Changes in gene expression and DNA methylation in the liver of broiler chickens after in ovo stimulation with sodium butyrate

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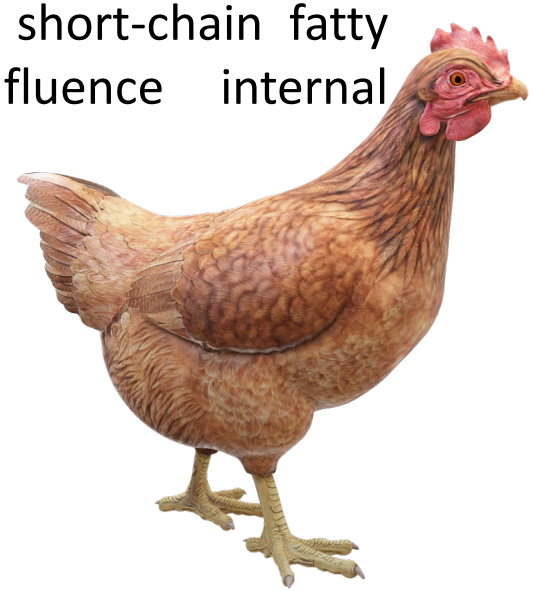
In ovo stimulation

- ***In ovo*** is an effective method of early stimulation of the host's microbiota, before direct contact of the newly hatched chicken with microorganisms from the external environment.

- Changes in **microbiota** communication can lead to modification of **gene expression** driven by epigenetic mechanisms.

- The **gut microbiota produces** bioactive peptides, short-chain fatty acids, hormones and amino acids that influence internal communication with liver.



Sodium butyrate

- lower intestinal pH,
- control intestinal colonization and growth of harmful microorganisms,
- stimulate intestinal villi growth,
- regulate gene expression,
- increase SCFA production.

Parameter	Sodium butyrate (%)			
	0	0.1	0.3	0.5
Cecum				
pH	7.48	7.32	7.14	7.40
Aacetic acid	45.95	58.22	44.71	47.35
Propionic acid	11.99^{a}	19.00^{b}	18.59^{b}	12.71^{a}
Isobutyric acid	1.04	0.98	1.00	1.33
Butyric acid	10.58^{ab}	12.51^{b}	8.92^{a}	7.51^{a}
Isovaleric acid	0.92	0.86	1.08	1.42
Valeric acid	0.91	1.21	1.18	0.97
Total SCFA	71.38	92.78	75.48	71.30

Figure 2. Intestinal digesta pH and SCFA concentrations in cecal.

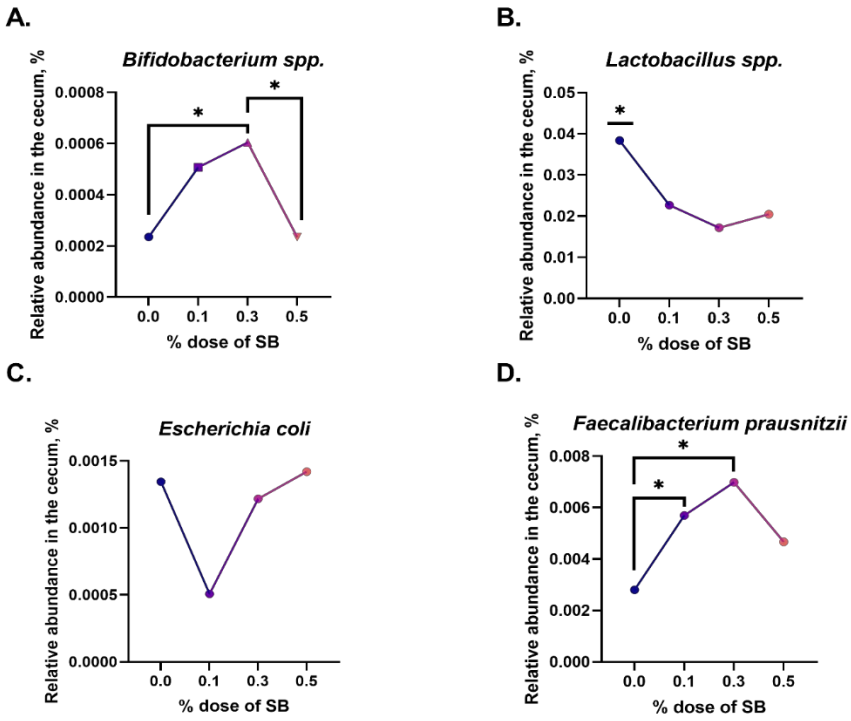
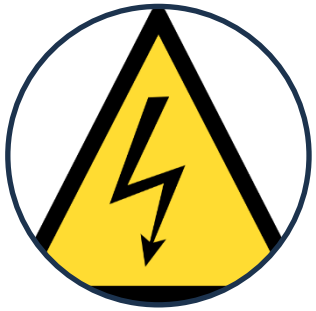


Figure 1. The relative abundance of bacteria in cecal.

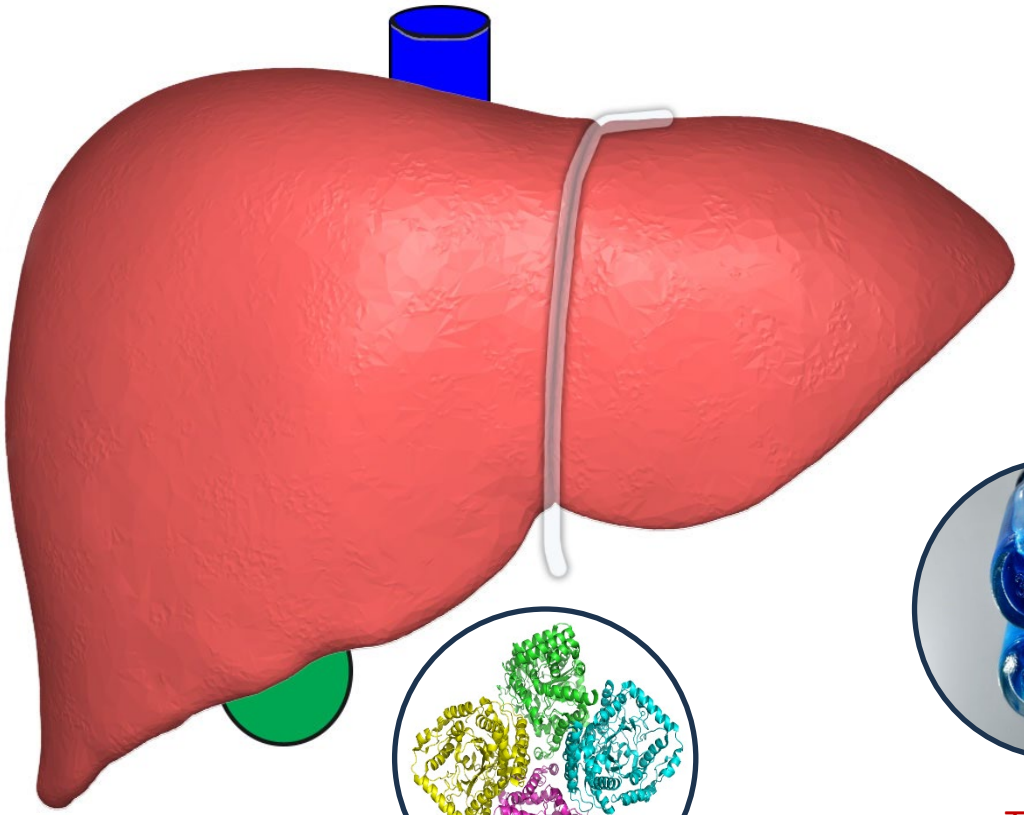
All results are from publication „The effect of sodium butyrate administered in ovo on the health status and intestinal response in broiler chicken” - https://doi.org/10.1016/j.psj.2024.104108

Liver functions



ENERGY PRODUCTION

BILE ACID PRODUCTION



METABOLIC PROCESSES CONTROL

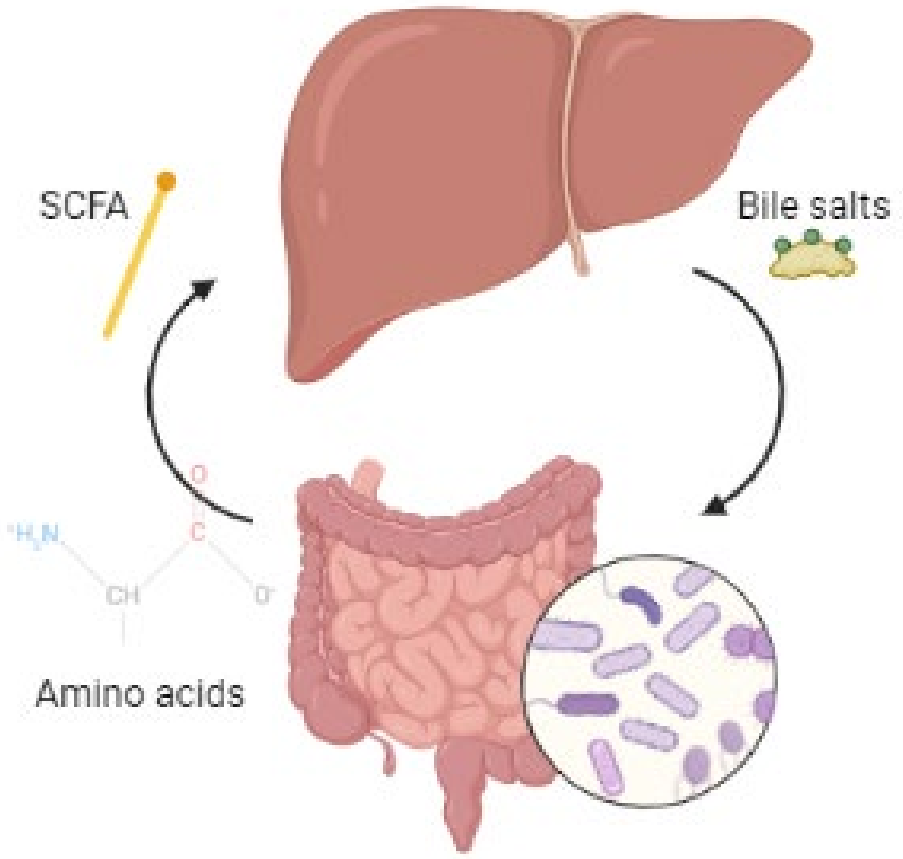
ANTI-INFLAMMATORY STIMULATION

TOXINS DEACTIVATION

Picture 1. Liver functions scheme.

Gut – liver axis

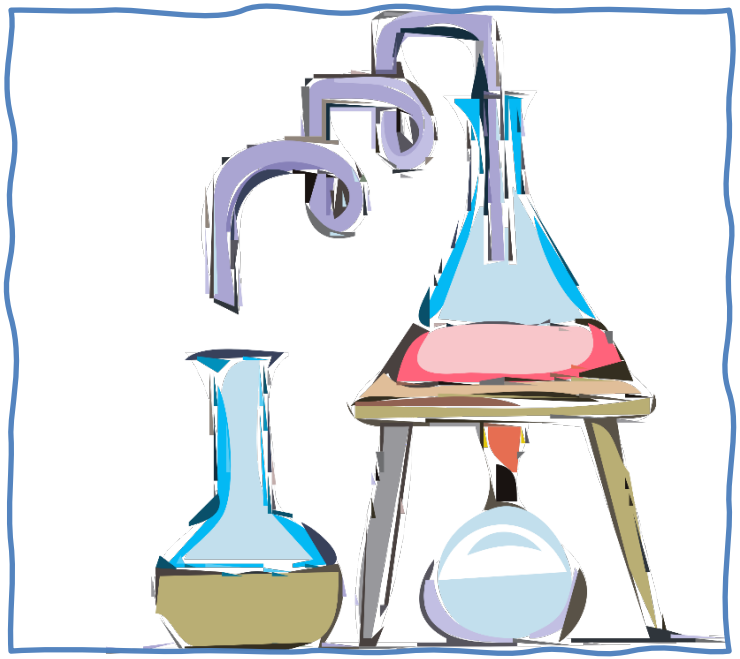
- Two-way relationship,
- communicate through the portal vein,
- intestines produce metabolism,
- liver produce and transports bile salts.



Picture 2. Gut-liver axis scheme.

Aim of the study

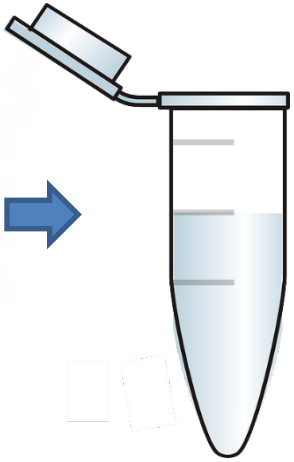
The aim of the study was to analyze the gene expression and methylation in the liver after *in ovo* stimulation by sodium butyrate on day 12 of egg incubation in broiler chicken.

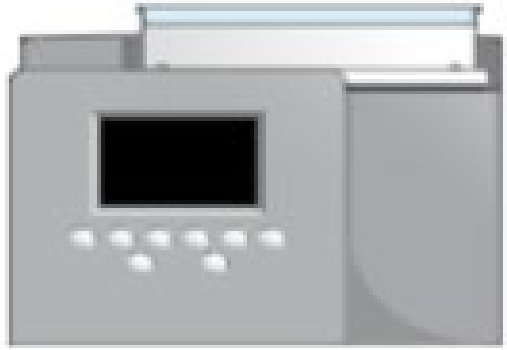


Materials and methods

Gene panel comes from a publication published in the journal Genes. Gene panel was developed based on the results from microarrays in which bioactive substances were used in the experiment.

in ovo stimulation on the day 12 of incubation





ROSS 308 eggs stimulated by
a) Postbiotic, sodium butyrate in three different doses 0.1%, 0.3%, 0.5%

chicken sacrificed on the day 42 post-hatching

Liver tissue
- RNA isolation for gene expression,
- DNA isolation for methylation analysis

analysis by qPCR (LightCycler instrument)

Picture 3. Materials and methods scheme used in experiments.

Results - angiopoietin like 4

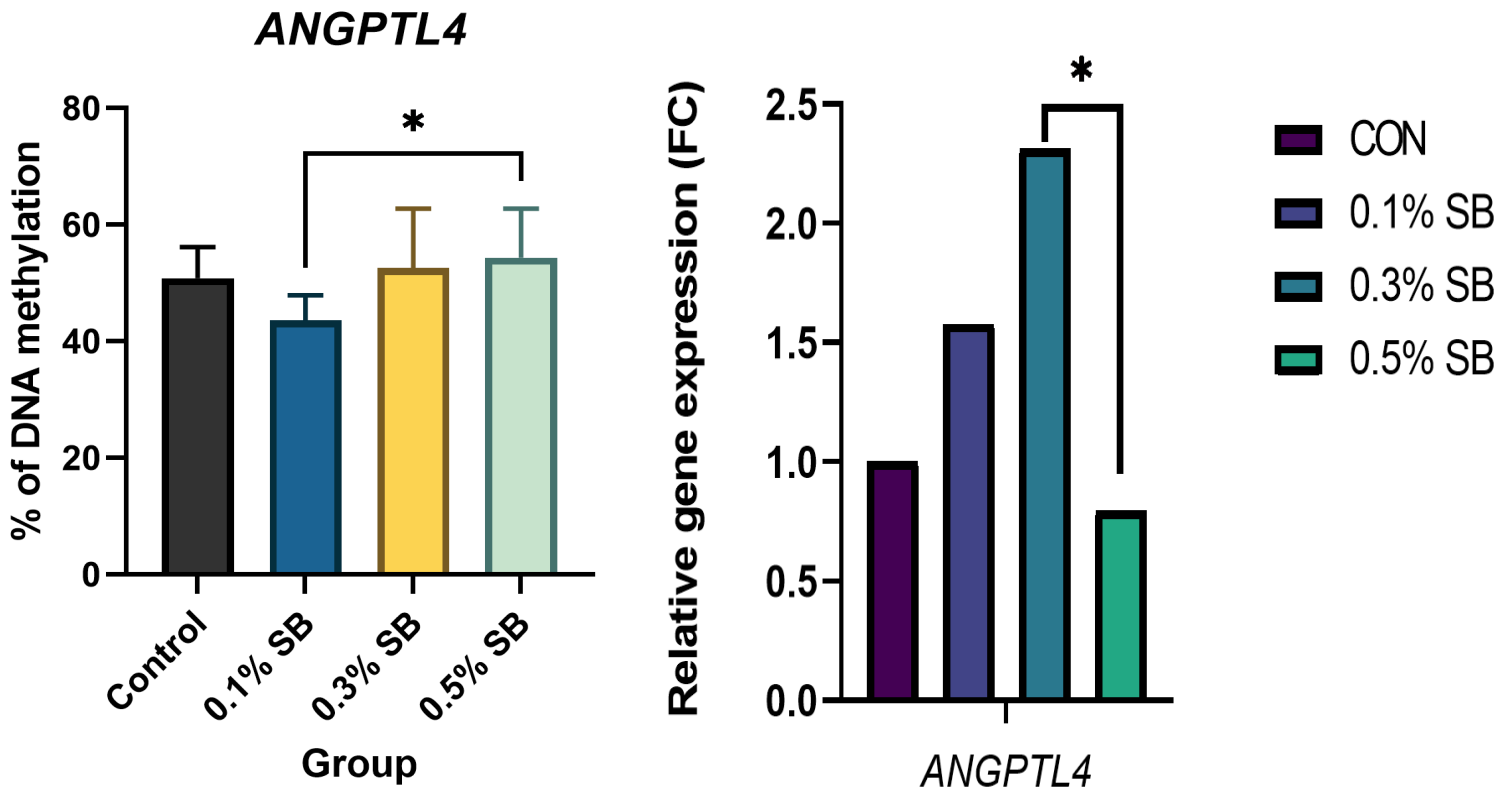


Figure 3. The relative gene expression was calculated by the $^{\Delta\Delta}C_T$ algorithm and the amount of the target gene was calculated by the $2^{-\Delta\Delta CT}$ formula. Asterisks indicate means that differ significantly from the control group at $P \leq 0.05$ (*). SB: 0.1%, 0.3%, 0.5% means different doses of sodium butyrate.

The relative DNA methylation level [%] was calculated from the melting curve results for each sample using the formula *% of methylation = 100 x (M/(M+U)).* Asterisks indicate means that differ significantly from the control group at $P \leq 0.05$ (*). SB: 0.1%, 0.3%, 0.5% means different doses of sodium butyrate.

Results - cysteine rich angiogenic inducer 61

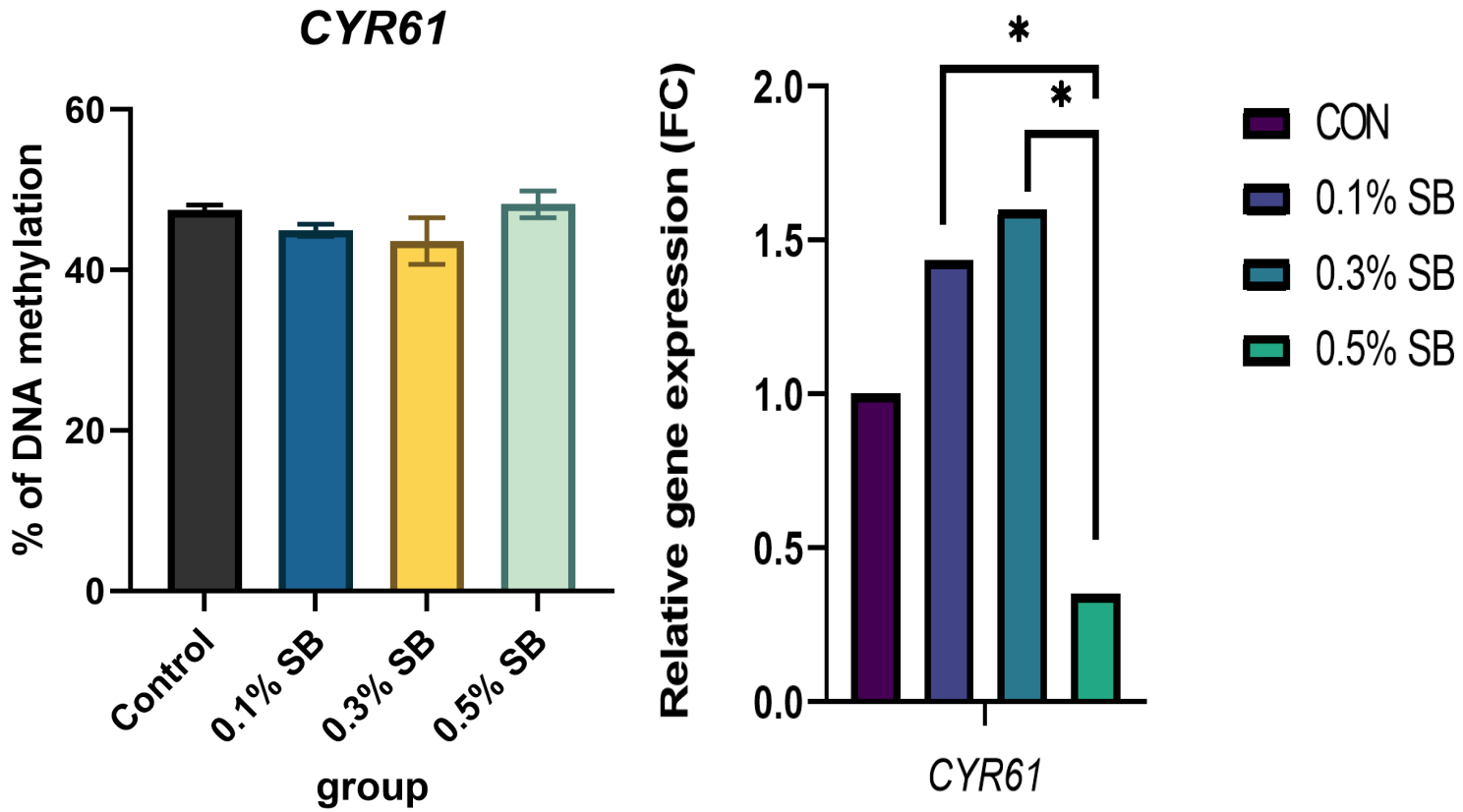


Figure 4. The relative gene expression was calculated by the $^{\Delta\Delta}C_T$ algorithm and the amount of the target gene was calculated by the $2^{-\Delta\Delta CT}$ formula. Asterisks indicate means that differ significantly from the control group at $P \leq 0.05$ (*). SB: 0.1%, 0.3%, 0.5% means different doses of sodium butyrate.

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Results - kelch like family member 6

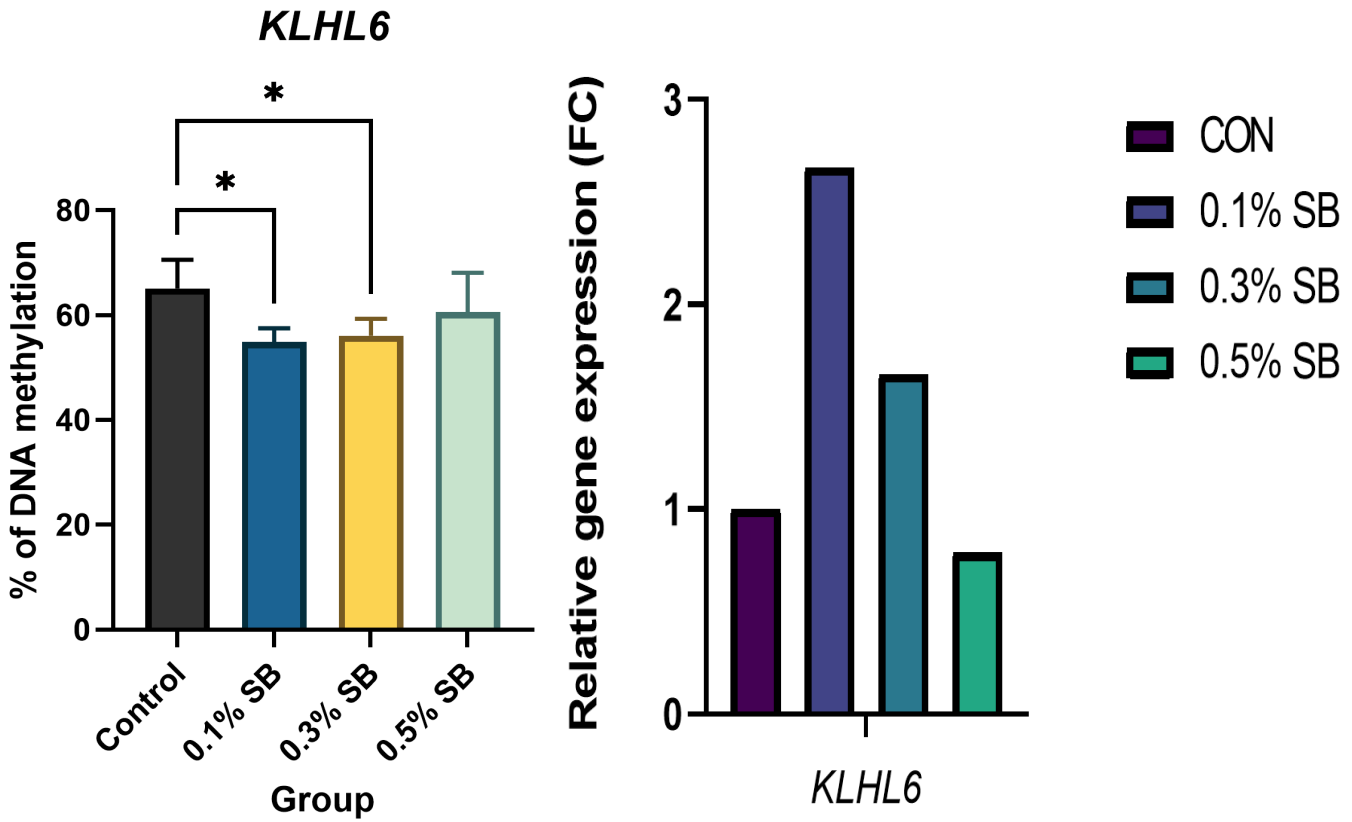

Figure 5. The relative gene expression was calculated by the $^{\Delta\Delta}C_T$ algorithm and the amount of the target gene was calculated by the $2^{-\Delta\Delta CT}$ formula. Asterisks indicate means that differ significantly from the control group at $P \leq 0.05$ (*). SB: 0.1%, 0.3%, 0.5% means different doses of sodium butyrate.

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Results – nuclear receptor

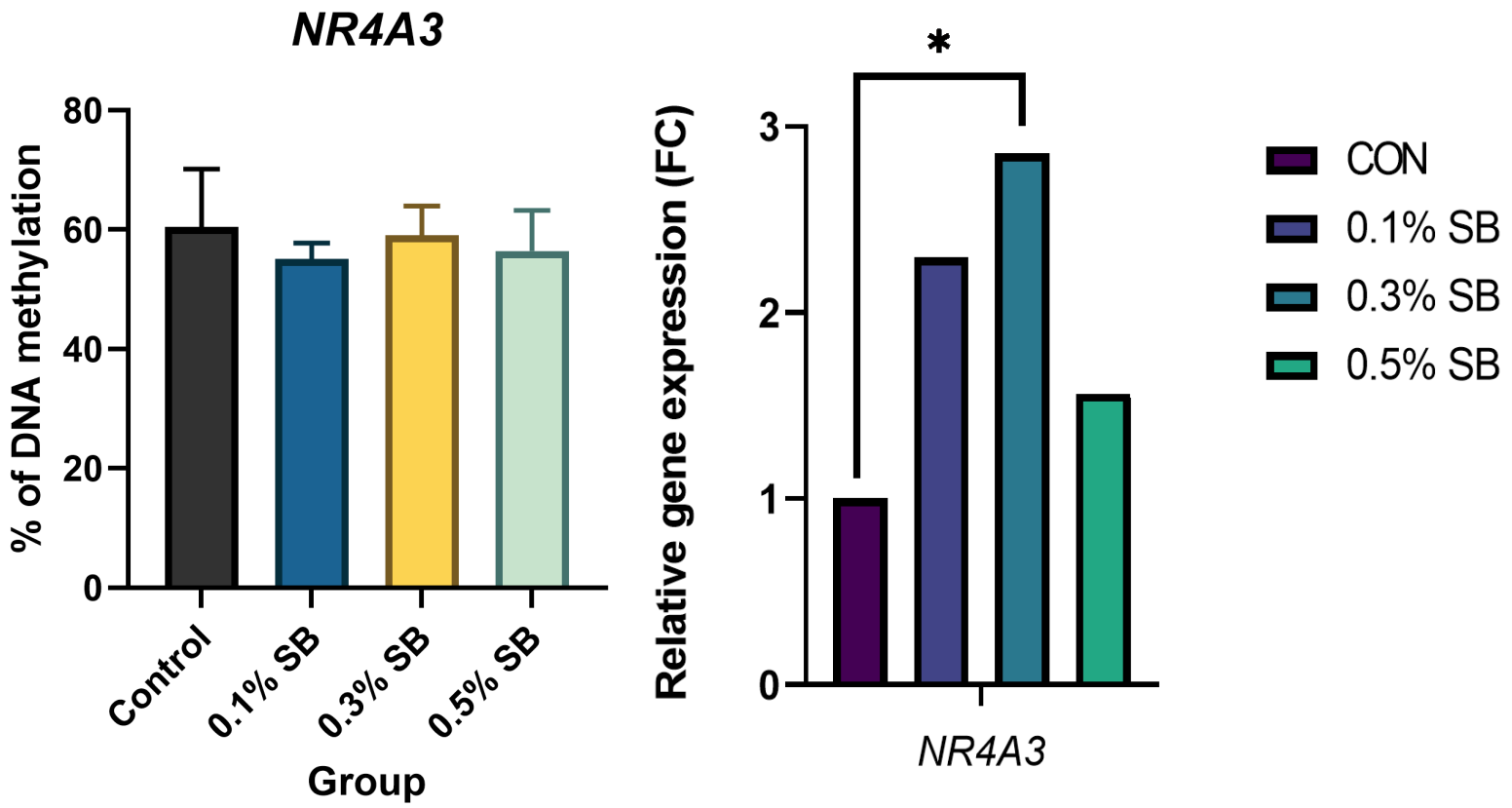

Figure 6. The relative gene expression was calculated by the $^{\Delta\Delta}C_T$ algorithm and the amount of the target gene was calculated by the $2^{-\Delta\Delta CT}$ formula. Asterisks indicate means that differ significantly from the control group at $P \leq 0.05$ (*). SB: 0.1%, 0.3%, 0.5% means different doses of sodium butyrate.

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Results – spleen-associated tyrosine

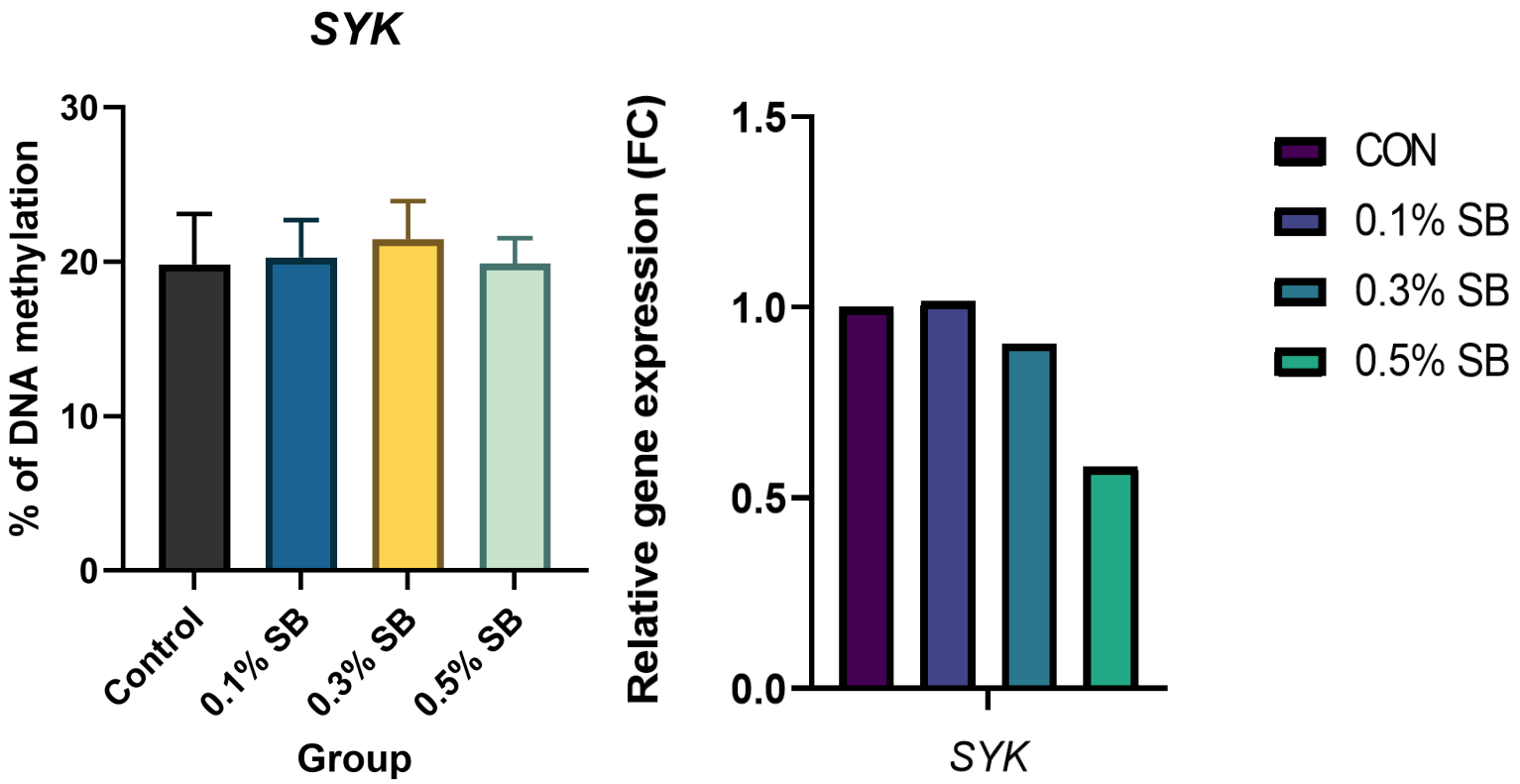

Figure 7. The relative gene expression was calculated by the $^{\Delta\Delta}C_T$ algorithm and the amount of the target gene was calculated by the $2^{-\Delta\Delta CT}$ formula. Asterisks indicate means that differ significantly from the control group at $P \leq 0.05$ (*). SB: 0.1%, 0.3%, 0.5% means different doses of sodium butyrate.

The relative DNA methylation level [%] was calculated from the melting curve results for each sample using the formula *% of methylation = 100 x (M/(M+U)).* Asterisks indicate means that differ significantly from the control group at $P \leq 0.05$ (*). SB: 0.1%, 0.3%, 0.5% means different doses of sodium butyrate.

Conclusions

- Sodium butyrate affects changes in the level of gene expression and methylation in the liver.
- In the case of DNA methylation, its level is usually correlated with the level of gene expression.
- RNAseq analysis is planned as a further research direction in order to develop an optimal gene panel.
- The liver will be subjected to global methylation analysis.

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