BIOLEX® MB40

BIOLEX MB 40®– POSITIVE EFFECT ON MICROBIAL COMPOSITION AND ACTIVITY IN THE CANINE GASTROINTESTINAL TRACT

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Five bacterial strains constitute 99% of the canine intestinal flora: Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria and Fusobacteria. Clostridia, Lactic Acid bacteria and Proteobacteria predominate in the small intestine, while Clostridiales, Bacteroidetes, Prevotella and Fusobacteria predominate in the large intestine. Today, a microbial imbalance (dysbiosis) is associated with a variety of diseases such as rheumatism, obesity, diabetes or various allergies (SUCHODOLSKI, 2018). Overweight dogs (KIELER et al., 2017) seem to have a different microbial composition than normal-weight dogs. The microbiome can even have a negative effect on the ability to reduce weight. The daily diet of the dog thus has a very large influence on the health and well-being of the animal.

Studies show that naturally fed dogs often have higher levels of potentially pathogenic microbes than commercially fed dogs. Mannan oligosaccharides (MOS) are known for their binding capacity to type 1 fimbria of the intestinal mucosa and the reduction of pathogens in the gastrointestinal tract. Thus STRICKLING et al. (2000) discovered a reduction of Clostridium perfringens in the faeces of MOS fed dogs. MIDDELBOS et al. (2007) found a numerical reduction of E. coli in the faeces of adult dogs and both BROWN & GORDON (2005) and EL KHOURY et al. (2012) found a positive effect on lactobacillus and on bifid bacteria, by MOS administration respectively. SUCHODOLSKI (2018) reports changes in the microbial community associated with inflammatory gastrointestinal diseases such as Inflammatory Bowel Disease (IBD). A reduction of Firmicutes and Bacteroidetes with a simultaneous increase of E. coli is most frequently reported. RYCHLIK et al. (2013) fed a yeast cell wall product (MOS) with high β-glucan levels, to dog patients suffering from IBD. During the test, a decrease in proinflammatory interleukin IL-6 and an increase in anti-inflammatory interleukin IL-10 were observed, as well as a drastic reduction in clinical IBD symptoms and IBD index relative to the individual animal.

The following study should determine the prebiotic activity of **Biolex® MB40** in the gastrointestinal tract of the dog. According to According to GIBSON & ROBERFROID (1995), the prebiotic effect in the gastrointestinal tract can be demonstrated by evaluating the growth of health-promoting bacteria, the reduction of pathogenic germs and the increase in the production of health-promoting metabolic products such as acetates, lactates, propionates and butyrates, which have among other things a positive effect on intestinal health.

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Study design:

Biolex® MB40 was tested *in vitro* for seven weeks at three different doses (0.5g/day – 1.0g/day – 2.0g/day). The SCIME® (Simulator of the Canine Intestinal Microbial Ecosystem) accurately reflects the conditions within the different intestinal regions such as stomach, small intestine and large intestine. The use of SCIME® allows the complete intestinal microbiota to be cultivated over a long period of time under representative conditions within the various intestinal segments without having to work on the animal. Relevant aspects such as temperature, retention time, pH and nutrient substrates are adapted to mimic the canine intestinal tract. The colon region is simulated by two regions (proximal and distal). The system was incubated with fresh Beagle faeces and fed with a specific medium simulating daily feeding. To this medium, **Biolex® MB40** and a negative control were added. The microbiota profile of each colon region was then mapped at the luminal level by 16S-targeted Illumina sequencing.

Results:

Biolex® MB40 results in a dose-dependent fermentation by the canine intestinal microbiota, proven by the test results of acidification, gas production and SCFA production and here in particular by the production of acetates, propionates and butyrates. More detailed information on the results of **Biolex® MB40** regarding metabolic products can be found in the report: "Evaluation of the prebiotic effect of **Biolex® MB40 using** SCIME®" more specifically the short version (see Fig. 1).

Fig. 1: Results "metabolic markers" (s. Report I): Assessment of the prebiotic effect of **Biolex® MB40** using SCIME® in the *in-vitro*-system:

1. All three **Biolex® MB40** doses (0.5g/day – 1.0g/day – 2g/day) result in a dose-dependent fermentation due to the canine microflora. (Measured values: pH value, gas production, acid-base titration).

2. All three **Biolex® MB40** doses significantly increase propionate production in the PC (proximal colon) and DC (distal colon). The increase in propionate production is dose-dependent with the greatest effect at the highest dosage of 2g/day.

3. A **Biolex® MB40** dose at 1g and 2g per day show a statistically significant increase in acetate and butyrate production in the PC and DC. Likewise with a dose-dependent effect.

4. Lactate initially shows a significantly higher concentration in the PC, which then decreases in the further course (DC). Justified by the so-called "cross-feeding effect" between lactate-producing and lactate-consuming bacteria.

5. The significant increase in SCFA production in the DC shows that **Biolex® MB40** is selectively fermented, especially in the rear colon section (DC).

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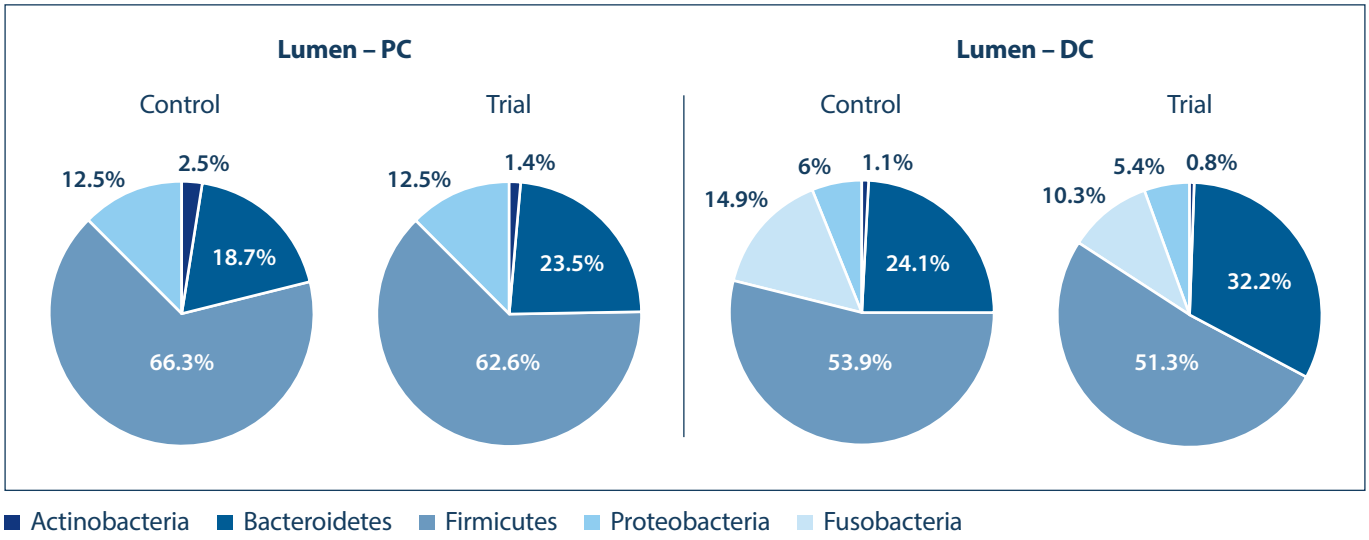
Fig. 2: Evaluation of the microbial composition by 16S Illumina sequencing. Abundance (%) of the microbial phylum level in the lumen of the proximal (PC) and distal (DC) colon of the gastrointestinal tract of the dog over the control period (control) and treatment duration (trial). For the different tested concentrations (0.5g/day, 1g/day and 2g/day) the average of the three test concentrations is shown (n = 3).



The analysis of the microbial composition by SCIME® showed at the phylum level a typical canine microbiota (KIM et al., 2017), with 60.1% Firmicutes, 21.4% Bacteroidetes, 9.3% Proteobacteria, 7.4% Fusobacteria and 1.8% Actinobacteria.

Each of the three **Biolex® MB40** doses was colonized by reproducible microbes with respect to their diversity (Simpson Diversity Index), both in the proximal colon (PC) and in the distal colon (DC).

In the luminal phylum-level test materials, **Biolex® MB40** measured a steady increase in Bacteriodetes (see Fig. 2). There are many propionate producers within the Bacteriodetes family. This would explain the strong dose-dependent increase in propionate production in both the PC and DC by **Biolex® MB40**. The proliferation of Bacteriodetes in the DC was at the expense of Fuso-bacteria.
The steady increase in **Bacteroidetes** (phylum levels) in SCIME® was mainly due to changes in three families (see Fig. 3). Indeed, a consistent decrease in the amount of Bacteroidaceae was observed in both colon regions (PC and DC) after addition of **Biolex® MB40**. In contrast, Porphyromonadaceae and Prevotellaceae increased strongly and specifically during supplementation. Further analysis of the data at the lowest phylogenetic level (OTU level) within the Porphyromonadaceae family was followed by two OTUs, which were mainly responsible for the observed increases. These two OTUs were related to Parabacteroides merdae (OTU5) and an unclassified Porphyromonadaceae species (OTU19).

Within the **Firmicutes** strain, the family of Veillonellaceae was found to be the main family. However, this family remained unaffected by the addition of **Biolex® MB40**. Other families within the Firmicutes strain, such as Lactobacillaceae and Lachnospiraceae, decreased after

the addition of **Biolex® MB40**. On the other hand, the Enterococcaceae family in the PC continuously increased, with the strongest increase observed at doses of 0.5g/day and 1.0g/day.

The main family belonging to **Proteobacteria** was the Enterobacteriaceae family, which decreased in both colon sections after product supplementation. This effect was masked at the phylum level due to the increase of other Proteobacteria families such as Pseudomonadaceae, Succinivibrionaceae, Sutterellaceae and Xanthomonadaceae. The **Actinobacteria** strain consists primarily of Bifidobacteriaceae and Coriobacteriaceae. With the addition of **Biolex® MB40** a slight decrease in the abundance of Bifidobacteriacae could be observed. As observed at the phylum level (Fusobacteria), **Biolex® MB40** also reduced the abundance of the Fusobacteriaceae family in the DC.

Fig. 3: Microbial composition at the family level. Abundances (%) of the dominant families Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes and Fusobacteria phylum, evaluated by 16S Illumina sequencing, during control period (C) and treatment (TR) in luminal samples within the proximal (PC) and distal colon (DC) at three different test concentrations (0.5g/day, 1g/day and 2g/day) (n = 1). The intensity of shading indicates the absolute frequency displayed for each of the different families.

Phylum	Family	PC						DC					
		0.5g/day		1g/day		2g/day		0.5g/day		1g/day		2g/day	
		C	TR	C	TR	C	TR	C	TR	C	TR	C	TR
Actinobacteria	Bifidobacteriaceae	2.8	2.2	2.2	1.8	2.7	0.2	0.7	1.4	0.9	0.8	1.8	0.2
Bacteroidetes	Bacteroidaceae	10.2	3.6	22.2	2.2	14.7	1.6	19.5	13.1	9.7	7.0	11.7	1.3
	Porphyromonadaceae	0.3	2.9	0.7	4.1	0.5	1.9	3.2	15.8	5.9	20.7	10.3	14.8
	Prevotellaceae	2.0	6.3	1.0	25.7	4.7	22.2	1.9	2.2	7.2	7.9	2.8	14.0
Firmicutes	Acidaminococcaceae	0.2	0.6	0.7	0.8	0.3	0.3	0.9	0.6	1.0	1.2	0.9	1.2
	Clostridiales unclassified	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0
	Enterococcaceae	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Erysipelotrichaceae	0.6	1.5	0.2	1.6	0.4	0.2	0.1	0.4	0.1	0.7	0.2	0.2
	Lachnospiraceae	5.0	1.6	0.9	0.9	1.5	0.6	2.1	1.5	2.6	2.2	2.9	1.1
	Lactobacillaceae	3.0	0.1	0.7	0.0	1.3	0.0	0.1	0.0	0.0	0.0	0.0	0.0
	Ruminococcaceae	0.2	0.1	0.3	0.1	0.2	0.1	0.2	0.1	0.3	0.2	0.3	0.2
	Veillonellaceae	63.9	72.3	62.2	47.8	57.1	58.6	51.7	49.5	50.5	42.4	47.5	52.1
Fusobacteria	Fusobacteriaceae	0.0	0.0	0.0	0.0	0.0	0.0	15.7	10.8	15.9	10.6	13.0	9.4
Proteobacteria	Enterobacteriaceae	11.2	1.6	6.9	2.3	15.1	3.2	1.6	0.3	1.9	0.6	4.9	1.3
	Pseudomonadaceae	0.5	5.5	1.8	11.5	1.3	9.6	1.5	2.6	2.9	4.4	2.1	2.4
	Succinivibrionaceae	0.0	0.7	0.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Sutterellaceae	0.0	0.2	0.1	0.5	0.0	0.1	0.7	1.3	1.0	1.3	1.4	1.4
	Xanthomonadaceae	0.1	0.3	0.1	0.1	0.3	1.2	0.0	0.3	0.0	0.0	0.0	0.3

The results of the microbial composition are directly related to the results of the production of health-related metabolic metabolites such as acetate, propionate and butyrate (SCFA).

The increase in **propionate concentration** was shown by:

- Reduction of Bacteroidaceae in the PC and DC
- Increase of Porphyromonodaceae and Prevotellaceae (propionate producers)
- Stability of Veillonellacae (propionate producer):
  - Strong increase in OTU due to Megamonas funiformis/ rupellensis
  - Strong increase in OTU due to Megamonas hypermegal

The increase in **butyrate concentration** was demonstrated by the Porphyromonodacae family:

- Increase in Parabacteroides merdae, which raise the propionate level
- Increase in Poryphyromonadaceae species, which raise butyrate levels

The increase in Porphyromonodaceae species must be greater than that of other butyrate-producing species, i.e. Lachnospiraceae and Lactobacilli (phylum Firmicutes).

The butyrate was increased due to the so-called "**cross feeding effect**". Lactate and acetate are converted to butyrate. The cross feeding effect was observed on the microbial level due to increase of Enterococcaceae in the PC (lactate producers). A very strong increase in lactate was observed especially at a **Biolex® MB40** dosage of 0.5g/ day and 1.0g/day.

- The fact that **lactate** increased in the PC only for the **Biolex® MB40** dosage of 0.5g/day and 1.0g/day reinforces the hypothesis that the stimulation of Enterococcaceae by **Biolex® MB40** is responsible for the increased lactate level.

According to GIBSON & ROBERFROID (1995) and their definition of the prebiotic effect, the use of **Biolex® MB40** showed both a growth of health-promoting bacteria and an increase in the production of health-related bacterial metabolites (SCFA), but also a reduction of **intestinal pathogens** (see Figure 4). These were indicated in particular by:

- A decrease of Fusobacteriaceae in the DC
- A decrease of Enterobacteriaceae, which contain potentially opportunistic pathogens, in the PC and DC. This effect was masked at the phylum level (Proteobacteria) due to an increase in other Proteobacteria families during treatment such as Pseudomonodaceae, Succinivibrionaceae, Sutterellaceae and Xanthomonadaceae

Discussion:

Stimulation of acetate, propionate and butyrate production after repeated **Biolex® MB40** supplementation in all colon regions was observed in a dose-dependent manner during product administration. All three concentrations tested showed a significant increase in propionate production in the PC and DC. It has already been shown in other studies that the addition of MOS (mannan oligosaccharides) from the yeast cell wall of *Saccharomyces cerevisiae* increases propionate production in adult dogs (STRICKLING et al., 2000). Propionate production is believed to influence the reduction of cholesterol and fatty acid synthesis in the liver (LIN et al., 2007, BERGGREN et al., 2007), influence glucose metabolism (BERGGREN, 2007) and regulate immune status in adipose tissue (AL LAHHAN et al., 2010 & 2012).

In dogs, propionate production has been shown to play a role in the stimulation of gastrointestinal saturation hormones such as GLP-1 and PYY. MASSIMIO et al. (1998) reported that the secretion of GLP-1 by enteroendocrine L-cells, which are predominantly present in the distal part of the gastrointestinal tract of the dog (HOLST et al., 2007), was increased with dietary supplements in healthy

dogs. In addition, PAPPAS et al. (1986) showed that the perfusion of fatty acids increased the peripheral PYY concentration in the dog's intestine. LE PAUL (2003) also suspects that propionate is involved in stimulating PYY release by activating receptors GRP41 and GRP43, both expressed by enteroendocrine L-cells in the distal part of the gastrointestinal tract. MUSCO et al. (2016) compared six different cell wall products based on *Saccharomyces cerevisiae*, which differed in MOS and glucan content and in the production process (ethanol yeast – baker's yeast – brewer's yeast). Both cell wall products based on brewer's yeast showed the best results in terms of gas production and SCFA production among other things.

When **Biolex® MB40** was administered, propionate stimulation correlated with the increased occurrence of Bacteroidetes, depending on the dose. The application of 16S-based Illumina sequencing ensured the conclusion that the increase within this strain was related to an increase in Porphyromonadaceae and Prevotellaceae, two families with potent propionate producers (SAKAMOTO et al., 2014, KRIEG et al., 2015). With respect to other saccharolytic metabolites, this resulted in **Biolex® MB40** initially yielding higher lactate concentrations, suggesting stimulation of lactate-producing substrate degraders. Indeed, the fermentation of yeast cell wall products by the microbiota of the dog has also been shown to increase lactate concentration *in vitro*, suggesting the ability of lactate-producing species to use yeast cell wall products as primary substrate (HUSSEIN et al., 2001).

The use of molecular tools showed that Enterococcaceae as the main producer of lactate was probably stimulated in contrast to Lactobacilli and Bifidobacteria. KIM et al. (2017) showed that Enterococcaceae do indeed belong to the nuclear microbiome of dogs, whereas Bifidobacteriaceae and Lactobacillaceae do not. Lactate is considered a health-promoting metabolite in the intestine, as it exerts strong antimicrobial effects against pathogens (ALAKOMI et al. 2000, RAYBAUDI-MASSILIA et al., 2009). In addition to its antipathogenic effect, lactate can contribute to butyrate production by cross feeding (BOURRIAUD et al., 2005). In the current study, this was observed at the highest dose tested (2.0g/day), by which lactate levels decreased in the last weeks of treatment, leading to significantly increased butyrate concentrations.

Other changes during the test included decreases in the Enterobacteriaceae and Fusobacteriaceae families, both of which contain several potentially opportunistic pathogens. It is known that several Enterobacteriaceae species cause disease in dogs, including Escherichia coli, which is believed to be responsible for urinary tract infections in dogs (SYKUS et al., 2013). Earlier studies showed that the supplementation of the spray-dried yeast cell wall in the diet of adult dogs reduces the concentration of Escherichia coli in faeces in a dose-dependent manner (MIDDELBOS et al., 2007). Overall, the decrease of the above-mentioned bacterial groups can be regarded as a beneficial effect of **Biolex® MB40** on the microbial composition.

In addition, increased SCFA production in the DC confirms that part of the product was able to withstand fermentation in the PC and was transferred to the DC. CALABRÒ et al. (2013) have shown that the dietary supplementation of the dried yeast cell wall to the diet favours a stronger distal fermentation in the gastrointestinal tract of the dog *in vitro*. Since saccharolytic fermentation takes place mainly in the proximal regions of the colon, fermentation in the distal colon is mainly proteolytic (WILLIAMS et al., 2001). Considering that proteolytic fermentation can lead to the formation of potentially toxic metabolites such as indoles and ammonia, there is great interest in finding prebiotics with biological activity in the distal colon (TERPEND et al., 2013). Although markers of proteolytic fermentation (ammonium and branched SCFA) with **Biolex® MB40** in the distal colon did not decrease significantly, the distal production of SCFA was an interesting finding from this perspective.

Summary:

Biolex® MB40 can positively influence the microbial activity and composition of the intestine in the gastrointestinal tract of the dog at different doses (0.5g/day – 1.0g/day – 2.0g/day). Changes in metabolic activity were associated with specific microbial changes at the family level, such as specific stimulation of the propionate-producing families Porphyromonadaceae and Prevotellaceae after administration of **Biolex® MB40**. **Biolex® MB40** is slowly and selectively fermented, the growth of health-promoting bacteria and the production of health-related bacterial metabolites is promoted, whereas intestinal pathogens are reduced.

A prebiotic effect of Biolex® MB40 on the gastrointestinal tract of the dog has been clearly demonstrated! Based on these results we recommend a Biolex® MB40 dosage at 0.05–0.2% in the feed.

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