Alpha-amylase microspheres at low dose in weaning rabbit feed

Scocca S[1], Torre ML[2], Faustini M[1]*, Russo V[1], Villani S[2], Conte U[2], Michelis M[2], Marchese L[2], Torri G[1], Vigo D[1]

*- massimo.faustini@unimi.it;
[1] University of Milan, Milan, Italy;
[2] University of Pavia, Pavia, Italy;

Introduction

Feed-added enzymes have been proposed in farm animals to optimize the digestive performances and enhance food conversion index, mainly in peri-weaning monogastric species. In fact, the suckling animal do not dispose of a full set of digestive enzymes, and precocious weaning can lead to gastrointestinal pathologies as well as a delay of growth (Debray et al., 2003).
The efficacy and the optimal dose of such integration is controversial, since great variability in outcomes has been reported (Close, 1995). This feature could be due to the damaging effect of gastric acidic medium and pepsin proteolytic activity that induces structural and functional alterations, often irreversible, on the oral administered enzymes. The answer to this problem is generally a huge dose of enzyme integration.
Multiparticulate delivery systems are frequently employed to enhance the bioavailability of oral administrated proteins. Gastroresistant microparticles for enzymes targeting the intestinal environment have been recently proposed (Vigo et al., 2004; Scocca et al.,2005): *in vitro* dissolution tests show an efficacious enzyme protection in the gastric milieu, and a fast release in the enteric environment.
The aim of this work was to verify the nutritional efficacy of α-amylase gastroresistant microspheres in rabbits at low dose in weaning food, with respect to a free enzyme integration.

Materials and methods

Weaning food preparation

Commercial food was milled and added of free alpha-amylase (Free), or alpha-amylase microspheres (Micro) at the concentration of 180 UI/kg food. The resulting mix was tableted (punch diameter 10 mm). As control (Ctrl) milled food was tableted without any supplementation. Microspheres were prepared by spraying an aqueous suspension (sodium alginate 2.5%, methacrylic acid copolymer (Eudragit® S100) 5%, lactose 2.25%, alpha-amylase 0.3%) in a physiological solution 0.3M of $CaCl_2$. Microspheres were harvested by filtration, rinsed with physiological solution and then freeze dried. Microparticle composition was: alpha-amylase 3%; calcium alginate 24.8%; Eudragit S®100 49.9%, lactose 22.3%. Microsphere mean diameter was 153±76 μm (Beckman Coulter LS230).

Animal management

At weaning (35th day from birth), 6 animals from each of 9 litters (total 54 rabbits) were randomly selected, and each group of six subjects randomly allocated in one of three feed groups: Free, Micro and Ctrl. The food regimen lasted 2 weeks, from day 35 to day 50 of life. Litter daily food intake was evaluated as the difference between total and unconsumed food amounts. Animals were individually weighed at t_0 and then on day 5, 8, 12, 15 and 50. The overall food conversion index (food intake/ weight increase) was calculated for each group.

Statistical analysis

Individual body weight and food consumption were analysed using two-way ANOVA; the weight increases were evaluated with one-way ANOVA.

Results and Discussion

Food intake, expressed as kg/group/day (figure 1) showed a significant lower consumption in Micro group ($P<0.02$); moreover, this trend is not accompanied by a slower growth rate, since mean weight values are higher for Micro with respect to the other treatments ($P=0.08$) (figure 2).

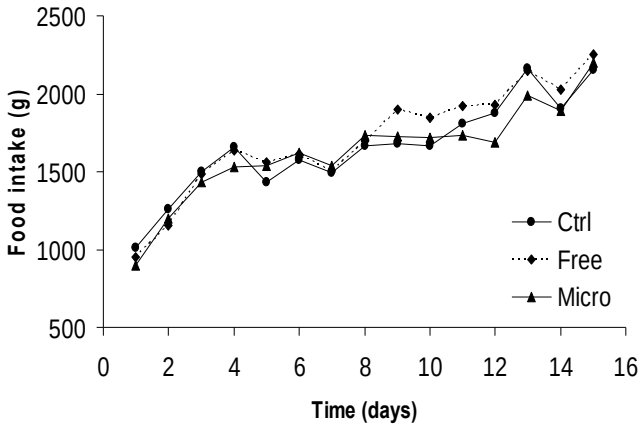

Figure 1: Daily food intake (g per treatement group) for the 15-day treatment period.

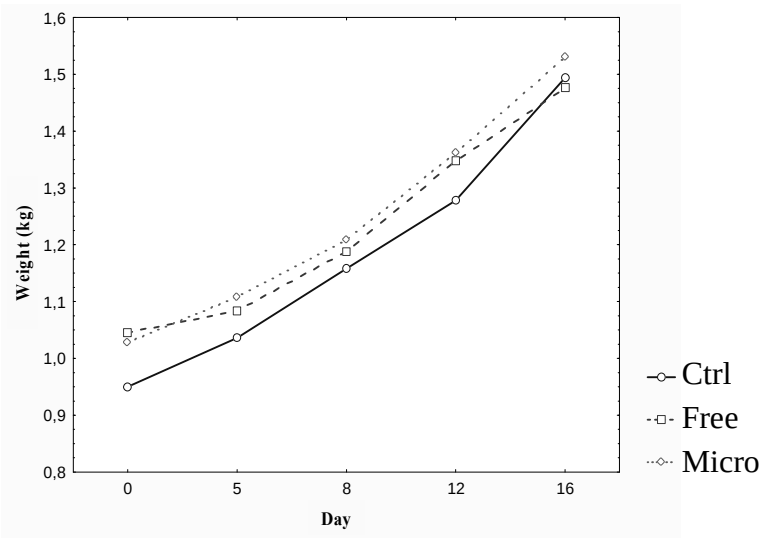

Figure 2. Weight trend for the three treatement groups.

Mean weight increase (figure 3) for the Micro group lies between the other treatments until day 12, but the last period of treatment (12-16 days) overtakes the other two ($P=0.08$). Seen as a whole, a lower food consumption and similar growth rate justify the better food conversion index in Micro

group (Ctrl: 3.07; Free: 3.46; Micro: 2.98).This could be due to a better utilization of food. It is, indeed, recognised that rabbits adapt their food intake in relation to the energy supply in the diet.
A wide variability in weights and growth rates of the animals in the Ctrl and Free groups has been noted: in Micro treated rabbits, smaller variability in growth and a greater homogeneity was detected. This confirms the large inter-subject differences and the individual variability in the development of the digestive capacity. Microencapsulaed alpha-amylase supplementation shows a "smoothing" effect in growth performances.

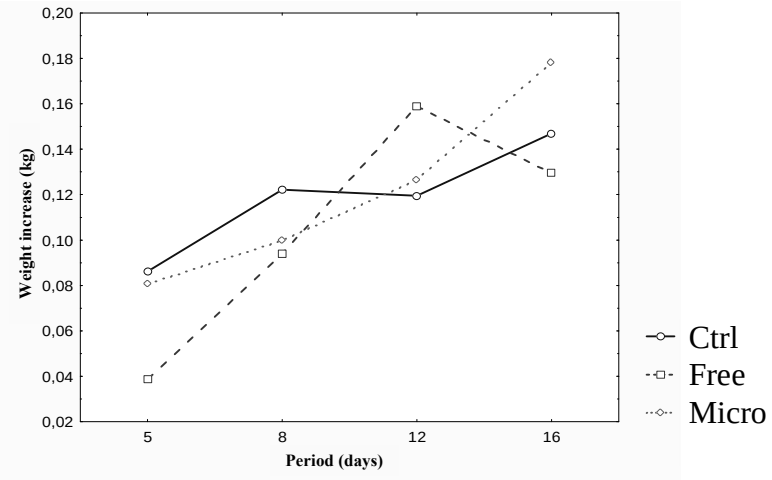

Figure 3. Weight increase for the three groups in the different periods.

Conclusions

A low dosage administration of alpha-amylase loaded in multiparticulate delivery systems leads to a decreasing in the food conversion index, but only a slight increase in growth profile was obtained. This in field trial represents the starting point for determining the minimal efficacious enzyme integration dosage, as well as treatment length to optimize the digestive performances in periweaning monogastric species.

References

Debray L. et al. (2003) Comparative Biochemistry and Physiology, Part A, 135, 443-455.
Close, W. (1995) Enzymes-Nature's Catalyst., special issue, 17-20.
Vigo D. et al. (2004) European Patent Application WO 2005/123034 (29/12/2005)
Scocca S et al. (2005) Proceedings of 15th International Symposium on Microencapsulation, Parma, Italy, September 18-21 2005, 215-216.

Acknowledgements

PRIN 2004; FIRST ex 60% Milan University and Pavia University FAR.