

Impact of probiotic supplementation on the skin-mucus microbiota, health, and growth performance of reared Baltic salmon

Vjačeslavs Revins*(vjaceslavs.revins@bior.lv), Olga Revina, Žanna Bertaite, Santa Purviņa, Justīne Padrevica, Kristofers Millers, Rainers Džeriņš

Introduction

The skin of teleost fish, including reared Baltic salmon (*Salmo salar*), serves as a flexible, scaled barrier that protects against external environmental threats. The outermost layer of this skin is coated with mucus, which not only acts as the first line of defense against infectious pathogens but also hosts a diverse bacterial community, comprising both commensal and opportunistic species. Despite its critical role, there is limited knowledge about the skin-mucus microbiome in reared Baltic salmon, particularly in relation to overall health.

To enhance the health of reared salmon in an environmentally sustainable manner, fish farms should consider incorporating probiotics into aquaculture practices. These beneficial microorganisms can naturally and effectively improve fish health. This study investigates the composition of the salmon skin mucus microbiota and evaluates the impact of probiotic supplementation on the microbial community within the fish skin mucus and its effect on growth performance.

Materials and methods

Experimental Design. The experiment lasted for two weeks and involved 9,000 Baltic salmon psmolts (34.39 ± 6.07 g mean weight $\pm$ SE) reared in a flow-through system across six 1.8 m³ tanks (n = 1500 per tank). Fish were divided into three experimental groups with duplications:

• **Control group (D1):** fed a basal diet.

• **Probiotic feeding group (D2):** basal diet + 1 ml kg⁻¹ Smart Fishery probiotics (Baltic Probiotics, Ltd., Latvia).

• **Probiotic bathing group (D3):** basal diet, with 15 ml m⁻³ for 15 minutes Smart Fishery probiotics baths administered weekly (two baths total).

Fish were fed daily according to standard salmon feeding norms. Probiotics were administered daily in the feed for D2, while D3 received probiotic baths once per week.

Growth performance was assessed by measuring the length and weight of 50 randomly selected fish from each tank at the start and after 14 days. Parameters calculated included weight gain (WG) and Fulton's condition factor (K).

For bacterial isolation and identification, pooled mucus samples from the skin and gills of ten fish per tank were collected at the beginning and after 14 days. Samples, taken with sterile cotton swabs, were transported in semisolid Amies transport medium to the RI BIOR. They were cultured on tryptone soya agar, blood agar, and MacConkey agar, incubated at 22°C and 37°C for 24-48 hours. Bacterial colonies were sub-cultured until pure colonies were obtained, and identification was based on colony morphology, growth temperature, and phenotypic tests (catalase, oxidase, indole), with species-level confirmation via MALDI-TOF Biotyper.

Antimicrobial susceptibility testing was performed on one isolate from each culture using the disk diffusion method (CLSI 2014). Isolates were tested against four antibiotics: doxycycline, enrofloxacin, florfenicol, and oxytetracycline. Bacterial suspensions were inoculated on agar plates and exposed to antimicrobial disks. After incubation (22°C for *Aeromonas salmonicida* and 37°C for other bacteria), inhibition zones were measured, and bacteria were classified as susceptible or resistant based on CLSI standards. The multiple antibiotic resistance (MAR) index was calculated, with values above 0.2 indicating high contamination potential.



Baltic salmon (*Salmo salar*)

Results

Growth performance of Baltic salmon

Group	Parameter					
	W _i	W _f	L _i	L _f	W _G	K
D1 Control	34.50 ± 6.46	39.00 ± 5.82	14.81 ± 1.29	15.36 ± 1.02	4.50	1.03 ± 0.16
D2	33.99 ± 5.74	45.27 ± 5.54	15.01 ± 1.24	15.91 ± 0.84	11.26	1.09 ± 0.08
D3	34.67 ± 5.98	43.93 ± 5.14	14.99 ± 1.32	15.97 ± 0.64	9.26	1.11 ± 0.09

Values are mean $\pm$ SE. Mean values with different superscript letters in a row are significantly different ($P < 0.05$). Abbreviations: W_i = initial mean weight [g], W_f = final mean weight [g], L_i = initial mean length [cm], L_f = final mean length [cm], W_G = weight gain [g], K = condition factor.

Isolated bacteria and antimicrobial susceptibility

GROUP	ISOLATE	ANTIBIOTICS				MAR
		Doxycycline	Enrofloxacin	Florfenicol	Oxytetracycline	
Beginning of trial						
D1 Control	<i>A.salmonicida</i>	☹	☹	☹	☹	0
	<i>A.veronii</i>	☹	☹	☹	☹	0,25
	<i>Pseudomonas spp.</i>	☹	☹	☹	☹	0
D2	<i>A.salmonicida</i>	☹	☹	☹	☹	0,5
	<i>A.veronii</i>	☹	☹	☹	☹	0,5
	<i>Pseudomonas spp.</i>	☹	☹	☹	☹	0,5
D3	<i>A.salmonicida</i>	☹	☹	☹	☹	0
	<i>A.bestiarium</i>	☹	☹	☹	☹	0
	<i>A.veronii</i>	☹	☹	☹	☹	0
End of trial						
D1 Control	<i>A.bestiarium</i>	☹	☹	☹	☹	0
D2	<i>A.bestiarium</i>	☹	☹	☹	☹	0
D3	<i>A.salmonicida</i>	☹	☹	☹	☹	0

☹ - Susceptible; ☹ - Resistant

Conclusion

This study demonstrated that the commercial probiotic Smart Fishery (Baltic Probiotics, Ltd., Latvia) significantly enhances the growth of Baltic salmon by improving digestion and feed assimilation. Fish in both the probiotic feeding (D2) and probiotic bathing (D3) groups showed higher weight gain (WG) and better Fulton's condition factor (K) compared to the control group (D1), indicating improved overall health.

Bacterial analysis revealed dynamic changes in the skin-mucus microbiota, while antimicrobial testing showed no development of antibiotic resistance. Incorporating probiotics into aquaculture promotes growth, supports a healthy microbiome, and reduces antibiotic reliance, making it a sustainable solution for improving fish health and productivity.

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