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ABSTRACT Butyrate, a short-chain fatty acid produced by intestinal bacteria, serves as an energy source for intestinal cells and exhibits anti-inflammatory effects. Sodium butyrate enhances poultry production and modulates the immune response and gut microbiota, affecting the gut-brain axis. In this study, sodium butyrate was administered *in ovo* to Ross 308 broiler eggs to analyze the effect on gene expression in the brain. Dose-dependent decrease in the expression levels of various genes was demonstrated. The optimal dose of sodium butyrate 0.3% indicated significant changes in the gut microbiota, indirectly affecting gene expression in the brain.

Introduction

Butyrate, which belongs to the SCFAs, is produced by bacteria residing in the intestine. Intestinal cells can use **butyrate as an energy source**. Butyrate has **anti-inflammatory effects** mediated by signaling pathways, such as modulation of pro-inflammatory cytokines through inhibition of NF- κ B activation. Recent years of research indicate that sodium butyrate **improves poultry production and influences the immune response and microorganisms in the gastrointestinal tract**, especially in the intestinal.

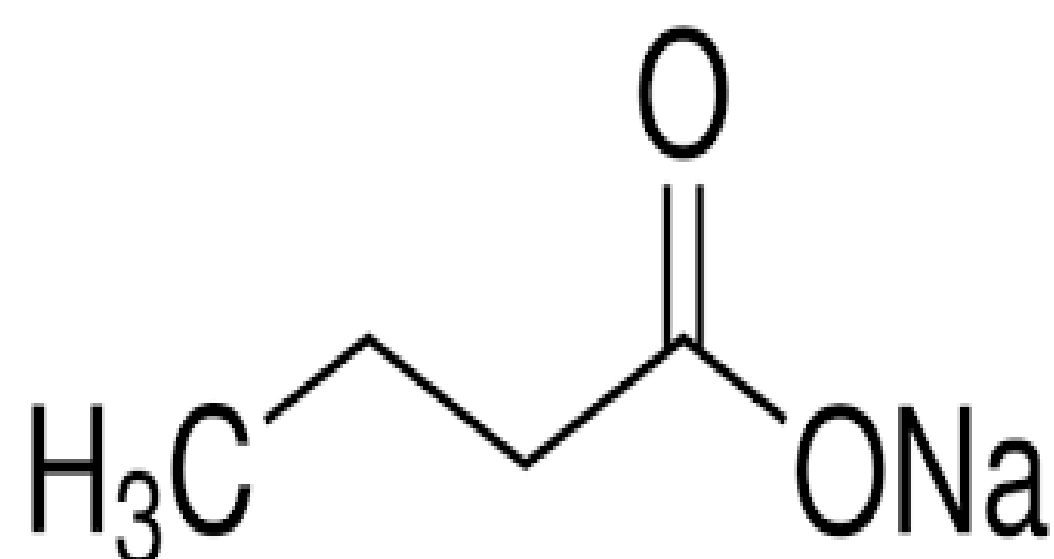


Fig. 1 Sodium butyrate chemical structure



Fig. 2 Ross 308 broiler chicken

Gut - brain axis

Changes in the gut microbiota profile can affect gut-brain response in poultry. The brain and intestines participate in bidirectional communication with the help of the endocrine and nervous systems. **The aim of the study was to analyze the effect of *in ovo* stimulation with sodium butyrate on gene expression level in the brain of broiler chickens.**

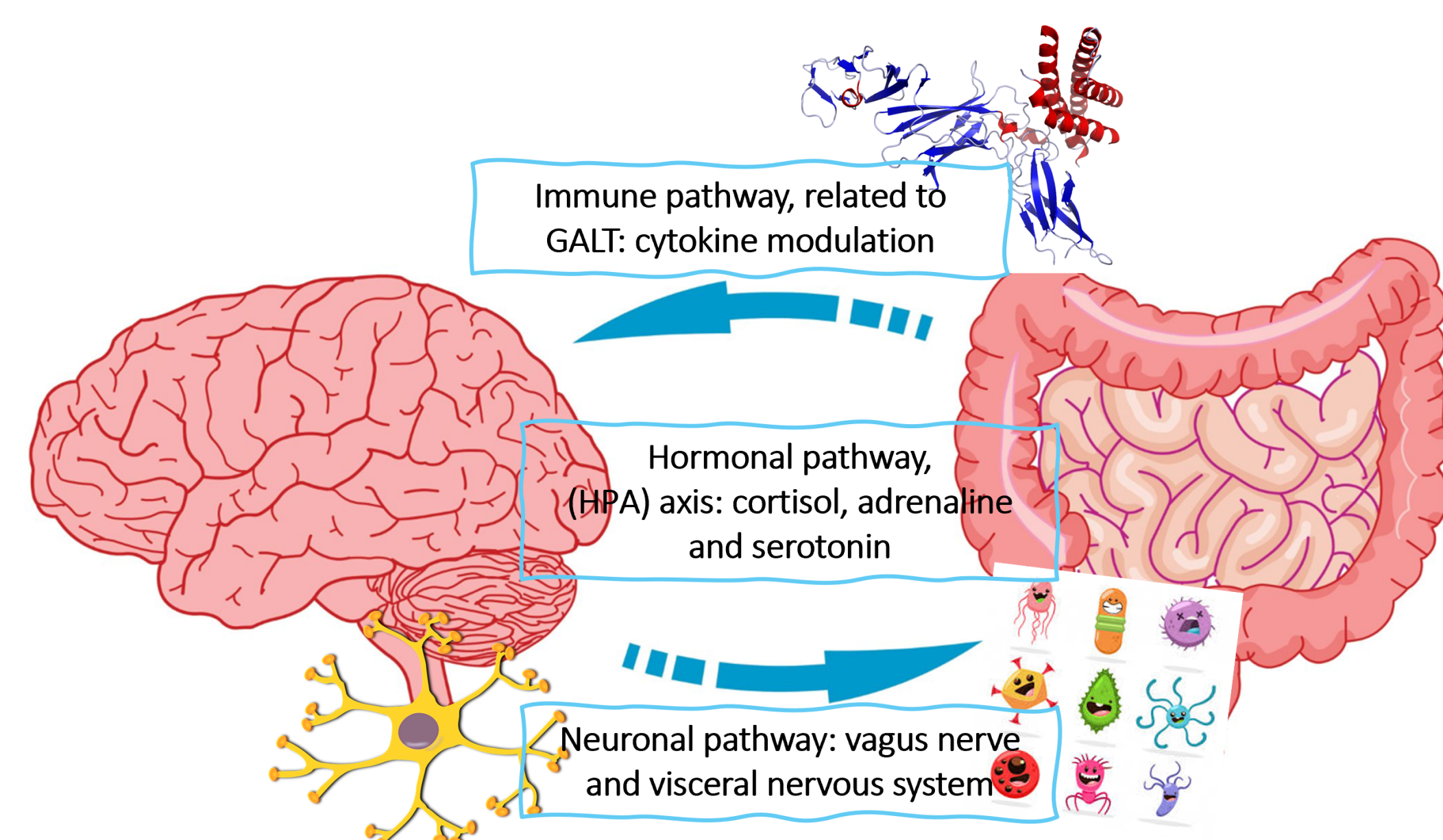


Fig. 3 Gut-brain axis

Materials and methods

1000 incubated eggs of **Ross 308 broiler chickens** on day 12 of incubation were injected with saline in the control group and with **postbiotic sodium butyrate (SB)** (three doses: 0.1%, 0.3%, 0.5%) in the experimental groups.

The brains were collected post-mortem on day 42 after hatching from 8 randomly selected individuals from each group for RNA extraction.

The panel of genes was selected based on the literature. It included: **immunity-related genes** (*IL-1 β* , *IL-2*, *IL-4*, *IL-6*, *IL-8*, *IFN γ* , *IFN β*), **neurotransmission-related genes** (*BDNF*, *GSK3 β*), **heat shock genes** (*HSPB1*, *HSPB5*, *HSPB8*, *HSPB9*, *HSP70*) and other mostly **metabolism-related genes** (*TLR4*, *GR*, *NR2A*, *CRH*, *NF- κ B p65*).

FINANCED

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Results

The results showed a decrease in expression for all genes, at different levels depending on the concentration of butyrate in the experimental groups. Gene expression was down-regulated in the dose with the highest concentration (0.5%). The 0.1% dose did not generate significant differences compared to the control. The most optimal effect was demonstrated by a dose of 0.3%, in which changes in the abundance of bacteria in the intestines contributed to changes in the gene expression level.

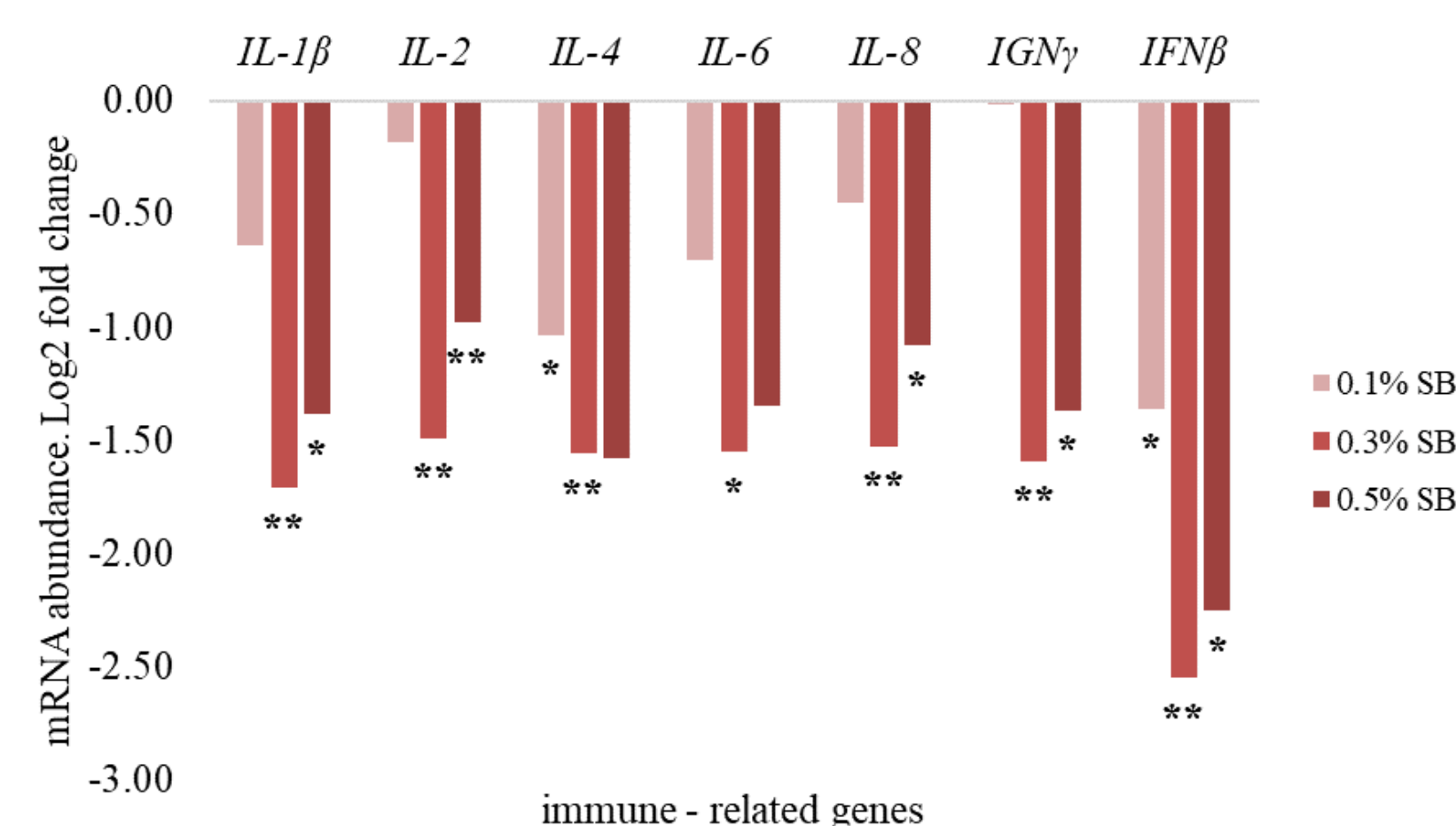


Fig. 4 Results of expression of immune-related genes in the brain

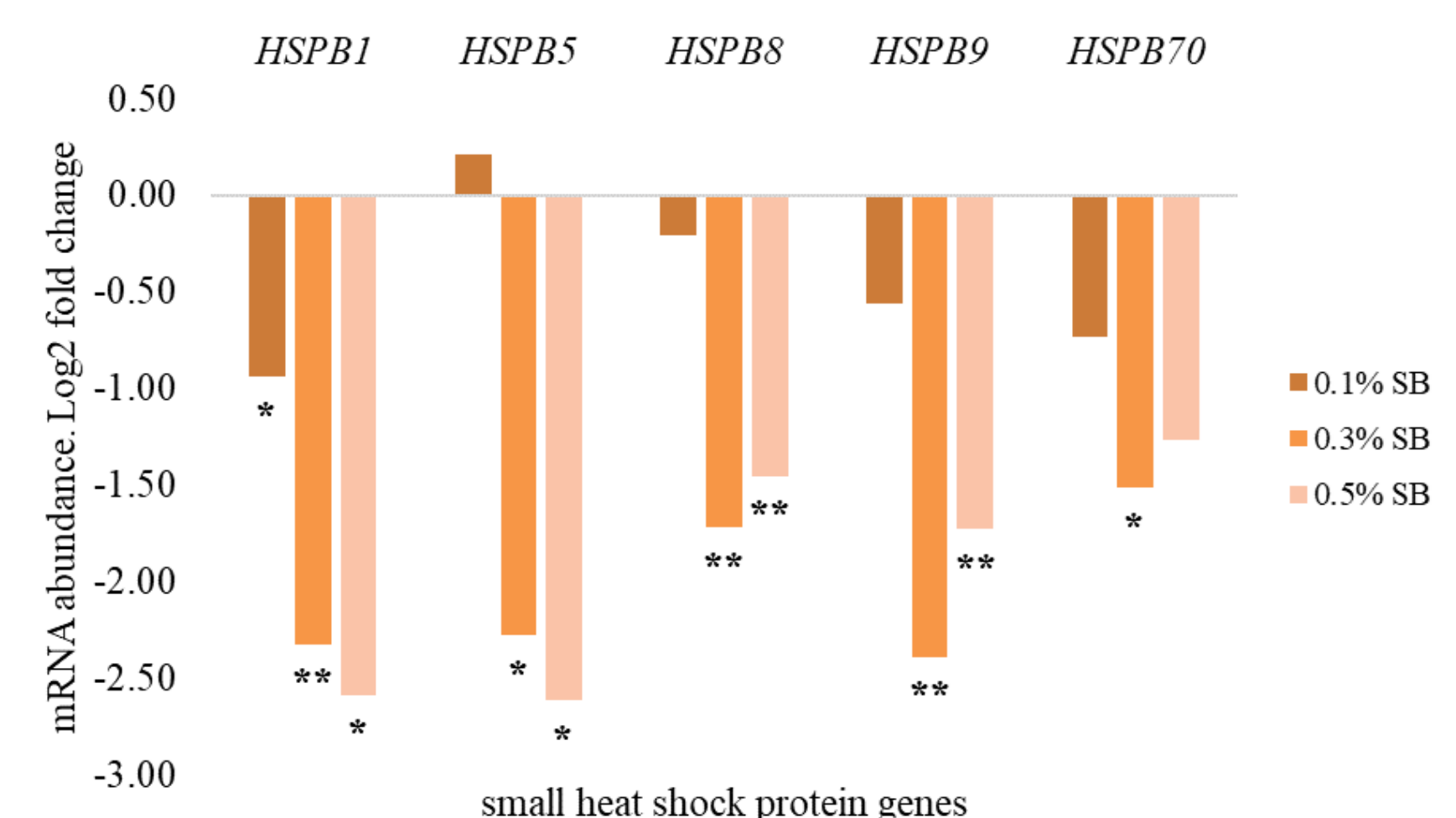


Fig. 5 Results of expression of heat shock protein genes in the brain

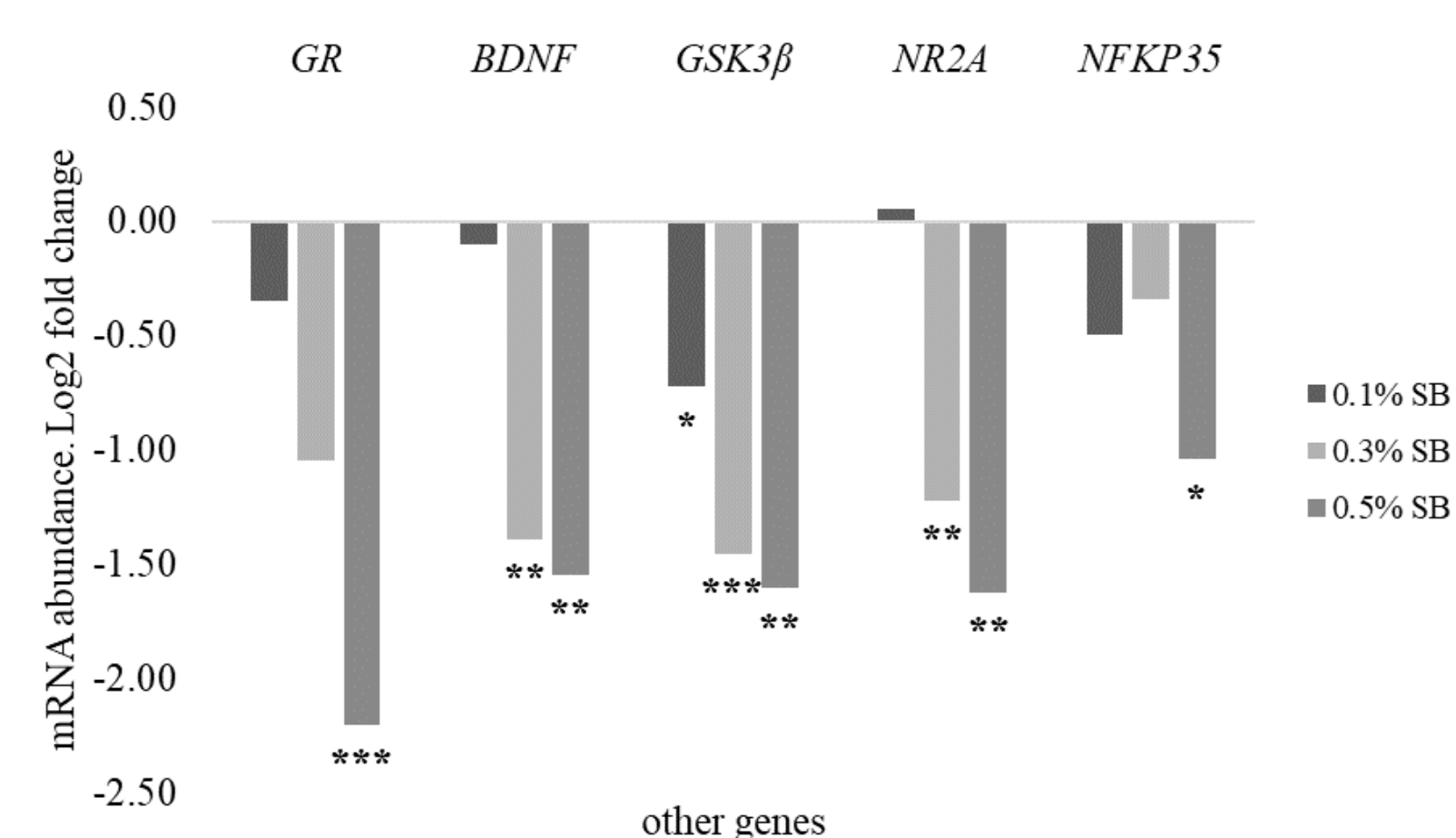


Fig. 6 Results of the gene expression in the brain

Conclusion and discussion

The above results can be identified as an effect of the gut-brain axis in which **sodium butyrate directly affected the gut and indirectly affected the brain**.

It can be assumed that as the amount of research increases to understand the functioning of this relationship, it will be possible to reduce or eliminate poultry diseases in the future.

Dose with butyrate concentration of **0.1%** shows little or no difference compared to the control, suggesting that the dose is too low.

The dose of **0.5%** causes a significant reduction in the expression of all genes, with may cause disruptions in the functioning of the host body.

The dose of **0.3%** showed the most optimal effect. The obtained results show differences related to the dose of the administered substance.

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