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Different carbohydrate sources affect swine performance and post-prandial glycaemic response

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ABSTRACT

The type of starch and fibre in the diet affects several parameters, including glycaemic and insulin response, that are involved in pig growth performance. Four experimental diets for growing pigs differing for carbohydrates source (corn, barley, faba bean and pea) were tested. The diets were analysed *in vitro* to assess the carbohydrates characteristics, and they were administered to 56 crossbreed growing pigs (Landrace × Large White) randomly divided into four groups (mean age of 95 ± 6 days; body weight 80 kg ± 4 days). Clinical examination and average daily gain were performed before recruitment and after 40 days of experiment. The metabolic effects were investigated by blood count and serum biochemical parameters and by the glycaemic and insulin post-prandial response. The study revealed substantial differences among the diets, suggesting that alternative feedstuffs for swine affect several parameters, including glycaemic and insulin response, with no negative effects on growing performance. The Barley group showed the highest daily weight gain ($p < .05$) associated with the highest glycaemic ($p < .05$) and insulin response at 1 and 2 h post-prandial ($p < .01$), suggesting that the barley-based diet can support performance comparable to that of the corn-based diet in growing pig. By contrast, the lowest glycaemia was observed in the Faba bean group ($p < .05$), confirming the capacity of this legume to modulate post-prandial glucose levels. Moreover, the ability of some ingredients in lowering glucose and insulin response enriches the knowledge on functional nutrients for animal diets and to prevent the incidence of enteric diseases.

HIGHLIGHTS

- The type of starch and fibre used in the diet highly affected some blood parameters, such as glycaemic and insulin responses.
- The Barley group showed the highest daily weight gain .
- Lower glycaemia levels were observed in the Faba bean group compared to the Corn one.
- Alternative protein sources for swine diets can limit the glycaemic and insulin response with no negative effects on growing performance.

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Introduction

Starch is the most represented nutrient in pig diets and its structure is considered to have a great impact on its digestion in terms of amount and rate. Nevertheless, the dietary starch sources (i.e. cereals, tubers or legume grains) differ for physical and chemical characteristics of the polysaccharides, thus affecting its potential digestion (Giuberti et al. 2012). The kinetics of complex carbohydrates digestion represent an important issue in pig nutrition, affecting the productive performance, nutrient digestibility, feed intake, carcass composition and physiological responses (Giuberti et al. 2012).

Several factors, such as the shape and size of crystal structure present in the starch granules, protein and lipid matrices, as well as the utilised feed-processing methods, may influence post-prandial glucose response (Sun et al. 2007). Starch is a polysaccharide characterised by a semi-crystalline structure and mainly composed by amylose and amylopectin that are both glucose polymers but differ for structure and molecular weight.

Corn is the main energy source in swine diet (Lammers et al. 2007) and its association with barley is widely used to increase the lysine and digestible phosphorus amount in the diet (Wang et al. 2017).

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Soybean meal (SBM) is the most used source of plant origin to guarantee protein requirements in livestock nutrition. The use of other protein sources, such as peas or faba beans, also implies a starch input (Mateos et al. 2007). The crude protein content of legume grains is twice than cereals and the complexes of amylose with surrounding lipids and proteins may influence their digestibility, thus lowering the rate of starch digestion (Witczak et al. 2016). Pea grains contain, on average, the 25% of crude protein (CP) and starch varying from 33% to 45% (Shen et al. 2016). Faba bean seeds, despite a similar CP content (20–25%), has a higher starch (39–49%) amount (Masoero et al. 2005).

The sources affect the proportion between amylose and amylopectin in the starch. High amylose starch was observed to be more slowly hydrolysed compared with the amylopectin fraction. In legume grains, amylose varies from 14% to 88% of molecular structure of starch, in function of the botanical species (Tayade et al. 2019).

In the past, in swine production, structural carbohydrates higher than 5% (Proto 1984) were considered inadvisable because of their negative effects on feed intake as well as on nutrients digestibility (de Lange 2000). On the other hand, during the last 15 years the presence of dietary fibre (insoluble and soluble) in the swine diet was demonstrated to reduce pollutant excretion, improving micronutrients absorption and limiting ammonia emission (Aarnink and Verstegen 2007; Vastolo et al. 2019). Soluble dietary fibre improves gut health and reduces the glycaemic index (Musco et al. 2015; Zhao et al. 2019), hence, it is nowadays considered a functional bioactive ingredient (Musco et al. 2016; Zijlstra et al. 2012) both in swine and in humans.

The types of starch and fibre used in the diet highly influence the digestion and absorption of nutrients, affecting several factors, including glycaemic and insulin responses that are involved in pig growing performance. In particular, a high glycaemic index is considered a tool that affect feed intake and performance in pigs (Giuberti et al. 2012). To this purpose, the impact of different ingredients on pig growing performance were evaluated using four diets differing for starch and protein sources (i.e. corn; barley, faba bean and pea).

Materials and methods

All the animals were treated according to the principles of the animal welfare stated by the EC Directive 63/2010/EEC regarding the protection of the animals used for experimental and other scientific purposes. All animal procedures were approved by the Ethical Animal Care and Use Committee of the University of Napoli Federico II (Prot. 15/0030057).

Experimental diets

A conventional diet for growing pigs based on corn and soybean meal was formulated and used as control (Corn diet). Three experimental diets were formulated according to NRC (2012) guidelines in order to satisfy the nutritional requirements. All the diets were isoenergetic and isoprotein. The main characteristics of the diets are reported in Tables 1 and 2.

In the Barley diet, corn was partially replaced with a barley flour with 13% of β-glucans (Beta Barley Flour 13 – Agroalimentare Sud, Melfi, Potenza, Italy). The Faba bean and Pea diets were formulated by

Table 1. Ingredients of the diets administered to the growing pigs (% as-fed).

Diets	Corn	Barley	Faba bean	Pea
Ingredients				
Corn	50.0	24.0	47.0	30.0
Wheat middlings	18.1	15.0	13.0	14.0
Wheat bran shorts	18.0	13.0	10.0	14.0
Soybean meal	9.5	9.0	–	–
Barley	–	35.0	5.0	10.0
Faba bean	–	–	21.15	–
Field peas	–	–	–	28.0
Soybean oil	0.7	0.7	0.7	0.7
Beet molasses	1.0	1.0	1.0	1.0
$CaCO_3$	1.5	1.5	1.0	1.4
Esterified palm oil	0.7	0.7	0.7	0.7
Supplements[a]	0.3	0.3	0.3	0.3
L-Lysine	0.3	0.3	0.3	0.3
NaCl	0.25	0.25	0.25	0.25
Monocalcium phosphate	0.2	0.1	0.2	0.2
DL-methionine	0.1	0.1	0.1	0.1

[a]Supplements: vitamins A 10,000 UI, D3 1000 UI, E 40 mg, B1 0.24 mg, B2 4.8 mg, B6 0.24 mg, B12 0.02 mg, K 0.2 mg, Niacin 0.02 mg, Folic acid 0.01 mg and Choline Chloride 400 mg. Microminerals Fe 100 mg, I 1.2 mg, Cu 12 mg, Mn 48 mg, Zn 72 mg, Se 0.20 mg.

Table 2. Nutritional characteristics of the diets administered to the growing pigs (%, as-fed).

Diets	Corn	Barley	Faba bean	Pea
Chemical composition				
DM	89.80	91.02	91.16	90.91
CP	14.16	14.41	14.54	14.33
EE	3.92	3.17	2.79	2.74
NDF	18.76	19.64	19.16	19.12
Ash	6.05	5.54	5.56	5.69
TDF	2.18	2.35	2.42	2.33
IDF	1.94	1.79	1.78	1.95
SDF	0.24	0.56	0.64	0.48
DE	14.91	14.81	14.90	14.90
ME	14.61	14.51	14.60	14.60

DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fibre; TDF: total dietary fibre; IDF: insoluble dietary fibre; SDF: soluble dietary fibre; DE: digestible energy; ME: metabolisable energy.

substituting in small part the corn with Beta Barley Flour 13 (5 and 10%, respectively) and completely replacing the soybean meal with whole faba bean (var. Torrelama) and pea (var. Lavagna). The botanical varieties of faba bean and pea were selected in function of their low levels of antinutritional factors (i.e. tannins, phytic acid, lectins, trypsin inhibitors).

The digestible and metabolisable energy of the diets were calculated using the equations proposed by Noblet and Perez (1993) and NRC (2012):

$$\mathrm{DE\ (kcal/kg)} = 4.168 - (91 \times \mathrm{Ash}) + (19 \times \mathrm{CP}) + (39 \times \mathrm{EE}) - (36 \times \mathrm{NDF})$$

$$\mathrm{ME\ (kcal/kg)} = \mathrm{DE} - (68 \times \mathrm{CP})$$

Chemical composition

The diets were ground at 1.1 mm and analysed for chemical composition according to AOAC (2005). In particular, crude protein, ether extract, dry matter and ash were determined (ID numbers: 954.01, 920.39 C, 934.01 and 942.05; respectively). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Van Soest et al. (1991).

Carbohydrates characterisation

The different carbohydrates fractions were used to determine total dietary fibre (TDF), insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) content following the indications of Prosky (1990) and Lee and Prosky (1995). For the separation of SDF from IDF fraction, the MES-TRIS buffer was utilised, and each residual was filtered through pre-weighed glass crucibles (Scott Duran porosity #3). The values of the three dietary fibre fractions were calculated subtracting the residual ash and the crude protein content (Musco et al. 2017).

Three aliquots for each diet were taken and analysed in order to fractioned the starch according to Englyst et al. (1992) and van Kempen et al. (2010) in: rapidly digestible starch (RDS, within 20 min of incubation), slowly digestible starch (SDS, between 20 and 120 min) and resistant starch (RS) not further hydrolysed. In particular, ground samples were incubated in quadruplicate with pepsin EC (Sigma- Aldrich P-7000) and guar gum in 0.05 mol/L HCl for 30 min at 37 °C into a water bath with constant agitation. The enzyme mixture contained pancreatin (Sigma-Aldrich P-7545), amyloglucosidase (Megazime E-AMGDF), and invertase (Sigma-Aldrich P-57629). For stopping starch digestion absolute ethanol was added to one millilitre of solution and glucose released was measured colorimetrically at 540 nm as reported on the brief of the glucose oxidase kit (Sigma-Aldrich GAGO20).

The values for total starch (TS), RDS, SDS and RS were calculated using the values of released glucose after 20 min (G20), 120 min (G120), FG (free glucose) and TG (total glucose) according to the methods proposed by Englyst et al. (1992):

$$\mathrm{TS} = (\mathrm{TG} - \mathrm{FG}) \times 0.9$$
$$\mathrm{RDS} = (\mathrm{G20} - \mathrm{FG}) \times 0.9$$
$$\mathrm{SDS} = (\mathrm{G120} - \mathrm{G20}) \times 0.9$$
$$\mathrm{RS} = (\mathrm{TG} - \mathrm{G120}) \times 0.9$$

In vivo *trial*

The trial was carried out for 40 days (10 days of adaptation + 30 days of experiment) in a commercial pig farm located in Apollosa (BN, Italy). A total of 56 crossbreed growing pigs (Landrace x Large White Commercial Hybrid "Ibrido Nazionale '88") homogeneous for age (95 ± 6 days) and body weight (BW, 80 ± 4 kg) were recruited after health assessment by a clinical examination and by the evaluation of haematological and biochemical profiles. The pigs were randomly assigned to one of the four experimental groups (Corn, Barley, Faba bean and Pea, $n = 14$ for each group) and housed in pens (2.0 m × 2.2 m). Each diet was administered in ratio of 197 kcal of ME per kg $\mathrm{BW}^{0.60}$. Artificial lights were used from 6:30 a.m. to 8:00 p.m. Environmental temperature was kept at 20 ± 2° C throughout the 40-days trial. The animals were fed twice per day at 8:30 a.m. and 3:30 p.m. and had free access to water. Individual BW was recorded at the arrival of the pigs in the pen and the end of the trial, and the cumulative data recorded were used to calculate daily weight gain (DWG). Dry matter intake (DMI) per pen was recorded daily during the trial.

Blood analysis

At recruitment and after 40th day of diet administration, blood samples were collected before feed administration from the jugular vein into tubes with and without K3-EDTA and immediately transported to the laboratory. Blood samples were centrifuged at 1200 × *g* for 15 min in order to obtain the sera that was divided in aliquots and frozen at −20 °C. Complete blood count was performed on whole EDTA blood by the IDEXX Lasercyte TM haematology analyser (IDEXX Laboratories, Inc., Westbrook, Maine, USA) (red blood cells (RBC), haematocrit (HCT), haemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), red blood cells distribution width (RDW), reticulocyte (RETIC), white blood cells (WBC), neutrophils (NEU), lymphocyte (LYM), monocyte (MONO), eosinophils (EOS), basophils (BASO), platelets (PLT)). Blood chemistry parameters were assayed using an automatic biochemical analyser AMS AUTOLAB (Rome, Italy) using reagents purchased from Spinreact (Girona, Spain) to determine: glucose (GLU), triglycerides (TRI), cholesterol (CHO), total proteins (TP), albumin (ALB), blood urea nitrogen (BUN), creatinine (CREA), aspartate amino transferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), creatine kinase (CK) and lactic dehydrogenase (LDH).

Glycaemic and insulin post-prandial response

After 40 days of trial, blood samples (5 mL/pig) were taken from seven pigs per group from the jugular vein before feed administration (time 0) and every 1 h for four times after feed assumption in order to evaluate the carbohydrates metabolism. Blood was centrifuged at 1200 × *g* for 15 min in order to obtain the sera that was immediately frozen at −20 °C until analysis. Sera glucose was determined as previously described, and insulin was measured by an AIA®-360 benchtop analyser (Carolina Liquid Chemistries Corp. 313 Gallimore Dairy Rd Greensboro, NC) using a specific pack (ST AIA-PACK IRI, Tosoh Bioscience, Tokyo, Japan).

Statistical analysis

Blood count and chemistry parameters, growth performance, dry matter intake and *in vivo* glycaemia and insulin data were statistically analysed using by ANOVA in order to evaluate the diet effects according to the following equation:

$$Yij = \mu + \alpha i + \varepsilon ij$$

where y is the dependent variable, μ is the mean, α is diet effect (i: Corn, Barley, Faba bean, Pea) and ε is the error term.

Different means were compared using Tukey's multiple comparison test. The differences were considered significant at $p < .01$. All statistical procedures were performed using JMP version (14 SW) SAS Institute Inc., Cary, NC.

Results

Carbohydrates characterisation

The characterisation of the starch in the diets is reported in Table 3. The total content and the relative fractions significantly varied ($p < .01$) among the diets. In particular, RDS was the least represented fraction in all the diets, except for the Pea one, where SDS showed the lowest level.

RS was the more represented fraction, except for the Faba bean diet.

Comparing the diets, the highest ($p < .01$) TS content was found in the Faba bean diet whereas the lowest value was detected in the Pea diet (390 and 346 g/kg DM, respectively). Barley and Corn diets showed intermediate and similar values (about 380 g/kg DM). Concerning the different fractions of starch, Barley and Faba bean diets showed a fair distribution among the three fractions (RDS, SDS, RS). On the contrary, in Corn diet, RDS resulted poorly represented (19.19% TS) and in Pea diet the distribution was clearly defined: RS > RDS > SDS. The resistant starch significantly ($p < .01$) varied among the diets: in Pea diet it represented almost the half of the total starch (48.38% TS), followed by Corn and Barley diets (40.79 and 38.08% TS, respectively), whereas Faba bean diet showed the lowest values (35.26% TS).

In vivo trial

Despite a similar dry matter intake, significant differences ($p < .05$) were observed for daily weight gain (Table 4). In particular, Barley group showed the highest DWG (622 g/day) compared to the other groups,

Table 3. Characterisation of starch in the diets.

Diets		Corn	Barley	Faba bean	Pea	RMSE
TS	DM g/kg	380.11^{B}	380.20^{B}	389.98^{A}	346.10^{C}	0.681
RDS	% DM	8.18^{C}	11.10^{B}	11.25^{B}	12.06^{A}	1.09
SDS	% DM	17.06^{A}	14.77^{B}	16.42^{A}	7.59^{C}	1.91
RS	% DM	17.39^{B}	15.90^{C}	15.07^{D}	18.42^{A}	1.15

TS: total starch; RDS: rapidly digestible starch; SDS: slowly digestible starch; RS: resistant starch. RMSE: root mean square error.
$^{A-D}$Within a row, values with different letters differ significantly at $p < .01$.

Table 4. Growth performance of the growing pigs ($n = 56$: 14/group).

Diets	Corn	Barley	Faba bean	Pea	RMSE
Initial BW kg	80.43	80.71	80.31	81.14	1.99
Final BW kg	95.26	99.39	97.80	98.87	2.29
Mean DMI kg/day	2.44	2.47	2.47	2.40	0.49
Mean DWG g/day	495^{c}	622^{a}	583^{b}	581^{b}	39

BW: body weight; DMI: dry matter intake; DWG: daily weight gain. RMSE: root mean square error.
$^{a-c}$Values within a row with different letters differ significantly at $P < .05$.

Table 5. Blood count in growing pigs before and after 40 days of experimental diets administration.

Diets		T40	Corn	Barley	Faba bean	Pea	RMSE
RBC	10^6/μL	7.21	7.16	7.27	6.92	7.45	0.564
HCT	%	36.91	39.37	38.46	36.47	38.91	2.308
HGB	g/dL	12.23	12.88	12.44	13.21	13.04	0.692
MCV	fL	51.33	55.05	53.38	52.76	52.34	2.507
MCH	pg	17.12	18.00	17.40	19.09	17.63	1.303
MCHC	g/dL	32.46	32.75^B	32.57^B	36.24^A	33.61^B	1.473
RDW	%	19.78	17.42	17.55	17.14	17.71	0.670
RETIC	%	0.97	0.98	1.00	1.00	0.91	13.91
WBC	%	12.26	11.15	11.65	12.94	12.69	1.581
NEU	%	50.25	44.77	55.34	52.33	48.56	1.146
LYM	%	40.09	48.28	33.32	36.64	42.13	1.287
MONO	%	8.32	5.57^B	10.28^A	9.76^A	7.69^{AB}	0.263
EOS	%	1.01	0.80	1.80	0.40	1.04	0.080
BASO	%	0.59	0.58	0.66	0.53	0.60	0.0157
PLT	10^3/μL	259	259	291	321	280	68.64

RBC: red blood cells; HCT: haematocrit; HGB: haemoglobin; MCV: mean corpuscular volume; MCHC: mean corpuscular haemoglobin concentration; RDW: red blood cells distribution width; RETIC: reticulocyte; WBC: white blood cells; NEU: neutrophils; LYM: lymphocyte; MONO: monocyte; EOS: eosinophils; BASO: basophils; PLT: platelets. RMSE: root mean square error; T40: mean values of the 4 experimental groups at the end of the trial.
$^{A–B}$Values within a row with different letters differ significantly at $P < .01$.

Table 6. Blood chemistry in growing pigs before and after 40 days of experimental diets administration.

Diets		T40	Corn	Barley	Faba bean	Pea	RMSE
GLU	mg/dL	86.5	92.33^{ab}	103.50^a	86.50^b	95.57^{ab}	8.777
TRI	mg/dL	54.04	39.50	38.17	47.60	50.57	11.01
CHO	mg/dL	133	98.00	96.67	87.00	87.86	9.010
TP	g/dL	9.56	10.03^{AB}	9.35^B	9.24^B	11.30^A	1.027
ALB	g/dL	4.75	5.28^{ab}	4.87^b	5.06^{ab}	5.52^a	0.390
BUN	mg/dL	24.3	27.00^A	26.33^A	21.71^{AB}	17.57^B	4.966
CREA	mg/dL	2.80	2.42	2.55	2.21	2.17	0.394
AST	U/L	55	56.00	60.67	60.86	59.28	6.841
ALT	U/L	66.6	71.83^{ab}	62.83^b	76.42^a	73.14^{ab}	8.279
GGT	U/L	37.3	29.00	46.33	36.80	44.43	12.247
CK	U/L	1374	779	1136	871	1035	236.71
LDH	U/L	1163	829	805	845	942	79.45

GLU: glucose; TRI: triglycerides; CHO: cholesterol; TP: total proteins; ALB: albumin; BUN: blood urea nitrogen; CREA: creatinine; AST: aspartate amino transferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl-transferase; CK: creatine kinase; LDH: lactic dehydrogenase. RMSE: root mean square error; T40: mean values of the 4 experimental groups at the end of the trial.
$^{A–B}$Values within a row with different letters differ significantly at $P < .01$.
$^{a–b}$Values within a row with different letters differ significantly at $P < .05$.

Faba bean and Pea groups showed intermediate and similar values (583 and 581 g/day, respectively), whereas the administration of Corn diet resulted in significant lower DWG respect to all the other diets.

Regarding haematology on pigs at 40 days (Table 5), only two blood parameters were significantly affected by the experimental diets. In particular, MCHC resulted significantly higher (36.24 g/dL; $p < .01$) in Faba bean compared to the other groups, and Corn group showed the significantly lower MONO values (5.57 K/μL; $p < .01$) respect to Barley and Faba bean groups (10.28 and 9.76 K/μL, respectively).

Blood chemistry parameters (Table 6) showed significant diet effects. Total protein resulted significantly higher ($p < .01$) in the Pea group (11.30 g/dL) compared to the Barley and Faba bean one (9.35 and 9.24 g/dL, respectively) and BUN was significantly higher ($p < .01$) in Corn and Barley groups (27.00 and 26.33 mg/dL, respectively) respect to Pea group (17.57 mg/dL). Instead, significant differences ($p < .05$) emerged for GLU, that resulted higher in the Barley group (103.50 mg/dL) respect to the Faba bean one (86.50 mg/dL). The Pea group showed the highest ALB (5.52 g/dL) compared to Barley group (4.87 g/dL). The Faba bean diet showed a higher ALT level (76.42 U/L) respect to the Barley group (62.83 U/L).

Glycaemic and insulin post-prandial response

Blood glucose and insulin trends are reported in Table 7. All the diets showed the highest glucose levels 2 h after feeding, with the exception of the Pea group which showed the highest value after 1 h. For insulin, the trend was similar in all the groups: the values rapidly increased from 0 to 1 h post feeding and then slowly decreased.

Table 7. *In vivo* glucose and insulin response in growing pigs.

Time, h	0	1	2	3	4
Glucose, mg/dL					
Corn	84.00^{ab}	84.86^a	93.71	83.14	84.57
Barley	85.42^a	82.57^{ab}	89.57	74.83	80.86
Faba bean	75.71^b	76.83^b	79.43	76.14	74.71
Pea	82.28^{ab}	84.28^{ab}	79.70	81.67	83.00
RMSE	6.35	5.19	22.1	8.0	10.8
Insulin, μU/mL					
Corn	8.57	11.00^B	7.64^B	7.30	9.74
Barley	13.15	20.95^A	14.23^A	11.16	9.33
Faba bean	7.28	10.24B	8.35^B	8.70	11.41
Pea	8.89	8.80B	4.34^B	5.93	6.21
RMSE	3.800	5.010	3.820	4.596	4.530

RMSE: root mean square error.
$^{a–b}$Values within a column with different letters differ significantly at $P < .05$.
$^{A–B}$Values within a column with different letters differ significantly at $P < .01$.

Glycaemia resulted significantly ($p < .05$) higher for the Barley group (85.42 mg/dL) respect to the Faba bean group before feeding (75.71 mg/fL) and Corn group showed a higher glycaemia value at 1 h post-feeding compared to the Faba bean group (84.86 and 76.83 mg/dL, respectively). Insulin production was significantly ($p < .01$) increased in Barley group at 1 and 2 h post-feeding (20.95 and 14.23 μU/mL, at 1 and 2 h, respectively) compared to all the other groups.

Discussion

With regards to the *in vitro* characterisation of starch, in all diets the sum of rapidly and slowly soluble fractions (RDS + SDS) was higher than 50%. Despite that, in the Pea diet the breakdown between the rapidly and slowly soluble starch was the opposite compared to the other three diets. In particular, more than 30% of the Pea diet starch was hydrolysed within the first 20 min. After 120 min of hydrolysis, the Pea diet showed the highest proportion of no-hydrolysable starch. This resistance to amylase activity could be partially explained by the crystalline structure and the high amylose proportion of pea starch granules (Hoover and Zhou 2003; Sun et al. 2007). The higher proportion of RS could improve colon health, because of its fermentability in the large intestine (Hoover and Zhou 2003; Sandhu and Lim 2008) improving the resistance to infective diseases (Mateos et al. 2007). As described by Murphy et al. (2008) the starch resistant to digestion is referred to as resistant starch type 2 (RS 2) found in some matrices, such as raw potato and legumes. Doti et al. (2014) reported similar results: these authors found *in vitro* comparable levels of resistant starch in a pig diet based on pea as starch source, compared to other experimental on cereals-based diets. Previous studies (Prandini et al. 2015, 2016) reported that the starch sources rich in amylopectin and soluble starch increase the feed efficiency and growth performance in pigs. In some cereals and legumes, the starch granules have adjacent proteins in the endosperm, or sometimes indented in the starch surface, thus providing a continuous protein matrix, that plays as further barrier against amylase (Berg et al. 2012).

Before the start of the trial, all the growing pigs showed haematological and biochemical profiles within the physiological ranges reported by Semiadi and Nugraha (2009) and Cooper et al. (2014). The blood count at 40 days of dietary treatment showed significantly higher corpuscular haemoglobin concentration (MCHC) levels in pigs fed the Faba bean diet. A similar increase of MCHC was described in humans (Lessire et al. 2017) after faba bean intake due to the lipaemia, albeit it can be associated to haemolysis due to the to the presence, in the faba bean seed, of glucopyranosides such as vicine and convicine that could be converted in redox-active derivatives divicine (D) and isouramil (I), respectively, and enter the blood compartment. The Faba bean diet also showed the lowest levels of serum glucose at fasting; this result is in accordance with Martinezm and Macarulla (1992), who found a reduction in serum glucose by feeding growing mice with a diet containing field beans as protein source. In humans, Fabek et al. (2017) tested the effect of faba bean flour inclusion into wheat flour crackers on glucose control; the authors concluded that the legume may help to modulate post-prandial glucose control.

Serum albumin and total proteins, responsible of osmotic pressure balance and transport of substances in the blood, resulted significantly higher in the group fed the Pea diet. Such results are in agreement with Bingol et al. (2016) who found higher blood total protein concentration in broilers fed a diet with 20% of pea replacing soybean. It may be assumed that pea aminoacidic composition influenced total protein concentration. This is different among pea, faba bean and soybean (Gorissen et al. 2018) and it was due to the differences among globulin fractions. The high blood urea nitrogen levels in Corn and Barley groups are in agreement with the results reported by Wu et al. (2015), who found significantly higher ($p < .05$) BUN levels in pigs fed a diet based on corn and soybean. Serum biochemical parameters are important indicators of physiological and metabolic functions and represent a tool to evaluate animal health. Serum BUN is the final product of the Krebs–Henseleit cycle, from the protein and amino-acid metabolism (Sun et al. 2007). The relatively lower BUN levels observed in the Pea group suggest that the diet feeding patterns contributed to ameliorate the possible negative effects of protein catabolism.

The Faba bean diet showed higher ALT levels, but the results fall in the physiological range for this parameter. Zhou et al. (2013) observed similar cases in human patients with normal glucose regulation and hypothesised that it was due to an unfavourable nocturnal glucose profile and they suggested that liver enzymes may be a marker of an adverse glucose profile.

Concerning pig performance, literature data are controversial. The parameters of voluntary ingestion and the daily weight gain recorded with all the diets are in agreement with those of Knap (2009). The dry matter intake was not affected by the nutritional treatment: the Corn diet showed the lowest value, on the contrary, the Barley diet showed the highest one, intermediate values were observed for both legumes-based diets. Prandini et al. (2011), testing in growing-finishing heavy pig three diets differing for protein sources (soybean meal vs. pea or faba bean), did not find significant differences in blood analysis, but the DWG resulted significantly ($p < .05$) higher in the Faba bean group than in the other diets. Harrold et al. (1989) demonstrated that a barley-based diet can support performance comparable to a corn-based diet in growing pigs. On the contrary, Tuśnio et al. (2017)

found significantly higher daily gain and feed conversion ratio in male swine fed a diet with pea as protein source for four weeks, compared to soybean. Stein et al. (2004) and Prandini et al. (2005) showed that pea may be included from 15 up to 20% in the diets offered to post weaning pigs without negative effects on growth performance. Partanen et al. (2003) showed that faba beans can be used up to 20% in diets formulated for growing swine, but a higher quantity can negatively affect the growth performance. In our study, the inclusion of faba bean and pea in the diets was 21 and 28%, respectively, without negative effects on growth pigs performance.

The apparent disagreement among the reported studies could be related to the different age of the swine utilised in the trials.

Regarding the glucose and insulin responses, the Corn diet induced a faster and higher post-prandial glycaemia increase compared to the Faba bean diet. The rate of starch digestion is one of the principal factors in determining glucose and insulin post-prandial response, for this reason it is extensively studied in humans, also for the relationship with metabolic diseases (Mathers and Daly 1998). According to our results concerning the Corn diet, starch digestion into the small intestine resulted in a slow release of glucose in the circulatory stream, which was extended up to 2 h. Similarly to Corn diet, the Barley diet caused a rapid glycaemic increase, but, in this case, it was associated with a more intense insulin response, probably because the soluble fibre, mainly represented by β-glucans in this diet, modified hormonal release, as suggested by Glore et al. (1994). De Vries et al. (2016), evaluating the effects of different dietary fibres in cannulated pigs, showed that β-glucans and resistant starch altered nutrients digestion and fermentation of diet complex carbohydrates. Miao et al. (2015) reported that SDS could be useful to prevent hyperglycaemia diseases.

The Faba bean diet caused a contained glycaemic response and the Pea diet caused a lower but faster glycaemic increase, characterised by a moderate insulin response compared to the other diets. Many studies indicated that a high level of resistant starch decreases post-prandial glucose and insulin response in subjects with either normal or impaired glucose tolerance (Behall et al. 2006). According to Deng et al. (2010) the lower blood glucose and insulin levels in diets rich in resistant starch, such as the Pea diet, suggest that the metabolisable energy supply was lower for this treatment compared with the other treatments in pig nutrition. These results are partially agreed with previous studies conducted in pigs (Regmi et al. 2010): a lower postprandial glucose response was observed with diets characterised by a low rapidly and slowly soluble starch levels and a high resistant starch content (such as Corn and Pea diets). On the other hand, it is recognised that the consumption of diets containing high RS levels can decrease postprandial plasma glucose (Deng et al. 2010), reducing the metabolism and, consequently, feed efficiency. Comparing the *in vitro* starch digestibility and the glycaemic and insulin post-prandial responses obtained *in vivo* in our trial, the Barley and Pea diets showed similar trends. In contrast, the Corn and Faba bean diets showed results similar to those obtained by the *in vitro* digestibility, with significant differences for the glycaemic response but not for the insulin one, probably because of the different proportion of soluble fraction in dietary fibre that resulted more than double in the Faba bean than in the Corn diets (Glore et al. 1994). The previous described discrepancies could be due to the interaction among nutrients (e.g. amylose/amylopectin ratio, protein-starch bonds, and chemical links) or, probably, to the short period of observation, that didn't allow to observe significant effects on metabolic process. Indeed, as reported by Regmi et al. (2010) the *in vivo* results are not always consistent and may vary considerably because of the confounding effect of nutrients other than starch or when the range of digestibility is not sufficiently wide.

Conclusions

The study revealed substantial differences among the diets and only partially it confirmed the results obtained *in vitro* suggesting the potential use of alternative feedstuffs for swine diets. In addition, the use of legume grains as protein sources alternative to soybean meal, as well as the use of barley grain in partial replace to corn, can represent an economic advantage for farmers, due to their local production. Soluble dietary fibre, mainly represented by barley β-glucans and resistant starch in legume grains, may guarantee a slower intestinal transit rate, with an improvement in micronutrients absorption. The results of this *in vitro* study contribute to determine the digestion rates of alternative feeds that can be included in pig diet either to encounter farmers' demand and to improve animal performance. Moreover, considering the ability of some ingredients in lowering glucose and insulin response, these results also enrich the knowledge on functional diets.

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Author contributions

Conceptualisation MIC and PL; Formal analysis SC, NM, RT and AV; Methodology VM, PL, NM; Writing – original draft NM, FI, MIC and SC; Writing – review & editing NM, PL and FI.

Ethical approval

All animal procedures were approved by the Ethical Animal Care and Use Committee of the University of Napoli Federico II (Prot. 15/0030057).

Disclosure statement

No potential conflict of interest was reported by the author(s).

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