World Rabbit Science

World Rabbit Sci. 2016, 24: 191-200
doi:10.4995/wrs.2016.3991

EFFECT OF DIET SUPPLEMENTATION WITH LIVE YEAST *SACCHAROMYCES CEREVISIAE* ON GROWTH PERFORMANCE, CAECAL ECOSYSTEM AND HEALTH OF GROWING RABBITS

BELHASSEN T.*, BONAI A.†, GERENCSÉR ZS.†, MATICS ZS.†, TUBOLY T.‡, BERGAOUI R.*, KOVACS M.†#

*Department of Animal Production, National Institute of Agronomy of Tunis, 43, avenue Charles Nicolle 1082, Mahrajène, Tunisia.
†Kaposvár University, Faculty of Animal Science, Guba S. u. 40, Kaposvár, Hungary.
‡Faculty of Veterinary Science, Szent István University, István 2, 1078 Budapest, Hungary.
#MTA-KE Mycotoxins in the Food Chain research Group, Guba S. u. 40, Kaposvár, Hungary.

Abstract: The aim of this study was to determine the effect of the live yeast *Saccharomyces cerevisiae* on the growth performance, caecal ecosystem and overall health of growing rabbits. A control diet was formulated (crude protein: 15.9%; neutral detergent fibre: 31.6%) and another diet obtained by supplementing the control diet with 1 g of *Saccharomyces cerevisiae* (6.5×10^9 colony-forming units) per kg of diet. Ninety 35-d old rabbits were allotted into 3 groups: TT (rabbits offered the supplemented diet from 17 d of age onwards), CT (rabbits offered supplemented diet from 35 d) and CC (rabbits fed non-supplemented diet). Body weight (BW) and feed intake were measured weekly and mortality was controlled daily. At 35, 42 and 77 d of age, 6 rabbits from each group were slaughtered and digestive physiological traits, serum clinical chemistry parameters, fermentation traits, and the composition of caecal microbiota examined. At 42 and 56 d of age, 10 rabbits from each group were injected intraperitoneally with 100 µg/animal of ovalbumin and blood samples were collected for examination of plasma immunological parameters. Throughout the experiment (5-11 wk), weight gain and feed intake (37.8 and 112.6 g/d, on av.) were not affected by yeast, except for weight gain in the first week after weaning, which was the highest in TT animals among the 3 groups (48.1 *vs*. 43.9 and 44.2 g/d for TT, CC and CT, respectively; P=0.012). This may be due to the increased trend in feed intake (P=0.072) in the TT group (96.4 g/d) compared to the others. Mortality (5/90) was low and did not differ among the 3 groups. Treatments had no effect on slaughter traits at the 3 sampling dates (35, 42 and 77 d). Only the weight of the empty caecum (% BW) was higher (P=0.02) in CC (2.2%) and CT (2.3%) than in TT group (1.8%) at 77 d of age. Treatments did not overtly affect the caecal microbiota, although the number of total anaerobic bacteria and *Bacteroides* were lower (10^8 and 10^7/g caecal digesta, respectively) in rabbits from CC group compared to those of CT and TT groups at 42 d of age (P=0.03). No difference between groups was observed for caecal short chain fatty acids profile, blood traits, or IgG and cytokine profile. In conclusion, supplementation of feed with yeast did not modify growth traits and resulted in only a temporary increase in weight gain and a slightly altered caecal microbiota after weaning.

Key Words: live yeast, digestive physiology, growth, fattening rabbit.

INTRODUCTION

The caecal microbiota and fermentation processes taking place in the caecum play a key role in the digestion and digestive health of rabbits (Carabaño *et al.,* 2006). One of the main hypotheses to explain the origin of digestive disturbances in young animals is an imbalance in the intestinal microflora (dysbiosis) caused by several stressors. These include pathogenic agents (*E. coli*) and nutritional or breeding stress (Jérome *et al.,* 1996). As the incidence of digestive pathology is higher in young rabbits than in adult rabbits, the maturation of the caecal ecosystem and its stability or biodiversity may be implicated in resistance to digestive diseases, a phenomenon known as "barrier flora effect" (Carabaño *et al.,* 2006). The flora also interacts with and affects maturation of the intestinal mucosa

Correspondence: T. Belhassen, takoubel@yahoo.fr. Received *July 2015* - Accepted *February 2016.*
doi:10.4995/wrs.2016.3991

and interacts with the local immune system (Fortun Lamothe and Boulier, 2007). Antibiotics are still widely used to reduce mortality in developing rabbits, although there are increasing concerns over drug residues in meat products and increases in bacterial resistance due to the prophylactic use of antibiotics. Due to this, the use of alternatives to antibiotics has received renewed emphasis (Trocino *et al.,* 2005; Falcão-e-Cunha *et al.,* 2007; Bovera *et al.,* 2012a). Among these alternatives, live yeast is widely used in various livestock species to control intestinal microbiota and enhance immune function. In rabbits, live yeast supplementation provided some positive effects on growth performance and health status, especially when animals were kept under sub-optimal environmental and sanitary conditions with high stocking density and low hygiene control (Maertens and De Groote, 1992). However, some studies found no significant effect of yeast on various production parameters (Kimsé *et al.,* 2012). The effect of yeast on growth performance and health status varies depending on the dose, age, livestock conditions, and even between studies. However, yeast's mechanism of action remains unknown. This study aimed to acquire more knowledge on the mode of action of yeast supplementation on the caecal ecosystem and the health of rabbits by determining certain immunological and physiological parameters.

MATERIALS AND METHODS

Diet

A control diet (C) containing 15.9% crude protein (CP) and 31.6% neutral detergent fibre (NDF) was formulated (Table 1). Another diet (T) was obtained supplementing the control diet with 1 g of *Saccharomyces cerevisiae* (Actisaf P (CNCMI-4407), Lesaffre, France) per kg of commercial diet (6.5×10^9 colony-forming units (CFU)/kg feed). This was coated with a matrix of fat and added to a vitamin and mineral premix and included in the diet before pelleting. No growth promoters, therapeutic antibiotics or coccidiostats were added to diet or administered in water along experiment. The ingredients and chemical composition of the diets are shown in Table 1.

Table 1: Ingredients and chemical composition of control diet.

Ingredients	%
Barley meal	5.0
Wheat bran	20.0
Dehydrated alfalfa meal	37.0
Soybean oil	2.0
Sunflower meal (36% CP)	10.0
Skimmed milk powder	2.0
Beet pulp dried	10.9
Beet molasses	2.0
Dried apple	8.9
Calcium diphosphate	0.5
Vitamin and minerals mixture[1]	0.5
Limestone	0.5
Salt	0.5
DL-methionine	0.1
HCl-lysine	0.1
Chemical composition and nutrients	
Ash	7.8
Crude protein	15.9
Crude fat	4.2
Neutral detergent fibre	31.6
Crude fibre	18.8

*Premix provided per kg of diet: 11000 IU vitamin A, 2000 IU vitamin D_3, 2.5 mg vitamin B_1, 4 mg vitamin B_2, 1.25 mg vitamin K, 15 mg niacin, 0.3 mg folic acid, 600 mg choline, 3 mg Cu, 50 mg Fe, 15 mg Zn, 60 mg Mn, 0.5 mg I, 0.5 mg Co, 0.5 mg lysine and 0.5 mg methionine.

Analyses of the control diet were carried out in duplicate using AOAC (2000) methods to determine the concentrations of CP (2001.11), crude fibre (CF; 978.10) and ash (967.05). Crude fat was determined after acid-hydrolysis (EC, 1998). NDF was analysed according to Mertens (2002) using the sequential procedure and the filter bag system (Ankom Technology, New York).

Experimental Design, Animal Management, and Housing

A total of 90 Pannon white rabbits were individually marked (with ear tattoos) at 28 d of age, weaned, moved from the maternal sector to the fattening sector, and allotted at the age of 35 d into 3 trial groups taking body weight (863±68 g) into account (30 rabbits/group): CC (Control group without yeast in feed), CT (supplemented with yeast from 35 d of age) and TT (rabbits started to eat diet supplemented with yeast from the age of 17 d). In the TT group, the yeast (Actisaf P-Lesaffre, France) was added to the does' diet for the suckling rabbits to also consume. The rabbits were given both water and feed *ad libitum.*

The rabbits were housed in wire net cages (0.61×0.32 m, 3 animals per cage). The temperature was kept between 16 and 20°C and the light period was 16 h long (6:00-22:00). The experiment was carried out in the rabbit farm of Kaposvàr University (Hungary).

During the experimental period (between 35 and 77 d of age), body weight (BW) and feed consumption were measured weekly. Mortality was controlled daily.

At 42 d of age, 10 rabbits from each group were injected intraperitoneally with 100 µg/animal ovalbumin (Sigma-Aldrich, Hungary) dissolved in 400 µL phosphate buffered saline (PBS, Sigma-Aldrich, Hungary) and 400 µL incomplete Freund's adjuvant (Sigma-Aldrich, Hungary). This injection was repeated after 14 d (at 56 d). Blood samples were collected from the animals at 42, 56 and 77 d of age and used for immunological measurements.

Samplings

Blood samples for serum clinical chemistry were taken from the marginal ear vein into native tubes at 35, 42, 56, and 77 d of age, while immunological parameters were determined in blood samples collected into heparinised tubes at 42, 56 and 77 d.

Plasma was obtained by centrifugation (Janetzky T23, VEB, Leipzig, Germany) at 3000 rpm for 15 min, and used for immunology. Serum was used for the determination of clinical biochemical parameters. All samples were stored deep-frozen (–27°C) until analysed.

At 35, 42 and 77 d of age, 6 healthy animals from each group were randomly selected and slaughtered at 13:00 h. BW was recorded after exsanguination. The digestive tract was removed immediately and the caecum and the stomach were separated, emptied and weighed. The caecal and gastric contents were weighed and pH registered. Caecal contents were collected for the determination of caecal microbiota and short-chain fatty acid (SCFA) concentration.

Methodology for yeast enumeration

Yeast enumeration was performed according to method EN15789 (AFNOR, 2009). The enumeration of yeast in feed samples consisted of the following steps: Preparation of sterile and dry poured agar plates, and sterile molten agar at 48±1°C for poured plates. Drawing of a representative test sample under sterile conditions. Preparation of the initial suspension to obtain a homogeneous distribution of yeast-like cells from the test portion. Preparation of further decimal dilutions of the initial suspension to reduce the number of microorganisms per unit volume, to allow the counting of colonies after incubation. Inoculation of the prepared plates with an aliquot of the optimum dilutions and dispersion of the inoculum using a sterile spreader, or poured plate. Aerobic incubation of inverted spread plates for 3 d at 30±1°C, or 35±1°C for 2 d for poured plates. Counting of typical colonies, considering the specific properties of yeast. Morphological verification of isolates of yeast by microscopic analysis. Calculation of the colony count per g or kg of feed sample.

Laboratory Analyses

Serum biochemistry

The concentration of total protein (TP), albumin (ALB), glucose (G), cholesterol (CHOL), urea and creatinine (CREA), and the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) were determined in a professional veterinary laboratory (Vet-Med Laboratory, Budapest, Hungary) using the Roche Hitachi 912 Chemistry Analyzer (Hitachi, Tokyo, Japan) with commercial diagnostic kits (Diagnosticum, Budapest, Hungary).

Determination of immune parameters

For specific IgG detection, ELISA plates (SIGMA-Aldrich) were coated with 100 µL of an ovalbumin solution (100 µg/mL ovalbumin in PBS) per each well and incubated overnight at 4°C in a humidified environment. Excess protein was removed by washing 3 times with 150 µL PBS-Tween 20 (Sigma-Aldrich). Serum samples were two-fold serially diluted in the washing solution, starting with 1:50, in a separate plate. Each dilution (100 µL) was transferred to the ELISA plate (2 wells for each dilution) and incubated for 1 h at room temperature (RT). Wells were then washed again

3 times with PBS-Tween and anti-rabbit IgG-HRPO conjugate (SIGMA-Aldrich, 1:10000) was then added to each well, followed by incubation at RT for 1 h. The washing step was then repeated and the appropriate substrate added.

Cytokine detection by reverse transcriptase real-time polymerase chain reaction (rt-RT-PCR)

Cytokine production was measured by real-time quantitative PCR, detecting mRNA of IL-2 and interferon gamma (IFN-γ). Whole blood was used for total RNA isolation with the Analytik Jena innuPREP Blood RNA Kit (Biometra, Germany) following the instructions of the producer. RNA templates were transcribed into cDNA using the Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Fermentas, Lithuania) according to the protocol of the manufacturer. Amplification using cytokine-specific primers was performed in an Eppendorf Realpex^2 Mastercycler. Primers for IL-2 were designed using the Primer3 Programme (Rozen and Skaletsky, 2000) and available Genbank sequences. For IFN-γ, published primers (Godornes *et al.,* 2007) were used. The mixture used consisted of the following: 2 µL cDNA solution, 5 µL 10× DreamTaq Buffer, 2 µL of 25 pmol/µL of each primer, 1 µL of 1 mmol dNTP Set (Fermentas), 2.5 µL EVA Green (Biotium, USA) and 0.2 DremTaq™ DNA Polymerase, 5U/µL DremTaq™ DNA Polymerase (5U/µL, Fermentas) in a final volume of 20 µL. The PCR reactions used were: preheating at 95°C for 5 min, 40 cycles of 54°C for 30 s, and 72°C for 45 s, followed by a final extension step of 72°C for 7 min.

Forward (F) and reverse (R) primers used:
IFG-F: 5'-CCTGTCACTTCGACCTTAGA-3', IFG-R: 5'-CAGTAACAGCCGTAAGAACC-3',
IL2-F: 5'-ACCTCTGGAGGAAGTGCTTA-3', IL2-R: 5'-ACTCGATGCTGAGATGATGC-3',

Cloned cytokine DNA was used as control, and diluted tenfold from 10^{-1} to 10^{-8}. The relative amounts were determined by comparing Ct (cycle threshold, the number of cycles required for the fluorescent signal to cross the threshold) values to the standard curve of the amplified control DNA.

Microbiological culturing techniques

From 1 g of fresh matter of caecal digesta, serial dilutions (1 g caecal sample+9 mL diluent [0.9% NaCl]) were made immediately after sampling and used for microbiological determination. Anaerobic bacteria were cultured on Schaedler's agar (Sharlan Chemie, Barcelona, Spain), the selectivity of which was increased by the addition of esculin (Merck, Darmstadt, Germany), neomycin (Merck, Darmstadt, Germany), and iron ammonium citrate (Sharlan Chemie, Barcelona, Spain). Gamma-sterile Petri dishes (Biolab, Budapest, Hungary) were placed into Anaerocult culture dishes (Merck, Darmstadt, Germany) in which anaerobic conditions were ensured using an "Anaerocult A" (Merck, Darmstadt, Germany) gas-producing bag. Subsequently, the samples were incubated in an LP 104 type thermostat (LMIM, Esztergom, Hungary) at 37°C for 96 h. Total aerobe germ count was determined on media supplemented with 5% calf blood. The samples were incubated at 37°C for 72 h. *E. coli* and other coliform bacteria were cultured on Cromocult differentiation medium (Merck, Darmstadt, Germany). The samples were incubated at 37°C under aerobic conditions for 24 h. After the incubation time had elapsed, the colonies were counted according to standard (ISO 4833:2003) with the Acolyte colony counter (Aqua-Terra Lab, Veszprem). The colony counts were expressed in $\log_{10}$ CFU related to 1 g of sample.

Determination of total volatile fatty acid concentration

About 3 g of caecal chyme was homogenised with 4.5 mL metaphosphoric acid (4.16%), then centrifuged at 10000×*g* for 10 min and filtered. The concentration of short chain fatty acid was measured by gas chromatography (Shimadzu GC 2010, Japan). Parameters: Nukol 30 m×0.25 mm×0.25 µm capillary column (Supelco, Bellefonte, PA, USA), FID detector, 1:50 split ratio, 1 µL injected volume, helium 0.84 mL/min. Detector conditions: air 400 mL/min, hydrogen 47 mL/min, temperature: injector 250°C, detector 250°C, column 150°C. 2-ethyl-butyrate (FLUKA Chemie GmbH, Buchs, Switzerland) was used as internal standard.

Statistical Analysis

Statistical analysis of the data obtained was carried out using the Statistical Package for Social Sciences (SPSS, version 19.0). Effect of treatment, age, and their interaction was analysed by the following GLM:

$$Y_{ijk}=\mu+T_i+A_j+TA_{ij}+e_{ijk}$$

Where μ=mean, T_i=effect of treatment (groups), A_j=effect of age, TA_{ij}=interaction of treatment and age and e_{ijk}=random error.

The significance of differences was tested using the least significant difference *post hoc* test. Productive data were analysed by means of one-factor analysis of variance (ANOVA). In the case of feed intake and feed conversion, the experimental unit is the cage but the animal for growth rate and other measurements. Mortality in groups was compared by chi-squared analysis. The model of repeated measurement was used for immunological parameters.

RESULTS

The concentration of dietary yeast after granulation was 2×10^5 CFU/g. No effect of yeast addition could be observed on any of the growth traits of fattening rabbits except weight gain in the first week after weaning. This was highest in TT animals compared to the other groups (48.1 *vs.* 43.9 and 44.2 g/d; P=0.012 for CC and CT respectively) (Table 2). The rate of mortality was low 5.5% (5/90) for the entire experimental period, with no differences observed between the groups, and can be ascribed to the hygienic conditions of the experimental animal house in which the trial was conducted.

There were no differences in the composition of caecal microbiota among dietary treatments at 35 and 77 d of age (Table 3). However, at 42 d of age, more anaerobic bacteria and *Bacteroides* were measured in CT animals compared to control groups (10^9 and 10^8/g of caecal digesta *vs.* 10^8 and 10^7/g of caecal digesta; P<0.05). Total anaerobic bacteria was also higher also in TT groups compared to the control group (10^9/g of caecal digesta *vs.* 10^8/g of caecal digesta; P<0.05). Overall, the number of total anaerobic and aerobic bacteria tended to be constant from weaning until slaughter, except the slight temporary variation described above.

No effects of yeast supplementation on slaughter traits were found (Table 4) at the 3 sampling dates except for the weight of the empty caecum, which was higher in CC and CT groups (P=0.02) at 77 d of age. BW after bleeding, weight of gastric content, weight of the empty stomach, and weight of the empty caecum all varied with age (Table 4).

Dry matter content of the caecal chyme varied between 21 and 24% and tended to be constant from 35 to 77 d of age (Table 5). The concentration of total SCFA ranged from 34 to 54 mmol/kg, with acetic acid the most predominant SCFA, followed by butyric and propionic acid (Table 5). There were no great differences between groups, although total volatile fatty acid concentration was lower in CT group at 77 d of age compared to the other groups (35.4 mmol/kg *vs.* 44.6 and 51.2 mmol/kg for CC and TT groups, respectively, P<0.05).

Dietary treatment did not affect any of the blood traits examined (Table 6), with all blood traits varying with age, while there were no differences in serum immunological parameters among groups (Table 7).

Table 2: Effect of live yeast *Saccharomyces cerevisiae* on production parameters (mean±SEM) and mortality rate (%).

		Group				
	Age (wk)	CC	CT	TT	SEM	*P*-value
Body weight (g)	5	860	860	869	7.2	NS
	11	2410	2420	2480	31.4	NS
Weight gain (g/d)	5-6	43.9[a]	44.2[a]	48.1[b]	0.6	0.012
	5-11	36.8	37.7	38.8	0.6	NS
Feed intake (g/d/rabbit)	5-6	90.0	89.7	96.4	1.3	NS
	5-11	110.7	110.4	116.8	2.3	NS
Feed conversion (g/g)	5-6	2.1	2.0	2.0	0.02	NS
	5-11	3.0	3.1	3.1	0.1	NS
Mortality (%)	5-11	6.7 (2/30)	3.3 (1/30)	6.7 (2/30)		NS

SEM: standard error of mean. [a,b]Different superscripts indicate significant P<0.05 differences among treatments. NS: no significant (P>0.05). CC: control group, CT: supplemented feed from the age of 35 d, TT: supplemented feed from the age of 17 d. n=30 rabbits/treatment.

Table 3: Effect of live yeast *Saccharomyces cerevisiae* on composition of the caecal microbiota expressed in colony-forming units (CFU) $\log_{10}$/g dry matter caecal digesta (mean±SEM).

Treatment	Age	*E. coli*	Total anaerobic	*Bacteroides*	Total aerobic
CC	Average	3.2	8.2^{A}	7.6^{B}	5.2
CT	Average	3.0	8.3^{B}	7.5^{A}	5.0
TT	Average	2.8	8.5^{B}	7.5^{A}	5.3
Average	35	3.0	8.6	8.1^{b}	5.5
	42	2.9	8.4	7.4^{a}	5.1
	77	3.0	8.2	7.3^{a}	5.0
SEM[1]		0.2	0.04	0.1	0.1
P treatment		NS	0.02	0.03	NS
P age		NS	NS	0.004	NS
P treatment×age		NS	NS	0.01	NS

SEM: standard error of mean. Different superscripts indicate significant $P<0.05$ differences among ^{a,b} ages and ^{A,B} treatments. NS: no significant ($P>0.05$). CC: Control group, CT: supplemented feed from the age of 35 d, TT: supplemented feed from the age of 17 d. *on day 35 groups CC and CT should be considered as identical. [1]n=6 rabbits for each combination treatment×age.

DISCUSSION

The results of our study confirm those from the previous studies of Chaudhary *et al.* (1995) and Kimsé *et al.* (2012), where no improvement on growth performance in fattening rabbits was noted with 5×10^{8} CFU of live yeast/animal and 10^{7} CFU/g feed, respectively. Similarly, under optimal breeding conditions, no significant differences in rabbit performance were observed by Maertens and De Groote (1992). Likewise, Hollister *et al.* (1990) noted that the dietary supplementation of Lacto-Sacc did not affect the weight gain and feed consumption of rabbits. However, Onifade *et al.* (1999) and Shanmuganathan *et al.* (2004) recorded a favourable impact of yeast on feed intake, feed conversion, and body weight gain in fattening rabbits. The mortality rate of fattening rabbits was not different between groups. This result is in agreement with that of Maertens and De Groote (1992) were Biosaf was used under optimal breeding conditions and Trocino *et al.* (2005) who used 2 doses of *Bacillus cereus* (2×10^{5} and 1×10^{6}) CFU/g of diet. However, Kimsé *et al.* (2012) recorded lower mortality when rabbits were fed 10 g of Biosaf/kg of diet due to diarrhoea between 42 and 56 d of age. Maertens and De Groote (1992) reported a lower mortality of fattening rabbits under less favourable housing conditions. However, it is important to note that the mortality rate recorded in our study on healthy rabbits was low (<10%), so any improvements through the addition of yeast were difficult to observe. In this regard,

Table 4: Effect of live yeast *Saccharomyces cerevisiae* on slaughter traits and caecal pH (means±SEM).

Treatment	Age	Body weight after bleeding (g)	Weight of the gastric content (g)	Weight of the empty stomach (g)	pH of the gastric content	Weight of the caecal content %BW	Weight of the empty caecum %BW	Caecal pH
CC	Avg	1404.4	61.7	19.7	1.6	5.8	1.9^{B}	6.1
CT	Avg	1802.8	70.6	22.0	1.7	5.9	2.0^{B}	6.1
TT	Avg	1488.9	69.9	18.4	1.9	5.8	1.8^{A}	5.9
Average	35	825.8^{a}	45.6^{a}	14.2^{a}	1.5^{a}	5.4	1.8^{b}	6.1
	42	1150.1^{b}	57.2^{a}	17.1^{a}	1.9^{b}	6.3	1.7^{a}	6.2
	77	2358.3^{c}	92.7^{b}	26.8^{b}	1.7^{a}	5.6	2.1^{c}	6.0
SEM[1]		28.2	2.0	0.4	0.05	0.2	0.03	0.04
P treatment		NS	NS	NS	NS	NS	0.02	NS
P age		≤0.001	≤0.001	≤0.001	0.03	NS	≤0.001	0.05
P treatment×age		NS	0.03	NS	NS	NS	NS	NS

SEM: standard error of mean. Average: Avg. Different superscripts indicate significant $P<0.05$ differences among ^{a,b,c} ages and ^{A,B} treatments. NS: no significant ($P>0.05$). CC: Control group, CT: supplemented feed from the age of 35 d, TT: supplemented feed from the age of 17 d. *on day 35 groups CC and CT should be considered as identical. [1]n= 6 rabbits for each combination treatment×age. %BW: % body weight.

Table 5: Effect of live yeast *Saccharomyces cerevisiae* on dry matter content (%), total volatile fatty acids (mmol/kg) and their molar proportions (%) (mean±SEM).

Treatment	Age	Dry matter content (%)	Total volatile fatty acids (mmol/kg)	Acetic acid (%)	Propionic acid (%)	Butyric acid (%)
CC	Avg	23.1	44.0^{B}	71.0	9.5	18.3
CT	Avg	22.9	34.8^{A}	68.8	10.0	20.3
TT	Avg	22.9	47.0^{B}	69.6	9.8	20.0
Average	35	23.0	53.5^{b}	75.5^{b}	9.1^{ab}	15.3^{a}
	42	22.9	36.6^{a}	65.8^{a}	12.8^{b}	20.9^{b}
	77	23.3	44.8^{ab}	71.4^{b}	8.0^{a}	19.5^{b}
SEM[1]		0.2	1.4	0.6	0.3	0.4
P treatment		NS	0.03	NS	NS	NS
P age		0.05	0.004	≤0.001	≤0.001	≤0.001
P treatment×age		0.01	NS	NS	NS	NS

SEM: standard error of mean. Average: Avg. Different superscripts indicate significant $P<0.05$ differences among ^{a,b} ages and ^{A,B} treatments. NS: not significant ($P>0.05$). CC: Control group, CT: supplemented feed from the age of 35 d, TT: supplemented feed from the age of 17 d. *on day 35 groups CC and CT should be considered as identical. [1]n=6 rabbits for each combination treatment×age.

Bovera *et al.* (2010, 2012b) observed that when no pathogenic events occur in a rabbit farm, the administration of antibiotics, or their alternatives, has no effects on animal mortality and growth performance. So, the contradictory results in the literature could be explained by differences in experimental design concerning breeding conditions, breed of rabbit, weaning age, composition of diet and strain, dose and period of administration of microorganisms used, as well as the hygienic levels during the experimental period.

The *Bacteroides* count was in accordance with data reported in the literature (Gouet and Fonty, 1979). Kimsé *et al.* (2012) reported that live yeast had no impact on the structure of the bacterial community after 11 d of yeast presence in the caecum, while the bacterial diversity tended to be higher. Likewise, live yeast seemed to promote the establishment of fibrolytic bacteria in the study of Gidenne *et al.* (2006), which noted a higher proportion of *Ruminococcus albus* in the caecum of 28 and 42 d old rabbits fed supplemented diet with *S. boulardii*. The counts of coliforms and total aerobic bacteria in caecum were low.

Table 6: Effect of live yeast *Saccharomyces cerevisiae* on serum clinical chemistry parameters (mean±SEM).

Treatment	Age	AST (IU/L)	ALT (IU/L)	GGT (IU/L)	TP (g/L)	Alb (g/L)	Glu (mmol/L)	CHOL (mmol/L)	CREA (µmol/L)
CC	Avg	40.5	21.6	7.1	56.5	34.7	8.2	2.3	70.1
CT	Avg	30.0	20.9	10.1	58.2	35.8	8.2	2.3	79.6
TT	Avg	35.0	21.7	7.3	56.7	34.7	8.4	2.5	73.6
Average	35	66^{a}	13.2^{a}	5.2^{a}	45.6^{a}	31.0^{a}	9.2^{a}	2.8^{a}	62.4^{a}
	42	37.3^{b}	25.0^{b}	9.1^{b}	50.0^{a}	33.6^{b}	9.3^{a}	2.3^{bd}	69.8^{a}
	56	27.8^{c}	22.0^{bc}	7.5^{ab}	60.0^{b}	35.0^{bc}	6.8^{b}	2.3^{bc}	66.9^{a}
	77	32.3^{cb}	22.1^{bc}	9.3^{cb}	66.7^{bc}	38.9^{d}	9.3^{a}	2.1^{cd}	99.9^{b}
SEM[1]		1.4	0.8	0.3	0.5	0.3	0.1	0.05	1.5
P treatment		NS	NS	NS	NS	NS	NS	NS	NS
P age		≤0.001	≤0.001	0.004	≤0.001	≤0.001	≤0.001	0.001	≤0.001
P treatment×age		NS	NS	NS	NS	NS	NS	NS	NS

SEM: standard error of mean. Average: Avg. AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma-glutamyl transferase, TP: total protein, Alb: albumin, Glu: glucose, CHOL: cholesterol, CREA: creatinine. Different superscripts indicate significant $P<0.05$ differences among ^{a,b,c} ages. CC: Control group, CT: supplemented feed from the age of 35 d, TT: supplemented feed from the age of 17 d. *on day 36 groups CC and CT should be considered as identical. [1]n=6 rabbits for each combination treatment×age.

Table 7: Effect of live yeast *Saccharomyces cereviasiae* on plasma immunological parameters.

	Treatment						
Age 1*	CC	CT	TT	SEM[1]	*P* treatment	*P* age	*P* age×treatment
IFN-γ	14.1	14.0	13.7	0.7	NS	NS	NS
IL-2	12.0	13.3	13.1	0.3	NS	NS	NS
Ig G	7.0	7.2	7.2	0.3	NS	NS	NS
Age 2**							
IFN-γ	16.0	16.4	16.8	0.5	NS	NS	NS
IL-2	11.5	11.8	11.7	0.2	NS	NS	NS
Ig G	8.3	7.7	8.7	0.2	NS	NS	NS

* Age 1: 42 d old: before vaccination, 56 d old: 14 d after vaccination. ** Age 2: 77 d old: 15 d after the second vaccination. CC: Control group, CT: supplemented feed from the age of 35 d, TT: supplemented feed from the age of 17 d. NS: not significant ($P>0.05$). [1]n=6 rabbits for each combination treatment×age.

Related to the effect of yeast supplementation on slaughter traits, Rotolo *et al.* (2014) reported no effect on the weight of the full and empty caecum. However, Maertens *et al.* (1994) observed that caecal weight was higher in rabbits fed the Paciflor® (Bacillus) diet. Additionally, Kermauner and Struklec (1996) noted that an effect of 0.5% probiotic Acid Pack 4 Way (*Lactobacillus acidophilus, Streptococcus faecium*) was only observed in the decreased proportion of stomach and increased proportion of caecum of gastrointestinal weight.

The values for dry matter content, total SCFA, and molar proportions of acetic, butyric and propionic acids are all in agreement with those obtained by Garcia *et al.* (2002) and Gidenne *et al.* (2008). Kimsé *et al.* (2012) and Rotolo *et al.* (2014) did not report any effect of live yeast (*Saccharomyces cerevisiae*) on volatile fatty acid molar proportions. Furthermore, Maertens *et al.* (1994) and Bonai *et al.* (2008) did not note any effect of Bacillus on the fermentation pattern.

Serum biochemical analysis is used to determine heart attack parameters and liver damage and to evaluate protein quality and amino acid requirements in animals (Ewuola and Egbunike, 2008). All values for the clinical chemistry parameters were within the physiological ranges reported in several reports (Yu *et al.,* 1979; Archetti *et al.,* 2008). In this study, dietary yeast supplementation did not affect blood traits. Similar results were obtained by Seyidoglu and Galip (2014) when applying 3 g of yeast/kg of rabbit diet and Ozsoy and Yalcin (2011) with *Saccharomyces cerevisiae* in broiler turkey. However, Onifade *et al.* (1999) reported that serum protein and albumin level were increased and the serum levels of cholesterol, ALT, AST and ALP were decreased with dietary yeast in rabbits. The differences in the enzymatic activity may be due to animal species and probiotic interventions. Paryad and Mahmoudi (2008) noted lower plasma cholesterol and triglyceride concentration and higher total plasma protein, albumin and globulin concentration in broiler chicks fed 1.5% *Saccharomyces cerevisiae*.

Recent studies have indicated that yeast cell wall components may modulate the immune response of the animal to maintain health and improve growth performance by reducing pathogenic bacteria (Shen *et al.,* 2009). Seljelid *et al.* (1987) reported that β-glucan could stimulate macrophage activity in mice, whereas Djeraba and Quere (2000) reported that mannans increased the macrophage activities in chickens. However, there is a lack of information on the effect of live yeast *Saccharomyces cerevisiae* on immune response of fattening rabbits. In this study, there was no effect of yeast on IgG serum concentration or cytokines profile (IL-2 and IFN-γ) in the treated group. Shen *et al.* (2009) reported that 5 g of yeast culture/kg of feed decreased plasma IFN-γ concentration in nursery pigs and Haghighi *et al.* (2005) reported that the oral administration of 0.5 mL of probiotic containing 10^5 bacteria *Lactobacillus acidophilus, Bifidobacterium bifidum* and *Streptococcus faecalis* did not affect the serum level of IgA and IgG in chickens.

CONCLUSION

We conclude that the addition of 6.5×10^9 CFU/kg feed of live *Saccharomyces cerevisiae* yeast to fattening rabbit diets did not cause any significant changes in fattening rabbit growth performance, caecal microbiota or SCFA profile, with the exception of temporary increase in weight gain and slight alteration of the caecal microbiota after weaning.

Serological data from the present study also showed that yeast supplementation did not alter the blood parameters assessed or the immune response. Although non-significant results were observed for the examined parameters, further studies are needed to understand the effects of live yeast and to clarify the effect on the immune response in growing rabbits.

Acknowledgements: The authors thank S.I. LESAFFRE for providing Actisaf Sc 47 and counting the viability of yeast cells after pelleting.

REFERENCES

AFNOR, 2009. European standard EN 15789, Isolation and enumeration of yeast probiotic strains. 1-17.

AOAC, 2000. Official Methods of Analysis, 17th ed. *Association of Official Analytical Chemists, Arlington, VA, USA.*

Archetti I., Tittarelli C., Cerioli M., Brivio R., Grilli G., Lavazza A., 2008. Serum chemistry and hematology values in commercial rabbits: preliminary data from industrial farms in northern Italy. *9th World Rabbit Congress, June 10-13, Verona, Italy*, *1147-1152.*

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. In: *Proceedings of the 9th World Rabbit Congress, June 10-13, Verona, Italy, 561-565.*

Bovera F., Nizza S., Marono S., Mallardo K., Piccolo G., Tudisco R., De Martino L., Nizza A. 2010. Effect of mannan oligosaccharides on rabbit performance, digestibility and rectal bacterial anaerobic populations during an episode of epizootic rabbit enteropathy. *World Rabbit Sci., 18: 9-16. doi:10.4995/wrs.2010.18.02.*

Bovera F., Lestingi A., Iannaccone F., Tateo A., Nizza A. 2012a. Use of dietary mannanoligosaccharides during rabbit fattening period: Effects on growth performance, feed nutrient digestibility, carcass traits, and meat quality. *J. Anim. Sci., 90: 3858-3866. doi:10.2527/jas.2011-4119*

Bovera F., Lestingi A., Marono S., Iannaccone F., Nizza S., Mallardo K., de Martino L., Tateo A. 2012b. Effect of dietary mannan-oligosaccharides on *in vivo* performance, nutrient digestibility and caecal content characteristics of growing rabbits. *J. Anim. Physiol. Anim. Nutr., 96: 130-136. doi:10.1111/j.1439-0396.2011.01134.x*

Carabaño R., Badiola I., Licois D., Gidenne T. 2006. The digestive ecosystem and its control through nutritional feeding strategies. *In: Recent Advances in Rabbit Sciences (Maerten L. and Coudert P.), 211-227.*

Chaudhary 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. doi:10.4995/wrs.1995.235*

Djeraba A., Quere P. 2000. In vivo macrophage activation in chickens with Acemannan, a complex carbohydrate extracted from *Aloe vera. Int. J. Immunopharmaco., 22: 365-372. doi:10.1016/S0192-0561(99)00091-0*

Ewuola E.O., Egbunike G.N. 2008. Haematological and serum biochemical response of growing rabbit bucks fed dietary fumonisin B1. *Afri. J. Biotechnol., 7: 4304-4309.*

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. doi:10.4995/wrs.2007.597*

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. doi:10.1016/j.livsci.2006.09.005*

García J., Gidenne T., Falcão-e-Cunha L., De Blas C. 2002. Identification of the main factors that influence caecal fermentation traits in growing rabbits. *Anim. Res., 51: 165-173. doi:10.1051/animres:2002011*

Gidenne T., Bennegadi-Laurent N., Bayourthe C., Monteils V., Fonty G. 2006. Post-weaning maturation of rabbit caecal microbial communities: impact of live yeast intake. In: Louis, P., Flint, H.J. (Eds.), Rowet Research Institute - Institut National de la Recherche Agronomique Conference, *5th Biennial Meeting Aberdeen, Scotland.*

Gidenne T., Combes S., Licois D., Carabaño R., Badiola I., García J. 2008. The caecal ecosystem and the nutrition of the rabbit: interaction with digestive health. *INRA Productions Animales. 21: 239-249.*

Gouet Ph., Fonty G. 1979. Changes in the digestive microflora of holoxenic * rabbits from birth until adulthood. *Ann. Biol. Anim. Bioch. Biophys., 19: 553-566. doi:10.1051/rnd:19790501*

Haghighi H.R., Grong J., Gyles C.L., Hayes M.A., Sanei B., Parvizi P., Gisavi H., Chambers J.R., Sharif S. 2005. Modulation of Antibody-Mediated Immune Response by Probiotics in Chickens. *Clin. Diagn. Lab. Immunol., 12: 1387-1392. doi:10.1128/cdli.12.12.1387-1392.2005*

Hollister A.G., Cheeke P.R., Robinson K.L., Patton N.M. 1990. Effects of dietary probiotics and acidifiers on performance of weanling rabbits. *J. Appl. Rabbit. Res., 13: 6-9.*

Jerome N., Mousset J.L., Lebas F., Robart P. 1996. Effect of diet supplementation with oxytetracycline combined or not with different feed-additives on fattening performance in the rabbit. *6th World Rabbit Congress*, *July 9-12, 1996, Toulouse, France, 205-210.*

Kermauner A., Struklec M. 1996. Addition of probiotic to feeds with different energy and ADF content in rabbits. 1. Effect on the digestive organs. *World Rabbit Sci., 4: 187-193. doi:10.4995/wrs.1996.293*

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. doi:10.1016/j.anifeedsci.2012.01.012*

Maertens L., De Groote G. 1992. Effect of dietary supplementation of live yeast on the zootechnical performances of does and weanling rabbits. *J. Appl. Rabbit Res., 15: 1079-1086.*

Maertens L., Van Renterghem R., De Groote G. 1994. Effects of dietary inclusion of Paciflor R (Bacillus Cip 5832) on the milk composition and performances of does and caecal and growth parameters of their weanlings. *World Rabbit Sci., 2: 67-73.*

Mertens D.R. 2002. Gravimetric determination of amylase-treated neutral detergent fibre in feeds with refluxing beakers or crucibles: collaborative study. *J. AOAC Int.* 85: 1217-1240.

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 diet with a culture of "*Saccharomyces cerevisiae*" using growth performance, blood composition and clinical enzyme activities. *Anim. Feed Sci. Technol.*, *77: 25-32. doi:10.1016/S0377-8401(98)00244-2*

Özsoy B., Yalçin S. 2011. The effects of dietary supplementation of yeast culture on performance, blood parameters and immune system in broiler turkeys. *Ankara Üniv. Vet. Fak. Derg.*, *58: 117-122. doi:10.1501/Vetfak_0000002460*

Paryad A., Mahmoudi M. 2008. Effect of different levels of supplemental yeast (*Saccharomyces cerevisiae*) on performance, blood constituents and carcass characteristics of broiler chicks. *Afr. J. Agr. Res.*, *3: 835-842.*

Rotolo L., Gai F., Peiretti P.G., Ortoffi M., Zoccarato I., Gasco L. 2014. Live yeast (*Saccharomyces cerevisiae* var. *boulardii*) supplementation in fattening rabbit diet: effect on productive, performance and meat quality. *Livest. Sci.*, *162: 178-184. doi:10.1016/j.livsci.2014.01.022*

Rozen S., Skaletsky H. 2000. Primer3 on the WWW for general users and for biologist programmers. *Methods Mol. Biol., 132: 365-386.*

Seljelid R., Rasmussen L.T., Larm O., Hoffman J. 1987. The protective effect of beta 1–3d-glucan-derivatized plastic beads against *Escherichia coli* infection in mice. *Scand. J. Immunol. 25: 55-60. doi:10.1111/j.1365-3083.1987.tb01046.x*

Seyidoğlu N., Galip N. 2014. Effects of *Saccharomyces cerevisiae* and *Spirulina platensis* on Growth Performances and Biochemical Parameters in Rabbits. *Kafkas Univ. Vet. Fak. Derg., 20: 331-336. doi:10.9775/kvfd.2013.9988*

Shanmuganathan T., Samarasinghe K., Wenk C. 2004. Supplemental Enzymes, Yeast Culture and Effective Micro-organism Culture to Enhance the Performance of Rabbits Fed Diets Containing High Levels of Rice Bran. *Asian-Aust. J. Anim. Sci.*, *17: 678-683.*

Shen Y.B., Piao X.S., Kim S.W., Wang L., Liu P., Yoon I., Zhen Y.G. 2009. Effects of yeast culture supplementation on growth performance, intestinal health, and immune response of nursery pigs. *J. Anim. Sci., 87: 2614-2624. doi:10.2527/jas.2008-1512*

Trocino A., Xiccato G., Carraro L., Jiménez 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.*

Yu L., Pragay D.A., Chang D., Wicher K. 1979. Biochemical Parameters of Normal Rabbit Serum. *Clin. Biochem., 12: 83-87. doi:10.1016/S0009-9120(79)80071-5*