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

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Abstract

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

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

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

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

Bacillus cereus or *Pediococcus acidilactis*.

Supplementation with probiotic sources of yeast origin has been evaluated on rabbits (Maertens and De Groote, 1992; Onifade et al., 1999). Only the NCYC Sc 47 strain of *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-pathogenic yeast widely used in human medicine to prevent and treat intestinal disorders, such as 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 additive in husbandry conditions have shown its positive effects on weaned pigs (Le Bon et al., 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, Lallemand Sas, Blagnac, France) on the apparent digestibility, growth performance, caecal fermentation, carcass characteristics and meat quality of broiler rabbits.

2. Materials and Methods

2.1. Animals, housing and diets

The study was carried out at the Department of Agriculture, Forest, and Food Sciences 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 three groups and fed isocaloric and isonitrogenous diets *ad libitum*, supplemented with LSB [$1x10^{10}$ colony forming unit (CFU)/g] at 0, 300 and 600 mg/kg of diet, respectively. After pelleting, one sample (500 g) of each diet was collected and stored in a plastic box at ambient temperature for yeast analysis. The analyses showed a concentration corresponding to 0, $3x10^6$ and $6x10^6$ CFU/g 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

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*
105 The nutrient digestibility coefficients of each diet were determined in the second week of the
106 growing trial (with 10 rabbits aged 44d). The faeces were individually collected for five days using
107 a nylon net placed under the floor of each cage, to avoid urine contamination. The faeces were
108 collected daily, at approximately 0900 h, before the daily ration was provided. Each faecal sample
109 was immediately weighed and then placed in a two-layer plastic bag to prevent loss of moisture and
110 immediately frozen at -20°C. The frozen samples, upon arrival at the laboratory, were dried in a
111 draft oven at 80°C to constant weight and then ground in a homogenizer (Tecator, Herndon, VA,
112 USA) and stored at - 20°C for chemical analysis.
113 All the analyses were carried out according to recommendations of the European Group on
114 Rabbit Nutrition (EGRAN, 2001) on three replicates of each feed and two replicate of each faeces
115 sample. Diets and faeces were analyzed to determine total N content according to AOAC method
116 #984.13 (AOAC, 2000), ash by ignition to 550°C, and ether extract by AOAC method #945.16
117 (AOAC, 2000), using the Soxlet method without previous acid hydrolysis. Neutral detergent fiber
118 (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).

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 -20ºC until chemical and microbiological analyses were performed. The remaining empty caecum was washed with distilled water, dried with blotting paper and weighed.

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 was centrifuged for 5 min at 3000xg and then filtered through a Schleicher and Schull membrane filter (BA-83, 0.2 µm). Using an on-column technique with an auto-sampler (Dani Instruments SpA, ALS 1000, Cologno Monzese, Italy), a 1 µL aliquot of the extract was injected into a wide-bore capillary column (SGE BP21 25m x 0.53 mm internal diameter and 0.5 µm film thickness; P/N 054474, SGE International, Ringwood, Victoria, Australia) installed in a gas chromatograph (Dani GC 1000 DPC), running in a temperature-programmed mode and equipped with a flame ionization

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 conditions. Caecal content was weighed in a sterilized bag and homogenized in 0.90 g/L sterile saline solution for 2 min in a stomacher (PBI International, Milan, Italy), in accordance with the 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}, and 10^{-4}), for total viable counts (TVC) and total anaerobes (10^{-3}, 10^{-4}, and 10^{-5}) using 0.90 g/L sterile saline solution and plated in 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 incubated at 25 °C for 72-110 h. TVC were enumerated by the pour-plate method using Plate Count Agar (Oxoid Ltd, Cambridge, UK). Plates were incubated at 30 °C for 48 h. Total anaerobes were enumerated by the inclusion method using Violet Red Bile Glucose agar (Oxoid Ltd, Cambridge, UK). Plates were incubated at 37 °C for 24 h. The number of colonies was expressed as log CFU per gram of chymus. All microbiological analyses were performed in duplicate.

2.5. Meat quality analyses

2.5.1. Sample preparation

After chilling for 24 h in a refrigerated room (+ 4°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°C until analyses were performed.

2.5.2. pH and Colour measurements

pH_{24} 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_{65} 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 [$C^* = (a^{*2} + b^{*2})^{1/2}$] and Hue [$H^0 = \tan^{-1}(b^*/a^*)$] were calculated according to Boccard et al. (1981). Values were the mean of two different measurements per meat sample.

2.5.3. Cooking losses

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

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.

2.6. Statistical analysis

Statistical analyses were performed using the SPSS software package (IBM SPSS, 2012). Mortality rate differences amongst groups were tested with the Fisher exact test (R Core Team, 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.

3. Results and discussion

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) did not modify the total digestibility of nutrients. Similarly, Chaudary et al. (1995) found that oral administration of yeast culture had no effect on the digestibility of nutrients in rabbits fed diets with different fibre content. Kamra et al. (1996) reported that feeding probiotics (*Lactobacillus 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 White rabbits under Indian hot climate environmental conditions. Oso et al. (2013) found that ADF, NDF and other nutrient digestibility values were not affected by dietary inclusion of probiotics of bacterial origin (*Pediococcus acidilactis* or *Bacillus cereus*) in mixed breed weaner rabbits. In contrast, in weaned piglets in a feeding trial lasting 35d, Giang et al. (2010) found that a basal diet supplemented with 0.2% yeast and a mixture of lactic acid bacteria improved the apparent total tract digestibility of CP, crude fibre and organic matter.

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 10^6 CFU/ g of *Saccharomyces cerevisiae* NCYC Sc 47 strain (Activesaf®) significantly halved mortality over the whole fattening period, compared to the control.

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

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 supplemented with 222 g/t of active dry yeast, *Saccharomyces cerevisiae boulardii*. Similarly, in the same species, Le Bon et al. (2010) found that dietary supplementation of *Saccharomyces cerevisiae boulardii* CNCM I-1079 strain ($2x10^9$ kg/feed) followed by *Pediococcus acidilactici* significantly improved FCR. On the contrary, Oso et al. (2013) found poor growth response in rabbits fed diets containing the probiotic of bacterial origin *Pediococcus acidilactis* or *Bacillus cereus*, while the inclusion of the prebiotics mannan and arabinoxylans oligosaccharides showed an improved growth 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 review, Falcão-e-Cunha et al. (2007) summarized that dietary inclusion of feed additives containing yeast generally improve ADG in rabbits, although results concerning FCR and mortality were partially contradictory. *Saccharomyces cerevisiae* ($5x10^8$ CFU per d) orally supplemented as yeast culture, did not improve growth performance in 6-week-old New Zealand White mash-fed rabbits (Chaudary et al., 1995). Similarly, Maertens and De Groote (1992) reported no significant difference in rabbit performance. Conversely, Onifade et al. (1999) found that rabbits fed 3.0 and

1.5 g/kg of *Saccharomyces cerevisiae* (Yeasacc$^{10261®}$), 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 of *Saccharomyces cerevisiae boulardii* on carcass characteristics, as most of the works are related to probiotic mixtures or generally indicate *Saccharomyces cerevisiae* supplementation. Onbaşilar and Yalçin (2008) found no differences in weight percentages of lung, heart, kidney and small intestine in New Zealand White rabbits fed 1g of probiotic (Bioteksin^{TM})/kg diet, but the liver percentage was affected in animals fed 1g of probiotic + 66 mg of anticoccidial agent (Robenine hydrochloride)/kg diet. Similarly, Tripathi and Karim (2011) showed that carcass traits did not change in lambs fed diets supplemented by *Saccharomyces cerevisiae* and other yeast cultures.

3.3. Caecal activity

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

The full and empty weights of the caecum and its contents were not affected by treatment and these values were similar to those reported by Cesari et al. (2009) and Gallois et al. (2005) on growing rabbits. The pH value of the caecum content was about 6.3–6.5, similar to values obtained 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 the treatment and were in accordance with those reported by Gidenne et al. (2000) who pointed out that acetic acid concentration of the caecum ranged between 78.0 and 82.5%, while butyric acid and propionic acid concentrations ranged from 13.1 to 16.9% and from 3.9% and 4.7%, respectively. Similar values were observed by Kimsé et al. (2009) in rabbit: acetate (77%), butyrate (17%) and propionate (5%).

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.

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

within the normal range for rabbit meat (Dalle Zotte and Szendro, 2011).

4. Conclusions

Protected live yeast (*Saccharomyces cerevisiae boulardii*, CNCM I-1079 strain) was resistant to the pelleting process and to the passage through the rabbit digestive tract as far as the caecum where it showed an 86% survival rate in the 600 mg/kg supplementation level group. Although the caecal population of yeast 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 characteristics, caecal fermentation and meat quality of broiler rabbits reared in standard farming 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.

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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.

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.

Czerucka, D., Piche, T., Rampal, P., 2007. Review article: yeast as probiotics – *Saccharomyces boulardii*. Aliment. Pharmacol. Ther. 26, 767–778.

Dalle Zotte, A., Szendro, Z., 2011. The role of rabbit meat as functional food. Meat Sci. 88, 319–331.

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.

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 of lactic acid bacteria and *Saccharomyces boulardii* on growth performance and total tract digestibility in weaned pigs. Livest. Res. Rural Develop. 22, http://www.lrrd.org/lrrd22/10/gian22179.htm

Gidenne, T., Pinheiro, V., Falcao-E-Cunha, L., 2000. A comprehensive approach of the rabbit

digestion: consequences of a reduction in dietary fibre supply. Livest. Prod. Sci. 64, 225–237.

Gidenne, T., Jehl, N., Segura, M.,Michalet-Doreau, B., 2002. Microbial activity in the caecum of the rabbit around weaning: impact of a dietary fibre deficiency and of intake level. Anim. Feed Sci. Technol. 99, 107–118.

IBM SPSS, 2012. IBM SPSS Statistics 20.0 SPSS Inc., Chicago, IL, USA.

Kamra, D.N., Chaudhary, L.C., Singh, R., Pathak, N.N., 1996. Influence of feeding probiotics on growth performance and nutrient digestibility in rabbits. World Rabbit Sci. 4, 85–88.

Kermauner, A., Strucklec, M., Marinsek-Logar, R., 1994. Influence of a probiotic added to different feed mixtures on the microbial metabolism in caecum of growing rabbits. Znan. Prak. Poljopr. Tehnol. 24, 120–127.

Kimsé, M., Monteils, V., Bayourthe, C., Gidenne, T., 2009. A new method to measure the redox potential (EH) in rabbit caecum: relationship with pH and fermentation pattern. World Rabbit Sci. 17, 63–70.

Kimsé, M., Bayourthe, C., Monteils, V., Fortun-Lamothe, L., Cauquil, L., Combes, S., Gidenne, T., 2012. Live yeast stability in rabbit digestive tract: Consequences on the caecal ecosystem, digestion, growth and digestive health. Anim. Feed Sci. Technol. 173, 235–243.

Kovács, M., Szendro, Z., Milisits, G., Bóta, B., Bíró-Németh, E., Radnai, I., Pósa, R., Bónai, A., Kovács, F., Horn, P., 2006. Effect of nursing methods and faeces consumption on the development of the bacteroides, lactobacillus and coliform flora in the caecum of the newborn rabbits. Reprod. Nutr. Dev. 46, 205–210.

Le Bon, M., Davies, H.E., Glynn, C., Thompson, C., Madden, M., Wiseman, J., Dodd, C.E.R., Hurdidge, L., Payne, G., Le Treut, Y., Craigon, J., Tötemeyer, S., Mellits, K.H., 2010. Influence of probiotics on gut health in the weaned pig. Livest. Sci. 133, 179–181.

Luick, B.R., El-Sayaad, G.A.E., Cheeke, P.R., 1992. Effect of fructooligosaccharides and yeast culture on growth performance of rabbits. J. Appl. Rabbit Res. 15, 1121–1128.

401 Maertens, L., De Groote, G., 1992. Effect of a dietary supplementation of live yeast on the
402 zootechnical performances of does and weanling rabbits. J. Appl. Rabbit Res. 15, 1079–1086.
403 Maertens, L., Van Renterghem, R., De Groote, G., 1994. Effects of dietary inclusion of Paciflor ®
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.
406 Maertens, L., Falcão-e-Cunha, L., Marounek, M., 2006. Feed additives to reduce the use of
407 antibiotics. In: Recent advances in rabbit sciences. Maertens L., Coudert P. (Eds.), Melle,
408 Belgium, pp. 259-265.
409 Mourão, J.L., Pinheiro, V., Alves, A., Guedes, C.M., Pinto, L., Saavedra, M.J., Spring, P., Kocher,
410 A., 2006. Effect of mannan oligosaccharides on the performance, intestinal morphology and
411 cecal fermentation of fattening rabbits. Anim. Feed Sci. Technol. 126, 107–120.
412 Ng, S.C., Hart, A.L., Kamm, M.A., Stagg, A.J., Knight, S.C., 2009. Mechanisms of action of
413 probiotics: recent advances. Inflamm. Bowel Dis. 15, 300–310.
414 Onbaşilar, I., Yalçin, S., 2008. The effects of dietary supplementation of probiotic and anticoccidial
415 additives on performance and blood parameters in growing rabbits. Revue Méd. Vét. 159,
416 570–574.
417 Onifade, A.A., Obiyan, R.I., Onipede, E., Adejumo, D.O., Abu, O.A., Babatunde, G.M., 1999.
418 Assessment of the effects of supplementing rabbit diets with a culture of *Saccharomyces*
419 *cerevisiae* using growth performance, blood composition and clinical enzyme activities.
420 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
423 fermentation, ileal morphology and caecal microflora of growing rabbits fed diets containing
424 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. http://www.R-project.org/. 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.
430 Tripathi, M.K., Karim, S.A., 2011. Effect of yeast cultures supplementation on live weight change,
431 rumen fermentation, ciliate protozoa population, microbial hydrolytic enzymes status and
432 slaughtering performance of growing lamb. Livest. Sci. 135, 17–25.
433 Trocino, A., Xiccato, G., Carraro, L., Jimenez, G., 2005. Effect of diet supplementation with
434 Toyocerin® (*Bacillus cereus* var. *toyoi*) on performance and health of growing rabbits. World
435 Rabbit Sci. 13, 17–28.
436 Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent
437 fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–
438 3591.
439 Zoccarato, I., Barbera, S., Tartari, E., 1995. Effetto dell'impiego di mangime contenente
440 un'associazione antibiotico-probiotico sulle performance del coniglio all'ingrasso (Effect of
441 dietary inclusion of antibiotic-probiotic combination on fattening rabbit performance).
442 Zootec. Nutr. Anim. 21, 297–304.
443

444 Table 1

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[1]	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[2], MJ/kg DM	10.2
Digestible protein[3], g/kg	114.8
DP/DE[4], g/MJ	11.3

446 [1] per kg of diet: Vit. A 200 IU; α-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

448 [2] The digestible energy content of the basal diet was calculated according to Fernández-Carmona et

449 al. (1996)

450 [3] 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 [4] DP/DE= Digestible protein/Digestible energy

453

Table 2

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

	LSB[1] (mg/kg)			*P*-value
	0	300	600	
Dry matter, %	62.6±0.4	60.6±0.4	61.0±1.0	0.051
Organic matter, %	62.0±0.2	60.7±0.6	61.9±0.9	0.297
Crude protein, %	67.6±1.3	68.2±1.1	68.1±1.3	0.934
Ether extract, %	69.6±3.0	74.2±1.7	71.8±3.1	0.497
Neutral detergent fibre, %	29.1±0.9[a]	23.8±0.6[b]	27.7±1.1[a]	0.001
Acid detergent fibre, %	29.5±1.0[a]	22.2±0.6[c]	25.9±1.3[b]	0.001

[1] LSB= yeast commercial product (LEVUCELL® SB10 ME TITAN)

[a,b,c] Means in the same row with unlike superscripts differ (P<0.05)

Table 3

Mortality and growth performance (means ± S.E.) of rabbits (n=50 per group) fed experimental diets

	LSB[1] (mg/kg)			*P*-value
	0	300	600	
Mortality				
37-84d, %	10	8	6	1.00
Growth performance				
IBW[2], g	1240±26	1218±23	1266±27	0.407
FBW[3], g	2870±54	2864±48	2911±52	0.805
ADFI[4], g	141.1±2.6	140.9±2.6	138.1±3.0	0.699
ADG[5], g	34.7±0.9	35.0±0.9	35.0±1.0	0.996
FCR[6]	4.2±0.1	4.2±0.2	4.0±0.1	0.675

[1] LSB= yeast commercial product (LEVUCELL® SB10 ME TITAN)

[2] IBW: initial body weight

[3] FBW: final body weight

[4] ADFI: average daily feed intake

[5] ADG: average daily gain

[6] FCR: feed conversion ratio

469 Table 4

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

	LSB[1] (mg/kg)			*P*-value
	0	300	600	
Slaughter weight (SW), g	2801±40	2829±52	2974±69	0.076
Dressing out, %	56.4±0.7	55.5±0.7	56.4±0.6	0.673
Full gastrointestinal tract, g/100g SW	17.1±0.4	17.8±0.4	17.0±0.5	0.377
Liver, g/100 g SW	2.70±0.08	2.95±0.15	2.85±0.18	0.481
Kidneys, g/100 g SW	0.58±0.02	0.62±0.03	0.58±0.03	0.504
Heart and lungs, g/100 g SW	1.17±0.08	1.23±0.06	1.28±0.15	0.295

471 [1] LSB= yeast commercial product (LEVUCELL® SB10 ME TITAN)

472

473 Table 5

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

	LSB[1] (mg/kg)			*P*-value
	0	300	600	
Full caecum, %BW[2]	6.13±0.46	6.52±0.36	5.96±0.45	0.903
Empty caecum, %BW	1.94±0.03	1.99±0.13	2.04±0.12	0.347
Caecal content, %BW	4.20±0.43	4.53±0.29	3.92±0.33	0.802
pH	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±0.06	0.57±0.03	0.50±0.03	0.301
Total VFA[3], mg/kg DM	12.0±2.8	12.0±1.3	11.0±1.3	0.268
Acetic acid, mg/kg DM	8.96±2.00	9.36±0.88	8.62±0.94	0.269
Propionic acid, mg/kg DM	1.02±0.17	0.92±0.07	0.83±0.06	0.132
Butyric acid, mg/kg DM	1.82±0.73	1.61±0.32	1.43±0.29	0.406
Valeric acid, mg/kg DM	0.22±0.06	0.13±0.02	0.12±0.01	0.114

475 [1] LSB= yeast commercial product (LEVUCELL® SB10 ME TITAN)

476 [2] BW= Body Weight

477 [3] VFA: volatile fatty acids

478

Table 6

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

	LSB[1] (mg/kg)			*P*-value
	0	300	600	
Yeast and moulds	0.00[a]	4.16±0.18[b]	4.76±0.16[c]	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

[1] LSB= yeast commercial product (LEVUCELL® SB10 ME TITAN)

[a,b,c] Means in the same row with unlike superscripts differ (P<0.05)

485 Table 7

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

487 muscle of rabbits (n=10 per group) fed experimental diets

	LSB[1] (mg/kg)			*P-value*
	0	300	600	
pH_{24}	5.74±0.02	5.74±0.03	5.76±0.02	0.632
L*[2]	56.9±0.5	57.1±0.4	57.9±0.5	0.616
a*[3]	2.68±0.23	2.61±0.26	2.50±0.20	0.395
b*[4]	2.81±0.20	2.41±0.20	2.96±0.20	0.517
Chroma	3.92±0.28	3.64±0.29	3.93±0.25	0.698
Hue	47.2±1.8	44.4±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±0.15	0.67±0.16	0.75±0.16	0.184
Ash, %	1.24±0.06	1.23±0.03	1.23±0.03	0.625

488 [1] LSB= yeast commercial product (LEVUCELL® SB10 ME TITAN)

489 [2] L*: lightness

490 [3] a*: redness

491 [4] b*: yellowness