

## DIETARY SUPPLEMENTATION WITH MANNANOLIGOSACCHARIDES AND $\beta$ -GLUCANS IN GROWING RABBITS. 1. GROWTH PERFORMANCE, HEALTH STATUS AND CARCASS TRAITS

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### ABSTRACT

A total of 384 rabbits weaned at 32 d of age were assigned to six experimental groups of 64 rabbits each, homogeneous in live weight (average and variability) and housed in individual cages. Animals fed *ad libitum* until slaughter (75 d) six diets which differed for the type and level of the supplemented additive: diet C without additives; diets G1 and G2 supplemented with two levels of  $\beta$ -glucans from *Saccharomyces cerevisiae* (100 and 200 mg/kg, respectively); diets M1 and M2 supplemented with mannanoligosaccharides (Actigen, Alltech; 400 and 800 mg/kg, respectively); and diet GM (100 mg  $\beta$ -glucans + 400 mg mannanoligosaccharides/kg). Health status of animals was good (average mortality 3.5%) and both caecal fermentation traits and nutrient apparent digestibility were not affected by the inclusion level and type of additive. Growth performance of rabbits was not affected by the treatments, but daily weight gain (55.6 vs. 55.2 g/d; P=0.07) and live weight (2131 vs. 2100 g; P=0.07) values of rabbits at the end of the first period after weaning (55 d of age) were slightly higher in rabbits fed  $\beta$ -glucans diets compared with those fed mannanoligosaccharides diets. The slaughter traits were similar among the experimental groups. In conclusion, under the rearing conditions of the present trial and in absence of epizootic rabbit enteropathy, the dietary supplementation with mannanoligosaccharides and/or  $\beta$ -glucans did not produce any appreciable advantage in growth performances and carcass quality of meat rabbits.

**Key words:** Mannanoligosaccharides,  $\beta$ -glucans, performance, health status, growing rabbits.

### INTRODUCTION

The diffusion of Epizootic Rabbit Enteropathy (ERE) and the limits imposed by EU legislation on the use of antibiotics have stimulated research on feeding strategies to prevent digestive diseases. Some substances, like mannanoligosaccharides (MOS) and  $\beta$ -glucans, have recognized prebiotic effects (Falcão-e-Cunha *et al.*, 2007). The MOS exert their action by preventing gram-negative pathogens to colonize intestinal mucosa; in rabbits, their dietary inclusion may increase villi height and volatile fatty acids (VFAs) production (Pinheiro *et al.*, 2004; Mourão *et al.*, 2006; Guedes *et al.*, 2009), improve nutrient digestibility and decrease mortality due to ERE (Bovera *et al.*, 2010), even if it does not generally affect performance (Scapinello *et al.*, 2001; Di Meo *et al.*, 2009; Pinheiro *et al.*, 2009).  $\beta$ -glucans have a recognized activity of immune-modulation, but no effect of their dietary inclusion was proved on performance of rabbit does and their litters (Wu *et al.*, 2010). Scarce information is available on the effect of MOS and  $\beta$ -glucans on carcass traits and meat quality. The present study aimed at evaluating the effect of dietary supplementation with MOS and/or  $\beta$ -glucans on diet utilization, performance, caecal fermentation and slaughter traits in growing rabbits.

## MATERIALS AND METHODS

### Animals and experimental diets

A total of 384 rabbits of both genders from a cross-bred line (Hyplus, Grimaud Frères, France) were reared in individual cages from weaning (33 d) until slaughter (75 d). The rabbits were divided into six groups of 64 animals and fed with diets C (control diet without additives), G1 and G2 (the control diet supplemented with 100 and 200 mg/kg of  $\beta$ -glucans), M1 and M2 (with 400 and 800 mg/kg of MOS) and GM (with 100 mg  $\beta$ -glucans/kg + 400 mg MOS/kg). The diets were based on dehydrated alfalfa meal (30%), wheat bran (28%), barley (18%), dried sugar beet pulp (8%), soybean (4%) and sunflower meal (8%) and differed only in the type and level of additives. A commercial product (Antigen, Alltech, Spain) was used for supplementing diets with MOS.  $\beta$ -glucans were supplemented by the inclusion of a mixture made up of 40% pure  $\beta$ -glucans (extracted from *Saccharomyces cerevisiae* by the Instituto de Estudios Biofuncionales, Madrid) and 60% calcium carbonate. The diets were free from other additives or antibiotic drugs, but contained coccidiostat (Diclazuril).

### Recordings

Individual live weight and feed intake were recorded once a week and the health status of rabbits was monitored daily from weaning until 74 d of age. The digestibility coefficients of the experimental diets were measured by an *in vivo* digestibility assay carried out on 78 rabbits among those on trial (12 animals of both genders per diet) from 52 to 56 d of age according to the European standardized method (Perez *et al.*, 1995). Ninety-six rabbits were slaughtered at 45-46 d of age (16 rabbits per experimental diet) to sample caecal content. At 75 d of age, 150 rabbits (25 per experimental group), representative in terms of average weight and variability, were selected and slaughtered in a commercial slaughterhouse. The carcasses were chilled at 4° C for 24 hours, weighted and dissected according to Blasco *et al.* (1993).

### Chemical analysis

Diets and faeces were analyzed to determine the content of dry matter, ash, crude protein, and starch following the procedures described by Trocino *et al.* (2011). Fibre fractions, that is aNDF (without sodium sulphite), ADF, and lignin (sa), were analyzed according to Mertens (2002), and Van Soest *et al.* (1991), respectively, using the sequential procedure and the filter bag system. The total dietary fibre (TDF) was determined with a gravimetric enzymatic procedure with  $\alpha$ -amylase, protease, and amyloglucosidase treatments (Method AOAC 991.43). The soluble fibre content was calculated by subtracting aNDF after correction for protein and ash from TDF (Van Soest *et al.*, 1991). The gross energy was measured with an adiabatic bomb calorimeter. Caecal N-ammonia was determined with a pH meter equipped with ammonia specific electrode and volatile fatty acid (VFA) concentration was measured on a cross bond capillary column by the procedures described by Trocino *et al.* (2011).

### Statistical Analysis

The data recorded was analysed by a one-way ANOVA (type of diet) and with weaning weight as a covariate. The GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA) was used for all analyses. Orthogonal contrasts were used to test the effect of the inclusion of additives, the type of additives and the level of additives.

## RESULTS AND DISCUSSION

The diets, formulated to meet the nutritional requirements of growing rabbits, were similar at the chemical analysis since they differed only in the inclusion type and level of the additives. In fact, crude protein averaged 15.1%, starch content averaged 16.0%, TDF remained in the range 39.2-40.1%, ADF in the range 18.0-18.3 and lignin (sa) in the range 4.1-4.5%.

The dietary treatment did not modify the digestive utilization of the main nutrients (Table 1). Only the apparent digestibility of aNDF (P=0.01) and ADF (P=0.04) were lower in diets supplemented with both

$\beta$ -glucans and MOS compared to diets containing  $\beta$ -glucans or MOS alone. Some authors did not find differences in digestibility (Pinheiro *et al.*, 2009), while others described an increase of crude protein and crude fibre digestibility depending on the inclusion of MOS (Bovera *et al.*, 2010). On the contrary, Volek *et al.* (2007) found a negative effect on cellulose apparent digestibility due to MOS.

**Table 1:** Apparent digestibility coefficients (%) of experimental diets.

	Diet						Diet <sup>1</sup>	Probability					RSD <sup>7</sup>
	C	G1	G2	M1	M2	GM		C vs. others <sup>2</sup>	C vs. MOS <sup>3</sup>	C vs. $\beta$ -G <sup>4</sup>	$\beta$ -G vs. MOS <sup>5</sup>	GM vs. G+M <sup>6</sup>	
Dry matter	63.0	62.7	62.9	63.0	63.4	62.8	0.69	0.73	0.46	0.75	0.47	0.48	0.55
Crude protein	76.6	76.5	76.5	76.7	76.3	77.4	0.97	0.95	0.81	0.93	0.97	0.39	1.52
aNDF	29.5	29.1	30.3	29.3	31.8	28.2	0.11	0.77	0.07	0.50	0.31	0.01	1.44
ADF	22.3	21.4	22.1	21.3	23.1	19.6	0.37	0.53	0.59	0.92	0.69	0.04	1.88
Soluble fibre	76.0	73.5	72.2	70.2	75.7	76.9	0.10	0.24	0.90	0.14	0.95	0.18	2.89
Gross energy	62.5	62.3	62.5	62.4	62.9	62.2	0.70	0.84	0.40	0.87	0.35	0.27	0.53

<sup>1</sup>Effect of diet. <sup>2</sup>Diet C vs. the others diets. <sup>3</sup>Diet C vs. diets containing MOS. <sup>4</sup>Diet C vs. diets containing  $\beta$ -glucans. <sup>5</sup>Diets containing  $\beta$ -glucans vs. diets containing MOS. <sup>6</sup>Diets containing  $\beta$ -glucans+MOS vs. diets containing  $\beta$ -glucans or MOS alone. <sup>7</sup>Residual standard deviation.

Growth performance of rabbits was similar among groups in the whole trial, even if during the post-weaning period (33-55 days of age) the supplementation of diets with  $\beta$ -glucans slightly stimulated daily growth (55.6 vs. 55.2 g/d; P=0.07) and feed intake (134 vs. 130 g/d; P=0.02) compared with rabbits fed diets supplemented with MOS (Table 2). For this reason, feed intake in the whole period was slightly higher in the rabbits fed with  $\beta$ -glucans diets compared with those receiving MOS supplementation (147 vs. 144 g/d; P=0.10). Final live weight, conversion index, and slaughter results did not change with the dietary treatments. Average slaughter weight of 2823±191 g, dressing out percentage of 61.9±1.5% and average reference carcass weight of 1461±114 g were recorded (data not reported in tables).

**Table 2:** Productive performance from 33 to 74 day of age.

	Diet						Diet <sup>1</sup>	Probability					RSD <sup>7</sup>
	C	G1	G2	M1	M2	GM		C vs. others <sup>2</sup>	C vs. MOS <sup>3</sup>	C vs. $\beta$ -G <sup>4</sup>	$\beta$ -G vs. MOS <sup>5</sup>	GM vs. G+M <sup>6</sup>	
Rabbits, n	46	44	47	46	46	46							
Live weight, g													
33 d	915	905	904	903	912	904	0.96	0.41	0.79	0.77	0.81	0.47	77
55 d	2091	2139	2122	2106	2094	2106	0.35	0.24	0.95	0.93	0.07	0.21	114
74 d	2874	2830	2890	2844	2833	2877	0.45	0.51	0.63	0.28	0.42	0.67	180
1 <sup>st</sup> period (33-55 d)													
Weight gain, g/d	53.8	56.0	55.2	54.5	53.9	54.5	0.35	0.24	0.95	0.93	0.07	0.21	5.17
Feed intake, g/d	131	136	133	130	131	133	0.20	0.33	0.81	0.98	0.02	0.28	11
Feed conversion	1.89	1.84	1.86	1.87	1.87	1.87	0.77	0.33	0.89	0.64	0.29	0.38	0.16
2 <sup>nd</sup> period (55-74 d)													
Weight gain, g/d	41.2	36.4	40.5	38.9	38.9	40.6	0.02	0.07	0.49	0.13	0.65	0.62	7.19
Feed intake, g/d	165	159	166	161	160	166	0.34	0.39	0.31	0.18	0.45	0.93	20
Feed conversion	2.45	2.42	2.42	2.41	2.46	2.43	0.81	0.42	0.87	0.95	0.72	0.49	0.20
Whole trial (33-74 d)													
Weight gain, g/d	48.0	46.9	48.4	47.2	47.0	48.0	0.48	0.51	0.63	0.28	0.42	0.67	4.40
Feed intake, g/d	147	147	148	145	144	148	0.51	0.87	0.40	0.34	0.10	0.56	13
Feed conversion	3.06	3.14	3.07	3.06	3.07	3.09	0.19	0.36	0.52	0.73	0.13	0.86	0.17

<sup>1</sup>Effect of diet. <sup>2</sup>Diet C vs. the others diets. <sup>3</sup>Diet C vs. diets containing MOS. <sup>4</sup>Diet C vs. diets containing  $\beta$ -glucans. <sup>5</sup>Diets containing  $\beta$ -glucans vs. diets containing MOS. <sup>6</sup>Diets containing  $\beta$ -glucans+MOS vs. diets containing  $\beta$ -glucans or MOS alone. <sup>7</sup>Residual standard deviation.

This performance was consistent with the genetic type used and the slaughter age of animals. Our results confirm previous findings that both MOS and  $\beta$ -glucans supplementation have no effect on performance of growing rabbits (Scapinello *et al.*, 2001; Volek *et al.*, 2007; Pinheiro *et al.*, 2009) or does and their litters (Wu *et al.*, 2010). Other authors (Mourão *et al.*, 2006; Di Meo *et al.*, 2009; Bovera *et al.*, 2010) observed a positive effect of MOS inclusion on feed conversion ratio of growing rabbits.

Health condition of rabbits was rather good during the whole experimental trial and mortality averaged 3.5% without any significant difference between treatments. Some authors described a positive effect of MOS on mortality of growing rabbits (Mourão *et al.*, 2006; Bovera *et al.*, 2010), while others did not (Volek *et al.*, 2007; Pinheiro *et al.*, 2009; Bovera *et al.*, 2011).

In our trial, the caecal traits measured at 45-46 d of age did not differ depending on additive supplementation (Table 3) as found also by Volek *et al.* (2007) and Pinheiro *et al.* (2009). The caecal pH averaged 6.00, for a total production of volatile fatty acids of 61.6 mmol/L and the typical fermentation pattern with the dominance of acetate, followed by butyrate and propionate. Differently from our results, some authors (Mourão *et al.*, 2006; Guedes *et al.*, 2009) described increased volatile fatty acid content in the caecum of rabbits fed MOS supplemented diets.

**Table 3:** Caecal traits at 45-46 day of age.

	Diet						Diet <sup>1</sup>	Probability					RSD <sup>7</sup>
	C	G1	G2	M1	M2	GM		C vs. others <sup>2</sup>	C vs. MOS <sup>3</sup>	C vs. $\beta$ -G <sup>4</sup>	$\beta$ -G vs. MOS <sup>5</sup>	GM vs. G+M <sup>6</sup>	
Rabbits, n	16	16	16	16	16	16							
pH	5.98	6.01	5.97	6.05	6.02	6.00	0.95	0.65	0.95	0.67	0.49	0.94	0.25
NH <sub>3</sub> , mol/L	5.73	5.22	5.86	5.94	5.67	5.16	0.92	0.81	0.42	0.95	0.67	0.88	2.47
Total VFA, mmol/L	62.8	67.2	58.5	62.3	60.9	57.6	0.54	0.73	0.65	0.73	0.74	0.44	15.3
C <sub>2</sub> , % mol, VFA	82.8	82.8	81.8	82.2	82.7	82.7	0.71	0.49	0.53	0.87	0.81	0.18	2.19
C <sub>3</sub> , % mol, VFA	3.77	3.88	4.30	4.32	3.80	3.65	0.19	0.39	0.16	0.93	0.89	0.11	0.92
C <sub>4</sub> , % mol, VFA	12.9	12.7	13.2	12.8	12.8	13.1	0.97	0.92	0.87	0.91	0.72	0.62	2.07
C <sub>5</sub> , % mol, VFA	0.52	0.63	0.67	0.76	0.71	0.56	0.62	0.23	0.32	0.22	0.43	0.33	0.43
C <sub>3</sub> /C <sub>4</sub>	0.30	0.31	0.34	0.36	0.30	0.28	0.39	0.56	0.29	0.94	0.95	0.30	0.11

<sup>1</sup>Effect of diet. <sup>2</sup>Diet C vs. the others diets. <sup>3</sup>Diet C vs. diets containing MOS. <sup>4</sup>Diet C vs. diets containing  $\beta$ -glucans. <sup>5</sup>Diets containing  $\beta$ -glucans vs. diets containing MOS. <sup>6</sup>Diets containing  $\beta$ -glucans+MOS vs. diets containing  $\beta$ -glucans or MOS alone. <sup>7</sup>Residual standard deviation.

## CONCLUSIONS

In the present trial, under the rearing conditions of an experimental farm and in absence of epizootic enteropathy, the dietary supplementation with MOS and/or  $\beta$ -glucans did not produce any appreciable advantage in terms of growth performance, digestive efficiency or caecal fermentation activity.

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