

TOLERANCE OF RABBITS TO A DIETARY OVERDOSE OF LIVE YEAST (BIOSAF SC 47)

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Abstract - Two groups of 5 does were fed a control diet or the same diet supplemented with 5% Biosaf, from the 14th day of gestation till weaning of their youngs. This dose corresponds with a dietary concentration of $50 \cdot 10^7$ living yeast cells/g or 50 times the usual dose. Twelve weaned rabbits from each group were further fed the experimental diets until 56 d of age. At weaning 2 does of the control group and 3 does of the Biosaf group were euthanized for histopathologic evaluation of lungs, kidneys, liver and spleen. The same number of fatteners was euthanized at 56 d of age. Equal weight development was obtained for the 2 diets during both pregnancy and lactation. Feed intake of Biosaf fed does even exceeded controls. The fattening trial confirmed that an overdose of 50 times the usual one, had no depressing effects on feed intake and weight gain. On the contrary, a tendency to increased performances was observed both before and after weaning when fed the Biosaf diet. Mortality did not occur in does, their offspring and the fatteners of the Biosaf treatment. In the controls, mortality was limited to one suckling young and one fattening rabbit. Histopathology did not reveal typical or more frequent lesions when fed the 5% Biosaf diet, both in does and fattening rabbits.

INTRODUCTION

Since January 1st 1996, live micro-organisms are recognized as feed additives in Europe. In order to protect the animals, humans and the environment, the supplier must describe the identity and prove the efficacy and security as it was already the case for the other feed additives: growth factors, coccidiostats, antibiotics (directive 70/524/CEE).

Live yeast *Saccharomyces cerevisiae* Sc 47 (BIOSAF Sc 47, S.I. Lesaffre, concentration of 10^{10} CFU/g) had been the subject of several tests demonstrating a positive effect on zootechnical and sanitary performances of rabbits (MAERTENS and DE GROOTE, 1992) and other livestock (BALLARINI *et al.*, 1992 ; AERTS *et al.*, 1993 ; FIEMS *et al.*, 1993); this positive effect was more pronounced when animals were placed under suboptimal sanitary and breeding conditions (MAERTENS & DE GROOTE, 1992). The enhancement of the gut microflora balance is the most probable mode of action of the tested live yeast, which could explain the positive zootechnical effects (GEDEK, 1989).

The objective of the present test was to check the absence or presence of any negative effect in case of an overdosage (50 times the recommended dose) both on lactating and fattening rabbits.

MATERIAL AND METHODS

Diets

A standard reproduction diet was prepared at our Institute (CP: 17.5%, DE: 10.5 MJ/kg), using conventional raw materials. Before granulation, the total quantity was separated in two parts. One half was used as control diet. The other half was supplemented with 5% of BIOSAF (SC 47, 10^{10} living yeast cells/g) delivered by the company Lesaffre S.I., in order to obtain a dietary inclusion of $50 \cdot 10^7$ CFU/g (experimental diet). Both diets were cold pelleted (≈ 3.2 mm) at the Institute. The temperature was always lower than 65°C during the granulation process. Diets were fed always *ad libitum* throughout the experiments.

Rabbits and housing

Experiments were performed in October - November 1995. Multiparous does from the female line of the Institute were inseminated with sperm of the male line of the Institute. They were housed on flat-deck cages (45 x 60 cm) in

a heated and ventilated compartment of rabbit house 13 and maintained at $18 \pm 2^\circ\text{C}$. Fourteen days post insemination, the experimental diets were already fed to the does. At parturition, 10 does with 8 young (2 x 5 does) were selected for the experiment.

Does received in total during 44 days their corresponding experimental diet.

At weaning (30 d of age), out of 4 litters (2 from does which have received the control diet and 2 from the experimental diet) 6 rabbits/litter were housed under the same conditions and received further the experimental diets *ad libitum* till the age of 8 weeks. These rabbits were caged per 3, so that 2 x 4 cages were fed the control diet and Biosaf diet, respectively.

Fatteners received already before weaning their experimental diet or in total they received one of both diets during 35 days.

Recordings

Does were weighed at parturition and at 3 and 4 weeks post parturition. Feed intake was determined in the corresponding periods. Young were weighed at parturition and at the age of 21 and 26 days. Feed intake and weight gain of weanlings was recorded weekly till the age of 56 days.

Data were interpreted by an analysis of variance, using the Statgraphics® Package version 5 (1991).

Histopathologic determinations

At the end of the experimental period 5 does (2 controls and 3 from the BIOSAF group) and 5 fatteners (2 controls, 3 Biosaf group) were transferred to the Department of Avian Pathology, Faculty of Veterinary Medicine of the University of Ghent. Ear tagged rabbits were provided blind. After euthanasia, portions of lungs, kidneys, liver and spleen were fixed in 10% buffered formalin (v/v) and processed for histopathology according to standard paraffin embedding and haematoxylin and eosin staining techniques.

RESULTS AND DISCUSSION

Zootechnical performances

Table 1 : Weight and feed intake of does and their young fed during 44 days a control or a 5% BIOSAF supplemented diet (mean \pm SD)

	Controls	BIOSAF 5%	Stat. sign.
Number of does	5	5	-
Mean weight of does (kg)			
- 14 d post insemination	4.81 \pm 0.28	4.60 \pm 0.19	>0.1
- at parturition	4.66 \pm 0.25	4.44 \pm 0.14	>0.1
- 3 weeks post parturition	4.76 \pm 0.42	4.70 \pm 0.16	>0.1
- at weaning	4.65 \pm 0.42	4.64 \pm 0.21	>0.1
Litter size			
- at parturition	9.6 \pm 0.8	9.4 \pm 1.5	>0.1
- after standardization	8.0 \pm 0.0	8.0 \pm 0.0	-
- at weaning	7.8 \pm 0.4	8.0 \pm 0.0	>0.1
Mean weight of young (g)			
- at parturition	57 \pm 6	65 \pm 8	>0.1
- after standardization	59 \pm 7	68 \pm 10	>0.1
- at 3 weeks	375 \pm 15	409 \pm 43	>0.1
- at weaning	565 \pm 67	647 \pm 36	0.03
Mean feed intake of does (g/day) ¹			
- during pregnancy	177 \pm 20	161 \pm 20	>0.1
- between parturition - 3 weeks PP	341 \pm 24	376 \pm 17	>0.1
- between 3 weeks - weaning	457 \pm 58	563 \pm 34	0.02

Weight development and feed intake of does are shown in Table 1. Does fed the diet with 5% of BIOSAF had a comparable weight development during the reproduction cycle. At the end of the experimental period weight in both groups was 4.6 kg.

Mortality of does and in their offspring was not observed in the BIOSAF group, while one young died in the control litters. Already at parturition, offspring of BIOSAF does showed a higher weight (+14%). This difference in favour of the experimental group was even more pronounced at weaning (+14.5%; $P < 0.05$). Does fed the BIOSAF diet showed normal

intake. Their daily intake during the lactation period exceeded that of the control does ($P < 0.05$).

The fattening performances of the young after weaning are summarized in Table 2. Rabbits fed the diet with 5% BIOSAF showed on average better performance than the controls. The weight difference at weaning (67 g) increased to 177 g at 54 d of age ($P < 0.01$) in favour of the BIOSAF group. Because the higher growth rate was

associated with a comparable increase in feed intake, feed conversion was quite the same in both experimental groups. No indication of increased mortality was observed with the overdosage of BIOSAF, because none of the rabbits died or showed clinical symptoms.

Table 2 : Weight gain and feed intake of weanling rabbits fed a control diet or a 5% BIOSAF supplemented diet (mean \pm SD)

	Controls	BIOSAF 5%	Stat. Sign.
Number of rabbits	12	12	-
Mean weight of rabbits (g)			
- at weaning (30 d of age)	780 \pm 78	847 \pm 104	>0.1
- final weight (56 d of age)	1840 \pm 82	2017 \pm 86	0.007
Mean daily weight gain after weaning (g)			
- week 1	42.0 \pm 5.4	48.9 \pm 2.0	0.06
- week 2	36.8 \pm 7.4	38.7 \pm 12.0	>0.1
- week 3	40.9 \pm 4.1	45.1 \pm 2.3	>0.1
Total period (30-56 days)	40.8 \pm 3.5	45.0 \pm 4.2	0.09
Mean daily feed intake (g)			
- week 1	76.9 \pm 9.1	91.4 \pm 9.1	>0.1
- week 2	93.3 \pm 9.4	97.7 \pm 16.4	>0.1
- week 3	116.5 \pm 15.3	132.5 \pm 12.0	>0.1
Total period (30-56 days)	102.5 \pm 6.7	115.7 \pm 3.8	>0.1
Feed conversion			
- week 1	1.85 \pm 0.20	1.88 \pm 0.19	>0.1
- week 2	2.58 \pm 0.27	2.67 \pm 0.46	>0.1
- week 3	2.99 \pm 0.12	2.93 \pm 0.25	>0.1
Total period (30-56 days)	2.52 \pm 0.16	2.59 \pm 0.24	>0.1
Mortality	1/12	0/12	-

Histopathology

Histopathology observations of all rabbits is summarized in Table 3. All rabbits showed minimal lesions typical for the pathology seen in the experimental unit. However, no indication was found of specific or more frequent lesions which could be associated with the feeding during several weeks of the 5% BIOSAF diet.

Table 3 : Histopathologic observations in different organs of controls and Biosaf treated rabbits

	Controls ¹	Biosaf 5% ¹
LIVER:		
- Congestion of the capillaries in the periphery of the lobules	3/4	5/6
- Congestion of the capillaries with occasional hemorrhages	1/4	1/6
- Areas with accumulation of glycogen-like material in the cytoplasm of hepatocytes	4/4	6/6
LUNG:		
- Mild congestion of the interalveolar capillaries	4/4	6/6
- Severe congestion of the interalveolar capillaries	1/4	2/6
- Mild interstitial inflammatory lesions with atelectasis	3/4	6/6
- Interstitial pneumonia	1/4	-
SPLEEN:		
- Congestion of the red pulp	4/4	6/6
- Occasional hemorrhages	1/4	1/6
- Activated periarteriolar lymphoid sheaths with enlarged germinal centers	-	1/6
KIDNEY:		
- Mild congestion of the capillaries	4/4	6/6
- Vacuolated tubular epithelial cells in foci	4/4	6/6
- Occasional focus of lymphoid cell infiltrate	1/4	1/6
- Some hyaline cylinders and calcium deposits in some tubular epithelial cells	-	1/6

¹ 4 controls and 6 Biosaf treated rabbits were observed

Based on these observations the following conclusions could be drawn :

- (i) when used with an overdosage (50 times the recommended dose), no depressing effects were observed both on feed intake and weight developments of does and their progeny.
- (ii) the histopathologic determinations on different organs confirmed the absence of any negative effect on the health status of rabbits fed the diet with an overdosage of BIOSAF. Thus, the experiments demonstrate that *Saccharomyces cerevisiae* Sc 47 live yeast can be considered as an acceptable feed additive regarding its safety and efficacy.

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La tolérance des lapins à un surdosage de levures vivantes (S.c. 47 Biosaf) - Deux lots de 5 femelles ont été alimentés d'un régime témoin ou du même régime supplémenté avec 5% de Biosaf, depuis le 14^{ème} jour de gestation jusqu'au sevrage de leurs lapereaux. Cette dose correspond à une concentration de $50 \cdot 10^7$ cellules vivantes de levure ou à 50 fois la dose recommandée. Douze lapins sevrés de chaque lot ont été par la suite alimentés avec ces mêmes régimes expérimentaux jusqu'à l'âge de 56 jours. Au sevrage, 2 lapines du lot témoin et 3 lapines du lot Biosaf ont été sacrifiées pour l'évaluation histopathologique des poumons, reins, foie et rate. Le même nombre de lapins sevrés à été eutanasié à l'âge de 56 jours.

L'évolution du poids était similaire pour les 2 régimes pendant les périodes de gestation et lactation. La consommation d'aliment des femelles du lot Biosaf était même supérieure à celle du lot témoin. L'essai engraissement confirmait qu'un surdosage de 50 fois la dose recommandée n'a aucune répercussion négative sur la consommation d'aliment et le gain de poids. Aucune mortalité n'est apparue chez les lapines, leurs portées et leurs lapins en engraissement dans le lot Biosaf. Dans le lot témoin, la mortalité était limitée à un lapereau non sevré et à un lapin en engraissement.

Les résultats histopathologiques n'ont pas révélé, chez les lapines ou les lapins d'engraissement alimentés du régime contenant 5% de Biosaf, une fréquence ni une spécificité de lésions plus importante que chez les témoins.
