Dietary protected sodium butyrate (Butirex® C4) supplementation modulates transcriptomic responses in the intestine of rainbow trout (Oncorhynchus mykiss): immunity, antioxidant defense and tight junction Proteins

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SUMMARY

This study was aimed to evaluate the effect of Na-butyrate (BUTIREX C4) on the intestinal integrity of rainbow trout (Oncorhynchus mykiss), specifically on intestinal immune response and expression of immune, antioxidant, tight junction related genes and intestinal histology.

METHOD AND MATERIAL

The study was carried out by the Department of Aquatic Animal Health, University of Tehran. A total of 240 rainbow trout (34.97 gr.) were distributed in 12 tanks appointed to four treatments supplemented with different levels of Butirex C4 during 45 days. During the trial, animals were hand-fed at a rate of 4% of BW, thrice a day. **Experimental diets:**

- Control Group
- 1.5 kg Butirex C4
- 2.5 kg Butirex C4
- 5 kg Butirex C4

Intestinal innate immune responses →Lysozyme, total protein, Alternative Lisozyme activity, Alternative Complement activity and Bactericidal activity in the intestine.

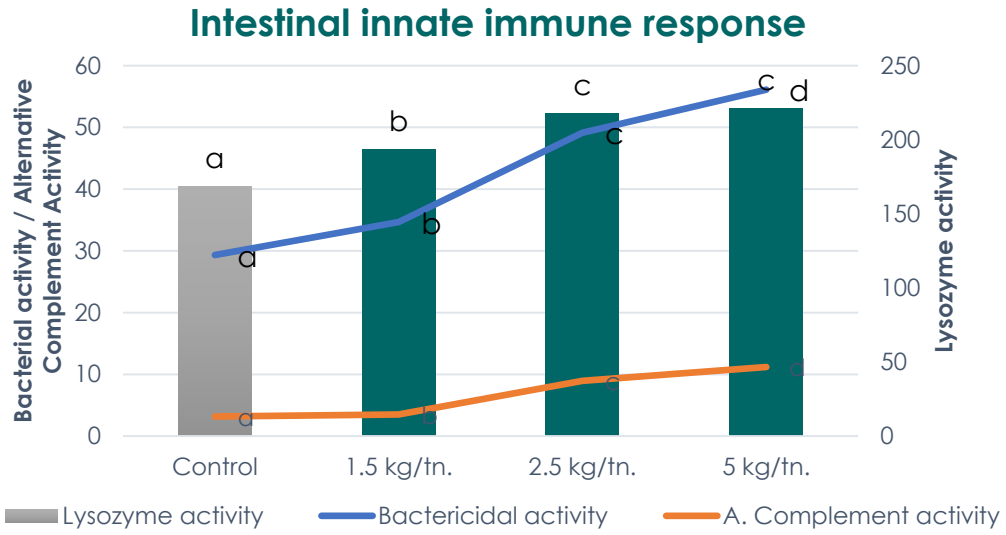
Intestinal Immune-related genes expression → Pro-inflammatory cytokines (TNF-a, IFN-g2, IL-1β, IL-8) mRNA and anti-inflammatory cytokines (TGF-β, IL-10) mRNA were analyzed.

Intestinal antioxidant-related genes expression → Expression of mRNA levels of antioxidant intestinal enzymes (SOD1, CAT, GPx) were analyzed.

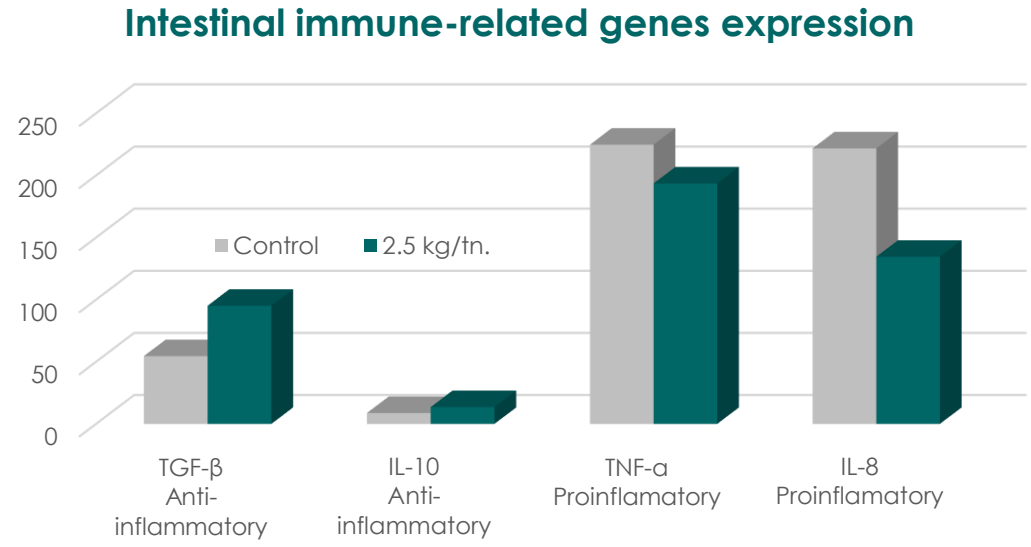
Intestinal tight junction protein genes expression → the tight junction genes of Claudin-3, Claudin-12, Occludin and ZO-1 proteins were measured.

Intestinal histology parameters → the villi length, villus thickness and muscular thickness.

RESULTS



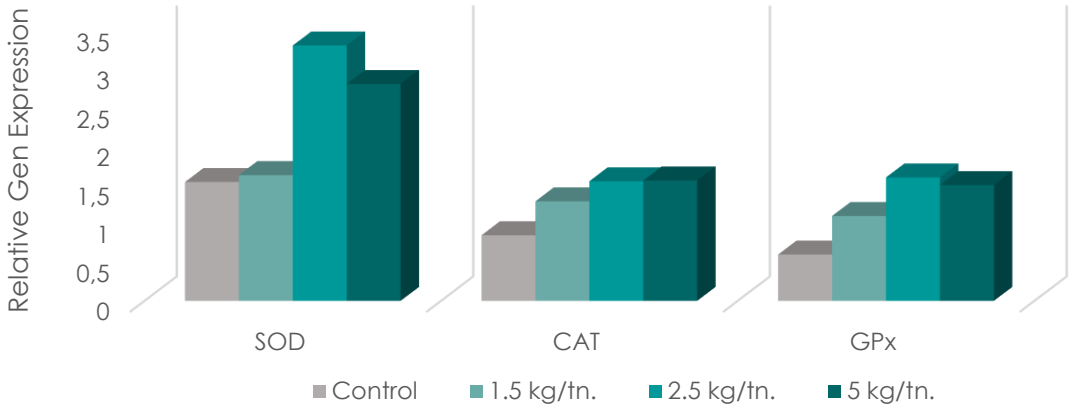
Fish fed with Butirex C4 diet showed significant **higher Lysozyme** in intestine than control, especially at 2.5 kg/ton of feed. Also, Butirex C4 in the diet, demonstrated **higher bactericidal activities and complement activity** compared with control in lineal effect with the inclusion.



Cytokine response is regulated by **anti-inflammatory and pro-inflammatory gens expression.** Butirex C4 **up-regulated the anti-inflammatory cytokines** (IL-10, TGF-β) mRNA levels in the intestine. Butirex C4 **down-regulated the pro- inflammatory cytokines** (TNF-a, IL-8) mRNA levels.

Butirex C4 help to avoid the inflammatory Cytokine expression gens in the intestine. Maximum expression of immune gens was obtained at 2.5 kg/ton of feed.

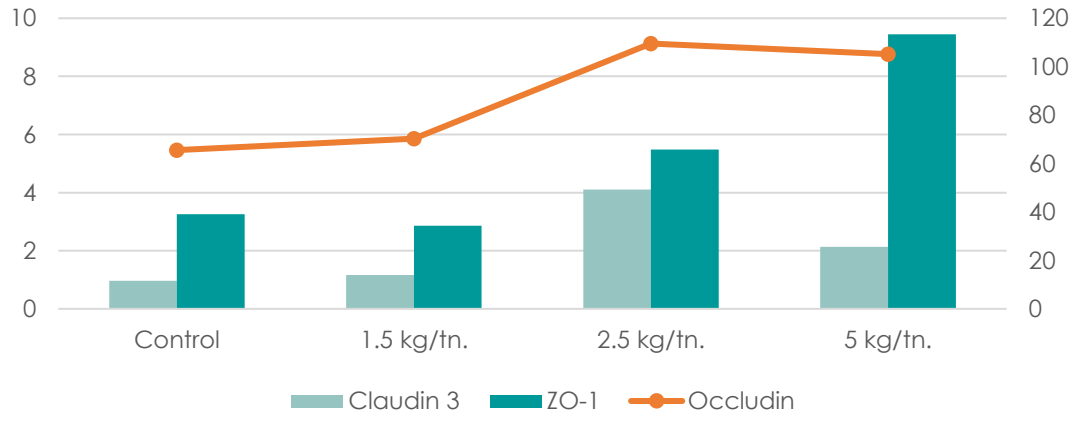
Intestinal Antioxidant-related genes expression



Intestinal oxidation has a negative impact in the epithelium integrity, in the present trial Butirex C4 increases antioxidant gene expression for antioxidant enzymes such SOD, CAT and GPx at the intestinal level. The inclusion of 2.5 kg/tn was the optimal inclusion rate for the antioxidant effect.

Intestinal barriers regulate defense mechanism in the intestinal mucosa, tight junction are proteins that are regulated by some genes expressions. Butirex C4 improved tight junction protein genes expression like Claludin 3, Occludin up to 2.5 kg/mt; and ZO-1 was linearly increase up to 5 kg/ mt.

Intestinal tight junction protein genes expression



Intestinal villi growing and development, as expression of intestinal health were measure at the end of the trial. Villi parameters were significantly difference with the inclusion of Butirex C4. Longest and thickest intestinal villi were found at 2.5 kg/tn of Butirex C4 inclusion. This benefit suggest a diet with Butirex C4 result in a healthier and better-feed absorption of the feed in fish.

Intestine histology parameters (µm)	Level of Butirex C4 kg/tn			
	0	1.5	2.5	5
Villi length	377.5 a	386.2 a	443.0 c	410.2 b
Villi thickness	33.0 a	32.16 a	45.16 b	41.16 b
Muscular thickness	160.7 a	160.8 a	169.7 ab	178.0 b

CONCLUSION

The results of this study reveal that Butirex C4 at 2.5 kg/mt of aqua culture feed. Butirex C4 has as positive effect on intestinal health, improving innate and gens immune function; with up-regulation anti-inflammatory cytokines and down-regulation pro-inflammatory cytokines; showing a good response on intestinal antioxidant capacity and tight junctions' genes expression; and promoting a better villi development in the intestine.