



# AperTO - Archivio Istituzionale Open Access dell'Università di Torino

## Live yeast (Saccharomyces cerevisiae var. boulardii) supplementation in fattening rabbit diet: Effect on productive performance and meat quality

## This is the author's manuscript

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/142522 since 2016-10-10T14:29:02Z

Published version:

DOI:10.1016/j.livsci.2014.01.022

Terms of use:

**Open Access** 

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)



# UNIVERSITÀ DEGLI STUDI DI TORINO

2 3 4 5 6 7 8	This Accepted Author Manuscript (AAM) is copyrighted and published by Elsevier. It is posted here by agreement between Elsevier and the University of Turin. Changes resulting from the publishing process - such as editing, corrections, structural formatting, and other quality control mechanisms - may not be reflected in this version of the text. The definitive version of the text was subsequently published in:
9 10 11	Livestock Science, Volume 162, April 2014, Pages 178–184
12 13 14	Live yeast ( <i>Saccharomyces cerevisiae</i> var. <i>boulardii</i> ) supplementation in fattening rabbit diet: Effect on productive performance and meat quality
15 16	Authors: L. Rotolo, F. Gai, P.G. Peiretti, M. Ortoffi, I. Zoccarato, L. Gasco
17 18	doi:10.1016/j.livsci.2014.01.022
19	The definitive version is available at:
20	La versione definitiva è disponibile alla URL:
21 22 23 24	http://www.sciencedirect.com/science/article/pii/S1871141314000560
25	

- Live yeast (Saccharomyces cerevisiae var. boulardii) supplementation in fattening rabbit diet:
   Effect on productive performance and meat quality
- 28

29 L. Rotolo<sup>a</sup>, F. Gai<sup>b</sup>, P.G. Peiretti<sup>b</sup>, M. Ortoffi<sup>b</sup>, I. Zoccarato<sup>a</sup>, L. Gasco<sup>a\*</sup>

30

<sup>a</sup> Dept. of Agricultural, Forest, and Food Sciences, University of Torino, Grugliasco, Italy

<sup>32</sup> <sup>b</sup> Institute of Science of Food Production, National Research Council, Grugliasco, Italy

33

\* Corresponding author: Dept. of Agricultural, Forest, and Food Sciences, University of Torino, Via
Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy - Tel: +39 011 6708574 - Fax: +39 011
6708563 - Email address: laura.gasco@unito.it

37

### 38 Abstract

The effects of dietary supplementation with Saccharomyces cerevisiae boulardii (CNCM I-1079 39 strain, LSB) at 0, 300 and 600 mg/kg on apparent digestibility, growth performance, caecal 40 fermentation, carcass characteristics and meat quality of broiler rabbits were studied from 37 to 84 41 days of age. One hundred and fifty New Zealand White rabbits were single housed and randomly 42 43 allotted into three groups. Animals were fed isocaloric and isonitrogenous basal diets ad libitum, supplemented with different levels of concentrated live yeast LSB (0, 3x10<sup>6</sup> and 6x10<sup>6</sup> colony 44 forming unit (CFU)/g diet, respectively). Protected LSB was resistant to the pelleting process and to 45 passage through the rabbit digestive tract as far as the caecum, where it showed an 86% survival 46 rate in the 600 mg/kg supplementation level group. Significant differences were found only for the 47 fibrous fractions digestibility that were lowest (P=0.001) in the animals fed 300 mg/kg 48 supplemented diet, while yeast and mould populations in the caecum increased (P=0.001) in the 49 animals fed 300 and 600 mg/kg supplemented diets (4.16 and 4.76 log CFU/g, respectively). 50

51 Mortality did not differ amongst dietary treatments being 10, 8 and 6% for groups fed LSB at 0, 300 52 and 600 mg/kg, respectively.

53

54 Keywords: Rabbit; Probiotic; Performance, Saccharomyces cerevisiae boulardii; Yeast

55

### 56 1. Introduction

In commercial production, health problems related to intestinal pathology are a major cause of mortality and reduced growth rates, especially in growing rabbits. In 2006, a complete ban on the use of antibiotics as growth promoters focused attention on probiotics as possible alternatives for improving production and health status in livestock (Maertens et al., 2006).

Probiotic provision has been effective in rabbits and other species when the animals are raised in unfavourable conditions (Zoccarato et al., 1995; Trocino et al., 2005), although the mechanism underlying this improved performance and welfare remains partially unexplained. There is evidence that probiotics act mainly by competing with enteric pathogens, balancing colonic microbiota, modulating the systemic and mucosal immune systems and influencing the intestinal barrier (Fortun-Lamothe and Boullier, 2007; Ng et al., 2009).

Among probiotic sources tested in rabbit rearing, many strains are of bacterial and yeast 67 origin, including the colonising (Lactobacillus and Enterococcus spp) and non-colonising (Bacillus 68 spp, Saccharomyces cerevisiae) microorganisms. Bovera et al. (2012) tested the effect of 69 Lactobacillus plantarum spray application on suckling New Zealand White rabbits and observed 70 changes in caecal microflora and a significantly lower mortality. Maertens et al. (1994) studied the 71 effect of dietary supplementation of Bacillus cereus (strain CIP5832) on caecal and growth 72 parameters of weanling rabbits. This work showed that the addition of this probiotic improved the 73 weaning weight and feed efficiency while no effect on mortality was observed. Oso et al. (2013) 74 reported poor growth response in growing rabbits fed a basal diet supplemented with 0.5 g/kg of 75

#### 76 Bacillus cereus or Pediococcus acidilactis.

Supplementation with probiotic sources of yeast origin has been evaluated on rabbits 77 (Maertens and De Groote, 1992; Onifade et al., 1999). Only the NCYC Sc 47 strain of 78 79 Saccharomyces cerevisiae has been approved in the European Union for the fattening period (Falcão-e-Cunha et al., 2007). Saccharomyces cerevisiae boulardii (CNCM I-1079 strain) is a non-80 pathogenic yeast widely used in human medicine to prevent and treat intestinal disorders, such as 81 82 infectious and antibiotic-associated diarrhoea (Buts and De Keyser, 2006; Czerucka et al., 2007). Its role in gut function has also been highlighted in physiological studies on swine, and trials as a feed 83 additive in husbandry conditions have shown its positive effects on weaned pigs (Le Bon et al., 84 85 2010). The aim of this preliminary study was to investigate the effect of increasing dietary supplementation of Saccharomyces cerevisiae boulardii (LSB, LEVUCELL® SB10 ME TITAN, 86 Lallemand Sas, Blagnac, France) on the apparent digestibility, growth performance, caecal 87 88 fermentation, carcass characteristics and meat quality of broiler rabbits.

89

#### 90 2. Materials and Methods

#### 91 2.1. Animals, housing and diets

92 The study was carried out at the Department of Agriculture, Forest, and Food Sciences 93 experimental rabbitry in Carmagnola (Turin, Italy). One hundred fifty New Zealand White rabbits were single housed in triple deck cages from 37 to 84 d of age. Rabbits were randomly allotted into 94 three groups and fed isocaloric and isonitrogenous diets *ad libitum*, supplemented with LSB  $[1x10^{10}]$ 95 colony forming unit (CFU)/g] at 0, 300 and 600 mg/kg of diet, respectively. After pelleting, one 96 sample (500 g) of each diet was collected and stored in a plastic box at ambient temperature for 97 yeast analysis. The analyses showed a concentration corresponding to 0,  $3x10^6$  and  $6x10^6$  CFU/g 98 99 diet, respectively. The LSB strain utilised was the CNCM I-1079, that is a concentrated live yeast supplied in a micro-encapsulated formulation and pre-mixed with barley meal. The ingredients and 100

101 composition of the basal diet are shown in Table 1. Diets did not contain antibiotics or 102 coccidiostatics.

103

## 104 *2.2. Digestibility trial*

The nutrient digestibility coefficients of each diet were determined in the second week of the 105 growing trial (with 10 rabbits aged 44d). The faeces were individually collected for five days using 106 107 a nylon net placed under the floor of each cage, to avoid urine contamination. The faeces were collected daily, at approximately 0900 h, before the daily ration was provided. Each faecal sample 108 was immediately weighed and then placed in a two-layer plastic bag to prevent loss of moisture and 109 110 immediately frozen at -20°C. The frozen samples, upon arrival at the laboratory, were dried in a draft oven at 80°C to constant weight and then ground in a homogenizer (Tecator, Herndon, VA, 111 USA) and stored at - 20°C for chemical analysis. 112

All the analyses were carried out according to recommendations of the European Group on Rabbit Nutrition (EGRAN, 2001) on three replicates of each feed and two replicate of each faeces sample. Diets and faeces were analyzed to determine total N content according to AOAC method #984.13 (AOAC, 2000), ash by ignition to 550°C, and ether extract by AOAC method #945.16 (AOAC, 2000), using the Soxlet method without previous acid hydrolysis. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest et al. (1991).

119 The apparent digestibility of the rations was calculated using total collection of faeces for 120 each rabbit and for each diet according to the following equation:

121 Digestibility = (ingested amounts – excreted amounts) / ingested amounts

122

### 123 2.3. Growth performance and carcass traits

124 The rabbit weight and feed intake were recorded every 14 days, except for the last period 125 which lasted 17 days. Mortality was recorded daily throughout the experimental period. Average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) were
calculated. Data from animals that died were excluded from the calculations of growth performance
parameters.

At the end of the experimental period, ten rabbits per group were weighed, stunned and slaughtered. The carcasses were prepared by removing non edible parts, as recommended by Blasco et al. (1993), and the gastrointestinal tract was weighed. After chilling for 24 h, weight of carcasses (with head, liver, kidneys, thoracic organs) were recorded and dressing out percentage was calculated. Liver, kidneys, heart and lungs were separated from the chilled carcass and weighed. The weights of the full gastrointestinal tract, liver, kidneys, heart and lungs were expressed as a percentage of slaughter weight (SW).

136

#### 137 *2.4. Caecal content analyses*

On five animals per group, the caecum was separated from the digestive tract, weighed and the pH value of the fresh caecal content was determined directly using a Crison MicropH 2001 pH meter (Crison Instruments, Barcelona, Spain). Caecal content was then removed, put into plastic bottles, and stored at -20°C until chemical and microbiological analyses were performed. The remaining empty caecum was washed with distilled water, dried with blotting paper and weighed.

143 Alcohol and volatile fatty acid (VFA) concentrations were determined on aqueous extracts of caecal content. One g of sample was extracted with 5 mL of distilled water at 20°C. The mixture 144 was centrifuged for 5 min at 3000xg and then filtered through a Schleicher and Schull membrane 145 filter (BA-83, 0.2 µm). Using an on-column technique with an auto-sampler (Dani Instruments 146 SpA, ALS 1000, Cologno Monzese, Italy), a 1 µL aliquot of the extract was injected into a wide-147 bore capillary column (SGE BP21 25m x 0.53 mm internal diameter and 0.5 µm film thickness; P/N 148 054474, SGE International, Ringwood, Victoria, Australia) installed in a gas chromatograph (Dani 149 GC 1000 DPC), running in a temperature-programmed mode and equipped with a flame ionization 150

detector and a PTV injection port, used in split mode, with a split vent flow of 100 mL/min. The injector and detector ports were set at 230°C and 240°C, respectively. Helium was used as the carrier gas and the oven temperature was programmed to increase from 60°C to 200°C at 5°C per min and held for 2 min giving a run time of 30 min. The peak area was measured using a Dani Data Station DDS 1000. Each peak was identified and quantified according to pure standards (Sigma Chemical, St. Louis, MO, USA).

Microbiological analyses were carried out on 10 g of caecal content taken under sterile 157 conditions. Caecal content was weighed in a sterilized bag and homogenized in 0.90 g/L sterile 158 saline solution for 2 min in a stomacher (PBI International, Milan, Italy), in accordance with the 159 160 methods proposed by Kovács et al. (2006) and Mourão et al. (2006). From the resulting dilution, decimal dilutions were prepared for yeast and moulds  $(10^{-2}, 10^{-3}, \text{ and } 10^{-4})$ , for total viable counts 161 (TVC) and total anaerobes  $(10^{-3}, 10^{-4}, \text{ and } 10^{-5})$  using 0.90 g/L sterile saline solution and plated in 162 163 duplicate to enumerate the following microorganisms: yeast and moulds were enumerated using the surface-plate method on Sabouraud Dextrose Agar (Oxoid Ltd, Cambridge, UK). Plates were 164 incubated at 25 °C for 72-110 h. TVC were enumerated by the pour-plate method using Plate Count 165 Agar (Oxoid Ltd, Cambridge, UK). Plates were incubated at 30 °C for 48 h. Total anaerobes were 166 enumerated by the inclusion method using Violet Red Bile Glucose agar (Oxoid Ltd, Cambridge, 167 UK). Plates were incubated at 37 °C for 24 h. The number of colonies was expressed as log CFU 168 per gram of chymus. All microbiological analyses were performed in duplicate. 169

170

## 171 2.5. Meat quality analyses

172 2.5.1. Sample preparation

After chilling for 24 h in a refrigerated room  $(+ 4^{\circ}C)$ , the carcasses were halved and the two *longissimus dorsi* (LD) muscles were excised. The left LD muscle was divided into two parts. The fore part was used to measure pH, colour and cooking losses. The hind part of the left LD and the whole right LD were vacuum-packed, frozen and stored at  $-20^{\circ}$ C until analyses were performed.

177 2.5.2. pH and Colour measurements

pH<sub>24</sub> was measured on the LD with a Crison MicropH 2001 (Crison Instruments, Barcelona,
Spain) provided with a combined electrode and an automatic temperature compensator.

Meat colour was measured at room temperature (20 °C) using a portable Minolta CR-331C Minolta Colorimeter (Minolta Camera, Osaka, Japan) with D<sub>65</sub> illuminant and 2° standard observer. The results were expressed in terms of lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ) in the CIELAB colour space model (CIE, 1976). Chroma [C\*= ( $a^{*2}$ +  $b^{*2}$ )<sup>1/2</sup>] and Hue [H<sup>0</sup>= tan<sup>-1</sup> ( $b^*/a^*$ )] were calculated according to Boccard et al. (1981). Values were the mean of two different measurements per meat sample.

186 2.5.3. Cooking losses

187 Samples of LD from each rabbit were weighed (F), vacuum packed in plastic bags and 188 cooked at 80 °C for 1 h by immersion in a water bath (Ramírez et al., 2004). Cooked samples were 189 cooled under running water for 30 min. The samples were then removed from the bags, blotted and 190 weighed (C). Cooking losses were calculated as (F - C)  $\times$  100/F.

191 2.5.4. Chemical composition

LD muscles were analyzed to determine moisture according to AOAC method #950.46 (AOAC, 2000), total N content by AOAC method #928.08 (AOAC, 2000), ether extract by AOAC method #960.39 (AOAC, 2000), and ash by AOAC method #920.153 (AOAC, 2000). Values were expressed on a fresh matter basis.

196

```
197 2.6. Statistical analysis
```

198 Statistical analyses were performed using the SPSS software package (IBM SPSS, 2012). 199 Mortality rate differences amongst groups were tested with the Fisher exact test (R Core Team, 200 2013). Bacterial numbers were not normally distributed and were log transformed to create a normal distribution prior to analysis. Analysis of variance was used to evaluate the effect of
different LSB levels on the nutrient digestibility coefficients, growth performance, caecal activity,
carcass characteristics and meat quality of broiler rabbits. Differences among treatment means were
determined using Duncan's test at a probability level of 0.05.

205

### 206 3. Results and discussion

#### 207 *3.1. Digestibility trial*

Apparent digestibility coefficients are reported in Table 2. The results show that the supplementation of LSB does not modify the dry matter intake and digestibility of dry matter, organic matter, EE, and CP. Differences (P < 0.001) were found for both NDF and ADF. Animals fed 300 mg/kg supplemented diet had the lowest value of both fibrous fraction digestibilities, while the ADF digestibility coefficient of animals fed 600 mg/kg supplemented diet showed an intermediate value between the other two groups.

Kimsé et al. (2012) found that adding live yeast (Saccharomyces cerevisiae NCYC Sc 47) 214 did not modify the total digestibility of nutrients. Similarly, Chaudary et al. (1995) found that oral 215 administration of yeast culture had no effect on the digestibility of nutrients in rabbits fed diets with 216 different fibre content. Kamra et al. (1996) reported that feeding probiotics (Lactobacillus 217 218 acidophillus, L. casei and Saccharomyces cerevisiae ITCCF 2094) have no significant effect on growth performance and NDF, ADF, hemicellulose and cellulose digestibilities in New Zealand 219 White rabbits under Indian hot climate environmental conditions. Oso et al. (2013) found that ADF, 220 NDF and other nutrient digestibility values were not affected by dietary inclusion of probiotics of 221 bacterial origin (Pediococcus acidilactis or Bacillus cereus) in mixed breed weaner rabbits. In 222 contrast, in weaned piglets in a feeding trial lasting 35d, Giang et al. (2010) found that a basal diet 223 supplemented with 0.2% yeast and a mixture of lactic acid bacteria improved the apparent total tract 224 digestibility of CP, crude fibre and organic matter. 225

226

# 227 *3.2.Health status and growth performance*

Mortality percentages were: 10, 8 and 6% for groups fed 0, 300 and 600 mg/kg LSB respectively. There were no significant differences among dietary treatments. Kimsé et al. (2012) stated that on growing rabbits from day 35 to day 70, the supplementation of 10<sup>6</sup> CFU/ g of *Saccharomyces cerevisiae* NCYC Sc 47 strain (Activesaf<sup>®</sup>) significantly halved mortality over the whole fattening period, compared to the control.

233 No growth performance parameters were affected by live yeast addition (Table 3) There 234 were also no differences in carcass characteristics among treatments (Table 4).

235 In a recent trial, aiming to study the response to Escherichia coli lipopolysaccharide administration, Collier et al. (2011) reported greater ADG than the controls in pigs whose diet was 236 supplemented with 222 g/t of active dry yeast, Saccharomyces cerevisiae boulardii. Similarly, in the 237 238 same species, Le Bon et al. (2010) found that dietary supplementation of Saccharomyces cerevisiae boulardii CNCM I-1079 strain (2x10<sup>9</sup> kg/feed) followed by *Pediococcus acidilactici* significantly 239 improved FCR. On the contrary, Oso et al. (2013) found poor growth response in rabbits fed diets 240 containing the probiotic of bacterial origin Pediococcus acidilactis or Bacillus cereus, while the 241 242 inclusion of the prebiotics mannan and arabinoxylans oligosaccharides showed an improved growth 243 and gut morphology in growing rabbits. Giang et al. (2010) showed that a mixture of lactic acid bacteria complex and Saccharomyces boulardii improved overall live performance. In an exhaustive 244 review, Falcão-e-Cunha et al. (2007) summarized that dietary inclusion of feed additives containing 245 yeast generally improve ADG in rabbits, although results concerning FCR and mortality were 246 partially contradictory. Saccharomyces cerevisiae ( $5 \times 10^8$  CFU per d) orally supplemented as yeast 247 culture, did not improve growth performance in 6-week-old New Zealand White mash-fed rabbits 248 (Chaudary et al., 1995). Similarly, Maertens and De Groote (1992) reported no significant 249 difference in rabbit performance. Conversely, Onifade et al. (1999) found that rabbits fed 3.0 and 250

1.5 g/kg of *Saccharomyces cerevisiae* (Yeasacc<sup>10261®</sup>), had higher body weight and feed intake with
a better feed conversion than the un-supplemented group.

In addition to growth performance, there is a lack of specific studies for rabbits on the effect 253 of Saccharomyces cerevisiae boulardii on carcass characteristics, as most of the works are related to 254 probiotic mixtures or generally indicate Saccharomyces cerevisiae supplementation. Onbaşilar and 255 Yalcin (2008) found no differences in weight percentages of lung, heart, kidney and small intestine 256 in New Zealand White rabbits fed 1g of probiotic (Bioteksin<sup>TM</sup>)/kg diet, but the liver percentage 257 was affected in animals fed 1g of probiotic + 66 mg of anticoccidial agent (Robenine 258 hydrochloride)/kg diet. Similarly, Tripathi and Karim (2011) showed that carcass traits did not 259 260 change in lambs fed diets supplemented by Saccharomyces cerevisiae and other yeast cultures.

261

#### 262 *3.3. Caecal activity*

263 Caecum content characteristics and its contents were reported in Tables 5 and 6, 264 respectively.

The full and empty weights of the caecum and its contents were not affected by treatment 265 and these values were similar to those reported by Cesari et al. (2009) and Gallois et al. (2005) on 266 growing rabbits. The pH value of the caecum content was about 6.3-6.5, similar to values obtained 267 268 by Bónai et al. (2008) who studied the effect of Bacillus cereus var. toyoi on caecal microflora in growing rabbits. The concentration of total VFA and the individual VFA values were unaffected by 269 the treatment and were in accordance with those reported by Gidenne et al. (2000) who pointed out 270 that acetic acid concentration of the caecum ranged between 78.0 and 82.5%, while butyric acid and 271 propionic acid concentrations ranged from 13.1 to 16.9% and from 3.9% and 4.7%, respectively. 272 Similar values were observed by Kimsé et al. (2009) in rabbit: acetate (77%), butyrate (17%) and 273 propionate (5%). 274

275

Oso et al. (2013) found that rabbits fed diets containing probiotics (Pediococcus acidilactis

and *Bacillus cereus*) had the lowest VFA concentration compared to dietary inclusion of prebiotics
and symbiotics, while the concentrations of the acetic, propionic and butyric acid produced were not
affected by dietary inclusion of probiotics.

There was no live yeast in the caecal contents of the rabbits fed the LSB 0 diet while, as expected, rabbits consuming LSB-supplemented diets showed higher (P<0.001) yeast concentrations of 4.16 and 4.76 log CFU/g of chyme, respectively (Table 6).

Live yeast concentration fell slightly after pelleting (-0.7 log CFU/g DM), at 70-80°C. Yeast survival rate, measured as the ratio between yeast intake and yeast excreted, was high and increased significantly from 81 to 86% with increasing yeast addition. A similar result was observed by Kimsé et al. (2012) who found that the survival rate of yeast increased from 90 to 97% with increasing yeast supplementation in rabbit digestive tract.

Luick et al. (1992) found that yeast culture and fructooligosaccharides did not affect caecal fermentation. Similarly, addition of probiotics (yeast or lactobacilli) seemed to not greatly modify caecal fermentation as measured *in vivo* by Kermauner et al. (1994). The germ counts of total anaerobes growing on Schaedler agar in caecal content were similar to those in previous experiments (Bónai et al., 2008).

Gidenne et al. (2002) found that the microflora in the caecum of rabbits is established at weaning, and only minor changes occur with age. Abecia et al. (2005) demonstrated by molecular microbiological tools that bacteria are the main constituents in the rabbit caecum, but Bennegadi et al. (2003) also reported a significant community of archea among other constituents of gut microbiota in rabbits.

297

## 298 3.4. Meat quality traits

Meat traits and chemical composition of LD muscle of rabbits are reported in Table 7. No significant effects of LSB supplementation were observed. Chemical composition of LD muscle fell 301 within the normal range for rabbit meat (Dalle Zotte and Szendro, 2011).

302

#### **4. Conclusions**

Protected live yeast (Saccharomyces cerevisiae boulardii, CNCM I-1079 strain) was 304 resistant to the pelleting process and to the passage through the rabbit digestive tract as far as the 305 caecum where it showed an 86% survival rate in the 600 mg/kg supplementation level group. 306 307 Although the caecal population of veast increased in the rabbits fed LSB supplemented diets, the supplementation at a dose of up to 600 mg/kg did not affect the productive performance, carcass 308 characteristics, caecal fermentation and meat quality of broiler rabbits reared in standard farming 309 310 conditions. No significant differences were found for nutrient digestibilities except for NDF and ADF values. These were lowest in animals fed a 300 mg/kg supplemented diet. 311

312

#### 313 Acknowledgments

The authors thank Dr. Nicole Hocke (Lallemand SAS, Blagnac, France) for providing the LEVUCELL® SB10 ME TITAN yeast, Mrs. Vanda Malfatto for her technical support, Mr. Luciano Sola, Mr. Dario Sola and Mr. Mario Colombano for rabbit care.

317

#### 318 **References**

- Abecia, L., Fondevila, M., Balcells, J., Edwards, J.E., Newbold, C.J., McEwan, N.R., 2005.
   Molecular profiling of bacterial species in the rabbit caecum. FEMS Microbiol. Lett. 244,
   111–115.
- AOAC, 2000. Official Method of Analysis, 17th Edn. Association of Official Analytical Chemists,
   AOAC, Arlington, VA.
- Bennegadi, N., Fonty, G., Millet, L., Gidenne, T., Licois, D., 2003. Effects of age and dietary fibre
  level on caecal microbial communities of conventional and specific pathogen-free rabbits.

- 326 Microb. Ecol. Health Dis. 15, 23–32.
- Blasco, A., Ouhayoun, J., Masoero, G., 1993. Harmonization of criteria and terminology in rabbit
  meat research. World Rabbit Sci. 1, 3–10.
- Boccard, R., Butcher, L., Casleels, E., Cosentino, E., Dransfield, E., Hood, D.E., Joseph, R.L.,
  MacDougall, D.B., Rhodes, D.N., Schön, I., Tinbergen, B.J., Touraille, C., 1981. Procedures
  for measuring meat quality characteristics in beef production experiments. Report of a
  working group in the Commission of the European Communities (CEC), beef production
  research programme. Livest. Prod. Sci. 8, 385–397.
- Bónai, A., Szendrő, Zs., Matics, Zs., Fébel, H., Pósa, R., Tornyos, G., Horn, P., Kovács, F., Kovács,
- M., 2008. Effect of *Bacillus cereus* var. *toyoi* on caecal microflora and fermentation in rabbits.
  Proc. 9th World Rabbit Congress, Verona, Italy, pp. 561–565.
- Bovera, F., Iannaccone, F., Mastellone, V., Nizza, S., Lestingi, A., De Martino, L., Lombardi, P.,
  Mallardo, K., Ferrara, M., Nizza, A., 2012. Effect of spray application of *Lactobacillus plantarum* on *in vivo* performance, caecal fermentations and haematological traits of suckling
  rabbits. Ital. J. Anim. Sci. 11, 145–149.
- Buts, J.P., De Keyser, N., 2006. Effects of *Saccharomyces boulardii* on intestinal mucosa. Dig. Dis.
  Sci. 51, 1485–1492.
- Cesari, V., Grilli, G., Ferrazzi, V., Toschi I., 2009. Influence of age at weaning and nutritive value of
  weaning diet on growth performance and caecal traits in rabbits. World Rabbit Sci. 17, 195–
  205.
- Chaudary, L.C., Singh, R., Kamra, D.N., Pathak, N.N., 1995. Effect of oral administration of yeast
   (*Saccharomyces cerevisiae*) on digestibility and growth performance of rabbits fed diets of
   different fibre content. World Rabbit Sci. 3, 15–18.
- CIE, 1976. Commission Internationale de l'éclairage. Colorimetry. Publication 15, Bureau Central
  de la CIE, Vienna, Austria.

- Collier, C.T., Carroll, J.A, Ballou, M.A., Starkey, J.D., Sparks, J.C., 2011. Oral administration of
   *Saccharomyces cerevisiae boulardii* reduces mortality associated with immune and cortisol
   responses to *Escherichia coli* endotoxin in pigs. J. Anim. Sci. 89, 52–58.
- 354 Czerucka, D., Piche, T., Rampal, P., 2007. Review article: yeast as probiotics *Saccharomyces* 355 *boulardii*. Aliment. Pharmacol. Ther. 26, 767–778.
- 356 Dalle Zotte, A., Szendro, Z., 2011. The role of rabbit meat as functional food. Meat Sci. 88, 319–
  357 331.
- EGRAN (European Group on Rabbit Nutrition), 2001. Technical note: attempts to harmonise
   chemical analyses of feeds and faeces, for rabbit feed evaluation. World Rabbit Sci. 9, 57–64.
- 360 Falcão-e-Cunha, L., Castro-Solla, L., Maertens, L., Marounek, M., Pinheiro, V., Freire, J., Mourão,
- J.L., 2007. Alternatives to antibiotic growth promoters in rabbit feeding: a review. World
  Rabbit Sci. 15, 127–140.
- Fernández-Carmona, J., Cervera, C., Blas, E., 1996. Prediction of the energy value of rabbit feeds
  varying widely in fibre content. Anim. Feed Sci. Technol. 64, 61–75.
- Fortun-Lamothe, L., Boullier, S., 2007. A review on the interactions between gut microflora and
   digestive mucosal immunity. Possible ways to improve the health of rabbits. Livest. Sci. 107,
   1–18.
- Gallois, M., Gidenne, T., Fortun-Lamothe, L., Le Huerou-Luron, I., Lalles, J., 2005. An early
  stimulation of solid feed intake slightly influences the morphological gut maturation in the
  rabbit. Reprod. Nutr. Dev. 45, 109–122.
- Giang, H.H., Viet, T.Q, Lindberg, J.E., Ogle., B., 2010. Effects of microbial enzymes and a complex 371 of lactic acid bacteria and Saccharomyces boulardii on growth performance and total tract 372 Develop. 22. 373 digestibility in weaned pigs. Livest. Res. Rural http://www.lrrd.org/lrrd22/10/gian22179.htm 374
- 375 Gidenne, T., Pinheiro, V., Falcao-E-Cunha, L., 2000. A comprehensive approach of the rabbit

376	digestion: consequences of a reduction in dietary fibre supply. Livest. Prod. Sci. 64, 225–237.
377	Gidenne, T., Jehl, N., Segura, M., Michalet-Doreau, B., 2002. Microbial activity in the caecum of
378	the rabbit around weaning: impact of a dietary fibre deficiency and of intake level. Anim.
379	Feed Sci. Technol. 99, 107–118.
380	IBM SPSS, 2012. IBM SPSS Statistics 20.0 SPSS Inc., Chicago, IL, USA.
381	Kamra, D.N., Chaudhary, L.C., Singh, R., Pathak, N.N., 1996. Influence of feeding probiotics on
382	growth performance and nutrient digestibility in rabbits. World Rabbit Sci. 4, 85-88.
383	Kermauner, A., Strucklec, M., Marinsek-Logar, R., 1994. Influence of a probiotic added to different
384	feed mixtures on the microbial metabolism in caecum of growing rabbits. Znan. Prak. Poljopr.
385	Tehnol. 24, 120–127.
386	Kimsé, M., Monteils, V., Bayourthe, C., Gidenne, T., 2009. A new method to measure the redox
387	potential (EH) in rabbit caecum: relationship with pH and fermentation pattern. World Rabbit
388	Sci. 17, 63–70.
389	Kimsé, M., Bayourthe, C., Monteils, V., Fortun-Lamothe, L., Cauquil, L., Combes, S., Gidenne, T.,
390	2012. Live yeast stability in rabbit digestive tract: Consequences on the caecal ecosystem,
391	digestion, growth and digestive health. Anim. Feed Sci. Technol. 173, 235–243.
392	Kovács, M., Szendro, Z., Milisits, G., Bóta, B., Bíró-Németh, E., Radnai, I., Pósa, R., Bónai, A.,
393	Kovács, F., Horn, P., 2006. Effect of nursing methods and faeces consumption on the
394	development of the bacteroides, lactobacillus and coliform flora in the caecum of the newborn
395	rabbits. Reprod. Nutr. Dev. 46, 205–210.
396	Le Bon, M., Davies, H.E., Glynn, C., Thompson, C., Madden, M., Wiseman, J., Dodd, C.E.R.,
397	Hurdidge, L., Payne, G., Le Treut, Y., Craigon, J., Tötemeyer, S., Mellits, K.H., 2010.
398	Influence of probiotics on gut health in the weaned pig. Livest. Sci. 133, 179–181.
399	Luick, B.R., El-Sayaad, G.A.E., Cheeke, P.R., 1992. Effect of fructooligosaccharides and yeast
400	culture on growth performance of rabbits. J. Appl. Rabbit Res. 15, 1121–1128.
	16

- Maertens, L., De Groote, G., 1992. Effect of a dietary supplementation of live yeast on the
  zootechnical performances of does and weanling rabbits. J. Appl. Rabbit Res. 15, 1079–1086.
- 404 (*Bacillus* CIP 5832) on the milk composition and performances of does and on caecal and 405 growth parameters of their weanlings. World Rabbit Sci. 2, 67–73.

403

Maertens, L., Van Renterghem, R., De Groote, G., 1994. Effects of dietary inclusion of Paciflor ®

- Maertens, L., Falcão-e-Cunha, L., Marounek, M., 2006. Feed additives to reduce the use of
  antibiotics. In: Recent advances in rabbit sciences. Maertens L., Coudert P. (Eds.), Melle,
  Belgium, pp. 259-265.
- 409 Mourão, J.L., Pinheiro, V., Alves, A., Guedes, C.M., Pinto, L., Saavedra, M.J., Spring, P., Kocher,
- A., 2006. Effect of mannan oligosaccharides on the performance, intestinal morphology and
  cecal fermentation of fattening rabbits. Anim. Feed Sci. Technol. 126, 107–120.
- Ng, S.C., Hart, A.L., Kamm, M.A., Stagg, A.J., Knight, S.C., 2009. Mechanisms of action of
  probiotics: recent advances. Inflamm. Bowel Dis. 15, 300–310.
- Onbaşilar, I., Yalçin, S., 2008. The effects of dietary supplementation of probiotic and anticoccidial
  additives on performance and blood parameters in growing rabbits. Revue Méd. Vét. 159,
  570–574.
- Onifade, A.A., Obiyan, R.I., Onipede, E., Adejumo, D.O., Abu, O.A., Babatunde, G.M., 1999.
  Assessment of the effects of supplementing rabbit diets with a culture of *Saccharomyces cerevisiae* using growth performance, blood composition and clinical enzyme activities.
  Anim. Feed Sci. Technol. 77, 25–32.
- 421 Oso, A.O., Idowu, O.M.O., Haastrup, A.S., Ajibade, A.J., Olowonefa, K.O., Aluko, A.O., Ogunade,
- 422 I.M., Bamgbose, A.M., 2013. Growth performance, apparent nutrient digestibility, caecal
- fermentation, ileal morphology and caecal microflora of growing rabbits fed diets containing
  probiotics and prebiotics. Livest. Sci. 157, 184–190.
- 425 R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for

- 426 Statistical Computing, Vienna, Austria. <u>http://www.R-project.org/</u>. Accessed January 2014.
- 427 Ramírez, J.A., Oliver, M.A., Pla, M., Guerrero, L., Ariño, B., Blasco, A., Pascual, M., Gil, M.,
- 428 2004. Effect of selection for growth rate on biochemical, quality and texture characteristics of
  429 meat from rabbits. Meat Sci. 67, 617–624.
- Tripathi, M.K., Karim, S.A., 2011. Effect of yeast cultures supplementation on live weight change,
  rumen fermentation, ciliate protozoa population, microbial hydrolytic enzymes status and
  slaughtering performance of growing lamb. Livest. Sci. 135, 17–25.
- Trocino, A., Xiccato, G., Carraro, L., Jimenez, G., 2005. Effect of diet supplementation with
  Toyocerin® (*Bacillus cereus* var. *toyoi*) on performance and health of growing rabbits. World
  Rabbit Sci. 13, 17–28.
- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent
  fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–
  3591.
- Zoccarato, I., Barbera, S., Tartari, E., 1995. Effetto dell'impiego di mangime contenente
  un'associazione antibiotico-probiotico sulle performance del coniglio all'ingrasso (Effect of
  dietary inclusion of antibiotic-probiotic combination on fattening rabbit performance).
  Zootec. Nutr. Anim. 21, 297–304.

445 Ingredients and proximate composition of basal diet

Ingredients	%
Alfalfa meal (16% CP)	30
Barley meal	20
Dried beet pulp	14
Wheat bran	20
Soybean meal (44% CP)	6
Sunflower meal (30% CP)	6
Soybean oil	1
Molasses	1.5
Dicalcium phosphate	0.5
Vitamin-mineral premix <sup>1</sup>	0.94
DL-methionine	0.06
Proximate composition on dry matter basis	
Dry matter, %	90.2
Crude protein, %	16.5
Ether extract, %	3.1
Ash, %	7.0
Neutral detergent fibre, %	33.7
Acid detergent fibre, %	22.3
Digestible energy <sup>2</sup> , MJ/kg DM	10.2
Digestible protein <sup>3</sup> , g/kg	114.8
DP/DE <sup>4</sup> , g/MJ	11.3

<sup>1</sup> per kg of diet: Vit. A 200 IU;  $\alpha$ -tocopheryl acetate 16 mg; Niacin 72 mg; Vit. B6 16 mg; Choline

447 0.48 mg;; Ca 500 mg; P 920 mg; K 500 mg; Na 1 g; Mg 60 mg; Mn 1.7 mg; Cu 0.6 mg

<sup>448</sup> <sup>2</sup> The digestible energy content of the basal diet was calculated according to Fernández-Carmona et

449 al. (1996)

450 <sup>3</sup> The digestible protein content of the basal diet was calculated as crude protein content multiplied

451 by the apparent digestibility coefficient of the protein

452 <sup>4</sup> DP/DE= Digestible protein/Digestible energy

0	300	600	<i>P</i> -value	
(2)	0 300 600			
$62.6 \pm 0.4$	$60.6 \pm 0.4$	$61.0{\pm}1.0$	0.051	
$62.0\pm0.2$	$60.7 \pm 0.6$	$61.9 \pm 0.9$	0.297	
67.6±1.3	$68.2 \pm 1.1$	68.1±1.3	0.934	
69.6±3.0	$74.2 \pm 1.7$	71.8±3.1	0.497	
29.1±0.9 <sup>a</sup>	$23.8 \pm 0.6^{b}$	27.7±1.1 <sup>a</sup>	0.001	
$29.5 \pm 1.0^{a}$	$22.2\pm0.6^{\circ}$	$25.9 \pm 1.3^{b}$	0.001	
-	$67.6\pm1.3$ $69.6\pm3.0$ $29.1\pm0.9^{a}$ $29.5\pm1.0^{a}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	

455 In vivo apparent digestibility (means  $\pm$  S.E.) of rabbits (n=10 per group) fed experimental diets

457  $^{a,b,c}$  Means in the same row with unlike superscripts differ (P<0.05)

460	Mortality and growth	performance	(means ± S.E.)	of rabbits (	(n=50 per	group) fe	ed experimental
-----	----------------------	-------------	----------------	--------------	-----------	-----------	-----------------

461 diets

		D volue		
	0	300	600	<i>P</i> -value
Mortality				
37-84d, %	10	8	6	1.00
Growth performance				
$IBW^2$ , g	$1240 \pm 26$	1218±23	1266±27	0.407
FBW <sup>3</sup> , g	$2870 \pm 54$	$2864 \pm 48$	2911±52	0.805
ADFI <sup>4</sup> , g	$141.1 \pm 2.6$	$140.9 \pm 2.6$	138.1±3.0	0.699
$ADG^5$ , g	$34.7 \pm 0.9$	35.0±0.9	35.0±1.0	0.996
FCR <sup>6</sup>	4.2±0.1	$4.2 \pm 0.2$	$4.0\pm0.1$	0.675

462 <sup>1</sup>LSB= yeast commercial product (LEVUCELL<sup>®</sup> SB10 ME TITAN)

- 464 <sup>3</sup> FBW: final body weight
- 465 <sup>4</sup> ADFI: average daily feed intake
- 466 <sup>5</sup> ADG: average daily gain
- 467 <sup>6</sup> FCR: feed conversion ratio

<sup>463 &</sup>lt;sup>2</sup> IBW: initial body weight

		$LSB^1$ (mg/kg)		<i>P</i> -value
	0	300	600	<i>r</i> -value
Slaughter weight (SW), g	2801±40	2829±52	2974±69	0.076
Dressing out, %	$56.4 \pm 0.7$	$55.5 \pm 0.7$	$56.4 \pm 0.6$	0.673
Full gastrointestinal tract, g/100g SW	17.1±0.4	$17.8 \pm 0.4$	$17.0\pm0.5$	0.377
Liver, g/100 g SW	$2.70 \pm 0.08$	$2.95 \pm 0.15$	$2.85 \pm 0.18$	0.481
Kidneys, g/100 g SW	$0.58 \pm 0.02$	$0.62 \pm 0.03$	$0.58 \pm 0.03$	0.504
Heart and lungs, g/100 g SW	$1.17 \pm 0.08$	$1.23 \pm 0.06$	$1.28 \pm 0.15$	0.295

470 Carcass characteristics (means  $\pm$  S.E.) of rabbits (n=10 per group) fed experimental diets

471  $^{1}$ LSB= yeast commercial product (LEVUCELL<sup>®</sup> SB10 ME TITAN)

		$LSB^{1}$ (mg/kg)			
	0	300	600	<i>P</i> -value	
Full caecum, %BW <sup>2</sup>	6.13±0.46	6.52±0.36	5.96±0.45	0.903	
Empty caecum, %BW	$1.94 \pm 0.03$	1.99±0.13	2.04±0.12	0.347	
Caecal content, %BW	$4.20 \pm 0.43$	4.53±0.29	3.92±0.33	0.802	
рН	6.3±0.1	6.3±0.1	6.5±0.1	0.292	
DM caecal content, %	22.7±0.4	22.5±0.5	23.0±0.3	0.903	
Propanol, mg/kg DM	$0.44 \pm 0.06$	$0.57 \pm 0.03$	$0.50 \pm 0.03$	0.301	
Total VFA <sup>3</sup> , mg/kg DM	$12.0{\pm}2.8$	12.0±1.3	$11.0{\pm}1.3$	0.268	
Acetic acid, mg/kg DM	$8.96 \pm 2.00$	9.36±0.88	$8.62 \pm 0.94$	0.269	
Propionic acid, mg/kg DM	$1.02 \pm 0.17$	$0.92 \pm 0.07$	$0.83 \pm 0.06$	0.132	
Butyric acid, mg/kg DM	$1.82 \pm 0.73$	$1.61 \pm 0.32$	1.43±0.29	0.406	
Valeric acid, mg/kg DM	$0.22 \pm 0.06$	$0.13 \pm 0.02$	$0.12 \pm 0.01$	0.114	

474 Caecal content characteristics (means  $\pm$  S.E.) of rabbits (n=5 per group) fed experimental diets

475  $^{1}$ LSB= yeast commercial product (LEVUCELL<sup>®</sup> SB10 ME TITAN)

476  $^2$  BW= Body Weight

477 <sup>3</sup> VFA: volatile fatty acids

480 Caecum microflora population (log CFU/g; means ± S.E.) of rabbits (n=5 per group) fed

481 experimental diets

		$LSB^{1}$ (mg/kg)		
	0	<i>P</i> -value		
Yeast and moulds	0.00 <sup>a</sup>	4.16±0.18 <sup>b</sup>	4.76±0.16 <sup>c</sup>	0.001
Total Viable Counts	4.24±0.13	4.49±0.13	4.73±0.24	0.159
Total anaerobes	4.49±0.16	4.27±0.18	4.57±0.14	0.287

482  $^{-1}$ LSB= yeast commercial product (LEVUCELL<sup>®</sup> SB10 ME TITAN)

483  $^{a,b,c}$  Means in the same row with unlike superscripts differ (P<0.05)

486 Meat traits and chemical composition (on a fresh matter basis; means  $\pm$  S.E.) of *longissimus dorsi* 

<sup>487</sup> muscle of rabbits (n=10 per group) fed experimental diets

		P-value		
	0	300	600	<i>P</i> -value
pH <sub>24</sub>	5.74±0.02	5.74±0.03	5.76±0.02	0.632
L* <sup>2</sup>	56.9±0.5	57.1±0.4	57.9±0.5	0.616
a* <sup>3</sup>	$2.68 \pm 0.23$	2.61±0.26	$2.50 \pm 0.20$	0.395
b* <sup>4</sup>	$2.81 \pm 0.20$	$2.41 \pm 0.20$	$2.96 \pm 0.20$	0.517
Chroma	$3.92 \pm 0.28$	3.64±0.29	3.93±0.25	0.698
Hue	$47.2 \pm 1.8$	$44.4 \pm 2.9$	50.6±1.9	0.158
Cooking losses, %	32.2±0.4	32.4±0.5	31.4±1.1	0.626
Chemical composition				
Moisture, %	74.3±0.5	74.9±0.1	74.7±0.2	0.334
Crude protein, %	22.5±0.3	22.2±0.3	22.6±0.5	0.164
Ether extract, %	$0.56 \pm 0.15$	$0.67 \pm 0.16$	$0.75 \pm 0.16$	0.184
Ash, %	$1.24 \pm 0.06$	$1.23 \pm 0.03$	$1.23 \pm 0.03$	0.625

488 <sup>1</sup>LSB= yeast commercial product (LEVUCELL<sup>®</sup> SB10 ME TITAN)

489  $^{2}$  L\*: lightness

490 <sup>3</sup> a\*: redness

491 <sup>4</sup> b\*: yellowness