The effects of interfering with *gs* and *gls* genes on intestinal genes expression and metabolic pathways in *Trachinotus blochii*

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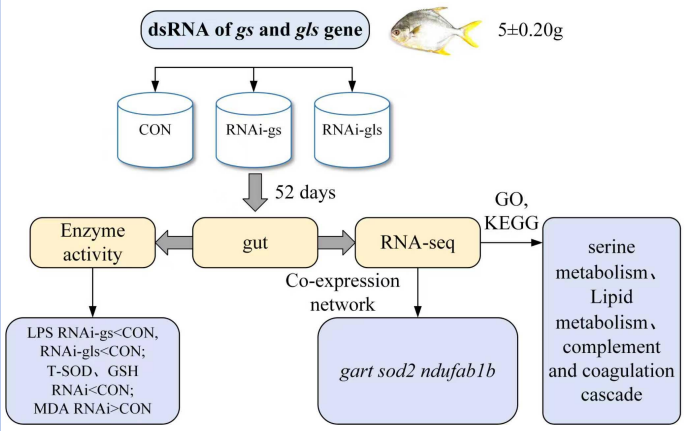
1. Introduction

This research utilized RNA interference technology to achieve sustained suppression of the *gs* and *gls* genes in live *T. blochii*. After 52 days of interference, we analyzed the growth and development of *T. blochii*, intestinal morphology, digestive and antioxidant enzyme activity, and intestinal transcriptome. The development of *T. blochii* was markedly inhibited by interference, with the intestinal diameter, villus length, width, and muscle layer in the interference group being greatly reduced compared to the control group. Measurements of intestinal enzyme activity showed a significant decrease in LPS content in *T. blochii* following interference, and antioxidant-related enzyme activities (T-AOC and GSH) were lowered, and MDA content was significantly raised. Transcriptome analysis indicated that following the knockdown of the *gs* and *gls* genes, there was a significant enrichment of growth-related serine metabolism pathways and lipid metabolism pathways, as well as immune inflammation-related pathways, including the complement and coagulation cascade and PPAR signaling pathway. Co-mRNA network analysis revealed a significant enrichment of genes associated with purine de novo synthesis and the coenzyme Q oxidoreductase family.

2. Methods



- Synthesis of *T. blochii gs* and *gls* dsRNA
- Detection of intestinal enzyme activity
- Preparation of *T. blochii* intestinal sections
- Transcriptome analysis

3. Result

It can be seen that as the injection and breeding time continued (52 days), the weight of the experimental group was significantly lower than that of the control group, but there was no significant difference in growth between the experimental groups (P<0.05) (Fig.1).

The intestinal diameter, villus height, villus thickness, and muscle layer thickness of the experimental group were considerably greater than those of the two control groups (P<0.05) (Fig.2).

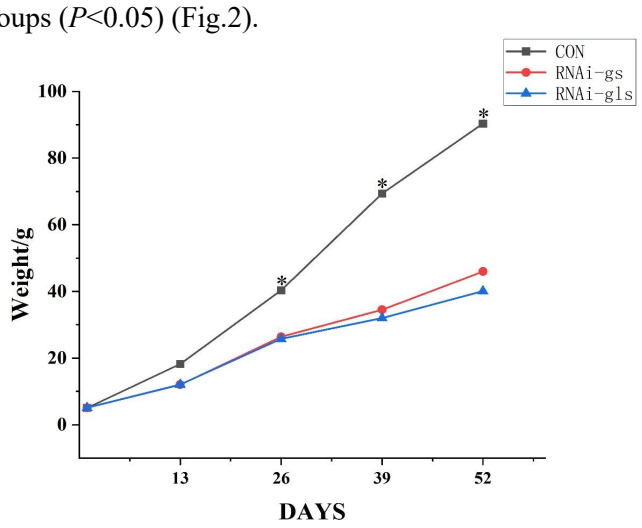


Figure .1. Growth trend of *T.blochii*.

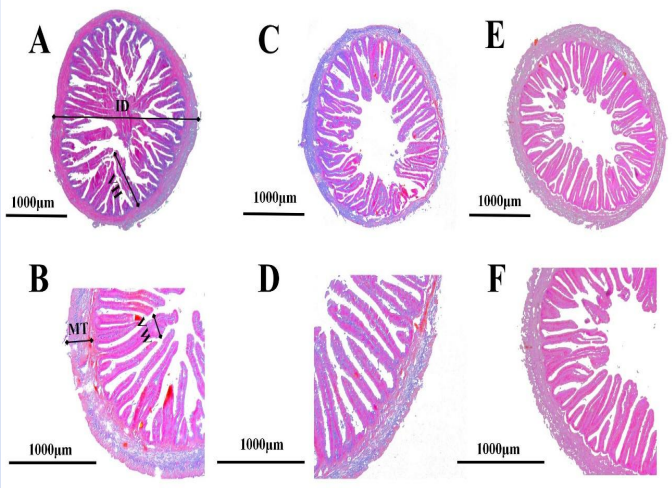


Figure .2. Morphological structure of the Intestinal sections of *T.blochii*. (A-B) Intestinal section of the control group. (C-D) Intestinal section of the RNAi-*gs* group. (D-E) Intestinal section of the RNAi-*gls* group. ID: Intestinal diameter, VH: Villus height, VW: Villus width, MT: Muscle layer thickness.

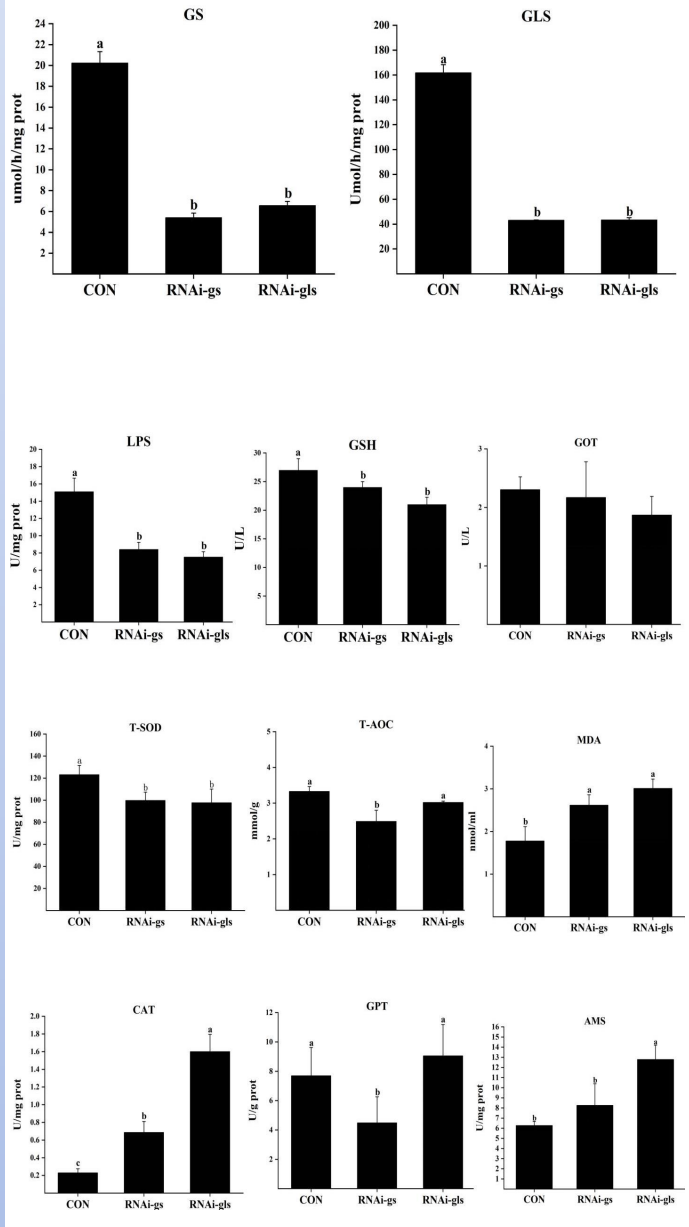


Fig .3. Enzyme activity indicators of *T.blochii*

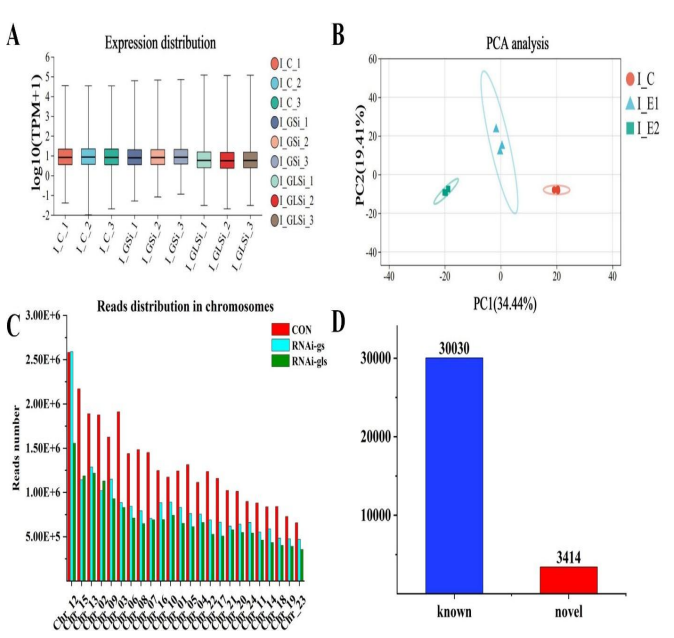


Fig .4. Characterization of intestinal mRNAs. (A) Box plots showing the levels of mRNA expression in different groups. (B) PCA results of the 9 datasets (n = 3). (C) Reads distribution in chromosomes. (D) Number of the known and novel mRNAs.

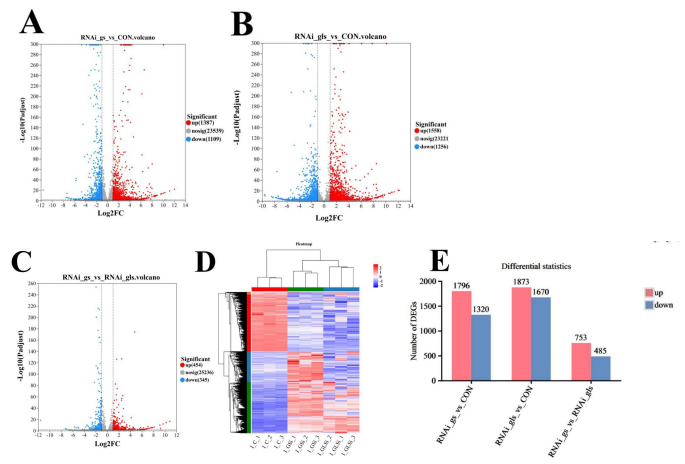


Fig .5. Differential expression analysis. (A-C) Volcano plot showing up-regulated and down-regulated DEGs in different comparison. (D)Upset plot of differentially expressed DEGs in different comparisons. (E) Statistical histogram of DEGs across three groups.

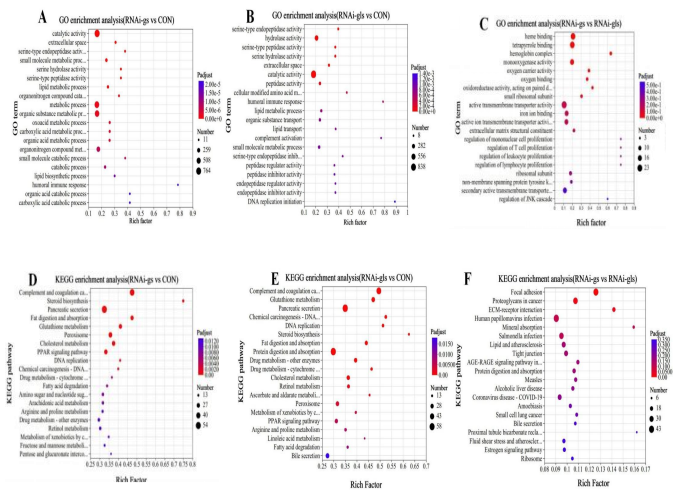


Fig .6. The DEGs GO and KEGG enrichment analysis in *T. blochii* transcriptome

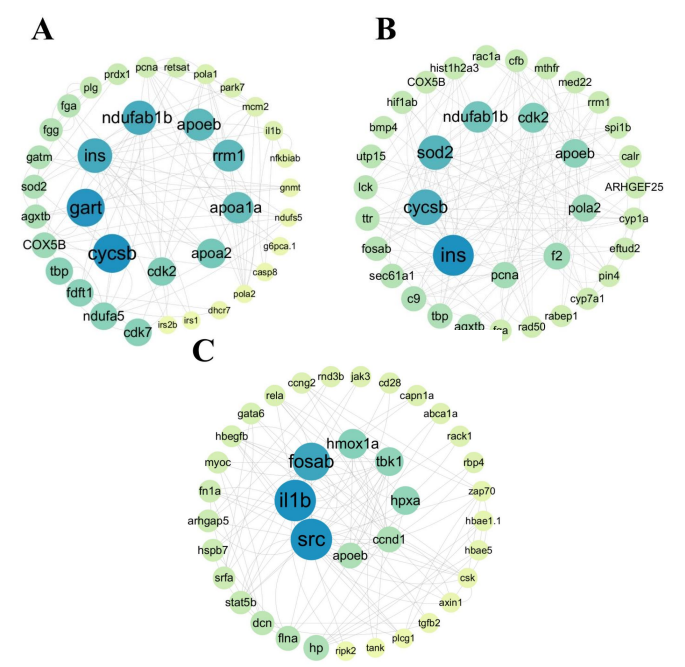


Fig .7. The DEGs network analysis in *T. blochii* transcriptome.

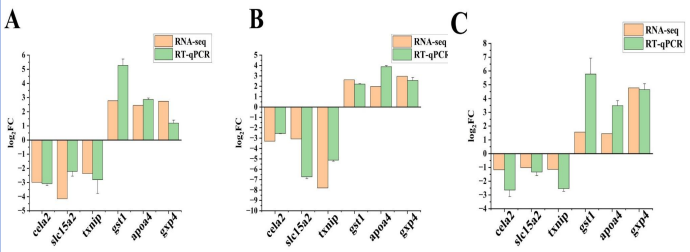


Fig .8. qRT-PCR analysis of genes for the validation of RNA-Seq data.

4. Conclusion

- A 52-day gene interference experiment involving *gs* and *gls* genes, caused intestinal structure damage, and decreased antioxidant capacity of the intestinal tissue.
- DEGs involved in serine metabolism pathways and lipid metabolism pathways were significantly down-regulated under interference.
- Knockdown of the *gs* and *gls* genes disrupts de novo purine synthesis pathways and tricarboxylic acid cycle, thereby inhibiting the growth of *T.blochii*.