TOTAL AND ILEAL DIGESTIBILITY AND CAECAL BACTERIAL FIBROLYTIC ACTIVITY IN ADULT RABBIT FED WITH RESISTANT STARCH

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ABSTRACT

The effect of intake of resistant starch on the digestive response of the rabbit was analysed by comparing a low, medium, or a high intake of resistant starch obtained by replacing wheat starch (WS) by raw potato starch (PS) in diets. Three diets, with 0, 7 or 14% of PS (resp. WS, PS7 and PS14) and having a high and similar concentration of starch (20%), were given to adult rabbits. The ileal, caecal and total digestibility, and the bacterial enzymatic activity (cellulase, xylanase and pectinase) have been measured in ileo-cannulated animals. In adult rabbit the ileal digestibility of starch was over 95%, while that of protein averaged 69%. Only the ileal starch digestibility was slightly reduced (98.3 for 96.7%; P=0.001) when 14% of potato starch replaced wheat starch. The bacterial fibrolytic activity in the caecal content was not significantly affected by level of resistant starch. Consequently, we assume that even for a high intake of resistant starch, the ileal starch concentrations and flow remained low, and should not be a risk factor for the microbial activity and balance in the caecum.

Key words: Adult rabbits, potato starch, digestibility, caecal activity

INTRODUCTION

Starch is the major available energy producing carbohydrate in the monogastric diets. Wheat starch is commonly used in diets for rabbits. It is predominantly amylopectin and can be almost fully digested in the small intestine, since it contains small amounts of resistant starch. Potato raw starch have granules with large size and typical B-crystalline structure (Noda *et al.*, 2008) what will difficult the access of enzymes and decreases the digestibility. In vitro hydrolysis with amylolytic enzymes have indicated extremely low digestibility of raw potato starch (Bauer *et al.,* 2003). It is expected that incorporating resistant starch in growing rabbit feeds could lead to digestive disturbances, as a result of a high starch flow in the caecum that may unbalance the microbiota and its activity (Cheeke and Patton, 1980). A review on the digestion of starch in the rabbit (Blas and Gidenne, 2010), indicated that few studies have addressed the problem of its digestion in the small intestine. The only available studies on starch effects compare mainly diets based wheat, barley, pea and maize (Gidenne and Perez, 1993; Xiccato *et al.*, 2002; Gutierrez *et al.*, 2002).Therefore, our study aimed to test the effect of high intake of resistant starch on diet digestion and caecal microbial activity of adult rabbit.

MATERIALS AND METHODS

Diets and experimental design

The trials were carried out in the animal facilities of the University of Trás-os-Montes e Alto Douro (UTAD) at Vila Real, Portugal, and of INRA Toulouse (TANDEM), according to the principles for the care of animals in experimentation. Three experimental diets were formulated, to obtain a linear replacement of wheat by purified raw potato starch "PS" (Roquette SA, Lestrem, France) at a level of

0, 7 and 14%, respectively for diets WS, PS7 and PS14 (Table 1). Among diets, the level of starch, fibre and crude protein were similar. In each experimental unit, 20 cannulated rabbit does with 4.4+-0.3kg, were divided in two groups according the scheme presented in Table 2. All does were individually housed in metabolic cages with water and feed *ad libitum*.

Table 1: Major ingredients and chemical composition of diets.

	Diets		
	WS	PS7	PS14
Ingredients (g/kg feed)			
Potato Starch [1]	0	70	140
Wheat	310	205	100
Soya	160	180	195
Wheat bran	95	105	130
Dehydrated lucerne meal	170	175	180
Dehydrated beet pulp	190	190	180
Wheat straw	55	55	55
Minerals, Vitamins and additives [2]	20	20	20
Chemical analysis (g/kg air dry basis)			
Organic matter	853	845	846
Crude protein (N×6.25)	169	167	165
Starch	198	195	200
Neutral-detergent fibre (NDF)	327	320	305
Acid-detergent fibre (ADF)	155	156	149
Nutritive value [3] (Fresh feed)			
Digestible protein (%)	12.2	12.4	12.4
Digestible energy (kcal/kg)	2634	2660	2675

[1] Chemical composition (%): humidity 20.5; crude protein 0.3; crude fibre 0.1; crude fat 0.1; crude ash 0.3; crude energy 3400 $kcal.kg^{-1}$ (data from Roquette SA); about 70% of resistant starch, acording Liu *et al.* (2007). [2] calcium phosphate 300 mg, calcium carbonate 400mg, methionine 200mg, cupper 17.4 mg; iron 64 mg; Zinc. 37.3 mg; Manganese 18.4 mg; Vit. A 900000U.I. ; Vit. D3 100000 U.I. ; Vit. E 16,5mg.; Vit. B1 1mg; Vit. C 50mg; Robenine 66mg (per kg of feed). Cr_2O_3 maker at 0.3%. [3] Calculated values according to Maertens *et al.*, 2002.

Measurements

Individual feed intake and live weight were recorded weekly. Digestibility coefficient for the whole tract was measured according to Perez *et al.* (1995) and ileal digestibility was calculated according the method described by Blas *et al.* (2000), using chromium oxide as marker. The fibrolytic activity of the caecal microbiota was measured in caecotrophes, by analysing the concentration of enzymes synthesized specifically by bacteria, as described previously (Gidenne *et al.*, 2002). The enzymes were extracted from microorganisms and the polysaccharidase activity was determined by measuring the release of reducing sugars, quantified by spectrophotometry, from the polysaccharide purified substrate (carboy methyl cellulose, poplar xylane, apple pectins).

Table 2: Experimental scheme followed in this study

Local	Group	Period I	Period II
INRA	I	Diet WS	Diet PS7
	II	Diet PS14	Diet PS7
UTAD	I	Diet WS	Diet PS14
	II	Diet PS7	Diet PS14

Chemical analyses

Samples of feed and faeces were analyzed for dry matter (DM), organic matter and ash, crude fat and crude protein (total nitrogen×6.25) according to harmonized procedures (EGRAN, 2001). Content of NDF was determined without sodium sulphite and with a heat-stable amylase (Van Soest *et al.*, 1991). Acid detergent fiber (ADF) was determined by sequential analysis of the residual NDF and expressed exclusive of residual ash. Nitrogen was determined by DUMAS combustion method. Starch was hydrolyzed enzymatically and the resultant glucose was measured by using the hexokinase (EC 2.7.1.1) G6PDH (NAD) (EC 1.1.1.49) system (Boëhringer Mannheim).

Statistical analysis

In the first step we studied the influence of the experimental unit (effect "unit"), comparing the results obtained with the diet WS in UTAD and INRA. In the second step we investigated the effect "group", comparing the values obtained with the same food (PS7 and PS14 in the INRA and UTAD) in period two, separately for each unit. In the third step we analyzed the effect of "period", comparing the results obtained with the same food in periods one and two through a bifactorial variance analysis ("diet" and "period"). The effect of diet was analyzed in the fourth step. Analysis of variance was performed by the GLM procedure of SAS and in the multiple comparison of means were used the Scheffe test.

RESULTS AND DISCUSSION

The effects of "group", "period" and "animal" were not significant, and results were presented according to the diet effect only. The feed intake was not affected by the incorporation of RS in the diets (table 3). The amount of caecotrophes produced with diet PS14 showed a trend (P = 0.07) to be higher (+ 4.6g DM/d) than in WS or PS7 groups. The amount of collected caecotrophes (12 to 16g DM/d) is slightly lower than the 20g DM/d, referenced by Carabaño *et al*. (2000), but seems to be acceptable, because those authors reported a range of variation between 6 and 47g DM/d. The variability of the measures was higher for ileal digestibility than for total digestibility, which is in agreement with previous work (Blas *et al.*, 2000).

Table 3: Total, ileal and caeco-rectal digestibility's in adult rabbits according to potato starch level.

	Diets			MSE	Prob.	L [1]
	WS	PS7	PS14			
Feed intake (g DM/d/kg PV)	*20.5*	*21.1*	*25.4*	*2.90*	*ns*	-
Caecotrophes production (g DM/d)	11.6	11.5	16.2	1.65	0.07	-
Total tract apparent digestibility (%)						
Dry matter	70.3	69.9	69.2	0.42	ns	ns
Organic matter	71.7	71.3	70.4	0.43	ns	0.163
Energy	69.8	69.5	68.8	0.46	ns	-
Crude protein	72.9	72.8	72.7	0.86	ns	ns
Starch	99.7	99.6	99.6	0.04	ns	ns
NDF	40.5	40.2	39.3	0.91	ns	ns
Ileal digestibility (%)						
Dry matter	42.9	42.9	43.0	1.29	ns	ns
Organic matter	47.9	47.2	46.9	1.14	ns	ns
Energy	50.1	49.9	49.4	1.11	ns	ns
Crude protein	70.6	67.7	68.3	1.42	ns	
Starch	98.3 ^{A}	97.4 ^{B}	96.7 ^{C}	0.23	0.001	<0.001
NDF	26.4	28.3	28.2	2.03	ns	-
Caeco-rectal digestibility (%)						
Dry matter	27.5	27.0	26.2	1.16	ns	ns
Organic matter	23.8	24.1	23.5	1.03	ns	ns
Energy	19.6	19.6	19.4	1.03	ns	ns
Crude protein	2.4	5.1	4.4	1.5	0.092	-
Starch	1.7	2.1	3.0	0.37	0.154	0.009
NDF	14.2	11.9	11.2	1.89	ns	-

Means with different letters on the same row differ significantly (Tukey test); [1] L: Linear effect of starch nature. MSE : mean square error. n=11 in WS diet and n=15 in PS7 and PS14 diets; NS: P>0.05.

In total, ileal and caecal digestibility only the ileal starch digestibility were affected by diet. The starch ileal digestibility decreased with the addition of potato starch (P = 0.001). When we analyzed the linear effect of the nature of the starch, only caecal and ileal digestibility of starch were significantly influenced by diet; the incorporation of potato starch decreased the ileal digestibility and increased the caecal-rectal digestibility. Consequently, the whole tract digestion of starch was unaffected by RS incorporation.

The ileal digestibility of starch was very high (> 96%), even in animals that received diet with 14% of potato starch, and the ileal digestibility of this diet was lower in only 1.6 percentage points, when compared to the control diet. The ileal digestibility of starch obtained in rabbits was higher than in pigs and was less influenced by the incorporation of RS in the diet (Martin *et al.*, 1998), which can be due to the caecotrophy in rabbits that allows a greater caecal predigestion, by the action of salivary, pancreatic and microbial amylases (Scapinello *et al.,* 1999).

The bacterial enzyme activities of cellulase, xylanase and pectinase were not influenced by RS in diets (Figure 1). Of the three enzymes studied, the pectinase showed the highest activity, followed by cellulase and xylanase (317, 85 and 16 mmol of reducing sugars released per gram of dry matter and hours, respectively). The enzymatic activity of bacterial enzymes studied presented a scaling similar to that observed by Marounek *et al.* (1995). The activity of these enzymes was not influenced by diet, because the amounts of fiber, protein and energy that reached the caecum were similar. Conversely, the differences in the flow of starch entering the caecum were very weak, and thus would not be able to significantly alter the microbial population and caecal activity.

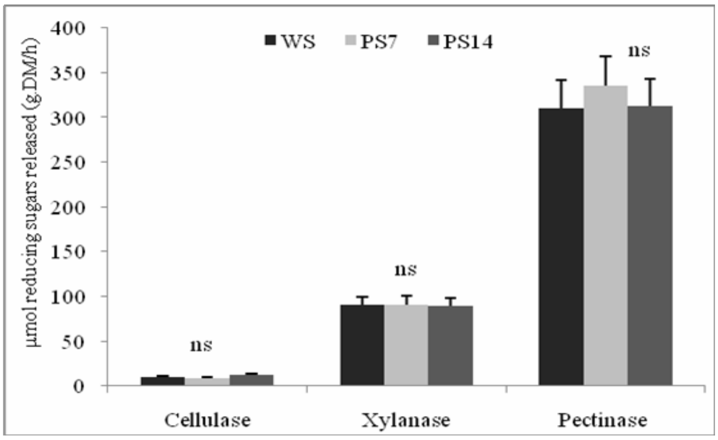

Figure 1: Bacterial fibrolytic activity (mean±sd) of the caecal flora of adult rabbit (n=8/diet) according to potato starch level.

CONCLUSIONS

Even for a high intake of potato raw starch, the ileal starch concentrations and flow remained low, that reflect the high digestive efficiency for starch in the small intestine of the rabbit. Consequently, an overload of starch in the caecum is not likely a factor of microbial unbalance in the caecum.

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