EFFECTS OF PURIFIED BREWER'S YEAST (*Saccharomyces cerevisiae*) ADDITIVES ON THE MUCOSAL HEALTH OF ATLANTIC SALMON PARR

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Excellence in Yeast

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1 INTRODUCTION

Restrictions on the use of antibiotic growth promoters in animal feed have necessitated a shift towards functional feed additives. Cell wall components of ***Saccharomyces cerevisiae*** (rich in **β-1,3 and -1,6-glucans and mannan oligosaccharides**) in isolated or whole forms have been demonstrated to confer immunomodulatory effects in fish (Rawling *et al.,* 2021). These benefits are at least partially induced by improvements of intestinal health. Despite the reported benefits, many knowledge gaps exist with regards to the optimal form and dosage.

2 AIMS

- The overall aim of this study was to investigate the effect of brewer's yeast derived **β-glucans and mannan oligosaccharides** on the mucosal health of Atlantic salmon. Specific aims were to investigate the impacts on skin and intestine:
  - Gross and ultrastructural morphology
  - Goblet cell abundance
  - Transcriptional response of targeted immunological and barrier regulating genes

3 METHODOLOGY

Table 1: Ingredient and nutrient composition in % of diet

Ingredient	Control	PβG	WYCW
Soy protein concentrate 62	30.00	30.00	30.00
LT Fishmeal	15.00	15.00	15.00
Soybean meal 48*	13.00	13.00	13.00
Wheat Gluten meal	13.85	13.85	13.85
Fish Oil	8.00	8.00	8.00
Sunflower oil	8.72	8.72	8.72
Sunflower meal	8.70	8.68	8.50
Fish Premix	0.50	0.50	0.50
Purified B-glucans (PβG)**	-	0.02	-
Whole Yeast Cell Wall (WYCW) **	-	-	0.2
Proximate composition (%)			
Dry Matter	96.5 ± 0.1	96.3 ± 0.1	97.2 ± 0.1
Crude Protein	49.2 ± 0.3	49.1 ± 0.8	48.9 ± 1.1
Fat	20.1 ± 0.4	18.6 ± 1.8	19.3 ± 1.3
Ash	5.8 ± 0.1	5.8 ± 0.0	5.8 ± 0.0

All ingredients except otherwise stated were sourced from BioMar Ltd, Scotland, UK
** Skretting Ltd*
*** Leiber GmbH, Germany*

Fish Source: Landcatch Natural Selection Ltd, Scotland, UK
N_{fish} = 120 Atlantic salmon parr
N_{tank} = 20 fish/tank
Av. Initial weight = 21 ± 0.4 g
Feeding rate: 1.5% BW/day
Duration: 4 weeks
Sampling: intestinal tissues at week 4 (Table 3)

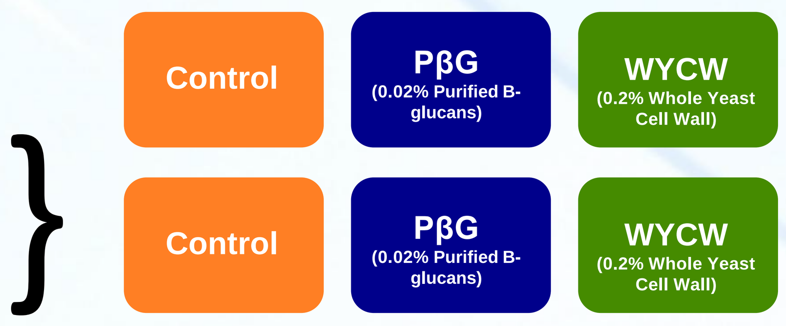

Table 2: Average water quality parameters

DO (mg L^{-1})	pH	Temperature (℃)	NH_3 (mg L^{-1})	NO_2^- (mg L^{-1})	NO_3^- (mg L^{-1})
9.6 ± 0.1	6.96 ± 0.11	16.6 ± 0.2	0.02 ± 0.01	<0.001	6.4 ± 1.6

Table 3: Samples and methods

Technique	Data	Protocols	References
Histology	Gross morphology, goblet cell abundance, microvilli length and density	Light microscopy, electron microscopy	Leclercq *et. al.* (2020)
Gene expression	Transcriptional response of target immunomodulatory and genes	RNA extraction, Real-time PCR	Rawling *et. al.* (2021)

RESULTS 4

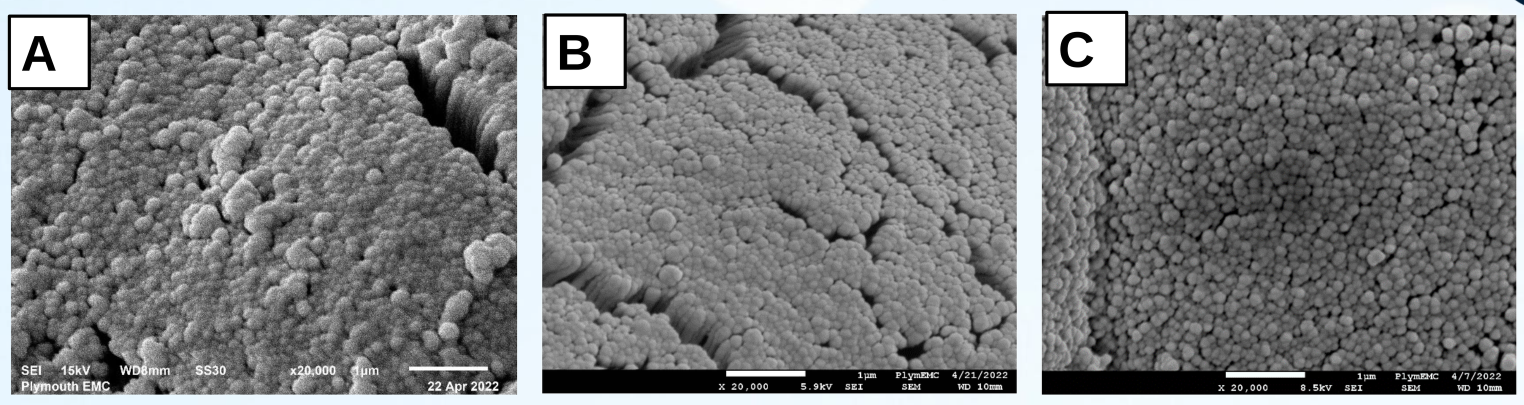

Plate 1: Representative scanning electron micrographs of the microvilli from the distal intestine of Atlantic salmon parr subjected to (A) Control (B) PβG and (C) WYCW treatments.

Table 4: Histological appraisal of intestine and skin of fish at week 4

	Control	PβG	WYCW	P-Value
Distal Intestine				
Goblet cell counts	10.5 ± 0.1[a]	10.8 ± 1.4[a]	14.6 ± 1.3[b]	0.0422
Lamina Propria width (µm)	28.1 ± 1.5	30.4 ± 1.2	27.6 ± 2.5	0.4940
Microvilli length (µm)	1.58 ± 0.04[a]	1.86 ± 0.03[b]	1.46 ± 0.03[c]	<0.0001
Microvilli density (per µm^2)	142.7 ± 4.7[a]	191.5 ± 5.6[b]	178.4 ± 10.4[b]	0.0001
Skin				
Goblet cell counts	22.7 ± 2.7[a]	33.8 ± 3.3[b]	27.0 ± 2.6[ab]	0.0459

Figures with different superscripts are significantly different at $P < 0.05$

DISCUSSION 5

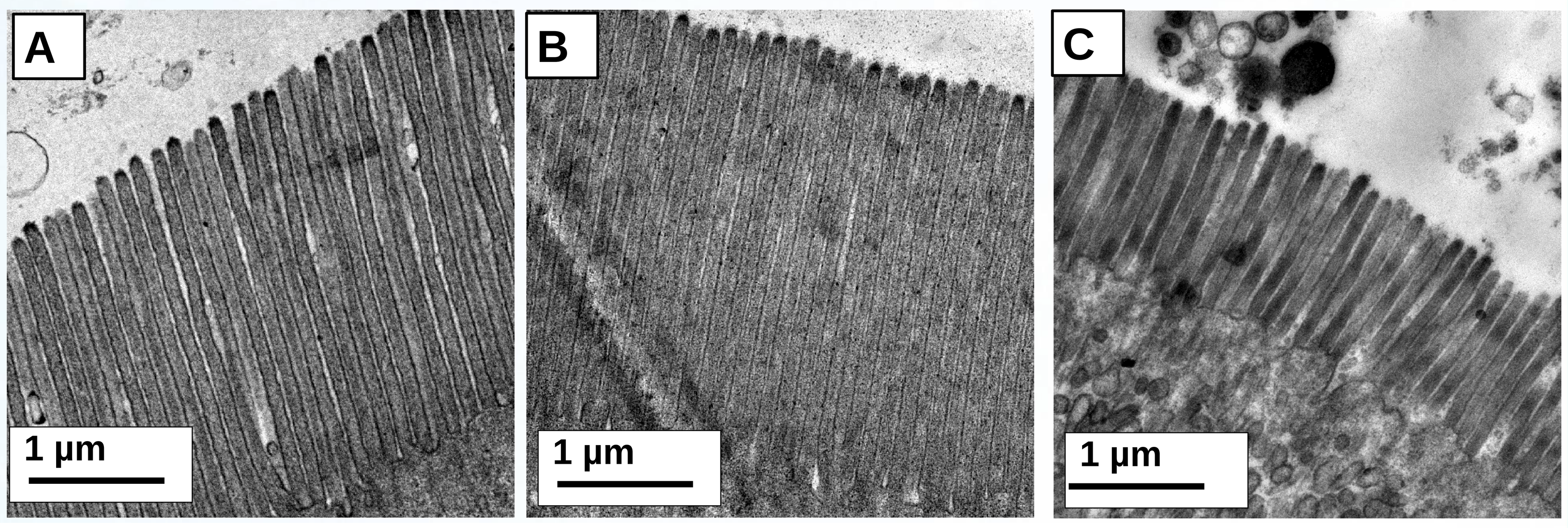

Plate 2: Representative transmission electron micrographs of the microvilli from the distal intestine of Atlantic salmon parr subjected to (A) Control (B) PβG and (C) WYCW treatments. Scale bars = 1 µm.

At the end of the four week experiment, although all treatments showed positive growth trends (average FCR = 0.8 ± 0.1), there was no significant difference in growth parameters among all treatments ($P > 0.05$). However, histological appraisal revealed goblet cell abundance was significantly increased (+39%) in the distal intestine of fish fed the WYCW and in the skin (+49%) of fish fed the PβG treatment when compared to the control group. Goblet cells are mucin-producing cells found on epithelial surfaces including the skin and intestine of fish. Their major function is the secretion of mucus, which forms a protective gel-like physical barrier against luminal threats. Our results supports the existing literature that suggests that yeast cell wall extracts may increase the proliferation of goblet cells in both skin and intestine of fish – an essential precursor for robust barrier defences (Merrifield *et. al.,* 2011; Micallef *et. al.,* 2017; Rawling *et. al.,* 2019).

In addition, transmission electron microscopy (TEM) analysis of the distal intestine revealed significantly different microvilli morphometrics. Fish fed the PβG treatment had significantly longer (+20%) and more densely packed (+34%) microvilli than the other treatment groups. Fish fed the WYCW treatment had significantly denser (+25%) microvilli arrangement than the control group. Ongoing analysis includes gene expression profiling of immunomodulatory and barrier function genes.

In conclusion, both dietary products demonstrated the potential to enhance the epithelial barriers of Atlantic salmon parr.

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