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Comparison of three patterns of feed supplementation with live *Saccharomyces cerevisiae* yeast on postweaning diarrhea, health status, and blood metabolic profile of susceptible weaning pigs orally challenged with *Escherichia coli* F4ac

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1 **Running head:** Yeast reduces ETEC effect in challenged pigs

2

3 **Comparison of three patterns of feed supplementation with live *Saccharomyces***
4 ***cerevisiae* yeast on post-weaning diarrhea, health status and blood metabolic profile of**
5 **susceptible weaning pigs orally challenged with *Escherichia coli* F4ac^{1,2}**

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17

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21 *Saccharomyces cerevisiae* CNCM I-4407

22

23

24 **Abstract**

25 The development of more effective feeding strategies to reduce the detrimental effect of
26 enterotoxigenic *Escherichia coli* F4ac (ETEC) plays a crucial role in reducing the occurrence
27 of therapeutic intervention with antibiotics in livestock. The ability of *Saccharomyces*
28 *cerevisiae* CNCM I-4407 (*Sc*), supplied in different patterns to counteract ETEC infection in
29 weaned pigs, was evaluated. Fifty pigs were weaned at 24 days and were then divided into
30 five groups: control (CO), CO + colistin (AB), CO + 5×10^{10} CFU of *Sc*/kg feed, from d 0–
31 21 (PR), CO + 5×10^{10} CFU of *Sc*/kg feed from d 7–11 (CM) and CO + one shot of 2×10^{11}
32 CFU of *Sc* when the first diarrhea appeared (CU). On d 7 post-weaning, all the pigs were
33 orally challenged with 10^8 CFU of ETEC. Blood samples were taken from the pigs (d 7, d 8,
34 d 12, d 21) while the fecal excretion of ETEC was assessed on d 7 and d 10. Fecal
35 consistency was scored from 12 h before infection to 144 h post-infection (p.i.). On d 21, the
36 piglets were sacrificed. The *in vitro* adhesion test on the intestinal villi confirmed individual
37 susceptibility to ETEC, excluding the presence of resistant pigs. Growth performance did not
38 differ between the treatments. Mortality was reduced in the AB group ($P < 0.01$) and,
39 marginally, in the PR group ($P = 0.089$) when compared to CO group. The CO group had a
40 higher fecal score than AB during the entire period of observation (from $P = 0.01$ to $P <$
41 0.001). Conversely, yeast administration reduced the fecal score when compared to CO group
42 12 h and 48 h after infection ($P = 0.04$). Total IgA never differed among the experimental
43 groups, but the ETEC-specific IgA concentration was lower in the AB group than in the CO
44 group ($P = 0.04$) at d 12. Four days p.i., the subjects fed with live yeast had reduced ETEC
45 excretion as compared with the CO group ($P = 0.05$). Blood metabolite concentrations of
46 C12:1 ($P < 0.01$), C5DC (C6-OH) ($P = 0.02$), PC_aa_C40:1 and PC_aa_C40:6 ($P = 0.01$ and
47 $P < 0.01$, respectively) and alpha-AAA ($P < 0.01$) were reduced in the AB group as

48 compared with the CO group; PR+CM reduced the concentration of SM_C18:0 ($P = 0.02$)
49 and increased the concentration of C10:2 ($P = 0.02$), vs. CO. Furthermore, the CM group had
50 an increased concentration of C10:2 ($P < 0.01$) as compared with the PR group. In
51 conclusion, the administration of live yeast, even in concomitance with ETEC infections,
52 reduces pig illness and mortality. Moreover, the strain of *Sc* tested did not show a therapeutic
53 effect.

54

55 Introduction

56 In 2006, the European Union banned the use of antibiotics as growth promoters; there is
57 diffuse agreement that a strong restriction of the use of therapeutics in livestock feed may
58 reduce the risk of spreading bacterial antibiotic resistance. This implies significant changes
59 in animal feeding. Developing new feeding strategies is particularly important in reducing
60 post-weaning digestive disorders, which are a relevant cause of illness in pigs fostered by
61 intensive feeding practices (Heo et al., 2013). The most important etiological agent is
62 *Escherichia coli* F4 (ETEC) (Nagy and Fekete, 2005) and the response to feeding strategies
63 may vary due to the existence of different phenotypes for ETEC adhesion on the intestinal
64 villi of pigs (Sellwood et al., 1975).

65 The concept of probiosis originated approximately a century ago, but its use in animal
66 production is still valid in reducing the detrimental effects of pathogen infection (Armstrong
67 et al., 2014). *Saccharomyces* spp. is the yeast most studied for counteract intestinal disorders
68 in young mammals (Farthing et al., 2013; Shan et al., 2013). The administration of
69 *Saccharomyces cerevisiae* (*S. cerevisiae*) modulates the activation of inflammation in mice
70 infected with *Salmonella enterica* serovar Typhimurium (Martins et al., 2011). Moreover, in
71 the pig model, *S. cerevisiae* yields positive effects in controlling ETEC infection, reducing
72 the severity of diarrhea in weaned piglets (Trckova et al., 2014).

73 For the first time, the effectiveness of *S. cerevisiae* CNCM I-4407 dosed in different
74 patterns **was compared** to counteract the detrimental effect of ETEC on the health status of
75 weaned pigs orally challenged with this pathogen. **Moreover, considering that exposure to**
76 **post-weaning stress and challenge with pathogenic *E. coli* affect several metabolites**
77 **(Sugiharto et al., 2014),** the blood metabolic profile of the pigs **was evaluated** to determine
78 the interaction among the yeast, ETEC and the host.

79

80 **Materials and Methods**

81 The procedures complied with Italian law pertaining to experimental animals and were
82 approved by the Ethic-Scientific Committee for Experiments on Animals of the University
83 of Bologna, Italy.

84

85 **General experimental design**

86 Fifty piglets were obtained from a commercial piggery **where ETEC infections had been**
87 **reported; this indicated the presence in the herd of pigs susceptible to ETEC. During the**
88 **suckling period, no creep feed was supplied. At 24 ± 2 days of age (d 0), the pigs were**
89 **weaned and moved to the experimental farm, divided into five groups balanced for litter and**
90 **body weight and were housed in pens with a mesh floor. The pigs were kept at a controlled**
91 **temperature (30°C at the beginning and 25°C at the end of the experiment, with a 1°C**
92 **decrease every 3 d). Infrared lamps were located above the piglets for the first 7 days. The**
93 **piglets had free access to feed and water throughout the experimental period; feed was**
94 **supplied in a dry feeder.** On d 7 post-weaning, all the pigs were orally dosed with 1.5 mL
95 suspension containing 10^8 CFU of ETEC O149/mL. The bacteria solution was prepared as
96 described by Bosi et al. (2004). **The product tested was a lyophilized live yeast strain**

97 (Actisaf; Lesaffre Feed Additives, France) of *S. cerevisiae* CNCM I-4407 (Sc) mixed in the
98 diet formula.

99 The piglets were assigned to one of five diets: control (CO, typical weaning diet – Table 1),
100 CO + 1 g colistin/kg of feed (AB), CO + 5×10^{10} colony-forming units (CFU) of Sc/kg of
101 feed, from d 0 to d 21 (PR, preventive dose), CO + 5×10^{10} CFU of Sc/kg of feed from d 7
102 (day of infection with ETEC) to d 11 (CM, competitive dose) and CO + 1 shot of 2×10^{11}
103 CFU of Sc/kg of feed when the first diarrhea appeared (CU, curative dose). Colistin
104 treatment was used as a positive control because it is active against the ETEC strain used for
105 the challenge. Colistin has strong properties against gram-negative bacteria and it is
106 frequently used for this purpose in other trials involving an ETEC challenge (Torrallardona
107 et al., 2003; Bosi et al., 2004). The pigs were individually penned in cages, except for the
108 first 2 days when they were kept in groups of two having the same dietary treatment for the
109 purpose of improving their adaptation and feed intake.

110

111 **Experimental Procedure**

112 Starting on d 0, each group received its experimental diet. The pigs were sacrificed at the
113 end of the trial (d 21). At slaughter, the animals were deeply anesthetized with sodium
114 thiopental (10 mg/kg body weight) and sacrificed via an intracardiac injection of Tanax (0.5
115 mL/kg BW).

116

117 **Experimental Controls**

118 The pigs were weighed individually at the start of the trial, on d 7 (pre-challenge), on d 14
119 and at sacrifice (d 21). The feed intake of each pig was recorded individually.

120 Blood was sampled on d 7 (pre-challenge), d 8, d 12 and on d 21 (day of sacrifice) by

121 venipuncture of the vena cava, centrifuged at $3,000 \times g$ for 10 min at 4°C; the serum was

122 then removed. The serum samples collected on d 7, d 12 and d 21 were inactivated at 56°C
123 for 30 min and stored at -20°C until analysis. On the other hand, the serum collected at d 8
124 was stored at -80°C after centrifugation. Individual fecal samples were obtained on d 7 (pre-
125 challenge) and d 10 for the ETEC plate counts following the protocol described by Bosi et
126 al. (2004). The severity of the diarrhea was evaluated daily in each subject by five point
127 fecal scores (1 to 5): 1 = hard, 5 = watery feces.) and by the same operator from 12 h before
128 to 144 h after infection.
129 On d 21, the piglets were sacrificed in order to collect a sample from the distal jejunum to
130 determine the phenotype for adhesion of the ETEC to the intestinal villi, as described in
131 Trevisi et al. (2009).

132

133 **Total IgA and *Escherichia coli* F4ac-specific IgA titers**

134 Total IgA determination was carried out by ELISA, using Pig Immunoglobulin Reference
135 Serum (Bethyl laboratories, Montgomery, TX) as the specific antibody for the standard
136 curve, Goat anti-Pig IgA-HRP conjugate (Bethyl Laboratories) as a secondary antibody and
137 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) (Roche Diagnostics, San
138 Francisco, CA) for chromogenic detection. The concentration was expressed as micrograms
139 per milliliter ($\mu\text{g mL}^{-1}$). The ETEC-specific IgA quantification was carried out by ELISA
140 according to Van den Broeck et al. (1999), using F4 fimbriae isolated from ETEC cultures
141 as reported by Bosi et al. (2004). Briefly, the F4 antigen was added at a concentration of 50
142 mg mL^{-1} in ELISA-diluted buffer to coated wells with F4 fimbrial adhesin Mab (CVL,
143 Addlestone, UK). Pooled serum obtained from five subjects, all ETEC-challenged and
144 positive for the ETEC adhesion test was used as a calibrant. The concentration values of
145 specific IgA were expressed as arbitrary units per gram (AU mg^{-1}) of total IgA.

146

147 **Metabolic profile of blood serum**

148 A targeted metabolic technique, designed to quantify the concentration of 188 endogenous
149 metabolites from 5 different compound classes taken from 10 μ L plasma, was performed
150 using the AbsoluteIDQ p180 Kit, (BIOCRATES, Life Science AG, Innsbruck, Austria).
151 Sample analyses were carried out on the API 4000 QTrap LC/MS/MS System (Applied
152 Biosystems, Foster City, CA.). Measurements were carried out on the same plate and
153 analyzed by MetIQ software packages, which are an integral part of the AbsoluteIDQ Kit.

154

155 **Statistical analysis**

156 Performance data were analyzed by ANOVA using the general linear model (GLM)
157 procedure of SAS (SAS Inst., Inc., Cary, NC) with a completely randomized design, two
158 blocks (time), sows within block and five dietary treatments. Degrees of freedom for the
159 dietary treatments were used to test the following orthogonal contrasts: CO vs. YEAST (PR,
160 CM, CU), PR vs. (CM and CU), CM vs. CU and CO vs. AB. However, for pre-challenge
161 observations, the CM and CU groups received the same diet and, thus, the contrasts were PR
162 vs. (CM+CU+CO), PR vs. AB and AB vs. CO.

163 $P < 0.05$ was statistically significant and $0.05 < P < 0.10$ was considered a trend .

164 For mortality data, Fisher's exact test were carried out comparing CO with each of the other
165 dietary treatments.

166 The metabolomic data were analyzed using linear mixed models (Pinheiro and Bates, 2009),
167 taking the concentration of a given metabolite as a dependent variable and including a
168 random effect for litter. Body weight at d 7 and fecal score were considered to be possible
169 confounding factors, the latter taken after centering with respect to the diet-specific mean
170 fecal score. In order to establish which of these factors should be included in the model for
171 each metabolite, a backward elimination procedure, based on bootstrap testing (Davison and

172 Hinkley, 1997) was carried out on the corresponding linear mixed model. The analysis was
173 focussed on diets AB, CO, CM and PR, and examined the following contrasts: AB vs. CO,
174 CM + PR vs. CO and CM vs. PR. For each null hypothesis, a Leave-One-Out (LOO)
175 procedure was implemented (Hastie et al., 2009) in order to account for the possible
176 presence of influential observations (Cook and Weisberg, 1982). The applied procedure
177 consisted of testing the given null hypothesis on 38 different datasets, each one obtained
178 after excluding one animal at a time; finally, the rejection of the null hypothesis was deemed
179 to be “most stable” when it occurred on each one of the 38 different LOO datasets.

180

181 **Results**

182 **Growth performances**

183 No difference in growth performance was observed among the experimental groups. The
184 average daily gain (ADG) was 72.0, 71.0, 63.4, 86.1 and 95.2 g (SEM = 17.4), from d 0 to d
185 7 (before the challenge), and 197, 188, 210, 204 and 202 g (SEM = 73.2), from d 7 to d 21,
186 for groups CO, AB, PR, CM and CU, respectively.

187

188 **Severity of diarrhea and mortality**

189 The *in vitro* tests confirmed the presence of specific receptors for ETEC on the intestinal
190 villi of all pigs.

191 Table 2 lists the number of pig deaths during the trial for each group. Mortality in the
192 CO group was significantly higher than in the AB group ($P < 0.01$) and a trend of reduction
193 was seen also for PR ($P = 0.089$). Figure 1 shows the time course of pig survival during the
194 trial. Twenty-four hours after infection (d 8), the first pig died in the CO group; in the PR
195 and CM groups, the first pig died on d 10. In the AB group, only one pig died on d 11.

196 Finally, in the CU group, even if the pigs started to die on d 10 as in the other yeast-treated
197 groups, the survival curve **decreased** faster than in the PR and CM groups.

198

199 **Fecal scores**

200 **Table 3 shows the effect of the dietary supplementation with live yeast on the fecal scores of**
201 **weaned pigs challenged with ETEC at different times and doses.** Before the challenge, the
202 maximum fecal score was 2.4, indicating that no diarrhea occurred and no differences
203 emerged among the groups. From 12 h to 144 h after infection, the CO group showed a
204 higher fecal score than the AB group (from $P = 0.01$ to $P < 0.001$). Conversely, the
205 administration of yeast significantly reduced the fecal score **as** compared with the CO diet
206 12 h and 48 h after infection ($P = 0.04$ and $P = 0.04$, respectively), and a tendency to reduce
207 this parameter against the same groups was seen 24 h post-challenge ($P = 0.08$). Moreover,
208 during the entire **period of** observation, no significant differences were **observed** among the
209 groups supplied with live yeast, even if 96 h after the infection, **the PR group tended to**
210 **reduce the fecal score as compared with the CM and CU groups ($P = 0.08$).**

211

212 **Immune response and *Escherichia coli* F4ac shedding in feces**

213 Table 4 shows the data related to the IgA concentration in the blood serum and **to** the fecal
214 excretion of ETEC. The total IgA never differed among the experimental groups at **any of**
215 **the time points considered.** Moreover, before the challenge, no difference was observed in
216 ETEC-specific IgA concentration among the experimental groups. At d 12, the ETEC-
217 specific IgA concentration was lower in the AB group than in the CO group ($P = 0.04$), and
218 the administration of live yeast tended to reduce the specific IgA concentration **as** compared
219 with the CO group ($P = 0.10$).

220 On d 7 (before the challenge), no pigs were found to be positive for fecal excretion of ETEC
221 while, four days after infection, the subjects fed with live yeast excreted less ETEC as
222 compared with the CO group ($P = 0.05$). No other significant differences among the groups
223 were observed.

224

225 **Blood metabolic profile**

226 The differences between the most stable metabolites (i.e. the metabolites for which a given
227 null hypothesis was rejected in all 38 LOO datasets) in the blood serum 24 h after infection
228 with ETEC in weaned pigs are shown in Table 5. Compared with the antibiotic-treated pigs,
229 in the CO group, there were increased concentrations of Dodecenoyl-L-carnitine (C12:1) (P
230 < 0.01), Glutaryl-L-carnitine/Hydroxyhexanoyl-L-carnitine (C5DC (C6-OH)) ($P = 0.02$),
231 Phosphatidylcholine diacyl C 40:1, phosphatidylcholine diacyl C 40:6 (PC_aa_C40:1 and
232 PC_aa_C40:6 (C 40 stands for total carbon numbers of the couples of acyls, and :1 and :6
233 for total double bond numbers) ($P = 0.01$ and $P < 0.01$, respectively). Moreover, the
234 concentration of the alpha-amino adipic acid (alpha-AAA) was also higher in the CO group
235 than in the AB group ($P < 0.01$), but this difference was affected by the fecal score factor.
236 In CM+PR vs. CO, the fecal score was responsible for the decreasing concentration of
237 Sphingomyelin-Ceramide (SM_C18:0) ($P = 0.02$) in the yeast-treated pigs. On the other
238 hand, the yeast treatments increased the concentration of Decadienyl-L-carnitine (C10:2) (P
239 $= 0.02$). However, when compared with the PR group, the CM group exhibited an increased
240 concentration of C10:2 ($P < 0.01$).

241

242 **Discussion**

243 This study evaluated the protective effect of three different patterns of *S. cerevisiae* CNCM
244 I-4407 supplementation in the feed of sensitive ETEC-challenged piglets: the preventive, the

245 competitive and the curative; a group treated with the antibiotic colistin, frequently used
246 against Gram-negative enterobacteria, was also included as a positive reference.

247 Due to experimental design, the absence of differences for growth parameters is not
248 surprising. An experiment on a larger scale is necessary to evaluate growth performance
249 differences in susceptible challenged pigs fed live yeast.

250 However, in experimental challenge trials with ETEC, health parameters provided relevant
251 indications regarding the entire effect of testing feeding practices; of these, mortality was an
252 important parameter to be evaluated (Fairbrother et al., 2005). Moreover, a proper
253 evaluation of the sensitivity of the animals used in the trials is a prerequisite for avoiding
254 false negative responses. In the present study, specific receptors for ETEC on the intestinal
255 villi were present in all the piglets, strengthening the relevancy of the experimental results.

256 Furthermore, the ETEC strain used to infect the piglets was proven to be sensitive to the
257 antibiotic used here as a positive control. The low mortality rate of the pigs, the low
258 concentration of specific IgA against ETEC in the blood serum and the lowest diarrhea
259 score compared with the CO group confirm the effectiveness of the antibiotic. Only one pig
260 in the AB group died as a result of diarrhea immediately after weaning as a consequence of
261 the reduction in feed intake and the subsequent reduction in antibiotic ingestion. Between
262 the three feeding strategies studied in the trial supplying *S. cerevisiae* CNCM I-4407 in the
263 feed, the preventive method was the classic method of supplying probiotics to livestock feed
264 in order to protect animals against the risk of pathogenic infection. In the literature, there is
265 evidence of the preventive effect of *S. cerevisiae* spp. supplied in weaned pigs challenged
266 with lipopolysaccharide (LPS) from *E. coli* (Collier et al., 2011) in order to reduce the
267 inflammatory response and mortality in pigs. Moreover, a protective effect of *S. cerevisiae*
268 on porcine epithelial cell lines reducing the increased expression of genes related to
269 inflammation upon ETEC stimulation was observed (Badia et al., 2012). Furthermore, a

270 continuous supply of *S. cerevisiae* CNCM I-4407 to the sows from late gestation and to the
271 piglets, before and after weaning, reduced the severity and duration of diarrhea upon ETEC
272 challenge (Trckova et al., 2014).

273 In the present trial, 70% of untreated piglets died after infection with ETEC while *S.*
274 *cerevisiae* CNCM I-4407 halved pig mortality when administered in a preventive way.
275 Similarly, Collier et al. (2011) reported that *S. cerevisiae* var. *boulardii* reduced the
276 mortality of LPS-challenged pigs by 20%. Furthermore, an examination of the time course
277 of pig survival reveals that, when yeast is supplied after weaning, a reduction in diarrhea
278 severity is associated with delayed mortality. From a practical point of view, this fact
279 implies a delay in the appearance of pig cachexia, and more time for eventual therapeutic
280 intervention. The protective effect in the PR group could also be ascribed to the ability of *S.*
281 *cerevisiae* CNCM I-4407 to modulate the immune response in the gut mucosa, as reported
282 by *in vitro* tests (Zanello et al., 2011a,b).

283 Currently, precision feeding is a new targeted technique for modern livestock production in
284 order to reduce the environmental footprint and improve growth efficiency; feed additives
285 should also be utilized in a similar manner, to be supplied ideally only when it is necessary.
286 For this reason, the competitive and curative uses of a probiotic product in piglet feeding
287 were tested. To our knowledge, this is the first trial aimed at studying pigs exposed to an
288 ETEC challenge and the ability of *S. cerevisiae* CNCM I-4407 to compete with the
289 pathogen. Furthermore, focusing on the potential therapeutic properties of *S. cerevisiae*
290 CNCM I-4407 when diarrhea was already present was really challenging and innovative.
291 The *S. cerevisiae* CNCM I-4407 used in the diet of the present trials was lyophilized. The
292 pig survival curve of the CM group, which shows an effect comparable to that of the PR
293 group, may be explained by the sudden activation of the yeasts in the gastrointestinal tract
294 (GIT). There is evidence of the capability of *S. cerevisiae* to produce ethanol along the

295 intestinal tract, fermenting the sugar derived from the digestive process or provided by the
296 diet (Etienne-Mesmin et al., 2011). The ethanol concentration in the gut was not quantified
297 in this study. However, on the basis of the data of Bode et al. (1984), *S. cerevisiae* CNCM I-
298 4407 should be able to produce ethanol in the stomach by means of the fermentation of the
299 sugar provided by the milk-derived product supplied with the feed formula. This, in turn,
300 could have reduced the quantity of viable ETEC available to adhere to the intestinal
301 receptors and/or the gut sensitivity to the bacterial toxins, as demonstrated in macrophages
302 *in vitro* or in the liver of mice challenged with *E. coli* lipopolysaccharide (Nishiyama et al.,
303 2002). Moreover, the continuous supply of live yeast for an additional four days in the CM
304 group may have been responsible for containing the inflammation of the intestinal mucosa,
305 thereby reducing the consequences of the ETEC challenge (Zanello et al., 2011b).

306 Other studies in the scientific literature targeted to human gut health and therapy against
307 diarrhea suggest a curative approach using probiotics. In clinical trials on children,
308 *Lactobacillus rhamnosus* GG seems to shorten the duration of acute diarrhea (Shornikova et
309 al., 1997; Guandalini et al., 2000). On the other hand, *Saccharomyces* spp. are considered to
310 be broad-spectrum probiotics because they are not commonly found on or adherent to the
311 mucosa of the GIT in mammals (Blehaut et al., 1989). Thus, an interspecific effect is
312 conceivable, as suggested by the positive results obtained with the same yeast strain in
313 human and animal models (McFarland, 2010; Kurugöl and Koturoğlu, 2005). Our
314 therapeutic dose of *S. cerevisiae* CNCM I-4407 was one shot, four times more concentrated
315 than the dose used in the PR and CM groups, but the resulting health data did not show any
316 reduction in the detrimental effects of ETEC infection. This suggests that, when ETEC has
317 already exerted its pathogenicity adhering to the mucosa and producing its toxins, yeast is
318 not capable of interfering with the pathogenic mechanisms of ETEC. This finding partially
319 disagrees with the meta-analyses of Szajewska et al. (2007) which indicated a moderate

320 clinical benefit of *S. cerevisiae* boulardii therapy in infants and children with acute
321 gastroenteritis, with a shortened duration of diarrhea; nevertheless, the same authors
322 indicated some methodological limitations in the study. We observed only a slight delay in
323 the time course of mortality in comparison to untreated animals; the number of dead piglets
324 did not differ between the CU and CO groups. As a confirmation of the general effect of *S.*
325 *cerevisiae* CNCM I-4407 against ETEC, there is a global lowering effect of the yeast
326 treatments on the specific IgA against ETEC, even if the greatest effect was attributable to
327 the PR group. This fact could indirectly indicate the ability of the yeast to reduce the
328 antigenic presence in the gut, reducing the antigen exposure and the specific immune
329 response.

330 In the present study, the blood plasma metabolic profile was considered to support the
331 clinical evidence and to reveal the metabolic effects resulting from the interaction among
332 ETEC, yeast and the host. In pigs, abrupt modifications in the microbial population in the
333 GIT can occur after weaning with a negative impact on the mucosal homeostasis and
334 consequently on the blood metabolic profile (Wikoff et al., 2009; Campbell et al., 2013). In
335 this study, a sudden impact of ETEC infection was observed on some bioactive metabolites
336 involved in cell signals and in the activation of immune pathways. In the CO group, two
337 phosphatidylcholine diacyls (C40:1, and C40:6) and 2-Aminoadipic acid were
338 upregulated. Phosphatidylcholine is by far the most abundant phospholipid component in
339 plasma and is largely found in diacylated form (Flögel et al., 2013). Lipopolysaccharide, a
340 bioactive component of the cell wall of gram-negative bacteria, stimulates
341 phosphatidylcholine breakdown in macrophages (Grove et al., 1990). T cells, by means of
342 acyltransferases, and phospholipases, manipulate phospholipid composition upon
343 stimulation (Robichaud et al., 2013). No specific reference to the two diacyl compounds
344 which were affected herein is reported; however, due to the time proximity to the ETEC

345 challenge, it can be hypothesized that this was related to the metabolic action of ETEC on
346 inflammatory or immune cells, and that this action was reduced by the antibiotic. Alpha-
347 AAA is a product of lysine degradation in tissues after oxidant stress (Sell et al., 2007) and
348 the higher blood values in the CO group may agree with the clinical observations and
349 indirectly indicate that ETEC infection stimulated the inflammatory pathways with
350 additional oxidative stress. Moreover, in all the experimental groups except for the AB
351 group, the carnitine metabolism was affected by an increase in the concentration of medium-
352 chain acylcarnitine compounds in the blood plasma. This finding agrees with the results of
353 Bene et al. (2006) regarding the increase in the level of decadienyl-L-carnitine in patients
354 affected by an acute inflammation of the hindgut. Moreover, increases in the acylcarnitine
355 compounds in the CO and CM groups, supported by evidence of their involvement in the
356 activation of the pro-inflammatory signaling pathways (Rutkowsky et al., 2014), indicated
357 the low protection rate against ETEC in these groups. Conversely, ceramide, a sphingolipid
358 involved in the regulation of cell growth, survival, immune cell trafficking and epithelial
359 integrity, which plays a key role in inflammation (Maceyka and Spiegel, 2014), was
360 downregulated in the PR and CM groups as compared with animals fed the control diet.
361 These findings, particularly in the CM group, indicate regulation between pro- and anti-
362 inflammatory signals which helps to explain the survival curve when pigs are fed *S.*
363 *cerevisiae* CNCM I-4407 in a competitive way.

364 In summary, our results demonstrated the effectiveness of *S. cerevisiae* CNCM I-4407 in
365 delaying cachexia in ETEC-susceptible piglets, providing a window for therapeutic
366 intervention. Moreover, preliminary evidence was provided regarding new perspectives for
367 the use of live yeast in livestock to reduce the use of antibiotics. Unfortunately, our evidence
368 suggested that this yeast strain alone is not completely capable of exerting a therapeutic

369 action when ETEC has already adhered to its specific receptors but it is, however, effective
370 as preventive treatment.

371

372 Literature cited

373 Armstrong, D., T.W. Bennedsgaard, L. Boyle, D. Burch, B. Debski, J. Dewulf, and R.

374 Wregor. 2014. EIP-AGRI Focus Group-Reducing antibiotic use in pig farming: Final

375 Report. European Commission, Brussels, Belgium.

376 Badia, R., G. Zanello, C. Chevaleyre, R. Lizardo, F. Meurens, P. Martínez, J. Brufau, and H.

377 Salmon, 2012. Effect of *Saccharomyces cerevisiae* var. *Boulardii* and b-galactomannan

378 oligosaccharide on porcine intestinal epithelial and dendritic cells challenged in vitro

379 with *Escherichia coli* F4 (K88). *Vet. Microbiol.* 43:4–15.

380 Bene, J., K. Komlósi, V. Havasi, G. Talián, B. Gasztonyi, K. Horváth, G. Mózsik, B.

381 Hunyady, B. Melegh, and M. Figler. 2006. Changes of plasma fasting carnitine ester

382 profile in patients with ulcerative colitis. *World. J. Gastroenterol.* 12:110–113.

383 Bode, J. C., S. Rust, and C. Bode. 1984. The effect of cimetidine treatment on ethanol

384 formation in the human stomach. *Scand. J. Gastroenterol.* 19:853–856.

385 Bosi, P., L. Casini, A. Finamore, C. Gremokolini, G. Merialdi, P. Trevisi, F. Nobili, and E.

386 Mengheri E. 2004. Spray-dried plasma improves growth performance and reduces

387 inflammatory status of weaned pigs challenged with enterotoxigenic *Escherichia coli*

388 K88. *J. Anim. Sci.* 52: 1764–1772.

389 Blehaut, H., J. Massot, G. W. Elmer, and R. H. Levy. 1989. Disposition kinetics of

390 *Saccharomyces boulardii* in man and rat. *Biopharm. Drug. Dispos.* 10:353–64.

391 Campbell, J. M., J. D. Crenshaw, and J. Polo. 2013. The biological stress of early weaned

392 piglets. *J. Anim. Sci. Biotechnol.* 4:19.

393 Collier, C. T., J. A. Carroll, M. A. Ballou, J. D. Starkey, and J. C. Sparks. 2011. Oral
394 administration of *Saccharomyces cerevisiae* boulardii reduces mortality associated with
395 immune and cortisol responses to *Escherichia coli* endotoxin in pigs. *J. Anim. Sci.*
396 89:52–58.

397 Cook, R.D, W. Sanford. 1982. *Residuals and Influence in Regression*. Chapman and Hall.
398 Press, New York, NY.

399 Davison, A. C., and D. V. Hinkley. 1997. *Bootstrap methods and their application*.
400 Cambridge University Press, New York, NY.

401 Etienne-Mesmin, L., V. Livrelli, M. Privat, S. Denis, J-M. Cardot, M. Alric, and S.
402 Blanquet-Diot. 2011. Effect of a new probiotic *Saccharomyces cerevisiae* strain on
403 survival of *Escherichia coli* O157:H7 in a dynamic gastrointestinal model. *Appl.*
404 *Environ. Microb.* 77:1127–1131.

405 Fairbrother, J. M., É. Nadeau, and C. L. Gyles. 2005. *Escherichia coli* in postweaning
406 diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies.
407 *Anim. Health. Res. Rev.* 6:17–39.

408 Farthing, M., M. A. Salam, G. Lindberg, P. Dite, I. Khalif, E. Salazar-Lindo, B. S.
409 Ramakrishna, K-L. Goh, A. Thomson, A. G. Khan, J. Krabshuis, and A. LeMair. 2013.
410 Acute diarrhea in adults and children: a global perspective - WGO Global Guideline
411 Acute Diarrhea. *J. Clin. Gastroenterol.* 47:12–20.

412 Flögel A., N. Stefan, Z. Yu, K. Mühlenbruch, D. Drogan, H-G. Joost, A. Fritsche, H-U.
413 Häring, M. H. de Angelis, A. Peters, M. Roden, C. Prehn, R. Wang-Sattler, T. Illig,
414 M.B. Schulze, J. Adamski, H. Boeing and T. Pischon. 2013. Identification of serum
415 metabolites associated with risk of type 2 diabetes using a targeted metabolomic
416 approach. *Diabetes.* 62:639–648.

417 Grove, R.I., N.J. Allegretto, P.A. Kiener, and G.A. Warr. 1990. Lipopolysaccharide (LPS)
418 alters phosphatidylcholine metabolism in elicited peritoneal macrophages. *J. Leukocyte*
419 *Biol.*, 48:38-42.

420 Guandalini, S., L. Pensabene, M. A. Zikri, J. A. Dias, L. G. Casali, H. Hoekstra, S. Kolacek,
421 K. Massar, D. Micetic-Turk, A. Papadopoulou, J. S. de Sousa, B. Sandhu, H.
422 Szajewska, and Z. Weizman. 2000. Lactobacillus GG administered in oral rehydration
423 solution to children with acute diarrhea: a multicenter European trial. *J. Pediatr. Gastr.*
424 *Nutr.* 30:54–60.

425 Hastie, T., R. Tibshirani, J. Friedman. 2009. The elements of statistical learning. *Data*
426 *mining, inference, and prediction.* 2nd Edition, Springer Science. Press, New York, NY.

427 Heo, J. M., F. O. Opapeju, J. R. Pluske, J. C. Kim, D. J. Hampson, and C. M. Nyachoti.
428 2013. Gastrointestinal health and function in weaned pigs: a review of feeding strategies
429 to control post-weaning diarrhoea without using in-feed antimicrobial compounds. *J.*
430 *Anim. Physiol. Anim. Nutr.* 97:207–237.

431 Kurugöl, Z., and G. Koturoğlu. 2005. Effects of *Saccharomyces boulardii* in children with
432 acute diarrhea. *Acta Paediatr.* 94: 44–47.

433 Maceyka, M., and S. Spiegel. 2014. Sphingolipid metabolites in inflammatory disease.
434 *Nature* 510:58-67..

435 McFarland, L. V. 2010. Systematic review and meta-analysis of *Saccharomyces boulardii* in
436 adult patients. *World J. Gastroenterol.* 16:2202–2222.

437 Martins, F., S. D. A. Elian, A. T. Vieira, F. C. P. Tiago, A. K. S. Martins, F. C. P. Silva, É.
438 L. S. Souza, L. P. Sousa, H. R. C. Araújo, P. F. Pimenta, C. A. Bonjardimb, R. M. E.
439 Arantes, M. M. Teixeira, and J. R. Nicoli. 2011. Oral treatment with *Saccharomyces*
440 *cerevisiae* strain UFMG 905 modulates immune responses and interferes with signal

441 pathways involved in the activation of inflammation in a murine model of typhoid fever.
442 Int. J. Med. Microbiol. 301:359–364.

443 Nagy, B., and P. Z. Fekete. 2005. Enterotoxigenic *Escherichia coli* in veterinary medicine.
444 Int. J. Med. Microbiol. 295:443–454.

445 Nishiyama D., K. Ikejima, H. Honda, M. Hirose, Y. Takei, and N. Sato. 2002. Acute ethanol
446 administration down-regulates toll-like receptor-4 in the murine liver. Hepatol. Res.
447 23:130–137.

448 Pinheiro, J., and D. Bates. 2009. Mixed-Effects models in S and S-PLUS, Springer-Verlag,
449 New York, NY.

450 Robichaud, P.P., K. Boulay, J.É. Munganyiki and M.E. Surette. 2013. Fatty acid remodeling
451 in cellular glycerophospholipids following the activation of human T cells. J. Lipid Res.
452 54:2665-2677.

453 Rutkowsky, J. M., T. A. Knotts, K. D. Ono-Moore, C. S. McCain, S. Huang, D. Schneider,
454 S. Singh, S. H. Adams, and D. H. Hwang. 2014. Acylcarnitines activate
455 proinflammatory signaling pathways. Am. J. Physiol-Endoc. M. 306:E1378–E1387.

456 Sell, D. R., C. M. Strauch, W. Shen, and W. M. Monnier. 2007. 2-Amino adipic acid is a
457 marker of protein carbonyl oxidation in the aging human skin: effects of diabetes, renal
458 failure and sepsis. Biochem. J. 404:269–277.

459 Sellwood, R., R. A. Gibbons, G. Jones, and J. M. Rutter. 1975. Adhesion of
460 Enteropathogenic *Escherichia coli* to pig intestinal brush borders: the existence of two
461 pig phenotypes. J. Med. Microbiol. 8:405–411.

462 Shan, L-S., P. Hou, Z-J. Wang, F-R. Liu, N. Chen, L-H. Shu, H. Zhang, X-H. Han, X-X. Ca,
463 Y-X. Shang, and Y. Vandenplas. 2013. Prevention and treatment of diarrhoea with
464 *Saccharomyces boulardii* in children with acute lower respiratory tract infections. Benef.
465 Microbes. 4:329–334.

466 Shornikova, A., E. Isolauri, L. Burkanova, S. Lukovnikova, and T. Vesikari. 1997. A trial in
467 Karelian republic of oral rehydration and Lactobacillus GG for treatment of acute
468 diarrhea. *Acta Paediatr.* 86:460–465.

469 Sugiharto, S., M. S. Hedemann, and C. Lauridsen. 2014. Plasma metabolomic profiles and
470 immune responses of piglets after weaning and challenge with *E. coli*. *J. Anim. Sci.*
471 *Biotechnol.* 5:17.

472 Szajewska, H., A. Skórka, and M. Dylag. 2007. Meta-analysis: *Saccharomyces boulardii* for
473 treating acute diarrhoea in children. *Aliment. Pharm. Therap.* 25:257–264.

474 Torrallardona, D., M.R. Conde, I. Badiola, J. Polo, and J. Brufau. 2003. Effect of fishmeal
475 replacement with spray-dried animal plasma and colistin on intestinal structure,
476 intestinal microbiology, and performance of weanling pigs challenged with *Escherichia*
477 *coli* K99. *J. Anim. Sci.* 81:1220-1226.

478 Trckova, M., M. Faldyna, P. Alexa, Z.S. Zajacova, E. Gopfert, D. Kumprechtova, E. Auclair,
479 and R. D’Inca. 2014. The effects of live yeast *Saccharomyces cerevisiae* on postweaning
480 diarrhea, immune response, and growth performance in weaned piglets. *J. Anim. Sci.*
481 92:767–774.

482 Trevisi, P, D. Melchior, M. Mazzoni, L. Casini, S. De Filippi, L. Minieri, G. Lalatta-
483 Costerbosa and P. Bosi. 2009. A tryptophan-enriched diet improves feed intake and
484 growth performance of susceptible weanling pigs orally challenged with *Escherichia*
485 *coli* K88. *J. Anim. Sci.* 87:148-156.

486 Van den Broeck, W., E. Cox, and B. M. Goddeeris. 1999. Receptor-dependent immune
487 responses in pigs after oral immunization with F4 fimbriae. *Infect. Immun.* 67:520–526.

488 Wikoff, W. R., A. T. Anfora, J. Liu, P. G. Schultz, S. A. Lesleyb, E. C. Peters, and G.
489 Siuzdak. 2009. Metabolomics analysis reveals large effects of gut microflora on
490 mammalian blood metabolites. *PNAS.* 106:3698–3703.

491 Zanello, G., F. Meurens, M. Berri, C. Chevaleyrea, S. Melo, E. Auclair, and H. Salmona.
492 2011a. *Saccharomyces cerevisiae* decreases inflammatory responses induced by F4+
493 enterotoxigenic *Escherichia coli* in porcine intestinal epithelial cells. *Vet. Immunol.*
494 *Immunop.* 141:133–138.

495 Zanello, G., M. Berri, J. Dupont, P-Y. Sizaret, R. D’Inca, H. Salmon, and F. Meurens.
496 2011b. *Saccharomyces cerevisiae* modulates immune gene expressions and inhibits
497 ETEC mediated ERK1/2 and p38 signaling pathways in intestinal epithelial cells. *PLoS*
498 *ONE* 6(4):e18573. doi:10.1371/journal.pone.0018573.

499

500 **Table 1.** Ingredients and calculated composition of the basal diet (% as-fed basis).

Ingredient	%	Calculated composition ¹	% or otherwise
Wheat shorts	20	CP	18.13
Corn	17	Crude fat	6.01
Barley	15	Total Lys	1.28
Barley, extruded	15	Total Thr	0.87
Soybean meal, 50	13.4	Total Met	0.50
Whey, dehydrated, skimmed	6	Total Met+Cys	0.81
Potato, protein concentrate	4	Total Trp	0.28
Vegetable oil	4	DE, growing pig, kcal/kg	3355
Beet pulp, dehydrated	2	NE, growing pig kcal/kg	2424
Calcium carbonate	1.38		
Monosodium phosphate hydrated	0.6		
L-Lysine HCl	0.4		
Sodium chloride	0.3		
DL-Methionine	0.2		
L-Threonine	0.15		
L-Tryptophan	0.07		
Vitamin and trace mineral mixture ²	0.5		

501 ¹Values were estimated by the EvaPig[®] database (Noblet et al., 2008); ² Provided per
502 kilogram of diet: vitamin A, 9000 IU; vitamin D₃, 1500 IU; vitamin K₃, 2 mg; vitamin E, 50
503 mg; vitamin B₁, 2 mg; vitamin B₂, 4 mg; vitamin B₆, 4 mg; vitamin B₁₂, 0.04 mg; niacin, 55
504 mg; biotin, 0.15 mg; d-pantothenic acid, 30 mg; folacin, 2 mg; choline chloride, 400 mg; iron
505 as FeSO₄, 150 mg; zinc as ZnSO₄, 110 mg; copper as CuSO₄, 25 mg; manganese as MnSO₄,

506 70 mg; iodine as KI, 1 mg; selenium as Na_2SeO_4 , 0.3 mg.

507 **Table 2.** Effect of dietary supplementation with *Saccharomyces cerevisiae* CNCM I-4407 on
 508 different patterns regarding the mortality of weaned pigs challenged with ETEC.

	Diet ¹					P – Fisher’s exact test			
	CO	AB	PR	CM	CU	CO vs.	CO vs .	CO vs.	CO vs.
						AB	PR	CM	CU
Alive	3	9	7	6	4				
Dead	7	1	3	4	5	<0.01	0.089	0.181	0.430

509 ¹ **CO:** no live yeast + F4 challenge; **AB:** antibiotic + F4 challenge; **PR:** Preventive
 510 administration pattern of live yeast (5×10^{10} CFU/kg of feed from d 0 to d 21) + F4
 511 challenge; **CM:** Competitive administration of live yeast (5×10^{10} CFU/kg of feed from d 7
 512 to d 11) + F4 challenge; **CU:** Curative administration of live yeast (1 shot of 2×10^{11} CFU
 513 when the first diarrhea appears) + F4 challenge.

515 **Table 3.** Effect of dietary supplementation with *Saccharomyces cerevisiae* CNCM I-4407 with different patterns on the fecal score of weaned
 516 pigs challenged with ETEC.

Hours	Diet ¹					SEM	AB vs. CO	YEAST vs. CO	PR vs. CM	PR vs. CM+CU	CM vs. CU
	CO	AB	PR	CM	CU						
- 12 ⁴	2.4	2.0	2.1	1.9	1.7	0.2	0.89	0.77 ²	-	-	-
12	2.9	2.2	2.5	2.3	2.3	0.2	0.02	0.04 ³	0.51	-	-
24	3.7	2.4	3.0	3.2	2.8	0.3	0.001	0.08 ³	0.57	-	-
48	4.2	2.4	3.4	3.7	3.2	0.3	<0.001	0.04	-	0.93	0.38
72	4.1	2.6	3.4	3.9	3.5	0.4	<0.05	0.41	-	0.67	0.42
96	4.0	2.5	2.9	3.9	3.5	0.4	0.01	0.38	-	0.08	0.60
120	3.3	1.9	3.5	3.9	3.7	0.3	0.01	0.42	-	0.58	0.81
144	3.0	1.9	3.2	3.3	3.1	0.3	0.02	0.73	-	0.93	0.77

517 ¹ **CO:** no live yeast + F4 challenge; **AB:** antibiotic + F4 challenge; **PR:** Preventive administration pattern of live yeast (5×10^{10} CFU/kg of feed
 518 from d 0 to d 21) + F4 challenge; **CM:** Competitive administration of live yeast (5×10^{10} CFU/kg of feed from d 7 to d 11) + F4 challenge; **CU:**
 519 Curative administration of live yeast (1 shot of 2×10^{11} CFU when the first diarrhea appears) + F4 challenge; ² CM and CU were **combined** with

520 CO because the pigs had not yet been given yeast;³ YEAST includes PR and CM only while CU was not considered in the contrast; ⁴ Contrast
521 before the challenge.

522 **Table 4.** Effect of dietary supplementation with *Saccharomyces cerevisiae* CNCM I-4407 with different patterns on total and specific
 523 immunoglobulins against ETEC and on the fecal excretion of ETEC of weaned pigs challenged with this strain.

Diet ¹						PR vs.	PR	AB	YEAST	PR	CM	
						CM+	vs.	vs.	vs.	vs.	vs.	
						SME	CU+CO ³	AB	CO	CO ⁴	CM+CU ⁴	CU ⁴
	CO	AB	PR	CM	CU							
Total IgA (mg/L)												
d7 ²	400	391	439	344	371	51	0.25	0.70	0.90	-	-	-
d12	801	717	666	1045	711	102	-	-	0.61	0.96	0.28	0.11
d21	1090	1312	1203	1298	1269	227	-	-	0.53	0.60	0.76	0.93
Specific IgA against ETEC (UI)												
d7 ²	0.5	0.21	0.18	0.14	0.33	0.12	0.13	0.56	0.12	-	-	-
d12	88.3	13.2	11.7	26.3	66.1	21.4	-	-	0.04	0.10	0.16	0.26
d21	182	45	340	228	210	114	-	-	0.23	0.97	0.93	0.98
ETEC fecal counts (log₁₀ CFU/g)												

d10 ⁵	8.9	8.4	7.3	8.2	7.7	0.5	-	-	0.52	0.05	0.45	0.54
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525

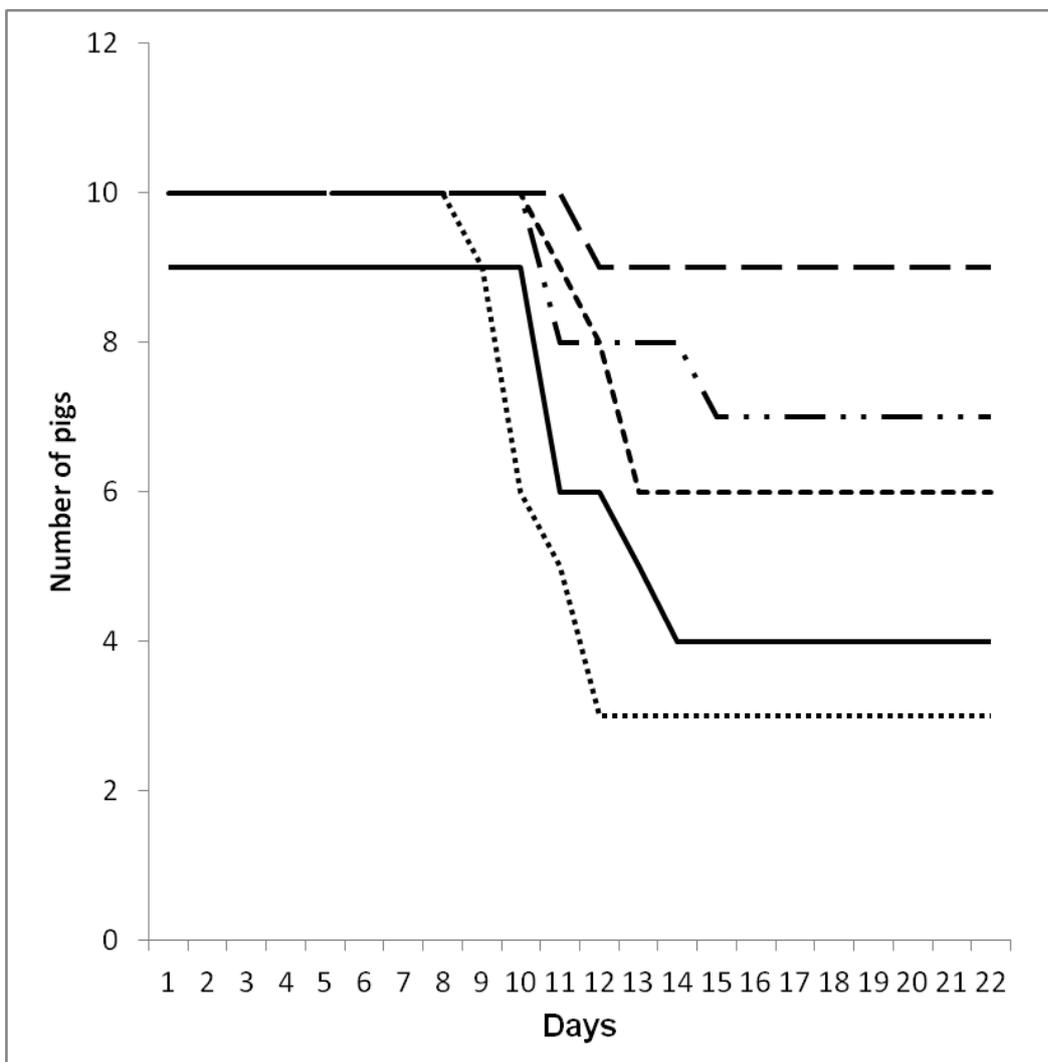
526 ¹ **CO**: no live yeast + F4 challenge; **AB**: antibiotic + F4 challenge; **PR**: Preventive administration pattern of live yeast (5×10^{10} CFU/kg of feed
527 from d 0 to d 21) + F4 challenge; **CM**: Competitive administration of live yeast (5×10^{10} CFU/kg of feed from d 7 to d 11) + F4 challenge; **CU**:
528 Curative administration of live yeast (1 shot of 2×10^{11} CFU when the first diarrhea appears) + F4 challenge; ² Contrast before the challenge; ³
529 CM and CU were **combined** with CO because the yeast **had not yet been given** to the pigs; ⁴ Contrast after the challenge; ⁵ Four days post-
530 challenge.

531 **Table 5.** Effect of *Saccharomyces cerevisiae* CNCM I-4407 on blood metabolic profile
 532 metabolites 24 h after infection with ETEC in weaned pigs.

Diet ¹ / Metabolites	P-value	Direction
AB vs CO		
C12:1 ²	<0.01	CO ↑
C5DC (C6-OH) ³	0.02	CO ↑
PC_aa_C40:1 ⁴	0.01	CO ↑
PC_aa_C40:6 ⁵	<0.01	CO ↑
alpha-AAA ^{6,9}	<0.01	CO ↑
CM+PR vs. CO		
SM_C18:0 ^{7,9}	0.02	CM+PR ↓
C10:2 ⁸	0.02	CM+PR ↑
CM vs. PR		
C10:2 ⁸	<0.01	CM ↑

533 ¹ **CO:** no live yeast + F4 challenge; **AB:** antibiotic + F4 challenge; **PR:** Preventive
 534 administration pattern of live yeast (5×10^{10} CFU/kg of feed from day 0 to day 21) + F4
 535 challenge; **CM:** Competitive administration of live yeast (5×10^{10} CFU/kg of feed from d7
 536 to d11) + F4 challenge; ²Dodecenoyl-L-carnitine; ³Glutaryl-L-carnitine / Hydroxyhexanoyl-
 537 L-carnitine; ⁴Phosphatidylcholine diacyl C 40:1; ⁵Phosphatidylcholine diacyl C 40:6; ⁶alpha-
 538 amino adipic acid; ⁷Shingomyeline-Ceramide; ⁸Decadienyl-L-carnitine; ⁹Affected by the
 539 confounding factor “fecal score”

540 **Figure 1.** Effect of dietary supplementation with *Saccharomyces cerevisiae* CNCM I-4407
 541 at different times and doses on the survival of weaned pigs challenged with ETEC (····· **CO**:
 542 no live yeast + F4 challenge; — — **AB**: antibiotic + F4 challenge; —·—· **PR**: Preventive
 543 administration pattern of live yeast (5×10^{10} CFU/kg of feed from d 0 to d 21) + F4
 544 challenge; - - - **CM**: Competitive administration of live yeast (5×10^{10} CFU/kg of feed
 545 from d 7 to d 11) + F4 challenge; ——— **CU**: Curative administration of live yeast (1 shot of
 546 2×10^{11} CFU when the first diarrhea appears) + F4 challenge).



547