Inflammation and the microbiome

Flavobiotics change the microbiome, regenerate the intestinal barrier and activate the immune system

CLINICAL STUDY

- 12 weeks
- parallel
- randomized
- double-blinded
- placebo-controlled
- on a set of 50 patients

Flavobiotic: hesperidin+naringin

The flavobiotic in this study is from citrus fruits - orange and grapefruit. It contains specifically active flavonoids and through them directly uses the potential of the intestinal microbiome and **improves the function of the intestinal barrier**. It is specially designed to positively support **the activity of the immune system.**

In 2019, a clinical study was conducted with a daily dose of 500 mg of Mikrobiom+® to evaluate the benefit for the composition of the intestinal microbiome. The results showed a significant change in the composition of short-chain fatty acids (SCFA). This is a direct effect of the change in the composition of the intestinal microbiome. In addition, a strong trend towards a decrease in fecal calprotectin was demonstrated, demonstrating immunomodulation. Both results strengthen the hypothesis that citrus polyphenolic compounds are able to modulate the composition and function of the gut microbiota, thereby promoting gut and host health through their anti-inflammatory effects.

Perbiotix
microbiome solutions

Perbiotix, s.r.o. | Litovelská 670/27
02401 Kysucké Nové Mesto | www.perbiotix.sk

Introduction

Developed countries are successfully fighting infectious diseases, but the prevalence of metabolic, cardiovascular and autoimmune diseases is increasing (1).

The increase in inflammatory and autoimmune diseases is related to the changed "Western" diet, which is an important factor influencing the intestinal ecosystem, which can be very important in the development of human diseases. Recent data suggest that various polyphenolic compounds, such as those derived from citrus fruits, are able to modulate the structure and function of the gut microbiome, thereby beneficially affecting gut health (2).

Flavobiotic is extracted from citrus fruits with excellent stability and a high concentration of two basic flavonoids: **hesperidin** and **naringin**. The benefits of Flavobiotic were evaluated in a clinical study that demonstrated the double action of the component:

1. Active flavonoids act directly in the intestinal lumen by **protecting and significantly improving the function of the intestinal barrier**.

2. They activate the immune system and reduce intestinal inflammation with a positive change in the composition of the microbiome: as a result, **the level of butyrate increased** (P = 0.031) and **the level of calprotectin decreased**, indicating a lower pathogen load.

Goals

The primary objective of the clinical study was to investigate the effect of 12 weeks of Flavobiotic supplementation on the intestinal ecosystem and on calprotectin levels in overweight individuals with an unbalanced lipid profile. Since the participants were overweight, they likely exhibited dysbiosis at the microbiome level and low chronic inflammation in the gut.

Methodology

A 12-week randomized, parallel, double-blind, placebo-controlled study was designed to investigate the effects of a daily dose of 500 mg of Flavobiotic on gut microbiome composition and calprotectin levels. The study was carried out on a group of 50 subjects with excess weight and thus with an altered microbiota (Table 1). Each subject completed three testing days. After an overnight fast, a stool sample was collected from the subjects on the first and last test days. Blood pressure was measured on all three test days.

	Total file (n = 50)	Placebo (n = 27)	Flavobiotic (n = 23)
Age	51 ± 13	50 ± 13	52 ± 11
Sex	18/32	9/14	9/18
WHR index	0.93 ± 0.07	0.91 ± 0.07	0.94 ± 0.07
BMI, kg/m2	30.8 ± 3.8	31.4 ± 4.2	30.0 ± 3.2

Table 1. Basic characteristics of study participants

Results

1) Mastné kyseliny s krátkym reťazcom

Po 12 týždňoch denného príjmu Flavobiotika sa podiel butyrátu na celkovom SCFA významne zvýšil (*P* = 0,031) (*obrázok 1*).1) Short chain fatty acids

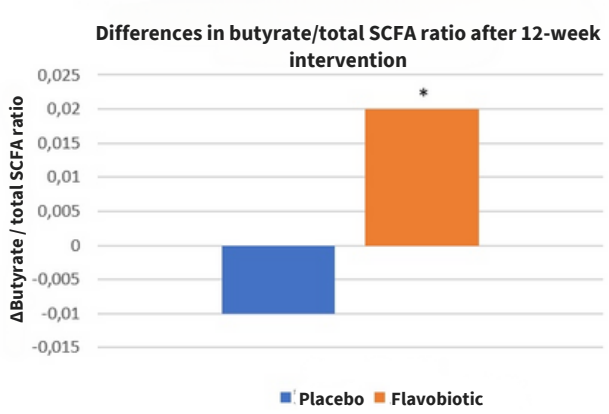

Figure 1. Difference in Butyrate / Short Chain Fatty Acid Ratio

In addition, the ratio of butyrate to acetate was significantly increased in the Flavobiotic group compared with the placebo group (P = 0.020).

2) Changes in the microbiome

After a 12-week intervention between the Flavobiotic and placebo groups, an increase in butyrate-producing ***Clostridium cluster XIVa*** was observed with a change from baseline of 43.9% in the intervention compared to 15.9% in the placebo group. (Figure 2).

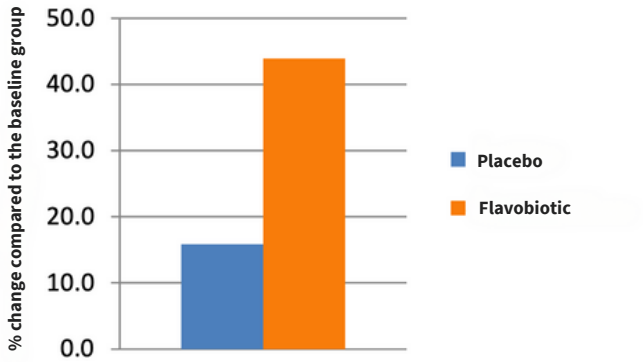

Figure 2. Relative differences in Clostridium cluster XIVa abundance (% change from baseline) between placebo and Flavobiotic at 12 weeks.

3) Calprotectin levels

After 12 weeks of daily Flavobiotic supplementation, a strong reduction in fecal calprotectin was shown compared to the placebo group (P = 0.058) (Figure 3). This reduction reflects a reduction in the pathogen load in the gut.

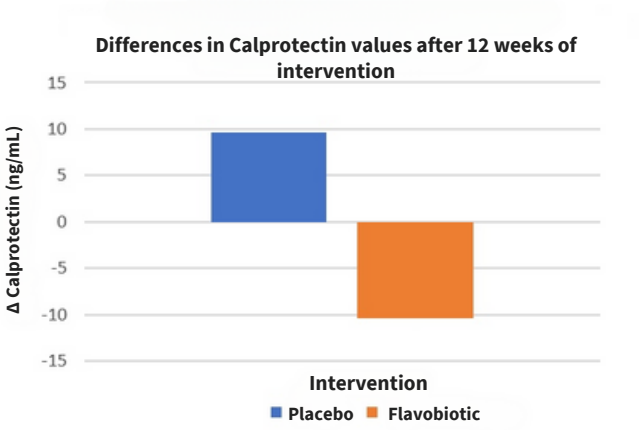

Figure 3. Differences in calprotectin values

IN-VITRO STUDIES

Goals

The aim of the in vitro study was to observe the immediate effect of Flavobiotic at lower doses in the TIM-2 model. The TIM-2 model is a well-validated in-vitro colon model.

In this controlled experiment, two different doses (250 mg and 350 mg) of Flavobiotics were administered over 72 hours to a TIM-2 intestinal model containing a representative sample of human microbiota from 6 healthy and elderly volunteers.

Results

1) Effect on fatty acid. with a short chain

After daily intake of Flavobiotic for 72 hours, an increase in SCFA was observed over time. The 350 mg dose produced the greatest increase (Figure 4).

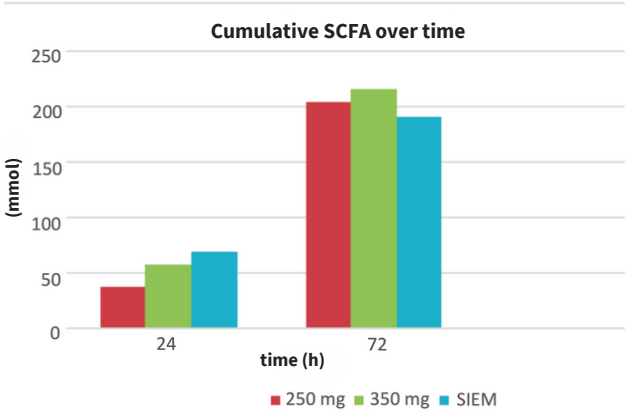

Figure 4. Effect on total short chain acids - SFCA

2) Difference in butyrate, acetate and propionate

After 12 weeks of daily intake of Flavobiotic, an increase in butyrate, acetate and propionate was observed over time, with acetate showing the greatest quantitative increase compared to the control. **Butyrate** and **propionate** regulate the immune system and are also important satiety modulators. **Acetate** plays an important role in appetite suppression by stimulating the release of the satiety hormones PYY and GLP-1.

3) Effect on the composition of the microbiota

Roseburia spp. is a member of the Clostridium XIVa cluster and is a butyrate-producing bacterial strain that is associated with weight reduction and reduced insulin resistance (prediabetes). After daily intake of Flavobiotic for 12 weeks, a significant increase in ***Roseburia spp.*** in the microbiome (Figure 5).

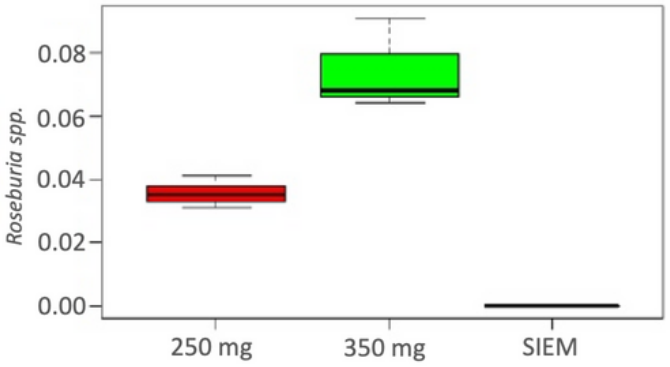

Figure 5. Effect on Roseburia spp.

B. eggerthii is a species within the bacteroidetes phylum, specifically the bacteroides fragilis phylum, which plays a role in acetate production and prevention of intestinal inflammation. As shown in the figure below, there was a significant increase in ***B. eggerthii*** in the microbiome after daily intake of Flavobiotic (Figure 6).

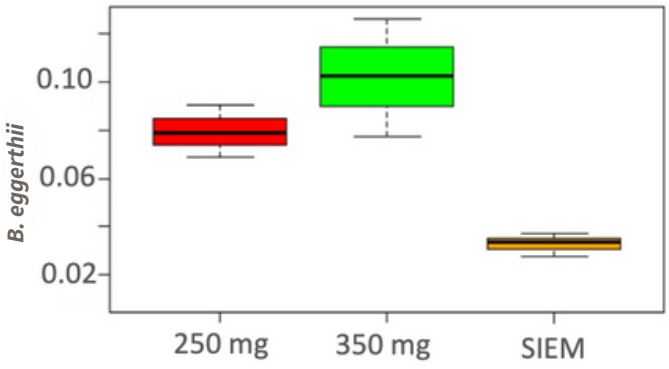

Figure 6. Effect on B. eggerthii

4) Changing the microbiome to a similar as we observe with a plant-based diet

At the phyla level, the Flavobiotic intervention resulted in a relative increase in Bacteroidetes and a relative decrease in Firmicutes. Such a shift in the Bacteroidetes / Firmicutes ratio is often associated with a plant-based diet that is typically high in fiber and low in fat. Similarly, the increase of ***Roseburia spp.*** reverses the trend that we usually observe with animal food.

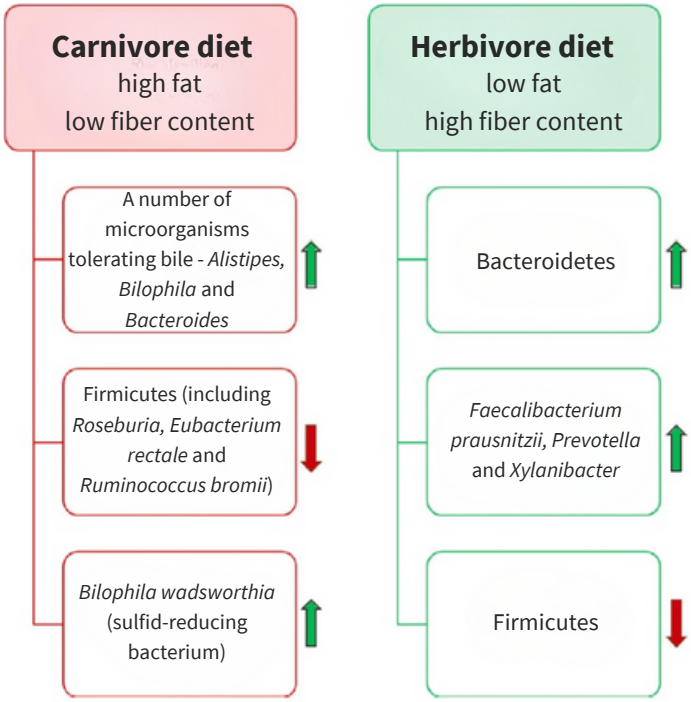

Figure 7. Typical increases and decreases in microbiota with animal and plant-based diets. From: Kumar et al, Nutrition and Healthy Aging (2016)

EFFECT ON THE IMMUNE SYSTEM

More than 80% of immune cells reside in the large intestine and it is the single largest organ with a direct function in our immune system.
The flavobiotic exerts its positive effects precisely in this important part of our immune system in several ways:

1) Mediates the change of microbiota

In vitro studies show that Flavobiotic can significantly modulate the intestinal microbiota.
It affects the growth of specific bacterial groups, especially ***Clostridium cluster XIVa***, capable of providing health benefits through SCFA production (e.g. butyrate, acetate, propionate).

2) Improves the functions of the intestinal barrier

In vitro experiments have shown that butyrate reduces permeability and improves intestinal barrier function (measured as transepithelial resistance). Specifically, butyrate mediates the formation of tight junction proteins, such as claudins and occludins, through AMPK.
Formation of tight junction proteins is a dynamic process that is critical in establishing and maintaining intestinal barrier function.

3) Reduces intestinal inflammation

As shown in the first clinical study, calprotectin – a clinical marker of intestinal inflammation – was reduced as a result of Flavobiotic consumption.
In collaboration with Maastricht University, a mechanistic study was performed on an innovative 2-dimensional in-vitro model of Caco2 cell lines combined with LPS-stimulated macrophages (Figure 8).

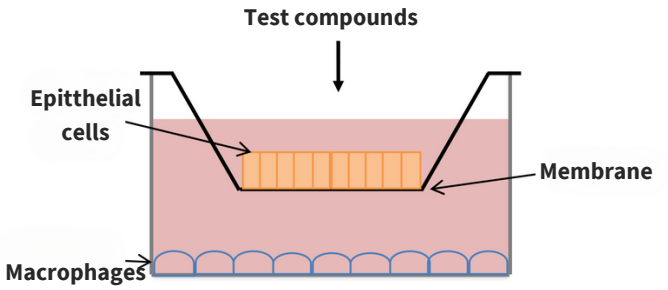

Figure 8. Co-culture of Caco2 and macrophages

The purpose of the study was to determine whether specific flavonoid metabolites in Flavobiotic have an effect on mediators of inflammatory reactions, such as **TNF-α**. **Hesperetin and Naringenin**, both direct metabolites of Flavobiotic, have a direct and significant effect on the release of TNF-α. By reducing the release of TNF-α, low-grade chronic inflammation in the gut can be counteracted (Figure 9 and 10).

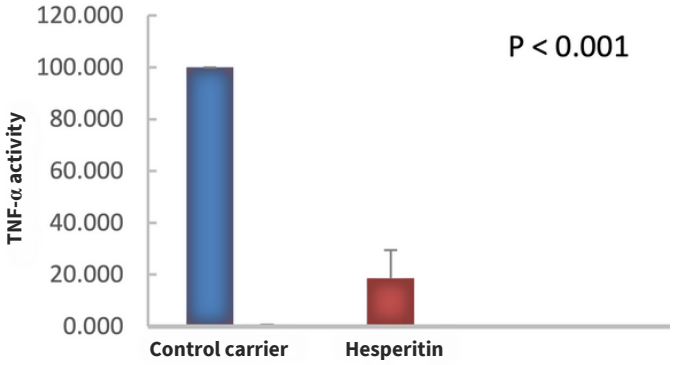

Figure 9. Effect of hesperetin on TNF-α activity

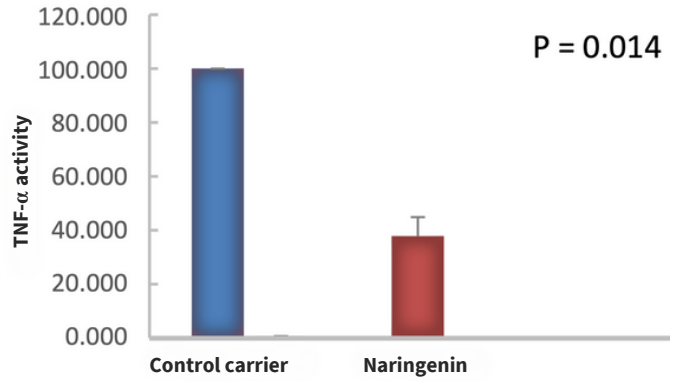

Figure 10. Effect of naringenin on TNF-α activity.

Conclusion

The flavobiotic supports gut and host health with its anti-inflammatory effects due to its ability to modulate the composition of the gut microbiome. A significant shift from acetate to the health-promoting butyrate and a significant increase in the share of butyrate in the total SCFA are the result of a changed composition of the microbiome.
Butyrate positively affects the function of the intestinal barrier and reduces intestinal inflammation, which is reflected in lower levels of calprotectin. Therefore, Flavobiotic is a first-class agent for strengthening the intestinal barrier and increasing immunity.

References

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Flavobiotic

- restores and significantly improves **intestinal barrier** function
- **develops the microbiome** with probiotic bacteria that cannot be produced
- activates and regulates **immunity**
- stronger **antioxidant** than vitamin C
- stops **inflammatory processes**