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Former food products have no detrimental effects on diet digestibility, growth performance and selected plasma variables in post-weaning piglets

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ABSTRACT

The effect of a diet based on former food products (FFP) as alternative feed ingredients on in vitro and in vivo digestibility of dry matter (DM), pig growth performance, and selected plasma biochemical variables were evaluated. After an adaptation period (7 d), 12 post-weaning piglets (28 days-old) were housed for 16 d in individual pens and assigned to two experimental groups: Control (n = 6), received a standard diet and FFP (n = 6), received a diet in which conventional cereals (wheat, barley, and corn), plasma powder and whey powder, were partially replaced by 30% FFP. During the experiment, stool and blood samples were also collected for DM in vivo digestibility and for plasma biochemical measurements, respectively. The results revealed that both in vitro and in vivo digestibility values were higher (p < .05) for FFP diets compared to the control diets. At the end of the experiment, no differences in BW were observed between groups (p=.61). ADG and ADFI were not affected by dietary treatment. Conversely, piglets on the FFP diet showed a lower FCR (p<.01). Finally, dietary treatment also affected plasma glucose and urea, which in pigs fed FFPs increased and decreased, respectively. Taken together, these results suggest that the use of FFPs up to a level of 30% in post-weaning diets has no detrimental effects on pig growth performance, although more studies are needed to confirm these results.

HIGHLIGHTS

- CFormer foodstuffs products (FFPs) are valuable alternative feed ingredients.
- FFPs can partially replace cereal grains in post-weaning piglet's diets without affecting growth performance.

Introduction

The livestock sector is highly dynamic worldwide. Human population growth, increasing urbanisation and rising incomes are predicted to double the demand for, and production of livestock and livestock products in developing countries over the next decades. By contrast, in developed countries, many production systems have been increasing their efficiency and environmental sustainability (Pinotti et al. 2016).

Sustainability includes the rational use of nonrenewable and renewable resources (i.e. energy and raw materials) to combat climate changes, a lower load on the environment due to manure production and contaminant excretion by animals, and enhanced animal welfare and profitability (Renaudeau et al. 2014; Pinotti et al. 2016). Animal feed is the most important livestock production cost factor and represents up to 85% of the farm gate value of poultry (FEFAC 2017). Appropriate feeding and nutrition have thus become increasingly important as livestock systems strive to become more efficient (Tretola, Ottoboni, et al. 2017).

Converting losses from the food industry into ingredients for the feed industry, thereby keeping food losses in the food chain (Pinotti et al. 2016; Tretola, Ottoboni, et al. 2017), can be considered a virtuous practice that should be implemented globally. Our research group (Pinotti and Dell'Orto 2011; Pinotti et al. 2014) has found that by using a balanced by-coproduct combination it is possible to partially substitute the 'classic' traditional source of energy and protein in animal diets. This substitution can be obtained

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without major changes in the composition of the diet, and cost effective, compared to traditional ingredients.

In this respect former food products (FFPs), represents a 'case study' in facilitating the transition to more sustainable material management and to a circular economy model. These concepts are also reported in the revised Waste Framework (European Commission 2018a), which clearly discerns FFPs from waste. FFPs are covered by feed legislation, they are placed on the market as safe feed materials and consequently they cannot be downgraded to waste (European Commission 2018a).

A further milestone is the European Commission (2018b) 'for the feed use of food no longer intended for human consumption', which highlights the importance of FFPs for the European Union (European Commission 2018b), not only in terms of implementing the circular economy but also in mitigating the competition for natural resources between human and animal nutrition (EFFPA 2018). The conversion of three million tons of losses from the food industry into ingredients for the feed industry has been estimated to save more than 350,000 hectares of wheat (EFFPA 2018). This data seems to be in line with various recent works (Pinotti et al. 2016; Giromini et al. 2017; Tretola, Di Rosa, et al. 2017; Tretola, Ottoboni, et al. 2017) which defined FFPs as a 'fortified version of cereals', with valuable energy content thanks to their sugar and high digestible starch content.

An adequate fat content can also contribute to conditioning the energy density of these materials. Dietary starch represents one of the major energy sources for monogastrics, and starch effective digestion has a great impact on the animal energy status. If an animal's digestion occurs in the small intestine, the final product for absorption is represented by glucose, resulting in improved energy-yielding pathways. The lower the starch digestibility due to the high resistant starch content, the higher the fermentation of the starch in the large intestine, leading to the production of volatile fatty acids, which are energetically less efficient than glucose (Gerrits et al. 2012). In general, the site, extent and rate of starch digestion are affected by intrinsic and external factors that may be interrelated and are thus not easily defined. It has been demonstrated that starch digestibility can be greatly altered by mechanical and/or thermal processing such as rolling, pelleting, flaking, extrusion and expander processing due to changes in physicochemical starch characteristics (Anguita et al. 2006; Alsaffar 2011).

Starchy food for human nutrition is usually subjected to heat treatments before consumption,

resulting in an edible product, with increased nutritive values (Alsaffar 2011). In fact, the processing techniques applied during the manufacture of cereal products tend to destroy the food matrix and lead to the gelatinisation of starch, thus increasing the susceptibility of starch to enzyme hydrolysis (Alsaffar 2011). Nevertheless, starches consumed by livestock are still fed largely untreated and/or in raw forms (Giuberti et al. 2014). A higher starch digestion rate can increase the plasma level of metabolites and nutrients, as well as the energy intake (Yin et al. 2010). Van Kempen et al. (2010) and Giuberti et al. (2012) measured the post-prandial blood glucose response in swine that were fed diets containing purified starches or different starch sources. The authors highlighted that high digestible starch diets (based on normal and low amylose purified rice starches) were associated with an increased blood glucose response over time, which can lead to a higher insulin secretion (Giuberti et al. 2014).

In this scenario, treated foodstuffs no longer intended for human consumption represent an attractive opportunity to introduce innovative and sustainable ingredients in pig nutrition. However, their use is still limited because of lack of information on their effects on pig growth and wellbeing. Nowadays, over many years of analysis of in-bound products, former foodstuffs producers owns enough experience to obtain consistent and standardised final products starting from a mixture of different substances with variable composition provided by different manufacturers. In order to increase the knowledge, and consequently the use of FFP in pig diets, this study evaluated for the first time the effects of a diet based on 30% FFP in pig diets, by assessing the impacts on in vitro and dry matter (DM) apparent total tract digestibility (ATTD), growth performance and haematological parameters in post-weaning piglets.

Materials and methods

Former food evaluation

A former foodstuffs producer based in the north of Italy has provided the complementary feed used for the formulation of the complete FFP diet. Based on our previous experience, the product with the highest *in vitro* digestibility (IVD) and the fastest carbohydrate digestion has been selected (Ottoboni et al. 2019), given the nutritional needs of post-weaning piglets. The complementary feed was composed by, in descending order of inclusion: leftovers from the food industry (bakery products, pasta and confectionary

Table 1. Analysed composition (g/100g on DM) of the pureFFP product.

| ltem | DM | Ash | EE | CF | NDF | СР | Starch | Sugar |
|-----------|------------|---------|-----------|--------|-----------|--------|------------|---------|
| Pure FFP | 91.30 | 3.22 | 11.00 | 4.41 | 15.70 | 11.50 | 42.30 | 20.20 |
| DM: drv m | natter: EE | : ether | extracts: | CF: cr | ude fibre | : NDF: | neutral de | teraent |

fibre; CP: crude protein; FFP: former foodstuffs products.

products); wheat by-products (e.g. bran); and wheat flour. Table 1 reports the nutrient composition of the complementary FFP used in this study. DM, ash, ether extract (EE), crude fibre (CF), neutral detergent fibre (NDF), crude protein (CP), starch and sugar are all expressed in g/100g on DM and were analysed as described below in the experimental diets section. The sugar content in the complementary feed has been evaluated as proposed by the Commission Regulation (EU) No. 152/2009. Additional information on the pure FFP used in this study (namely FFP2) can be found in Giromini et al. (2017) and Ottoboni et al. (2019).

Experimental diets

Experimental feed ingredients are reported in Table 2. In the FFP complete feed, 30% of conventional cereals (wheat, barley and corn) and whey powder were substituted with 30% FFPs. Feed samples of CTR and FFP diets were analysed for moisture, ash, CP, EE, CF, NDF and starch, as proposed by the Association of Official Analytical Chemists (AOAC) (2005) and the Commission Regulation (EU) No. 152/2009. The content of glucose, fructose and sucrose in the two diets has been analysed according to the method PT 119 NA-2017. Based on CTR and FFP composition data, DE and ME values for pigs were estimated using the following equations:

 $\begin{array}{l} \mathsf{DE} \ (\mathsf{kcal/kg}) = (53.7 \, \ast \, \% \, \mathsf{CP}) + (75.8 \, \ast \, \% \, \mathsf{EE}) \\ & + (41.1 \, \ast \, \% \, \mathsf{starch}) + (7.6 \, \ast \, \% \, \mathsf{NDF}) \\ & + (39.0 \, \ast \, \% \, \mathsf{residue}) \end{array}$

 $\mathsf{Residue} = \mathsf{OM} - \mathsf{CP} - \mathsf{EE} - \mathsf{starch} - \mathsf{NDF}$

ME(kcal/kg) = DE-(6.8 * % CP)

The DE equation was originally formulated by Sauvant et al. (2004), while ME was proposed by Noblet and Perez (1993) and further adapted by NRC (2011). Energy and chemical constituents are expressed on a DM basis in all equations. Table 3 presents the analysed chemical composition of the two experimental diets. The diets were iso-energetic (16.0 MJ/kg DM) and iso-nitrogenous (20.5% DM), and met NRC (2011) requirements (Table 3).

Table 2. Ingredient composition (g/100 g of diet) of the two experimental diets.

| Ingredients | CTR | FFPs |
|-------------------------------------|-------|-------|
| Former foodstuffs | - | 30 |
| Barley | 22.80 | 22.10 |
| Dextrose | 5 | 4.50 |
| Flaked decorticated barley | 4 | 0 |
| Corn | 6.50 | 4 |
| Flaked corn | 6.50 | 1 |
| Vegetable fibres | 1 | 1 |
| Wheat | 12.33 | 10.10 |
| Flaked wheat | 6 | 1 |
| Wheat bran | 3 | 2.48 |
| Vegetable oil | 1.50 | 0.50 |
| Soy oil | 1.50 | 0.50 |
| Fish meal (65% protein) | 2.50 | 2.60 |
| Plasma powder | 3.50 | 3.80 |
| Whey powder | 11 | 4.50 |
| Soy e.f. 50 ^a | 3.50 | 3.50 |
| Soycomil R ^b | 5.50 | 4.55 |
| L-lysine HCl | 0.55 | 0.55 |
| DL-methionine | 0.23 | 0.23 |
| L-threonine | 0.25 | 0.25 |
| L-tryptophan | 0.08 | 0.08 |
| Vitamin-mineral premix ^c | 2.76 | 2.76 |
| Total | 100 | 100 |

CTR: control diet; FFPs: former foodstuffs products diet ^aSov extraction flour 50%.

^bHigh quality soy protein concentrate.

^cProvided per 100 g of complete diet: 0.25 g vitaminic premix, 0.4 g benzoic acid, 0.5 g.

Hydrated dicalcium phosphate, 0.4g calcium carbonate, 0.15 sodium chloride, 0.8g acidifying mixture, 0.06g copper sulphate, 0.2g sodium butyrate.

 Table 3. Analysed composition (g/100 g or Mj/kg on DM) of the CTR and FFP diets.

| CTR | FFPs |
|-------|--|
| 90.90 | 90.30 |
| 5.60 | 5.40 |
| 20.90 | 20.60 |
| 5.94 | 5.92 |
| 4.23 | 2.79 |
| 13.07 | 9.56 |
| 36.20 | 42.60 |
| 6.00 | 6.17 |
| 0.13 | 0.41 |
| 1.07 | 3.11 |
| | |
| 16.00 | 16.00 |
| | CTR 90.90 5.60 20.90 5.94 4.23 13.07 36.20 6.00 0.13 1.07 16.00 |

CTR: control diet; FFPs: former foodstuffs products diet; CP: crude protein; EE: ether extracts; NDF: neutral detergent fibre.

Animal, housing and treatment

The *in vivo* trial was performed at the Experimental Animal Research and Application Centre in Lodi, at the University of Milan. The *in vivo* trial, which complied with Italian regulations on animal experimentation and ethics (DL 26/2014), was authorised by the Italian Health Ministry (number 711/-PR) in accordance with European regulation (Dir. 2010/6) and according to the principles of the 3Rs (Replacement, Reduction and Refinement). A conventional herd was selected to supply piglets. The herd was free from diseases according to the list of the International Office of Epizootic and

from Aujeszky's disease, atrophic rhinitis, transmissible gastroenteritis, porcine reproductive and respiratory syndrome and salmonellosis, without history of PWD and Oedema Disease (OD).

A total of 12 piglets (Large White \times Landrace) with a body weight (BW) of 8.52 ± 1.73 kg (mean \pm standard deviation) born from the selected sows, were used for this study. On arrival, individual faecal samples were collected in order to microbiologically verify the absence of haemolytic Escherichia coli strains. After an adaptation period of one week, post-weaning piglets (28 d old) were housed for 16 d in individual pens and randomly assigned to two experimental groups (CTR and FFPs) with similar initial body. Six piglets were assigned to the CTR group and received a standard diet, while six other piglets were assigned to the FFP group and received a diet in which 30% of conventional cereals (wheat, barley and corn) were substituted for 30% former food (Table 2). All pens were in the same room with the same environmental conditions. All pigs always had ad libitum access to the feed and water. Individual feed intake (FI) was recorded daily, piglet bodyweight (BW, kg) was recorded on days 0, 5, 9 and 16 of the experiment. Both individual feed intake and BW were used to calculate the average daily feed intake (ADFI kg/day), average daily gain (ADG kg/day), and Feed Conversion Ratio (FCR kg/kg). Specifically, ADFI, ADG and FCR were calculated as the means of the entire experimental period (16 d).

In vitro digestibility

The IVD was serially performed on each experimental diet sample three times (three biological replicates); in each digestion series, CTR diet, FFP diet, internal control (flaked wheat) and blank (digestion enzymes alone) samples were included in triplicate (three technical replicates). A flaked wheat sample was included as an internal control feed ingredient because of the similar food-technology processes it had undergone (e.g. heat treatments, steam flaking and pelleting) compared to FFPs and in order to compare IVD values obtained in previous studies (Giromini et al. 2017). The IVD of experimental diets was performed according to the protocol described by Boisen and Fernández (1997) and Regmi et al. (2009) with minor adaptations. Neither protocols include mouth digestion since it is known that in vivo food is quickly swallowed and passed along the oesophagus to the stomach, where the pH is unfavourable for salivar alpha-amylase activity (McDonald et al. 2011).

Briefly, 500 g of sample were mixed with 0.1M phosphate buffer (pH 6.0). Subsequently, the pH of the solution was lowered with 10 mL of 0.2 M hydrochloric acid solution and adjusted to pH 2.0. A total of 1 mL of freshly prepared pepsin (P7000 Sigma-Aldrich, St. Louis, MO) solution (25 mg mL⁻¹) was added. In order to minimise bacterial fermentations during digestion, a 0.5 mL chloramphenicol solution (5 mg mL⁻¹ ethanol) was added to the mixture. The bottle was placed in a shaking water bath at 39°C for 2 h (simulation of the gastric phase). Subsequently, 10 mL of 0.2 M phosphate buffer at pH 6.8 was added, followed by 5 mL of 0.6 M NaOH and the pH was adjusted to 6.8 with 1 M hydrochloric acid or 1 M NaOH. In addition, 3 mL of freshly prepared pancreatin (P3292; Sigma-Aldrich) solution (100 mg/3 mL) were added to the mixture. The bottle was placed in a shaking water bath at 39°C for 4h (simulation of the small intestinal phase).

At the end of the second incubation, 10 mL of 0.2 M EDTA was added to the mixture and the pH was reduced to 4.8 with a 30% acetic acid solution. Next, 0.5 mL of Viscozyme (V2010; Sigma-Aldrich) were added to each bottle and incubated in a shaking water bath at 39 °C for 18 h (to simulate the fermentation process). To enable the precipitation of undigested soluble proteins, 5 mL of 20% sulphosalicylic acid was added and the bottle was kept at RT for 30 min. The undigested fraction (UF) was then collected in a filtration unit using a porcelain filtration funnel lined with pre-weighed filter paper (Whatman no. 54; Whatman Inc., Florham Park, NJ, USA). The UF, along with the filter paper, were dried overnight at 65 °C.

In vitro DM digestibility (%) was calculated as follows:

$$\label{eq:IVD} \begin{split} \mathsf{IVD}\,(\%\,\mathsf{DM}) &= (\mathsf{sample}\,\,\mathsf{DM} - \mathsf{sample}\,\,\mathsf{UF}\,\,\mathsf{DM})/\\ (\mathsf{sample}\,\,\mathsf{DM})\,\times\,\,\mathsf{100}. \end{split}$$

Apparent total tract digestibility of DM

The ATTD of DM was determined using the acid-insoluble ash (AIA) method for the determination of DM digestibility coefficients, which is a reliable technique for the measurement of the digestibility of pig diets (Kavanagh et al. 2001). Faeces were thus collected before the morning feeding on days 4, 5 and 6, for the first monitoring period (initial ATTD), and on days 10, 12 and 16, i.e. at the end of the treatment (final ATTD). After collection, the faeces were immediately stored in a plastic bag and frozen pending analysis $(-20 \,^{\circ}\text{C})$. Faecal samples were pooled per pig per period, ground to pass through a 1-mm sieve, and sampled for analyses.

In accordance with the AIA analytical procedure reported below and based on Van Keulen and Young (1977), faeces were analysed as follows:

- Each duplicate 5 g sample of feed or faeces (dried and ground) was weighed in a 50 mL crucible, dried (2 h) in a forced air oven (135 °C), cooled in a desiccator to room temperature, re-weighed (Ws), and then ashed overnight at 450 °C.
- ii. The ash was transferred to a 600 mL Berzelius beaker (without a spout) and 100 mL of 4N HCI were added. The mixture was then boiled for 5 min on a hotplate.
- iii. The hot hydrolysate was filtered (Whatman No. 41) and washed free of acid with hot distilled water (85–100 °C). The ash and filter paper were then transferred back into the crucible and ashed overnight at 450 °C.
- iv. The crucible and content were cooled in a desiccator to room temperature, weighed while containing ash (Wf) and re-weighed immediately after emptying (We). Percentage AIA was calculated from the equation:

$$AIA = (Wf - We)/Ws \times 100$$

where Wf = weight of crucible with ash, We = weight of empty crucible and Ws = weight of sample DM. All weighings were recorded to the nearest 91 mg.

Blood sampling and analysing

For the plasma biochemical analyses, blood was collected in EDTA containing heparinised Vacutainers (Vacuette, Greiner Bio-One GmbH, Kremsmünster, Austria) after puncture of the jugular vein on days 0 and 16 of the trial. Blood samples were centrifuged (120 xg (14,000 rpm) for 15 min at 10 °C) to obtain plasma, which was stored at -20 °C pending analysis. Plasma total proteins, albumin, globulins, urea, as well as alanine aminotransferase (ALT-GPT), aspartate aminotransferase (AST-GOT), alkaline phosphatase (ALP), glucose, cholesterol, calcium, potassium and magnesium were measured via standard enzymatic colorimetric analysis using a multiparametric autoanalyser for clinical chemistry (ILab 650; Instrumentation Laboratory Company, Lexington, MA, USA) at 37 °C.

Statistical analysis

Data were analysed using IBM SPSS Statistics version 24 (SPSS, Chicago, IL). In the case of *in vivo* data, the pig was considered the experimental unit. Data were tested for normality with the Shapiro–Wilk test before statistical analysis. *In vitro* and *in vivo* digestibility values, growth performance data (BW, ADFI, ADG and FCR) and plasma biochemical values were analysed using one-way analysis of variance (ANOVA) in order to compare means. The analysis was performed using the following model:

$$yij = \mu j + \epsilon i j$$

where yij is the observation (values); μ j is the mean of the observations for the j-th group (sample); and ϵ ij represents the within-sample random variability. The day 0 plasma Mg and K concentrations differed among treatments and were used as a covariate in the model. Differences with *p* values <.05 were considered significant. IVD data are presented as means of triplicate stimulations from three independent experiments ± standard error of the mean (±SEM), while *in vivo* data are presented as least squares means and SEM.

Results

In-vitro digestibility, apparent total tract digestibility and growth performance

All animals remained in good health throughout the experiment and there were no morbidity or mortality issues. The IVD values recorded for the FFP diet (86.5%±1.21) and wheat (88.7%±0.7) were comparable (p>.05); by contrast IVD values were significantly lower (p<.05) for the CTR diet (83.8%±1.02) compared to the FFPs one. There were no differences in ATTD of the two experimental diets at the beginning of the trial, but the final ATTD was higher (p<.05) in piglets fed FFPs. Data are reported in Table 4.

Body weight measured at the piglets' arrival and at the end of the trial did not differ between diets (Table 4). Despite similar FI, piglets fed the FFP diet showed a lower feed conversion ratio (p<.01) compared to piglets fed the control diet.

Plasma biochemical variables

The plasma biochemical variables measured in piglets are presented in Table 5. After 16 d of the FFP diet, no differences in the analysed haematological parameters were observed, with the exception of an increased (p<.05) glucose and decreased (p<.05) urea concentration in piglets fed FFPs.

Discussion

Defining nutritional and functional proprieties of FFPs intended for pig nutrition is essential for establishing the best practices for their sustainable use (Pinotti and Dell'Orto 2011; Pinotti et al. 2014). However, the lack of knowledge regarding their effects on growth performance and animal wellbeing has limited their use in practise.

A concern in the use of FFP could be the variability in the composition, since they are a mixture of different substances. One load of product received by Former Foodstuffs Producers, in fact, can vary from the next even from the same manufacturer (e.g. biscuits may be plan on one day and chocolate covered on another), making a significant different in oil and energy. However, over many years of analysis of inbound products, the former foodstuffs processors are

 Table 4. Effects of partial replacement of conventional cereals

 by FFPs on growth and apparent total tract digestibility.

| ltem | CTR | FFPs | p Values |
|--------------------------------|--------------------------|---------------------------|----------|
| Initial BW (kg) | 9.20 ± 0.54 | 8.76 ± 1.84 | .680 |
| Final BW (kg) | 14.10 ± 0.82 | 13.60 ± 1.15 | .610 |
| Average daily gain (kg) | 0.29 ± 0.04 | 0.31 ± 0.04 | .520 |
| Average daily feed intake (kg) | 0.45 ± 0.04 | 0.43 ± 0.04 | .810 |
| FCR (kg/kg) | 1.55 ^A ± 0.04 | 1.39 ^B ± .04 | .002 |
| Initial ATTD (%) | 78.00 ± 0.45 | 81.20 ± 0.73 | .060 |
| Final ATTD (%) | $78.60^{a} \pm 0.45$ | 83.30 ^b ± 0.97 | .020 |

CTR: control diet; FFPs: former foodstuffs products diet; BW: body weight; FCR: feed conversion ratio; ATTD: apparent total tract digestibility Value for each item is the mean ± SEM (standard error of the mean).

^{A,B}Values within a row with different superscripts differ significantly

at p < .01. ^{a,b}Values within a row with different superscripts differ significantly at p < .05. able to predict the range of variation in analysis between different sources of product and between the same source and different loads. Moreover, has been demonstrated that differences among geographical regions in the chemical composition of bakery meals appear to be relatively small (Liu et al. 2018). This enables the processors to produce an average analysis of raw materials with very low statistical coefficients of variation, where this average values may be used to predict concentrations of nutrients in bakery meals (Liu et al. 2018). Consequently, the finished feed produced use raw materials whose nutrient data the producers are very confident with, and this is validated by finished product analysis. Summarising, a good nutrient analysis of in-bound FFP ensures a good mix on similar materials to ensure consistent analysis.

It is known that post-weaning piglets cannot utilise starch very effectively. Simple sugars and cooked cereals are generally more digestible than raw starch. It is, thus, widely accepted that piglets benefit from readily digestible carbohydrates until their digestive system is fully capable of utilising raw starch. Previous studies have demonstrated that candy co-products, and the related sugar-rich diet (total sugar content about 20% DM), can be used in a high percentage as an alternative carbohydrate source in diets for nursery pigs up to 12 days post-weaning (Guo et al. 2015).

This study provided data on the effects of partial replacement of conventional cereals with 30% FFPs on *in vitro* and *in vivo* digestibility, growth performance and selected plasma biochemical variables in postweaning piglets. The study indicated improved feed digestibility and no detrimental effect on growth performance and animal wellbeing in piglets fed FFP,

| Table 5. | Plasma | biochemical | variables | in | post-weaning | piglets | fed | CTR | and | FFP | diet | on | days | 0 |
|----------|--------|-------------|-----------|----|--------------|---------|-----|-----|-----|-----|------|----|------|---|
| and 16. | | | | | | | | | | | | | | |

| | C | TR | FF | Ps | | |
|----------------------|-------------------------|-------------------------|-------------------------|-------------------------|---------|--|
| ltem | d 0 | d 16 | d 0 | d 16 | p Value | |
| Total proteins (g/L) | 49.80 ± 1.59 | 51.50 ± 1.59 | 49.70 ± 1.10 | 49.60 ± 1.67 | .45 | |
| Albumin (g/L) | 27.70 ± 0.87 | 26.90 ± 1.17 | 26.50 ± 1.14 | 24.80 ± 0.85 | .22 | |
| Globulins (g/L) | 22.10 ± 1.78 | 24.60 ± 2.04 | 23.20 ± 0.97 | 24.70 ± 0.93 | .96 | |
| Urea (mmol/L) | $2.03^{a} \pm 0.34$ | $1.58^{a} \pm 0.15$ | $1.51^{a} \pm 0.32$ | $1.03^{b} \pm 0.08$ | .01 | |
| ALT:GPT (IU/L) | 26.70 ± 3.79 | 27.30 ± 2.04 | 24.30 ± 3.30 | 30.70 ± 2.53 | .34 | |
| AST:GOT (IU/L) | 45.50 ± 5.68 | 45.00 ± 4.92 | 70.80 ± 19.20 | 51.70 ± 7.75 | .50 | |
| ALP (mmol/L) | 2.21 ± 0.07 | 1.78 ± 0.11 | 1.90 ± 0.16 | 1.81 ± 0.08 | .80 | |
| Glucose (mmol/L) | 4.82 ^a ±0.11 | 5.08 ^a ±0.26 | 5.65 ^a ±0.45 | 6.18 ^b ±0.36 | .04 | |
| Cholesterol (mmol/L) | 2.05 ± 0.11 | 2.33 ± 0.07 | 1.71 ± 0.16 | 1.97 ± 0.16 | .10 | |
| Calcium (mmol/L) | 2.54 ± 0.04 | 2.56 ± 0.03 | 2.58 ± 0.04 | 2.64 ± 0.04 | .26 | |
| Phosphorus (mmol/L) | 3.00 ± 0.04 | 3.16 ± 0.07 | 2.46 ± 0.12 | 2.88 ± 0.08 | .13 | |
| Magnesium (mmol/L) | 0.96 ± 0.04 | 0.91 ± 0.04 | 0.79 ± 0.04 | 0.80 ± 0.04 | .69 | |

CTR: control diet; FFPs: former foodstuffs products diet

ALT: alanine aminotransferase; GPT: glutammate piruvate transaminase

AST: Aspartate amino transferase; GOT: glutammate-ossalacetate transaminase

ALP: Alkaline phosphatase

Value for each item is the mean \pm SEM (standard error of the mean). Values within a row with different superscripts differ significantly at p<.05.

although further evaluations are necessary. Although the weaning period, of course, do not resemble all the rearing phases (like growing, finishing, etc.) for which FFPs can be even more cost effective, the weaning period is the most critical. As consequence, most of the outcomes obtained in this phase can be rendered in other subsequent ones.

In vitro and apparent total tract digestibility of dry matter

The FFP-based diet showed increased values of both in vitro and in vivo digestibility compared to the conventional diet. In fact, the differences between the tested diets in absolute values were smaller (Δ 2.7%) in the case of IVD, compared to 4.7% for ATTD. The reason for this is unknown, although the 'maturation' of the digestive system in post weaning piglets, as well as the presence of highly digestible nutrients (probably starch) cannot be ruled out. An FFP-based diet contains a relative high concentration of FFPs (30%) characterised by a higher digestibility rate compared to the conventional ingredients commonly used in feed formulation. These results are in line with a previous study performed by Giromini et al. (2017) in which the authors observed that IVD values of FFP ingredients not included in a complete diet were more digestible than flaked wheat, particularly regarding FFP samples with low fibre and a high starch content. Consequently, the differences in the digestibility values could be explained by the higher starch and lower fibre fractions and ash content, which differentiate the FFP from the CTR diet. Another possible hypothesis is related to the nature of FFPs. In contrast to ingredients commonly used in feed production, FFPs are originally intended for human consumption and thus are subjected to a wide range of processing techniques to improve starch digestibility.

Compared to the untreated starch consumed by livestock (Giuberti et al. 2014), small food particles have a greater surface in contact with digestive enzymes, leading to a higher digestion rate (Alsaffar 2011). Also, processing such as extrusion can significantly increase the digestibility of starches (Altan et al. 2009). This is probably because of the loss of structural integrity of starch granules due to the increased shearing action and kneading in the extruder barrel, which increase starch susceptibility towards enzymatic attack.

It has also been demonstrated that the processing of starchy food can result in a decrease in anti-nutritional factors such as phytic acid and tannins (Rehman and Shah 2005). These authors suggested that the removal of tannins and phytic acid creates a large space within the matrix, which increases the susceptibility towards enzymatic attack and consequently improves starch digestibility (Rehman and Shah 2005).

As reported by Singh et al. (2010), the nutritional characteristics of the food and *in vitro* starch hydrolysis are strongly influenced by processing. Anguita et al. (2006) observed an increased degree of starch hydrolysis, with values higher than 90% (at the end of incubation with pancreatin) for wheat, barley and oats when subjected to different technological processes. Extrusion cooking has also been demonstrated to significantly increase the IVD of starches (Altan et al. 2009).

Starch and starchy food with different levels of digestibility are characterised by a different rate and duration of the glycaemic response (Giuberti et al. 2012). Several studies have proved a good relationship between the rate of *in vitro* and *in vivo* digestion and the glycaemic response (Bellmann et al. 2018). This evidence is in accordance with our haematological data which showed an increased plasmatic glucose concentration in pigs fed FFPs compared to CTR, together with a reduction in urea concentration, as detailed below.

Growth performance

The growth performance was within the expected range for the commercial breed chosen for the study. Although there was no significant difference in the growth rate (p=.45), in pigs fed FFPs, the feed conversion ratio (-10.3%, p < .01) was significantly improved. The reduction in feed conversion ratio was a combination of the small but non-significant changes in the average daily gain and variability within groups. However, these results could also be due to the different chemical composition of the diets, with special emphasis on starch content and its digestibility. Doti et al. (2014) argued that a high rate of starch digestion results in a sharp but short increase in blood glucose after feeding, facilitating fat deposition. In contrast, a gradual increase in blood glucose from the digestion of slowly-digestible starch gives rise to a prolonged release of insulin in the blood, resulting in an increased lean deposition (Li et al. 2017). In line with our findings, previous studies have demonstrated that other former food sources have no detrimental effects on growth performance in newly weaned pigs in commercial farm conditions. Guo et al. (2015) investigated the effects of supplemental candy co-products as an alternative carbohydrate source to lactose in nursery pigs. Similarly to our study, in which most of the whey powder was replaced by simple sugars present in FFPs, Guo et al. (2015) partially replaced lactose in nursery diets with candy co-products containing about 51% of sugars from the food and candy industries without impairing growth performance, feed intake and feed efficiency. The authors (Guo et al. 2015) speculated that partially replacing lactose with carbohydrates from candy co-products could increase feed intake in early nursery phases but may cause a decrease in weight gain in later nursery phases. In fact, as demonstrated by earlier studies, whey powder with sucrose or other sweeteners may increase the feed palatability compared to diets containing only whey powder. However, it may be less efficient in the production of lactic acid by lactic acid bacteria in the gut and in the protein accretion in tissues, resulting in a reduced weight gain (Guo et al. 2015). Further evaluations are thus necessary to clarify the role of FFPs on carcase composition and gut microbiota community.

Haematological parameters

According to Addass et al. (2012), nutrition affects the blood values of animals. The analysis of haematological parameters represents a readily available assessment of nutritional health status of animals on feeding trials and may be considered as appropriate measure of nutritional status (Olabanji et al. 2007) or to distinguish normal state from stress which could be nutritional stress (Etim, et al. 2014). Blood analyses showed that the FFP diet increased (p < .05) glucose and decreased (p < .05) urea concentrations compared to the CTR diet, which is indicative of changes in metabolism associated with the diet. We speculate that these changes are due to the higher digestibility of the starchy food present in FFPs and their higher glycaemic index. Ottoboni et al. demonstrated that FFP ingredients are characterised by a higher hydrolysis index and predicted glycaemic index, as well as the time trend in carbohydrate digestion compared to conventional cereal feed ingredients such as corn (Ottoboni et al. 2018). The high availability of glucose can lead to a reduction in the use of other energy sources such as a decreased deamination of amino acids via the urea cycle, as observed in an opposite scenario by Newman et al. (2017). It has also been demonstrated that, compared to less digestible starch sources such as trans-glycosylated starch, highly digestible starch is associated with reduced serum urea levels, which clearly suggests the decreased utilisation of amino acids (Newman et al. 2017). In addition, glucose, irrespectively of insulin levels, decreases hepatic amino nitrogen conversion resulting in a decreased plasma nitrogen urea concentration (Newman et al. 2017). However, these aspects merit further evaluation.

Conclusions

The increasing global need to find alternative ingredients to counteract food insecurity and the environmental impact of livestock sector, has promoted research in the field of non-conventional energy sources for feed production. In this study, for the first time FFPs were included up to a level of 30% in a complete diet. The results showed that FFPs can replace up to 30% of conventional cereal grains commonly used in pig nutrition without impairing growth performance and nutritional status in post-weaning piglets. Furthermore, the partial replacement of untreated starch with processed starch may increase the feed digestibility as a consequence of the nature of FFPs, which were originally intended for human consumption and subjected to a wide range of processing techniques. In conclusion, this study suggests that conventional cereal grains could be partially replaced by FFPs in pig production as a sustainable alternative energy and nutrient source to traditional feed ingredients. However, further larger and longer studies are necessary to confirm the present findings and also to evaluate FFPs effects on carcase composition and gut health.

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Ethical approval

All procedures carried out in this experiment were reviewed and approved by the Italian regulations on animal experimentation and ethics (DL 26/2014), was authorised by the Italian Health Ministry (number 711/-PR) in accordance with European regulation (Dir. 2010/6) and according to the principles of the 3Rs (Replacement, Reduction and Refinement).

Disclosure statement

No potential conflict of interest was reported by the authors.

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