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Plasma glucose response and glycemic indices in pigs fed diets differing in *in vitro* hydrolysis indices

G. Giuberti, A. Gallo and F. Masoero†

Faculty of Agriculture, Institute of Food Science and Nutrition, Università Cattolica del Sacro Cuore, via Emilia Parmense 84, 29100 Piacenza, Italy

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Different dietary starch sources can have a great impact in determining starch digestion potential, thus influencing the postprandial blood glucose response. Our objectives were to define: (i) the incremental plasma glucose response in pigs fed diets containing various sources of starch differing in in vitro *digestion patterns, (ii) the* in vivo *glycemic index (GI) values for the same diets, (iii) the possible relationship between* in vitro *and* in vivo *data. Diets, formulated with 70% of starch from five heterogeneous sources, were characterized in depth by using two distinct* in vitro *evaluations. The first one was based on the Englyst-assay for nutritional classification of starch fractions, whereas the second one was based on a time-course multi-enzymatic assay up to 180 min from which the hydrolysis indices (HIs) were calculated and used as a link between the physicochemical properties of starch from diets and the* in vivo *responses. For the* in vivo *study, five jugular-catheterized pigs (35.3 ± 1.1 kg body weight) were fed one of the five diets for 6-day periods in a 5 × 5 Latin square design. On day 5, blood was collected for 8 h postprandially for evaluating glucose appearance. On day 6, blood was collected for 3 h postprandially for the estimation of the GI. Starchy diets differed for rapidly digestible starch (from 8.6% to 79.8% of total starch (TS)) and resistant starch contents (from 72.5% to 4.5% of TS). Wide between-diets variations were recorded for all the kinetic parameters and for the HI calculated from the* in vitro *digestion curves ($P < 0.05$). *On the basis of the obtained HI, diets contained starch with a very low to a very high* in vitro *digestion potential (ranging from 26.7% to 100.0%;* $P < 0.05$). *The glucose response differed among diets* ($P < 0.05$), *with marked differences between 15 and 120 min postprandial. Overall, the ranking of incremental glucose appearance among diets agreed with their* in vitro *HI classification: high HI diets increased plasma glucose response more* ($P < 0.05$) *than low HI diets. Lastly, different* in vivo *GIs were measured (ranging from 30.9% to 100.0%;* $P < 0.05$). *The relationship between HI and GI showed a high coefficient of determination* ($R^2 = 0.95$; *root mean square error (RMSE)* = 15.8; $P < 0.05$). *In conclusion, diets formulated with starches with a wide range in HI potential can strongly affect the postprandial glucose response in pigs.*

Keywords: glucose, *in vitro* method, glycemic index, starch, pig

Implications

Starch is the main energy source for monogastric species and knowledge about its digestive behaviour can be important in order to understand the consequently metabolic and endocrine functioning in monogastrics. This study showed that diets formulated with heterogeneous sources of starch and characterized by a wide range in enzymatic starch digestion potential modulated the postprandial glucose response in pigs. A better understanding of the nutritive role of starch and its effect on metabolism is an important nutritional topic for monogastric animals.

† E-mail: francesco.masoero@unicatt.it

Introduction

Starch is the major energy and glucose source in pig diets and its structure, along with the shape and dimension of starch granules, particle size, protein and lipid matrices and the processing method, can have a strong effect on the rate and extent of digestion and on physiological responses (Sun *et al.*, 2006; Bach Knudsen, 2011). The kinetics of starch digestion are an emerging issue in swine nutrition (Menoyo *et al.*, 2011), because of their implications for productive performance (Mateos *et al.*, 2007), nutrient digestibility (Vicente *et al.*, 2008), metabolism of amino acids (Li *et al.*, 2008), feed intake (van Kempen *et al.*, 2007), carcass composition (Bach Knudsen, 2011) and physiological responses (Regmi *et al.*, 2011).

Although the effect of different starch sources with various physicochemical characteristics (such as starch structure, amylose content, resistant starch content (RS) and degree of gelatinization) on postprandial blood glucose appearance and physiological responses has been reported both for humans (Englyst *et al*., 1996) and pigs (Li *et al*., 2008; van Kempen *et al*., 2010), their contribution in the kinetics of the *in vivo* postprandial glucose response is not clearly understood. In particular, *in vivo* responses are not always consistent (Regmi *et al*., 2011), and results may vary considerably because of the confounding effect of nutrients other than starch or when the range of digestibility is not sufficiently wide (Noah *et al*., 2000; Regmi *et al*., 2010).

The glycemic index (GI) has initially been introduced to classify human starchy foods on the basis of their incremental postprandial blood glucose response after an equal-carbohydrate meal (Jenkins *et al*., 1981; Parada and Aguilera, 2011) and it is currently widely adopted (Ludwig, 2002; Monro and Mishra, 2010). Recently, Menoyo *et al*. (2011) introduced the concept of GI also for pig nutrition by ranking cereals on the basis of low and high GI. In their study, the authors reported that cereals with high GI led to an increased insulin response over time, which in turn resulted in a numeric increase in feed intake (+3.6%).

One of the approaches commonly used to estimate the *in vivo* GI response is through a hydrolysis index (HI) calculated from *in vitro* enzymatic starch digestion curves (Goñi *et al*., 1997). Although numerous studies have confirmed both the role that various starch sources play on *in vivo* GI (Parada and Aguilera, 2011) and the relationship between HI and GI for humans (Björck *et al*., 2000; Araya *et al*., 2002; Garsetti *et al*., 2005), limited information is currently available for pigs. Furthermore, to overcome the expense and difficulty of the *in vivo* analysis, an *in vitro* prediction of the GI for pigs, based on the released glucose throughout time after an enzymatic digestion, could be considered as a promising alternative to animal trials.

Therefore, five sources of dietary starch were used to test the hypothesis that diets containing starch with a wide range in starch degradation kinetics estimated by an HI approach could influence the postprandial glucose response in pigs. The HI approach was used as a link between the physicochemical properties of starch from diets and the *in vivo* responses.

The objectives of the study were to determine: (i) the incremental postprandial plasma glucose response in jugular-catheterized pigs fed diets containing starch with a wide range in *in vitro* digestion patterns; (ii) the *in vivo* GI values to the same diets in jugular-catheterized pigs; (iii) the possible relationship between *in vitro* HI and *in vivo* GI data.

Material and methods

Dietary treatments and preparation of the experimental diets
Purified Gelose 80 corn starch (Penford Food Ingredients, Centennial, Co, USA), purified Nastar pea starch (Cosucra Group Warconing, Warconing, Belgium), purified Remy B7 rice starch, purified Remyline AX-DR rice starch (Remy Industries, Leuven–Wijgmaal, Belgium) and milled white bread (purchased from a local commercial market), selected to cover a wide range of starch degradation kinetics, were used as the major source of carbohydrates for the formulation of five test starch diets (diet A, B, C, D and corresponding area for the reference diet (CTR), respectively). Using a 30 kg capacity twin-screw experimental mixer, five batches of about 15 kg per diet were prepared by mixing all the ingredients in the proportions reported in Table 1. The nutrient composition of the diets containing the five sources of starch met NRC requirements (Nutrient requirements for swine (NRC), 1998). The experimental diets were characterized for particle partition in agreement with the method reported by the American Society of Agricultural Engineers (ASAE, 1985). Briefly, 100 g of sample was sieved for 10 min by manual shaking and the residue retained over 1000 and 500 μm square diameter opening sieves was then measured.

Chemical analysis of purified starches and diets
Before inclusions in the experimental diets, purified starches were analysed for their amylose content using a Megazyme amylose/amylopectin assay kit (Megazyme Int. Bray, Ireland). Dried diets were ground through a 1-mm screen using a laboratory mill (Retsch grinder; model ZM1; Brinkman Instruments, Rexdale, Ontario, Canada) and stored until analysis. The samples were analysed for dry matter (DM; method 930.15 Association of Official Analytical Chemistry (AOAC), 2000) and for total starch content (TS) using an enzymatic method (Blasel *et al*., 2006) modified according to the procedure described by Masoero *et al*. (2010).

In vitro *study*
Two distinct evaluations were conducted to completely characterize the *in vitro* starch digestion potential of the five starch diets. Starch from each prepared diet was first characterized as RDS (rapidly digestible starch; as a percentage of TS), RS (as a percentage of TS) and total digestible starch (TDS, 100 − RS, as a percentage of TS) according to the procedure described by Englyst *et al*. (1996).

Subsequently, each diet was also characterized for its time-course starch digestion potential with a multi-enzymatic assay that mimics the digestive processes in the gastrointestinal tract of pigs, proposed by van Kempen *et al*. (2010) based on the *in vitro* technique initially described by Englyst *et al*. (1992). The enzymes used were: pepsin from Sigma (Sigma-Aldrich Co., Milan, Italy) (catalogue no. P-7000), pancreatin from Merck (Merck KGaA, Darmstadt, Germany) (catalogue no. 7133), amyloglucosidase from Sigma (catalogue no. A-7095) and invertase from Sigma (catalogue no. I-4504). In particular, to properly characterize the starch digestion rate, time points at which subsamples were taken for glucose analysis were 0, 15, 30, 60, 90, 120 and 180 min after incubation. The amount of glucose for each time interval was determined colorimetrically with a glucose oxidase kit (GODPOD; glucose SL reference 4058, Giesse Diagnostic SNC, Rome, Italy).

Using the data obtained from the multi-enzymatic *in vitro* assay up to 180 min, an *in vitro* digestion coefficient of starch for each time interval (DC_t, as a percentage of TS) was calculated by the following equation:

$$DC_t = 100 \times (((\text{glucose present at time } t \text{ '0' min} - \text{glucose release}) \times 0.9)/\text{total starch})$$

using a factor of 0.9 to convert monosaccharides to polysaccharides (Stevnebø *et al.*, 2006).

To describe the kinetics of starch digestion, a modified first-order kinetic model (Mahasukhonthachat *et al.*, 2010) was applied with time expressed in minutes and digested starch expressed as a percentage of TS. The model used is shown in equation (1):

$$DC_t = DC_0 + DC_\infty\ (1 - e^{(-kt)}) \qquad (1)$$

where DC_t corresponds to the *in vitro* digestion coefficient of starch at time t, DC_0 is the *in vitro* digestion coefficient at 0 min, DC_∞ is the *in vitro* digestion coefficient after 180 min, k is the digestion rate (min^{-1}) and t is the chosen time.

Areas under the *in vitro* digestion curves (AUC) were calculated using equation (3) proposed by Mahasukhonthachat *et al.* (2010) and an HI was then derived from the ratio between the AUC for each purified starch diet and CTR expressed as a percentage (Goñi *et al.*, 1997). Diets were considered to range from a very low to a very high *in vitro* digestion potential based on the *in vitro* obtained HI values.

In vivo *study*

The study was conducted in accordance with the European Community (EC) Council Directive guidelines for animals used for experimental and other scientific purposes (EC, 1986).

Five female pigs (average initial body weight 35.3 ± 1.1 kg; mean ± s.e.), housed individually in stainless steel metabolism crates in a temperature-controlled room (from 23°C to 26°C), were fitted with an indwelling jugular vein catheter (Deng *et al.*, 2010), which was carried out as described by Li *et al.* (2008). Only five pigs were used because of difficulties related to animal management. After recovery, pigs were fed one of the five diets in a 5 × 5 Latin square design. Feed intake was adjusted for individual pigs at the start of each experimental period to 2.6 times maintenance energy requirement (NRC, 1998) and feed was provided twice daily in two equal meals at 0800 and 2000 h, with water available at all times. Each experimental period consisted of 6 days: 4 days of adaptation and 2 days of blood collection, where day 5 was used for the evaluation of the incremental glucose appearance, whereas day 6 was used for the *in vivo* GI determination. Specifically, on day 5, jugular venous blood samples were collected before the meal and at 15, 30, 60, 90, 120, 240 and 480 min postprandially. Subsequently, at the end of the blood sampling procedures, animals were deprived of food overnight and then, on day 6 at 0800 h, they were fed an amount of their starch diet to obtain an ingestion of 50 g of TS. Pigs were sampled in the fasting state and at 15, 30, 60, 90, 120 and 180 min after feeding, and at the end of the sample collection procedures, they were fed the residual part of their daily meal.

Plasma from all the collected blood samples was analysed for glucose (intra- and inter-assay CV ⩽ 2.0; glucose oxidase kit, Diagnostics Chemicals Ltd, Charlottetown, Canada).

The GI for each diet per period was then calculated by expressing the incremental area under the glucose response curve (IAUC) for each purified starch diet (i.e. A, B, C and D) as a percentage of CTR (Wolever *et al.*, 1991). These values were then compared with the previously calculated *in vitro* HI.

Statistical analysis

Data were tested for normality using the Shapiro–Wilk test. *In vitro* kinetics parameters (DC_0, DC_∞ and k) and HI were analysed as a completely randomized design by using the GLM procedure of the SAS (Statistical Analytical System; 2003) according to the following model:

$$Y_{ij} = \mu + D_i + e_{ij}$$

where Y_{ij} = observations; μ = overall mean; D_i = effect of starchy diet ($i = 1$ to 5); and e_{ij} = residual error. The experimental unit was the batch.

In vivo data were analysed as a standard 5 × 5 Latin Square design by using the GLM procedure of SAS (2003) according to the model described below:

$$Y_{ijkl} = \mu + D_i + P_j + S_k + e_{ijkl}$$

where Y_{ijkl} = observations; μ = overall mean; D_i = effect of starchy diet ($i = 1$ to 5); P_j = effect of period ($j = 1$ to 5); S_k = effect of subject ($k = 1$ to 5); and e_{ijkl} = residual error. The experimental unit was the pig.

The least square means were compared by using LSMEANS statement of SAS (2003) and significance was declared at $P < 0.05$. The relationship between *in vitro* HI and *in vivo* GI was determined by using the REG procedure of SAS (2003).

Results

Chemical composition, starch chemistry and HIs of diets

The batches prepared for each experimental diet (i.e. 5 batches/diet) could be considered homogeneous, the within-diet variance being lower than 3.0% of total variance (i.e. 0.3%, 2.1%, 2.8% and 0.6% for DC_0, DC_∞, k and HI, respectively). In addition, the DM and TS contents and particle size partition could be considered similar for the five experimental diets, as shown in Table 1.

The different starch characteristics between diets were confirmed by the wide range in RDS, RS and TDS fractions (expressed on a TS basis), ranging from 8.6% to 79.8%, from 72.5% to 4.5% and from 27.5% to 95.5% for diet A and CTR, respectively (Table 1).

Wide between-diets variations for the *in vitro* starch digestion curves were observed (Figure 1). Specifically, across

Table 1 *Ingredient compositions, particle size[1], chemical analysis[1] and starch fractions[1] of the five experimental diets*

	Starch diets[2]				
Ingredients (g/kg of diet)	A	B	C	D	CTR
Gelose 80 cornstarch[3]	700	–	–	–	–
Nastar pea starch[3]	–	700	–	–	–
Remy B7 rice starch[3]	–	–	700	–	–
Remyline AX-DR rice starch[3]	–	–	–	700	–
White bread	–	–	–	–	800
Casein[4]	140	140	140	140	100
Fish meal[5]	74	74	74	74	40
Cellulose[6]	40	40	40	40	14
Canola oil	10	10	10	10	10
Limestone	10	10	10	10	10
$CaHPO_4$	8	8	8	8	8
NaCl	3	3	3	3	3
Vitamin premix[7]	5	5	5	5	5
Mineral premix[8]	8	8	8	8	8
K_2CO_3	2	2	2	2	2
Particle size (g/kg of diet)					
>1000 μm	9	8	9	10	8
<1000 μm and >500 μm	75	73	76	78	77
<500 μm	916	919	915	912	915
Chemical analysis					
DM (%)	89.5	90.0	90.6	89.9	90.3
TS (% of DM)	68.7	69.1	68.7	69.0	68.9
Starch fractions (analysed as TS basis)					
RDS (%)	8.6	11.1	33.4	51.4	79.8
RS (%)	72.5	53.5	25.3	20.1	4.5
TDS (%)	27.5	46.5	74.7	79.9	95.5

CTR = corresponding area for the reference diet; DM = dry matter; TS = total starch; RDS = rapidly digestible starch; RS = resistant starch; TDS = total digestible starch.
[1]Values are means, $n = 5$.
[2]Calculated digestible energy (Noblet *et al.*, 2003): 14.8, 14.8, 14.8, 14.8 and 14.9 MJ/kg for diets A, B, C, D and CTR, respectively.
[3]Amylose content (as percentage of DM): 64.1%, 26.8%, 18.6% and 3.1% for Gelose 80 cornstarch, Nastar pea starch, Remy B7 rice starch and Remyline AX-DR rice starch, respectively.
[4]Calcium caseinate (American Casein Company, Burlington, NY, USA).
[5]Menhaden fish meal (Omega Protein, Hammond, LA, USA).
[6]Solka-floc (International Fiber Corp., North Tonawanda, NY, USA).
[7]Provided per kg of diet: retinol, 2.5 mg; cholecalciferol, 20.6 μg; α-tocopherol, 2.7 μg; niacin, 35 mg; D-pantothenic acid, 15 mg; riboflavin, 5 mg; menadione, 4 mg; folic acid, 2 mg; thiamine, 1 mg; D-biotin, 0.2 mg; vitamin B-12, 0.05 mg.
[8]Provided per kg of diet: Zn, 100 mg as $ZnSO_4$; Fe, 80 mg as $FeSO_4$; Cu, 50 mg as $CuSO_4$; Mn, 25 mg as $MnSO_4$; I, 0.5 mg as $Ca(IO_3)_2$; Se, 0.1 mg as Na_2SeO_3.

starch diets, diet A was digested slowly, showing the lowest *DC* at all time intervals, whereas diet CTR was digested faster and to a higher extent for the entire incubation period.

In addition, diets influenced all the *in vitro* kinetic parameters (i.e. DC_0, DC_∞ and k) and the HI values calculated from the hydrolysis curves (Table 2). In particular, different DC_0 (ranging from 0.2% to 2.3% of TS), DC_∞ (from 33.1% to 96.4% of TS) and k (from 0.008 to 0.061 min^{-1}) values were recorded ($P < 0.05$). Lastly, on the basis of the HI, a distinction can be made between very low (i.e. A; HI = 26.7%), low (i.e. B; HI = 46.0%), moderate (i.e. C; HI = 77.0%), high (i.e. D; HI = 85.1%) and very high (i.e. CTR; HI = 100.0%) starch degradable diets ($P < 0.05$).

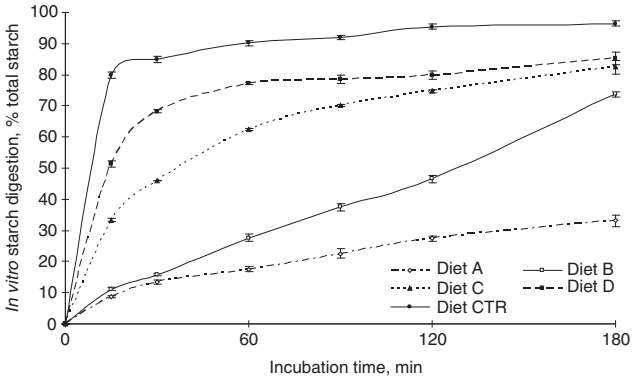

Figure 1 Effect of the five experimental diets on the time course of *in vitro* starch digestion. Values are means ($n = 5 \pm$ s.e.). CTR = corresponding area for the reference diet.

Incremental postprandial plasma glucose appearance and GI
Feed refusal and vomit events were not observed and all starch diets were promptly consumed by pigs within about 15 min after meal administration. Moreover, all pigs were healthy during the experiment as observed by normal appetite and growth, and no difference in basal jugular plasma glucose concentration was observed between diets (with an average value of 72.4 ± 2.2 mg/dl; $P > 0.05$).

Overall, the ranking of incremental plasma glucose concentration, as a consequence of feeding the five tested diets formulated with different sources of starch, was consistent for almost the entire postprandial period among diets. In particular, the glucose response differed among diets ($P < 0.05$), with marked differences observed between 15 and 120 min postprandial (Figure 2).

On average, plasma glucose peaked at approximately 46 ± 9 min postprandial at a maximal concentration that was higher for diets D and CTR when compared with diets A, B and C (55.4 and 64.2 mg/dl *v.* 15.0, 24.4 and 43.2 mg/dl, respectively; $P < 0.05$) (Table 3).

Moreover, diets A and B elicited the lowest rate of plasma glucose increment ($rate_{incr}$: 0.12 and 0.53 mg/dl per min *v.* 1.32, 1.50 and 1.83 mg/dl per min) when compared with diets C, D and CTR, respectively ($P < 0.05$), whereas no difference was recorded for the rate of plasma glucose decrement ($rate_{decr}$; $P > 0.05$).

The IAUCs after consumption of the same amount of TS differ ($P < 0.05$) between starch diets (ranging from 1623 to 5252 mg/dl per 180 min) and experimental periods (ranging from 3341 to 6623 mg/dl per 180 min). Lastly, the lowest GI values for diets A and B were recorded (30.9% and 43.3%) when compared with diets C, D and CTR (81.0%, 85.0% and 100.0%, respectively; $P < 0.05$) without the effect of period ($P > 0.05$) (Table 2).

Relationship between in vitro *hydrolysis and* in vivo *GIs*
The relationship between the *in vitro* HI and *in vivo* GI showed a high coefficient of determination ($R^2 = 0.95$; root

Table 2 In vitro *kinetic parameters*[1] *and hydrolysis index*[1] *of the five experimental diets*

	Starch diets					
	A	B	C	D	CTR	s.e.
In vitro kinetics parameters						
DC_0[2] (%)	0.3[a]	0.2[a]	1.6[b]	2.1[c]	2.3[c]	0.08
DC_∞[3] (%)	33.1[a]	73.7[b]	82.4[c]	85.2[d]	96.4[e]	1.60
k[4] (min^{-1})	0.008[a]	0.016[b]	0.024[c]	0.042[d]	0.061[e]	0.0016
HI[5] (%)	26.7[a]	46.0[b]	77.0[c]	85.1[d]	100.0[e]	1.06

CTR = corresponding area for the reference diet; DC_0 = *in vitro* digestion coefficient of starch at 0 min; DC_∞ = *in vitro* digestion coefficient of starch after 180 min; k = digestion rate; HI = hydrolysis index.
[a,b,c,d,e]Means in the same line with different letters differ at $P < 0.05$.
[1]Values are means, $n = 5$.
[2]DC_0 (as a total starch basis).
[3]DC_∞ (as a total starch basis).
[4]Rate of starch digestion.
[5]HI calculated by using diet CTR as reference meal.

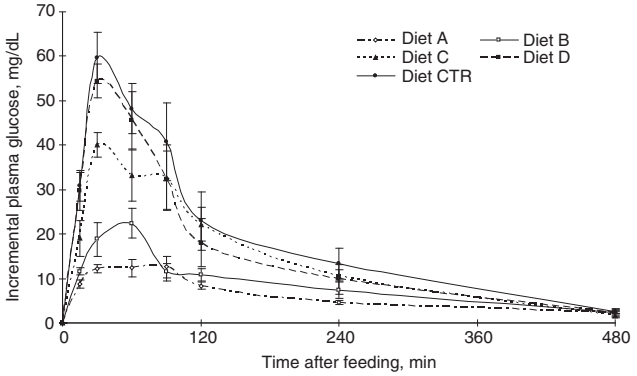

Figure 2 Effect of the five experimental diets on the incremental postprandial plasma glucose concentration in jugular-catheterized pigs. Values are means ($n = 5 \pm$ s.e.). CTR = corresponding area for the reference diet.

mean square error (RMSE) = 15.8; $P < 0.05$). The intercept was not significant; consequently, it was not retained in the regression analysis. As a result, the *in vivo* GI values can be predicted by the following linear regression equation (equation 2; Figure 3):

$$\text{GI (\%)} = 1.013 \times \text{HI (\%)} \qquad (2)$$

Discussion

This study evaluated the effect of five sources of dietary starch characterized by a wide range of HI values in jugular-catheterized pigs. Generally, heterogeneous starch types from different sources are considered to have a strong impact on the rate and extent of *in vivo* glucose response (Regmi *et al.*, 2010), even if misleading results were reported (Thorne *et al.*, 1983; Annison and Topping, 1994; Noah *et al.*, 2000), probably because of the confounding effects of starch-associated compounds (such as fat, fibre, protein and/or anti-enzymatic complexes; Parada and Aguilera, 2011) or because of experimental conditions (observation of the glucose response in peripheral circulation *v.* portal circulation or different coverage of *in vivo* postprandial duration) (Regmi *et al.*, 2011). As a consequence, additional information is currently warranted.

Moreover, in animal nutrition, there is presently a need for quick and reliable *in vitro* techniques, as an alternative to more expensive *in vivo* methodologies, for assessing the enzymatic degradation potential of dietary starches that may reflect the *in vivo* glucose responses. For this purpose, the separation of starch into different digestible fractions by enzymatic analysis (i.e. RDS, slowly digestible starch (SDS) and RS) has been proposed (Englyst *et al.*, 1992). However, because of the analysis of single-point measurements and the coverage of limited time (120 min) foreseen by this *in vitro* assay, whether these fractions accurately reveal the full digestion profile and may adequately estimate the *in vivo* response is controversial (Dona *et al.*, 2010; van Kempen *et al.*, 2010).

Consequently, we addressed these issues using three different approaches. First, the digestion profile of the five test diets was characterized in depth with an HI approach through an extended time-course enzymatic digestion up to 180 min, where digestion parameters can be estimated to better understand the digestion process. Second, purified starches were selected in order to avoid possible confounding effects of starch-associated compounds, whereas white bread was selected as the reference starch source (Goñi *et al.*, 1997). Third, the *in vivo* responses were observed up to 480 min, because a major proportion of starch digestion can take place after 120 min postprandial (van Kempen *et al.*, 2010). Lastly, no data are currently available on the direct measure of GI in jugular-catheterized pigs.

In vitro *enzymatic assessments and HI of starch diets*

To appropriately characterize the starch digestion profile, we ran an extended version of the method initially proposed by Englyst *et al.* (1992), characterized by seven incubations up to 180 min. Moreover, as a good estimation of the *in vitro* starch digestion potential requires sufficient measurements,

Table 3 *Effect of diets containing starches with varying* in vitro *degradation potential on* in vivo *parameters in jugular catheterized pigs*

	Starch diets						*P*-value[1]		
In vivo parameters[2]	A	B	C	D	CTR	s.e.	Diet	Period	Pig
Max[3] (mg/dl)	15.0[a]	24.4[b]	43.2[c]	55.4[d]	64.2[d]	3.00	*	*	ns
T_{peak}[4] (min)	60	48	42	36	42	8.8	ns	ns	ns
$Rate_{incr}$[5] (mg/dl per min)	0.12[a]	0.53[a]	1.32[b]	1.50[b]	1.83[b]	0.252	*	ns	ns
$Rate_{decr}$[6] (mg/dl per min)	−0.12	−0.13	−0.26	−0.27	−0.34	0.060	ns	*	ns
IAUC[7](mg/dl per 180 min)	1623[a]	2274[a]	4253[b]	4464[bc]	5252[c]	452.2	*	*	ns
GI[8] (%)	30.9[a]	43.3[a]	81.0[b]	85.0[bc]	100.0[c]	6.10	*	ns	ns

CTR = corresponding area for the reference diet; IAUC = incremental area under the glucose response curve; GI = glycemic index.
[a,b,c,d]Means in the same line with different letters differ at $P < 0.05$.
[1]Statistical significance of effects of diet, period and pig are indicated: ns = not significant; $*P < 0.05$. [2]Values are means, $n = 5$.
[3]Maximal plasma glucose concentration.
[4]Peak time.
[5]Rate of plasma glucose increment.
[6]Rate of plasma glucose decrement.
[7]IAUC obtained by feeding to pigs a portion of 50 g of total starch from each test diet.
[8]GI calculated by using diet CTR as reference meal.

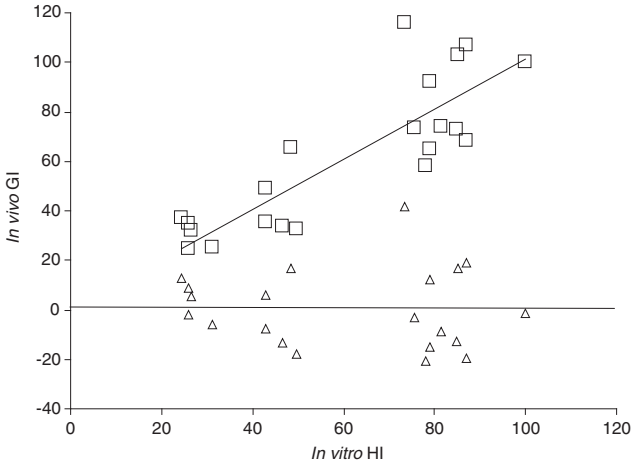

Figure 3 Plot of observed (□) and residuals (Δ) for *in vitro* HI (%; X-axis) *v. in vivo* GI (%; Y-axis) data (GI = 1.013 x HI; $R^2 = 0.95$; RMSE = 15.8; $P < 0.05$). HI = hydrolysis index; GI = glycemic index.

especially during the first 60 min (Weurding *et al*., 2001), four subsamples during the first hour were collected.

The ranking of the five test diets based on *in vitro* starch digestion agreed with their starch composition, in line with previous findings (van Kempen *et al*., 2010). The rate and extent of starch degradation depends on its botanical origin (Gallant *et al*., 1992), thus influencing the amylose : amylopectin ratio (Svihus *et al*., 2005) and also the structural characteristic of the starch granule (Anguita *et al*., 2006). In particular, purified starches used in the diet formulation contained a wide range of amylose content (ranging from 3.1% to 64.1% of DM for AX-DR rice and for Gelose 80 corn starch, respectively; Table 1). In general, the degradability of raw starches is inversely proportional to their amylose content (Svihus *et al*., 2005) because of decreased enzyme accessibility to the molecule (Tester *et al*., 2006). Moreover, rice starch is expected to be more available to enzyme action than corn or pea starches, because of the smaller size of starch granules (Tester *et al*., 2006). Smaller granules possess greater surface area and are therefore more susceptible to enzyme hydrolysis than larger granules (Lee *et al*., 2011). Lastly, the type of crystal structure may influence the degradation potential (Sajilata *et al*., 2006); for instance, pea starch exhibits a C-type crystal structure, which can make it resistant to enzyme action (Sun *et al*., 2006).

Back to experimental conditions, diet A expressed the lowest *in vitro* starch digestion curve among all the test diets ($P < 0.05$), probably because of its pronounced RS content (i.e. 72.5% of TS). Several studies reported that high RS values could limit the digestion or hydrolysis rates in food (Sáyago-Ayerdi *et al*., 2005) and feed (Sun *et al*., 2006). Moreover, amylose can enhance the formation of RS (Sajilata *et al*., 2006), which is consistent with the results of this study.

On the contrary, diet CTR had the highest *in vitro* digestion profile when compared with other diets. Heat-processed materials have been previously found to be more degradable than raw counterparts (Sun *et al*., 2006), because the starch gelatinization process enhances the surface of contact between the substrate and digestive enzymes (Dona *et al*., 2010; Parada and Aguilera, 2011). In particular, heat processing can modify the chemical structure of starch because of the loss of crystalline structures, exudation of amylose and an increase in viscosity, thus facilitating enzymatic degradation (Svihus *et al*., 2005). In addition, some authors have reported that heat processing improved starch digestibility (Vicente *et al*., 2009) and performances (Medel *et al*., 2004) in pigs, even if this effect may be strongly dependent on carbohydrate sources and processing conditions (Svihus *et al*., 2005; Sun *et al*., 2006).

Irrespective of the starch source, diets exhibited monophasic *in vitro* digestion profiles that were suitably described by equation (1) to indicate a first-order kinetics, in accordance with previous findings (Mahasukhonthachat *et al*., 2010). In addition, the inclusion of the DC_0 in the model was

justified by the fact that all the test diets contained soluble glucose at the start of incubation (DC_0 ranging from 0.2% to 2.3% of TS; $P < 0.05$). The soluble glucose must be considered in the kinetic model because it could be confused with the rate of digestion, its degradability being instantaneous.

The kinetics of enzymatic starch digestion greatly differed among diets ($P < 0.05$). In particular, a wide range in the rate of *in vitro* starch digestion (k, min^{-1}) was reported, with the highest value recorded for diet CTR ($0.061\ min^{-1}$; $P < 0.05$). A higher rate of starch digestion could have several implications for animal performance: it may lead to a more complete digestion of starch (Weurding *et al.*, 2001; Lee *et al.*, 2011) and could minimize starch fermentation in the small intestine (Sun *et al.*, 2006), ensuring a more efficient energy intake. Moreover, a high starch digestion rate could influence feed intake by minimizing the amount of undigested dietary material reaching the end of the small intestine (Al-Rabadi *et al.*, 2011).

HI is useful for the comparison of starch digestion values between samples of interest and a reference material. The HI values significantly differed among starch diets ($P < 0.05$). Different authors found that the digestion potential is largely affected by the starch source, which may be primarily related to starch chemistry, granule size, accessibility to starch granules, amylose/amylopectin ratio and crystallinity (Singh *et al.*, 2010; Regmi *et al.*, 2011). In this trial, diet CTR had the highest HI value ($P < 0.05$), confirming its high degradation potential due to the starch gelatinization process (Dona *et al.*, 2011). On the contrary, diet A had the lowest HI ($P < 0.05$), in line with its reduced starch degradation due to its high RS and amylose contents.

Incremental plasma glucose appearance and GI characterization

Glucose is considered to be the primary trigger for postprandial changes in metabolic and endocrine functioning (Buyse *et al.*, 2002; Hooda *et al.*, 2010). As a consequence, defining the postprandial potential glucose response after a meal is of great interest (Regmi *et al.*, 2010). To measure *in vivo* glucose levels, pigs were fitted with a jugular vein catheter. The choice to analyse the glucose response on peripheral circulation is in line with the original GI procedural protocol developed by Jenkins *et al.* (1981). Although this approach was sensitive for detecting differences in glucose responses in swine (Deng *et al.*, 2010), it could not account for hepatic glucose consumption (van Kempen *et al.*, 2010).

On the basis of *in vitro* HI data, an increased plasma glucose concentration in pigs was expected for high HI starch diets and indicated that gastric emptying did not confound the ranking of starch sources, in line with previous results (Regmi *et al.*, 2010). In particular, our results showed that diets with high HI increased the incremental plasma glucose appearance in jugular-catheterized pigs when compared with low HI diets in the same level of TS ($P < 0.05$). Different postprandial glucose responses were detected when pigs (Li *et al.*, 2008; van Kempen *et al.*, 2010) and humans (Behall *et al.*, 2002) consumed various sources of dietary starch with a wide range in starch digestion kinetics. The reason for the different incremental plasma glucose levels may be related primarily to the hydrolysis of starch in the digestive tract, starch hydrolysis being a rate-limiting factor for the appearance of glucose (Cummings and Englyst, 1995). Consistent with the results of previous studies conducted on pigs (Regmi *et al.*, 2010), a lower postprandial glucose response was observed with diets characterized by a low starch degradation potential and a high content in RS fraction (i.e. diet A and B). It is recognized that the consumption of diets containing RS can decrease postprandial plasma glucose (Deng *et al.*, 2010), because of a reduced kinetic of starch digestion (Morand *et al.*, 1992). Several studies (Reader *et al.*, 1997; Murray *et al.*, 1998) also found a reduction in the postprandial glucose response after the ingestion of a commercial RS ingredient. On the contrary, diets with high starch digestion kinetics were associated with a rapid increase in glucose concentration (Dona *et al.*, 2010; van Kempen *et al.*, 2010).

In general, the GI, which ranks carbohydrates according to their effect on postprandial glucose levels, is utilized for defining the effect of starch digestion (Parada and Aguilera, 2011). The GI calculation was carried out in agreement with the original procedure initially proposed for humans, where the use of a fixed amount of carbohydrates (Jenkins *et al.*, 1981) or starch (Granfeldt *et al.*, 1994) is recommended. This is because the glycemic response increases linearly as carbohydrate ingestion increases from 0 to 50 g, whereas it tends to level off when carbohydrate ingestion increases from 50 up to 100 g (Wolever *et al.*, 2003).

Although GI values differ among starch sources in humans (Dona *et al.*, 2010), no data are currently available for pigs. The different starch diets affected both the IAUC and the GI values in jugular-catheterized pigs. In particular, a period effect was observed for IAUC, but not for GI values. This was probably because of a uniform increase of IAUC values among periods, either for pigs fed the experimental or control diets. As a consequence, the GI could be considered as a mathematical measurement more inherent to starchy diets rather than to the animal metabolic response to the same diet.

The *in vivo* GI ranking seems to corroborate the previous *in vitro* HI classification, even if the GI was found to be less distinctive when compared with HI in discerning the starch sources utilized for the diet formulation. These discrepancies may reflect the higher complexity of the *in vivo* evaluation compared with the *in vitro* technique (Parada and Aguilera, 2011). Recently, Hasjim *et al.* (2010), studying the structure of starch granule during both *in vitro* and *in vivo* pig digestions, indicated that *in vitro* methods might not fully reproduce the heterogeneous and complex conditions taking place during *in vivo* digestion. In particular, *in vitro* experiment involves the interaction of only the substrate and purified enzymes and the entire sample is incubated with an optimized cocktail at time 0 (van Kempen *et al.*, 2010). In contrast, the *in vivo* digestion is a dynamic process that involves animal variation, chewing, bowel movement and other enzymes such as salivary amylases and glucose carriers

(Lee *et al*., 1985; Weurding *et al*., 2001; Hasjim *et al*., 2010). Moreover, the lack both in the simulation of the effect of diets on passage rate and viscosity and in the removal of digestion products foreseen for the *in vitro* analyses could further explain these differences (Weurding *et al*., 2001).

Relation of in vitro *HI with* in vivo *GI*

There are many reports detailing the role of GI both in human and animal nutrition (Ludwig, 2002; Menoyo *et al*., 2011); however, the *in vivo* study being laborious and expensive, interest in an *in vitro* methodology to estimate glycemic response has recently increased. The HI was previously found to be a good predictor of glycemic response to food ingestion and highly correlated with the GI in humans (Goñi *et al*., 1997). In addition, the authors reported in their final report, a linear equation that was widely adopted to convert HI to GI data in human studies (Chung *et al*., 2008; Germaine *et al*., 2008). However, whether this equation could be applied to pigs is questionable, because monogastric animals have a digestive system only roughly similar to human beings (Parada and Aguilera, 2011). Moreover, the equation proposed by Goñi *et al*. (1997) is mainly based on cooked starch-containing food, whereas the starch consumed by domestic animals is largely uncooked. In our experimental conditions, it was confirmed that the *in vitro* HI was a good predictor of the *in vivo* GI determined in jugular-catheterized pigs, as evidenced by regression analysis (equation 2). However, a high prediction error was reported (RMSE = 15.8), indicating that further study needs to be done to minimize the discrepancy between *in vivo* and *in vitro* digestion evaluations for practical purposes (Hasjim *et al*., 2010). Moreover, this approach was mainly focussed on pig nutrition with limited relevance to humans, because uncooked starches were used.

Conclusions

This study showed that different sources of dietary starch affected the kinetics of starch digestion, the incremental plasma glucose response and the GI in pigs. In concert with chemical composition and classical criteria (as the ileal or the faecal digestibility), the *in vitro* procedure described for the estimation of the starch hydrolysis potential may be useful for the assessment of the GI in pigs, in order to provide valuable laboratory tools for screening the nutritional value of starch-rich materials. However, for practical purposes, further studies are required to optimize the *in vitro* conditions.

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References

Al-Rabadi GJ, Torley PJ, Williams BA, Bryden WL and Gidley MJ 2011. Effect of extrusion temperature and pre-extrusion particle size on starch digestion kinetics in barley and sorghum grain extrudates. Animal Feed Science and Technology 168, 267–279.

ASAE (American Society of Agricultural Engineers) 1985. Methods of determining and expressing fineness of feed materials by sieving. American society of agricultural engineers standard S 319. American Society of Agricultural Engineers Yearbook Standards, ASAE, St Joseph, MI, USA.

Anguita M, Gasa J, Martín-Orúe SM and Pérez JF 2006. Study of the effect of technological processes on starch hydrolysis, non-starch polysaccharides solubilization and physicochemical properties of different ingredients using a two-step *in vitro* system. Animal Feed Science and Technology 129, 99–115.

Annison G and Topping DL 1994. Nutritional role of resistant starch: chemical structure and physiological function. Annual Review of Nutrition 14, 297–320.

Araya H, Contreras P, Alviña M, Vera G and Pak N 2002. A comparison between an *in vitro* method to determine carbohydrate digestion rate and the glycemic response in young men. European Journal of Clinical Nutrition 56, 735–739.

AOAC (Association of Official Analytical Chemistry) 2000. Official methods of analysis, 17th edition. AOAC, Gaithersburg, MD, USA.

Bach Knudsen KE 2011. Triennial growth symposium: effect of polymeric carbohydrates on growth and development in pigs. Journal of Animal Science 89, 1965–1980.

Behall KM, Scholfield DJ and Hallfrisch J 2002. Plasma glucose and insulin reduction after breads varying in amylose content. European Journal of Clinical Nutrition 56, 913–920.

Björck I, Liljeberg H and Ostman E 2000. Low glycemic-index foods. British Journal of Nutrition 83, 149–155.

Blasel HM, Hoffman PC and Shaver RD 2006. Degree of starch access: an enzymatic method to determine starch degradation potential of corn grain and corn silage. Animal Feed Science and Technology 128, 96–107.

Buyse J, Janssens K, van der Geyten S, vas As P, Decuypere E and Darras VM 2002. Pre- and post-prandial changes in plasma hormone and metabolite levels and hepatic deiodinase activities in meal-fed broiler chicken. British Journal of Nutrition 88, 641–653.

Chung HJ, Shin DH and Lim ST 2008. *In vitro* starch digestibility and estimated glycemic index of chemically modified corn starches. Food Research International 41, 579–585.

Cummings JH and Englyst HN 1995. Gastrointestinal effects of food carbohydrate. American Journal of Clinical Nutrition 61, S938–S945.

Deng J, Wu X, Bin S, Li TJ, Huang R, Liu Z, Liu Y, Ruan Z, Deng Z, Hou Y and Yin YL 2010. Dietary amylose and amylopectin ratio and resistant starch content affect plasma glucose, lactic acid, hormone levels and protein synthesis in splanchnic tissues. Journal of Animal Physiology and Animal Nutrition 94, 220–226.

Dona AC, Pages G, Gilbert RG and Kuchel PW 2010. Digestion of starch: *in vivo* and *in vitro* kinetics models used to characterize oligosaccharide or glucose release. Carbohydrate Polymers 80, 599–617.

Dona AC, Pages G, Gilbert RG and Kuchel PW 2011. Starch granule characterization by kinetic analysis of their stages during enzymic hydrolysis: 1H nuclear magnetic resonance studies. Carbohydrate Polymers 83, 1775–1786.

Englyst HN, Kingman SM and Cummings JH 1992. Classification and measurement of nutritionally important starch fractions. European Journal of Clinical Nutrition 46, S33–S50.

Englyst HN, Veenstra J and Hudson GJ 1996. Measurement of rapidly available glucose (RAG) in plant foods: a potential *in vitro* predictor of the glycaemic response. British Journal of Nutrition 75, 327–337.

European Community (EC) 1986. Council directive of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. 1986/609/European Community Official Journal L358, 1–28.

Gallant DJ, Bouchet B, Buleon A and Perez S 1992. Physical characteristic of starch granule and susceptibility to enzymatic degradation. European Journal of Clinical Nutrition 46, S3–S13.

Garsetti M, Vinoy S, Lang V, Holt S, Loyer S and Brand-Miller JC 2005. The glycemic and insulinemic index of plain sweet biscuits: relationship to *in vitro* starch digestibility. Journal of the American College of Nutrition 24, 441–447.

Germaine KA, Samman S, Fryirs CG, Griffiths PJ, Johnson SK and Quail KJ 2008. Comparison of *in vitro* starch digestibility methods for predicting the glycaemic

index of grain foods. Journal of the Science of Food and Agriculture 88, 652–658.

Goñi I, Garcia-Alonso A and Saura-Calixto F 1997. A starch hydrolysis procedure to estimate glycemic index. Nutrition Research 17, 427–437.

Granfeldt Y, Drews A and Björck I 1994. Arepas made from high amylose corn flour produce favorable low glucose and insulin responses in healthy humans. Journal of Nutrition 125, 459–465.

Hasjim J, Lavau GC, Gidley MJ and Gilbert RG 2010. *In vivo* and *in vitro* starch digestion: are current *in vitro* techniques adequate? Biomacromolecules 11, 3600–3608.

Hooda S, Matte JJ, Vasanthan T and Zjilstra RT 2010. Dietary oat beta-glucan reduces peak net glucose flux and insulin production and modulates plasma incretin in portal-vein catheterized grower pigs. Journal of Nutrition 140, 1564–1591.

Jenkins DJA, Wolever TMS, Taylor RH, Barker H, Fielden H and Baldwin JM 1981. Glycemic index of foods: a physiological basis for carbohydrate exchange. American Journal of Clinical Nutrition 34, 362–366.

Lee PC, Brooks SP, Kim O, Heitlinger LA and Lebenthal E 1985. Digestibility of native and modified starches: *in vitro* studies with humans and rabbit pancreatic amylases and in vivo studies in rabbits. Journal of Nutrition 115, 93–103.

Lee TT, Huang YF, Chiang CC, Chung TK, Chiou PWS and Yu B 2011. Starch characteristics and their influences on *in vitro* and pig prececal starch digestion. Journal of Agriculture and Food Chemistry 59, 7353–7359.

Li TJ, Dai QZ, Yin YL, Zhang J, Huang RL, Ruan Z, Deng Z and Xie M 2008. Dietary starch sources affect net portal appearance of amino acids and glucose in growing pigs. Animal 2, 723–729.

Ludwig DS 2002. The glycemic index: physiological mechanisms relating to obesity, diabetes and cardiovascular disease. Journal of the American Medical Association 287, 2414–2423.

Mahasukhonthachat K, Sopade PA and Gidley MJ 2010. Kinetics of starch digestion in sorghum as affected by particle size. Journal of Food Engineering 96, 18–28.

Masoero F, Gallo A, Zanfi C, Giuberti G and Spanghero M 2010. Chemical composition and rumen degradability of three corn hybrids treated with insecticides against the European corn borer (*Ostrinia nubilalis*). Animal Feed Science and Technology 155, 25–32.

Mateos GG, Martin F, Latorre MA, Vicente B and Lázaro R 2007. The effect of inclusion of oat hulls in pig diets based on raw or cooked rice and maize. Animal Feed Science and Technology 135, 100–112.

Medel P, Latorre MA, de Blas JC, Lázaro R and Mateos GG 2004. Heat processing of cereals in mash or pellet diets for young pigs. Animal Feed Science and Technology 113, 127–140.

Menoyo D, Serrano MP, Barrios V, Valencia DG, Lázaro R, Argente J and Mateos GG 2011. Cereal type and heat processing of cereal affect nutrient digestibility and dynamics of serum insulin and ghrelin in weanling pigs. Journal of Animal Science 89, 2793–2800.

Monro JA and Mishra S 2010. Database values for food-based dietary control of glycaemia. Journal of Food Composition and Analysis 23, 406–410.

Morand C, Rémésy C, Levrat MA and Demigé C 1992. Replacement of digestible wheat starch by resistant corn starch alters splanchnic metabolism in rats. Journal of Nutrition 122, 345–354.

Murray SM, Patil AR, Fahey Jr GC, Merchen NR, Wolf BW, Lai CS and Garleb KA 1998. Apparent digestibility of a debranched amylopectin-lipid complex and resistant starch incorporated into enteral formulas fed to ileal-cannulated dogs. Journal of Nutrition 128, 2032–2035.

Noah L, Krempf M, Lecannu G, Maugére P and Champ M 2000. Bioavailability of starch and postprandial changes in splanchnic glucose metabolism in pigs. American Journal of Physiology, Endocrinology and Metabolism 278, 181–188.

Noblet J, Bontems V and Tran G 2003. Estimation de la valeur énergétique des aliments pour le porc. INRA Productions Animales 16, 197–210.

Nutrient requirements for swine (NRC) 1998. NRC, 10th edition. Academic Press, Washington, DC, USA.

Parada J and Aguilera JM 2011. Review: starch matrices and the glycemic response. Food Science and Technology International 17, 187–204.

Reader D, Johnson ML, Hollander P and Franz M 1997. *The Elycemic and Insulinemic* response of resistant starch in a food bar vs. two commercially available bars in person with type II diabetes mellitus (abstract). Diabetes 46, 254A.

Regmi PR, Matte JJ, van Kempen TATG and Zijlstra RT 2010. Starch chemistry affects kinetics of glucose absorption and insulin response in swine. Livestock Science 134, 44–46.

Regmi PR, van Kempen TATG, Matte JJ and Zijlstra RT 2011. Starch with high amylose and low *in vitro* digestibility increases short-chain fatty acid adsorption, reduces peak insulin secretion, and modulates incretin secretion in pigs. Journal of Nutrition 19, 1–8.

SAS (Statistical Analytical System) 2003. SAS/SAT guide for personal computers, version 9.13. SAS Institute Inc., Cary, NC, USA.

Sáyago-Ayerdi SG, Tovar J, Osorio-Dìaz P, Paredes-López O and Bello-Pérez LA 2005. *In vitro* starch digestibility and predicted glycemic index of corn tortilla, black beans, and tortilla-bean mixture: effect of cold storage. Journal of Agriculture and Food Chemistry 53, 1281–1285.

Singh J, Dartois A and Kaur L 2010. Starch digestibility in food matrix: a review. Trends in Food Science and Technology 21, 168–180.

Sajilata MG, Singhal RS and Kulkarni PS 2006. Resistant starch – a review. Comprehensive Reviews in Food Science and Food Safety 5, 1–17.

Stevnebø A, Sahlström S and Svihus B 2006. Starch structure and degree of starch hydrolysis of small and large starch granules from barley varieties with varying amylose content. Animal Feed Science and Technology 130, 23–38.

Sun T, Lærke HN, Jørgensen H and Bach Knudsen KE 2006. The effect of extrusion cooking of different starch sources on the *in vitro* and *in vivo* digestibility in growing pigs. Animal Feed Science and Technology 131, 66–85.

Svihus B, Uhlen AK and Harstad OM 2005. Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: a review. Animal Feed Science and Technology 122, 303–320.

Tester RF, Qi X and Karkalas J 2006. Hydrolysis on native starches with amylases. Animal Feed Science and Technology 130, 39–54.

Thorne MJ, Thompson LU and Jenkins DJ 1983. Factors affecting starch digestibility and the glycemic response with special reference to legumes. American Journal of Clinical Nutrition 38, 481–488.

van Kempen TATG, Pujol S, Tibble S and Balfagon A 2007. *In vitro* characterization of starch digestion and its implications for pigs. In Paradigms in pig science (ed. J Wiseman, MA Varley, S McOrist and B Kemp), pp. 515–525. Nottingham University Press, Nottingham, UK.

van Kempen TATG, Regmi PR, Matte JJ and Zijlstra RT 2010. *In vitro* starch digestion kinetics, corrected for estimated gastric emptying, predict portal glucose appearance in pigs. Journal of Nutrition 140, 1227–1233.

Vicente B, Valencia DG, Pérez-Serrano M, Lázaro R and Mateos GG 2008. The effects of feeding rice in substitution of corn and the degree of starch gelatinization of rice on the digestibility of dietary components and productive performance of young pigs. Journal of Animal Science 86, 119–126.

Vicente B, Valencia DG, Serrano PM, Lázaro R and Mateos GG 2009. Effect of feeding rice and the degree of starch gelatinization of rice on nutrient digestibility and ileal morphology of young pigs. British Journal of Nutrition 101, 1278–1281.

Weurding RE, Veldman A, Venn WAG, van der Aar PJ and Verstegen MWA 2001. *In vitro* starch digestion correlates well with rate and extent of starch digestion in broiler chickens. Journal of Nutrition 131, 2336–2342.

Wolever TM, Jenkins DJ, Jenkins AL and Josse RG 1991. The glycemic index: methodology and clinical implication. American Journal of Clinical Nutrition 54, 846–854.

Wolever TMS, Vorster HH, Björck I, Brand Miller J, Brighenti F, Mann JI, Ramdath DD, Granfeldt Y, Holt S, Perry TL, Venter C and Wu X 2003. Determination of the glycemic index of foods: interlaboratory study. European Journal of Clinical Nutrition 57, 475–482.