

## EFFICACY OF AMYLOFEED® IN GROWING RABBIT DIETS

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**ABSTRACT:** The efficacy of Amylofeed® (an amylase,  $\beta$ -glucanase and  $\beta$ -xylanase enzyme complex) was evaluated on growing rabbit performance. Three isonutritive diets (medicated with bacitracine) were formulated corresponding to the levels of Amylofeed®: 0 (diet A), 400 (diet B) and 500 (diet C) ppm. Four hundred and fifty six and four hundred and eighty New Zealand White x Californian rabbits weaned at 35 days of age, weighing 910 and 960 g as average, respectively, were fed with the experimental diets for three weeks after weaning in two experiments. From 56 to 65 days of age animals were fed with a common non-medicated diet. In the first assay, the mortality during the experimental period was very low (2.6%) and treatments had no effect on any of the performance traits studied. In the second assay a higher incidence of diarrhoea and associated mortality occurred with respect to the first. In these circumstances, the supplementation of feed with 400 or 500 ppm of Amylofeed® significantly ( $P=0.03$ ) reduced mortality from 27.0 to 19.7 and 14.8%, respectively. An improvement of feed efficiency (by 16%;  $P=0.03$ ) was observed in animals fed with the two enzyme supplemented diets in the overall period (35 to 65 of age) with respect to the animals fed with the control diet. It is concluded that enzyme supplementation has beneficial effects on mortality, feed efficiency and growth of fattening rabbits when the incidence of intestinal disorders and mortality in the control group are of relevance.

**Key words:** enzyme supplementation, growing rabbits, performance.

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## INTRODUCTION

Enzymes are proteins which are able to catalyse specific chemical reactions with a minimum energy waste. The use of exogenous enzymes in animal nutrition has increased in the last years as alternative natural products that might improve animal productivities and reduce the morbidity and the mortality in intensive farms.

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The main objective of the enzyme supplementation is to complement the endogenous enzymatic capability of the animal and to increase the nutritive value of the feed. Their use has special interest in young animals, which do not have a completely mature digestive tract, in diets with antinutritive factors resulting in poorer digestion and feed utilisation, and to increase the availability of some nutrients. Recent studies in poultry (BEDFORD, 2000; CHESSON and STEWARD, 2001) have demonstrated that enzyme addition improves the feed utilization in the small intestine, changing the substrate that arrives to the hindgut. This change affects the development of the microbiota population in this area and seems to favour the health status of the animals.

Several works have already investigated the efficacy of digestive enzymes in 35 to 49 days old rabbits (FERNANDEZ *et al.*, 1996; REMOIS *et al.*, 1996; SEQUEIRA and VILLAMIDE, 1999). The results of these studies have shown little or no effects of enzyme supplementation on fattening rabbit performance. However in younger animals, from 25 to 39 days of age, addition of enzymes has improved daily gain (by 3.1%), feed efficiency (by 3.7%) and the mortality in the starter (by 53%) and in the whole fattening period (by 50%) (GUTIERREZ *et al.*, 2002).

The aim of the present investigation was to determine the effect of dietary supplementation with 400 or 500 ppm of Amylofeed® (an enzyme complex containing  $\alpha$ -amylase,  $\beta$ -glucanase and  $\beta$ -xylanase) on the productive parameters of growing rabbits.

## MATERIALS AND METHODS

### Diets

A basal diet (A) was formulated according to the nutrient recommendations of DE BLAS and MATEOS (1998). The ingredient composition of the basal diet is shown in Table 1. Another two diets were formulated by adding 400 (B) and 500 ppm (C) of Amylofeed® to the basal diet. Amylofeed® is an enzyme complex containing 3100 U/g of  $\alpha$ -amylase (EC 3.2.1.1), 275 U/g of  $\beta$ -glucanase (EC 3.2.1.6) and 400 U/g of

**Table 1:** Ingredient composition (%) of the basal diet (diet A).

Wheat	12.00
Barley	12.25
Wheat bran	23.00
Alfalfa hay	15.00
Sunflower meal	13.60
Cereal straw	7.70
Grape seed meal	3.30
Soybean hulls	3.00
Palmkernel meal	2.48
Barley rootless	3.00
Soybean meal, 44% CP	1.00
Molasses	1.00
L-Lysine HCl, 0.78% pure	0.16
DL-Methionine, 0.99% pure	0.03
L-Threonine	0.03
Calcium carbonate	1.40
Salt	0.50
Choline-75%	0.03
Vitamin and mineral premix <sup>1</sup>	0.17
Robenidine premix	0.10
Antibiotic <sup>2</sup>	0.20

<sup>1</sup> Vitamin and mineral premix supplied for 1 kg of complete diet: Vitamin A: 11.390 IU; Vitamin D<sub>3</sub>: 1.360 IU; Vitamin E: 47.6 IU; Vitamin K<sub>3</sub>: 1.7 mg; Thiamine: 1.7 mg; Riboflavin: 4.3 mg; Pantothenic acid: 13.6 mg; Pyridoxine: 1.7 mg; Biotin: 85 µg; Folic acid: 850 µg; Vitamin B12: 13.6 µg; Fe: 47.6 mg; Cu: 17 mg; Zn: 68 mg; Mn: 22.7 mg; Co: 595 µg; Se: 140 µg; I: 1.2 mg.

<sup>2</sup> Bacipremix 50 (bacitracin 5%): 100 ppm.

$\beta$ -xylanase (EC 3.2.1.8). These diets were supplemented with bacitracin because of the present incidence of mucoid enteropathy. Feeds were pelleted at a temperature between 50 and 60°C. The chemical composition of the diets is shown in Table 2. Another non-medicated diet and without enzyme addition was formulated and offered from 56 to 65 days of the fattening period. The principal ingredients of this diet were: wheat bran (23.12%), alfalfa hay (18%), sunflower meal (12.7%), wheat (10%), cereal straw (7.7%), soybean hulls (6%), distiller's grains and solubles dehydrated from barley (5%), grape seed meal (3.3%), palmkernel meal (3%), molasses (2.5%), gluten feed (2%), lard (1.98%), citrus pulp (1.5%) and soybean meal, 44% (1.2%).

### **Animals**

Four hundred and fifty six and four hundred and eighty New Zealand White  $\times$  Californian rabbits weaned at 35 days of age, weighing 910 and 960 g as average, respectively, were fed *ad libitum* with the experimental diets for three weeks after weaning in two experiments. Rabbits were caged in groups of eight rabbits resulting in 19 and 20 replicates per treatment in experiments 1 and 2, respectively, and results were calculated per cage. From 56 to 65 days of age animals were fed with a common non-medicated diet. Feed intake and live weight of the rabbits at 56 day of age and at the end of the experimental period (65 days of age) were recorded. Morbidity and mortality were controlled daily throughout the experiment. Feed efficiency was calculated as the ratio between weight gain and total feed consumption per cage. No corrections were made because of mortality, so that this trait takes into account the actual negative effect of mortality on feed efficiency.

### **Housing**

Animals were housed in collective flat-deck cages measuring 41 x 90 x 35 cm until they reached the 65 day slaughter age. Building heating systems and forced ventilation maintained the temperature between 12 and 20° C in the two experiments. A cycle of 12 h light and 12 h dark was used throughout the experiments. Animals were handled according to the principles for the care of animals in experimentation published by the SPANISH ROYAL DECREE 223/88.

## Analytical Methods

Chemical analysis of diets was made using the sequential procedure of VAN SOEST *et al.* (1991) for neutral detergent fibre, acid detergent fibre and acid detergent lignin. Procedures of the AOAC (2000) were used for starch, dry matter, crude fibre, ether extract, ash, calcium, total phosphorus and crude protein.

## Statistical Analysis

Data (averaged by cage) of the two experiments were analysed as a completely randomised design with weaning weight as linear covariate and type of diet as the main source of variation by using the General Linear Model (GLM) procedure of SAS (1990). The results are presented as least-square means. Contrasts were used for mean comparisons. Normality was assumed for mortality because of the large number of rabbits controlled per treatment.

**Table 2:** Chemical composition of the experimental diets (%).

	Diets		
	A	B	C
Amylofeed® (ppm)	0	400	500
Dry matter	88.3	88.3	88.8
Crude protein	15.6	15.4	15.9
Crude fibre	15.1	14.5	15.0
Neutral detergent fibre	36.0	35.0	37.7
Acid detergent fibre	19.2	18.1	19.7
Acid detergent lignin	5.10	5.00	5.80
Ether extract	2.00	2.00	2.00
Starch	21.1	20.8	20.7
Ash	7.90	7.80	7.70
Calcium	1.20	1.20	1.20
Total phosphorus	0.49	0.45	0.45

## RESULTS AND DISCUSSION

Results from the first experiment indicate that mortality during the first period (35 to 56 days of age) was low (2.6%), and especially in the control group (1.9%). The use of Amylofeed® did not affect any of the performance traits studied. Rabbits fed diets supplemented with enzymes (diets B and C) showed similar ( $P>0.05$ ) growth rate (35.2 vs 35.1 g/d), feed intake (103.5 vs 104.0 g/d) and feed efficiency (0.341 vs 0.342 g/g) to the control diet A. The results from 56 days of age until the end of the experiment are not shown due to the occurrence of a higher than usual mortality (myxomatosis) not related to experimental treatments.

The results of the second experiment are shown in Table 3. In this assay a higher incidence of diarrhoea and associated mortality (20.5% as average) occurred compared with the previous experiment. In these circumstances, the supplementation of feed with 400 or 500 ppm of Amylofeed® reduced mortality rate ( $P=0.03$ ) from 27.0 to 19.7 and 14.8%, respectively. In comparison with the control, feed intake was lower for rabbits fed the diet with 400 ppm enzyme supplementation, both in the first three weeks after weaning ( $P=0.04$ ) and in the whole fattening period ( $P=0.07$ ). The use of Amylofeed® increased daily gain of rabbits due to the reduced mortality and morbidity as well as improved feed conversion associated with the enzyme supplementation. Rabbits fed diet containing 500 ppm of Amylofeed® grew faster than controls over the 35 to 56 days period ( $P=0.01$ ) although the difference decreased thereafter. The final effect was an improvement of feed efficiency (by 16%) in animals fed with the two supplemented diets in the overall period (35 to 65 of age) with respect to the animals fed with the control diet.

These results are globally similar to those observed by GUTIERREZ *et al.* (2002) who used an enzyme complex that contained  $\alpha$ -amylase,  $\beta$ -glucanase and  $\beta$ -xylanase. In their trial, mortality in the control group was 13.9% which decreased to 6.9% when the diets were supplemented with enzymes. The reduction of the mortality during the fattening period with enzyme supplementation was associated with a decrease in ileal starch concentration that might limit the growth of the pathogenic

**Table 3:** Effect of Amylofeed® supplementation on fattening performance in experiment 2.

	Diets			SEM	P-value	Contrast		
	A	B	C			A vs B	A vs C	B vs C
	0	400	500					
<i>First period (35 to 56 days)</i>								
Amylofeed® supplementation (ppm)	0	400	500					
Average daily gain (g)	31.8	34.2	36.3	1.20	0.02	NS	0.01	NS
Average daily feed intake (g) <sup>1</sup>	136	121	126	5.06	0.10	0.04	NS	NS
Feed efficiency (g gain/g intake)	0.239	0.290	0.293	0.013	0.01	0.008	0.005	NS
Mortality (%)	24.4	16.6	14.1	3.28	0.08	0.10	0.03	NS
<i>Whole fattening period (35 to 65 days)</i>								
Average daily gain (g)	29.8	31.2	32.1	0.96	0.11	NS	0.10	NS
Average daily feed intake (g) <sup>1</sup>	130	118	122	4.41	0.15	0.07	NS	NS
Feed efficiency (g gain/g intake)	0.233	0.271	0.268	0.011	0.03	0.02	0.03	NS
Mortality (%)	27.0	19.7	14.8	3.10	0.03	0.10	0.008	NS

<sup>1</sup> Including feed intake of dead animals.

SEM = Standard error of the mean (20 cages with 8 rabbits per cage/treatment).

NS = Non significant ( $P > 0.15$ ).

flora (*E. coli* and *Clostridium*), which depend, partially, on the starch contribution as substrate in the hindgut (CHEEKE and PATTON, 1980; BORRIELLO and CARMAN, 1983; BLAS and GIDENNE, 1998). Furthermore, the enzymatic complex used in this work also contains other hydrocarbonases ( $\beta$ -glucanases and  $\beta$ -xylanases) that might contribute to reduce the amount of substrate that reaches the caecum. Some recent studies carried out in broiler (APAJALAHTI and BEDFORD, 1998; DÄNICKE *et al.*, 1999; CHESSON and STEWARD, 2001) suggest that addition of  $\beta$ -xylanases could modulate gut microflora, enhancing growth performances and health status of the animals.

From these assays we can conclude that Amylofeed® supplementation has beneficial effects on mortality, feed efficiency and growth for growing rabbits when higher incidence of intestinal disorders and mortality occur. This situation is usual during the post-weaning period in commercial conditions. Accordingly, these results suggest the interest of the enzyme supplementation in rabbit diets, especially in the post-weaning period, to reduce mortality and to improve performance.

**Acknowledgements:** This work was supported by a Project signed by Fundación Premio Arce with COREN and Materias y Actividades, S. L.

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