IMPROVING THE NUTRITIVE VALUE OF LUPIN SEED FOR GROWING RABBITS: α-GALACTOSIDASE ENZYMES VS. WASHING

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ABSTRACT

To improve the nutritive value of lupinus seed for growing rabbits, four diets containing 30% of lupin (*Lupinus albus*) and similar levels of NDF and CP were formulated. In diet TS, lupin was not supplemented with enzymes or washed. Diets TE_1 and TE_2 were supplemented with 0.1 and 0.25% of a α-galactosidase preparation (500 GALU/g), respectively. Diet TM contained lupins which had been previously washed during 5 days (tenfold volume of water, stirred frequently, and changed daily), then dried. Each diet was fed *ad libitum* to 12 rabbits from 27 to 63 days of age. Intake was measured three times a week, live weight weekly. Caecotrophs were collected at the ninth day of the trial, faeces during the fourth week, and the caecal contents after the rabbits were slaughtered. Caecotrophs were tested for DM and fibre-degrading activity, caecal contents for DM, fibre-degrading activity, pH, and VFA. Although feed intake was lower ($P<0.05$) with diet TM (130.1 g/d) than with diets TS (141.3 g/d), TE_1 (141.0 g/d) and TE_2 (137.7 g/d), both the ADG (about 43 g/d) and the FCR (about 3.2) were not statistically different across all four treatments. Diet TM had generally higher digestibility of nutrients than the other three diets: higher digestibility of DM, OM, energy (about 59% vs. about 56% in the other three), NDF (27.7 vs. 23.6, 20.4 and 20.2% for TS, TE_1 and TE_2 respectively) and hemicelluloses (estimated as NDF-ADF) (41.3 vs. 31.6, 29.1 and 26.4% for TS, TE_1 and TE_2, respectively). CP digestibility (about 80%) did not vary across diets. On the other hand, both the fibre-degrading activities of the caecotrophs and caecal contents and the VFA levels of the latter were higher ($P<0.05$) in both enzyme-supplemented diets. We can conclude that both α-galactosidase and washing had some statistically significant effects on nutrients digestibility and caecal enzyme activities, however neither of the two treatments had any important practical effect on the rabbit performance.

Keywords: Growing rabbits, *Lupinus albus*, α-galactosidase, Washing lupins.

INTRODUCTION

Lupins represent an interesting alternative when soybeans cannot be economically produced. They are nitrogen-fixing plants, and they can be particularly interesting in the so-called biological animal production systems. In countries such as Portugal, lupin seeds have been used as food and feed for a long time, despite their content of bitter alkaloids. In the past these antinutritional factors were removed by keeping the lupin seeds under running water for some time. Later, the geneticists obtained so-called sweet varieties of lupins, which are practically devoid of these alkaloids. However, both the conventional and the sweet varieties of lupins also contain α-galactosides, which can have a negative effect on performance of farm animals.

Adding exogenous α-galactosidases showed positive effects in some cases (e.g. improving the DM digestibility in piglets, as shown by Gdala *et al.*, 1997b, or the performance of growing pigs, as reported by Froidmont *et al*., 2005).

The traditional treatment of lupin can also lead to a loss of others seed components, mainly soluble sugars and water-soluble vitamins, although this may help to expose the grains to digestive enzymes and increase the availability of others seed components.

Thus, we fed diets containing 30% of either untreated lupins, washed lupins, or lupins supplemented with α-galactosidase, to growing rabbits; and followed the rabbits performance, digestibility of feeds, and the fermentation activity and pattern in the caecotrophs and the caecal contents.

MATERIALS AND METHODS

Animals, feeds and experimental design

Four diets were formulated, all containing 30% of lupin (*Lupinus albus*), and similar levels of NDF and CP. Diet TS was the control diet. Diets TE_1 and TE_2 were respectively supplemented with 0.1 and 0.25% of a α-galactosidase preparation, containing 500 GALU/g. Diet TM contained lupin grains which had been previously washed and dried. In order to simulate the traditional washing, grains were kept in a tenfold volume of water for five days, and water was stirred about ten times a day, and changed daily. Grains were then dried in an oven at 60ºC. Table 1 shows ingredients and chemical compositions of the four diets.

Table 1: Ingredients and chemical composition of the experimental diets

Diets	TS	TE_1	TE_2	TM
Ingredients (%)				
Lupinus albus	30.5	30.5	30.5	30.5
Galactosidase preparation	-	0.1	0.25	-
Corn	16.2	16.2	16.2	16.2
Alfalfa + wheat straw	49.5	49.5	49.5	49.5
Molasses	1.72	1.62	1.47	1.72
SMV*	1.8	1.8	1.8	1.8
DL-Methionine	0.12	0.12	0.12	0.12
L-Lysine	0.16	0.16	0.16	0.16
Chemical composition (%)				
Dry matter	88.7	88.7	88.8	86.0
Ash	8.1	8.1	8.6	8.1
Crude protein	15.3	15.8	16.5	16.4
NDF	38.1	37.4	36.6	36.2
ADF	22.4	22.1	22.4	22.0
Energy (MJ/kg)	16.14	16.21	16.06	15.69
Sugars **	2.5	2.2	2.3	0.9

*Mineral and vitamin mixture supplied per kg of diet: vitamin A 10,000 IU, vitamin D_3, 1,800 UI; vitamin E, 15 mg; vitamin K_3, 4.5 mg; vitamin B1, 0.5 mg; vitamin B2, 4 mg; vitamin B12, 0.001 mg; folic acid, 0.1 mg; pantothenic acid, 7 mg; nicotinic acid, 20 mg; I, 1 mg; Mn, 60 mg; Cu, 5.5 mg, Zn, 75 mg; Fe, 40 mg; Co, 0.3 mg; Se, 0.08 mg; Robenidine, 52.8 mg, antioxidant, 0.250 mg** raffinose family

Each diet was fed *ad libitum* to twelve rabbits. Groups of rabbits were designed according to litter and weight. At 27 days of age, each rabbit was housed in an individual metabolism cage, where it stayed until 63 days of age. Feed intake was measured three times a week, and live weight was measured weekly.

Nine days after the beginning of the trial, neck collars were put to rabbits at around 08:00 am, to block caecotrophy. The collars were kept just long enough to collect a sufficient amount of caecotrophs for further analyses. Faeces were collected during the fourth week of the trial, and digestibility measured according to Perez *et al.* (1995). In the end, rabbits were slaughtered, and their contents weighed and stored at –20ºC, to be analyzed later.

Chemical analyses

Feeds were milled through a 1 mm screen, faeces were thawed, dried at 70°C for 48 hours, and then also milled using the same screen. All analyses were performed in triplicate on feed and in duplicate on samples of faeces. DM was measured by oven-drying during 24 hours at 103ºC, ash by burning overnight at 550°C, CP by the Kjeldahl method, and NDF, ADF and ADL according to Van Soest *et al.* (1991). Hemicellulose and cellulose were calculated as the differences NDF-ADF and ADF-ADL, respectively. The energy of feeds and faeces was measured in a bomb calorimeter (Parr model 1261). Raffinose family sugars were analysed in diets by the raffinose assay procedure (K-RAFCA 10/04), Megazyme International Ireland. The pH of caecal contents was measured with a glass electrode pH meter (WTW pH522), their VFA levels by gas chromatography, according to Jouany *et al.* (1982). Bacterial fibre-degrading activities in the caecotrophos and in the caecal contents were assessed according to Falcão-e-Cunha *et al.* (2004).

Statistical analysis

The dietary effects of on growth performance, total tract apparent digestibilities, and intestinal microbial activity were compared by the contrasts method, using the GLM procedure of SAS (SAS, 1991).

RESULTS AND DISCUSSION

Table 2 summarizes the performance of rabbits, according to phase: first period (post-weaning, 27-42 d), second period (42-63 d), and whole trial (27-63 d). Diets mostly did not differ significantly.

Table 2: Effects of washing or adding α-galactosidase on the rabbit performance

	Diets				Contrasts[1]					RSD[2]
	TS	TE_1	TE_2	TM	1	2	3	4	5	
1st period (27- 42 days old)										
Initial live weight (g)	576	576	576	573	NS	NS	NS	NS	NS	71.8
Daily feed intake (g)	101.0	101.9	96.8	88.6	NS	NS	0.044	0.047	NS	14.4
Daily weight gain (g)	44.4	44.5	44.9	41.6	NS	NS	NS	NS	NS	6.0
Feed conversion rate	2.30	2.30	2.16	2.14	NS	NS	NS	NS	NS	0.28
2nd period (42-63 days old)										
Initial live weight	1197	1199	1211	1156	NS	NS	NS	NS	NS	113.2
Daily feed intake (g)	168.1	163.3	164.1	157.8	NS	NS	NS	NS	NS	17.7
Daily weight gain (g)	44.3	40.2	43.4	42.2	NS	NS	NS	NS	NS	4.7
Feed conversion rate	3.81	4.10	3.78	3.74	NS	NS	NS	NS	NS	0.33
Whole period										
Final live weight (g)	2127	2079	2123	2043	NS	NS	NS	NS	NS	128.4
Daily feed intake (g)	141.3	141.0	137.7	130.1	NS	NS	0.040	0.052	NS	12.5
Daily weight gain (g)	44.3	42.9	44.0	42.0	NS	NS	0.102	NS	NS	3.3
Feed conversion rate	3.19	3.30	3.12	3.10	NS	NS	NS	NS	0.048	0.20

(1) Contrasts: 1– TS vs. (TE_1, TE_2, TM), 2 – TS vs. (TE_1 e TE_2), 3 – TS vs. TM, 4 - (TE_1 e TE_2) vs. TM, 5 – TE_1 vs. TE_2
(2) RSD: Residual standard deviation

As expected the TM diet with washed lupin had a lower level of raffinose sugars. Nevertheless the daily feed intake was significantly lower ($P<0.05$) at diet TM (88.6 g/d) compared to the untreated (101.0 g/d) or the enzyme-supplemented diets (99.4 g/d) during the first period. But this difference had no significant effect on either daily growth rate (about 43 g/d) or feed conversion ratio (about 3.2). No differences at all could be detected during the second period, and as such, most differences in the whole trial are just the result of differences which occurred in the first period.

Significant differences in TTAD (Table 3) could however be detected. Diet TM, containing washed lupins, had higher digestibility of DM, OM and energy than the other three diets, which were similar in this regard. On the other hand, while washing also increased the digestibility of NDF and hemicellulose, relative to untreated lupins, α-galactosidase had just the opposite effect. If the effect of washing may possibly be explained by the combined effects of the washing itself and the lower intake – and thus a longer retention time, a relationship which has been reported by several authors – the negative effects of the α-galactosidase are difficult to explain, especially as far as hemicelluloses are concerned.

Table 3: Effects of washing or adding α-galactosidase on total tract apparent nutrient digestibility

	Diets				Contrasts [1]					
	TS	TE_1	TE_2	TM	1	2	3	4	5	RSD[2]
Coefficients of total tract apparent digestibility (%)										
Dry matter	56.0	55.9	55.5	59.5	NS	NS	0.012	0.002	NS	3.2
Organic matter	56.3	56.2	56.0	59.7	NS	NS	0.013	0.003	NS	3.2
Crude protein	80.1	80.0	80.2	80.2	NS	NS	NS	NS	NS	2.3
NDF	23.6	20.4	20.2	27.7	NS	NS	0.096	0.001	NS	5.7
ADF	18.1	14.4	16.4	19.0	NS	NS	NS	NS	NS	6.2
Hemicellulose (NDF-ADF)	31.6	29.1	26.4	41.3	NS	0.056	0.0002	<0.0001	NS	5.6
Cellulose (ADF-ADL)	20.0	15.6	17.4	19.0	NS	NS	NS	NS	NS	6.1
Energy	56.9	56.5	56.6	59.1	NS	NS	NS	0.036	NS	3.2

(1) Contrasts: 1– TS vs. (TE_1, TE_2, TM), 2 – TS vs. (TE_1 e TE_2), 3 – TS vs. TM, 4 - (TE_1 e TE_2) vs. TM, 5 – TE_1 vs. TE_2
(2) RSD: Residual standard deviation

In pigs, the effects of α-galactosidase on lupin protein utilization have been generally unconclusive. Some positive reports are available: Gdala *et al.* (1997a) detected an effect on the ileal digestibility of almost all amino-acids; however Pires *et al.* (2007) did not observe any effect on the apparent ileal digestibility of protein and amino-acids. In our trial, protein TTAD was about 80%, and was not affected by any of the treatments.

The results of digestibility trial are apparently contradictory with both the fibre-degrading activity of the caecotrophs (Table 4) and the fermentation activity in the caecal contents (Table 5). Both the caecotrophs and the caecal contents of the rabbits which were fed the α-galactosidase supplement had higher ($P<0.05$) enzyme activities and VFA levels than the ones from rabbits fed untreated or washed lupins, although the caecal pH was the same in all cases.

Table 4: Effects of washing or adding α-galactosidase on fibre-degrading activities in caecotrophs (measured on the 9th day of the trial)

					Contrasts					
	TS	TE_1	TE_2	TM	1	2	3	4	5	RSD
Enzymatic activity (mg sugar h^{-1} g^{-1} DM)										
Pectinase	100.7	132.9	132.4	127.7	0.0265	0.0264	0.0983	0.7168	0.9762	36.58
Xylanase	39.1	69.9	78.1	55.2	0.0173	0.0065	0.2570	0.1176	0.546	32.07
Cellulase	36.9	59.2	57.2	58.8	0.0048	0.0077	0.0162	0.9307	0.8108	30.03
Dry matter (%)	29.1	28.4	27.6	25.8	0.585	0.2827	0.0067	0.0234	0.4659	2,54

(1) Contrasts: 1– TS vs. (TE_1, TE_2, TM), 2 – TS vs. (TE_1 e TE_2), 3 – TS vs. TM, 4 - (TE_1 e TE_2) vs. TM, 5 – TE_1 vs. TE_2
(2) RSD: Residual standard deviation

Table 5: Effects of washing or adding α-galactosidase on the caecal fermentative activity

	Diets				Contrasts [1]					
	TS	TE_1	TE_2	TM	1	2	3	4	5	RSD[2]
Enzymatic activity (mg sugar/h/g DM)										
Pectinase	60.5	69.1	76.2	63.5	NS	0.051	NS	NS	NS	16.6
Xylanase	33.4	62.4	63.4	49.5	0.045	0.026	NS	NS	NS	36.2
Cellulase	38.2	35.6	41.3	37.3	NS	NS	NS	NS	NS	17.2
DM caecal contents (%)	21.4	20.5	20.0	19.2	0.003	0.028	0.0006	0.050	NS	1.4
pH	6.42	6.41	6.41	6.39	NS	NS	NS	NS	NS	0.3
Total VFA (mmol/l)	41.1	52.4	47.8	44.3	0.098	0.048	NS	NS	NS	12.4
Molar proportions of individual VFA										
C2 (mol/100 mol)	81.0	78.6	79.6	80.4	NS	0.047	NS	NS	NS	2.6
C3 (mol/100 mol)	9.0	10.1	9.2	10.0	NS	NS	NS	NS	NS	1.8
C4 (mol/100 mol)	10.0	11.3	11.1	9.7	NS	0.102	NS	0.058	NS	2.1

(1) Contrasts: 1– TS vs. (TE_1, TE_2, TM), 2 – TS vs. (TE_1 e TE_2), 3 – TS vs. TM, 4 - (TE_1 e TE_2) vs. TM, 5 – TE_1 vs. TE_2
(2) RSD: Residual standard deviation

It is possible that the α-galactosidase had an effect on water-soluble NSP, which are high in lupins (Knudsen, 1997), making them an easier substrate for caecal microorganisms than the hemicelluloses, which we estimated by subtracting ADF from NDF. Differences in molar VFA proportions suggest that α-galactosidase possibly alters substrates that reach the caecum, which is an idea supported by differences in DM of caecotrophs and caecal contents.

CONCLUSIONS

In the present experiment, washing or supplementation of lupins with α-galactosidase influenced both the digestibility of cell wall components and the caecal fermentation activity to some extent. No significant influence on the rabbit performance, however, was observed.

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