CEFI® PRO

CEFI® PRO HAS A POSITIVE INFLUENCE ON THE INTESTINAL MICROBIOTA OF DOGS

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The ability to influence the intestinal microbiota is considered as an essential feature for the health-supporting effect of brewers' yeast in dogs. A positive effect on the composition and metabolic activity of the intestinal microbiota leads to the expectation that fewer problems will result from digestion disorders, such as diarrhea. This allows the development of positive effects on the immune system of dogs, based on the positive influence of lymphatic tissue (GALT – gut-associated lymphoid tissue). The so-called GALT is considered as the most important source of immune cells that monitor the intestinal mucosa, which is the first protection barrier to e.g. pathogens and/or mycotoxins.

As an autolysed brewers' yeast, **CeFi® pro** undergoes a special production process that leads to the availability of valuable components for the animal in an open structure, and therefore significantly more readily available. In addition to nutritional components such as a high content of protein, peptides and amino acids, **CeFi® pro** additionally contains functional components such as:

- β-glucans (immunomodulators)
- Mannan-oligosaccharides (MOS/prebiotic)
- Nucleotides (cell regeneration)

Nucleotides are considered as non essential nutritional components, but they play an important role in many metabolic processes or partially in important life stages. Nucleotides can be absorbed directly by enterocytes in the intestine. They are made available in the body via the so-called "salvage pathway". This is a conversion process intrinsic to the body that uses the components of nucleotides from metabolic processes, dead cells or also nucleotide-rich foods such as **CeFi® pro**, and making these available for cell regeneration or cell production. However, the body's own production can be limited for any number of reasons. This is the case, for example, for the reproduction of intestinal epithelial cells, blood cells, hepatocytes and immune cells or in stress situations such as in young or old animals in acute or chronic illnesses.

The aim of the present study was to investigate whether the supplementary feeding of an autolysed and thus nucleotide-rich brewers' yeast (**CeFi® pro** leads to a favourable effect on the intestinal microbiota in dogs, and whether the effects differ from those of a unextractred brewers' yeast.

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Material and Methods:

Twenty-one female and male dogs of the Malamute and Husky breeds were randomly divided into three groups. The average weight of the dogs was between 21.2 and 39.4 kg. The dogs lived at a private home and were kept in groups of seven dogs each in kennels with a free run. The dogs were given a commercial, dry, complete feed that, according to the declaration, was free of yeast ingredients. All dogs were individually fed, so that a controlled intake of the basic feed and the supplements took place. For a period of 30 days, **CeFi® pro**, at 1.5g per day and Leiber unextracted brewers' yeast at 3g per day were fed directly to the animals.

The following parameters were determined during the trial:

- Body Weight monitoring, feed intake during the entire period of the trial and acceptance
- Consistency of faeces and dry matter content of the faeces samples
- Examination of the intestinal microbiota: 16sRNA sequencing
- An examination of microbial metabolic activities based on the concentrations of short chain fatty acids (SCFA) in the faecal samples

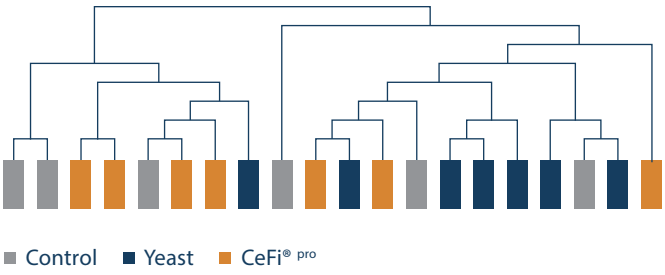
Results:

During the entire trial period, **CeFi®pro** and also pure brewers' yeast showed outstanding acceptance and no negative effects on body weight, faecal consistency or quantity of faeces.

The composition of the microbiota was considered on various levels, from strain (phylum) to order then down genus level.

With regard to diversity (similarity dendrogram), two groups emerged: as shown in figure 1, the left group with primarily **CeFi® pro**, and a right group with six of seven animals receiving brewers' yeast. In addition, four of seven animals in the **CeFi® pro** group formed a separate main cluster within the left group, while four of seven animals demonstrated a separate subcluster in the right group after the administration of brewers' yeast.

Figure 1: Pure brewers' yeast and **CeFi® pro** showed clear differences in the bacterial profile.



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In accordance with expectations, the phylum Firmicutes dominated at the upper level (phylum) in all three groups. Generally speaking, an increase in the Bacteroidetes emerged after the administration of unextracted brewers' yeast and **CeFi® pro** in comparison to the control. The order level (Table 1) showed a significant decrease in Clostridiales in the control group and in the **CeFi® pro** group. The **CeFi® pro** group showed a significant decrease in the Coriobacteriales, this was compensated by a clear increase in Lactobacillus. Furthermore, significant differences with unextracted brewers' yeast and **CeFi® pro** were apparent for Bacteriodales, and for Bifidobacteriales with **CeFi® pro**.

Table 1: Excerpt of relative abundance [%] of the dominant order in the faeces of dogs (n = 21)

	Control		Unextracted brewers' yeast		CeFi® pro	
	before	after	before	after	before	after
Clostridiales	**75.88 (± 7.76)**	**59.59 (± 15.40)**	72.51 (± 11.5)	69.27 (± 9.8)	**74.94 (± 4.34)**	**49.08 (± 17.41)**
Coriobacteriales	3.33 (± 2.47)	1.77 (± 1.51)	2.88 (± 2.12)	2.55 (± 1.14)	**5.14 (± 2.34)**	**2.52 (± 2.36)**
Lactobacillales	7.33 (± 6.88)	15.96 (± 15.81)	1.52 (± 1.11)	3.74 (± 2.87)	4.49 (± 2.75)	22.89 (± 19.67)
Bacillales	2.95 (± 2.56)	5.15 (± 2.87)	5.60 (± 3.83)	4.16 (± 3.00)	4.36 (± 4.28)	6.54 (± 4.06)
Bifidobacteriales	**0.004 (± 0.002)**	**0.44 (± 0.41)**	0.78 (± 0.89)	0.04 (± 0.03)	4.04 (± 6.51)	5.06 (± 5.76)
Erysipelotrichales	**3.24 (± 0.87)**	**0.89 (± 0.72)**	3.42 (± 1.85)	5.46 (± 2.66)	3.70 (± 2.09)	3.71 (± 3.12)
Fusobacteriales	3.40 (± 2.21)	2.42 (± 2.52)	3.88 (± 2.15)	4.54 (± 3.40)	1.56 (± 1.04)	2.74 (± 2.40)
Bacteroidales	**1.52 (± 1.73)**	**2.84 (± 2.17)**	2.60 (± 2.79)	7.94 (± 8.05)	1.34 (± 1.05)	5.03 (± 5.65)

(Significant differences within an experimental group are printed in bold; Mann-Whitney before/after; $p \leq 0.1$)

At the genus level (Table 2), the control group showed a significant decrease in Blautia sp. and a non-classified genus from the Lachnospiraceae family, and a significant increase in Lactobacillus sp. and Selenomonas sp. A significant decrease in Bifidobacterium sp. was observed with the administration of the unextracted brewers' yeast. A significant decrease in Peptostreptococcus sp. and Hespellia sp. was observed after the administration of **CeFi® pro**. In the **CeFi® pro** group, the increase in Lactobacillales at the order level could be traced back to the drastic increase in the genus Lactobacillus. A tendency to an increase in the Bifidobacterium was determined with the administration of **CeFi® pro**, and a tendency to a decrease in Clostridium and Collinsella.

Table 2: Excerpt of the relative abundance of the dominant genera in the faeces of dogs (n = 21)

	Control		Unextracted brewers' yeast		CeFi® pro	
	before	after	before	after	before	after
Clostridium	31.04 (± 10.02)	31.11 (± 6.26)	30.66 (± 10)	29.54 (± 6.08)	29.20 (± 5.99)	24.11 (± 10.62)
Blautia	**17.87 (± 2.53)**	**10.15 (± 4.74)**	11.98 (± 7.17)	12.25 (± 6.08)	13.79 (± 6.86)	9.34 (± 2.88)
Collinsella	3.01 (± 2.30)	1.52 (± 1.32)	2.47 (± 1.9)	2.22 (± 1.01)	4.45 (± 1.93)	2.37 (± 2.28)
Lactobacillaceae	**7.29 (± 6.89)**	**15.29 (± 16.02)**	1.32 (± 1.11)	3.43 (± 2.81)	4.44 (± 2.75)	22.63 (± 19.79)
Hespellia	5.26 (± 4.31)	0.64 (± 0.50)	3.08 (± 3.2)	3.79 (± 3.87)	**4.37 (± 3.56)**	**2.27 (± 2.71)**
Bifidobacterium	0.004 (± 0.002)	0.44 (± 0.41)	**0.78 (± 0.89)**	**0.035 (± 0.03)**	4.04 (± 6.51)	5.06 (± 5.76)
Unclassified Erysipelotrichaceae	3.23 (± 0.87)	0.89 (± 0.73)	3.41 (± 1.85)	5.46 (± 2.66)	3.69 (± 2.09)	3.69 (± 3.12)
Peptostreptococcus	0.21 (± 0.16)	0.12 (± 0.11)	0.14 (± 0.1)	0.14 (± 0.11)	**2.64 (± 4.62)**	**0.11 (± 0.07)**
Bacteroidetes	1.29 (± 1.31)	2.46 (± 1.88)	1.99 (± 1.96)	6.21 (± 6.84)	0.98 (± 1.05)	3.86 (± 4.14)
Unclassified bacteria	1.75 (± 2.17)	10.43 (± 16.35)	2.46 (± 2.21)	0.62 (± 0.49)	0.7 (± 0.73)	0.52 (± 0.57)
Prevotella	0.27 (± 0.44)	0.24 (± 0.26)	0.45 (± 0.82)	1.39 (± 1.1)	0.32 (± 0.36)	0.96 (± 1.41)
Selenomonas	**0.11 (± 0.18)**	**0.36 (± 0.39)**	0.24 (± 0.29)	0.55 (± 0.54)	0.21 (± 0.22)	0.10 (± 0.12)
Unclassified Lachnospiraceae	**0.23 (± 0.21)**	**0.057 (± 0.041)**	0.32 (± 0.33)	0.17 (± 0.1)	0.19 (± 0.22)	0.14 (± 0.19)
Peptococcus	0.24 (± 0.33)	0.30 (± 0.47)	0.71 (± 0.64)	0.41 (± 0.26)	0.087 (± 0.092)	0.25 (± 0.19)

(Significant differences within an experimental group are printed in bold; Mann-Whitney before/after; $p \leq 0.1$)

The distribution pattern for the modular portion of the short chain fatty acids (SCFA) showed several interesting differences between unextracted brewers' yeast and **CeFi® pro** (Table 3). A noticeably significant increase in the lactate concentration emerged after the administration of **CeFi® pro**. This verified the visible increase in lactobacillus on the order level and the drastic numeric increase in the genus Lactobacillus upon examination of the intestinal microbiota through 16sRNA sequencing. Compared to the other groups, the administration of the unextracted brewers' yeast showed a significant increase in the acetic acid concentration (acetate).

Table 3: Dry substance content and bacterial metabolites in the faeces of dogs [g/kg or µmol/g sample]

	Control		Unextracted brewers' yeast		CeFi® pro	
	before	after	before	after	before	after
DS [g/kg]	353.6 (± 92.6)	286.7 (± 46.9)	348.0 (± 47.9)	420.7 (± 60.2)	384.7 (± 152.7)	297.1 (± 77.4)
L-lactate	**0.3 (± 0.3)**	**1.9 (± 1.5)**	0.2 (± 0.3)	0.5 (± 0.4)	**0.7 (± 0.9)**	**3.6 (± 4.1)**
D-lactate	**0.1 (± 0.1)**	**1.2 (± 1.1)**	**0.1 (± 0.2)**	**0.4 (± 0.3)**	**0.5 (± 0.8)**	**3.4 (± 4.3)**
Total lactate	**0.4 (± 0.4)**	**3.2 (± 2.6)**	0.6 (± 0.4)	1.1 (± 0.5)	2.3 (± 1.6)	7.0 (± 8.4)
Acetate	87.3 (± 2.9)	78.5 (± 15.7)	**63.0 (± 14.1)**	**88.0 (± 15.6)**	79.3 (± 13.7)	69.2 (± 16.5)
Propionate	28.5 (± 4.5)	20.2 (± 9.8)	20.1 (± 7.1)	27.8 (± 6.9)	29.2 (± 10.6)	24.1 (± 9.4)
Total SCFA	135.3 (± 6.5)	114.6 (± 22.8)	**99.0 (± 22.7)**	**137.5 (± 28.1)**	128.0 (± 15.2)	110.7 (± 34.4)

(Significant differences within an experimental group are printed in bold; Mann-Whitney before/after; $p \leq 0.1$)

Discussion:

Compared to Leiber® unextracted brewers' yeast, **CeFi® pro** (autolysed brewers'yeast) showed a signi-ficant decrease in bacteria of the order Clostridiales, which at the genus level led to significant reductions in the dominant Clostridiales genera Hespellia and Pepto-streptococcus. Compensating this, the genus lactobacillus clearly gained in significance and this was also shown by a significant increase in lactate concentrations.

Clostridiales are the dominant order in the lower digestive tract of many animals. They include many species that are important for the utilization of undigested carbohydrates. The fermentation of carbohydrates leads to the release of metabolites such as acetate (pH reduction) or n-butyrate (substrate for epithelial cells), which is assessed overall as being positive. Moreover, the order Clostridiales also includes a series of pathogenic species or species that lead to increased protein fermentation. A reduction in Clostridiales is also not to be equated per se with a "healthy" microbiota. Compensating the reduction in Clostridiales in the **CeFi® pro** group, there was a drastic increase in Lactobacillales, more precisely of the genus Lactobacillus. Lactobacilli are generally assessed as being positive because they do not include pathogenic species and are known to suppress pathogenic species through the production of lactate and other antibacterial substances. It is furthermore known that some species interact with the host immune system. Lactobacillus sp. are infrequently dominant in the lower digestive tract where they do dominate in the upper digestive tract which is the site of action of many pathogenic bacteria and viruses. All essential digestive processes also take place there. An increase in Lactobacilli in the faeces indicates that the administration of **CeFi® pro** resulted in a change in the microbiota in the upper digestive tract in contrast to unextracted brewers' yeast. One reason for this is assuredly the extraction procedure (autolysis) of the yeast cells in **CeFi® pro**, which, in contrast to the normal Leiber unextracted brewers' yeast, provides bacteria with specific growth factors such as amino acids, peptides and nucleotides. That **CeFi® pro** is active in particular in the upper digestive tract is additionally clear through the changes in the dominant genera of the lower digestive tract, which showed a significant reduction in the genus Peptostreptococcus in the **CeFi® pro** group as compared to the administration of Leiber unextracted brewers' yeast. Peptostreptococcus sp. are strictly peptidolytic as they ferment only protein or peptides

and amino acids. A reduction in Peptostreptococcus sp. with **CeFi® pro** shows that the growth conditions for Peptostreptococcus sp. have deteriorated. The fermentation of proteins in the lower digestive tract is undesirable because of the possible production of biogenic amines, ammonium or non-utilizable metabolites. **CeFi® pro** provides great added value here in terms of the digestive physiology and relieves the metabolism.

Additionally, **CeFi® pro** contains immunologically active substances such as β-glucans. The immune effect of various Leiber brewers' yeast products was examined in an *in vitro* study of canine cells (Leiber GmbH 2017). Corresponding to their natural content of 1.3/1.6-β-D-glucans, the brewers' yeast products showed variable activity in the canine immune system here. While highly purified β-glucans such as **Leiber® Beta-S** showed immunomodulating effects on the innate and on the adaptive immune system, **CeFi® pro** in particular positively influenced the interleukins, such as IL-6, IL-8 und IL-17A. Among others, interleukins serve as markers of inflammation (IL-6) and are liberated in acute inflammations, or as a key element (IL-8) in chronic inflammatory reactions because it attracts inflammatory cells to the location of the infection, or also as a signalling cytokine (IL-17A) that is associated with various autoimmune diseases such as chronic inflammatory skin disease or IBD (inflammatory bowel disease). **CeFi® pro** was able to reduce all three proinflammatory interleukins by 200 µg/ml and thus reduce inflammatory reactions.

Conclusions:

In comparison to the use of unextracted brewers' yeast, **CeFi® pro** demonstrated clearer and different types of changes in the faecal microbiota in dogs. The main effect relates to the drastic increase in lactic acid bacteria, in particular lactobacillus sp. An increased occurrence and activity of lactobacilli is to be assessed basically as a positive change in the microbiota. An increase in Lactobacilli in the faeces along with a simultaneous decrease of strictly peptidolytic bacteria shows that **CeFi® pro** is digested in the upper digestive tract, unextracted brewers' yeast, in contrast, is more likely to be digested in the lower digestive tract. In contrast to normal, unextracted brewers' yeast, **CeFi® pro** provides specific growth factors such as amino acids, peptides and nucleotides.

Leiber CeFi® pro

- ✓ clearly difference in bacterial profile (diversity)
- ✓ drastic increase in Lactobacillaceae e.g. Lactobacillus
- ✓ significant increase in lactate concentration
- ✓ significant reduction in peptidolytic bacteria e.g. Peptostreptococcus
- ✓ reduced risk production of biogenic amines and ammonium
- ✓ digested in the upper digestive tract
- ✓ reduced proinflammatory interleukines IL-6, IL-8 and IL-17A
- ✓ provides specific growth factors: amino acids, peptides and nucleotides

Reference: Free University of Berlin, Department of Veterinary Medicine, Institute for Animal Nutrition, Prof. Dr. Jürgen Zentek (2019)

Literature on request.

For more information:

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