

## EFFICACY OF *SACCHAROMYCES BOULARDII* AS A PROBIOTIC IN DOGS WITH LINCOMYCIN INDUCED DIARRHOEA

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### Abstract

Twenty-four dogs were divided into three equal groups. Lincomycin was administered intramuscularly to all groups. However, group II was given *S. boulardii* after lincomycin induced diarrhoea occurred, and in group III, lincomycin was administered together with *S. boulardii*. Lincomycin caused diarrhoea in groups I (in 7.8±1.6 d) and II (in 6.9±2 d), but it did not cause diarrhoea in group III. The durations of diarrhoea were significantly different ( $P<0.05$ ) between group I (6.5±1.2 d) and group II (2.9±0.4 d). The total short chain fatty acid (TSCFA) level was significantly lower ( $P<0.001$ ) compared with their baselines in groups I and II at the time of the diarrhoeas' onset and cessation. Additionally, there were differences between groups at the time of diarrhoea cessation and 1 week after the treatment ( $P<0.05$ ). In conclusion, lincomycin decreased TSCFAs causing diarrhoea in the dogs when given alone, and *S. boulardii* was effective in treating lincomycin induced diarrhoea and to prevent the occurrence of diarrhoea when given together with lincomycin.

**Key words:** dog, diarrhoea, *Saccharomyces boulardii*, lincomycin, short chain fatty acids.

Antibiotics may cause side effects as seen with other drugs (14). Diarrhoea as a complication is observed in 5%-25% of humans who take antibiotics (3). Ampicillin, lincomycin, clindamycin, and cephalosporins were reported as a cause of diarrhoea (27). Some antibiotics and chemicals cause the deterioration in normal intestinal flora and result in the growth of some pathogenic microorganisms (4, 24). *Clostridium difficile* (*Cl. difficile*), growing faster than other pathogens existing in the colon, has been the most frequently seen pathogen in antibiotic induced diarrhoea (24). Besides, it was noted that the other pathogens (3%) cause diarrhoea as well (23).

*In vitro* studies showed that the short chain fatty acid (SCFA) values decreased, when most of the

antibiotics reach sufficient concentration levels in the colon (19). By decreasing the SCFA content, absorption of water and electrolytes decrease and diarrhoea occurs. When the level of carbohydrates that cannot be absorbed in the intestine lumen increases, they take water by an osmotic effect and cause diarrhoea (38).

*Saccharomyces boulardii* (*S. boulardii*), a member of *Saccharomycetaceae* family, is yeast that has an ability of suppression and fermentation of the carbohydrates showing Gram<sup>+</sup> staining property. As yeast, it is resistant to antibiotics showing a difference from probiotics of a bacterial origin. It is not affected by gastric acid and bile secretion (12). *S. boulardii* competes for food and mucosal receptors with pathogenic microorganisms in the intestine lumen (15), and destroys bacterial toxins and their receptor sides by releasing proteases (11).

In humans, one of the treatments of choice is *S. boulardii* as a probiotic for the therapy of antibiotic induced diarrhoea (5, 37). An increase in aminopeptidase and disaccharidase levels was detected in rats given *S. boulardii* (9). However, to our knowledge there is no report on the use of *S. boulardii* as a probiotic in such cases in dogs. The purpose of this study was to investigate the efficacy of *S. boulardii* in dogs with lincomycin induced diarrhoea.

### Material and Methods

The Ethic Committee of Animal Care and Use in the Faculty of Veterinary Medicine in Ankara University approved this experimental protocol (Decision No. 2004/11). Different breeds of dogs, 14 male and 10 female, aging 1-5 years were used in the study. Vaccination and anti-parasite drug administration were applied to all dogs. The dogs were fed a commercial dog feed and had water *ad libitum*.

The dogs were divided into three equal groups. Dogs in the groups I and II were injected intramuscularly (i.m.) with 150 mg/kg/d of lincomycin (Linkoles, Aroma, Turkey) until diarrhoea occurred. After the onset of diarrhoea, group II was given 1000 mg/d of *S. boulardii* (Reflor, Dif, Turkey) orally for ten days. Group III was given i.m. for ten days 150 mg/kg/d of lincomycin together with an oral dose of 1000 mg/d of *S. boulardii*.

Sampling and clinical examinations in groups I and II were carried out four times, *i.e.* before the experiment (baseline), just after the diarrhoea occurred (onset of diarrhoea) and stopped (diarrhoea cessation), and a week after the diarrhoea stopped (1 week after treatment). Sample collection and examinations in group III was performed twice, *i.e.* before the experiment (baseline) and on the 10<sup>th</sup> d of the study. Clinical findings included respiration and pulse rates, body temperature, appearance, and the existence of diarrhoea, dehydration, appetite, and behaviour. Haemogram, SCFAs, and *Cl. difficile* toxins (Ridascreen *Cl. difficile* toxin-A/B EIA) were analysed in faeces on the related days. The isolations of pathogenic microorganisms such as *E. coli*, *Shigella*, *Salmonella*, and *Campylobacter* were performed by culturing the faeces. The faecal smears were examined for possible spores of *Cl. perfringens* as well (25).

The analyses of total short chain fatty acids (TSCFAs) were carried out using gas chromatography (Agilent Technologies 6890 Network GC system). A sample of 2-5 g faeces was homogenised with 4 ml of 2-ethylbutiric acid (3 mmol/L) and 1 ml of H<sub>2</sub>SO<sub>4</sub> (0.5 mmol/L), and then the vacuum distillation was performed. Two micro litre samples were used by an automatic integrator to obtain the peak values of each short chain fatty acid. Comparing the peak values of standards and samples each to other, TSCFAs were calculated in mmol/L units (21). TSCFAs included all of the SCFA measurements.

Statistical analysis was done using a One-Way and Two-Way ANOVA test, incorporating a repeated measures design and if indicated by a significant F-statistic, differences in specific means were sought by a Tukey's post hoc test requiring a P<0.05 for significance.

## Results

Six out of 8 dogs (75%) in the group I, 7 (87.5%) in group II, and none in group III, developed diarrhoea. The time for the onset of diarrhoea after lincomycin administration and duration of diarrhoea for groups I and II, are shown in Table 1. The time for the onset of diarrhoea in both groups was noted as similar. The duration of diarrhoea in group II was significantly (P<0.05) shorter than that of group I, showing the effect of the treatment.

**Table 1**

Mean days for the onset of diarrhoea after lincomycin administration and duration of diarrhoea in groups I and II

Time	Group I	Group II
Onset of diarrhoea	7.8 ± 1.6	6.9 ± 2.0
Duration of diarrhoea	6.5 ± 1.2	2.9 ± 0.4

±: standard deviations of the means

In the physical and haematological examination of the groups I and II, no abnormalities were detected, except for diarrhoea; and any abnormalities (data are not shown) were displayed in group III. No *Cl. difficile* toxin was found, and no pathogen isolation took place in any of the groups throughout the experiment.

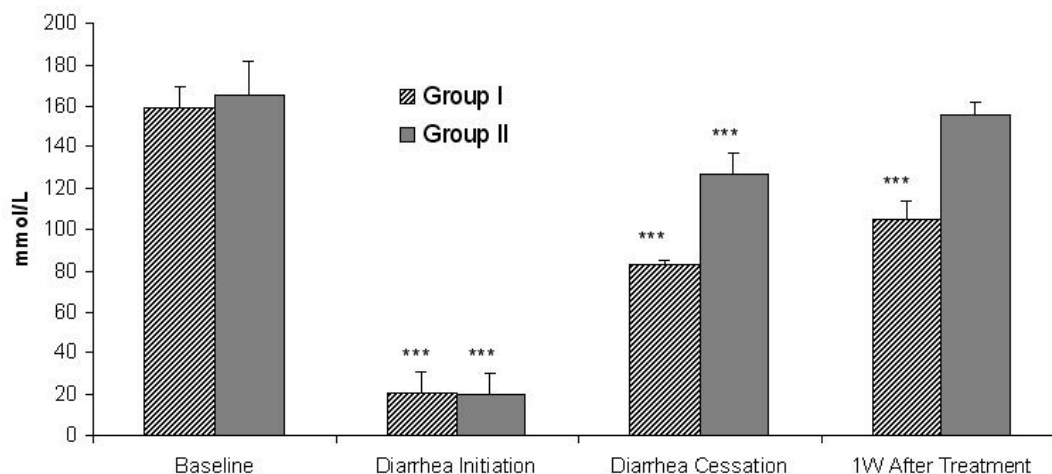
The mean values of TSCFAs in groups I and II are shown as the time-points of baseline, onset, and cessation of diarrhoea, and 1 week after the treatment (Fig. 1). The mean values of TSCFAs in both groups decreased significantly (P<0.001) at the time of the onset of diarrhoea and slightly increased with the same manner during the course of the experiment for up to 1 week after the treatment. However, TSCFA levels in the group I was lower than its baseline 1 week after the treatment, while that in the group II reached the steady position of its baseline (P=0.127). When comparing both groups, the values of TSCFAs were higher in group II than in group I at the time-points of diarrhoea cessation and 1 week after the treatment (P<0.05).

The mean values of TSCFAs in group III were demonstrated for the time-points before the experiment (baseline) and on the 10<sup>th</sup> d of its course (Fig. 2). There were no statistically significant differences between the time-points of data collection (P=0.1).

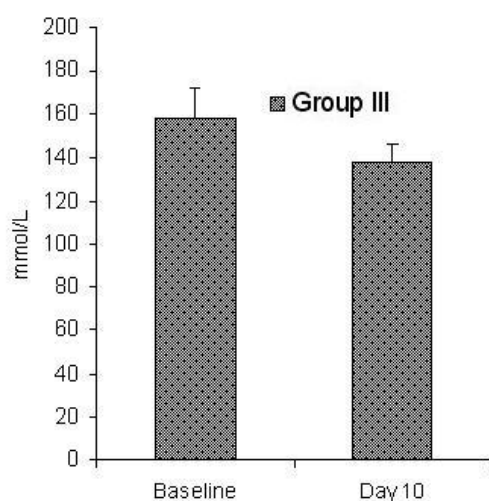
## Discussion

Berezin (4) reported that diarrhoea might usually develop in 3-9 d after commencement of the administration of antibiotics. Most of the dogs in the presented study exhibited diarrhoea in the first and second group as a side effect of lincomycin. A significant difference (P<0.05) occurred between both groups concerning the duration of diarrhoea, suggesting that *S. boulardii* is an effective cure for the induced diarrhoea.

Various antibiotics given to hamsters (6), pigs (36), bears (30), penguins (18), mares and their offspring, adult horses, rats (2), rabbits (33), and stray dogs (28) cause diarrhoea, and in some cases, *Cl. difficile* toxins have been isolated as an active agent. Some researchers (7, 16, 34, 40) claimed that *Cl. difficile* toxins were rarely encountered in diarrhoea caused by antibiotics in dogs. In this study, *Cl. difficile* toxins were not isolated in the cases of diarrhoea caused by lincomycin. Boon and Beale (6) indicated that a dose related *C. difficile* toxin appeared in a hamster.



**Fig. 1.** Mean values of total short chain fatty acids of baseline, diarrhoea initiation, diarrhoea cessation, and 1 week after treatment. \*\*\*  $P < 0.001$  compared with the baseline in each group. The bars represent standard deviations of the means.



**Fig. 2.** Mean values of total short chain fatty acids of baseline and 10<sup>th</sup> d of the experiment. The bars represent standard deviations of the means.

It has also been mentioned that, apart from *Cl. difficile*, some other pathogenic organisms could cause diarrhoea by multiplying (3%) themselves in the intestines. *E. coli*, *Salmonella*, *Campylobacter*, *Cl. perfringens*, and *Shigella* sp. have been considered as enteric pathogenic microorganisms that could cause diarrhoea in dogs (26). The fact that more than five *Cl. perfringens* spores were found in a microscopic immersion field of faecal smears obtained from the dogs with diarrhoea; suggests that diarrhoea may be associated with *Cl. perfringens* (39). Any faecal pathogenic microorganism did not multiplied in the cultures and no *Cl. perfringens* spores was detected in any of the faecal smears in the dogs given lincomycin.

Further studies in dogs should be addressed to highlight the effects of a longer duration and/or a higher dosage of lincomycin than the experimental design was used here, in order to reveal the potential existence of pathogenic organisms given above.

Acute infections, foreign proteins, tissue damage, blood loss, and bacterial and chemical toxins would increase the number of white blood cells, especially neutrophils, particularly in acute infections (29). Furthermore, haematological findings of this study were within normal limits in all of the groups during the course of the experiment.

Hove *et al.* (19) demonstrated that TSCFAs in faeces of a man taking antibiotics were below 10 mmol/L. In addition, Hoverstad *et al.* (20) reported that after application of clindamycin in man, TSCFA values declined from 62.9 to 7.3 mmol/L during the treatment, after using ampicillin from 62.4 to 47.8 mmol/L, there were no significant differences between groups given metronidazol. Similarly, Rao *et al.* (32) found that TSCFA values decreased in humans given vancomycin and bacitracin. Furthermore, Gustafsson *et al.* (17) found that TSCFA values were 14.46 mmol/L in patients positive for *Cl. difficile* toxin and with diarrhoea due to antibiotic usage, and 9.15 mmol/L in patients with diarrhoea due to antibiotic usage, but negative for *Cl. difficile* toxin. They also found TSCFA values to be much lower in patients with diarrhoea due to antibiotic usage than in healthy individuals. In accordance with the findings of a previous reports (17, 19), TSCFA values of groups I and II on the day of the onset of diarrhoea were low, as compared with their baseline ( $P < 0.001$ ).

Wisker *et al.* (41) found out that succinate and lactate may be converted into TSCFAs in the colon in cases of diarrhoea in pigs caused by antibiotics. Sucrose, maltase, and lactase disaccharide activities were found to be increased in 7 healthy men given lyophilised *S. boulardii* (8) and sucrose and maltase activities in rats given 100 mg/d of *S. boulardii* (10). Zaouche *et al.* (42)

claimed that significant increases occurred in enzymatic activities of sucrose, glucoamilase, and n-aminopeptidase in rats given *S. boulardii*. Yeast cultures have an appetising effect with their attractive natural taste. They contain vitamin B and an unknown growth factor as well as separate vitamins from the chelate form making them ready for the absorption, secreting such digestive enzymes as protease, lipase, proteinase invertase, cellulase, initiating growth of cellulotic bacteria, and synthesising acetate, which is a pre-substance for TSCFAs in fermentation (1, 13). The efficacy of *S. boulardii* in dogs with lincomycin induced diarrhoea in this study, may be explained by the above-mentioned reports, as seen in humans. It has been established that oral use of *S. boulardii* in humans and rats caused a notable increase in both specific and total activities of enzymes without causing any changes in intestinal mucosal membrane, and these enzymes enable breaking indigestible carbohydrates into TSCFAs, making them absorbable.(22, 35, 37). In the presented study, the TSCFA values in group I were found to be rather low ( $P<0.001$ ), while values in group II given *S. boulardii* reached the values of its baseline 1 week after the treatment ( $P=0.127$ ). When the TSCFA values in dogs of groups I and II were compared, it was found out that there were no significant differences ( $P=0.336$ ) of the baseline and on the day of the onset of diarrhoea ( $P=0.878$ ). This data shows that the induction of diarrhoea fits well between groups. It was also established that there were significant differences between the day of diarrhoea cessation ( $P<0.05$ ) and one week after treatment ( $P<0.05$ ), which demonstrate the efficacy of the probiotic used in the study.

Rao *et al.* (32) and Hove *et al.* (19) mentioned that TSCFA values, which decreased as a result of the deterioration of carbohydrate metabolism in diarrhoea, caused by antibiotics, and would reach its normal level in 1-5 weeks. In this study, it was established in group I, one week after the diarrhoea ceased that TSCFA values were still low compared with the baseline, while they reached their baseline in the second week. *S. boulardii* as a yeast is said to be resistant to antibiotics, which makes it different from other probiotics of a bacterial origin. It is also said not to be affected by gastric acid and bile secretion and does not change its efficiency and activities (12, 31, 37). In this research, TSCFA values in group III, given *S. boulardii* and lincomycin together, were found to be decreased insignificantly ( $P=0.1$ ) from the baseline ( $157.5\pm 14.6$  mmol/L) to the 10<sup>th</sup> d ( $138\pm 8.1$  mmol/L). Since the TSCFA values in group III did not decrease significantly enough to induce diarrhoea, *S. boulardii* could not accomplish reducing the TSCFA values for preventing diarrhoea.

In conclusion, as it was demonstrated, a decrease in TSCFA content causes diarrhoea in the dog treated with lincomycin. Additionally, *S. boulardii* as probiotic was an effective cure for diarrhoea and to prevent its occurrence when given together with lincomycin.

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