LIVE YEAST STABILITY IN THE DIGESTIVE TRACT OF THE RABBIT: RELATIONSHIP WITH DIGESTION, GROWTH AND DIGESTIVE HEALTH

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ABSTRACT

Live yeast *Saccharomyces Cerevisiae* NCYC Sc 47 (Biosaf®) was added to the diet of weaning rabbits to evaluate the effects on growth, feed efficiency, health status, digestibility and caecal parameters. Two Biosaf® levels (1 and 10 g Biosaf®/kg feed, group C1 and C10 corresponding to 10^6 and 10^7 CFU/g of DM in diet) were compared to a control group (C0) without yeast addition. Thirty rabbits (3 groups of 10) were used to measure the digestibility and caecal parameters and to calculate the yeast survival rate in the digestive tract. Growth performances and health status were studied on 120 rabbits (3 groups of 40 cages individually). In diet, live yeast concentration fell slightly (-0.2 to 0.5 log CFU/g DM) after pelleting, although the pelleting temperature was high (70-80°C). The survival rate of yeast in digestive tract was high and increased from 90 to 97% with yeast supplementation, but this did not affect the faecal digestibility or the caecal pH. However, redox potential (Eh) of the caecal content increased significantly with yeast addition. Mortality was 50% lower with the highest yeast addition (C10) compared to C0 and C1 groups, while growth, intake or feed efficiency remained similar.

Key words: Probiotics, Yeast, Redox potential, Rabbit, Digestibility, Growth performance, Mortality.

INTRODUCTION

The action of probiotics is generally ascribed to their ability to stimulate the digestive processes or to improve the gut microbial balance. Effect of probiotics on rabbit performances or health was recently reviewed by Falcao E Cunha *et al.* (2007). The most beneficial effect of yeast supplementation would be on the digestive health in the young rabbit, that could be significant under non optimal breeding conditions (Maertens and De Groote, 1992), but their action mechanism remained to be elicited. The main impact of live yeast was supposed on digestive microorganisms, and for monogastric animal as rabbit, the targets for yeast role would be mainly the hind-gut (caecum or colon). However, live microorganism will be beneficial provided that they survive to the environmental conditions, such the incorporation in pelleted feed, or the transit through the gastrointestinal tract. Therefore, we aimed to measure the stability of Biosaf® live yeast during pelleting and in the rabbit digestive tract, and the effect of the yeast supplementation on the caecal biotope, growth performances and digestive health status in the rabbit after weaning.

MATERIALS AND METHODS

Trial 1: yeast stability, caecal pH and redox potential and nutrients digestibility

Diet and animal

A basal diet was formulated to cover requirements of growing rabbits (Table 1). It was divided into 3 equal portions before pelleting at the milling unit of INRA (UMR 1289 TANDEM): one portion without yeast (diet C0), and two portions supplemented with yeast (Biosaf®: *Saccharomyces Cerevisiae* NCYC Sc 47 coated with saccharides) at 1 or 10 g/kg of basal flour (diet C1 and C10

resp.). After pelleting, 3 samples (500 g) were collected and stored for yeast analysis. Each diet was assigned to 10 rabbits (New Zealand white x Californian rabbits). Rabbits were kept in individual metabolism cages (55 x 40 cm) from weaning (35 d) to 46 days of age, and submitted to a 12 h light (07:00 to 19:00 h) and 12 h dark schedule, at 18±2°C. They were given ad libitum access to water and pelleted diets. The weight and feed intake were daily recorded.

Digestibility, E_h and pH measurements in the caecum

After a 7 days adaptation period, the total daily excretion of hard faeces was individually collected for the 3 groups of 10 rabbits during 4 consecutive days, to estimate yeast survival rate and total tract digestibility of nutrients (Perez *et al*., 1995). After collection, the samples were stored at -20°C in plastic bags. In addition, for 6 rabbits per group, 10 g of freshly collected hard faeces (1st day) were analysed immediately for their concentration in yeast using culture method (Lesaffre Feed Additive, 2000). Feed samples were collected during faeces collection period and analysed for chemical composition. Immediately after the end of the faecal collection period (4th day), and for 6 rabbits by treatments, soft faeces were collected, then rabbits were sacrificed to measure the redox potential (E_h) and pH of the caecal content. The E_h and pH measurements were made with two electrodes connected to digital pH meter (Metrohm® model 713 CH-9101, Herisau, Switzerland). A glass pH and temperature electrode "Unitrode" (Pt1000/B/2/3MKCl; Metrohm®) and an E_h platinum electrode "Combined" (Pt-ring electrode; Pt/-2 to 80°C; Metrohm®) were used. The measurements were carried out during 25 min-period to reach stability before recording values (Kimsé *et al*., 2007). After these measurements caecal content was sampled to analyse the concentration in yeast and dry matter.

Table 1: Ingredients and chemical composition of the basal diet

Ingredients	%
Wheat	32.00
Barley	14.40
Lucerne meal	34.00
Wheat straw	4.10
Soya molasses	7.00
Sunflower meal	5.20
Soya bean hulls	2.00
Salt	0.50
Methionin	0.30
Vitamin premix	0.5
Chemical composition	**Content (g/kg raw basis)**
Dry matter	911
Organic matter	836
Crude ash	75
Crude protein	174
Crude fat	41
Crude fibre	143
Neutral-detergent fibre	375
Acid-detergent fibre	190
Acid-detergent lignin	47

Chemical analyses

Dry matter, ash, crude protein, crude fibre, neutral-detergent fibre, acid-detergent fibre and acid-detergent lignin of feeds were analysed according to E.G.R.A.N. (2001). Hard faeces were analysed for DM, CP and ash.

Yeast culture

Hard faeces or diet sample (10 g) were hydrated in a stomacher apparatus and incubated during 30 min at 37°C. The water ratio was 90 g for 10 g fresh faecal sample, which corresponded to 10^{-1} dilution. After the first incubation, a dilution series was made from 10^{-2} to 10^{-6}. Five ml of each serial dilution were pitched in 3 Petri dishes. Sterilized agar medium (Milieu YM Agar, 0712-17-0, DIFCO) was added to 2/3 of height of dish. Petri dishes were kept in an incubator at 37°C to 48 h, and then the number of yeast colonies was counted. Calculation of yeast concentration was done using the following formula, and expressed as CFU/g fresh matter: Revivifiable yeast concentration = Mean of each dilution/ Test portion (1 g) x Dilution factor (Values included counts ranged from 30 to 300 colonies in one Petri dish).

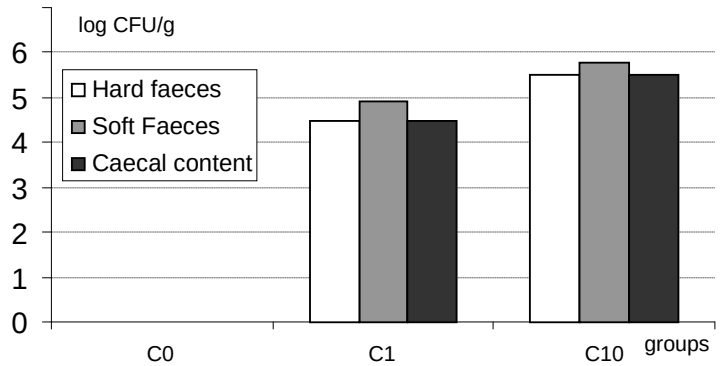

Figure 1: Live yeast concentration (log CFU/g) in hard and soft faeces and in the caecal content of the growing rabbit

Trial 2: effect of yeast addition on growth performances

A total of 120 New Zealand white x Californian rabbits weaned at 35 days of age were allotted in 3 equal groups (C0, C1 and C10) according to their weaning weight, and were fed freely the three experimental diets previously described. Rabbits were caged individually (70 x 25 x 34 cm) in a ventilated room (18±2°C) and under 12 h light (07:00 to 19:00 h). Live weight and feed intake were recorded weekly, while mortality was recorded daily.

Statistical analysis

Data were subjected to analysis of variance using the GLM procedure of SAS, and means were compared using the Scheffe test. Mortality was judged using the X^2-test and means comparisons were made using orthogonal contrasts.

RESULTS

Yeast stability and physico-chemical parameters of rabbit caecum

Before pelleting, the live yeast cells concentration was 2.5 Log (CFU/g) in flour of the basal diet (C0) indicating the presence of "wild" yeast in the medium (Table 2). During pelleting, temperature increased from 19°C (ambient temperature) to 80°C at end of pelleting, and this led to a reduction in "wild" yeast concentration (diet C0), but not for protected Biosaf® yeast (Log CFU/g DM, Table 2). There was no live yeast in the faeces and in caecal contents of control group (C0, Figure 1). As expected, live yeast concentration was about 10 times higher in C10 than in C1 group ($P<0.01$), whatever the sampling site: caecum, hard or soft faeces. The determination of live yeast concentration in hard faeces allows us to calculate the survival rate of yeast after passage in the rabbit digestive tract. Yeast survival rate was high, and increased significantly from 90 to 97% with yeast addition.

Table 2: Live yeast concentration in diet before and after pelleting, and survival rate in rabbit digestive tract

	Diet				
	C0	C1	C10	VC (%)	P level
Yeast level in diet before pelleting, Log (CFU/g DM)	2.5	5.7	7.0		
Yeast level in diet after pelleting, Log (CFU/g DM)	<2	5.6	6.5		
Yeast intake, Log (CFU/d)	*	7.6	8.5	1.0	<0.01
Yeast excretion, Log (CFU/d)	*	7	8.3	2.5	<0.01
Survival rate of yeast (% excretion/intake)**		90.3	96.9	2.3	<0.01

*Invalid calculation; VC: variation coefficient; **: calculated on 6 rabbits per group

Dry matter (DM) and pH of caecal content were similar among treatments (Table 3). The average DM was 23% and pH was 6.0. However, caecal redox potential (E_h) increased significantly with yeast concentration in diet ($P<0.01$).

Impact of yeast supplementation on digestion, growth and digestive health status

Yeast addition did not significantly modify the digestibility coefficients whatever nutrients assessed (Table 3). Averages values of digestibility coefficients were 62.9±1.7%, 62.7±1.7% and 74.7±2.5% respectively for dry matter, organic matter and crude protein. Whatever the age, the weight, weight gain, feed intake and feed efficiency were similar among groups (Table 4). Final body weight at 70 days of age was meanly 2615±247 g, while feed intake averaged 142±18 g/d. Results of mortality were arranged in 3 periods: 35 to 42 days of age, 42 to 56, and 56 to 70 days. During the first week of the experiment no mortality or morbidity was registered. Diarrhoea incidence increased sharply during the second and third week of the trial, with 11 and 14 dead rabbits in C0 and C1 groups (table 5), but only 4 dead in the C10 group ($P<0.05$). Effect of yeast supplementation was not significant during the third period (56-70 days of age), and for the whole fattening period mortality rate decreased by about 50% for the C10 group. However, yeast addition did not affect the morbidity from 35 to 70 d of age.

DISCUSSION

In the control diet, live yeast concentration was 2.5 Log (CFU/g) in flour. This indicates that live yeast ("wild" strain) colonises the air at a low but noteworthy level, as previously described (Harrison *et al*., 1988; Maertens and De Groote, 1992). But, we showed that without yeast addition in the feed, the rabbit hind gut did not harbour yeast, since no yeast was found in the caecal content of the control group.

Table 3: Effect of yeast addition on caecal parameters and faecal digestibility

	Groups				
	C0	C1	C10	VC (%)	P level
Caecum					
Number of rabbits	6	6	6		
DM (%)	23	23	23	9	ns
pH	5.9	6.0	6.1	7	ns
Eh (mV)	-251^{B}	-238^{A}	-213^{A}	10	<0.01
Digestibility					
Number of rabbits	8	9	9		
Dry matter (%)	62.4	63.3	63.5	2.6	ns
Organic matter (%)	62.0	63.1	63.0	2.7	ns
Crude protein (%)	74.9	75.0	74.2	3.4	ns

^{A,B}:Means with the same superscripts did not differ at the level P=0.05; ns: P>0.15; VC: variation coefficient (%)

The Biosaf® yeast appeared resistant to the hard conditions of pelleting (here 80°C), and also to the passage along the rabbit digestive tract with a high acidity in stomach (pH<2) and bile salts. The yeast survival rate to rabbit digestion increased when the yeast intake increased by one Log (10^7 to 10^8). The product used here, corresponding to a specific strain and to a specific protection of the yeast (coating with saccharides), seemed more resistant than those used previously by Maertens and De Groote (1992).

The main effect founded in this study was an improvement of digestive health of the growing rabbit. Even with a limited number of rabbit, the mortality rate was significantly reduced when a high level of Biosaf® (10%) was added to the feed, since the mortality rate was high in our conditions. In the study of Maertens and De Groote (1992), a similar high concentration of yeast (Biosaf® 1%) in diet did not improve mortality rate compared to low concentration (Biosaf® 0.15%). This improvement of digestive health must be thus confirmed using a larger number of animals, caged collectively. The favourable effect of yeast found here, could be linked to the higher level of yeast found in the caecum of supplemented animals. Furthermore, this high yeast level in the caecum was associated with an increased redox potential (E_h). Relationship between yeast supplementation and ruminal E_h and pH of dairy cow was recently showed by Marden (2007). who found that yeast (Biosaf®) supplementation reduced E_h of rumen and increased the pH. On the contrary in our study, rabbit caecal E_h increased with yeast supplementation and the pH remained unchanged. The rumen is not a totally anaerobic compartment with digestive flora adapted with strictly anaerobic and facultative anaerobic species, while the caecum is a more strictly anaerobic compartment. This difference among rumen and caecal biotopes probably supports a differential role of yeast in the digestive ecosystem.

Table 4: Effect of yeast on intake, growth performance and feed efficiency of healthy rabbits*, from 35 to 70 days of age

	Groups				
	C0	C1	C10	VC (%)	P level
Number of rabbits	29	26	36		
Feed intake (g/d)	145	143	138	12	ns
Initial body weight 35 days (g)	1030	1030	1030	12	ns
Final body weight 70 days (g)	2650	2663	2558	9	ns
Daily gain 35-70 days (g/d)	46	46	44	16	ns
Feed efficiency	3.2	3.2	3.1	15	ns

VC, variation coefficient (%), ns:P>0.15; * Rabbits sick or dead are excluded

For all other parameters, there was no significant effect of yeast supplementation. Feed intake, feed efficiency, and digestibility were similar even with high yeast incorporation rate (10 g/kg). Consequently, body gain and final weight were similar among treatments. Several reasons could explain this result. First, the yeast concentration (dose) in the diet may not be sufficiently high to detect an effect on performances. Secondly, the level of growth performances recorded here on healthy rabbits was already high (mean daily gain=45 g/d for 35-70 d

period), and thus improvement in growth or feed efficiency was hard to obtain, contrary to the health status which was poor during the second and third week of the trial. Similarly, for optimal breeding conditions, no significantly difference of rabbit performances (daily weight gain, feed efficiency and mortality) was observed by Maertens and De Groote (1992). Reversely, for lower level of performances, the effect of a yeast addition in feed could be detectable as shown by Onifade *et al*. (1999), for rabbits having relatively low intake (70 g/d) and daily gain (20 g/d).

CONCLUSIONS

Protected live yeast (Biosaf®) was resistant to the pelleting process and to the passage along the rabbit digestive tract. Dietary supplementation with yeast at a dose of 10^6 CFU/g reduced the mortality of the growing rabbit, and increased the redox potential in the caecal content, while digestion and growing performances remained unaffected.

Further research is necessary to understand the role of yeast within the caecal biocenosis (biodiversity or stability), particularly to explain improvement in digestive health around the weaning period.

Table 5: Effect of yeast on rabbit health status from weaning (35 days) to 70 days of age

	Groups			
	C0	C1	C10	P-level
Mortality: number of animals				
35 to 42 d	0	0	0	
42 to 56 d	11^{AB} (n=40)	14^{A} (n=40)	4^{B} (n=40)	0.04**
56 to 70 d	7 (n=29)	3 (n=26)	5 (n=36)	ns
35 to 70 d	18	17	9	0.08*
Morbidity: number of animals				
35 to 70 d	8 (n=40)	8 (n=40)	9 (n=40)	ns

^{A,B}Means with the same superscripts did not differ at the level P=0.05; ns:P>0.15; VC: variation coefficient (%)
ns: P>0.15; n: initial number of rabbit, within a period.
**Contrast C10 vs. (C1 + C0): P=0.02; *Contrast C0 vs. (C1 + C10): P=0.03

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REFERENCES

E.G.R.A.N. 2001. Technical note: Attempts to harmonise chemical analyses of feeds and faeces, for rabbit feed evaluation. *World Rabbit Sci., 9, 57-64.*

Falcao-e-Cunha L., Maertens L., Marounek M., Pinheiro V., Freire J., Mourao J.L. 2007. Alternatives to antibiotic growth promoters in rabbit feeding: A review. *World Rabbit Sci., 15, 127-140.*

Harrison G.A., Hemken R.W., Dawson K.A., Harmon R.J., Barker K.B. 1988. Influence of addition of yeast culture supplement to diets of lactating cows on ruminal fermentation and microbial populations. *J. Dairy Sci., 71, 2967-2975*.

Kimsé M., Gidenne T., Bayourthe C., Monteils V. 2007. Mesure du potentiel redox dans le cæcum du lapin: premiers résultats méthodologiques. *In: Proc. 12èmes Journées de la Recherche Cunicole, Le Mans, France, 81-84.*

Lesaffre Feed Additive 2000. Dénombrement des levures revivifiables, milieu YM. *In: Lesaffre internal report, pp. 1-4.*

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.*

Marden J.P. 2007. Contribution à l'étude du mode d'action de la levure Saccharomyces cerevisiae Sc 47 chez le ruminant: Approche thermodynamique chez la vache laitière. PhD thsesis, Inst. National Polytechnique, pp 232.

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.*

Perez J.M., Lebas F., Gidenne T., Maertens L., Xiccato G., Parigi-Bini R., Dalle Zotte A., Cossu M.E., Carazzolo A., Villamide M.J., Carabaño R., Fraga M.J., Ramos M.A., Cervera C., Blas E., Fernandez Carmona J., Falcao E Cunha L., Bengala Freire J. 1995. European reference method for in-vivo determination of diet digestibility in rabbits. *World Rabbit Sci., 3, 41-43.*