# EFFECT OF ENZYMES ADDITION IN DIET ON PROTEIN AND ENERGY UTILIZATION IN RABBIT

BOLIS S.[1], CASTROVILLI C.[1], RIGONI M.[1], TEDESCO D.[2], LUZI F.[3]

[1] Istituto di Zootecnia Generale - Facoltà di Agraria - Via Celoria, 2 - 20133 Milano - Italy
[2] Istituto di Alimentazione Animale - Facoltà di Medicina Veterinaria - Via Celoria, 10 - 20133 Milano - Italy
[3] Istituto di Zootecnica - Facoltà di Medicina Veterinaria - Via Celoria, 10 - 20133 Milano - Italy

**Abstract** - The effect of enzymes addition (glycosidase and protease) in rabbit diets on nutrients digestibility, nitrogen balance and energy utilization was studied.
9 adult rabbits were fed a commercial diet containing 15.5% of crude protein and 23% of crude fibre (basal diet, diet A). Then, the same animals were fed the same diet, with the addition (per kg as fed) of 100 IU of cellulase (diet B), or 300 IU of cellulase (diet C), or 0.25 g of a pool of glycosidase and protease (diet D) or 0.75 g of the same pool (diet E) or with mean levels of cellulase and pool (diet F).
From the obtained results, the digestibility increased significantly for NDF (+5%) and for ADF (+13%), with mean levels of cellulase and pool (diet F). The digestibility of the other nutrients was never improved by enzymes addition. Nitrogen balance was always lower in animals fed enzymes, with negative values for diets D, E and F; energy digestibility and metabolism activity in animals fed diets with the pool of enzymes were significantly lower than in the control group.
The negative effect of enzymes addition could depend on the change in rabbit digestive physiology and particularly in caecotrophy, that allows the reutilization of the enteric fermentation products that supplies energy and proteins.

## INTRODUCTION

The double digestive process by which normal feed intake is followed by caecotrophy, makes the rabbit completely different from other herbivores.
In fact, fibre utilization in rabbits is not comparable to ruminants, and neither to monogastrics, because the fibrous parts are more quickly eliminated (RIGONI *et al.*, 1993). In spite of this, fibre plays an indispensable role: it favours intestinal motility, supplies a *substratum* adverse to the development of *Clostridium* and *Coli*, and facilitates the reutilization of ammonium (De BLAS, 1992; GIDENNE, 1992, 1994; GIDENNE *et al.*, 1991; MORRISSE *et al.*, 1985; PEETERS *et al.*, 1994).
A low fibre level, normally concomitant with an excess of starch, causes constipation and enteritis by abnormal selection towards the caecum of thinly dispersed substances; an excess of fibre in the diet increases *digesta* flow, reduces caecal fermentation, VFA synthesis and therefore reduces energy availability (BJÖRNHAG, 1972; LAPLACE, 1978; Lebas and LAPLACE, 1977 a, b; MORRISSE and Cheeke, 1985-1986; RUCKEBUSCH *et al.*, 1981).
Since fibre acts through both miomotory activity of the VFA and the mechanical action of lest, it would seem a good idea to use feeds with a high fibre level and to increase their digestive utilization with enzymes. There has been evidence for this topic over 25 years (AUXILIA, 1983; BONSEMBIANTE and PARIGI-BINI, 1970; CALAFAT FRAU and PUCHAL SABARTES, 1993; GIPPERT and CSIKVARY, 1988; MAKKAR and SINGH, 1987 a, b; YU and TSEN, 1991, 1993), but considering both zootechnical performance and nutrients digestibility, the problem is still complex.

## MATERIAL AND METHODS

### Animal housing and diets

The trial was performed on 9 adult New Zealand White male rabbits having an average weight of 4 kg, individually housed in metabolism cages in an air conditioned recovery. A commercial diet for adult rabbits (basal diet) was given. Enzymes were added to the basal diet, obtaining other five diets as follows:

| A | B | C | D | E | F |
|---|---|---|---|---|---|
| *basal diet* | *basal diet* + 100 IU/kg cellulase * | *basal diet* + 300 IU/kg cellulase | *basal diet* + 0.25g/kg pool ** | *basal diet* + 0.75g/kg pool | *basal diet* + 200 IU/kg cellulase + 0.50g/kg pool |

* Cellulase: from a fermentative process operated by *Aspergillus*
** Enzymes pool: obtained by the aerobic fermentation of *Trichoderma longibrachiatum* and containing: xylanase, beta-glucanase, beta-glucosidase, pentosanase, cellulase, amyloglucosidase, acid protease, neutral protease. The enzymatic activity was evaluated in beta-xylanase and corresponds to 20,000 IU/g.

**Table 1: Basal diet**

| | |
|---|---|
| Ingredients (g/kg) | |
| Dehydrated grass | 400 |
| Wheat hard and soft middling | 210 |
| Maize | 180 |
| Soybean meal | 180 |
| Vitamins and minerals | 30 |
| Chemical analysis (g/kg DM, unless otherwise stated) | |
| Organic matter | 908 |
| Crude protein (Nx6.25) | 155 |
| Crude fibre | 234 |
| NDF | 525 |
| ADF | 260 |
| ADL | 54 |
| Ether extract | 28 |
| Ashes | 91 |
| Gross energy (MJ/kg DM) | 18.3 |

Ingredients and chemical composition of basal diet are shown in table 1.

All diets were pelleted at low temperature in order not to inactivate enzymes.
Animals behaviour and health conditions were daily watched; the presence of soft faeces in the collection tray was checked.

### Experimental procedure

After an adaptation period of 2 weeks to environmental condition, animals were fed the experimental diet *ad libitum* for 5 days. During this period feed intake was daily checked. In the following 11 days they were fed 90% of the quantity ingested during the first period and . faeces and urine were collected for the analysis in the last 6 days.
This sequence was repeated as many times as there were the diets to be tested, in the following order: diet A, B, C, D, E, F.
Faeces and urine of each animal were collected separately every day at 9 a.m. and the diet administered. 10% Sulfuric acid was added to urine to prevent ammonia losses. The individual *excreta* were kept at -20 °C until analysis.

### Chemical analysis

Samples of feeds and faeces were analysed to determine dry matter (PEREZ *et al.*, 1995), ashes using the A.O.A.C. method (1984), crude fibre using WEENDE method, fibrous fractions with Van SOEST and Robertson (1979) sequential method, energy content with an adiabatic calorimeter IKA C 400-Werk of Janke & Kunkel KG.
Samples of feeds, faeces and urine were analysed to determine nitrogen content using KJELDAHL method.
The metabolizable energy was estimated through regression with the following equation:

$$ME = DE - 37\ Nu - 6.6$$ (Bolis *et al.*, personal communication)

where: ME = kJ/day; DE = kJ/day; Nu = nitrogen content in urine (g/day).

### Calculations and statistical analysis

Apparent digestibility was calculated as the difference between intake and excretion, expressed as a percentage of the ingested quantity.
Retained nitrogen was determined as the difference in grams between N introduced with feed and N excreted with faeces and urine.
Body weight was recorded at the beginning and at the end of every trial period.
As feed intake was very different between the animals, the statistical analysis was carried out using the intake of nutrients as covariate. The recorded data were analysed by analysis of variance and covariance with the GLM procedure of SAS/STAT package (1992).
The general model was:

$$Y_{ijk} = \mu + D_i + A_j + b_1x_1 + \Sigma_{ijk}$$

where:

$Y_{ijk}$ = apparent digestibility of nutrients, metabolizable energy, absorbed N, retained N;
$\mu$ = overall mean;
$D_i$ = effect of the $i^{th}$ diet (i=1,...,6);
$A_j$ = effect of the $j^{th}$ animal (j=1,...,9);
$x_1$ = intake of nutrients;
$\Sigma_{ijk}$ = random error term with zero mean and variance.

Significant differences among means were determined by nonorthogonal contrasts (SAS, 1992).

## RESULTS

The analysis of covariance for effects of diet, animal and intake of nutrients is in table 2. Apparent digestibility of crude fibre was not different among the control group (diet A) and the groups with cellulase (diet B and C). In the group with both cellulase and pool (diet F) a higher digestibility of ADF (+13%) and NDF (+5%) was found (table 3).

**Table 2: The analysis of covariance of apparent digestibility, metabolizable energy, absorbed N and retained N.**

| | | Mean Squares | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Sources of variance | df | CP | CF | ADF | NDF | DE | ME | ABS. N | RET. N |
| diet | 5 | 11.178 *** | 44.872* | 84.898** | 35.869* | 154.440*** | 7.932*** | .018*** | 6.366*** |
| animal | 8 | 10.981 ** | 90.588 * | 87.260** | 67.175** | 28.750* | .988** | .017** | .144 |
| intake of nutrients | 1 | .175 | 1.648 | 1.807 | 1.108 | .380 | .018 | 2.746*** | .573*** |
| error | 39 | 16.567 | 140.35 | 137.752 | 103.825 | 50.934 | 1.4613 | .021 | 1.245 |

* $P \leq .05$; ** $P \leq .01$; *** $P \leq .001$.

**Table 3 : Apparent digestibility, energy utilization and nitrogen balance values[1]**

| | CP (%) | | CF (%) | | NDF (%) | | ADF (%) | | DE (%) | | ME (kJ/g DM) | | ABS. N (g/*day*) | | RET. N (g/*day*) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DIET | $\bar{y}$ | *SE* | $\bar{y}$ | *SE* | $\bar{y}$ | *SE* | $\bar{y}$ | *SE* | $\bar{y}$ | *SE* | $\bar{y}$ | *SE* | $\bar{y}$ | *SE* | $\bar{y}$ | *SE* |
| A | 71.6$^{a}$ | *.222* | 16.2$^{ab}$ | *.692* | 28.5$^{a}$ | *.575* | 18.2$^{a}$ | *.683* | 53.5$^{a}$ | *.385* | 9.24$^{a}$ | *.065* | 2.884$_{a}$ | *.008* | $_{a}$.746 | *.061* |
| B | 71.2$^{ab}$ | *.218* | 15.9$^{ac}$ | *.667* | 27.7$^{a}$ | *.558* | 17.4$^{a}$ | *.659* | 53.9$^{a}$ | *.381* | 9.23$^{a}$ | *.064* | 2.865$_{ab}$ | *.008* | $_{b}$.236 | *.060* |
| C | 70.7$_{bc}$ | *.217* | 16.5$^{ab}$ | *.646* | 27.7$^{a}$ | *.547* | 17.3$^{a}$ | *.639* | 52.9$^{a}$ | *.383* | 9.03$^{c}$ | *.065* | 2.847$_{bc}$ | *.008* | $_{b}$.195 | *.060* |
| D | 70.5$^{c}$ | *.221* | 15.4$^{a}$ | *.691* | 28.9$_{ab}$ | *.574* | 18.4$^{a}$ | *.682* | 49.9$^{b}$ | *.384* | 8.40$^{b}$ | *.065* | 2.836$_{c}$ | *.008* | -.172$_{c}$ | *.061* |
| E | 71.5$^{a}$ | *.219* | 17.5$^{bc}$ | *.635* | 29.1$_{ab}$ | *.543* | 17.0$^{a}$ | *.629* | 50.1$^{b}$ | *.388* | 8.39$^{b}$ | *.066* | 2.876$_{a}$ | *.008* | -.185$_{c}$ | *.060* |
| F | 71.7$^{a}$ | *.222* | 18.2$^{b}$ | *.694* | 30.3$^{b}$ | *.576* | 21.0$^{b}$ | *.685* | 50.3$^{b}$ | *.384* | 8.45$^{b}$ | *.065* | 2.886$_{a}$ | *.008* | -.305$_{c}$ | *.061* |

[1]Least squares means and standard error.
Means with the same letter are not significantly different ($P \leq .05$).

Apparent digestibility of proteins was significantly lower in the groups with a higher dose of cellulase (diet C) and a lower dose of pool (diet D).

Nitrogen retention was always lower in treated groups, with negative values in the animals treated with the addition of the lower dose of pool (diet D) and still more with higher dose of pool (diet E) and both pool and cellulase (diet F).

Digestible and metabolizable energies were always significantly lower in groups with pool addition (diet D, E and F), as was found for dry matter and organic matter apparent digestibility (Castrovilli *et al.*, 1995).
The weight of animals fed control diet was almost constant. Slight losses were observed in groups with higher dose of pool. Soft faeces were found sometimes in important quantities in the groups fed diets with the higher cellulase addition (diet C and F).

## DISCUSSION

The results of this trial showed that enzymes addition in rabbit diet didn't cause any significant improvement in energy and protein utilization. This was not clear for the following reasons:
- diets were pelleted at low temperature and immediately used;
- rabbit gastric pH may have only slightly reduced the activity of enzymes, for the characteristics of enzymes of fungal origin (Mc NAB, 1993) and also for the treatments of manufacturing industry, but not specifically stated.

Furthermore some results regarding nitrogen metabolism appeared unusual: in fact we observed a significantly minor retention in all groups fed with enzymes. Protein digestibility was significantly lower than in other groups only with addition of higher dose of cellulase (diet C) and with lower dose of pool (diet D) as for absorbed nitrogen.

The use of adult animals and a high fibre diet, should have increased the effect of the overall enzymatic activity, with apparent higher digestibility and greater nutrients utilization.
Useful for providing clues about the causes of these results, so different from those expected, was the remarks on soft faeces presence in *excreta*. It may be an interaction between the exogenous enzymes activity and the digestive physiology of rabbit.
Crude fibre digestibility in the group with pool addition at low dose (diet D) was significantly lower than with higher doses of pool (diet E and F). ADF and NDF digestibilities showed differences in favour of the group with both pool and cellulase (diet F). This means that neither pool alone nor cellulase alone can improve the digestibility of fibre and its fractions.
However this effect did not significantly influence energy utilization since all groups with pool showed a digestible energy lower than the control group and the groups with cellulase alone (table 3).
It is possible to suppose that added enzymes acted in advance from their usual site, thus depriving the large intestine flora of *substratum* for its development and activity. This arguments has already been proposed by KIRCHGESSNER *et al.* (1989) for pigs and by SALISH *et al.* (1991) for chickens.
Moreover cellulase, acting on a single principle, has not changed the bacteria activity because there were other carbohydrates as *substratum*. Probably enzymes pool influenced nutrients utilization also affecting the endogenous enzymes. In this case the pool acted negatively at low doses because it was not sufficient to achieve the normal digestion alone, while at higher doses the added enzymes substituted the activity of the endogenous ones.
The concomitant weight decrease further confirms a nitrogen loss due to a poor re-organisation of catabolites in intestinal lumen and/or to the lack of their ingestion with caecotrophy. Considering that soft faeces have an aminoacids content of about three times more than hard faeces (FERRANDO *et al.*, 1970), it seems that addition of enzymes, which could change the microbial activity of rabbit, causes lower protein efficiency in a way quite different to that observed by Mc NAB (1993) in chickens.

**Acknowledgments** - This research was supported by MURST 60% funds.

## REFERENCES

A.O.A.C. (1984). Official methods of analysis. 14th ed. Washington, DC: Association of Official Analytical Chemists.
AUXILIA M.T. (1983). Integrazione enzimatica delle diete per conigli da carne. *Riv.Coniglic.*, **20(7)**, 29-32.
BJÖRNHAG G. (1972). Separation and delay of contents in the rabbit colon. *Swedish J.Agric. Res.*, **2**, 125-136.
BONSEMBIANTE M., PARIGI-BINI R. (1970). L'integrazione enzimatica delle diete per conigli: effetti sull'accrescimento, sull'utilizzazione degli alimenti e sulla digeribilità delle sostanze nutritive e dell'energia. *Riv. Zoot.*, **43**, 587-595.

CALAFAT FRAU F,. PUCHAL SABARTES F. (1993). Utilizacion de complejos enzimaticos en cebo de conejos. *XVIII Symp. Cunic. Granollers*, 71-73.

CASTROVILLI C., TEDESCO D., BOLIS S,. RIGONI M. (1995). Effetto di addizioni enzimatiche sulla digeribilità della sostanza organica e dell'energia nel coniglio. *Atti XI Congr. Naz. ASPA*, Grado, 119-120.

DE BLAS C. (1992). The roles of fibre in rabbit nutrition. *J. Appl. Rabbit Res.*, 15, 1329- 1343.

FERRANDO R., WOLTER R., VITAT J.C,. MEGARD J.P. (1970). Teneur en acides aminés des deux catégories de fèces du lapin: caecotrophes et fèces dures, *C. R. Acad. Sci.*, Paris, **270**, *2202-2204*.

GIDENNE T. (1992). Effect of fibre level, particle size and adaptation period on digestibility and rate of passage as measured at the ileum and in the faeces in the adult rabbit. *Br. J. Nutr.*, **67**, 133-146.

GIDENNE T. (1994). Effects d'une réduction de la teneur en fibres alimentaires sur le transit digestif du lapin. Comparison et validation de modèles d'ajustement des cinétiques d'excrétion fécale des marqueurs. *Reprod. Nutr. Dev.*, **34**, 295-306.

GIDENNE T., SCALABRINI F,. MARCHAIS C. (1991). Adaptation digestive du lapin à la teneur en constituants parétaux du régime. *Ann. Zootech.*, **40**, 79-84.

GIPPERT T,. CSIKVARY L. (1988). Use of the cellulase enzymes « Phylacell » in rabbit feeding. *VI Arbeit. Peltz. Kaninch. Heimt. Produk. Krankeit.*, Celle, 147-157.

KIRCHGESSNER M., KREUZER M,. FOX F.X. (1989). Microbial turnover in the hindgut of sow as affected by intracecal infusion of large amounts of different pure substrates. *Landw. Forschg.*, **42**, 72-92.

LAPLACE J.P. (1978). Le transit digestif chez les monogastriques. III. - Comportement (prise de nourriture - caecotrophie), motricité et transit digestifs, et pathogénie des diarrhées chez le Lapin. *Ann. Zootech.*, **37(2)**, 225-265.

LEBAS F,. LAPLACE J.P. (1977 a). Le transit digestif chez le Lapin. 6) Influence de la granulation des aliments. *Ann. Zootech.*, **26**, 83-91.

LEBAS F,. LAPLACE J.P. (1977b). Le transit digestif chez le Lapin. 8) Influence de la source de cellulose. *Ann. Zootech.*, **26**, 575-584.

MAKKAR H.P.S,. SINGH B. (1987 a). Comparative enzymatic profiles of rabbit cecum and bovine rumen contents. *J. Appl. Rabbit Res.*, **10(4)**, 172-174.

MAKKAR H.P.S,. SINGH B. (1987 b). Distribution of some hydrolytic and ammonia assimilation enzymes in gastrointestinal tract of rabbits. *Nutr. Rep. Int.*, **36(4)**, 867-877.

MCNAB J.M. (1993). Optimal use of enzymes for special ingredients. *Proc. 1st Symp. on Enzymes in Animal Nutrition*, Kartause Ittingen, 97-124.

MORISSE J.P., BOILLETOT E,. MAURICE R. (1985). Alimentation et modifications du milieu intestinal chez le lapin (AGV, $NH_3$, pH, flore). *Rec. Med. Vet.*, **161**, 443-449.

MORISSE J.P,. CHEEKE T.R. (1985-1986). Alimentation et milieu intestinal. Echange d'informations. *Cuni Sci.*, **3(2)**, 29-34.

PEETERS J.E., ORSENIGO R., MAERTENS L., COLIN M. (1994). Promoting effect of dietary beet pulp on iotaenterotoxaemia (*C. spiriforme*) in rabbits at weaning. *VI Journ. Rec. Cunic. en France*, La Rochelle, 105-112.

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., CARABANO R., FRAGA M.J., RAMOS M.A., CERVERA C., BLAS E., FERNANDEZ J., FALCAO CUNHA L., BENGALA FREIRE J. (1995). European reference method for in vivo determination of diet digestibility in rabbits. *World Rabbit Sci.*, **3(1)**, 41-43.

RIGONI M., CASTROVILLI C., CICOGNA M. (1993). Confronto tra cavie e conigli nell'utilizzazione digestiva dei principi alimentari e dell'energia. *Atti X Congr. Naz. ASPA*, Bologna, 709-714.

RUCKEBUSH Y., BUENO L,. FIORAMONTI J. (1981). Constituants alimentaires et motricité digestive. *Reprod. Nutr. Develop*, **21 5B)**, 749-771.

SALIH M.E., CLASSEN H.L,. CAMPBELL G.L. (1991). Response of chickens fed on hull-less barley to beta-glucanase at different ages. *Anim. Feed Sci. Technol.*, **33**, 139-149.

SAS (1992). SAS Application Guide. Ver. 6.08, SAS Inst.Inc., Cary NC, USA.

VAN SOEST P.J,. ROBERTSON J. (1979). Systems of analysis evaluating fibrous feed. Workshop Ottawa, Canada, Dept. Anim. Sci. Cornell Univ., Ithaca, NY 14853.

YU B,. TSEN H.Y. (1991). Studies on digestive enzymes of domestic rabbits. 1 Carbohydrases. *J. Chin. Soc. Anim. Sci.*, **20(2)**, 145-158.

YU B,. TSEN H.Y. (1993). An in vitro assessment of several enzymes for the supplementation of rabbit diets. *Anim. Feed Sci. Technol.*, **40**, 309-320.

**Effetto dell'addizione di enzimi nella dieta sull'utilizzazione proteica ed energetica nel coni glio -**
E' stato studiato l'effetto dell'aggiunta nel mangime di enzimi (glicosidasi e proteasi) sulla digeribilità dei principi alimentari, sul bilancio azotato e sull'utilizzazione energetica nei conigli.

Nove conigli adulti sono stati alimentati con una dieta commerciale contenente 15,5% di proteine grezze e 23% di fibra grezza (dieta di riferimento - dieta A); successivamente gli stessi animali hanno ricevuto lo stesso alimento, ma addizionato, per kg di alimento tal quale, con 100 UI di cellulasi (dieta B), o con 300 UI di cellulasi (dieta C), o con 0,25 g di un pool di glicosidasi e cellulasi (dieta D) o con 0,75 g di pool degli stessi (dieta E) o con valori intermedi di cellulasi e di pool (dieta F).

*Dai risultati* è emerso un aumento significativo, rispetto al gruppo di controllo, della digeribilità dell'NDF (+5%) e dell' ADF (+13%) nel caso di aggiunta di cellulasi e pool a dosi interemedie (dieta F). La digeribilità degli altri principi non è mai stata migliorata dall'aggiunta di enzimi.

Il bilancio azotato è sempre risultato significativamente inferiore negli animali alimentati con enzimi, con valori negativi nei gruppi D, E ed F; è sempre risultata significativamente inferiore al controllo l'utilizzazione energetica, sia digestiva che metabolica, negli animali alimentati con diete addizionate con il pool di enzimi.

La risposta complessivamente negativa dell'aggiunta di enzimi alla dieta per conigli è da ricercarsi nella alterazione della normale fisiologia del digerente che, con la ciecotrofia, probabilmente compromessa dall'attività degli enzimi esogeni, riutilizza i prodotti di fermentazione enterica, sia energetica che proteica.