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**Original article**

# Fate of Levucell® SC I-1077 yeast additive during digestive transit in lambs

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**Abstract** – The fate of a live yeast strain, which was used as a feed additive for ruminants (Levucell® SC I-1077), was studied during digestive transit in two gnotoxenic lambs reared in a sterile isolator. The number of live yeast cells were counted in the rumen and in faeces after a single administration or a daily feeding of 100 mg of Levucell® SC. If the supplement was not renewed, the live yeast cells persisted in the rumen for approximately 30 h at a level close to the initial value. They were then gradually cleared. They began to be excreted with the faeces approximately 8 h after their consumption and were no longer detected after 102 h. Yeast additives did not colonize the rumen. As 17 to 34 % of yeast cells remained alive during their transit through the digestive tract, their effect might extend beyond the rumen the post-ruminal compartments. © Inra/Elsevier, Paris

**yeast additives / transit / rumen / gnotoxenic lambs**

**Résumé – Devenir de la levure additive Levucell® SC I-1077 pendant le transit digestif chez l'agneau.** Le devenir de levures vivantes utilisées comme additif alimentaire pour le ruminant (Levucell® SC I-1077) a été étudié au cours du transit digestif chez deux agneaux gnotoxéniques élevés en isolateur stérile. Les levures vivantes ont été dénombrées dans le rumen et les fécès en cinétique après avoir distribué soit une seule fois soit quotidiennement 100 mg de Levucell® SC. Si l'on ne renouvelle pas leur distribution, les levures vivantes se maintiennent dans le rumen pendant environ 30 h à un niveau proche du niveau initial, puis sont progressivement éliminées. Elles commencent à être excrétées avec les fécès 8 h environ après leur distribution, et ne sont plus détectées 102 h après l'arrêt de la supplémentation. Les levures additives ne colonisent donc pas le rumen. Le fait que 17 à 34 % des cellules vivantes de levure résistent pendant leur transit dans le tube digestif suggère que leur effet pourrait ne pas se limiter au rumen mais s'exercer également dans les compartiments post-ruminaux. © Inra/Elsevier, Paris

**levures additives / transit / rumen / agneaux gnotoxéniques**

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## 1. INTRODUCTION

During the last decade, microbial additives (probiotics), yeasts (*Saccharomyces cerevisiae*) or fungi (*Aspergillus oryzae*), have become increasingly widely used in animal nutrition. These additives are given to ruminants to optimize ruminal function and to improve the utilization of the energy contents and nutritional components of their diet, while at the same time avoiding nutritional disorders [10].

It was shown recently that Levucell® SC (*Saccharomyces cerevisiae* CNCM I-1077, Institut Pasteur, Paris, France) is able to stimulate the activities of certain ruminal micro-organisms in vitro. For example, the rate of cellulose breakdown by the anaerobic ruminal fungus *Neocallimastix frontalis* MCH3 [4], and the utilization of lactic acid by the bacterium *Megasphaera elsdenii* [6], are increased in the presence of this additive. In vivo, this yeast strain makes it possible to prevent the accumulation of lactic acid in the rumen of sheep adapting to starch-rich diets, thereby avoiding the strong decrease in ruminal pH [16]. Other yeast strains have increased the breakdown rate of hay in sacco [19], and stimulated the growth of cellulolytic [18] and lactate-utilizing microflora [11].

Generally, in ruminants [5, 9] and in monogastric animals [1], the stimulating effects of yeast additives depend on the yeast being live, although some effects can be observed with dead cells [5, 6].

*S. cerevisiae* does not belong to the native ruminal flora. Yeast additives seem unable to colonize the rumen [12, 17]. However, Dawson and Newman [8] found that the number of cells increased 6-fold in rumen fluid in vitro. Bruning and Yokoyama [3] suggested that yeast cells living in the rumen may have an anaerobic metabolism, which is demonstrated by an abundant alcohol production.

Optimizing the potential of yeast additions to feed and their in vivo efficiency requires a better knowledge of their behaviour in the rumen. To this end we decided to study the fate of live *S. cerevisiae* cells (Levucell® SC) during their transit in the digestive tract of lambs raised in sterile isolators.

## 2. MATERIALS AND METHODS

### 2.1. Animals

This experiment was conducted with two gnotobiotic lambs (A and B) which were 6 months old. They were born naturally, had been left with their dams for 1 day and then each of them had been reared in a sterile isolator. In this model, the rumen host a simplified bacterial flora at that time, since fungi, protozoa, cellulolytic bacteria and methanogenic archæa have not been allowed to become established normally [13]. Cellulolytic bacterial cultures (*F. succinogenes*, *R. flavefaciens* and *R. albus*) had been introduced in the rumen of these animals after 10 days of life to ensure efficient digestion of the cellulosic feed components in the diet. As it has been observed that wild yeast strains [15] as well as bacterial spores or fungi [2] could grow in feed ingredients such as forages or silages and then may be detected in the rumen of conventional ruminants, rearing the lambs in sterile isolators made it possible for the animals to be maintained in an environment that could be kept free of any contamination by yeast strains which might invalidate the results.

The lambs had been given sterile (UHT) cow's milk until age 70 days. From age 1 month they had been given access to dehydrated lucerne hay (ad libitum) in the form of 7-mm pellets sterilized by γ-irradiation. They had been fitted with a permanent ruminal plastisol cannula (25 mm diameter) at age 10 weeks.

### 2.2. Experimental design

#### *2.2.1. Period 1: control period, duration from D1 to D7*

The lambs were not given the additive. Absence of any yeast growth was verified.

### 2.2.2. *Period 2: fate of yeast given in a single dose, duration from D8 to D12*

The lambs were given one single dose of 100 mg of Levucell® SC, containing $2 \times 10^9$ CFU, along with their morning meal. During the subsequent days, they did not receive the additive anymore. The transit of yeasts through the digestive tract was then monitored for 4 days.

### 2.2.3. *Period 3: fate of yeast given in repeated daily doses, duration from D13 to D21*

The lambs were given 100 mg of Levucell® SC every morning, along with the meal for 5 consecutive days (D13 to D17). The transit of the yeast was monitored from D15 to D21.

## 2.3. Sampling and counting of yeast cells

Approximately 50 mL of rumen contents were withdrawn via the cannula, for each lamb, at different times, as follows:

– period 1: once daily for two consecutive days (D4 and D5);

– period 2: 2, 4, 6, 8, 12, 16, 24, 30, 36, 48, 72 and 96 h after consuming yeast additive;

– period 3: 4, 8, 12, 16 and 23 h after consuming the yeasts on D15, the same on D16, and then on D17, 4, 8, 12, 16, 24, 28, 32, 36, 48 and 102 h after the last dose of Levucell® SC.

Faeces were collected using the same schedule as was used for the rumen contents during period 1. During period 2 they were collected from the isolator 4, 8, 12, 16, 24, 30, 36, 48, 72 and 96 h after yeast consumption. During period 3, collection was 4, 8, 12, 16 and 23 h after Levucell® SC dispensing on D15, 8, 16 and 23 h after on D16, and then 8, 16, 24, 32, 48 and 102 h after the last dose of Levucell® SC on D17. At every collection time, all the faeces produced were weighed prior to mixing and withdrawal of a sample (1 g) necessary for microbiological analysis.

Live yeast cells were counted in Petri dishes on a malt agar medium containing 10 % (v/v) of an antibiotic solution (streptomycin 0.025 %, ampicillin 0.04 %, chloramphenicol 0.03 %, tetracycline 0.012 %, neomycin 0.03 %). The pH of the medium was adjusted to 4.5 with a sterile solution of 10 % (w/v) lactic acid.

The samples were then used for enumeration. Using 1 mL of rumen contents or 1 g of previously homogenized faeces, serial decimal dilutions were made in physiologically sterile water. Each dilution tube was vigorously shaken to avoid a possible entrappement of yeast cells in solid materials. The CFU value was determined after 48 h incubation at 30 °C.

Collection of all the faeces from the isolators for 102 h after the last dose of Levucell® SC (period 3) enabled us to calculate the percentage of yeast cells excreted relative to the number ingested, and to evaluate the quantity of live yeast cells able to survive transit through the digestive tract.

## 3. RESULTS AND DISCUSSION

Period 1: no yeast colonies grew from the samples either of rumen contents or faeces, indicating that all yeast colonies grown during periods 2 and 3 would only be of strain I-1077.

Period 2: in both lambs, the total counts of anaerobic bacteria were close to $10^{10}$ cells.mL$^{-1}$, cellulolytic bacteria had established normally and ruminal digestion of alfalfa pellets was efficient (data not shown). Yeast cells were found in the rumen of both lambs at levels close to those initially distributed, i.e. approximately $10^5$ CFU mL$^{-1}$, at 24 h after ingestion of Levucell® SC. This time is comparable to the residence time of the solid phase in the rumen, which means that the yeast cells would be associated with the solid phase, as already observed by Jouany et al. [14]. The yeast cells were then gradually eliminated. After 96 h, only $2 \times 10^3$ CFU mL$^{-1}$ were counted in lamb A and $1 \times 10^3$ CFU mL$^{-1}$ in lamb B (*figure 1*). Yeasts were then detected in the faeces 4 to 8 h after ingestion in lambs A and B, respectively, the yeast concentration in the faeces peaked 16 to 30 h after ingestion at $6.6 \times 10^5$

CFU $g^{-1}$ and $2.2 \times 10^6$ CFU $g^{-1}$ (*figure 2*). The yeast cell concentration in the faeces diminished after this time. No further yeasts were detected in lamb B at 96 h after ingestion, and only $1.2 \times 10^4$ CFU $g^{-1}$ were detected in lamb A. This kinetic pattern of excretion of yeast with the faeces was comparable to that obtained in cattle with spores of *Bacillus thermophilus*. These inert spores were used as a bacterial tracer to monitor the rate of excretion of *Clostridium tyrobutyricum* spores, an undesirable bacterium that grows in silage [7].

Period 3: during the time when there was daily ingestion of Levucell® SC, yeast cell counts were always close to $10^5$ CFU $mL^{-1}$ in the rumen and $10^6$ CFU $g^{-1}$ in the faeces. These levels continued until the time when no more yeast was dispensed. About 30 h after discontinuing supplementation, the CFU count diminished gradually (*figures 3* and *4*) and no further yeast cells were detected 102 h after the last dose was consumed.

The ratio of quantity of yeast excreted to quantity ingested showed that a large part of the yeast population disappeared during digestive transit. Only 17 and 34 % of the yeast consumed were recove-

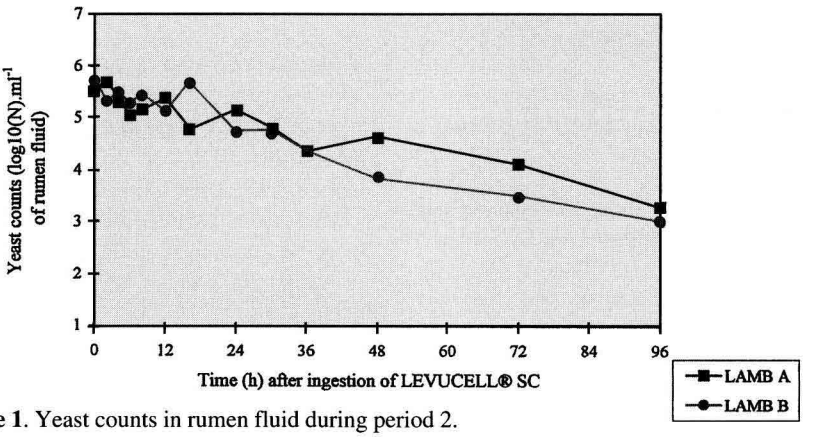


**Figure 1**. Yeast counts in rumen fluid during period 2.

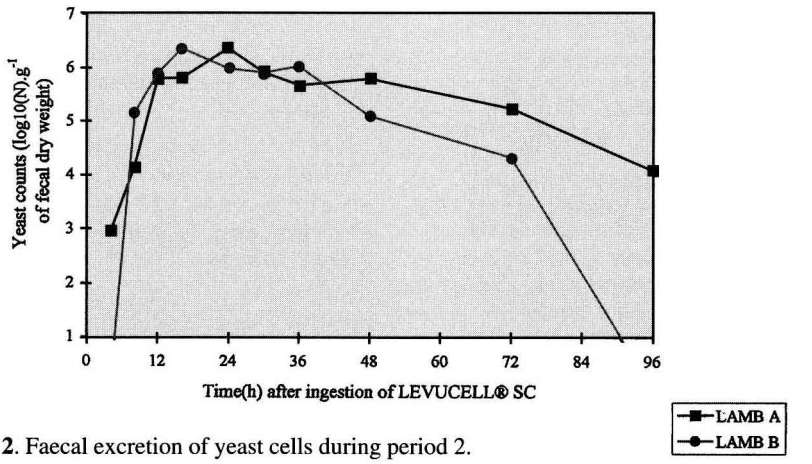


**Figure 2**. Faecal excretion of yeast cells during period 2.

red live in faeces from lambs A and B, respectively at the end of period 2.

These results showed that the strain of *S. cerevisiae* I-1077 in Levucell® SC was unable to colonize the digestive tract of lambs. When the cells were dispensed only once, or when the supplementation was discontinued, they stayed alive in the rumen for about 30 h, to an extent close to the initial value, but they did not multiply and were gradually cleared. Consequently the supplementation should be repeated at least daily. However, it is not known if yeast additives retain their metabolic activities and exert their effects on the ruminal microflora throughout the whole of this period. Hence dispensing schedules should be set carefully, and the optimal frequency of supplementation (once or several times daily) must be determined according to the effects sought on production or animal health.

In our study, a small proportion of yeast cells survived during the digestive transit and were found, still alive, in the faeces. Although this result has to be confirmed with conventional adult ruminants, it supported the hypothesis of Newbold et al. [17] and showed that live yeasts can play a nutritional role in compartments down-

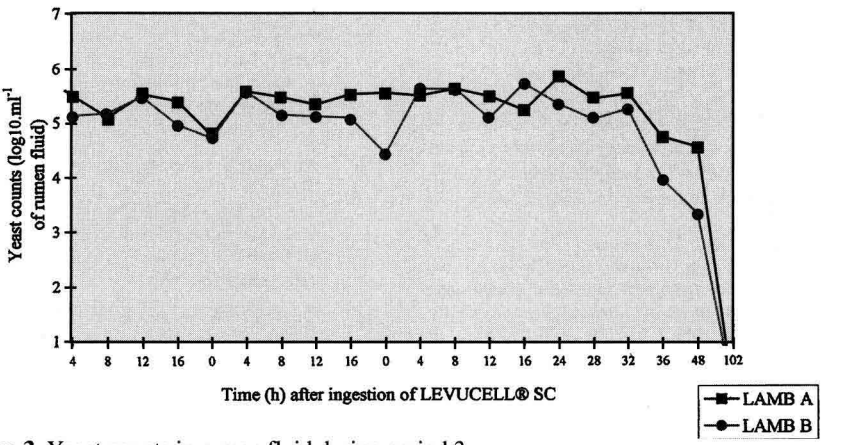


**Figure 3**. Yeast counts in rumen fluid during period 3.

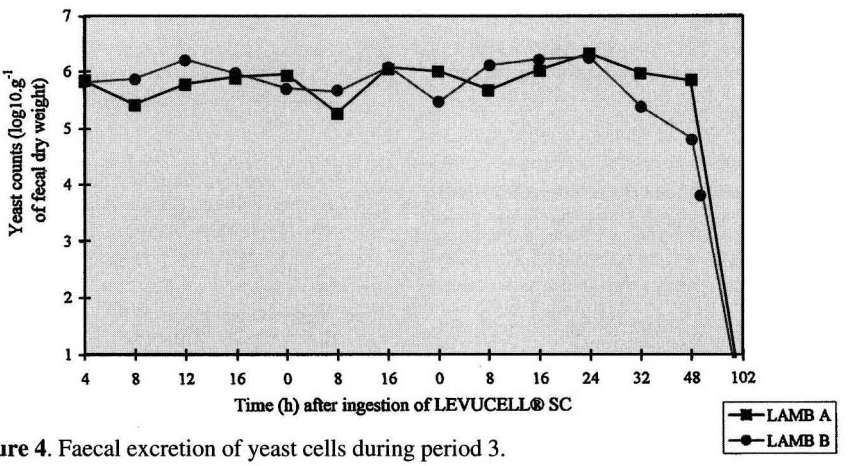


**Figure 4**. Faecal excretion of yeast cells during period 3.

stream of the rumen (duodenum, ileum, etc.). This role, however, remains to be fully elucidated.

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