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Sugary vs salty food industry leftovers in postweaning piglets: effects on gut microbiota and intestinal volatile fatty acid production

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ABSTRACT

Awareness of the need to improve the sustainability of livestock by reducing the loss of natural resources has increased significantly. This study investigated the effects of two categories of food industry leftovers, also referred to as former foodstuff products (**FFPs**), on pig gut microbiota and intestinal volatile fatty acid (**VFA**) production. Thirty-six female postweaning piglets (28 days old, Large White × Landrace, 6.5 ± 1.1 kg) were separated into three groups and fed a conventional diet (**CTR**), and diets in which cereals were partially replaced (30% w/w) by sugary confectionery products (**FFPs-C**) or salty bakery products (**FFPs-B**), respectively. After 42 days of dietary treatments, faeces were collected from the rectal ampulla, snap-frozen, and used for next-generation sequencing to analyse the composition and the alpha and beta diversity indexes of the microbial population. The concentration of VFAs in the intestinal content collected at the slaughterhouse was also analysed. The study demonstrated that balanced diets can be obtained by the inclusion of both FFPs-C and FFPs-B, with a similar chemical composition compared to traditional diets. Neither the FFPs-C nor FFPs-B diets affected the abundance and biodiversity indexes of the microbial community. Only a few taxa, normally attributed to a healthy gut, increased with FFPs-C and FFPs-B compared to the CTR. The experimental diets had no impact on the production of the VFAs in the faeces. Lastly, the inclusion at 30% (w/w) of both categories of FFP diets slightly affected the faecal microbiota. FFPs could thus be used as a promising alternative to traditional ingredients in pig diets; however, additional analyses are needed to further investigate the presence of potentially pathogenic bacteria. The effects of such ingredients on other markers of gut health, and on product quality when used in the fattening period also need to be investigated.

Implications

The re-use of former foodstuff products from the food industry to replace cereal grains in feed represents a promising strategy for sustainable food. Because of the limited information on their effects on animal health and performance, leftovers have not yet been completely accepted as a source of feed. This study demonstrates that sugary and salty food losses slightly influenced the gut microbial population but not their metabolites in faeces, thus suggesting no detrimental effects on the gut health. The study exploits food industry losses as feed in order to reduce the environmental and climate footprint of animal products together with the prevention of food waste.

Introduction

Food can be lost or wasted during different steps of the manufacturing chain. According to the definition of the Food and Agriculture Organisation of the United Nations, food losses should be defined as food unintentionally lost due to technical/logistical constraints impacting the storage, transportation, packaging, or inefficient marketing systems, mainly in the first phases of the food supply chain (McGuire, 2015). Worldwide, there is over one billion tonnes/year of produced but uneaten food (McGuire, 2015) with over 102 million tonnes/year in the EU (Girotto et al., 2015).

The 2030 Agenda for Sustainable Development proposed several actions to mitigate the loss of food. These include the alternative recycling of food losses, also called former foodstuff products (**FFPs**) such as its use as ingredients in animal nutrition, biogas production, fertilisers, and, as a last resort, disposed of and incinerated (FAO, 2019).

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By 2050, the demand for food is expected to have increased significantly, while at the same time, urbanisation is limiting the availability of natural resources (McMichael et al., 2007).

The recovery of FFPs as animal feed addresses both food reduction and food security challenges. The FFPs can be distinguished into two main categories: sugary confectionary FFPs (**FFPs-C**), which include chocolate sweets, biscuits, cakes, and candy from the confectionary industry, and salty FFPs from bakery production (**FFPs-B**) such as bread, pasta (Luciano et al., 2020).

Only a few studies have investigated the nutritional properties of FFPs *in vitro*, such as the chemical composition (Giromini et al., 2017), safety (Tretola et al., 2017), and predicted glycemic index (Ottoboni et al., 2019). Also *in vivo* studies have been carried out to explore the impact of FFPs on animal health and performance, on both postweaning piglets (Tretola et al., 2019c; Luciano et al., 2022) and ruminants (Kaltenegger et al., 2020). All these studies demonstrate the high potential of FFPs as sustainable ingredients for livestock, as their chemical characteristics are comparable with cereals or grains traditionally used in feed. Moreover, no adverse effects have been observed on growth performance when used at a specific percentage of inclusion.

To the best of our knowledge, the impact of partially replacing cereal with FFPs on the gut bacterial community has not yet been extensively examined, despite the potentially useful properties of these ingredients. With respect to pig health, concerns exist due to the possible greater incidence of nutritional diarrhoea because of the presence of high amounts of simple sugars (Pinotti et al., 2021). The higher digestibility and simple sugar content of FFPs compared to traditional and un-processed cereals could also influence the structure and biodiversity of the host's intestinal flora, disrupting the balance in the relative abundance of commensal and potential pathogenic species. This can lead to an overgrowth of harmful bacteria that induce oxidative stress-associated pathways in enterocytes, increasing the production of reactive oxidative species and damaging the intestinal epithelial barrier (Gresse et al., 2017).

A pilot trial conducted by our research group targeted at the postweaning period showed that in pigs, FFP-based diets decreased the richness and evenness of the intestinal bacteria, with only slight effects on the taxa composition (Tretola et al., 2019a). This latter study was conducted without discriminating between FFPs-C and FFPs-B and only during the first 16 days after weaning, in contrast to the present study.

Little information is thus available regarding these effects on a longer period such as the growing phase. Weaning is the most delicate phase in pig production, generally associated with enteric infections. During weaning, the gut microbiota is particularly susceptible to dysbiosis, leading to several infectious diseases in piglets and above all postweaning diarrhoea (Gresse et al., 2017). Postweaning and growing phases were therefore considered as the most appropriate periods to test the safety of FFPs in terms of the impact on the faecal microbial population.

The present study aims to test the hypothesis that both sugary and salty FFPs as diet ingredients for postweaning and growing pigs: (1) do not impair the composition, evenness, and biodiversity of the faecal microbiota; (2) do not impact on the production of intestinal volatile fatty acids.

Material and methods

Former foodstuff product ingredients used in our experimental diets

Two different types of FFP products were provided by an Italian FFP-processing plan to partially replace traditional feed ingredients in the two experimental diets. The two types of products differed in terms of the source of food used for their production. Specifically, sugary FFPs-C were mainly composed of chocolate, biscuits, and sweet snacks. On the other hand, bread, pasta, and salty snacks were used for the salty FFPs-B products. The chemical composition of the two FFP product types employed to obtain the final experimental diets is reported in Table 1.

Both the FFP products were characterised by a theobromine content below NOAEL of 7 mg/kg b.w. recommended for young growing pigs (EFSA, 2008). The contents of the three experimental diets were in accordance with NRC (2012) requirements, including a similar amount of energy (14.0 MJ/kg DM) and nitrogen (19% DM). A company in Italy mixed and prepared the FFPs-B and FFPs-C products and the complete diets. Details of the ingredients used for the three diets are reported in Table 2.

Animals, housing and treatment

The *in vivo* trial was conducted at the Experimental Animal Research and Application Center in Lodi (LO), University of Milan (Milan, Italy). It was conducted in full compliance with the Italian laws regarding ethical issues (DL 26/2014, protocol 711/-PR) and authorised by the Italian Health Ministry. The principles of the 3Rs were applied.

Thirty-six postweaning pigs (Large White × Landrace females – 28 days old, 6.5 ± 1 kg BW) were used. Animals were allotted to individual pens under controlled environmental conditions. Interaction between pigs was possible and plastic environmental enrichments were provided, following the animal welfare regulation and the European Directive (EC Directive 2008/120/EC). Access to freshwater was always possible, and an adaptation period of

Table 1
Analysed composition (g/100 g or MJ/kg of DM) of the two pure FFPs used for the FFPs-C and FFPs-B experimental complete pig diets.

	Experimental ingredients	
Item	Pure FFPs-C	Pure FFPs-B
DM	91.0	87.7
DE	19.6	19.4
CP	10.0	11.0
Ash	2.10	2.10
Crude fats (after hydrolysis)	9.59	7.50
CF	1.60	2.20
Starch	42.5	50.5
NFE	67.8	64.9
TS (in sucrose)	21.0	10.5
Fe (mg/kg)	41.7	95.0
Amino acids		
Arg	0.48	0.20
His	0.19	0.17
Ile	0.33	0.27
Leu	0.59	0.68
Lys	0.26	0.18
Met	0.05	0.13
Phe	0.40	0.50
Thr	0.25	0.31
Val	0.40	0.27
Ala	0.29	0.66
Asp	0.48	0.40
Cys	0.10	0.10
Glu	2.44	2.87
Gly	0.32	0.48
Pro	0.80	1.34
Ser	0.40	0.54
Tyr	0.22	0.19
Total	8	9.29

Abbreviations: FFPs-C = confectionary former food products; FFPs-B = bakery former foodstuffs products; DE = digestible energy; CF = crude fibre; NFE = Nitrogen-Free Extract; TS = Total sugar. Values are expressed on a DM basis.

Table 2
Ingredient Composition (g/100 g of diet on fresh matter) of the CTR, FFPs-C, and FFPs-B pig diets.

Ingredients	Experimental diets		
	CTR	FFPs-C	FFPs-B
Wheat	25	25	17
Pure FFPs-C	–	30	–
Pure FFPs-B	–	–	30
Wheat flaked and hulled	10	–	–
Barley flaked and hulled	10	–	–
Barley	14.1	6.1	10
Sweet whey	8	8	8
Whole soybeans flaked and ground	6.2	1	4
Bran	5	14	11
Fermented soy protein concentrate	5	3	5
Rice flakes	5	–	5
Vitamin premix	2.65	2.65	2.18
Fish Meal	2	2	2
Soybean Meal 47%	1.4	4.85	–
Soybean Oil	1.35	–	1.02
Sucrose	1	–	1
L-Lysine	0.72	0.78	0.85
Monocalcium phosphate	0.5	–	0.78
Calcium carbonate	0.5	0.5	0.5
Sodium chloride	0.5	0.1	0.1
L-threonine	0.3	0.4	0.4
DL-methionine	0.3	0.4	0.5
B vitamins	0.2	0.2	0.2
L-valine	0.19	0.34	0.36
L-tryptophan	0.06	0.09	0.16
Flavour	0.01	0.01	0.01

Abbreviations: CTR = control diet; FFPs-C = confectionary former foodstuff products diet; FFPs-B = bakery former foodstuff products diet. Values are expressed on fresh matter.

seven days was used to allow piglets to acclimatise to the new conditions.

Piglets were then randomly assigned to a standard postweaning diet (CTR), a sugary confectionary FFP-based diet (FFPs-C), or a salty bakery FFP-based diet (FFPs-B) for 42 days. Individual pig BW was measured every week, while feed intake was monitored every day. Individual daily feed intake was condensed into a weekly mean and used for statistical analysis. The feed conversion ratio (**FCR**), average daily gain (**ADG**) and average daily feed intake were calculated. These parameters were used to calculate the growth performance of the animals with regard to the three experimental feeds.

Slaughtering involved only six pigs per group at 42 ± 2.8 d of age at the research station abattoir after fasting for approximately 15 h. The pigs were walked ~100 m to the stunning area, and allowed to rest for 10 min before they were subjected to CO2 stunning for 100 s, after which they were exsanguinated. Approximately 50 g of intestinal digesta was collected from each animal in each group and preserved at −80 °C for VFA analysis. Other samples were collected from the scarified pigs and used for further investigation, not published herein.

Apparent total tract digestibility of DM

The apparent total tract digestibility (**ATTD**) of DM was determined in faeces collected for three days per week before feeding by the acid-insoluble ash method, as described in (Kavanagh et al., 2001). The amount of naturally occurring acid-insoluble ashes found in all the three diets (about 4 g/kg) is considered adequate for this method to be applied in pigs (Kavanagh et al., 2001). Briefly, faeces were weighed, dried at 80 °C for 48 h and then incinerated at 450 °C. A total of 4 N HCl was added to the ash and boiled for 5 minutes. The solution was filtered, and the filters, together with the ash, were incinerated at 450 °C. As reported in Kavanagh et al. (2001), the equation was used to calculate the percentage of insoluble acid ash. The indirect method proposed by Kavanagh et al. (2001) was also used to calculate the ATTD of DM.

Sample collection, DNA extraction, and sequencing

Faecal samples were collected from the rectal ampulla after 42 days of experimental diets. They were immediately snap-frozen in liquid nitrogen and stored at −80 °C until further analysis. Samples were sent for next-generation sequencing as described below. Starting with 200 μg of stool, the DNA was extracted with the QIAamp Fast DNA Stool Mini Kit (QIAGEN, Germantown, USA) following the manufacturer's instructions and quantified with Nanodrop ND2000. The universal primers for prokaryotic (341F/802R: CCTACGGGNGGCWGCAG/GACTACHVGGGTATC TAATCC, respectively) were used to amplify by PCR the V3 and V4 regions of the 16S rRNA gene. The amplicons were sequenced by BMR Genomics (Pavia, Italy) through the Illumina MiSeq platform and a v2 500 cycle kit (San Diego, CA, USA). The paired-end reads obtained were tested for chastity and subjected to demultiplexing and trimming by Illumina real-time analysis software v2.6. The read quality was checked by FastQC v0.11.8. USEARCH v11.0.667 was used to trim forward and reverse reads of the paired-end reads. The merged sequences were checked for errors and ambiguous bases and denoised by UNOISE to discard singletons and chimeras. The UNCROSS algorithm was used to filter the obtained OTU abundance table. Abundances were then adjusted for 16S copy numbers using the UNBIAS algorithm. The RDP database was used to predict the taxonomy of the OTUs obtained, with a threshold of 0.5 by the SINTAX algorithm (Girard et al., 2021).

Intestinal volatile fatty acid quantification

Reagents, materials, solutions

Acetate, propionate, butyrate and valerate were provided by Sigma. A 10% perchloric acid solution in water was prepared in the laboratory and used for the extraction of digesta samples. Headspace solid-phase microextraction was performed using a 75 μm Carboxen/polydimethylsiloxane fibre.

Procedure

Volatile fatty acids were determined by simultaneous Headspace solid-phase microextraction (VWR International, Leuven, Belgium) GC–MS analysis described by Fiori et al. (2018) with some modifications. Briefly, 1 g of digesta was combined with 5 ml perchloric acid solution (10% v/v in water), homogenised and centrifuged for 5 min, 4 °C, 15,000 rpm. Finally, an aliquot of 500 μL of supernatant was diluted 1:10 in distilled water to reach the final concentration and the solution was subjected to Headspace solid-phase microextraction extraction. VFA extraction conditions were as follows: 75 μm Carboxen / polydimethylsiloxane fibre, 10 min of equilibration, 70 °C, and 30 min of extraction. The analytes obtained were desorbed through the injector port of the gas chromatograph at 250 °C for 10 min, including fibre cleaning.

Statistical analysis

Data on growth performance, ADG and feed intake and VFA were analysed using IBM SPSS Statistics v. 27 (SPSS, Chicago, IL). Residuals were checked for normality and homoscedasticity before the statistics were analysed. BW, average daily feed intake, ADG, and FCR were analysed using ANOVA for repeated measurements to compare means. Differences were considered significant when *P*-values <0.05. All microbiota data analyses were run in R v4.0.3 (Boston, MA, USA). The R packages used were phyloseq v1.26.1, vegan v2.5–5, microbiome v1.12.0, and microbiomeutilities

v1.00.14. The alpha diversity indexes used were the observed OTUs and Chao1, Simpson, and Shannon (microbiome package, v.1.12.0). Both the weighted and unweighted Unifrac distances were calculated on rarefied OTUs. Both the variance (PERMANOVA) and similarities (ANOSIM) of the tested groups were also calculated.

The linear discriminant analysis effect size (**LEfSe**) between groups was calculated using the following conditions: alpha value <0.05 for the Kruskal–Wallis sum-rank test among the classes; threshold >3.0 on the logarithmic linear discriminant analysis score (Segata et al., 2011). To estimate the common core microbiota, the "microbiome" library was used (detection threshold: 0.001, prevalence: 80/100). Multivariate analysis was conducted using MaAsLin (Morgan et al., 2012) to investigate associations between microbial abundances (from the domain to genus taxonomic level) and faecal VFAs. Default settings were used for this analysis, specifically: maximum false discovery rate (significance threshold) = 0.05. Minimum for feature relative abundance filtering = 0.0001. Minimum for feature prevalence filtering = 0.01.

Results

The three diets did not show any effect on the growth performance (Fig. 1). No differences were found in ADG, average daily feed intake and FCR (data not shown).

Experimental diet composition

The three experimental diets were found to be iso-nitrogenous and iso-energetic, with a similar chemical composition (Table 3). The FFPs-B diet had a lower quantity of NDF compared to CTR and FFPs-C. As expected, the amount of simple sugars was higher in the FFPs-C diet compared to CTR and FFPs-B. Another slight difference was in the non-structural carbohydrate (**NSC**) content, which was higher in the FFPs-C diets, followed by the FFPs-B and CTR diets. The ATTD of DM was similar between the CTR and FPPs-B diets; however, in the FFPs-C diet, it was lower than the CTR and FPPs-B diets ($P < 0.05$).

Gut microbiota characterisation

A total of 4 435 844 sequences, 3 021 taxa by seven taxonomic ranks were obtained through the next-generation sequencing of 16S rRNA genes in the collected faeces, with a sparsity value of 0.87. The minimum number of sequences obtained in one sample was 22 385 which was used to obtain equal sample sums for downstream analysis of alpha diversity. Supplementary Fig. S1 reports the rarefaction curve and shows that the sequencing depth was sufficient for accurate data analysis.

The most representative phyla were Firmicutes, Bacteroidetes, Proteobacteria, Spirochaeta, and Tenericutes (Supplementary Fig. S2).

Diets did not affect the gut microbial community at the family level. In all the pigs, the most representative families were *Prevotellaceae, Ruminococcaceae, Lachnospiraceae, Veillonellaceae,* and *Lactobacillaceae* (Fig. 2).

No significant differences ($P > 0.05$) in the alpha diversity indexes analysed were observed between groups. All the results are summarised in Supplementary Table S1. No differences ($P > 0.05$) in the phylogenetic diversity were found between groups (data not shown).

Table 3
Analysed (g/100 g on DM basis) and measured (MJ/kg) composition of the three experimental diets in pigs.

	Experimental diets		
Items (%)	CTR	FFPs-C	FFPs-B
Analysed			
DM	90.1	90.2	88.8
CP	19.1	19.1	19.0
NSC	57.6	59.1	58.4
Ash	6.11	6.10	6.19
Crude fat	3.90	3.99	3.71
Starch	39.9	38.0	39.7
NDF	11.2	10.7	9.71
ADF	3.71	3.42	3.23
Simple Sugar	4.69	6.60	4.70
Ca	0.72	0.72	0.72
P	0.61	0.61	0.61
Fe (mg/kg)	0.13	0.13	0.13
Lys	1.52	1.52	1.52
Met	0.63	0.63	0.83
ATTD	82.3	80.7	82.1
Measured (MJ/kg)			
ME	3 131	3 100	3 090

Abbreviations: CTR = control diet; FFPs-C = confectionary former foodstuff products diet; FFPs-B = bakery former foodstuff products diet; NSCs = Non-structural carbohydrates; ATTD = apparent total tract digestibility; ME = Metabolisable energy. Values are expressed on DM basis.

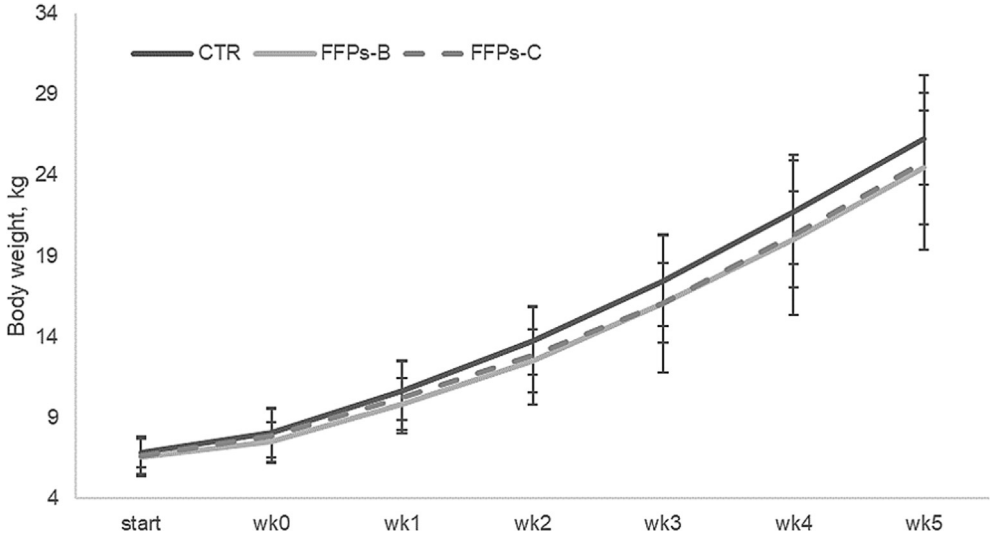

Fig. 1. Pig BW (kg). Data are presented as means by group and by dietary treatment ± SD. *Abbreviations:* CTR = Standard diet; FFPs-B = bakery former foodstuff products diet; FFPs-C = confectionary former foodstuff products diet; WK = week.

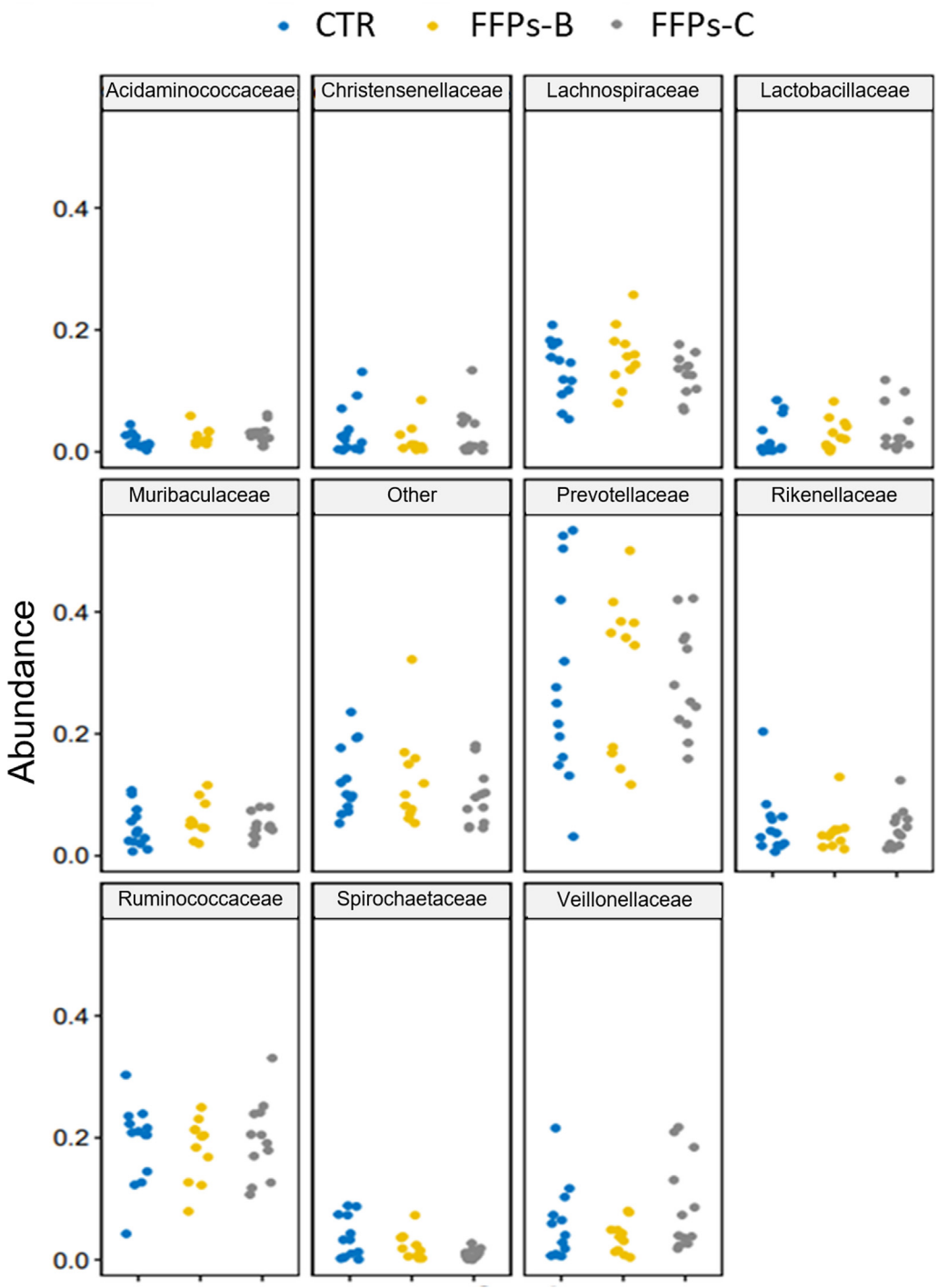

Fig. 2. The most representative families of gut microbiota in piglets fed a standard diet, bakery, and confectionary former foodstuff products diets for 42 days after weaning. *Abbreviations:* CTR = Standard diet; FFPs-B = bakery former foodstuff products diet; FFPs-C = confectionary former foodstuff products diet.

No differences were observed in either the Unweighted (PERMANOVA, $P = 0.16$, data not showed) or Weighted UniFrac beta diversity measures between groups (PERMANOVA, $P = 0.23$), where axes 1 and 2 explain 92.6 and 3.5% of the differences, respectively (Fig. 3A).

Different bacteria as potential biomarkers between the three groups were identified through the LefSe analysis at the end of the experiment. As shown in Fig. 3B and C, the *coprostanoligenes* group and U29_B03 taxa were more abundant in FFPs-C than in FFPs-B and CTR. The abundance of bacteria belonging to the phylum of Verrucomicrobia was higher in piglets fed the FFPs-B diet compared to the other two groups. On the other hand, bacteria belonging to the Treponema and Sutterella genera, together with members of the *erysipelotrichaceae UCG_004* family, were more abundant in the CTR group than in the other two categories of piglets.

Volatile fatty acid content in faeces, and correlations with gut microbiota

The volatile fatty acids acetate, propionate, butyrate, and valerate were quantified in the faeces of pigs belonging to the three

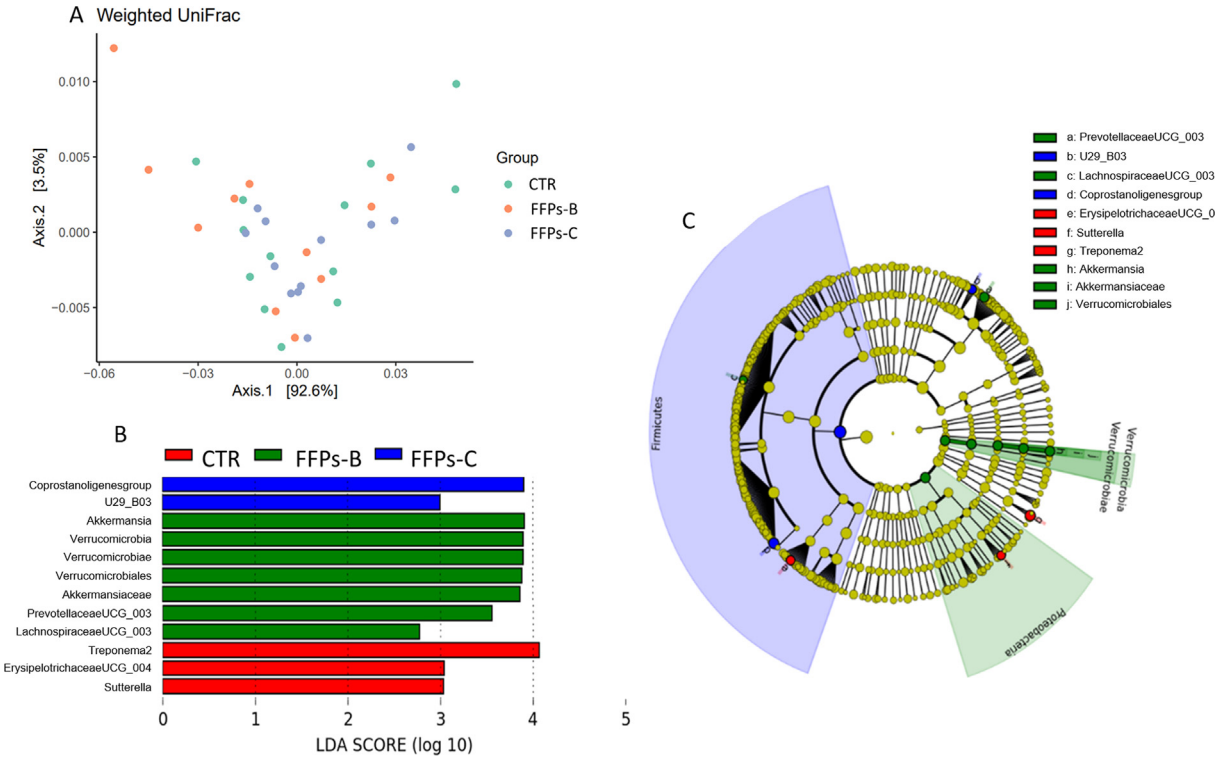

Fig. 3. (A) Weighted UniFrac beta diversity in the gut microbial population of piglets fed standard diet, bakery, and confectionary former foodstuff products diets at 42 days after weaning. (B) Linear discriminant analysis (LDA) coupled with effect size measurements (LEfSe); (C) Cladogram showing the distribution of the most differentially abundant taxa on the phylogenetic tree. *Abbreviations:* CTR = Standard diet; FFPs-B = bakery former foodstuff products diet; FFPs-C = confectionary former foodstuff products diet.

experimental groups. No significant differences ($P > 0.05$) were found between dietary treatments. Results are reported in Table 4. As reported in Fig. 4, the multivariate analysis by linear models found that the OTU corresponding to the genus *Ruminococcaceae UCG-008*, belonging to the family *Ruminococcaceae*, correlated positively ($P < 0.01$) with the faecal acetate concentration. In contrast, the genera *Oscillospira* and *Lachnoanaerobaculum*, belonging to the *Ruminococcaceae* and *Lachnospiraceae* families, respectively, showed a negative correlation ($P < 0.01$) with the faecal concentration of valerate.

Discussion

The ingredients and thus the nutrient composition of both sugary and salty FFPs can vary during the year. This seasonal variability also affects conventional feedstuffs. The variability that characterises crops can be determined by the genetics of plant species, climatic conditions, agronomic factors and harvesting and storage procedures. Regarding FFPs-C and FFPs-B, the processing can be a further variability factor (Zijlstra, 2006).

Although the formulation of feeds can be complicated due to the great variability of these ingredients, FFPs-C and FFPs-B products are very flexible, and can satisfy the nutrient/energy requirements of the target animals. FFP processors have gained experience regarding the processing of these products and they can predict the range in variation among different sources of products and the same source and different loads. These findings have enabled processors to produce raw materials with very low coefficients of variation. Such aspects are fully described in Pinotti et al. (2021). In a parallel study, we found that the replacement of common grains with FFPs-C or FFPs-B up to a level of 30% does not decrease the performance of pigs in the growing phase (Pinotti et al., 2020; Luciano et al., 2021).

Growth performance very much depends on gut health and gut microbiota, whose composition is closely linked to the diet (Fouhse et al., 2016). Although they were iso-energetic and iso-nitrogenous, the three diets of the present study had different ingredients used in their formulation. In the FFPs-C and FFPs-B diets, some of the un-processed ingredients such as wheat and barley were replaced by highly processed FFPs. The diets also slightly differed in terms of their chemical composition. Both FFPs-C and FFPs-B diets had a lower amount of NDF compared to the CTR. Due to the high content of confectionary products used to formulate the FFPs-C, the simple sugar and NSC content was higher in the FFPs-C diet compared to CTR and FFPs-B.

Despite the risk of osmotic diarrhoea due to the high amount of sugar in the FFPs-C diet, no signs of liquid faeces were observed during the trial in any of the three groups. It is well known that the gut microbiota structure is susceptible to changes in the diet. For example, the bacterial community depends on the amount

Table 4
Concentration of volatile fatty acids in the faeces of growing pigs. Results are reported in µmol/g as means and standard error of the means.

	Experimental diets				
Item	CTR	FFPs-B	FFPs-C	SEM	*P*-value
Volatile fatty acids					
Acetate	45.1	46.7	42.9	1.69	0.67
Propionate	8.01	9.01	7.64	0.51	0.55
Butyrate	10.9	11.8	10.4	0.87	0.81
Valerate	0.67	0.73	0.72	0.06	0.92

Abbreviations: CTR = control diet; FFPs-B = bakery former foodstuff products diet; FFPs-C = confectionary former foodstuff products diet.

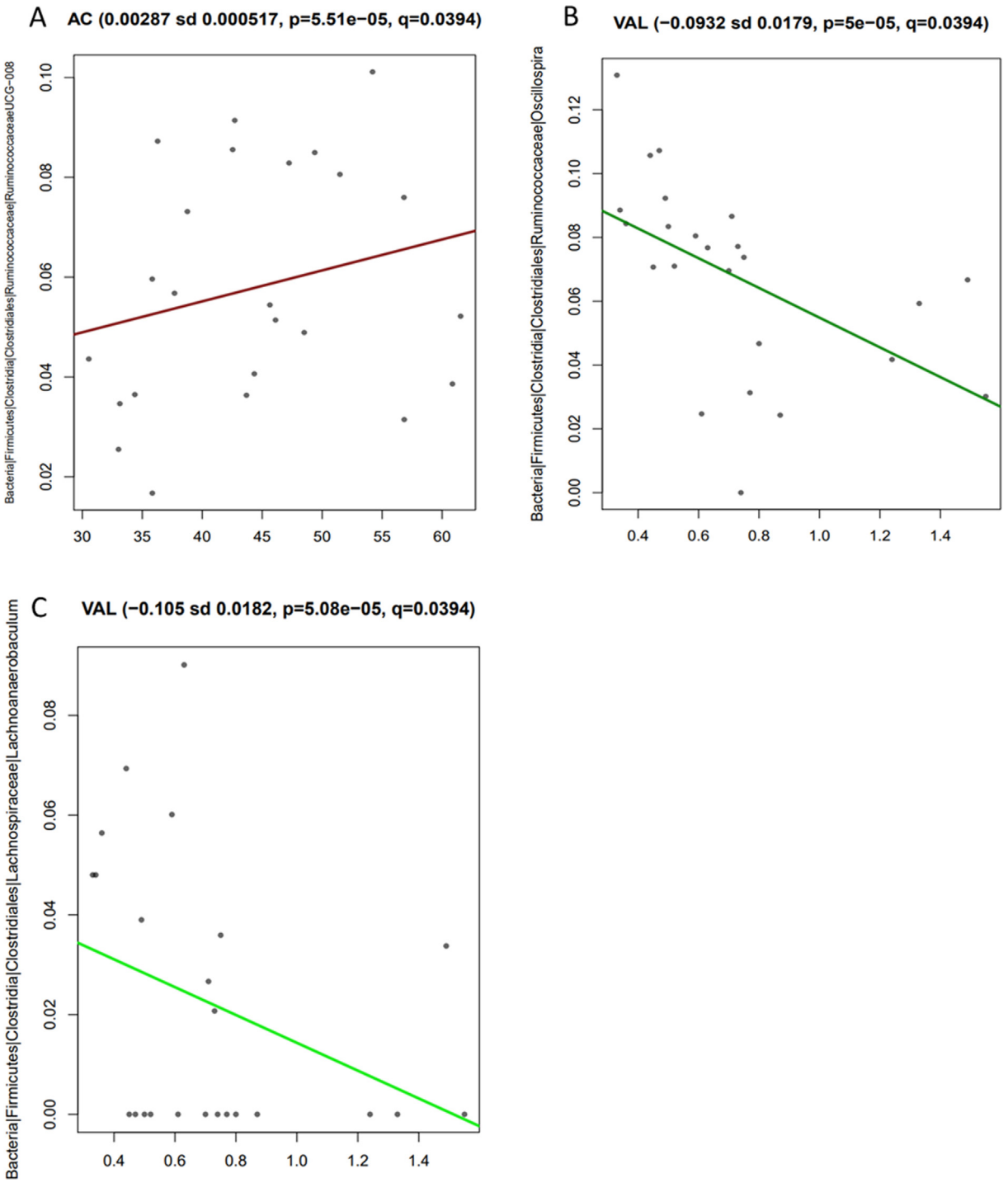

Fig. 4. Correlations between specific pig gut bacterial taxa and (A) faecal acetate and (B, C) valerate, irrespectively of the experimental diet. *Abbreviations:* AC = acetate; VAL = valerate.

and type of dietary fibres which increase the growth of bacteria with cellulolytic and xylanolytic activities (Durmic et al., 1998).

Other dietary treatments are known to affect the intestinal bacterial community, such as tannins (Tretola et al., 2019b) and several sources of carbohydrates (Guo et al., 2015; Tretola et al., 2019a).

Despite the different nature of the FFPs-C and FFPs-B ingredients compared to the traditional ingredients, no differences at the family level were found between the three dietary treatments. The phyla of Firmicutes, Bacteroidetes, Proteobacteria, Spirochaeta, and Tenericutes represented the largest proportion of the bacterial population, in accordance with the literature (Isaacson and Kim, 2012). The abundance and biodiversity of the gut microbiota were not affected by the different diets either and no clusters were observed by the beta diversity analysis. This thus indicates that these diets had no major effects on the microbial community in the faeces.

These results are in contrast with findings obtained in the previous study by our research group on postweaning piglets fed an FFP diet (Tretola et al., 2019a). The study found that when FFPs

were included in the diet to replace 30% of traditional ingredients, the bacterial abundance and biodiversity decreased (Tretola et al., 2019a). Compared to the diets previously used in postweaning piglets, in this study, the experimental diets were more similar in terms of their chemical composition. Accordingly, the ATTD values of the FFP diets did not improve, as found by Pinotti et al. (2020).

In a previous work, we speculated that the differences in the ATTD were due to the nature of the FFP ingredients. The unprocessed grains used for the standard feed formulation were partially replaced by highly processed and highly digestible FFPs originally produced for human consumption (Tretola et al., 2019c). In the present study, despite the partial replacement of grains with FFPs, the difference in the fibre content between the diets was low due to the higher amount of bran included in the FFPs-C and FFPs-B diets. It is thus possible that, by balancing the amount of fibre and consequently the ATTD, the potential negative effects of the FFP diets on the gut microbial community can be avoided due to the high ATTD that we observed in our previous study on postweaning piglets (Tretola et al., 2019a).

Diet digestibility and dietary fibres are in fact key dietary components for gut health. These results confirm that diets including both FFPs-C and FFPs-B need to be carefully formulated to correctly feed the gut microbiota and prevent major effects on the bacterial community. As shown by the LefSe analysis, only minor differences were observed in the gut microbiota between pigs fed the three diets.

As already mentioned, the chemical composition of the CTR, FFPs-C, and FFPs-B diets were similar. Minor differences were only observed in the NDF, NSC and simple sugar content. According to the LefSe analysis, the OTUs belonging to the genus of coprostanoligenes increased with the sugary FFPs-C diet. This taxon is a cholesterol-reducing bacteria (Freier et al., 1994) which ferments simple sugars such as fructose, glucose and mannose (Freier et al., 1994). U29-B03, a member of the Bacteroidetes phylum, was more abundant in the sugary FFPs-C group compared to the salty FFPs-B and CTR groups. However, no exhaustive information on the taxa is available in the literature, which is mainly on ruminants and environments undergoing complex carbon degradation (Hongoh et al., 2005).

Compared to the CTR and FFPs-C diets, the salty FFPs-B diet increased the abundance of the Akkermansia genus, belonging to the phylum of Verrucomicrobia, together with Proteobacteria, *Prevotellaceae* UCG-003 and Lachnospiraceae UCG-003. Members of the genus Akkermansia have been suggested to be biomarkers of a healthy intestine because of its abundance in healthy mucosa and the inverse correlation with several intestinal disorders (Belzer and De Vos, 2012). This mucin-degrading bacteria also produces acetate and propionate within the mucus layer, which are easily available for host absorption (Belzer and De Vos, 2012). Its abundance in the FFPs-B diet is therefore promising in terms of the effects of this FFP category on gut health. *Prevotellaceae* UCG-003 belongs to the Prevotella genus.

Prevotella is known to play an essential function in the metabolism of carbohydrates (for example, sugar, starch, and xylan), and bacteria belonging to this taxa can grow at a low pH (Adeyemi et al., 2020). The increased relative abundance of *Prevotellaceae* UCG-003 in the faeces of pigs fed the FFPs-B diet is thus probably due to the increased fermentation of NSCs, which were more abundant in the FFPs-B diet than in CTR. It is not clear why these taxa did not increase in the FFPs-C group, which had the highest NSC content. One hypothesis could be that a different cross-feeding relationship between bacteria was established in the FFPs-C and FFPs-B groups, which resulted in the growth of different taxa specialised in carbohydrate fermentation. The excreted products from one strain may be the preferred energy source for another strain, and this cross-feeding relationship can be particularly complex in environments such as the lower gut of pigs (Smith et al., 2019).

The Lachnospiraceae family belongs to the core of gut microbiota. It is usually associated with intestinal health and is the principal producer of short-chain fatty acids (Vacca et al., 2020). However, its impact on the host physiology is often inconsistent across studies (Vacca et al., 2020).

The CTR diet increased the abundance of Treponema, Erysipelotrichaceae UCG-004, and Sutterella. The genus Treponema contains both pathogenic and non-pathogenic species. Non-pathogenic bacteria can be found in the normal microbiota of the intestine, oral cavity, or genital tract (Radolf, 1996). *Erysipelotrichaceae UCG-004* are members of the Erysipelotrichi class, belonging to the Firmicutes phylum (Kaakoush, 2015). Their increased abundance in the GI tract has been associated with detrimental consequences on the host's health (Kaakoush, 2015). The capacity of the Erysipelotrichi class to improve cholesterol and lipid metabolism in the GI tract has also been reported (Parmentier-Decrucq et al., 2009). No information on the functional roles of the UCG-004 subtype has been reported in the literature. In addition, Sutterella, which was highest in the CTR group, seems to be associated with intestinal disease. Recent reports link Sutterella with gastrointestinal diseases, in particular with ulcerative colitis due to its capacity to degrade immunoglobulins (Kaakoush, 2020).

To summarise, pigs fed FFPs-C and FFPs-B diets had a similar microbiota composition, abundance, and biodiversity compared to pigs fed the standard diet. Minor modifications in specific bacterial taxa seem to indicate that both FFPs-C and FFPs-B increased the abundance of beneficial bacteria capable of fermenting carbohydrates and producing VFAs, and reduced the level of potentially pathogenic bacteria compared to the CTR group.

The main sources for the production of VFAs by intestinal bacteria fermentation are carbohydrates (Ríos-Covián et al., 2016).

Despite the differences in the carbohydrate content of the three diets, no differences were observed in faecal VFAs between the groups. In accordance with the literature (Ríos-Covián et al., 2016), acetate was the most abundant VFA produced, followed by butyrate, propionate, and valerate. The health benefits of VFAs are well known since they lead to a reduced luminal pH, resulting in the inhibition of pathogenic microorganisms and increased nutrient absorption (Macfarlane and Macfarlane, 2012). One example is the protection of Bifidobacteria from enteropathogenic infection through the production of acetate, which increases intestinal defence mediated by epithelial cells (Fukuda et al., 2011). Higher propionate production in the large intestine has been positively correlated to feed efficiency and reduced inflammatory response in pigs (Gardiner et al., 2020). Butyrate increases mucin production, which results in an enhanced tight-junction integrity (Peng et al., 2009). Other VFAs, such as valerate, contribute to ATP generation and affect cell metabolism in enterocytes when butyrate concentrations become low (Gardiner et al., 2020).

The production of VFAs is thus essential to maintain a proper gut barrier function. According to the similarities in the VFA concentration measured in faeces, we can assume that there is no risk of a deteriorated gut barrier function associated with the use of FFPs-C or FFPs-B in growing pig diets.

The results on the VFA production are in accordance with the lack of significant differences in the microbiota composition between the three groups.

Nutrition can influence the production of VFAs by modulating the intestinal microbiota composition. For example, a higher number of short-chain fatty acids is found in the faeces of animals fed a high fibre-low fat diet, in contrast with animals fed a diet with lower fibre content (De Filippo et al., 2010).

The differences in the NSC, simple sugar and NDF contents between the FFPs-C, FFPs-B, and CTR diets were insufficient to

impact the gut microbiota. Although FFPs-B and FFPs-C increased the abundance of some short-chain fatty acids that produce bacteria compared to the standard diets, these differences did not have any consequence on the intestinal production of VFAs. The taxa that have been found to positively or negatively correlate with acetate or valerate production (*Ruminococcaceae UCG-008*, *Oscillospira*, and *Lachnoanaerobaculum*) were not differentially expressed in the three dietary treatments. This supports the hypothesis that FFPs-C and FFPs-B can be used in growing pig diets without harmful effects on gut microbiota and the related VFA intestinal production and gut integrity.

Several other aspects, however, need to be further investigated. One example is the effect that the use of FFPs could have on the product quality in pigs. It is well known, for example, that the fat quality of pig carcasses is affected by the fatty acid composition of the feed. A high amount of dietary saturated and monounsaturated fatty acids increases the firmness of the back fat, improving the quality of the meat. In contrast, softback fat derives from the accumulation of dietary polyunsaturated fatty acids (Madsen et al., 1992).

Apart from starch, bakery products contain margarine, butter and partially hydrogenated vegetable oils which are therefore the main fat source. Saturated fatty acids are the main type of fat present in bakery and pastry products (Albuquerque et al., 2017). Their effects on animal performance, carcass, meat and fat quality of pigs fed FFPs during the whole grower-finisher period need to be assessed in further studies.

Conclusions

Confectionary and bakery leftovers can be used as ingredients for the formulation of FFPs-C and FFPs-B, respectively. No significant differences were observed between FFPs-C, FFPs-B, and standard diets on the gut microbiota composition and faecal concentration of VFAs. Minor modifications in specific bacterial taxa suggest the potential beneficial effects of FFPs-C and FFPs-B against the growth of potentially pathogenic bacteria. Based on our past and present findings, it can thus be concluded that industrial leftovers should not be considered as waste but as a valid alternative to common cereal grains for sustainable and safe diets in pig nutrition. However, more in-depth investigations regarding the abundance of potentially pathogenic bacteria, together with further markers of gut health, need to be addressed to draw more extensive conclusions.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2022.100584.

Ethics approval

The *in vivo* trial was authorised by the Italian Health Ministry and in accordance with Italian law (DL 26/2014, protocol 711/-PR). The principles of the 3Rs were also applied.

Data and model availability statement

No data were deposited in an official repository. Data are available from the authors upon request.

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Declaration of interest

The authors are unaware of any potential conflict of interest.

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