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Effect of substitution of wheat starch by potato starch on the performance, digestive physiology and health of growing rabbits

V. Pinheiro^{1†}, L. Falcão e Cunha², J. L. Mourão¹ and T. Gidenne^{3,4,5}

¹UTAD, CECAV – Department of Animal Science, University of Trás-os-Montes e Alto Douro, PO Box 1013, 5000-911 Vila Real, Portugal; ²UTL, ISA-CEER – Instituto Superior of Agronomia, Tapada da Ajuda, 1349-017 Lisboa, Portugal; ³INRA, UMR1289 Tissus Animaux Nutrition Digestion Ecosystème et Métabolisme, F-31326 Castanet-Tolosan, France; ⁴INPT ENSAT, UMR1289 Tissus Animaux Nutrition Digestion Ecosystème et Métabolisme, Université de Toulouse, F-31326 Castanet-Tolosan, France; ⁵ENVT, UMR 1289 Tissus Animaux Nutrition Digestion Ecosystème et Métabolisme, F-31076 Toulouse, France

(Received 20 April 2012; Accepted 28 November 2012)

The goal of this research was to study the effect of the substitution of wheat starch by potato starch (PS) on the performance, health and digestion of growing rabbits. Three experimental diets were formulated with 0%, 7% and 14% PS (PS0, PS7 and PS14, respectively) and similar starch contents (22% dry matter basis), proteins and fibre. The three diets were administered to three groups of 48 rabbits from weaning (28 days) to slaughter (70 days), and growth and health measurements were made. Another 10 rabbits per diet (30 rabbits at each age), reared under similar conditions, were slaughtered at 6 to 10 weeks of age, and the digesta were collected to analyse the caecal microbial activity (pH, volatile fatty acids (VFA) levels, fibrolytic activity) and the starch concentration in the ileal digesta. At the same ages, the whole tract digestibility coefficients were measured in 10 other rabbits for each treatment (30 rabbits). The feed intake between 28 and 42 days of age (days) increased by 11% ($P < 0.05$) in PS0 v. PS14. Over the whole growth period (28 to 70 days), weight gain was similar among diets (40.5 g/day), whereas the feed intake and feed conversion increased (8.5% and 5.2%, respectively; $P < 0.05$) with the PS14 diet. Mortality and morbidity were not affected by the diets. The starch concentration of the ileal contents increased ($P < 0.01$) with the addition of PS to the diet (0.39%, 0.77% and 1.08% for diets PS0, PS7 and PS14, respectively). Starch digestibility was 0.8 percentage units higher (99.8% v. 99.0%) with the PS0 diet than the PS14 diet ($P = 0.04$). The bacterial cellulolytic activity in the caecum tended to be higher with the PS14 diet ($P = 0.07$). The total VFA caecal concentration increased ($P < 0.01$) only in 6-week-old rabbits with PS7 compared with PS0 (54.7 v. 74.5 mmol/l). Protein digestibility and ileal starch concentration decreased ($P < 0.05$) with age (6 v. 10 weeks), and hemicelluloses digestibility increased ($P < 0.05$). At 10 weeks of age, rabbits showed a higher VFA pool (6.25 mol) and proportion of butyrate (15.9%) and a lower proportion of acetate (79.3%), ammonia level (7.5 mmol/l) and C3/C4 ratio (0.31) than at 6 weeks of age. The intake of potato starch had no effect on the performance, caecal microbial activity or digestive health of growing rabbits.

Keywords: growing rabbits, resistant starch, caecal microbial activity, health status

Implications

Because of the specific digestive physiology of rabbits, the dietary level and quality of starch can play an important role in their growth and health. The literature provides conflicting results regarding starch digestion and the unfavourable role of undigested starch on the caecal microbial activity and digestive health in growing rabbits. This work attempted to clarify this question by comparing the effects of highly digestible starch (from wheat) to those of digestion-resistant starch (from potato) on the growth and digestive processes in the rabbit.

Introduction

Starch is the primary source of carbohydrates and one of the main energy sources in monogastric diets, and it is generally hydrolysed by amylolytic enzymes in the small intestine. However, a fraction of starch, which is defined as resistant starch, is not digested in the small intestine and reaches the large intestine, where it is hydrolysed and fermented by micro-organisms. Potato starch (PS) is 20% amylose, which has large granules and a B-crystalline structure that is not generally accessible to digestive enzymes (Champ and Colonna, 1993; Noda *et al.*, 2008). *In vitro* hydrolysis of raw potato starch with amylolytic enzymes showed significant resistance to these enzymes (Lee *et al.*, 1985; Bauer *et al.*, 2003). Thermal

[†] E-mail: vpinheir@utad.pt

treatments can modify the structure of starch and decrease its resistance to amylases (Kingman and Englyst, 1994).

Wheat starch (WS) is commonly used in diets for rabbits. This starch is predominantly composed of amylopectin and can be almost fully digested in the small intestine (93% to 98%, Blas and Gidenne, 2010) because it contains only small amounts of resistant starch. Even in the case of highly digestible starch, such as raw wheat starch, several authors have hypothesised that an excess of starch in rabbit feeds could lead to an increase of undegraded starch flowing through the small intestine due to limited amylolytic capacity. When this undegraded starch reaches the caecum and the colon, it may unbalance the microbiota and cause digestive disturbances (Cheeke and Patton, 1980). However, Blas and Gidenne (2010) showed that there is no direct relationship between the dietary starch level and mortality, which depends instead on fibre intake (Gidenne *et al.*, 2010), namely, the type of fibre and feed composition (De Blas, 2012).

A review of the literature concerning the digestion of starch in the rabbit (Blas and Gidenne, 2010) indicated that few studies have examined the problem of its digestion in the small intestine and the effect of the nature of starch on the microbial activity and digestive health of the growing rabbit, particularly during the growing period. Moreover, only a small number of reports have studied the effect of the nature (origin) of the starch. The available studies on the effects of starch on rabbits mainly compared diets based on wheat, barley, maize and peas (Gidenne and Perez, 1993a; Gutiérrez *et al.*, 2002; Xiccato *et al.*, 2002). Studies in other species, mainly rats (Mathers *et al.*, 1997; Le Blay *et al.*, 2003) and pigs (Martin *et al.*, 1998; Hedemann and Knudsen, 2007; Bhandari *et al.*, 2009), primarily studied the effects of the incorporation of raw potato on the fermentative characteristics of starch in the large intestine.

Experiments were performed to determine the effects of high intake of starches with different origins (wheat and potato) on the growth performance, health, success in digestion, caecal fermentative traits and fibrolytic activity of growing rabbits.

Material and methods

Diets and experimental design

The trials were carried out in the animal facility of the University of Trás-os-Montes e Alto Douro at Vila Real. Rabbits were kept in a closed and temperature-controlled facility (18°C to 23°C and received 12 h of light daily (0700 to 1900 h). Animals were handled according to the principles of the care of animals in experimentation (Port. no 1005/92).

Three experimental diets were formulated (Table 1) to obtain a linear replacement of wheat starch by purified raw potato starch 'PS' (Roquette SA, Lestrem, France) at levels of 0%, 7% and 14% for the PS0, PS7 and PS14 diets, respectively. The level of starch, fibre and CP were similar in all diets (Table 1). The diets contained 16% acid detergent fibre (ADFom), corresponding to the minimal ADF levels required for growing rabbits (De Blas and Mateos, 2010; Gidenne *et al.*, 2010). To attempt to

Table 1 Major ingredients, chemical composition and nutritive value of experimental diets

	Diets		
	PS0	PS7	PS14
Ingredients (g/kg raw basis)			
PS ¹	0	70	140
Wheat (whole grain)	310	205	100
Soya bean meal 48% CP	160	180	195
Wheat bran	95	105	130
Dehydrated lucerne meal 14% CP	170	175	180
Dehydrated beet pulp	190	190	180
Wheat straw	55	55	55
Minerals and vitamins and additives ²	20	20	20
Chemical composition (g/kg DM)			
DM (g/kg feed)	899	905	897
Organic matter	949	934	943
CP (N × 6.25)	188	185	184
Starch	220	215	223
aNDFom	364	354	340
ADFom	172	172	166
Lignin	30	34	28
Hemicellulose (aNDFom–ADFom)	192	182	174
Cellulose (ADFom–lignin)	142	1380	138
Gross energy (MJ/kg DM)	18.4	18.2	18.3

PS = potato starch; DM = dry matter; aNDFom = neutral detergent fibre; ADFom = acid detergent fibre.

¹Chemical composition (%): humidity 20.5; CP 0.3; crude fibre 0.1; fat 0.1; minerals 0.3; gross energy 14.23 MJ/kg (according to the supplier).

²Calcium phosphate 300 mg, calcium carbonate 400 mg, methionine 200 mg, copper 17.4 mg; iron 64 mg; zinc 37.3 mg; manganese 18.4 mg; vitamin A 900 000 U.I.; vitamin D3 100 000 U.I.; vitamin E 16.5 mg; vitamin B1 1 mg; vitamin C 50 mg; robenidine 66 mg (per kg of feed according to supplier).

generate a potential detrimental effect of a high ileal flow of starch on caecal microbial activity and rabbits' health, the level of starch in diets was high (22%). In the PS7 and PS14 diets, ~1/3 and 2/3 of the dietary starch, respectively, came from raw PS (resistant starch).

Growth performances and health status

To measure growth performance and health status, the experimental diets were given *ad libitum* to three groups of 48 rabbits of both sexes (New Zealand × Californian), which were housed individually in cages, from weaning (28 days) to slaughter (70 days). Feed intake and live weight (LW) were recorded weekly. The results are summarised in the following three periods: for 28 to 42, 42 to 70 and 28 to 70 days.

Health status was recorded individually three times a week. All clinical signs of sickness (e.g. transitory diarrhoea, presence of mucus in excreta, abnormal behaviour) were registered. Morbidity was defined as the animals' having abnormally low intake or growth (<mean – 2.2 s.d.) or clinical signs of sickness. Animals with morbidity were not included in growth performance analyses. The sanitary risk index (SRI) was calculated as the sum of mortality and morbidity.

Whole tract digestive efficiency and digesta sampling

Whole tract digestibility was measured according to the method of Perez *et al.* (1995) between 37 and 41 days and between 65 and 69 days on three groups of 10 rabbits from the same litters. Rabbits were housed individually in metabolic cages from weaning (28 days) to 69 days and were fed the experimental diets. Data from rabbits with diarrhoea or other signals of sickness were rejected.

At 42 and 70 days, 10 rabbits per diet were slaughtered (between 1130 and 1230 h) by sudden cervical dislocation. For each rabbit, one sample of the contents of the terminal ileum (segment of 20 cm before the ileo-caecal junction) was taken to measure the starch level, and four samples of the caecal contents were taken to measure dry matter, volatile fatty acids (VFA), ammonia (NH₃) and fibrolytic activity. The samples for enzymatic fibrolytic activity were collected in precooled (4°C) tubes containing an anaerobic buffer and were stored at -80°C until further processing, and the other samples were stored at -20°C until analysis.

Chemical analysis and caecal fibrolytic activity

Samples of feed and faeces were analysed for ash, dry matter, organic matter, crude fat and CP (total nitrogen × 6.25) according to Gidenne *et al.* (2001). The content of neutral detergent fibre (aNDFom) was determined without sodium sulphite and with a heat-stable amylase (Van Soest *et al.*, 1991). ADFom and ADL (lignin) were determined by sequential analysis and expressed exclusive of residual ash (Van Soest *et al.*, 1991).

Nitrogen was determined by the Dumas combustion method using Leco apparatus (model FP-428, Leco Corporation, St. Joseph, MI, USA). Starch of all samples was hydrolysed enzymatically (Edwards, modified by Kozlowski, 1994), and the resultant glucose was measured using the hexokinase (EC 2.7.1.1) G6PDH (NAD) (EC 1.1.1.49) system (Boehringer Mannheim). The gross energy of the diets was measured with an adiabatic calorimeter (PARR Instrument; Moline, IL, USA). The *in vitro* assay of starch hydrolysis of samples of the raw material (WS and PS) used to prepare the diets was performed at 37°C and 60°C according to the method of Tollier and Guilbot (1974). This *in vitro* analysis was performed with diets only at 60°C. The raw material and the diets were incubated in triplicate with stirring, and samples of the solution (1 ml) were taken at different times (0, 5, 10, 20, 30, 60, 120 and 210 min) to measure reducing sugars. The results were expressed as a proportion of the starch incubated. The resultant reducing sugars were measured according to the DNS method (Miller, 1959).

The VFA of the caecal contents were analysed by gas liquid chromatography (CP9000, Chrompack, Middelburg, The Netherlands) on a semi-capillary column. The VFA pool was calculated by multiplying the VFA concentration (μmol/l) by the caecal contents (g) by the percentage of water in the caecal contents (Demigé and Rémésy, 1985). The caecal NH₃ measurements were performed using the colorimetric method of Verdouw *et al.* (1977).

The fibrolytic activity of the caecal contents was analysed by measuring the concentration of cellulase, xylanase and pectinase synthesised by the bacteria as described by Gidenne *et al.* (2002).

Statistical analysis

Data on the growth and feed intake of the rabbits were examined by one-way ANOVA with the GLM procedure of the Statistical Analysis System (SAS, online guide). The digestibility coefficients of the feeds, caecal characteristics, fibrolytic activity of caecal contents and ileal starch results were tested for the effect of age, diet and the interaction age*diet. Means were subsequently compared using the Scheffe test. When a significant interaction ($P < 0.05$) occurred between the effect of age and the effect of the diet, the data were further subjected to the following two types of statistical analyses: within the same age (among the three diets) and within the same diet (between the two ages). The catmod procedure (SAS) was utilised for the analysis of mortality, morbidity and sanitary risk. The linear effect of the potato starch level in the feed was analysed for all studied parameters of growth performance and digestibility. Differences among the means with $P < 0.05$ were accepted as representing statistically significant differences. Differences among means with $0.05 < P < 0.10$ were accepted as representing tendencies toward differences.

Results

Diets and starch *in vitro* hydrolysis

As expected, the diets had similar concentrations of starch, protein and fibre (Table 1). The gross energy of the diets was also analogous, varying from 18.2 to 18.4 MJ/kg.

The *in vitro* hydrolysis of the two types of starch (wheat and potato) at 37°C and 60°C revealed a lower speed of hydrolysis for potato starch compared with wheat starch (Figure 1). Within 210 min at 37°C, 30% of the wheat starch was hydrolysed compared with <2% of the potato starch. The increase in the incubation temperature increased the hydrolysis of both the potato starch and the wheat starch by a similar value (ca. 15% units). The *in vitro* hydrolysis at 60°C

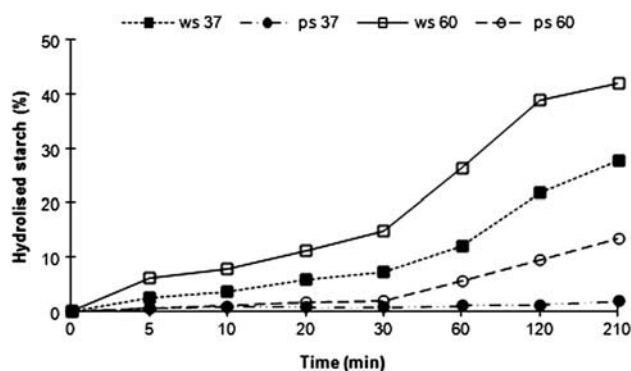


Figure 1 Comparative *in vitro* hydrolysis (at 37°C and 60°C) of WS or PSs ($n = 3$). WS = wheat starch; PS = potato starch.

exhibited lower values with the PS7 and PS14 diets over any time of incubation (Figure 2).

Growth performances and health status

The dietary level of potato starch had no significant effect on the LW of rabbits at slaughter (Table 2). However, at 42 days of age, rabbits fed diet PS14 showed a higher LW (+99 g) than rabbits fed diet PS0 ($P < 0.05$).

With the increase of the level of potato starch, feed intake increased 11% during the 2 weeks after weaning ($P < 0.05$). In the finishing period (42 to 70 days), the feed intake tended to a further increase (more 8%; $P = 0.09$). Over the total growing period, rabbits fed the PS14 diet showed a higher feed intake than those fed PS0 ($P < 0.05$). In all periods

studied, the addition of potato starch had a significant linear effect on daily feed intake ($P < 0.05$).

The daily weight gain during the post-weaning period increased with the incorporation of PS (+14% for PS14, $P < 0.01$). Over the whole growth period (35 to 70 days of age), weight gain was similar among diets.

In the finishing (42 to 70 days of age) and whole growth periods, feed conversion was higher with the PS14 than with PS0 diets ($P < 0.05$). In these periods, the potato starch level showed linear effects on feed conversion ($P < 0.05$).

Mortality and morbidity were not significantly different between diets (Table 3). In the period from 42 days to slaughter, the sanitary risk doubled with the PS7 diet (4% v. 8%) and tripled with the PS14 diet (4% v. 12%) with respect to the PS0 diet. Accordingly, the linear analysis showed that the SRI between 42 and 70 days old increased with the addition of PS to the diet ($P < 0.05$), and tended increase over the whole growth period ($P = 0.059$).

Digestibility coefficients

Except for starch and CP, whole tract digestibility was not affected by the incorporation of any level of potato starch in the diet (Table 4). The digestibility of starch was higher (+0.8 unit, $P = 0.04$) with the PS0 diet than with the PS14 (99.8% and 99.0%, respectively). The analysis of the effect of age on whole tract digestibility showed a higher protein digestibility at 6 weeks than at 10 weeks (+5 units, $P < 0.05$). At 10 weeks, a higher hemicellulose digestibility was found ($P < 0.05$) in addition to a trend for higher ADFom digestibility ($P = 0.08$).

The linear analysis of the effect of potato starch level on the whole tract digestibility indicated a significant negative

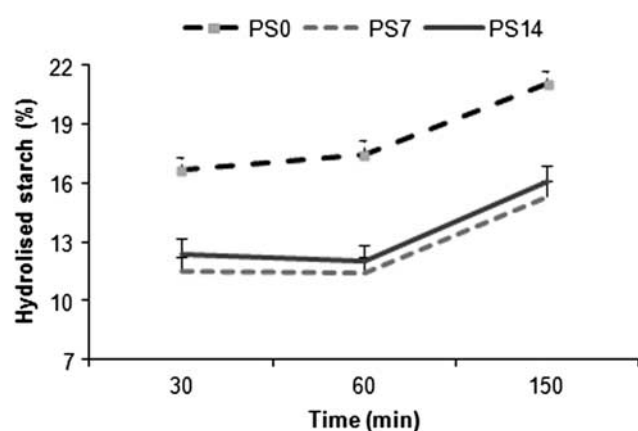


Figure 2 Comparative *in vitro* hydrolysis at 60°C of different diets ($n = 3$). PS = potato starch.

Table 2 Effect of crude PS dietary incorporation on growth and feed intake of rabbits from weaning (28 days) to slaughter (70 days) by ($n = 48$ animals per treatment)

	Diets			s.e.m.	P-level ¹	L ²
	PS0	PS7	PS14			
LW						
Weaning (g)	575	578	579	8.3	ns	ns
At 42 days (g)	1125 ^b	1175 ^{ab}	1224 ^a	15.9	*	*
At slaughter (g)	2225	2307	2320	27.3	ns	ns
Daily feed intake (g/day)						
Weaning – 42 days	63.4 ^b	65.4 ^b	70.9 ^a	1.29	*	*
42 days – slaughter	105.3	111.6	114.0	3.61	0.09	*
Weaning to slaughter	92.1 ^b	95.9 ^{ab}	99.9 ^a	2.16	*	*
Daily weight gain (g/day)						
Weaning – 42 days	39.2 ^B	42.0 ^{AB}	45.4 ^A	0.89	**	**
42 days – slaughter	39.5	40.0	38.8	0.65	ns	ns
Weaning to slaughter	39.4	41.0	41.8	0.68	ns	ns
Feed conversion ratio						
Weaning – 42 days	1.62	1.58	1.59	0.014	ns	ns
42 days – slaughter	2.72 ^b	2.77 ^{ab}	2.92 ^a	0.038	*	*
Weaning to slaughter	2.31 ^B	2.34 ^{AB}	2.43 ^A	0.021	**	*

PS = potato starch; s.e.m. = standard error of the mean; LW = live weight.

¹In the same line, means with different superscripts differed significantly (lowercase $P < 0.05$ and uppercase at $P < 0.01$).

²Linearity test of starch level.

effect on starch digestibility ($P < 0.05$) and a trend toward decreasing organic matter digestibility ($P = 0.09$).

Caecal microbiota activity and ileal starch concentration

A significant interaction ($P < 0.05$) between the effect of the age (6 v. 10 weeks) and the effect of the diets was observed for the caecal VFA concentration (Table 5 and Figure 3). In 6-week-old rabbits, the VFA level was higher with the PS0 diet than with the PS7 diet (68 v. 54 mmol/l, $P < 0.05$); conversely, at 10 weeks of age, no significant effect of the dietary treatments was observed.

Acetic acid (C_2) was the predominant VFA, representing ~80% followed by butyric (C_4) and propionic (C_3) acids. The proportions of acetic (pC_2) and butyric (pC_4) acids were affected by age ($P < 0.05$). The proportion of propionic (pC_3) was not affected. Ten-week-old rabbits had a higher

butyrate proportion that was compensated by a decrease in acetate. In the oldest rabbits, the NH_3 concentration and the C_3/C_4 ratio decreased ($P < 0.05$). The pool of VFA showed a trend to be higher in the 10-week-old rabbits (+43%; $P = 0.051$).

The weights of empty caecum and caecal content as a proportion of the rabbits LW (Table 5) decreased with age ($P < 0.01$).

A significant interaction ($P < 0.05$) between the effect of age (6 v. 10 weeks old) and the effect of diet was detected on the ileal starch concentration (Table 5 and Figure 4). In 6-week-old rabbits, the ileal starch concentration increased sharply ($P < 0.05$) from diet PS0 to PS7 to PS14 but remained $< 1.5\%$, even with diet PS14. At 10 weeks of age, the ileal starch concentration remained similar among diets (mean 0.5%). The starch concentration in the ileum decreased with age (6 v. 10 weeks) in rabbits fed diets PS7 and PS14 ($P < 0.05$).

The linear analysis of the effect of potato starch level only showed a significant effect on the NH_3 caecal concentration ($P < 0.05$) and the ileal concentration of starch ($P < 0.001$). In both cases, the values increase with the increase of potato starch level.

Treatments did not affect cellulase, xylanase and pectinase activities (Table 6).

Discussion

High starch intake and digestion in the growing rabbit

The knowledge on the effects of starch origin and quality on the digestive response of the growing rabbit remain limited because there are few studies that analyse the effect of origin (wheat, barley, corn, pea, potato) and because the studies are restricted to the effects on growth and digestibility (Lee *et al.*, 1985; Gidenne and Perez, 1993a and 1993b; Gutiérrez *et al.*, 2002; Xiccato *et al.*, 2002; Blas and Gidenne, 2010).

Raw potato starch is known to be resistant to digestion in the small intestine. Martin *et al.* (1998) observed that 56%

Table 3 Effect of crude PS dietary incorporation on health status (morbidity, mortality and SRI; number and (%)) of the growing rabbit ($n = 48$ animals per treatment)

	Diets			P-level ¹	L ²
	PS0	PS7	PS14		
Weaning to 42 days					
Mortality	2 (4.1)	5 (10.4)	1 (2.1)	ns	ns
Morbidity	7 (14.6)	6 (12.5)	9 (18.7)	ns	ns
SRI	9 (18.7)	11 (22.9)	10 (20.8)	ns	ns
42 days to slaughter					
Mortality	0 (0)	3 (7.0)	3 (6.4)	ns	ns
Morbidity	4 (8.7)	5 (11.6)	9 (19.1)	ns	ns
SRI	4 (8.7)	8 (18.6)	12 (25.5)	ns	*
Weaning to slaughter					
Mortality	2 (4.2)	8 (16.7)	3 (6.3)	ns	ns
Morbidity	11 (22.9)	11 (22.9)	18 (37.5)	ns	ns
SRI	13 (27.1)	19 (39.6)	22 (45.8)	ns	0.059

PS = potato starch; SRI = sanitary risk index.

¹Significance level.

²Linearity test of starch level.

Table 4 Effect of crude PS dietary incorporation and age on whole tract digestive efficiency ($n = 10$ animals per treatment)

	Diets			Age (weeks)		s.e.m. ¹	P-level ²			L ²
	PS0	PS7	PS14	6	10		Diet	Age	D × A	
Digestibility (%)										
DM	72.6	72.1	71.0	72.1	71.6	0.74	ns	ns	ns	ns
Organic matter	72.8	72.2	71.0	72.0	71.9	0.72	ns	ns	ns	0.09
CP	79.6 ^{ab}	80.3 ^a	77.9 ^b	81.8	76.6	0.63	0.05	***	ns	ns
aNDFom	41.0	40.4	38.4	38.4	41.5	0.98	ns	ns	ns	ns
ADFom	29.8	28.3	28.1	26.7	30.9	1.18	ns	0.08	ns	ns
Starch	99.8 ^a	99.5 ^{ab}	99.0 ^b	99.4	99.5	0.11	*	ns	ns	*
Cellulose	52.0	52.3	49.0	50.0	52.1	0.82	ns	ns	ns	ns
Hemicellulose	30.8	31.0	33.1	29.0	34.5	1.33	ns	*	ns	ns

PS = potato starch; DM = dry matter; s.e.m. = standard error of the mean; aNDFom = neutral detergent fibre; ADFom = acid detergent fibre.

¹In the same line, means with different superscripts differed significantly (lowercase $P < 0.05$ and uppercase at $P < 0.01$).

²Linearity test of starch level.

Table 5 Effect of crude PS dietary incorporation and age on caecal fermentative activity and starch concentration in the ileum of growing rabbits (n = 10 animals per treatment)

	Diets			Age (weeks)			P-level ¹			L ²
	PS0	PS7	PS14	6	10	s.e.m.	Diet	Age	D × A	
Caecal content characteristics										
VFA ²	61.3	67.6	62.8	64.6	63.2	3.21	ns	ns	*	ns
pC2	81.6	82.0	80.7	83.6	79.3	0.56	ns	***	ns	ns
pC3	5.0	4.7	4.7	4.9	4.7	0.28	ns	ns	ns	ns
pC4	13.3	13.5	14.7	11.7	15.9	0.46	ns	***	ns	ns
pVFAm	0.3	0.3	0.4	0.4	0.3	0.07	ns	0.09	ns	ns
'pool' VFA (mol)	5.4	5.4	5.1	4.4	6.2	0.29	ns	***	0.051	ns
NH ₃ (mmol/l)	8.3	8.4	8.5	9.3	7.5	0.45	ns	**	ns	*
C3/C4	0.4	0.4	0.3	0.4	0.3	0.04	ns	***	ns	ns
DM (%)	21.6	21.1	21.4	21.5	21.3	0.47	ns	ns	ns	ns
Caecum (% LW)										
Empty	1.7	1.6	1.6	1.7	1.5	0.07	ns	**	ns	ns
Contents	7.2	6.5	6.6	8.0	5.6	0.32	ns	***	ns	ns
Concentration in the ileum(% DM)										
Starch	0.4	0.8	1.1	0.9	0.6	0.07	***	*	0.047	***

PS = potato starch; s.e.m. = standard error of the mean; LW = live weight; DM = dry matter; VFA = volatile fatty acid; pC2 = proportion of acetic acid; pC3 = proportion of propionic acid; pC4 = proportion of butyric acid.

¹In the same line, means with different superscripts differed significantly (lowercase $P < 0.05$ and uppercase at $P < 0.01$).

²Linearity test of starch level.

Q4

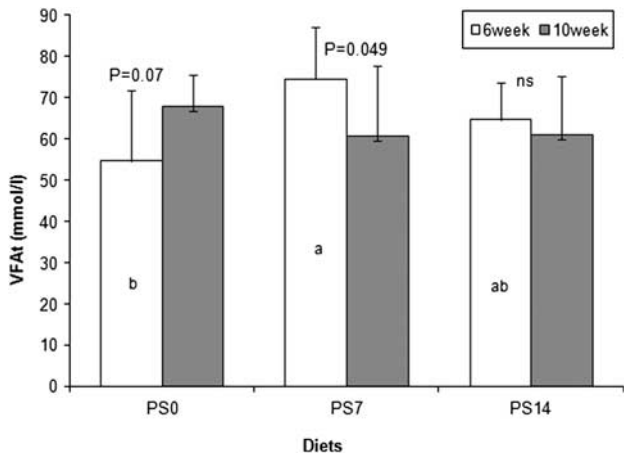


Figure 3 Effect of crude potato starch (PS) dietary incorporation and age on total volatile fatty acid (VFA) concentration in the caecum of growing rabbits. Within the same age, means with different superscripts (a, b) differ at the level $P = 0.05$ (effect of diet). Within the same diet, means with a different superscript differed at $P < 0.05$ (effect of age).

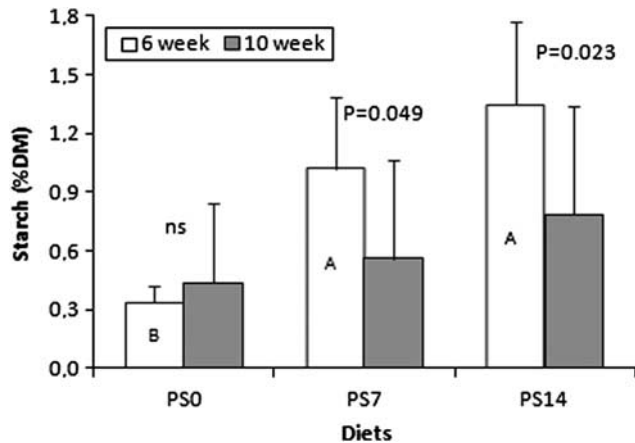


Figure 4 Effect of crude potato starch (PS) dietary incorporation and age on starch concentration in the ileum of growing rabbits. Within the same age, means with different superscripts (a, b) differ at the level of $P = 0.05$ (effect of diet). Within the same diet, means with different superscripts differed at $P < 0.05$ (effect of age).

of starch is not digested in the small intestine in pigs fed a diet containing 6.5% potato starch. The results obtained in the *in vitro* hydrolysis of potato starch by Lee *et al.* (1985) and in the present study confirm these findings.

Several studies have shown that thermal treatment of resistant starch can reduce its resistance to hydrolyse and can have a positive effect on starch digestibility (Kingman and Englyst, 1994; Sun *et al.*, 2006). The *in vitro* hydrolysis achieved in present work confirmed this effect, suggesting that the temperature of feed manufacture can have positive effect on starch digestibility. However, this effect does not

seem to be dependent on the starch origin. Consequently, it is expected that after granulation, diets with potato starch should maintain a high level of resistant starch. This finding was confirmed by the *in vitro* starch hydrolysis of the diets in which ~85% remained not hydrolysed. However, the *in vivo* study with rabbits exhibited notably high levels of starch digestibility (99%) of the diets with potato, which was only slightly lower than the starch digestibility of the diet with wheat. The results of *in vitro* starch hydrolysis of raw material at 37°C and 60°C and diets at 60°C seem to show that the high *in vivo* starch digestibility of the PS7 and PS14 diets are not simply due to a thermal effect of diet granula-

Table 6 Effect of crude PS dietary incorporation and age on bacterial fibrolytic activity in the caecum of growing rabbits (n = 10 animals per treatment)

	Diets			Age (weeks)		s.e.m.	P-level ¹			L ²
	PS0	PS7	PS14	6	10		Diet	Age	D × A	
Cellulase ³	14.9	13.2	18.4	15.2	15.3	1.52	0.07	ns	0.10	ns
Xylanase ³	58.6	50.3	63.0	62.1	52.5	7.23	ns	ns	ns	ns
Pectinase ³	189	143	200	179	175	18.4	ns	ns	ns	ns

PS = potato starch; s.e.m. = standard error of the mean.

¹In the same line, means with different superscripts differed significantly (lowercase P < 0.05 and uppercase at P < 0.01).²Linearity test of starch level.³mmol of reducing sugars by g DM/h.

tion on resistant starch. These results of *in vitro* hydrolysis show a similar positive effect of temperature on both types of starch (wheat and potato), although a higher fraction of potato starch remains resistant. Consequently, we can affirm that rabbits have a higher capacity to digest potato starch than what has been observed in other animal species, such as the pig.

The observed starch concentrations in the terminal ileum contents of rabbits fed diets with wheat or potato starch was low (0.4% to 1.2%). Blas and Gidenne (2010) found similar values when wheat was used as raw material and slightly higher values with corn. These values represent a marginal loss of starch in the faeces, lower than the faecal loss observed by Gidenne and Perez (1993a) in growing rabbits fed with diets containing barley starch (losses of 1%) and resistant corn starch (losses of 6%). Pinheiro and Gidenne (1999) reported 1.4% with a diet similar to PS0, which represents an estimated ileal starch loss lower than 2 g per day. These concentrations of starch in the ileum suggest that more than 90% of the starch was digested before the caecum, even when two-thirds of the starch ingested had potato origin (diet PS14). This high capacity of rabbits to digest starch, including resistant starch, could be explained by the endogenous amylase activity and also by amylase activity provided by the bacteria of soft faeces of previous intakes stored in fundus of the stomach (Scapinello *et al.*, 1999).

According to Gidenne and Perez (1993b) and Arruda *et al.* (2000), the increase in ileal starch flow would enhance fibre digestion in the caecum. The results of these authors seem to be related to a surprisingly low starch digestibility (75% to 86%). The results obtained by Xiccato *et al.* (2002), Gidenne *et al.* (2005a) and the results obtained in the present work do not support such a hypothesis because no change in fibre digestibility was observed. It is likely that the small increase of the quantity of undigested starch entering in the caecum with the PS7 and PS14 diets had a reduced effect on the caecal microbial population. The observed lack of effect of diets with potato starch on fermentative activity, weight of caecal contents and development of the caecum are in keeping with this small effect. Furthermore, the studies of Nizza and Moniello (2000) and Xiccato *et al.* (2002) failed to observe effects of the starch source (barley *v.* maize) on fermentative activity. It is probable that the potato starch

changed the microorganism profile, contributing to observed increase of the degrading enzymatic activity of pectinases and hemicellulases without affecting the cellulolytic activity. These effects of starch on enzymatic activity are in agreement with previous studies on rabbits (Marounek *et al.*, 1995; Gidenne *et al.*, 2000). In the present work, these changes in enzymatic activity were not enough to increase fibre digestibility.

According to Cheeke and Patton (1980), a high flow of starch into the caecum could impair gut health because it may imbalance the microbial population. However, in the present work, the increase of resistant starch in the diet had no effects on the frequency of digestive troubles. This may be related to the small effect of the increase in potato starch on the quantity of undigested starch entering in the caecum and on the caecal microbial population previously described. The preceding studies reported higher ileal starch concentrations (5 to 10 times higher, Gidenne *et al.*, 2005a) that were not associated with a higher frequency of digestive troubles in the growing rabbit (Gidenne *et al.*, 2005b). The small effect observed on digestive troubles could be linked to the similar fibre level among the PS0, PS7 and PS14 diets. Therefore, our results supported the previous works showing that fibre intake plays a primary role in the frequency of digestive troubles (Gidenne, 2003; Gidenne *et al.*, 2010). However, the significant linear effect between potato starch level and sanitary risk suggested that with high starch levels and minimal ADF levels (16%), detrimental effects of starch intake on the digestive health of the growing rabbit could be observed.

The post-weaning and complete growth performance confirmed that rabbits have the ability to efficiently use diets containing digestion-resistant starches, such as raw potato starch. However, high levels of PS in the diet (14%) increased feed intake and feed conversion as result of the slightly lower digestive efficiency of these animals. The previous results also showed a slight detrimental effect of maize starch (more resistant to digestion than wheat) on feed conversion and were associated with a higher intake (Gidenne *et al.*, 2005b).

Effect of age of rabbits

Starch digestibility in the whole digestive tract of rabbits remained notably high (over 99.5%) and was only slightly

lower at 42 days of age. Consequently, a higher value of ileal starch was found in rabbits at 6 weeks of age than at 10 weeks, although both values were low (0.9% and 0.6%). Blas *et al.* (1994) found ~7% starch in the ileum content of 5-week-old rabbits. The effect of rabbit age on ileal starch interacted with the nature of the starch. The effect of age on the starch in ileal contents, with higher levels being observed at 6 weeks of age than at 10 weeks, was evidenced only in rabbits fed diets with resistant potato starch and was not significant with the PS0 diet. This finding was in agreement with Gidenne *et al.* (2000), which reported similar starch levels of the ileal contents in rabbits fed a diet of wheat starch at 6 or 10 weeks. We measured a lower level of ileal starch (<1.3%), in 6-week-old rabbits fed the PS14 diet. The differences among the previous values may also be due to difficulties in the measurement of starch in the ileal contents of the rabbit due to the low quantities of samples available and variable intake pattern of rabbits fed *ad libitum*, thereby leading to high inter-individual variability ($\approx 20\%$).

From 6 to 10 weeks of age, VFA production by rabbits was similar, although there were changes in the fermentation pattern, with a higher proportion of butyrate and lower proportion of acetate being observed in agreement with previous studies (Gidenne *et al.*, 2002; Guedes *et al.*, 2009).

Conclusions

The rabbit has a high capacity to digest starch, even after weaning and when potato starch is present in the diet. Consequently, a high intake of potato starch had no major impact on caecal microbial activity, digestive health and growth performance of the growing rabbit.

Acknowledgements

The authors thank Muriel Ségura, Carole Bannelier and André Lapanouse (INRA, UMR 1289 TANDEM Toulouse) and António Silva (Universidade de Trás-os-Montes e Alto Douro, Vila Real) for their technical assistance.

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