Effects of live yeast dietary supplementation on nutrient digestibility and fecal microflora in beagle dogs

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ABSTRACT: The effects of live yeast Saccharomyces cerevisiae (strain CNCM I-4407; Actisaf Sc 47; Phileo Lesaffre Animal Care, Marcq-en-Barœul, France) administration on nutrient digestibility and fecal microflora in dogs were investigated. The study included 24 young beagle dogs. They were allocated in control and live yeast (LY) groups (6 males and 6 females in each). During the Adaptation (d 1 to 28) and Trial (d 29 to 70) periods, the dogs received a standard dry pelleted diet. In the Trial period, the LY dogs were given capsuled Actisaf Sc 47 at 1 g/kg live weight with Saccharomyces cerevisiae at $2.9 \times 10^8$ cfu/g. The control dogs received empty capsules. Live weight and feed consumption were recorded. Blood samples for complete blood count (CBC) and serum biochemistry (urea, creatinine, alkaline phosphatase, and alanine aminotransferase) and fecal samples for pH, microbiology, DM, lactic acid, and ammonia and digestibility evaluation were collected during the Trial period from each dog. The LY dogs had a higher ($P < 0.05$) weight gain during the Trial period than the control ones. Feed consumption was not adversely affected by LY. The CBC values and urea, creatinine, alkaline phosphatase, and alanine aminotransferase were not adversely affected by LY. Live yeast did not significantly influence pH of fresh feces. Fecal lactic acid and ammonia concentrations were not affected. The LY dogs showed lower ($P < 0.05$) Escherichia coli and fecal enterococci counts in feces than the control ones. Lactic acid bacteria, Clostridium perfringens, and total coliforms did not show any significant differences between the treatments. The LY dogs showed a higher ($P < 0.05$) apparent digestibility of NDF. Digestibilities of DM, ash, crude fiber, CP, and fat were not influenced.

Key words: dogs, feed supplement, live yeast, nutrient digestibility, Saccharomyces cerevisiae

INTRODUCTION

Live cultures of yeast Saccharomyces cerevisiae (live yeast [LY]) have been used as feed supplements in various animal species for their ability to support beneficial digestive microflora and suppress the growth of pathogens, thereby contributing to the improvement of performance and health of the host animal. There have been numerous reports of favorable effects of LY in ruminants, horses, swine, poultry, and rabbits (Bontempo et al., 2006; Jouany et al., 2008; Thrune et al., 2009; Agazzi et al., 2009; Monroy-Salazar et al., 2012; Kimsé et al., 2012; Trckova et al., 2014).

In ruminants, LY increases ruminal counts of cellulolytic microflora, improves ruminal anaerobiosis, and supports lactic acid utilizers while reducing lactic acid producers (Newbold et al., 1995). Live yeast favorably influences the ruminal digestion and contributes to pH stabilization (Marden et al., 2008; Thrune et al., 2009). In horses, LY was reported to increase digestibility of fiber fractions and improve stability of microflora in the large gut and cecum (Medina et al., 2002; Jouany et al., 2008; Agazzi et al., 2009). In rabbits, reduced mortality was observed as a result of LY supplementation (Kimsé et al., 2012). Live yeast is an important dietary supplement in pigs. Live yeast
supplements have been reported to support growth rate in piglets and reduce the occurrence of postweaning di­arrhea and increase immunoglobulin contents in sow’s milk (Jurgens et al., 1997; Monroy-Salazar et al., 2012; Jang et al., 2013). Live yeast and yeast derivatives also reduced the growth of intestinal pathogens and their adherence to the intestinal mucosa (Badia et al., 2010; Kiarie et al., 2011; Trckova et al., 2014).

So far, little has been published on effects of LY sup­plementation in dogs and cats. Middelbos et al. (2006, 2007) investigated the effect of spray-dried S. cerevisiae cell wall in dogs. They observed increased nutrient di­gestibilities and reduced Escherichia coli counts.

The aim of this study was to evaluate the effect of dietary supplementation with LY S. cerevisiae CNCM I-4407 on nutrient digestibility and fecal microflora in dogs fed a high-fiber diet.

**MATERIAL AND METHODS**

The study was performed at MediTox s.r.o., Konárvice, Czech Republic. The experiment included 24 healthy beagle dogs, 12 males and 12 females, at 5 to 9 mo of age, with initial BW ranging from 7.2 to 12.3 kg. The number and age of the animals used in this study complied with the Organisation for Economic Co-operation and Development 409 principles (OECD, 1998) to meet scientific and regulatory guidelines for this type of study. The dogs were handled in compliance with European Union Directive 86/609/EEC (European Council Directive, 1986) on the protection of animals used for experimental and other scientific purposes (European Treaty Series, 1986), Act number 246/1992 Coll. of Laws of the Czech Republic on the protection of animals against cruelty as amended.

**Animals and Housing**

The dogs were individually housed in stainless steel cages in a room with a controlled climate (air temperature 15–21°C, relative humidity 30–70%, and 12 h of light/d). The cages were cleaned daily, and feed and water bowls were sanitized twice a week. Each dog was ear tattooed for identification. The experimental dogs had been vaccinated with a combined vaccine against parvoviroisis, canine distemper, leptospirosis, parainflu­enza, canine hepatitis, and rabies and dewormed.

**Table 1.** Chemical composition of the diet\textsuperscript{1,2} fed to the young beagles from d 1 to 70

<table>
<thead>
<tr>
<th>Item</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>91.17</td>
</tr>
<tr>
<td>CP, %</td>
<td>27.96</td>
</tr>
<tr>
<td>Fat, %</td>
<td>11.16</td>
</tr>
<tr>
<td>Crude fiber, %</td>
<td>8.52</td>
</tr>
<tr>
<td>NDF, %</td>
<td>18.86</td>
</tr>
<tr>
<td>Ash, %</td>
<td>8.82</td>
</tr>
<tr>
<td>Calcium, g/kg</td>
<td>14.6</td>
</tr>
<tr>
<td>Phosphorus, g/kg</td>
<td>15.3</td>
</tr>
<tr>
<td>Copper, mg/kg</td>
<td>18.6</td>
</tr>
<tr>
<td>ME, kcal/kg</td>
<td>3,226.7</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Ingredient composition of the diet: wheat flour, wheat bran, pork meat and bone meal, pork lard, palatability enhancer, and preservative.

\textsuperscript{2}Added vitamins and minerals: 14,600 IU/kg vitamin A, 1,450 IU/kg vita­min D\textsubscript{3}, 130 mg/kg vitamin E (alpha tocopherol), 160 mg/kg vitamin E (alpha tocopherol), 0.4 mg/kg I (potassium iodide), 0.3 mg/kg Co (cobalt sulfate heptahydrate), 19.7 mg/kg Cu (copper sulfate pentahydrate), 80 mg/kg Mn (manganese oxide), 80 mg/kg Zn (zinc oxide), and 0.16 mg/kg Zn (zinc oxide).

\textsuperscript{3}NfE = nitrogen-free extract.

**Experimental Plan**

The entire experiment lasted 70 d, consisting of the Adaptation period (d 1 to 28) and the Trial period (d 29 to 70).

**Diet and Feeding**

During the Adaptation and Trial periods, the dogs received a dry pelleted diet with an increased propor­tion of fiber (manufactured by Delikan, s.r.o., Lipník nad Bečvou, Czech Republic) at a daily dose of 30 g/l kg of live weight. Individual daily doses were calcu­lated according to BW values measured weekly. The diet composition and analytical values are given in Table 1. The dogs were fed once a day, between 0730 and 0830 h. The diet given to the dogs prior to the experiment (Delikan, s.r.o.) contained 34% CP, 20% crude fat, 4.4% ash, and 2.9% crude fiber.

**Water**

Water of monitored quality was provided ad libi­tum. Water analyses were performed routinely twice a year to avoid toxic and microbial contamination. The following parameters were evaluated: coliform bac­teria, E. coli, color, turbidity, pH, conductivity, chemi­cal oxygen demand, Mn, Fe, ammonium ions, nitrates, nitrites, odor, and taste (Water Management Company Vrchlice-Maleč, a.s. Kutná Hora, Czech Republic). The water quality has been consistent in the long term.
**Dietary Treatments**

On d 21, the dogs were weighed and, according to their live weight and sex, randomized into 2 groups: LY (live yeast supplemented) and control. Twelve animals were allocated in each group (6 males and 6 females).

**Administration of Live Yeast.** From the start of the Trial period (d 29), the LY dogs received gelatinous capsules filled with Actisaf Sc 47, batch number 3640 (Phileo Lesaffre Animal Care, Marcq-en-Barœul, France), at a dose of 1 g/kg of live weight daily that contained LY *S. cerevisiae* CNCM I-4407 at $2.9 \times 10^8$ cfu/g. The dosage was modified according to the dogs’ BW measured weekly. The control dogs received empty capsules. The capsules were deposited on the root of tongue and each animal was watched until ingestion was completed. The dogs received their regular meal after the capsule ingestion.

**Live Weight and Feed Consumption**

During the whole experiment the dogs were weighed once a week (d 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, and 70). Feed consumption was recorded daily.

**Health Status**

During the Adaptation period, the dogs’ health status was evaluated once a day, and during the Trial period, the dogs’ health status was evaluated twice a day. The general evaluation included general appearance, mentation, posture and gait, appetite, and presence of clinical signs. A thorough physical examination was performed on the evaluation of general health status; mentation; rectal temperature; skin; muscle–skeletal, respiratory (thoracic auscultation), genital–urinary, digestive, neurologic, and cardiovascular systems; eyes; and visible mucosae was performed on d 1, 8, 29, and 70. The physical examination results were recorded.

**Complete Blood Count and Serum Biochemistry**

Complete blood count (CBC) and serum biochemistry analyses were performed on d 1, 28, and 70 to assess the health status of the dogs.

**Sample Collection.** Blood samples were taken by venipuncture from the vena cephalica antebrachii or vena saphena lateralis of each dog. For CBC, blood was collected into 2 Vacuette Greiner Bio-one tubes (Greiner Bio-One GmbH Kremsmünster, Austria), 1 of those containing K$_{3}$EDTA. For chemistry analyses, blood was placed in Tapval tubes (J. P. Selecta, s.a. Abrera (Barcelona), Spain), which did not contain anticoagulation agents. Serum was obtained via blood centrifugation at 6,000 rpm for 15 min $3622 \times g$, 20°C. Serum for biochemistry analyses was transferred into Eppendorf tubes (Eppendorf Czech & Slovakia s.r.o. Ričany u Prahy, Czech Republic), appropriately labeled, and sealed. The serum samples were frozen at −20°C.

**Hematology.** The CBC was performed, including red blood cells (RBC), hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC), and platelet count (PLT). Analyses were done with the automatic hematological analyzer Vet ABC Animal Blood Counter (ABX International S.A., Montpellier, France).

Differential leukocyte counts were determined by a microscope on peripheral blood smears dyed with May–Grunewald and Giemsa–Romanovski stains.

The hematological analyses were performed in the laboratory of hematology and biochemistry of the company MediTox s.r.o.

**Biochemistry.** Biochemistry analyses included urea, creatinine, alkaline phosphatase (ALP), and alanine aminotransferase (ALT), using the biochemistry analyzer Diimension RxL, Dade Behring (Siemens Healthcare Diagnostics Inc., Newark, DE 19714,). The analyses were performed at the Department of Clinical Biochemistry of the Pardubice Hospital, Pardubice, Czech Republic.

The reference ranges for hematology and biochemistry parameters used to evaluate the health status were based on The Merck Veterinary Manual (Kahn and Line, 2010).

**Fecal Analyses**

**Collection of Stools.** Stool samples for microbiology (about 10 g) were taken, during the Trial period, from each dog once a week (d 29, 36, 43, 50, 57, 64, and 70), at hourly intervals, from 0730 to 1330 h. Immediately after the collection, each stool specimen was covered with 25% aqueous solution of glycerol as cryoprotectant, divided into 2 aliquots, and frozen at −18°C. One of the aliquots was sent to the microbiological laboratory (Laboratoires Biocéane, Le Havre, France) on dry ice. The second aliquot was stored as a backup at MediTox s.r.o.

Stool samples (80–100 g) for determination of DM, VFA, lactic acid, and ammonia were collected during the trial period from all the dogs on the same days as those for microbiological examination. The samples were placed in plastic tubes, frozen, and stored at −18°C until the laboratory analyses by SOS s.r.o. (Skalice nad Svitavou, Czech Republic).

**Fecal pH.** All the fresh stool samples collected were subjected to pH measurement. Before each measuring session, the pH meter was calibrated with pH 4 and pH 7 buffers. Between the measurements, the
electrode was rinsed with demineralized water and its pH was checked using the pH 7 buffer.

**Fecal DM, Lactic Acid, and Ammonia Contents.**
For DM, 9 to 11 g of thawed feces was transferred into a vessel and placed in a hot air dryer and predried at 45°C for 2 to 3 d until reaching a constant weight. Afterward, the sample was cooled down and weighed. The dry sample was ground using the Retsch ZM200 crusher (Retsch Technology GmbH, Haan, Germany) with a 1-mm stainless steel screen at 18,000 rpm for 30 s. Then, the sample was transferred into a 60-mL flask. Ten milliliters of ultraclean water was added and ammonia was extracted for 20 s at 4°C. Subsequently, the sample was centrifuged (978.10; AOAC International, 2005), and ash (942.05; AOAC International, 2005), crude fiber (973.18; AOAC International, 2005), and ADF (973.18; AOAC International, 2005), CP (2003.05; AOAC International, 2005), crude fat (2001.11; AOAC International, 2005), and EE (2009.11; AOAC International, 2005). Total amounts of feces produced over 3 consecutive days were collected for every individual animal in every week of the Trial period (d 31–33, 36–38, 43 to 45, 50 to 52, 57 to 59, and 64 to 66. Total amounts of feces produced over 3 consecutive days were collected for every individual animal in every week of the Trial period (d 31–33, 36–38, 43 to 45, 50 to 52, 57 to 59, and 64 to 66. Total amounts of feces produced over 3 consecutive days were collected for every individual animal in every week of the Trial period.

Every day, collected feces were frozen at −18°C until laboratory analysis (SOS s.r.o.). In the laboratory, the stools were thawed and the consecutive 3-d productions were pooled and predried at 103°C for 48 h.

In feed and feces, the following parameters were measured: NDF according to Van Soest et al. (1991; ANKOM Technology Method 13; ANKOM, 2015) and ADF (973.18; AOAC International, 2005), CP (2001.11; AOAC International, 2005), crude fat (2003.05; AOAC International, 2005), crude fiber (978.10; AOAC International, 2005), and ash (942.05; AOAC International, 2005). Dry matter was obtained by drying samples at 105°C (National Forage Testing Association 2.2.2.5; Shreve et al. 2006). Feed ME was calculated according to the following Association of American Feed Control Officials (2008) equation: kcal ME/100 g = % CP × 3.5 + % crude fat × 8.5 + % nitrogen-free extract (NfE) × 3.5. Nitrogen-free extract was calculated using the NDF fiber amount recorded by analytical methods, according to the following equation: NfE = 100 − (moisture + CP + ash + fat + NDF).

Apparent digestibility (%) was calculated as follows:

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment GLM repeated measures</th>
<th>SEM</th>
<th>Treatment × sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, kg</td>
<td>Control1</td>
<td>LY2</td>
<td>Treatment</td>
</tr>
<tr>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>9.50</td>
<td>9.58</td>
<td>9.71</td>
<td>9.68</td>
</tr>
</tbody>
</table>

1 No supplementation.
2 LY = live yeast; supplementation of LY culture (Actisaf Sc 47; Phileo Lesaffre Animal Care, Marcq-en-Baroeul, France).
3 NS = nonsignificant.

**Digestibility**
Apparent digestibilities were measured by quantitative collection of feces. Feed intake was measured every week of the Trial period (presented as feed minus refusals) on d 29 to 31, 36 to 38, 43 to 45, 50 to 52, 57 to 59, and 64 to 66. Total amounts of feces produced over 3 consecutive days were collected for every individual animal in every week of the Trial period (d 31–33, 36–38, 43 to 45, 50 to 52, 57 to 59, and 64 to 66). Every day, collected feces were frozen at −18°C until laboratory analysis (SOS s.r.o.). In the laboratory, the stools were thawed and the consecutive 3-d productions were pooled and predried at 104°C for 48 h.

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Apparent digestibility (%) was calculated as follows:

**Table 2.** Counting media and references for fecal microflora examination methods (Laboratoires Biocéane, Le Havre, France)

<table>
<thead>
<tr>
<th>Flora</th>
<th>Medium1</th>
<th>Standard2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>TSN + thioglycolate</td>
<td>NF ISO 7937</td>
</tr>
<tr>
<td>Lactic acid bacteria</td>
<td>Rogosa</td>
<td>NF ISO 15214</td>
</tr>
<tr>
<td>Enterococci</td>
<td>BEA</td>
<td>NF ISO 7899-2</td>
</tr>
<tr>
<td><em>Clostridium</em></td>
<td>DRBC</td>
<td>NF ISO 16649-2</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>TBX</td>
<td>NF ISO 21527-1</td>
</tr>
<tr>
<td>Yeasts</td>
<td>RVS and ASAP</td>
<td>NF ISO 6579/Amd 1</td>
</tr>
</tbody>
</table>

1 TSN = Trypsincase Sulfite Neomycin; BEA = Bile Esculin Agar; VRBL = Violet Red Bile Lactose; TBX = Tryptone Bile X-Glucuronide; DRBC = Dichloran Rose Bengal Chlorotetracycline; RVS = Rappaport-Vassiliadis Soya Peptone; ASAP = ASAPTM , bioMérieux Industry, Hazelwood, Missouri.
2 NF = Norme francaise; ISO = International Organization for Standardization.

**Table 3.** Effects of live yeast oral supplementation on the BW of young beagles

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment GLM repeated measures</th>
<th>SEM</th>
<th>Treatment × sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, kg</td>
<td>Control1</td>
<td>LY2</td>
<td>Treatment</td>
</tr>
<tr>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>9.50</td>
<td>9.58</td>
<td>9.71</td>
<td>9.68</td>
</tr>
</tbody>
</table>

1 No supplementation.
2 LY = live yeast; supplementation of LY culture (Actisaf Sc 47; Phileo Lesaffre Animal Care, Marcq-en-Baroeul, France).
3 NS = nonsignificant.

frozen stool samples were thawed in cold water and weighed in the stomach filtration bag. Peptone water was added to each sample to reach 100 g (dilution step 1) and then homogenized for 10 sec. A gradual decimal dilution (log 10; dilution steps 2–9) was performed by transferring 1 mL of the solution into a tube containing 9 mL of saline. The cultivation media used, microorganisms, and standards are listed in Table 2.

**Fecal Microbiology.** The microbiological examination was performed by Laboratoires Biocéane. The
Table 4. Effects of live yeast oral supplementation on the hematological parameters of young beagles\(^1\)

<table>
<thead>
<tr>
<th>Item(^2)</th>
<th>Control(^3)</th>
<th>LY(^4)</th>
<th>SEM</th>
<th>Treatment</th>
<th>Sex</th>
<th>Treatment × sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC, 10^9/L</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>0.42</td>
<td>NS(^5)</td>
</tr>
<tr>
<td>RBC, 10^12/L</td>
<td>6.71</td>
<td>6.45</td>
<td>6.50</td>
<td>6.65</td>
<td>0.08</td>
<td>NS</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>159.12</td>
<td>150.68</td>
<td>158.90</td>
<td>159.13</td>
<td>1.67</td>
<td>NS</td>
</tr>
<tr>
<td>Hematocrit, L/L</td>
<td>0.47</td>
<td>0.45</td>
<td>0.46</td>
<td>0.47</td>
<td>0.06</td>
<td>NS</td>
</tr>
<tr>
<td>MCV, fl</td>
<td>70.35</td>
<td>69.27</td>
<td>70.91</td>
<td>70.46</td>
<td>0.18</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MCH, pg</td>
<td>23.59</td>
<td>23.62</td>
<td>24.15</td>
<td>23.92</td>
<td>0.11</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>MCHC, g/L</td>
<td>337.54</td>
<td>336.66</td>
<td>346.97</td>
<td>337.17</td>
<td>1.00</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Platelet count, 10^9/L</td>
<td>355.13</td>
<td>353.55</td>
<td>331.78</td>
<td>354.04</td>
<td>6.86</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^1\)Reference ranges for dogs used were according to The Merck Veterinary Manual (Kahn and Line, 2010): 5.0 × 10^9 to 14.1 × 10^9 white blood cells/L, 4.95 × 10^12 to 7.87 × 10^12 red blood cells/L, 119 to 189 g hemoglobin/L, 35 to 57% packed cell volume, 66 to 77 fl mean corpuscular volume, 21.0 to 26.2 pg mean corpuscular hemoglobin, 320 to 363 g/L mean corpuscular hemoglobin concentration, and 211 × 10^9 to 621 × 10^9 platelets/L.
\(^2\)WBC = white blood cells; RBC = red blood cells; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.
\(^3\)No supplementation.
\(^4\)LY = live yeast; supplementation of LY culture (Actisaf Sc 47; Phileo Lesaffre Animal Care, Marcq-en-Baroeul, France).
\(^5\)NS = nonsignificant.

Apparent digestibility (%) =
\[
\frac{\text{total nutrient in feed (g)} - \text{total nutrient in feces (g)}}{\text{total nutrient in feed (g)}}
\]

Statistical Analysis

For statistical analyses, the generalized linear model for repeated measures under SPSS version 11.5 (IBM, Armonk, NY) was used. Dog was the experimental unit. Data lacking normality (yeast and bacterial cell counts) were logarithmically transformed.

Treatment effect, sex effect, and interaction were tested, and differences were considered statistically significant at \(P < 0.05\). Covariates corresponding with the pre-experimental period were used, with measurements realized on d 1, 8, 15, and 22 for BW; d 29 and 36 for microbiology; and d 1 and 28 for hematology and biochemistry parameters. No covariate was used for digestibility data. Data in the tables are presented as means ± SEM.

RESULTS

Clinical Observations and Mortality

The health status of all the dogs was very good throughout the whole experiment, and no clinical symptoms and mortality were observed.

Body Weight and Feed Consumption

During the Adaptation period, a growth depression was observed in all the dogs in both the groups (average daily weight gain: 8 kg for LY and 7 kg for control). During the Trial period, BW increased (average daily weight gain: 26 kg for LY and 19 kg for control). Rates of the BW increase were sex dependent. The LY dogs (supplemented with LY *S. cerevisiae*) showed a significantly higher growth rate increase \((P < 0.05)\) than the control dogs, as shown in Table 3. All the dogs consumed all the feed presented to them.

Hematology

The treatment and sex effects on WBC and RBC counts, hemoglobin, and hematocrit were not statistically significant. Mean corpuscular volume was slightly lower \((P < 0.1)\) in females than in males and significantly higher \((P < 0.05)\) in LY dogs than in control dogs. The treatment × sex interaction did not show a significant effect. Mean corpuscular hemoglobin was slightly higher \((P < 0.1)\) in the LY dogs and sex effect was nonsignificant. Mean corpuscular hemoglobin concentration was sex dependent, significantly \((P < 0.05)\) higher in males than in females, and treatment dependent, with an interaction between sex and treatment \((P < 0.1)\). The LY dogs had significantly higher MCHC values \((P < 0.05)\) than the control ones. No significant differences in PLT were found between the control and LY animals during the Trial period. All the hematological parameters were within the reference ranges (see Table 4), and they were not adversely affected by the experimental treatment.

Biochemistry

No significant differences in urea and creatinine serum levels were found between the treatments.
Creatinine levels were significantly influenced by sex \( (P < 0.05) \), with males showing higher levels. Serum activity of ALP was not significantly influenced. In general, all the clinical chemistry parameters were within reference ranges (Table 5) and were not adversely affected by the experimental treatments.

**Feces Analyses**

**Fecal pH.** No statistically significant differences in the pH values of fresh feces were found between the LY and control dogs (Table 6).

**Fecal Chemistry.** Contents of VFA (acetate and propionate) were not significantly influenced by the treatment. Fecal lactic acid showed a significant treatment \( \times \) sex effect \( (P < 0.01) \). Live yeast males showed higher lactate values compared with the control ones, whereas the LY females showed lower values than the controls. The feces biochemistry results are given in Table 6.

**Fecal Microbiology.** The log cfu per gram values of the *S. cerevisiae* CNCM I-4407 were significantly higher in feces of the LY animals \( (P < 0.01) \). For *E. coli*, log cfu per gram values were significantly lower in the LY animals than in the control ones \( (P < 0.05) \). Fecal enterococci were also significantly lower in the female LY group \( (P < 0.05) \).

Lactic acid bacteria, *Clostridium perfringens*, and total coliforms did not show any significant differences between the treatments. Feces microbiology results are given in Table 7.

**Digestibility**

The LY supplementation had no significant effect on DM, ash, crude fiber, CP, and fat digestibility. However, NDF digestibility was significantly \( (P < 0.05) \) higher in the LY animals than in the control animals. The digestibility determination results are given in Table 8.

**DISCUSSION**

In this study, the effects of oral supplementation of Actisaf Sc 47, containing LY *S. cerevisiae* CNCM I-4407 at \( 2.9 \times 10^8 \) cfu/g to dogs on nutrient digestibility and profile of the fecal microflora were investigated. The study included 24 beagle dogs (12 males and 12 females) from 5 to 9 mo of age fed a diet with an increased fiber content. The dogs were randomized according to BW and sex into 2 treatments: LY (live yeast supplemented) and control (unsupplemented). The dogs showed no clinical signs during the Adaptation and Trial periods. During the Adaptation period, a slight growth depression was observed for each dog, most probably due to the dietary change from the pre-experimental to the experimental diet (2.9 vs. 8.52% crude fiber). During the Trial period (LY supplementation), BW increased with age for both the treatments, with the LY dogs showing a significantly \( (P < 0.05) \) greater increase in BW than the control ones. van Heugten et al. (2003) observed in postweaning piglets supplemented with LY *S. cerevisiae* SC47 \( 2.4 \times 10^7 \) cfu/g feed significantly higher mean daily weight gain compared with LY unsupplemented piglets. Similarly, Jurgens et al. (1997) and Bontempo et al. (2006) reported significantly higher weight gain in postweaning piglets receiving LY *S. cerevisiae*. However, the numerical differences between the treatments found in our study were not great and the effect of LY on growth would probably be greater over a longer period of time.

Each dog in both the treatments consumed all the feed presented every day, and feed consumption was not adversely affected by LY. In available literature sources, no adverse effects of LY supplementation to dogs and other animal species on growth rate and feed intake were described. Jurgens et al. (1997) did not report any effects of LY supplementation on feed intake. Middelbos et al. (2006) reported that the administration of autolyzed

**Table 5. Effects of live yeast oral supplementation on the blood chemistry parameters of young beagles**

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>LY</th>
<th>SEM</th>
<th>Treatment</th>
<th>Sex</th>
<th>Treatment</th>
<th>Sex</th>
<th>Treatment</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea, mmol/L</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>0.16</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>72.23</td>
<td>66.11</td>
<td>69.43</td>
<td>64.23</td>
<td>1.15</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ALP, μkat/L</td>
<td>0.58</td>
<td>0.56</td>
<td>0.54</td>
<td>0.50</td>
<td>0.02</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ALT, μkat/L</td>
<td>1.59</td>
<td>1.49</td>
<td>1.33</td>
<td>1.43</td>
<td>0.03</td>
<td>&lt;0.10</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

1Reference ranges for dogs used were according to The Merck Veterinary Manual (Kahn and Line, 2010): 2.86 to 10 mmol/urea L, 44.2 to 88.4 μmol creatinine/L, 0.02 to 1.9 μkat alkaline phosphatase/L, and 0.17 to 1.82 μkat alanine aminotransferase/L.
2No supplementation.
3LY = live yeast; supplementation of LY culture (Actisaf Sc 47; Phileo Lesaffre Animal Care, Marcq-en-Baroeul, France).
4NS = nonsignificant.
5ALP = alkaline phosphatase.
6ALT = alanine aminotransferase.
yeast cell wall to dogs did not influence their feed intake. van Heugten et al. (2003) even reported an increase in mean daily feed intake in LY-supplemented piglets.

The clinical state of the dogs was evaluated by regular observations, physical examinations, measurements of CBC, and urea, ALP, ALT, and creatinine values. No significant differences between the treatments were found in RBC, hemoglobin, hematocrit, and PLT. However, MCV and MCHC were significantly higher in the LY dogs than in the control ones, but both were within the reference ranges (Table 4). There is a connection between MCV, MCHC, and iron status (Haram et al., 1997). However, in this study, the dogs’ iron status was not investigated. Unlike Middelbos et al. (2006, 2007), who reported a decrease in WBC counts for dogs given autolyzed yeast cell wall, in this study, the LY supplementation influenced neither total leukocyte (WBC) counts nor differential counts of different types of leukocytes. Serum urea, creatinine, ALP, and ALT were chosen to assess the kidney and liver function. During the experiment, no significant differences between the treatments were found in these parameters and the values were within the reference ranges (Table 5), indicating that the kidney and liver function was not impaired. In the literature, little information on effects of LY cultures on biochemical parameters in blood is available. Shen et al. (2011) reported lower serum urea levels in pregnant sows given yeast cell wall–derived fermentation products than in control pregnant sows.

There were no significant differences found in pH of fresh feces between the LY and control treatments. On the contrary, Middelbos et al. (2007), whose study included 5 adult female dogs in a 5 × 5 Latin square with 14-d experimental periods, found lower fecal pH in dogs supplemented with autolyzed yeast cell wall. They suggested an association with increased Lactobacillus and Bifidobacterium spp. that produce lactic acid and short-chain fatty acids. In this study, fatty acids (acetate and propionate) were not significantly influenced by LY supplementation. No significant effect between the LY and control treatments was found. Fecal lactic acid contents showed a significant effect of treatment and sex interactions. The LY males showed higher fecal lactic acid than the control males, whereas the LY females showed lower lactate levels than the control ones.

For feces microbiology, the gradual dilution method and cultivation on appropriate cultivation media were used. Clostridium perfringens, lactic acid bacteria, enterococci, coliforms, E. coli, LY, and Salmonella spp. were determined in feces. No significant differences between the treatments were found in bacterial counts, except for LY, E. coli, and enterococci. Fecal counts (cfu/g) of S. cerevisiae CNCM I-4407 contained in the Actisaf Sc 47 were highly significantly higher in stools of LY dogs. Escherichia coli counts (cfu/g) were significantly lower in the LY dogs than in the controls. Fecal enterococci counts were also significantly lower in the LY treatment.

Many researchers reported a reduction in counts of E. coli, salmonellae, or other pathogens in dogs and pigs (Middelbos et al., 2006, 2007; Kiarie et al., 2011; Terekova et al., 2014). The mode of action is explained by the hypothesis that mannose residues in the yeast cell wall prevent the pathogens that possess fimbria type-1 from adherence to specific receptors in the intestinal mucosa of the host. Mannose is capable of occupying binding sites on fimbrial adhesins on the surface of bacteria. Due to the binding of bacterial fimbriae on mannose groups in the yeast cell wall bacteria are prevented from adherence to the surface of intestinal mucosa cell, therefore not being able to colonize the intestinal wall and penetrate into the circulation (Neese et al., 1986; Mann and Petri, 1995). This mode of action, however, is used only by some pathogens. Tiago et al. (2012) tested 11 species of enteropathogenic bacteria and found that only E. coli, Salmonella typhimurium, and Salmonella typhi (Gram-negative bacteria) adhered to the cell wall of selected strains of Saccharomyces boulardii and S. cerevisiae.
Some researchers (White et al., 2002; Swanson et al., 2002; Grieshop et al., 2004) reported that LY and yeast cell wall–derived products increased counts of lactobacilli present in the intestines, whereas in another study (van Heugten et al., 2003), a reduction in lactobacilli was observed in postweaning piglets that were given LY. In this study, lactobacilli were not influenced by LY supplementation in dogs; similarly, counts of lactobacilli were not influenced by the administration of autolyzed yeast cell wall to the dogs in the study published by Middelbos et al. (2007).

Live yeast has been observed to enhance fiber digestion. Lizardo et al. (2012) found a favorable effect of LY supplementation on NDF degradation in piglets. With the high-fiber diet we had chosen, we expected to better express the LY potential to improve fiber digestion. As animal protein sources are getting questionable, more and more pet food manufacturers use higher fiber contents in the product they put on the market. For fiber analysis and fiber digestibility assessment, we used crude fiber, NDF, and ADF. The LY dogs that received LY showed significantly higher digestibility of NDF than the control ones. For pet foods, total dietary fiber (Prosky et al., 1985), which includes both soluble and insoluble fiber, seems to be more suitable, as suggested by de Oliveira et al. (2012), who compared NDF and ADF and crude fiber in dog foods in a digestibility study with 6 dry dog foods and 6 dry cat foods and 36 beagle dogs and 36 cats. Crude fiber had, in general, the lowest apparent digestibility. Total dietary fiber and NDF apparent digestibilities were higher and were positively correlated in dogs ($r > 0.84$). High digestibility of NDF in dog kibbles can be due to interference with high starch and fat contents. Also, the large amount of gelatinized starch in kibble diets can interfere in the NDF analysis, which requires a pretreatment with amylase. However, in the Czech Republic, total dietary fiber is not commonly used for feed assessment and is not included in the list of feed analyses methods defined by the Czech legislation (Central Institute for Supervising and Testing in Agriculture; ÚKZÚZ, 2009).

No significant differences were found between the treatments in digestibility of other nutrients (DM, ash, crude fiber, CP, and fat). On the contrary, Middelbos et al. (2007) reported that yeast cell wall administration to dogs resulted in an increase in ileal digestibility of DM, OM, CP, and GE compared with control animals. The experimental diet also had rather low digestibility of DM (66.84 to 68.35%) and CP (64.36 to 66.54%). Generally,

### Table 7. Effects of live yeast oral supplementation on the fecal microbiology (feces, as-is basis) of young beagles

<table>
<thead>
<tr>
<th>Item</th>
<th>Control1</th>
<th>Treatment GLM repeated measures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae, log cfu/g</td>
<td>2.18</td>
<td>1.99</td>
</tr>
<tr>
<td>Lactic acid bacteria, log cfu/g</td>
<td>8.31</td>
<td>8.35</td>
</tr>
<tr>
<td>Fecal enterococci, log cfu/g</td>
<td>5.13</td>
<td>6.29</td>
</tr>
<tr>
<td>Coliforms, log cfu/g</td>
<td>5.43</td>
<td>5.66</td>
</tr>
<tr>
<td>Escherichia coli, log cfu/g</td>
<td>4.11</td>
<td>4.72</td>
</tr>
<tr>
<td>Clostridium perfringens, log cfu/g</td>
<td>3.14</td>
<td>3.35</td>
</tr>
</tbody>
</table>

1 No supplementation.
2 LY = live yeast; supplementation of LY culture (Actisaf Sc 47; Phileo Lesaffre Animal Care, Marcq-en-Baroeul, France).
3 NS = nonsignificant.

### Table 8. Effects of live yeast oral supplementation on the apparent total tract digestibility of nutrients in the diet fed to young beagles

<table>
<thead>
<tr>
<th>Item</th>
<th>Control1</th>
<th>Treatment GLM repeated measures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>DM, %</td>
<td>68.14</td>
<td>68.35</td>
</tr>
<tr>
<td>Ash, %</td>
<td>22.90</td>
<td>23.83</td>
</tr>
<tr>
<td>Crude fiber, %</td>
<td>53.28</td>
<td>51.43</td>
</tr>
<tr>
<td>NDF, %</td>
<td>35.63</td>
<td>36.77</td>
</tr>
<tr>
<td>CP, %</td>
<td>66.48</td>
<td>66.60</td>
</tr>
<tr>
<td>Fat, %</td>
<td>86.71</td>
<td>87.67</td>
</tr>
</tbody>
</table>

1 No supplementation.
2 LY = live yeast; supplementation of LY culture (Actisaf Sc 47; Phileo Lesaffre Animal Care, Marcq-en-Baroeul, France).
3 NS = nonsignificant.
values for DM and CP digestibility reported in the literature are higher. Murray et al. (1997) measured ileal digestibilities for different diets in dogs. Their values for DM and CP ranged from 68.3 to 77.3% and from 73.9 to 82.8%, respectively. The low DM digestibility contributed to the initial growth depression found in this study. Nguyen et al. (1998) suggested that the formation of semisolid gel from alkaline detergent glucan contained in the cell wall of S. cerevisiae might have led to a slower digesta passage in the small intestine, and therefore, a longer time was spent in the small intestinal milieu that is rich in enzymes. However, previous studies (Strickling et al., 2000; Swanson et al., 2002) where dogs were supplemented with yeast cell wall at a dietary concentration below 1% did not find any significant effects on ileal and total tract nutrient digestibility. On the contrary, Zentek et al. (2002) observed reduced digestibility in dogs receiving feed with 5% inclusion rate of yeast cell wall. In pregnant and lactating sows receiving feed supplemented with a yeast fermentation product, total nutrient digestibility was not influenced (Shen et al., 2011). van Heugten et al. (2003) reported reduced digestibility of DM, fat, and GE in postweaning piglets supplemented with LY; however, no effect on CP digestibility was observed. On the other hand, Kornegay et al. (1995) did not observe any effects of LY supplementation on nutrient digestibility in pigs fed different sources of fiber.

The results of this study did not indicate any adverse effects of LY S. cerevisiae as a dietary supplement in dogs as shown by the results of clinical observations, hematology, and blood chemistry. However, further studies going into greater details on blood chemistry are suggested, particularly to investigate how LY supplementation influences mineral and vitamin status in dogs. Live yeast seems to induce some shifts in fecal microflora. The reduction in fecal E. coli counts was in accordance with the previous findings in other species. However, the mode of action of LY on intestinal microflora still requires further research. Live yeast seems to increase the growth rate of young dogs because of improved fiber digestibility. However, more work is necessary to study effects of LY on nutrient digestibilities in connection with intestinal microflora profile shifts induced by LY.

LITERATURE CITED


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LITERATURE CITED


