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EFFECT OF INCLUSION OF GRAPE-SEED MEAL ON DISACCHARIDASE ACTIVITY IN THE SMALL INTESTINE OF GROWING RABBITS[1]

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ABSTRACT

Twenty New Zealand White x Californian rabbits were used to determine the influence of grape-seed meal (GSM) and sunflower hulls (SH) on disaccharidase activity in the small intestine. Two diets were formulated by supplementing these fibrous feeds with a concentrate containing starch, protein, fat and a vit/min mix. Sucrase specific activity was a 23 (jejunum) and a 80% (ileum) higher in animals fed GSM than in those fed SH ($P < 0.05$). Maltase specific activity in ileum also tended to increase a 31% in rabbits fed GSM ($P = 0.103$), whereas that in jejunum was similar to that obtained for animals fed SH (19420 mmol glucose/g protein and 30 min, on average). Another forty rabbits were used to study the effect of substitution of a 15.2% of a basal diet by GSM on disaccharidase activity in the small intestine. Substitution of 15.2% of the basal diet by GSM increased by 36% the specific activity of sucrase in ileum, and did not affect specific activities of maltase in ileum and those of sucrase and maltase in jejunum (25397, 4023 and 31876 mmol glucose/g protein tissue and 30 min, on average, respectively). From these results it can be concluded that inclusion of GSM in rabbit diets improve disaccharidase activity in the small intestine. However, more studies are needed to elucidate the effect of acid detergent lignin (lignin and cutin) on mucosal physiology.

INTRODUCTION

The inclusion of small amounts of high lignified fibrous by-products is usual in rabbit diets in Spain. However, García et al. (1997) established a negative effect of dietary acid detergent lignin proportion on specific activity of sucrase in the ileum, an enzyme implied in maltose digestion (Kidder and Manners, 1978). An increase of the ileal flux of glucose might lead to digestive troubles (Borriello and Carman, 1983). Besides, sucrase specific activity has been used to indicate maturity of enterocytes and functional capacity (Henning, 1985; Hampson and Kidder, 1986; Tang et al., 1999) as sucrases are contained in the brush border of the differentiated mucosal cells (Sernka, 1974). Thus, in a previous work it has been also observed that lignin supplementation compared to that of alfalfa caused significant damage of the villi surface in the duodenum and jejunum and shortened villus height in the jejunum and ileum (Chiou et al., 1994).

The aim of this work was to determine the effect of inclusion of grape-seed meal, a source of fibre highly lignified, on sucrase and maltase activities in the small intestine.

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MATERIAL AND METHODS

Trial 1

The effect of grape-seed meal (GSM) on disaccharidase activity was studied using sunflower hulls (SH) as a control. Sunflower hulls has a high NDF proportion, very closed to that of GSM. Degree of lignification of SH is high but lower than that of GSM, due to the high cutin content of GSM (García et al., 2000). Thus, two diets were formulated to contain 61.3% of grape-seed meal (GSM) or sunflower hulls (SH) as the sole source of fibre and a similar NDF concentration. To assure a minimum nutrient supply, both fibre sources were supplemented with a concentrate (15.3% casein, 18% wheat flour, 1.2% lard, 4.03% minerals and 0.17% vit/min premix) to obtain diets containing at least 18% crude protein and 12% starch. Chemical composition of diets is shown in Table 1.

A group of 20 New Zealand White x Californian rabbits (ten per diet) 46 days old and weighing 1324±36 (SE) g, were randomly allotted to the experimental diets without regard to gender. Animals were given ad libitum access to the feed. Following a 34-d period of adaptation to each diet, the animals were slaughtered by cervical dislocation at 19.00 h. They weighed 2261±58 g. The small intestine was divided in three segments of equal length. A portion of 5-7 cm was sampled in the middle of the second (jejunum) and third (ileum) segment. These portions were flushed with a saline solution, frozen on dry ice and kept at -20°C until prepared for analysis. The samples were homogenised with distilled water (50 mg intestine/ml). Homogenising was done by using a Polytron homogeniser.

Trial 2

A basal diet (BD) was formulated according to the recommendations of De Blas and Mateos (1998). The ingredient composition was (%): 30.3 alfalfa hay, 33 wheat bran, 5 wheat straw, 4 sugar-been pulp, 4 barley rootlets, 6.1 corn germ meal, 2 barley, 2.5 cane molasses, 1.12 lard, 2.9 sunflower seed meal, 1.9 soya-bean meal-44, 2 corn dry distillery grains and solubles, 2 corn gluten feed, 1.64 grape-seed meal, 0.01 Alimet (DL-Methionine. Novus. USA), 0.09 Lysine 50% and 0.04 threonine). Another diet was formulated by substituting 15.2% of the basal diet by grape-seed meal (15.2% GSM) to study the influence of GSM on the enzyme activity using a more common diet. Both diets were supplemented with (%): 0.6 calcium carbonate, 0.5 sodium chloride, 0.03 choline chloride, 0.1 robenidine premix and 0.17 vit/min premix. Chemical composition of diets is shown in Table 1.

A group of 40 New Zealand White x Californian weaned rabbits (twenty per diet) of 30 days old and weighing 672±12 g, were randomly allotted to the experimental diets without regard to gender. Animals were given ad libitum access to the feed. Animals were slaughtered by cervical dislocation at 19.30 h when they weighted 2062±89 g (62 days old). Small intestine was sampled and prepared as described before.

Housing.

Animals were housed in wire cages measuring 405 x 510 x 320 mm. A cycle of 12 h of light and 12 h of dark was used throughout the experiment. The light was switched on at 7.30h. Heating and forced ventilation systems allowed the building temperature to be maintained between 18 and 23°C throughout the experiment. Experimental procedures followed the principles for care of animals in experimentation (Spanish Royal Decree 223/88, 1988).

Analytical Methods.

Chemical analysis of diets was performed using the method of Van Soest et al. (1991) for NDF and Goering and Van Soest (1970) for ADF, ADL and acid detergent cutin (ADC). Neutral detergent fibre was determined directly, whereas ADF and ADL extracted successively. Acid detergent cutin was determined after extracting ADF, ADL and permanganate lignin. Procedures of AOAC (1990) were used for DM, ash and crude protein. Determination of intestinal sucrose and maltose activities were done according to Dahlquist (1964). This procedure involved incubation of intestinal homogenates in a solution of sucrose or maltose respectively at 37°C for 30 minutes. The glucose released by sucrose or maltose action was oxidised by glucose oxidase and measured spectrophotometrically. Protein concentration of intestinal segments was determined according to Schachterle and Pollack (1973).

Statistical Analysis

Data were analysed by using the GLM procedure of SAS (1990) with type of diet as main factor.

Table 1. Chemical composition of experimental diets (% DM basis).

	Trial 1		Trial 2	
	61.3% GSM	61.3% SH	Basal diet	15.2% GSM
Ash	7.60	5.86	8.6	8.6
Crude protein	24.0	19.4	17.4	16.7
Starch[1]	12.6	14.0	13.4	11.4
NDF	49.1	47.3	41.4	44.3
ADF	44.5	36.9	21.3	27.3
ADL	36.0	13.7	5.7	11.7
Cutin	27.7	5.89	2.5	8.5

[1] Calculated from tabulated ingredient composition (FEDNA, 1999).

RESULTS AND DISCUSSION

The results obtained with the 61.3% GSM and 61.3% SH diets are shown in Table 2. Sucrase specific activity was a 23 (jejunum) and a 80% (ileum) higher in animals fed 61.3% GSM than in those fed 61.3% SH ($P < 0.05$). Maltase specific activity in ileum also tended to increase a 31% in rabbits fed 61.3% GSM ($P = 0.101$), whereas that in jejunum was similar to that obtained for animals fed 61.3% SH (19420 mmol glucose/g protein and 30 min, on average). Disaccharidases specific activity decreased by 45 and 57% from jejunum to ileum in animals fed GSM or SH, respectively, as observed previously by García et al. (1997) and Dojana et al. (1998). Protein concentration in

jejunum and ileum averaged 11.6 g/100 g fresh tissue (Table 2).

The main factor that affects disaccharidase activity in the small intestine is the quantity of substrates present in the diet (Zabielski et al., 1999). However, in this study the animals with higher enzyme activity, those fed 61.3% GSM, had a similar or even lower starch intake than those fed 61.3% SH (19.0 vs 21.4 g/d). In this work, animals fed the diet with higher ADL content (61.3% GSM) also showed higher disaccharidase activity, although previous studies had determined a negative effect of lignin and ADL on this trait (Chiou et al., 1994; García et al., 1997). Acid detergent lignin includes several constituents (mainly lignin and cutin) that are not in the same proportions in GSM and in SH, and they might affect differently the small intestine physiology. Besides, type of lignin and cutin of GSM and SH probably differ and have a different effect on the small intestine physiology. Physical characteristics (particle size, shape of particles) of the fibrous sources might also affect intestinal morphology and influence disaccharidase activity (Nicodemus et al., unpublished data).

Table 2. Effect of grape-seed meal and sunflower hulls on feed intake and disaccharidase activity in the small intestine.

	61.3% GSM	61.3% SH	SEM[1]	P
Feed intake, g DM/d[2]	151	153	6.25	0.78
Jejunum				
Protein[3]	11.6	11.6	0.4	0.85
Glucose released by sucrases[4]	446	360	29	0.040
Sucrase specific activity[5]	3826	3111	211	0.024
Glucose released by maltases[4]	2392	2103	173	0.26
Maltase specific activity[5]	20622	18218	1517	0.28
Ileum				
Protein[3]	11.3	11.8	0.2	0.103
Glucose released by sucrases[4]	203	120	16	0.002
Sucrase specific activity[5]	1826	1012	154	0.001
Glucose released by maltases[4]	1409	1134	124	0.15
Maltase specific activity[5]	12687	9657	1240	0.101

[1] n = 10. [2] mean of the three days previous to slaughter. [3] g protein/100 g fresh tissue. [4] mmol glucose/g fresh tissue and 30 min. [5] mmol glucose/g protein and 30 min.

The results obtained with the 15.2% GSM and basal diets are shown in Table 3. Substitution of 15.2% of the basal diet by GSM increased by 36% the specific activity of sucrase in ileum, and did not affect specific activities of maltase in ileum and those of sucrase and maltase in jejunum (25397, 4023 and 31876 mmol glucose/g protein tissue and 30 min, on average, respectively). Disaccharidases specific activity decreased by 37 and 24% from jejunum to ileum in animals fed the basal diet or the 15.2% GSM diet, respectively. Protein concentration in jejunum and ileum averaged 8.43 g/100 g fresh.

Animals fed the 15.2% GSM diet had again a similar or even a lower starch intake than those fed the basal diet (17.1 vs 15.8 g/d). The positive effect of inclusion of GSM respect to that of SH (Trial 1) or that of a basal diet (Trial 2) on enzyme activity might indicate an improvement of mucosal functional capacity (Henning, 1985; Hampson and

Kidder, 1986; Tang et al., 1999). This result could partially explain the relatively higher digestible energy value obtained previously for GSM and higher fattening performance obtained with the 15.2% GSM diet respect to the basal diet (García et al., 1999[a]; García et al., 1999[b]).

The positive effect of GSM is more clearly observed with the sucrase than with the maltase activity, and in the ileum than in the jejunum. The higher sensibility of sucrase respect to maltase activity could be due to the reduced number of enzymes with sucrase activity (only maltase I) compared to that of maltase (maltase I, II and III. Kidder and Manners, 1978).

Table 3. Effect of inclusion of grape-seed meal on feed intake and disaccharidase activity in the small intestine.

	Basal diet	15.2% GSM	SEM[1]	P
Feed intake, g DM/d[2]	128	139	3.37	0.032
Jejunum				
Protein[3]	7.68	7.91	0.34	0.64
Glucose released by sucrases[4]	304	307	27	0.94
Sucrase specific activity[5]	4044	4003	381	0.94
Glucose released by maltases[4]	2502	2360	201	0.63
Maltase specific activity[5]	32953	30799	2662	0.58
Ileum				
Protein[3]	8.92	9.21	0.36	0.60
Glucose released by sucrases[4]	172	239	15	0.006
Sucrase specific activity[5]	1989	2704	215	0.031
Glucose released by maltases[4]	2248	2295	199	0.87
Maltase specific activity[5]	25014	25781	2156	0.81

[1] n = 20. [2] mean of the fattening period. [3] g protein/100 g fresh tissue. [4] mmol glucose/g fresh tissue and 30 min. [5] mmol glucose/g protein and 30 min.

From these results it can be concluded that inclusion of GSM in rabbit diets improve disaccharidase activity in the small intestine, which might also indicate a better carbohydrate digestion and a higher functional capacity of the small intestine. However, more studies are needed to elucidate the effect of acid detergent lignin (lignin and cutin) on mucosal physiology.

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