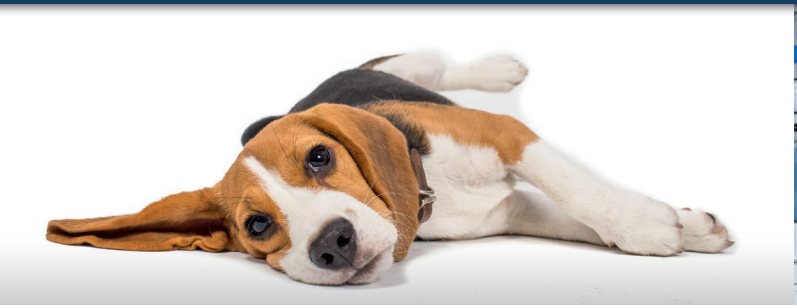
BIOLEX® MB40



ASSESSMENT OF THE PREBIOTIC EFFECT OF BIOLEX® MB40 WITH UTILISATION OF THE SCIME™ (Simulator of the Canine Intestinal Microbial Ecosystem) IN-VITRO SYSTEM

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Indigestible carbohydrates from the cell walls of brewers' yeast (*Saccharomyces cerevisiae*) – better known as "mannan oligosaccharides (MOS)" – have for many years on account of their prebiotic effect been successfully used in the feeding of livestock.

Initial MOS studies with dogs show comparable positive effects, in particular on gut health and the immune system. For example, in the review article "The Role of Yeast in Companion Animal Nutrition" (SWANSON K., 2006), yeast cell walls are described as a "useful prebiotic with a beneficial potential for gut health". MOS is characterised as a fermentable, prebiotic substrate, with the ability to binding E. coli and salmonella within the intestine, with a positive influence on the microflora in the gut, and a positive effect on the immune system (SWANSON et al., 2004). Studies which include the use of *in-vitro* systems (VICKERS et al., 2001; HUSSEIN & HEALY, 2001) have indicated positive influences on the SCFA concentrations and, in particular, on the lactate-producing bacteria.

Biolex® MB40 consists of the cell walls of real brewers' yeast (100% *Saccharomyces cerevisiae*) and is distinguished, amongst other properties, by its naturally high content of β-glucan and mannan oligosaccharides (MOS). In a study using porcine cells, **Biolex® MB40** demonstrated good binding to E. coli and salmonella (ZENTEK J., 2011). Feeding tests of **Biolex® MB40** with piglets demonstrated improved performance parameters as well as a positive influence on the consistency of their dung (FUCHS et al., 2011). Various studies with turkeys and broiler chickens describe, among other properties, improved immunity parameters (TYSON, 2014) and an improved gut morphology (NOVI SAD, 2014).

Investigations with humans indicated a positive effect on "gut barrier enforcement" and, in addition, an effectiveness in binding E. coli during diarrhoea (NIZO, 2014, unpublished). **Biolex® MB40** shows therefore numerous, scientifically demonstrated positive effects. The aim of the present study is to assess the potential of the prebiotic activity of **Biolex® MB40** within the canine gastrointestinal tract. To do this, an *in-vitro* system was used (SCIME™), which corresponds to the conditions in the various sections in the gastrointestinal tract (stomach – small intestine – large intestine) of a dog (see figure 1).

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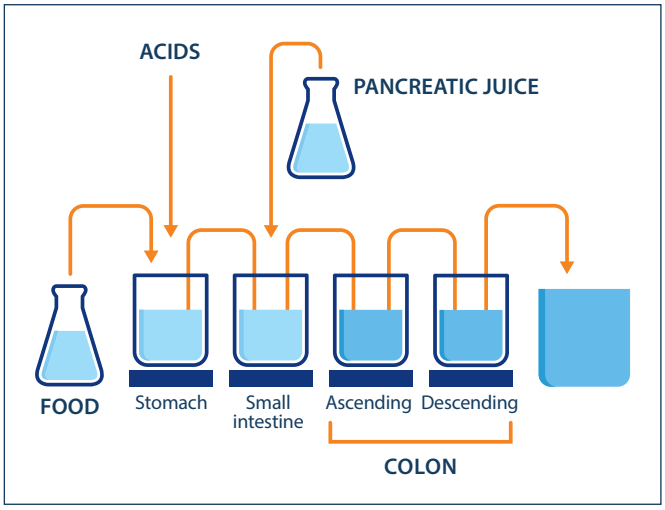
Experimental design:

In a seven-week-long experiment, **Biolex® MB40** was tested for three different doses (0.5g/day – 1.0g/day – 2.0g/day). Utilisation of the SCIME™ (Simulator of the Canine Intestinal Microbial Ecosystem) allowed the monitoring of the entire digestive tract with all the microbiological processes in it under controlled conditions without having to work directly with an animal. This means, that the SCIME™ cannot just provide detailed information about the prebiotic activity of a raw material but it can also localise more accurately the area where it is effective (see figure 2).

Figure 1: SCIME™ (Simulator of the Canine Intestinal Microbial Ecosystem)

During a two-week-long stabilisation period, the individual sections of a canine *in-vitro* system were matched to the relevant conditions (e.g. temperature, pH-profile, retention time). To do this, the large intestine section was subdivided into two sections: proximal colon (PC) and distal colon (DC). Afterwards, the colon system was injected with microbiota isolated from fresh faeces of beagles.

Figure 2: Standard setup of the Simulator of the Canine Intestinal Microbial Ecosystem (SCIME™), consisting of 4 sequential reactors, which simulate the different regions of the canine intestinal tract.



After the stabilisation period, a two-week control period followed. In this phase, the reference values (control) were determined, which would later be compared to the results gained during the three-week-long experimental period.

According to the GIBSON & ROBERFROID definition (1995), prebiotics are: "... non-digestible substances that provide a beneficial physiological effect to the host by selectively stimulating the favourable growth or activity of a limited number of indigenous bacteria." A prebiotic effect can be demonstrated by, among other factors, the enhancement of a positive microflora, the reduction of pathogens or by the influence of health-enhancing metabolites such as acetates, propionates, butyrates (SCFA) or the lowering of negative metabolites such as b-SCFA.

Experimental results:

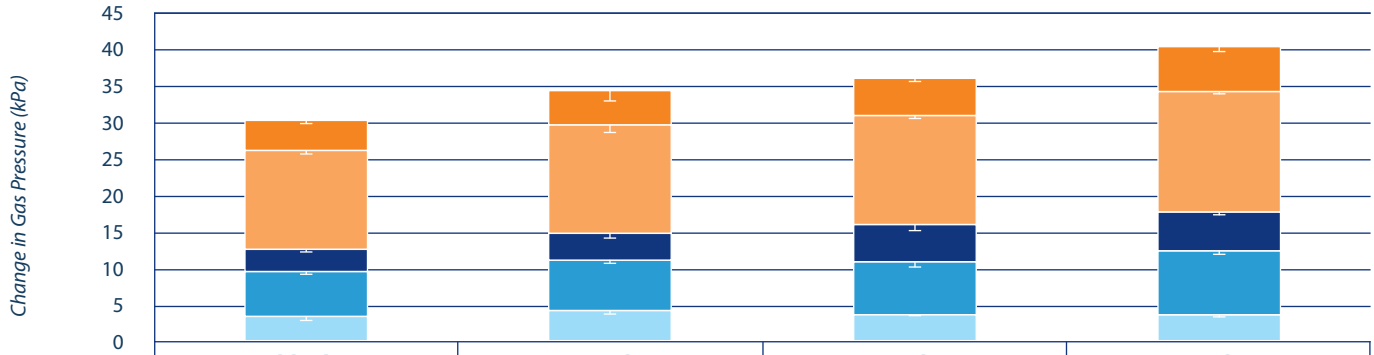
1. Gas production & measurement of the pH-values:

The measurement of gas produced was carried out in enclosed incubation systems. To do this, material from the proximal colon (PC) was removed during the control period and **Biolex® MB40**, corresponding to the three doses, was added separately.
The pH-value was determined at the same time. The degree of acidification at the end of the experiment is a measure of the intensity of the fermentation of the material to be tested. Changes in the gas production and the pH-value are good indicators for assessing the fermentation activity of the material to be tested, both with regard to the microbial activity itself and also with regard to its responsiveness.

- At all of the three doses, **Biolex® MB40** demonstrated a relatively low reduction in the pH-value. Within the period 0–24 h and at a dose of 2g/day, this reduction was significant compared to the control ($p > 0.05$).
- After 2 h, **Biolex® MB40** showed a tendency towards a dose-dependent increase in gas production.
- Highest gas production for a dose of 2g **Biolex® MB40**/day
- After 24 h, **Biolex® MB40** still showed a further increase in the gas production.

At all of the three doses, a measurable fermentation activity in the canine gut flora was determined. The dosing with 2g Biolex® MB40 shows the highest activity here.

Figure 3: Average gas production (kPa) during a control incubation an upon dosing three different concentrations of **Biolex® MB40** to the proximal colon microbiota (n=3) derived from the TripleSCIME during the control period.



	blank	0.5g/day	1g/day	2g/day
D 24–48	4,0	4,5	5,0	5,8
D 6–24	13,3	14,9	14,9	16,5
D 4–6	3,1	3,5	4,9	5,5
D 2–4	6,1	7,0	7,3	8,6
D 0–2	3,7	4,4	3,9	3,8

The various groups of bacteria appear, on the one hand, to be responsible for the gas production or the gas consumption and, on the other hand, for the SCFA/lactate (pH) production. This is clearly apparent by the limited pH-change while, at the same time, the gas production rises as a function of the dose. **Biolex® MB40** is fermented slowly by the microbiota in the colon and depends on the dose. A slower fermentation and therefore a moderate gas production inside the colon is, according to CALABRÒ et al. (2013), desirable in order to prevent flatulence or a softer consistency of the faeces.

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2. Acid/base titration:

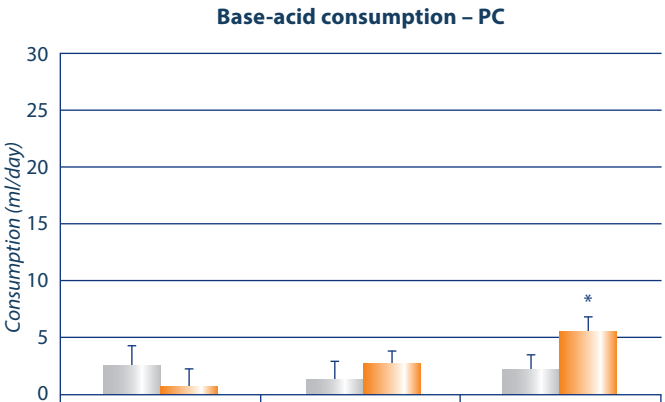
The formation of metabolites in the colon always changes the pH value. Without constant monitoring and adjustments, by adding acids and bases, the pH would change to the critical range. In the SCIME™, a controlled pH–system is present, which in the proximal colon (PC) keeps the pH value constant at 5.6 – 5.9 and, in the distal colon (DC), constant between 6.6 – 6.9. Only such a stable system will later allow the direct comparison between the three doses and the control. The measurement of the acid/base titration complements the results from the earlier pH-measurement and the determination of the gas production, again in order to be better able to assess the microbial activity of **Biolex® MB40** in the *in-vitro* system.

- **2g Biolex® MB40/day shows a significant increase of the acid/base titration in the proximal colon (PC) and the distal (DC) colon.**
- **1g Biolex® MB40/day shows a significant increase of the acid/base titration in the distal colon (DC).**

Overall, again, a dose-dependent, fermentation profile was found here for Biolex® MB40. The increase of the acid/base titration in the distal colon (DC) for 1g and 2g per day clearly indicate that Biolex® MB40 is selectively fermented in the large intestine. A significant quantity therefore passes through to the proximal colon without being digested (see figures 4 and 5)!

Even a dose of 1g **Biolex® MB40** per day supports the microflora in the rear section of the large intestine (DC). A specific support to both sections of the large intestine (PC and DC) appears therefore to be possible by increasing the dose to 2g per day or by a combination with other "rapidly fermentable" substrates, such as fructooligosaccharides (CALABRÒ et al., 2013), which are less selective with regard to the microflora and more effective in the forward part of the colon (PC).

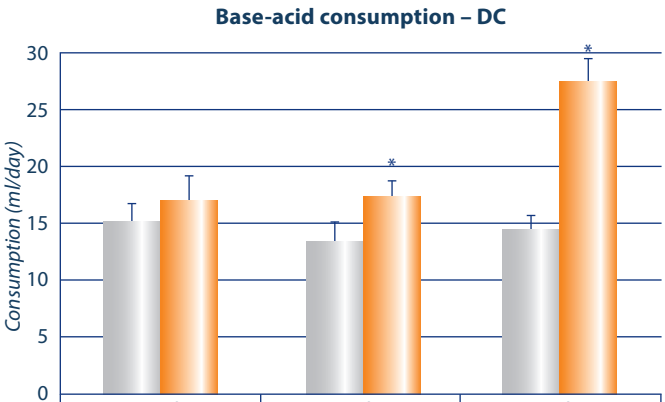
Figure 4: Averaged acid/base consumption over the control (n=6) and treatment (n=9) period



	0.5g/day	1g/day	2g/day
C	2,6	1,4	2,3
TR	0,8	2,7	5,6

(indicates the statistically significant differences relative to the preceding period)*

Figure 5: Averaged acid/base consumption over the control (n=6) and treatment (n=9) period



	0.5g/day	1g/day	2g/day
C	15,2	13,4	14,4
TR	17,1	17,3	27,5

(indicates the statistically significant differences relative to the preceding period)*

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3. SCFA production:

The production of SCFA (short-chain fatty acids) results from the metabolism of carbohydrates in the large intestine. The most common SCFA are acetates, propionates and butyrates. All three of them play an important role for gut health.

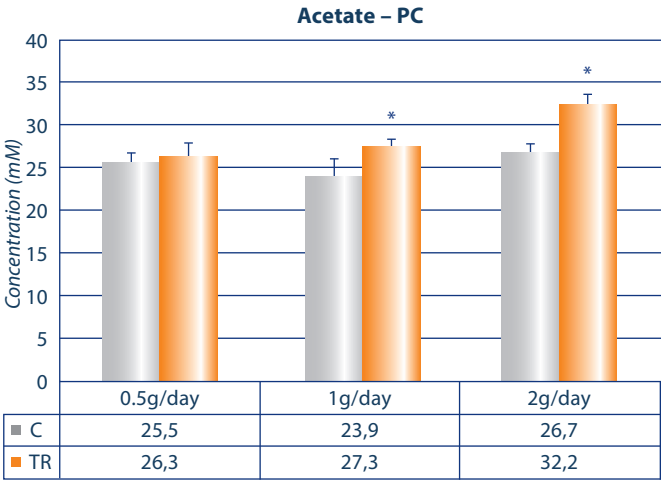
For example, acetates are used by the microflora for the generation of energy, but they are also a potential substrate for the lipometabolism in the body and an important by-product for the butyrate synthesis. Propionate and butyrate, however, are regarded as main energy sources for the intestinal epithelium (CUMMINGS, 1987). They not only support the gut health in general but also provide protection against inflammation. Propionates can also be transported to the liver, where they have an additional cholesterol-lowering effect (WRIGHT, 1990 & DEMIGNE, 1995) and are therefore supposed to have a positive influence on the glucose level (WONG, 2006).

A. Acetates

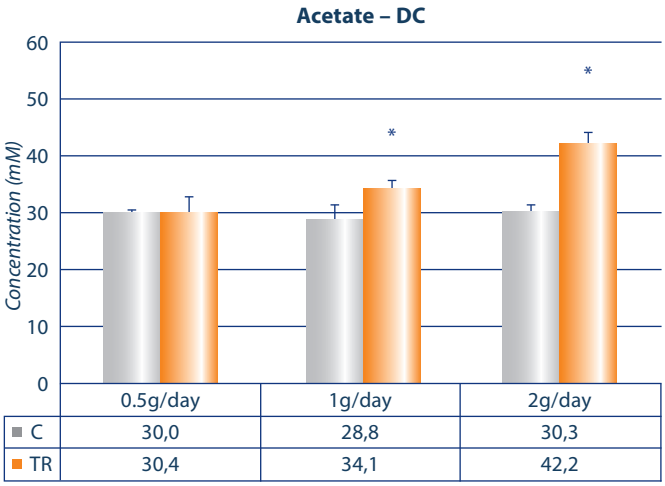
The results with **Biolex® MB40** with regard to acetate production show the following (see figure 6):

- **1g/day and 2g/day result in a statistically significant increase ($p < 0.001$) in the acetate production in the proximal colon (PC) and in the distal colon (DC).**
- **In both colon sections, a dose-dependent effect can be demonstrated.**
- **2g/day results in the highest acetate production.**

Figure 6: Average acetate production over the control (n=6) and treatment (n=9) period



	0.5g/day	1g/day	2g/day
C	25,5	23,9	26,7
TR	26,3	27,3	32,2



	0.5g/day	1g/day	2g/day
C	30,0	28,8	30,3
TR	30,4	34,1	42,2

*(*indicates the statistically significant differences relative to the preceding period)*

Once again it turned out that Biolex® MB40 is selectively fermented in the large intestine. Similar to the acid/base titration, again, a marked increase in the production of SCFA is found in the distal colon (DC) compared to the proximal colon (PC). A significant quantity of Biolex® MB40 therefore passes through the PC without being digested and is completely fermented in the DC.

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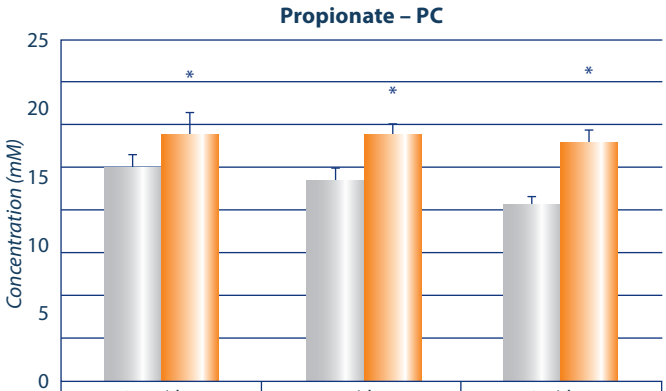
B. Propionates

Many different microbes in the gut produce propionates. The most important propionate producers include bacteroides (phylum bacteroidetes) and Akkermansia muciniphila (phylum verrucomicrobia). The following effects on the propionate production from **Biolex® MB40** could be identified (figure 7):

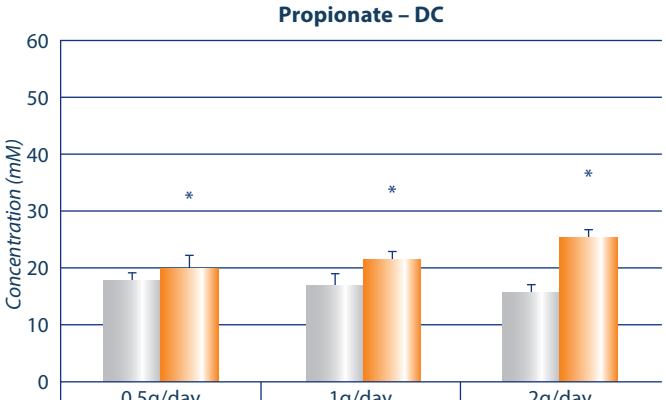
- **All three doses (0.5g/day, 1.0g/day, 2.0g/day) resulted in a statistically significant increase in the propionate production in the PC and in the DC ($p < 0.05$).**
- **In both sections of the large intestine, a dose-dependent effect is apparent.**

Again, production in the DC is higher compared to the PC. This is a further indication of the selective effect of Biolex® MB40, in particular on the rear part of the colon (DC).

Figure 7: Average propionate production over the control (n=6) and treatment (n=9) period



	0.5g/day	1g/day	2g/day
C	15,6	14,7	12,9
TR	18,0	18,0	17,4



	0.5g/day	1g/day	2g/day
C	18,1	16,9	16,0
TR	20,1	21,8	25,3

(indicates the statistically significant differences relative to the preceding period)*

HUSSEIN & HEALY (2001) described for the deployment of MOS a dose-dependent increase in the production of SCFA, in particular for acetate and propionate, but also lactate. **Biolex® MB40** also resulted in a significant, dose-dependent increase in propionates and acetates. Propionate is, in addition to acetate and butyrate, one of the main energy suppliers for the gut epithelium and therefore it contributes significantly to the health and protection of the gut.

Biolex® MB40 also acts selectively and specifically promotes, in contrast to other oligosaccharides, the microflora in the posterior colon (DC). **Biolex® MB40** could therefore be deployed prophylactically, both in low doses (1g/day), e.g. in combination with other oligosaccharides, but also directly in acute stress situations with 2g/day, in order to support or protect the microflora specifically in the rear gut section.

The advantages of a combination of MOS with other oligosaccharides has already been described in more detail in scientific studies. GRIESHOP et al. (2004) discovered in a feeding test with older dogs, that a combination of

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chicory and MOS has a positive influence both on the faecal microbiota and the immune system. Both led to an increase in the faecal bifidobacteria concentration, with MOS showing in a lowering in the faecal E. coli concentration. Similar results were found in a study by MIDDELBOS (2007a), in which FOS was combined with yeast cell walls and fed to dogs. Again, an increase in the faecal bifido and lactobacilli concentration was found, with a tendency for E. coli to be reduced by MOS and therefore a possible binding activity of MOS for pathogenic germs. SWANSON et al., (2002c, 2002b) also fed a MOS/FOS combination to dogs and also observed an increase in the faecal bifidobacteria and lactobacilli concentrations. Again, the author concludes that a positive effect on the intestinal health and the immune system is obtained by the combination of MOS with FOS.

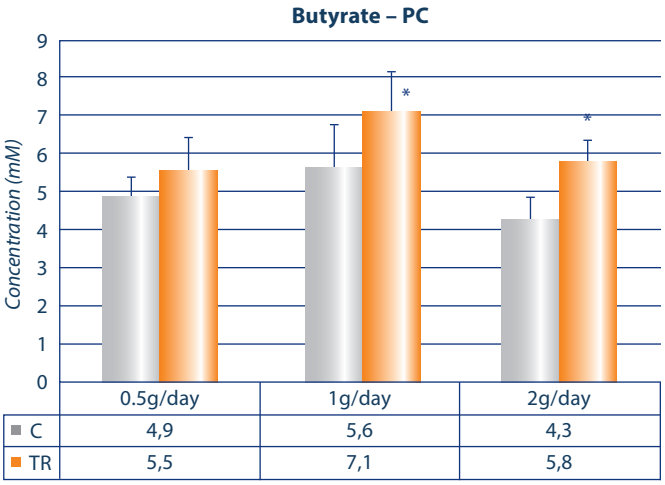
C. Butyrates

Butyrate is mostly produced by chlostridium clusters IV and XIVa (phylum firmicutes) by means of the so-called "cross-feeding effect". Here the butyrate is not produced directly but by conversion from acetate/lactate into butyrate. The following effects could be identified (figure 8):

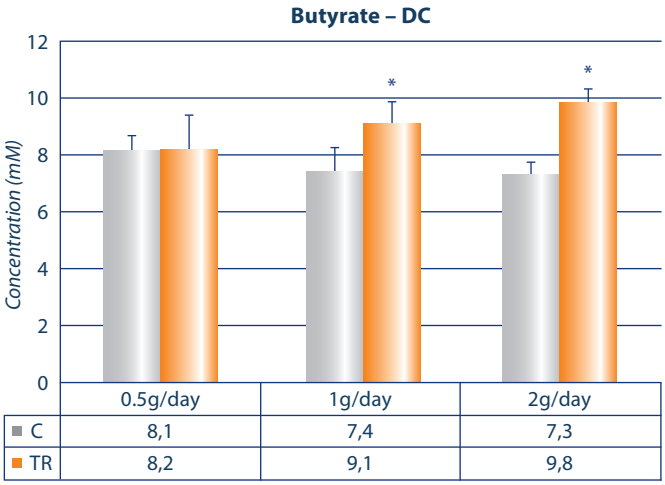
- **1g/day and 2g/day result in a statistically significant increase in butyrate production in the PC and the DC ($p < 0.05$)!**
- **A dose-dependent effect could be identified in the DC, with an increase in butyrate production at 2g/day.**

As mentioned above, butyrates are produced by firmicutes, which produce acetate and lactate and then convert these to butyrates. Biolex® MB40 appears to have a positive influence on this so-called "cross-feeding effect".

Figure 8: Average butyrate production over the control (n=6) and treatment (n=9) period



	0.5g/day	1g/day	2g/day
C	4,9	5,6	4,3
TR	5,5	7,1	5,8



	0.5g/day	1g/day	2g/day
C	8,1	7,4	7,3
TR	8,2	9,1	9,8

(indicates the statistically significant differences relative to the preceding period)*

Biolex® MB40 is presumably broken down by a proportion of the microorganisms and fermented into acetate and lactate, which are then consumed by other microbes, which then form propionate and butyrate (cross-feeding process). It has been observed several times that **Biolex® MB40** is fermented relatively slowly and selectively in the large intestine. The relatively long metabolic pathway associated with this would actually favour a "cross-feeding process".

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VICKERS et al. (2001) also describe an *in-vitro* experiment with various oligosaccharides, a moderate SCFA-production and a low increase of the lactate production if MOS is deployed. Again, in this situation the so-called "cross-feeding effect" or positive influence on the lactate producing or consuming microflora is described.

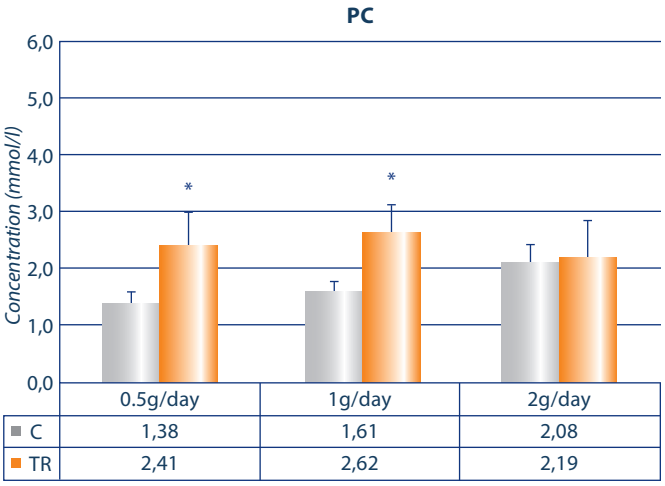
The production enhancing effect of **Biolex® MB40** is apparent for both butyrate and even more clearly for propionate, in particular in the DC. It is known that the so-called "cross-feeding pathway" is able to be saturated. If the production of acetate and lactate is increased so much by **Biolex® MB40** that the conversion process is "saturated", the butyrate production cannot be increased any further and more propionate is formed. If the results from the SCIME™ are compared to **Biolex® MB40**, it appears that on account of the saturation of the "cross-feeding pathway" more propionate is formed than butyrate.

D. Lactates

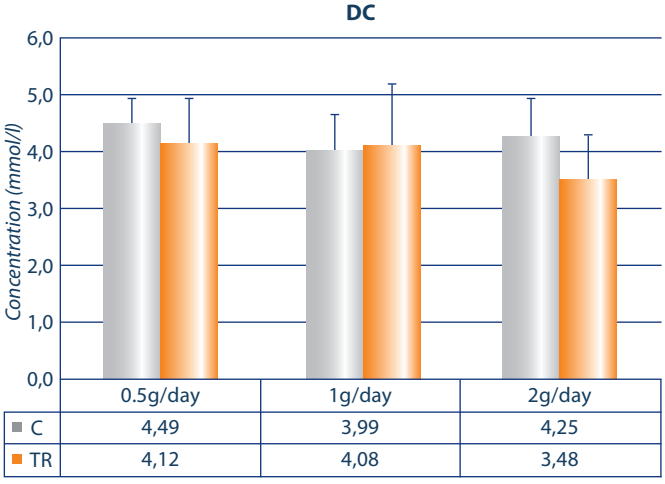
In the canine gut system both lactate-producing and lactate-consuming bacteria are found. Lactate production is always associated with a lowering of the pH-value. Lactates can have a strong anti-microbial effect against pathogens and thereby provide an important contribution to gut health. As mentioned above, specific lactate-consuming and butyrate-producing microbes can convert lactate to butyrate. A high lactate value can therefore mean either an increased lactate production itself or a lower conversion of lactate to butyrate (cross-feeding). **Biolex® MB40** led to the following results (figure 9):

- **0.5g/day and 1.0g/day show a statistically significant difference in the PC between the control and the test ($p < 0.05$).**

Abb. 9: Effect of **Biolex® MB40** on lactate production in the proximal (PC) and distal colon (DC) reactors. Top: averaged weekly lactate production (n=3), bottom: averaged lactate production over the control (n=6) and treatment (n=9) period



	0.5g/day	1g/day	2g/day
C	1,38	1,61	2,08
TR	2,41	2,62	2,19



	0.5g/day	1g/day	2g/day
C	4,49	3,99	4,25
TR	4,12	4,08	3,48

*(*indicates statistically significant differences)*

If the present lactate result is compared to the results from butyrate, it becomes clearly apparent that lactate (see PC) is converted to butyrate (see DC) by means of "cross-feeding".

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The present results for the butyrate production show a dose-dependent effect, in particular in the DC. In comparison, lactate shows a significant increase in concentration at low doses of **Biolex® MB40** in the PC and a falling lactate concentration in the DC, in particular also at the highest doses of 2g/day. It appears, therefore, that lactate is increasingly (see PC) converted to butyrate (see DC). In the literature, this type of conversion process is often associated with clostridium cluster IV and/or XIVa. But bifido bacteria also produce a lot of lactate.

Biolex® MB40 appears to have a positive influence in particular on the microorganisms producing propionate (phylum bacteroidetes) and those producing lactate or butyrate (phylum firmicutes).

In summary, the following can be stated for **Biolex® MB40** with regard to the SCFA production:

- **Acetates:** 1g/day and 2g/day result in a significant increase in the acetate production in the PC and in the DC ($p < 0.001$). A dose-dependent effect was identified!
- **Propionates:** The propionate production was increased significantly in the PC and in the DC ($p < 0.05$) for all three doses, with the highest values being reached in the DC. A dose-dependent effect was identified!
- **Butyrate:** 1g/day and 2g/day result in a significant increase in the production in the PC and in the DC ($p < 0.05$), with the higher values being reached at 2g/day. A dose-dependent effect was identified!
- **Lactate:** Lactate is converted to butyrate by the so-called "cross-feeding effect" because the butyrate value in the PC increases at 2g/day and, at the same time, the lactate value in the PC falls.

The proteolytic activity of **Biolex® MB40** was measured by determining b-SCFA and ammonium levels. Both measured values are often associated with direct or indirect harmful effects on health; therefore, the reduction of ammonium and b-SCFA is desirable.

Biolex® MB40 contains an increased protein content with 20–25% raw protein and is fermented in the rear section of the large intestine (DC). However, with **Biolex® MB40**, the indicators for the proteolytic activity tended to fall or did not increase in the DC. The reduction in b-SCFA and ammonium in the DC is an interesting result as, on account of the relatively high protein content of **Biolex® MB40**, a marked increase in the values might have been expected. This section of the intestine in particular is susceptible to chronic gut diseases such as IBD.

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Summary:

The present study serves to demonstrate the prebiotic effect of **Biolex® MB40** by deployment of the SCIME™ (Simulator of the Canine Intestinal Microbial Ecosystem) *in-vitro* system. The following results were found:

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

2. All three doses of **Biolex® MB40** significantly increase the propionate production in the PC and in the DC. The increase in propionate production turns out to be dose-dependent, with the greatest effect occurring at the highest dose of 2g/day.

3. 1g and 2g of **Biolex® MB40**per day result in a statistically significant increase in acetate and butyrate production in the PC and the DC. This is also a dose-dependent effect.

4. Lactate shows initially a much higher concentration in the PC, which then falls further on (in the DC). The reasons for this are the so-called "cross-feeding effect" between the lactate-producing and lactate-consuming bacteria.

5. The clearly increasing SCFA production in the DC indicates that **Biolex® MB40** passes through the PC largely without being digested in order to then be fermented, in particular by the microflora in the DC. **Biolex® MB40** acts therefore selectively, in particular, on the rear section of the large intestine (DC).

The present results show with their various indicators that **Biolex® MB40** is fermented both slowly by the canine microbiota in the large intestine, and also selectively in the distal colon. This effect was shown in many cases to be dose-dependent. The use of **Biolex® MB40** appears therefore to be beneficial for animals, both at a higher dosage (2g/day) in acute stress situations and as a prophylactic continuous administration with a lower dosage (1g/day).

Compared to other prebiotic substances such as FOS, which are fermented relatively quickly and especially in the PC (MCFARLANE & GIBSON, 1992 & CALABRÒ 2013), **Biolex® MB40**, is moderately and selectively fermented in the DC. This is a peculiarity, especially considering that proteolysis occurs in the DC, which is often associated with the production of toxic by-products such as ammonium and b-SCFA, which could, among other substances, be responsible for chronic bowel disease such as IBD (CASSIDY 1994). From this point of view, prebiotics that are active in the distal colon (DC) are very significant (MCFARLANE & GIBSON 1992; TERPEND 2013). The use of **Biolex® MB40**as a prebiotic, on its own or in combination with other oligosaccharides (SWANSON et al., 2002c; GRIESHOP et al., 2004; MIDDELBOS et al., 2007 a), appears to be a promising way of improving and protecting gut health.

A prebiotic effect from Biolex® MB40 on the canine gastrointestinal tract could therefore be clearly demonstrated!

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