity (ß-glucans in the YCW activate immune cells)

- Support and relief for the immune system → Activation of defense mechanisms
- Benchmarking trial against competitor product (2016); feeding of tilapia juveniles with 2 kg/to Biolex® MB40 vs. 4 kg/to competitor product (*n* = 21)

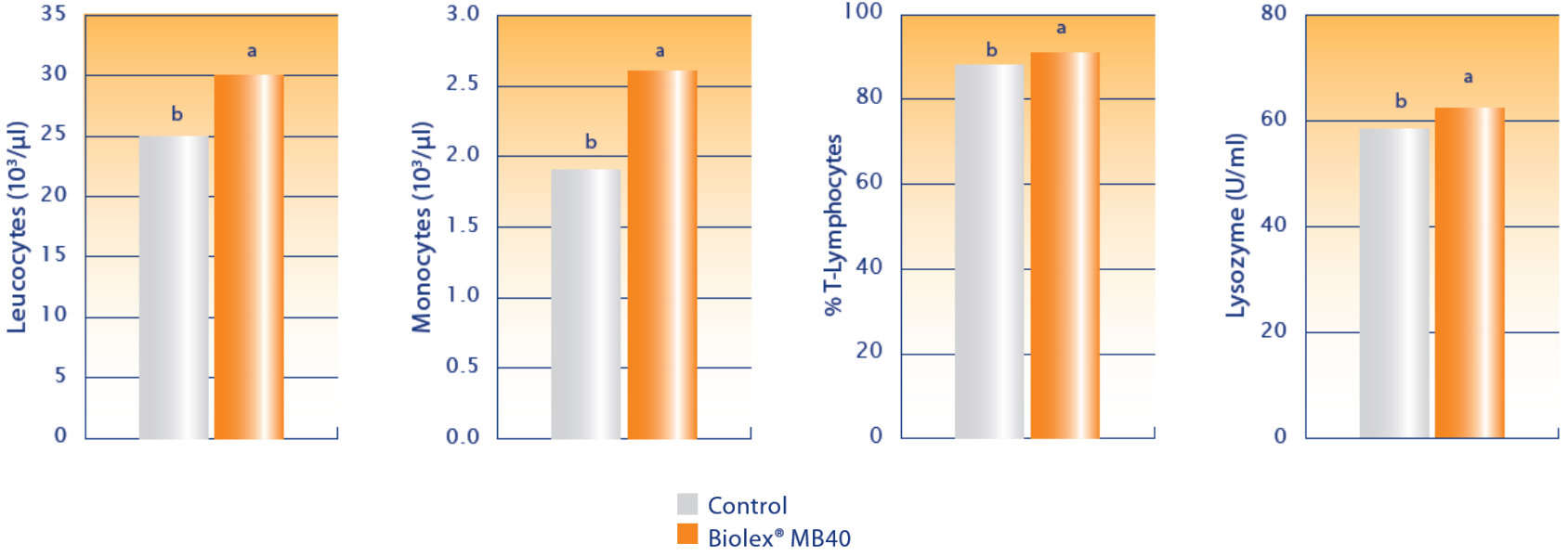


Respiratory burst analysis of whole blood after 6 weeks of feeding (*n* = 21). Results are presented as means with standard deviation. Different superscripts indicate a significant difference ($p < 0.05$)

Prebiotic brewer's yeast cell walls

Activation of immunity (ß-glucans in the YCW activate immune cells)

- Support and relief for the immune system → Activation of defense mechanisms
- Tyson Foods Inc. Research Farm (2011); feeding of broilers with 2 kg/to Biolex® MB40 (*n* = 2000)



*n=20
[a,b] significant difference between data with different letters (P < 0.05)

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Biofilm formation

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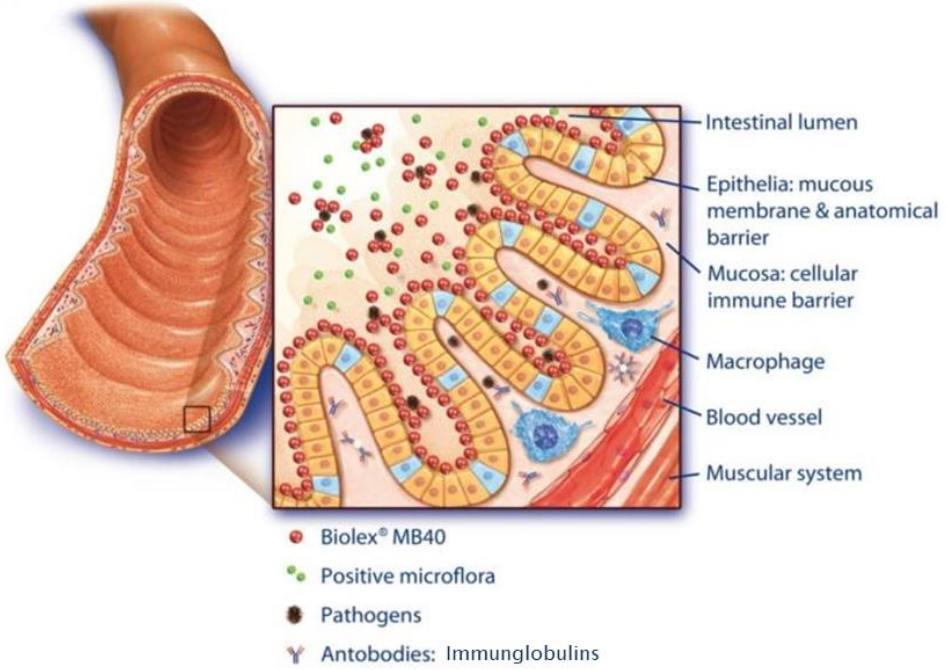
Formation of a biofilm on the gut mucosa

- Biofilm as infection barrier → forming an additional protective layer on the intestinal mucosa



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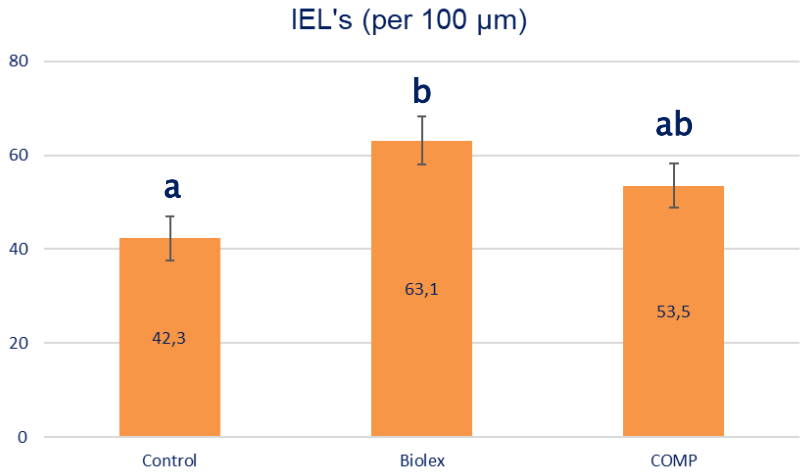
Improvements in gut morphology

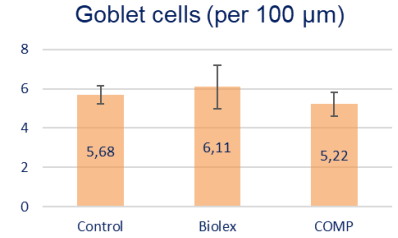
Prebiotic brewer's yeast cell walls

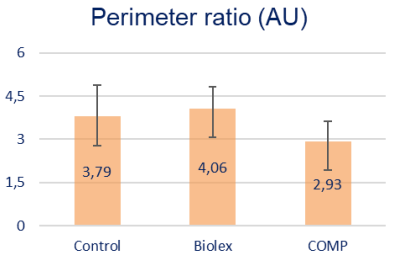
Improvements in gut morphology and GALT (gut-associated lymphoid tissue)

- Increases in absorptive surface area (perimeter ratio, villi length, etc.)
- Improvements in number of goblet cells and/or intraepithelial leukocytes (IEL's)
- Benchmarking trial against competitor product (2016); feeding of tilapia juveniles with 2 kg/to Biolex® MB40 vs. 4 kg/to competitor product (recommended dosages)



Intestinal morphometric analyses of tilapia mid intestine (n = 6). Results are presented as means with standard deviation. Different superscripts indicate a significant difference ($p < 0.05$)





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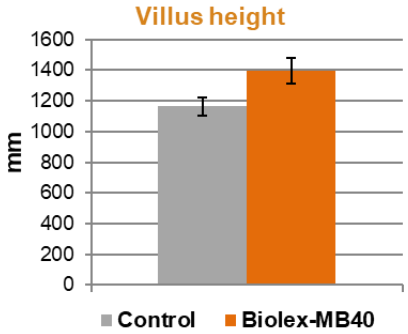
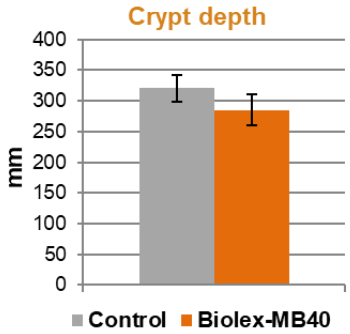
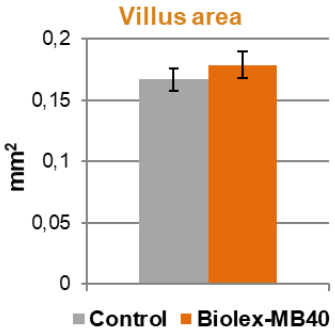
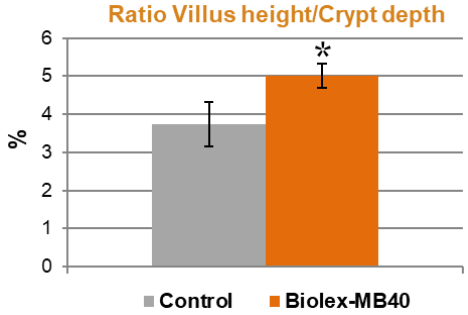
Improvements in gut morphology

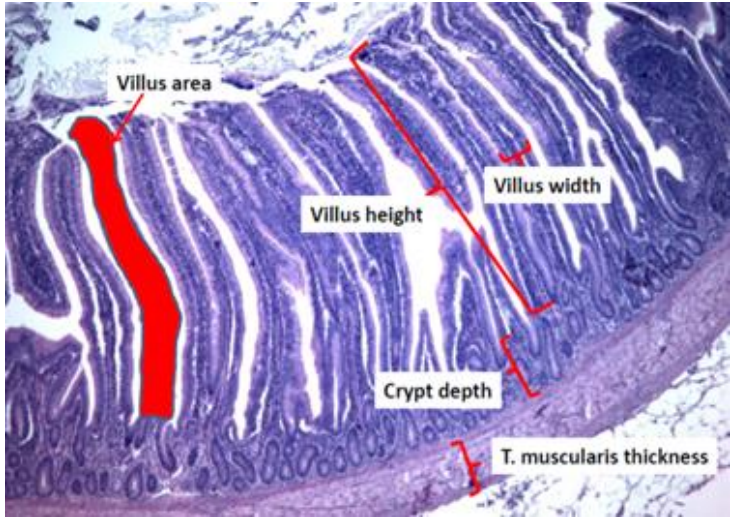
- Broiler feeding trial at University of Novi Sad, Serbia (2014); feeding of 2 kg/to Biolex® MB40 for 42 days











* significantly different ($p < 0.05$)

Prebiotic brewer’s yeast cell walls

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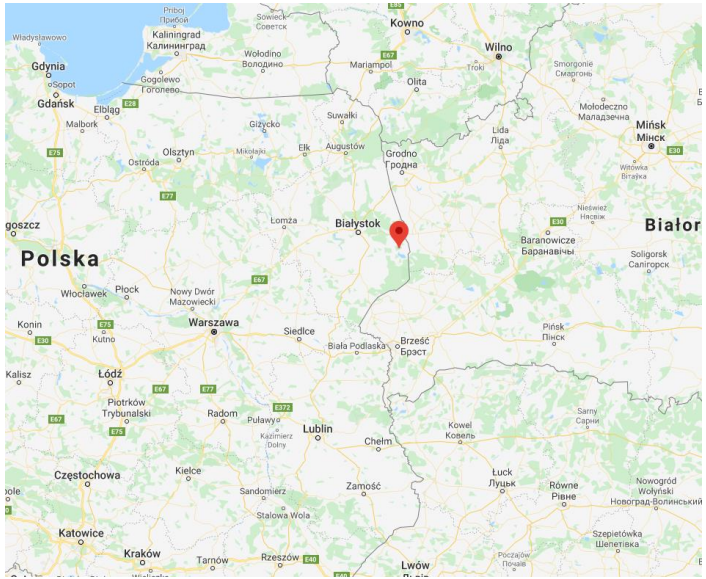
Polish field trial data in carp (*Cyprinus carpio*)

Biolex® MB40 – carp field trial data

- A field trial was conducted at the Fish Breeding station in Bachury, Poland
- 2 ponds (2 ha each) were used for the trial (one for control and one for Biolex MB40 treatment)
- Juvenile carp in good condition were stocked in ponds May 2019, trial finished mid-September 2019
- Initial average body weight of the carp was 50 g, the total stocking density was 5000 carp per pond



Biolex® MB40 – carp field trial data

- Feeds and feeding
  - Feeds produced by Probio sp.z.oo. (Plowce, Poland)
  - Ingredients: soybean meal, wheat flour, rapeseed meal, pea, corn, DDGS, sunflower oil, linseed, $CaCO_3$, NaCl (exact formula company secret)
  - 0.2 % Biolex® MB 40 was added to the feed mix and pelleted (2.5 and 3.5 mm)
  - Feeding 3 times/week (Mon, Wed, Fri)
  - Average body weight was calculated bi-weekly by catching carp with a cast-net from the ponds

Chemical composition of feed

Crude protein	29%	Vit A	8000 IU
Energy	12.5 MJ	Vit D3	1500 IU
Crude fiber	6 %	Vit E	100 mg
Crude fat	5 %	Cu	10 mg
Crude ash	5 %	Zn	70 mg
Ca	0.5 %	Mn	90 mg
Na	0.1 %	Fe	120 mg
P total	0.6 %	J	1.2 %
Lysine	1.48 %	Se	0.15 mg
Methionine	0.38 %	Aroma	200 mg
Threonine	0.95 %		

Biolex® MB40 – carp field trial data

Parameter	Control	0.2 % Biolex® MB40	Difference in %
Total initial body mass (5000 pc.)	250 kg	250 kg	
Ø initial body weight	50 g	50 g	
Total final body mass	1465 kg	1800 kg	+ 23 %
Ø final body weight	480 g	450 g	- 6 %
Feed conversion	2.47	1.93	- 22 %
Estimated losses	39 %	20 %	- 51 %
Total amount of feed used	3000 kg	3000 kg	
Feed costs/kg	0.53 €	0.54 €	
Feed costs/kg weight gain	1.31 €	1.05 €	- 20 %

Biolex® MB40 – carp field trial data

- Further remarks:
  - During harvest also grass carp (*Ctenopharyngodon idella*) were caught ~ 800 kg
  - Quite cold water temperature at trial start (start got delayed)
  - Some cormorant issues at the farm
  - Follow-up trial planned with 3rd-year carp from May-September (~ 450 g until market size)

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Conclusions and recommendations for use

Prebiotic brewer’s yeast cell walls

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Conclusions

- Biolex® MB40 has a strong bacteriostatic effect against highly antibiotic-resistant pathogens
- Biolex® MB40 strongly promotes beneficial and inhibits potentially pathogenic bacteria in the gut
- Biolex® MB40 most efficiently binds and deactivates ZON, potential to bind other mycotoxins
- Biolex® MB40 supports and relieves the immune system
- Biolex® MB40 forms an additional protective infection barrier / biofilm on the gut mucosa
- Biolex® MB40 leads to improvements in gut morphology and gut-associated lymphoid tissue

Prebiotic brewer’s yeast cell walls

Recommended usage

- 1 – 2 kg/to in feed (0.1 – 0.2%) → usually 2 kg/to feed
- To prevent or counteract impaired gut health

  → through gut dysbiosis - impaired gut microbial communities (e.g. after antibiotic treatment)

  → where challenging diets are used (high levels of plant ingredients, mycotoxins, etc.)
- Support of immunity in grow-out stages
- Maintaining high immune competence if purified ß-glucans were used in larval stages as immune boosters

  → follow-up treatment

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

General recommendation guideline for conceptual use in fish and shrimp

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PROBLEM	Diseases and stress Low immune competence	Immune status grow-out stages Impaired gut health	High cell turn-over Low feed attractability
	◆ High pathogen pressure ◆ Stress (handling, transport, ...) ◆ Environmental changes ◆ Limited immune capacity of larvae	◆ Gut dysbiosis (impaired gut microbial communities) ◆ Challenging diets (plant ingredients, mycotoxins, ...)	◆ Phases of high nucleotide demand (larvae growth, shrimp moulting, ...) ◆ In diets with attractability issues and negative effects on feed intake
SOLUTION	**Leiber® Beta-S/Beta-S Plus**	follow-up → **Biolex® MB40**	**CeFi® pro**
	◆ Prophylactic use 4 weeks in advance ◆ Dosage Leiber® Beta-S: 200 g/to ◆ Dosage Leiber® Beta-S Plus: 1 kg/to	◆ Follow-up from Leiber® Beta-S use ideal to maintain high immune competence ◆ Dosage Biolex® MB40: 1-2 kg/to	◆ Ideal for species with a short digestive tract, like shrimp ◆ Dosage CeFi® pro: 2-10 kg/to
BENEFIT	**Immunity Boost**	**Gut Eubiosis**	**Cell Health**
	◆ Increased immune competence ◆ Higher disease and stress resistance ◆ Reduced mortalities ◆ Improved build-up of immune status in larval and juvenile stages ◆ Adjuvant effect in vaccinations	◆ Binding of certain pathogens and mycotoxins in the gut ◆ Prebiotic effect on beneficial gut microbiota ◆ Improved gut morphology ◆ Immunity support	◆ High levels and bioavailability of nucleotides and nutrients ◆ Nucleotides promote cell division and regeneration, especially in gut tissue in larval stages ◆ Increased feed intake and growth

For more information please contact:
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