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Nutrient utilisation and intestinal fermentation are differentially affected by the consumption of resistant starch varieties and conventional fibres in pigs

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This study examined the influence of different resistant starch (RS) varieties and conventional fibres on the efficiency of nutrient utilisation and intestinal fermentation in pigs. Thirty-six pigs (30 kg) were fed poultry meal-based diets supplemented with 10 % granular resistant corn starch (GCS), granular resistant potato starch (GPS), retrograded resistant corn starch (RCS), guar gum (GG) or cellulose for 36 d according to a completely randomised block design. Distal ileal and total tract recoveries were similar ($P>0·05$) among the RS varieties. Distal ileal starch recovery was higher ($P<0·05$) in pigs consuming the RS diets (27–42 %) as compared with the control group (0·64 %). Consumption of GCS reduced ($P<0·05$) apparent total tract digestibility and whole-body retention of crude protein in comparison with the control group. Consumption of GPS reduced ($P<0·05$) total tract Ca digestibility and whole-body retention of Ca and P compared with the control group. However, consumption of RCS increased ($P<0·05$) total tract Ca digestibility compared with the control group. Caecal butyrate concentration was increased ($P<0·05$) following consumption of RCS and GG in comparison with the control group. Consumption of all the RS varieties reduced ($P<0·05$) caecal indole concentrations compared with the control. Caecal butyrate concentrations were positively correlated ($P<0·05$; r 0·63–0·83) with thermal properties among the RS varieties. We conclude that nutrient utilisation and intestinal fermentation are differentially affected by the consumption of different RS varieties and types of fibres. Thermal properties associated with different RS varieties may be useful markers for developing RS varieties with specific functionality.

Resistant starch and fibre: Nutrients: Butyrate and indole: Pigs

Despite a wealth of research in both human subjects[1,2] and various animal model species[3,4], the relevance of resistant starch (RS) to human health and disease prevention remains unclear. Much of this uncertainty likely stems from the exceptional diversity that is encountered among RS varieties. Although the heterogeneous nature of traditional dietary fibre supplements is well documented, it is equally important to recognise that RS varieties originating from different plant sources and manufactured with alternative processing technologies will possess unique physiochemical properties[5]. RS is generally classified into four distinct types: physically entrapped, inaccessible starch; native granular starch; retrograded starch; chemically modified starch[6].

Due to its physiochemical attributes, RS is a particularly useful functional food additive to manage weight loss[7], improve insulin sensitivity[8] and reduce dyslipidaemia[9]. Furthermore, RS consumption is believed to improve intestinal health and function by increasing micronutrient absorption and favourably altering bacterial fermentation patterns in the large intestine[10,11]. However, as literature reports do not often stress the importance of starch variety in contributing to physiological responses, it is not clear how nutrient digestion and fermentation characteristics are affected by different RS varieties. Thus, before the potential health benefits of RS can be realised, food technologists and nutritionists need clarification on how specific RS varieties influence intestinal function and systemic health.

Therefore, our objectives were to investigate changes in the efficiency of nutrient utilisation and intestinal fermentation in response to the consumption of three different varieties of RS in the pig model. To further evaluate the nutritional significance of RS, the physiological responses were compared with those associated with the consumption of guar gum (GG) and cellulose, which are well-characterised, conventional soluble and insoluble fibre supplements, respectively.

Materials and methods

Diets

In an effort to reasonably approximate a typical North American high-fat diet, a basal diet (control) was formulated with poultry meal (45 %), casein (4 %) and an exogenous animal and vegetable fat blend (15 %, Table 1). The experimental

Abbreviations: CP, crude protein; GCS, granular high amylose corn starch; GG, guar gum; GPS, granular potato starch; RCS, retrograded high amylose corn starch; RS, resistant starch.

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Table 1. Composition of experimental diets* (g/kg diet) for growing pigs¶

	Experimental diets					
	Con	GCS	GPS	RCS	GG	Cell
Poultry meal	450·00	450·00	450·00	450·00	450·00	450·00
Casein	40·00	40·00	40·00	40·00	40·00	40·00
Animal fat	150·00	150·00	150·00	150·00	150·00	150·00
DL-methionine	3·90	3·90	3·90	3·90	3·90	3·90
Sucrose	43·10	43·10	43·10	43·10	43·10	43·10
Cornstarch	300·00	78·00	160·02	58·70	200·00	200·00
Resistant starch	0·00	222·00	139·80	241·30	–	–
GG	–	–	–	–	100·00	–
Cellulose	–	–	–	–	–	100·00
Vitamin premix	3·00	3·00	3·00	3·00	3·00	3·00
Mineral premix†	1·00	1·00	1·00	1·00	1·00	1·00
Iodised salt†	5·00	5·00	5·00	5·00	5·00	5·00
Chromic oxide‡	3·00	3·00	3·00	3·00	3·00	3·00
Nutrient content						
DE (MJ/kg) §	174·20	157·40	157·40	157·40	157·40	157·40
Crude protein (g/kg diet)‖	338·78	340·12	352·31	354·81	368·59	371·72
Ca (g/kg diet)‖	10·39	9·34	9·81	12·53	11·04	10·9
P (g/kg diet)‖	9·50	8·80	9·20	9·40	9·70	9·90

Con, Control diet with normal corn starch; GCS, granular high amylose corn starch (45·04 % resistant starch content); GPS, granular potato starch (71·51 % resistant starch content); RCS, retrograded high amylose corn starch (41·45 % resistant starch content); GG, guar gum (as food grade G2-S SUPERCOL®-Guar Gum provided by Hercules Incorporated Aqualon Division. GG properties: average molecular weight of 1·5 million; granulation of medium coarse; viscosity on as-is basis reported at 4500 centi poise with the GG dispersed in cold water to form a 1 % solution measured at 25°C after by using a Brookfield RVT viscometer at 20 rpm); Cell, cellulose (powdered cellulose; Solka-Flock, International Fibre Corporation).

* On as-fed basis.

† Details of the mineral, vitamin and salt premixes were published in a previous publication[20].

‡ Fisher Scientific.

§ Calculated values based on National Research Council (1998). Does not include digestible energy (DE) contributions from fibre supplements.

‖ Analysed values.

¶ For details of animals and procedures, see Materials and methods.

diets were formulated by replacing the normal corn starch in the basal diet with 10 % granular high amylose corn starch (GCS), granular potato starch (GPS; Avebe), retrograded high amylose corn starch (RCS), GG (SUPERCOL®-Guar Gum; Hercules Incorporated Aqualon Division) or cellulose. As the amount of starch deemed resistant to enzymatic hydrolysis *in vitro* differed between the starch varieties (see Results), different concentrations of the RS preparations were supplemented into the respective diets to ensure that the final inclusion of 'resistant' starch was 10 %. Chromic oxide was included (0·3 %) as an indigestible marker to measure distal ileal and total tract nutrient digestibility. Commercial mineral and vitamin premixes were added to the diets to meet or exceed the concentrations recommended by the National Research Council[12].

Animals and experimental design

The experimental procedures for the care and treatment of the pigs were reviewed and approved by the Animal Care Committee at the University of Guelph. The pigs used in this experiment were cared for in accordance with the guidelines established by the Canadian Council of Animal Care[13]. Thirty-six Yorkshire pigs, with an average initial body weight of 30 kg, were acquired from the Arkell Swine Research Station at the university. The pigs were placed in an environmentally controlled room (20°C) and randomly assigned to individual stainless steel metabolic crates (height 83 cm; length 145 cm; width 86 cm). For the next 3 d, the pigs were fed a typical grower ration and acclimatised to the environment and research staff. During the first 14 d of each experimental period, the pigs were fed their respective diets at 09.00 and 16.00 hours at close to *ad libitum* intake (5·5 % body weight). During days 10–14, faecal and urine samples for the mass balance experiments were collected. During days 15–16, the pigs were surgically fitted with a distal ileal cannula according to previously established procedures[14]. Following a post-surgical recovery period (days 17–20), the pigs resumed their previous feeding schedule for the duration of the experiment. On days 30–34 of the experiment, distal ileal effluent samples were collected according to the procedures outlined later. On the last day (day 36) of the experiment, the pigs were anaesthetised with isoflurane (2·5 %) in O_2 (2·5 litres/min) and killed with an intracardial injection of sodium pentobarbital (50 mg/kg body weight; Schering) for collection of caecal digesta samples.

The experiment was carried out according to a randomised complete block design with six replications (blocks) and a total of thirty-six animals, according to the following model[15]:

$$\gamma_{ij} = \mu + t_i + \rho_j + \varepsilon_{ij},$$

where μ is the general mean, t_i is the treatment effect, ρ_j is the block effect, and ε_{ij} is the experimental error.

Sample collection and processing

Fresh faecal samples were collected at 2 h intervals in the daytime of the collection period, placed in containers with sealed lids and immediately stored at 4°C for the duration of the period. The distal ileal digesta samples were collected from the distal ileal cannula into plastic bags (length 10 cm; internal diameter 1·5 cm) containing 5 ml formic acid (2·86 M) to minimise bacterial fermentation. Digesta were immediately frozen at −20°C.

Urine samples were obtained using a funnel-shaped metal tray secured to the base of the webbed floor of the metabolic crate. Urine flowed from the metal tray into a collection container placed in an electronic cooler beneath the metabolic crate. The coolers were maintained at a temperature of 4°C to reduce microbial activity and urea degradation and NH_3 emission loss during the collection period. The total volume of urine collected each day was recorded. A representative urine sample (about 5 % of the daily collection) from each day was pooled and stored at 4°C for the remainder of the experimental period.

At the conclusion of the experiment, representative faecal and digesta samples from each pig were freeze-dried, ground into a homogenous mixture and stored at 4°C in sealed containers. Fresh caecal digesta samples were collected and stored in sealed plastic centrifuge tubes at −20°C until analyses. Dietary samples were ground and stored in a similar manner.

Chemical analyses

Analyses of DM were carried out according to Association of Official Analytical Chemists methods[16]. Chromic oxide concentration in diet, distal ileal digesta and faecal samples was analysed with an atomic absorption spectrometer (SpectrAA-10/20; Varian) at 375 nm with a slit width of 0·5 nm according to the procedures outlined by Saha and Gilbreath[17]. Total crude protein (CP) content (N × 6·25) in dietary, distal ileal digesta and faecal samples was analysed according to the combustion method (Dumas procedure) on a Leco FP-428 Nitrogen analyser (Leco Corporation). Urine samples were first diluted (5 ×) with sulfuric acid (0·20 M) to prevent NH_3 vaporisation, then analysed using sucrose (0·1 g) as the carrier compound. Total Ca and P contents in diet, distal ileal effluent, faecal and urine samples were analysed according to previously published procedures[18].

RS content in starch varieties and diet samples was measured following incubation with pancreatic α-amylase and amyloglucosidase for 16 h at 37°C with a commercial kit (Megazyme International Ltd.) according to an Association of Official Analytical Chemists method[19]. Total starch content in diet, ileal digesta and faecal samples was measured enzymatically with a commercial assay kit (Megazyme International Ltd.). GG content in diet, ileal digesta and faecal samples was measured with a commercial galactomannan assay kit (Megazyme International Ltd.). Cellulose content in diet, ileal digesta and faecal samples was measured according to official Association of Official Analytical Chemists methods[16] in an ANKOM fibre analyser (ANKOM Technology). Caecal SCFA were determined by GC-MS analyses according to previously established procedures[20].

Differential scanning calorimetry

Thermal analyses of selected starches were performed using a differential scanning calorimeter (2920 Modulated DSC; TA Instruments) equipped with a refrigerated cooling system. Samples of starch (12 mg) were weighed into high-volume pans. Distilled water was added using a micropipette to make suspensions with 70 % moisture content. Pans were sealed and equilibrated overnight at room temperature before heating in the differential scanning calorimeter. The measurements were carried out at a heating rate of 10°C/min from 5 to 190°C. The instrument was calibrated using indium and an empty pan as the reference. The change in enthalpy (ΔH) of phase transitions was measured from the endotherm of differential scanning calorimetry thermograms using software (Universal Analysis, version 2.6D; TA Instruments) based on the mass of dry solid. Onset and peak temperatures of phase transitions were also measured from differential scanning calorimetry thermograms.

Calculations and statistical analyses

The efficiency of digestive and post-absorptive utilisation, including digestibility and retention, of CP, Ca and P was calculated according to previously established equations[18,20]. Data were analysed with the mixed model of SAS according to a completely randomised block design. Multiple comparisons between treatments were made using Tukey's test. Treatment differences from the control were further analysed using Dunnett's test. Correlations between starch thermal properties and nutrient utilisation and fermentation characteristics were conducted using Pearson partial correlation analyses. Differences were considered significant at $P<0·05$.

Results

The pigs remained healthy and had similar feed intake ($P=0·55–0·89$) for the duration of the experimental periods. The fraction of starch analysed to be resistant to *in vitro* enzymatic hydrolysis was different among the selected starches, i.e. normal corn starch 0 (SEM 0·01) %, GCS 45·04 (SEM 0·29) %, GPS 71·5 (SEM 2·51) % and RCS 41·45 (SEM 0·39) %. As expected, the conventional starch in the control, GG and cellulose diets was almost completely digested by the end of the distal ileum (Table 2). The distal ileal starch concentrations (g/100 g DM) and recoveries (% RS intake) were higher ($P<0·05$) in pigs consuming the RS varieties compared with the control group (Table 2). Furthermore, faecal starch concentrations and RS recoveries (0·11–0·17 % RS intake), reflecting an almost complete fermentation in the hindgut, did not differ ($P=0·37–0·92$) among the RS varieties (Table 2). The distal ileal recovery of GG (62·26 (SEM 5·27)) was higher ($P<0·05$) than that of cellulose (37·91 (SEM 6·73)) and all three of the RS varieties (Table 2).

The efficiency of digestive and post-absorptive utilisation of CP in response to the consumption of RS, GG and cellulose is presented in Table 3. Consumption of the granular corn starch varieties (GCS and GPS) depressed ($P<0·05$) apparent total tract CP digestibility and increased ($P<0·05$) total tract CP loss in comparison with the control group. However, only the GCS variety reduced ($P<0·05$) apparent CP retention in

Table 2. Distal ileal and faecal starch characteristics following the consumption of different varieties of resistant starch (RS) and conventional fibres in the pig‡

(Values represent least squares means with their standard errors of the mean)

	Experimental diets						
	Con *n* 6	GCS *n* 5	GPS *n* 4	RCS *n* 5	GG *n* 5	Cell *n* 5	SEM *n* 6
Distal ileal characteristics							
Total starch concentration (g/100 g DM digesta)	1·09[b]	9·07[a*]	10·19[a*]	11·38[a*]	2·48[b]	1·26[b]	1·03
GG or Cell concentrations (g/100 g DM digesta)	–	–	–	–	16·38[a]	11·97[b]	1·01
Starch recovery (% of dietary starch or RS)†	0·64[b]	35·59[a*]	27·14[a*]	42·83[a*]	5·25[b]	1·88[b]	3·90
GG and Cell recoveries (% of dietary intake)	–	–	–	–	62·26[a]	37·91[b]	8·40
Faecal characteristics							
Total starch concentration (g/100 g DM digesta)	0·12	0·15	0·18	0·11	0·11	0·15	0·03
GG or Cell concentrations (g/100 g DM digesta)	–	–	–	–	6·27[a]	10·30[b]	0·83
Starch recovery (% of dietary starch or RS)†	0·03	0·17	0·18	0·11	0·13	0·20*	0·05
GG and Cell recoveries (% of dietary intake)	–	–	–	–	12·26	17·50	3·46

Con, control diet with normal corn starch; GCS, granular high amylose corn starch (45·04 % RS content); GPS, granular potato starch (71·51 % RS content); RCS, retrograded high amylose corn starch (41·45 % RS content); GG, guar gum; Cell, cellulose.

[a,b,c] Values within a row with unlike superscript letters are significantly different ($P<0·05$) as analysed with Tukey's multiple comparison test using PROC MIXED model of SAS.

Values are significantly different from the Con group (*$P<0·05$) as analysed with Dunnett's test using PROC MIXED model of SAS.

† Recoveries for the RS have been corrected for the contribution of the conventional starch measured from the control group. Values are % of dietary RS content (GCS, GPS and RCS diets) and % of dietary total starch content (GG and Cell diets).

‡ For details of animals and procedures, see Materials and methods.

comparison with the control group. The urinary:faecal CP ratio was decreased ($P<0·05$) in the pigs consuming GCS, GPS and GG in comparison with the control group.

The efficiency of digestive and post-absorptive utilisation of Ca in response to the consumption of RS, GG and cellulose is presented in Table 4. Consumption of GPS reduced ($P<0·05$) total tract Ca digestibility, increased ($P<0·05$) total tract Ca excretion and reduced ($P<0·05$) whole-body Ca retention in comparison with the control group. Consumption of RCS increased ($P<0·05$) total tract Ca digestibility and reduced total tract Ca loss but did not significantly affect ($P=0·42$) whole-body Ca retention in comparison with the control group. Among the three RS preparations, the pigs consuming RCS had the highest ($P<0·05$) total tract Ca digestibility and whole-body Ca retention.

The efficiency of digestive and post-absorptive utilisation of P in response to the consumption of RS, GG and cellulose is presented in Table 5. Pigs consuming RCS had higher ($P<0·05$) apparent total tract P digestibility and efficiency of whole-body P retention than the pigs consuming the other two RS preparations. Consumption of the GPS reduced ($P<0·05$) total tract P digestibility, increased ($P<0·05$) total P excretion and reduced ($P<0·05$) the efficiency of P retention in comparison with the control diet. Consumption of GG reduced ($P<0·05$) the ileal P digestibility but did not influence ($P=0·85–0·99$) total tract P digestibility or whole-body P retention efficiency in comparison with the control pigs.

Consumption of GG increased ($P<0·05$) the caecal production of butyric, hexanoic, isovaleric and valeric acids and resulted in a higher total SCFA production in comparison with the control group (Table 6). Consumption of RCS increased ($P<0·05$) the caecal production of butyrate in comparison with the control diet and the other RS diets. Furthermore, consumption of all three RS varieties reduced ($P<0·05$) the caecal concentration of indole compared with the control group (Table 6).

Table 3. Efficiency of digestive and post-absorptive utilisation of crude protein (CP) following the consumption of resistant starch (RS) and conventional fibres in the pig†

(Values represent least squares means with their standard errors of the mean)

	Experimental diets						
	Con *n* 6	GCS *n* 5	GPS *n* 4	RCS *n* 5	GG *n* 5	Cell *n* 5	SEM *n* 6
Efficiency of utilisation	% of total CP intake						
Apparent ileal CP digestibility	73·96	70·96	75·99	74·13	67·08	74·16	1·93
Apparent total tract CP digestibility	89·97[b]	82·41[a*]	83·38[a*]	89·55[b]	85·71[ab]	88·92[b]	1·19
Apparent total tract CP loss	10·04[b]	17·59[a*]	16·62[a*]	10·45[b]	14·29[ab]	11·08[b]	1·19
Urinary CP loss	20·78	19·92	18·19	18·77	20·28	19·99	1·27
Total CP loss	30·81[ab]	37·51[a*]	34·81[ab]	29·22[b]	34·57[ab]	31·07[ab]	1·76
Apparent CP retention	69·18[ab]	62·49[a*]	65·19[ab]	70·78[b]	65·43[ab]	68·93[ab]	1·76
Urinary CP:faecal CP	2·07[b]	1·19[a*]	1·14[a]	1·80[bc]	1·30[ac]	1·84[bc]	0·13

Con, control diet with normal corn starch; GCS, granular high amylose corn starch (45·04 % RS content); GPS, granular potato starch (71·51 % RS content); RCS, retrograded high amylose corn starch (41·45 % RS content); GG, guar gum; Cell, cellulose.

[a,b,c] Values within a row with unlike superscript letters are significantly different ($P<0·05$) as analysed with Tukey's multiple comparison test using PROC MIXED model of SAS.

Values are significantly different from the Con group (*$P<0·05$) as analysed with Dunnett's test using PROC MIXED model of SAS.

† For details of animals and procedures, see Materials and methods.

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Table 4. Efficiency of digestive and post-absorptive utilisation of Ca following the consumption of resistant starch (RS) and conventional fibres in the pig†

(Values represent least squares means with their standard errors of the mean)

	Experimental diets						
	Con *n* 6	GCS *n* 5	GPS *n* 4	RCS *n* 5	GG *n* 5	Cell *n* 5	SEM *n* 6
Efficiency of utilisation	% of total Ca intake						
Apparent ileal Ca digestibility	65·08	62·33	64·78	71·85	50·47	60·36	5·33
Apparent total tract Ca digestibility	54·00^{c}	39·79^{ac}	35·26^{a*}	72·44^{b*}	52·23^{ac}	57·26^{bc}	4·26
Apparent total tract Ca loss	46·00^{c}	60·21^{ac}	64·74^{a*}	27·56^{b*}	47·77^{ac}	42·74^{bc}	4·26
Urinary Ca loss	0·58	0·92	1·34	0·99	0·33	1·23	0·36
Total Ca loss	43·87^{bc}	61·13^{ac}	65·89^{a*}	28·32^{b}	48·01^{ac}	43·97^{bc}	4·22
Apparent Ca retention	56·13^{bc}	38·87^{ac}	34·10^{a*}	71·68^{b}	51·91^{ac}	56·03^{bc}	4·22

Con, control diet with normal corn starch; GCS, granular high amylose corn starch (45·04 % RS content); GPS, granular potato starch (71·51 % RS content); RCS, retrograded high amylose corn starch (41·45 % RS content); GG, guar gum; Cell, cellulose.

^{a,b,c} Values within a row with unlike superscript letters are significantly different ($P<0·05$) as analysed with Tukey's multiple comparison test using PROC MIXED model of SAS.

Values are significantly different from the Con group (*$P<0·05$) as analysed with Dunnett's test using PROC MIXED model of SAS.

† For details of animals and procedures, see Materials and methods.

Starch thermal properties were measured in order to examine the potential association between these commonly analysed physical–chemical endpoints and the physiological responses observed following consumption of the starch varieties (Table 7). The highest transition temperatures, i.e. onset and peak, were associated with the RCS variety while potato starch had the lowest transition temperatures. Large differences in transition temperatures and enthalpy changes were observed between normal corn starch and GCS (Table 7).

Endpoint changes in the efficiency of nutrient utilisation and intestinal fermentation were statistically analysed for their correlations with the thermal physical characteristics measured (Table 8). Apparent CP digestibility values were negatively correlated ($P<0·05$) with the enthalpy changes in the three RS preparations. Apparent Ca and P digestibility values were positively correlated ($P<0·001$ and 0·02, respectively) with onset and peak temperatures of the starch varieties. Caecal butyrate concentration was positively correlated ($P<0·001$, 0·001 and 0·004, respectively) with onset and peak temperatures as well as the enthalpy changes in the RS preparations.

Discussion

Distal ileal starch recovery

As evident from the rather low distal ileal recovery (27–42 %) of the selected RS varieties, a discrepancy exists between the amount of RS measured *in vitro* and the amount of RS recovered *in vivo*. In accordance with this observation, previous research with human subjects suggests that current *in vitro* RS analyses procedures are unreliable predictors of starch that is truly resistant under *in vivo* conditions[21,22]. Alternatively, Silvester *et al.*[23] have concluded that the amount of starch reaching the large intestine can be accurately estimated from *in vitro* measurements. Thus, the correlation between *in vitro* and *in vivo* RS measurements may be dependent on various physiological and methodological factors, including the animal model employed and the *in vitro* method used to analyse the RS fractions[24]. Additionally, the problem of *in vivo* RS analyses may be further complicated by the method used to recover intestinal starch. As concerns have been raised regarding the comparison of dietary RS concentrations obtained using ileostomy models and intubation techniques[21], it is possible that the intestinal cannulation procedures in the present study may

Table 5. Efficiency of digestive and post-absorptive utilisation of P following the consumption of resistant starch (RS) and conventional fibres in the pig†

(Values represent least squares means with their standard errors of the mean)

	Experimental diets						
	Con *n* 6	GCS *n* 5	GPS *n* 4	RCS *n* 5	GG *n* 5	Cell *n* 5	SEM *n* 6
Efficiency of utilisation	% of total P intake						
Apparent ileal P digestibility	76·98^{a}	73·39^{a}	77·49^{a}	76·87^{a}	63·81^{b*}	73·71^{a}	2·94
Apparent total tract P digestibility	72·22^{bc}	61·94^{ab}	55·99^{a*}	77·67^{c}	70·08^{bc}	72·63^{bc}	2·74
Apparent total tract P loss	27·78^{bc}	38·05^{ab}	44·01^{a*}	22·33^{c}	29·92^{bc}	27·37^{bc}	2·74
Urinary P loss	9·52	9·71	11·25	10·32	12·28	9·77	1·17
Apparent total P loss	37·30^{bc}	47·77^{ab}	55·27^{a*}	32·65^{c}	42·20^{bc}	37·14^{bc}	3·00
Apparent P retention	62·70^{bc}	52·23^{ab}	44·73^{a*}	67·35^{c}	57·80^{bc}	62·86^{bc}	3·00

Con, control diet with normal corn starch; GCS, granular high amylose corn starch (45·04 % RS content); GPS, granular potato starch (71·51 % RS content); RCS, retrograded high amylose corn starch (41·45 % RS content); GG, guar gum; Cell, cellulose.

^{a,b,c} Values within a row with unlike superscript letters are significantly different ($P<0·05$) as analysed with Tukey's multiple comparison test using PROC MIXED model of SAS.

Values are significantly different from the Con group (*$P<0·05$) as analysed with Dunnett's test using PROC MIXED model of SAS.

† For details of animals and procedures, see Materials and methods.

Table 6. Individual and total caecal SCFA concentrations following the consumption of resistant starch (RS) and conventional fibres in the pig†

(Values represent least squares means with their standard errors of the mean)

	Experimental diets						
	Con *n* 6	GCS *n* 5	GPS *n* 4	RCS *n* 5	GG *n* 5	Cell *n* 5	SEM *n* 6
	mg/g DM digesta						
Acetic acid	14·84	14·83	12·15	17·38	17·98	12·30	1·66
Butyric acid	4·98^{a}	6·37^{a}	6·18^{a}	11·89^{b*}	11·40^{b*}	8·45^{ab}	0·83
Hexanoic acid	6·80^{a}	7·14^{a}	7·94^{ab}	4·87^{a}	17·12^{b*}	8·63^{ab}	1·64
Indole	0·12^{a}	0·04^{b*}	0·02^{b*}	0·04^{b*}	0·06^{ab}	0·08^{ab}	0·01
Isobutyric acid	4·03^{ab}	3·04^{bc}	2·50^{bc}	1·98^{c*}	4·92^{a}	2·88^{bc}	0·35
Isovaleric acid	4·20^{a}	2·41^{a}	3·08^{a}	1·73^{a}	7·91^{b*}	3·92^{a}	0·68
p-cresol	0·38	0·51	2·43	0·080	3·28	0·047	1·01
Propionic acid	20·65^{ab}	14·79^{a}	20·66^{ab}	29·77^{b}	31·47^{b}	20·25^{ab}	2·42
Skatole	0·26	0·34	0·14	0·24	0·14	0·23	0·06
Valeric acid	8·62	7·65	7·42	8·47	13·49*	8·66	1·08
Total SCFA	64·11^{a}	56·29^{a}	59·25^{ab}	75·94^{ab}	104·24^{b*}	65·09^{ab}	7·40

Con, control diet with normal corn starch; GCS, granular high amylose corn starch (45·04 % RS content); GPS, granular potato starch (71·51 % RS content); RCS, retrograded high amylose corn starch; GG, guar gum; Cell, cellulose.

^{a,b,c} Values within a row with unlike superscript letters are significantly different ($P<0·05$) as analysed with Tukey's multiple comparison test using PROC MIXED model of SAS.

Values are significantly different from the Con group (*$P<0·05$) as analysed with Dunnett's test using PROC MIXED model of SAS.

† For details of animals and procedures, see Materials and methods.

have altered intestinal physiology and microbial fermentation patterns and ultimately affected the distal ileal starch recovery. Furthermore, a potential microbial contribution to the fermentation of RS in the small intestine is likely, as the distal ileal recovery of GG (38 %) and cellulose (62 %) was lower than expected (Table 2).

Nutrient utilisation

In protein nutrition, fibre supplements are traditionally considered anti-nutritional factors as they have been frequently shown to depress the efficiency of CP utilisation[25]. Previous work has demonstrated that the net efficiency of protein utilisation is reduced in rats fed potato starch compared with rats consuming corn starch[26]. In the current study, consumption of the two granular starch preparations was associated with increased total tract CP excretion and a reduced urinary: faecal CP ratio. Similarly, previous work confirms that there is a net transfer of plasma urea N to the large intestine with subsequent conversion to NH_3 and incorporation into bacterial protein in response to the consumption of RS type 2[27]. The efficiency of microbial protein synthesis is thought to be partially dependent on the amount and type of carbohydrate that enters the large intestine[28]. Under conditions where energy may be derived from a sufficient carbohydrate supply, bacterial growth is maintained through efficient N recycling by ureolytic bacteria[28]. Given the reduction in the urinary: faecal CP ratio observed in response to the granular starch preparations (Table 3), it is likely the granular RS varieties were preferentially utilised as bacterial energy sources and resulted in a greater caecal recycling of blood urea N in comparison with the RCS variety and the traditional fibre sources. However, without knowledge of how the RS preparations affected ileal digesta passage rates, it is difficult to make firm conclusions regarding the efficiency of caecal fermentation in response to the different starch varieties.

Literature reports concerned with the effects of RS and conventional fibre supplements on the efficiency of Ca utilisation have been inconsistent[29]. The increased apparent total tract Ca digestibility that we observed in response to the consumption of RCS confirms the observations of Morais *et al.*[30], who reported that feeding 7–10 d old piglets a cooked, cooled high amylose corn starch preparation increased apparent Ca absorption by 30 %. Alternatively, in agreement with previous findings[31], a reduction in total tract Ca digestibility was observed in response to the consumption of the GPS variety compared with the control group. As shown in Table 4, apparent ileal Ca digestibility was not affected by diets. Furthermore, the much lower apparent total tract Ca digestibility values than the corresponding apparent distal ileal values in GCS- and GPS-based diets would also suggest that the endogenous Ca secretion into the large intestine might have been enhanced by GCS and GPS feeding in the pig. The caecum is considered to be a major site of Ca absorption through both transcellular and paracellular routes[32]. SCFA produced by microbial fermentation enhance Ca absorption

Table 7. Thermal properties of starch varieties heated in the presence of excess water (70 %, w/w)*

(Values represent least squares means with their standard errors for three animals)

	Gelatinisation					
	T_0 (°C)		T_p (°C)		ΔH (J/g)	
Starch products	Mean	SE	Mean	SE	Mean	SE
Normal corn starch	64·5	0·3	71·1	0·2	10·9	0·3
Granular corn starch	78·1	1·6	104·4	0·5	22·5	0·0
Granular potato starch	61·0	0·0	66·6	0·2	21·2	0·2
Retrograded corn starch	103·8	0·0	123·8	1·5	22·6	1·5

T_0, onset temperature; T_p, peak temperature; ΔH, change in enthalpy.

* For details of animals and procedures, see Materials and methods.

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Table 8. Pearson correlations between intestinal endpoints and thermal properties of the starch varieties*

	Thermal properties of starch varieties					
	T_0		T_p		ΔH	
	R	*P*	*r*	*P*	*r*	*P*
Apparent total tract CP digestibility	0·26	0·22	0·13	0·59	−0·45	0·05
Apparent total tract Ca digestibility	0·66	<0·001	0·53	0·02	−0·06	0·80
Apparent total tract P digestibility	0·54	0·01	0·44	0·05	−0·22	0·35
Caecal butyrate concentration	0·83	<0·001	0·80	<0·001	0·63	0·004
Caecal indole concentration	−0·39	0·10	−0·44	0·06	−0·44	0·06
Caecal total short chain fatty acid concentration	0·34	0·16	0·09	0·71	0·28	0·25

T_0, onset temperature; T_p, peak temperature; ΔH, change in enthalpy; CP, crude protein.
r, Pearson partial correlation coefficients; *P*, probability of significance for six animals.
* For details of animals and procedures, see Materials and methods.

by decreasing caecal pH and solubilising luminal Ca complexes[33], enlarging the absorptive surface area of the caecum[34], increasing blood flow to the large intestine[35] and/or directly affecting the functional properties and the Ca transport pathways associated with the epithelial cell surface[36]. Increased butyrate production following RCS consumption may also help to explain the enhanced total tract Ca digestibility observed with this starch variety as compared with the other RS supplements. Both *in vitro*[36] and *in vivo*[37] investigations have suggested a relationship between butyrate production and caecal Ca absorption. However, if this is indeed the case, it is surprising that Ca absorption was not enhanced in the pigs consuming GG as caecal butyrate production was also high in this group. However, the effects of GG consumption on intestinal Ca absorption have been reported to be independent of caecal fermentation in rats[38].

Dietary P intake and intestinal P utilisation are important factors regulating whole-body Ca and P homeostasis and bone mineralisation[39]. Previous investigations have reported similar total tract absorption and retention of P in rats and pigs fed granular or retrograded corn starch preparations at a level of 6 %[40]. However, we observed a significant reduction in the efficiency of P retention in response to the consumption of the GPS variety in comparison with the control group (Table 5). Furthermore, the RCS variety significantly increased the apparent total tract P digestibility and the efficiency of whole-body P retention in comparison with the granular starch preparations (GPS and GCS) (Table 5). Differences in the basal diet formulation and fibre supplementation may be responsible for the apparent discrepancy between the results of the current study and the aforementioned report. Alternatively, the reduction in the apparent ileal P digestibility observed in the current study in response to GG consumption is consistent with previous findings[41] and is most likely associated with the viscous nature of GG. On the other hand, as shown in Table 5, the much lower apparent total tract P digestibility values than the corresponding apparent distal ileal values in GCS- and GPS-based diets would also suggest that the endogenous P secretion into the large intestine might have been enhanced by GCS and GPS feeding in the pig.

Intestinal fermentation endpoints

Butyrate is important in regulating intestinal function and systemic health and has been shown to act as a signalling molecule in a diverse range of metabolic events including: (i) the absorption and packaging of dietary fat by the intestinal enterocyte[42]; (ii) the modulation of pro-inflammatory cytokine expression for the prevention and treatment of inflammatory bowel disease[43]; (iii) the expression of intestinal epithelial antimicrobial peptide[44]; (iv) the inhibition of colonic crypt cell proliferation for prevention of colon-rectal cancer[45]; (v) the reduction of cell adhesion molecule expression in endothelial cells[46]; (vi) the modulation of arterial smooth muscle cell proliferation[47]. Thus, caecal butyrate production by bacterial fermentation may be a good indicator of intestinal function and systemic health in response to dietary fibre consumption. In the current study, consumption of both the RCS and GG diets significantly increased caecal butyrate concentrations. Therefore, as compared with the granular RS preparations (GCS and GPS), it appears that the retrograded RS variety (RCS) is the most effective RS for the enhancement of microbial butyrate production.

On the other hand, indoles and their derivatives are volatile odour-causing compounds that have been demonstrated to be carcinogenic[48]. Interestingly, all the RS varieties in the present study were shown to significantly reduce caecal indole concentrations and may therefore be a useful dietary therapy in the prevention and treatment of intestinal cancers.

Relationship between thermal properties of resistant starch and functionality

In the current study, feed intake was similar between the treatment groups and dietary RS supplementation was equalised by adjusting for the starch fraction resistant to *in vitro* enzymatic hydrolysis. Therefore, the different nutrient utilisation patterns that we observed are most likely related to the unique physiochemical, thermal and molecular structural properties associated with each starch variety. In agreement with previous findings[49], the different starch varieties used in the current study were characterised by unique thermal properties. In the food-processing industry, starch thermal properties are recognised as important parameters influencing the gelling ability and textural quality of food products[50]. Furthermore, although various starch thermal and structural parameters have been widely examined for their effects on α-amylase function and granular starch release[51], the relationship between these starch parameters and physiological responses is not known. To our knowledge, this is the first study to

demonstrate significant correlations between starch thermal parameters and nutrient digestibility values and caecal SCFA concentrations. Although a biological explanation for the relationship between the observed physiological responses and the starch thermal parameters is not readily apparent, one can speculate that these thermal properties are a reflection of the starch molecular and structural characteristics that influence the physical interaction between starch granules and pancreatic digestive enzymes, dietary and endogenous nutrients and microbial species within the intestinal lumen. A more comprehensive understanding of these correlations may help predict potential physiological responses based on routine thermal–physical analyses and facilitate the development of future RS-based functional food ingredients.

In conclusion, nutrient utilisation efficiency and intestinal fermentation are differentially affected by the consumption of granular and retrograded starch varieties. Apparent total tract Ca digestibility was increased following consumption of the retrograded starch variety (RCS) but was reduced with the consumption of GPS. Furthermore, consumption of the GPS variety was associated with a reduction in the efficiency of whole body Ca and P utilisation in comparison with the control group. Although all the RS varieties reduced the production of the odour-causing and carcinogenic compound indole, the retrograded starch variety (RCS) was the most effective in enhancing caecal butyrate production. Additionally, the thermal physical properties associated with unique RS varieties were correlated with important nutrient digestion and fermentation endpoints and therefore may be useful in the development of future RS varieties with desired functionality.

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