UNIVERSITÀ DEGLI STUDI DI MILANO

DOCTORAL PROGRAMME IN NUTRITIONAL SCIENCE

The use of former food in post-weaning pig diets: effects on growth performance, apparent total tract digestibility, gut health, feed safety and environmental impact.

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Dedicated to my family and friends.
To all their love and support. To their patience and presence.

Abstract

The demand for livestock products is expected to increase as much as 50 percent by 2050. This increase in the consumption will demand enormous resources. Animal feed is the most challenging, because of the limited availability of natural resources, climatic changes and food-feed-fuel competition. The costs of conventional feed resources, such soymeal, fishmeal and standard cereals like corn, are very high and moreover their availability in the future will be limited. For this reason, scientific research is focusing on the study of alternative sources (e.g., by/co-products from cereals process, former food products, seaweeds, insects) to standard feed ingredients. The focus of this dissertation will be on the use of former food as an alternative ingredient in animal nutrition, especially for pigs. Various subjects will be addressed concerning effects of these materials on pig growth, health and performance as well as their safety and sustainability.

Riassunto

Entro il 2050, si prevede che la domanda di prodotti di origine animale aumenterà fino al 50% in tutto il mondo. Questo aumento del consumo di prodotti di origine animale richiederà l’impiego di enormi risorse. Di tutto il comparto zootecnico, l'alimentazione animale è la più impegnativa e dispendiosa, a causa della limitata disponibilità di risorse naturali, dei cambiamenti climatici e della competizione cibo-mangime-carburante. Attualmente, i costi delle risorse alimentari convenzionali, come per esempio farina di soia, farina di pesce e cereali standard come il mais, sono molto elevati e inoltre la loro disponibilità in futuro sarà limitata. Per questo motivo, la ricerca scientifica si sta orientando sullo studio di fonti alternative (come ad esempio sottoprodotti e coprodotti che derivano dalla lavorazione dei cereali, ex-alimenti, alghe e insetti) agli ingredienti standard per mangimi. Il focus di questo elaborato sarà sull’utilizzo di former food – detti anche ex alimenti - come ingrediente alternativo in alimentazione animale, specialmente per i suini in post-svezzamento. Verranno affrontati diversi argomenti che riguardano la sostenibilità, la sicurezza e tutti gli aspetti legati alla salute intestinale e growth performance degli animali che assumono diete integrate con former food, nonché tutti gli aspetti che riguardano la sicurezza e sostenibilità di questi ingredienti.

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CHAPTER 1

General Introduction

1.1 CLIMATE CHANGE AND OTHER IMPLICATIONS OF ANIMAL PRODUCTION

The human population is expected to growth to almost 11 billion people by 2100 (UNPD, 2019). For this reason, also the demand for livestock products is expected to increase as much as 50 percent by 2050, especially in developing countries (Africa and Southern Asia) (FAO 2020a). As a result of increasing incomes, urbanization, environment and nutritional concerns and other anthropogenic pressures, the global food system is undergoing a profound change. Worldwide, there is a huge rise in the demand for food of animal origin, this is happening for a variety of reasons, not only caused by growth population, but also urbanization and economic enlargement in developing countries. In the wake of the COVID-19 pandemic, the deep interconnection between human, animal, plant and environmental health has emerge clearly; much of the world has become aware of the importance of biosecurity in protecting global health. This accentuates the urgency to transform the planet's agri-food systems: it was so before the pandemic, it is even more now. In any case, it is time to take action to reinvent our approach to animal production and animal nutrition (Hulme, 2020).

Livestock animals contribute to food security, nutrition, poverty alleviation, and economic growth. Moreover, animal feed plays a vital role in the global food security, and it is conceived to ensure the sustainable production of safe and affordable animal proteins. With the increase of the animal production, it will be necessary for more feed to be produced, which will be safety certified. Consequently, new and old feed sources are being controlled for hazards and critically analyzed to guarantee feed safety for animal consumption. However, the food safety regulatory framework is not fully harmonized between the countries, creating a lack in feed safety chain, increasing the animal health risks and the animal consumption by the humans (FAO, 2018a)

Beyond food production, farm animals play other important economic, cultural and social roles and provide multiple functions and services. Farms are an essential part of agro-ecosystems. Furthermore, this increase in demand for animal products stems from the fact that millions of people are changing and will change their basic diet in the future, shifting from plant-based diets to more intensive demand for animal products like meat, dairy and eggs; obviously this could provide opportunities for economic growth for developing countries (Delgado et al., 1999; Cassidy et al., 2013). Globally, 34% of global food protein comes from livestock animals. Worldwide, will have to face the challenge of increasing the number of farms and also, at the same time, increasing the production of cereals to support the diets, both for humans and for animals (FAO 2020b). Nowadays, industrialization and globalization of livestock sector is centered on advances that have improved feed-to-meat conversion efficiency, animal health, reproduction rates, transport costs and liberalization of world-trade (Naylor et al., 2005). The most significant shift has been towards the production of monogastric animals, such as chickens and pigs, which are able to use concentrated feed more efficiently than cattle (or sheep) and which have short life cycles that accelerate genetic improvements (Muir and Aggrey, 2013). For example, the average time it takes to produce a broiler chicken in the United States has been reduced from 72 days in 1960, 48 days in 1995, to 42-46 days today, and the slaughter weight has gone from 1.8 to 2.2 to 2.9kg today (USDA, 2020). While, another important indicator is the feed conversion ratio (FCR) that measure the efficiency of feed turned into a kg of animal product, and as reported from FEFAC (2021), from 2000 to 2019, the FCR improvement for fattening pigs went from 3.03 to 2.68 kg feed/kg body weight (11% of improvement), and for salmon it went from 1.57 to 1.31 kg feed/kg body weight (16% of improvement). However, in the meantime, FCR were reduced to 15% for broilers and more than 30% for laying hens (USDA, 2020). The annual growth of pig and poultry production in developing countries was double the world average in the 1990s. In 2001, three countries - China, Thailand and Vietnam - counted for more than half of the pigs and one third of the chickens produced all over the world. Another major meat producing country, that is expected to become the largest meat exporter in the world, is Brazil. Precisely, as a consequence of the world population growth and the increase of demand of food in many developing countries, in fact, a rapid growth in meat consumption is expected. Already in the last 50 years, the annual per capita consumption of meat in the world has gone from 23 kg in 1961 to 43 kg in 2018 (FAOSTAT, 2018). In particular, emerging countries have seen an increase in consumption of meat, especially from monogastric livestock (i.e., pig and poultry). In general, animal production can be expressed as per protein basis, allowing comparisons between species and products. For example, Asia, with about 19 million tons of protein, is the region with the highest production, mostly represented by monogastric species. Otherwise, Western Europe, North America, Latin America and the Caribbean and South Asia have comparable production levels, between 12 and 10 million tons of protein. In Latin and North American the main role is play by beef, milk and chicken, while in Western Europe, animal production is primarily driven by dairy cattle; the major role in South Asia is conducted by buffalo production. Finally, the Near East and North Africa, Sub-Saharan Africa, Eastern Europe, Oceania and the Russian Federation produce between 4 and 1.6 million tons of protein, with a lower individual share on a global scale (Figure 1) (FAOSTAT, 2018).

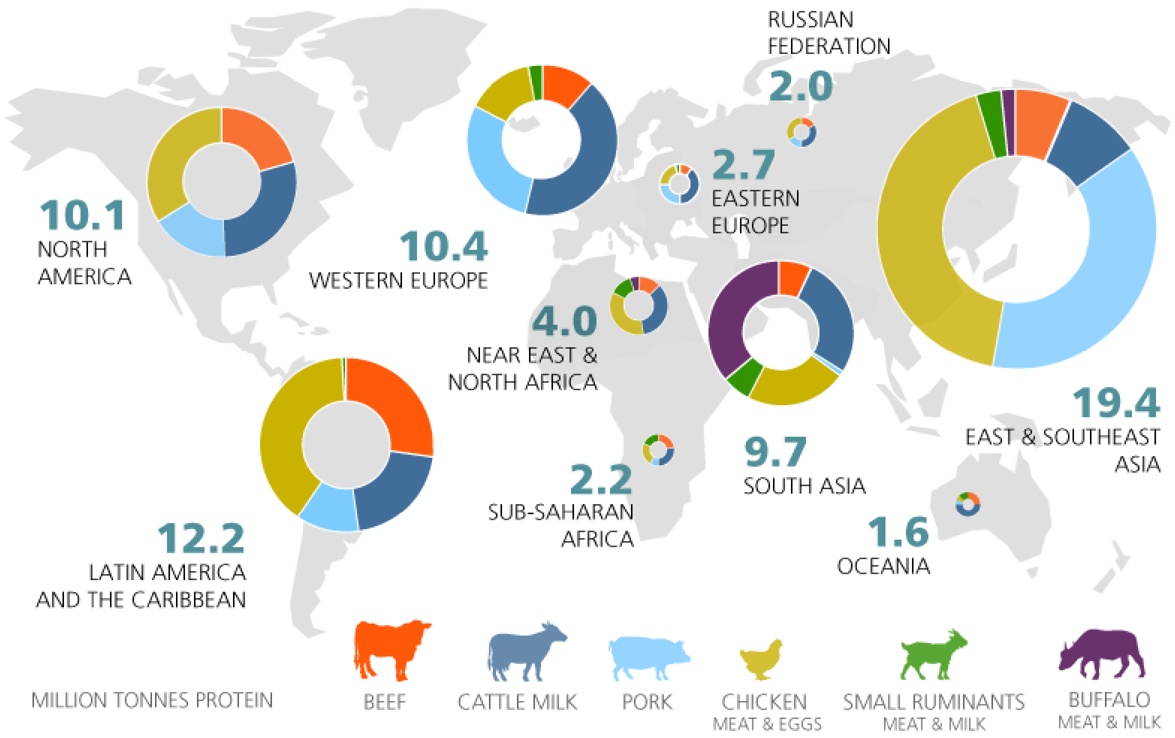

Figure 1. Regional total animal production. Meat production in protein basis was calculated by using data on dressing percentages, carcass to bone-free meat and average bone free meat protein content. Milk from all species was converted into fat and protein corrected milk. Eggs production is also expressed in protein terms (FAO, 2017)

Livestock systems have a significant impact on the environment (air, land, soil, water and biodiversity). With the increase in number of farms and livestock animals, one of the major aspects to consider will be the climate change. Undoubtedly, climate change will affect livestock production, changing the quality and quantity of feed (Thornton et al., 2013). According to Steinfeld et al (2006), livestock takes 70% of all agricultural land and 30% of the planet’s land surface. According to the estimates of the Intergovernmental Panel on Climate Change (IPCC), the warming of the air, in the next period, could be between 1.1 and 2.9° in the best scenario, while in the worst scenario, it could be between 2.4 and 6.4° (IPPC, 2017). Unfortunately, climate change will modify the biodiversity and genetic resources of the various cultivable species and this will affect the digestibility and nutritional quality of forages and other feed ingredients. In the near future, due to climate change, security and availability of water source will be priorities for humanity (Thornton et al., 2010). The effects of climate change will be different all over the world and may be beneficial in some countries and disadvantageous in others; only few researches studied the effect of climate change in tropical regions, and they have looked at the impacts in temperate regions (Easterling et al., 2007; Nardone et al., 2010). As long as the water needs are satisfied, the increase in temperature and concentrations of CO2 and nitrogen can be advantageous, by increasing in some areas of the world, primary production in pastures and allowing the cultivation of even more productive forage species (Thornton et al., 2013). Climate change will have a greater impact on grassland-based systems than on cropland because growing conditions on croplands could be more easily manipulated (e.g., through irrigation and/or wind protection) (FAO 2020b). Some countries, like North America, Northern Europe, Northern Asia and the Mediterranean basin, have a high farm animal density and a high-level animal production. These farms are based on industrialized livestock systems, farming high selected pigs, poultry, meat and dairy cows (Nardone et al., 2010). Climate change will also affect animals, through increased heat stress, change in water availability and more livestock diseases and disease carriers (Thornton et al., 2010). Therefore, the real challenge of the future will be to balance the increase in the number of animals farming, with the demand of raw materials for feed production and the productivity of farms, while improving the sustainability of all the livestock sector. Moreover, livestock sector is the largest land-use system and it occupies 30% of the world's ice-free surface, contributes 40% of global agricultural gross domestic product, and provides income for more than 1.3 billion people and nourishment for at least 800 million food-insecure people, all the while using vast areas of range lands, one-third of the freshwater, and one-third of global cropland as feed (Herrero et al., 2013). The cultivation of crops requires the input of manure and fertilizers as well as energy carriers, water, crop protection products and auxiliary materials and may involve land transformation (Thornton et al., 2013). The most crucial aspect of land use is deforestation, with livestock playing a major role through the creation of new pastures or expansion of arable land to produce crops like soybeans and other cereals, important to support the global intensification of livestock feeding (Herrero et al., 2009, Naylor et al., 2005). While cattle ranching is considered as the major proximate cause of forest clearing in the Legal Amazon, soy cultivation often expanded into areas previously used as pastures, thereby indirectly triggering forest-to-pasture conversion elsewhere (Barona et al., 2010). Moreover, soy production may have contributed to deforestation by other indirect pathways, such as boosting land prices and infrastructure development (Barona et al., 2010, Fearnside, 2001, Fearnside, 2005, Nepstad et al., 2006). This creates a great pressure on agriculture and natural resources. This will be crucial, as today, because the animals reared and then slaughtered contribute directly or indirectly to 18% of greenhouse gases (GHG) emission and 9% of CO_2 total emissions (FAO, 2018b; Nardone et al., 2019). Both, ruminants and non-ruminants are responsible of enteric fermentation during the digestive process. These animals produce especially methane, but the quantity is lower in monogastric species, compared to ruminants. Poorly digestible rations, like highly fibrous ingredients, produce higher enteric methane emissions (FAO, 2018b). Livestock sector emitted an estimated total of 8.1 gigatons CO_2-eq in 2010. Methane (CH_4) accounts for about 50% of the total; while nitrous oxide (N_2O) and carbon dioxide (CO_2) represent almost equal shares with 24% and 26%, respectively (FAO 2017). Manure is a source of both methane and nitrous oxide. Methane is released during anaerobic decomposition of organic matter. Nitrous oxide is mainly generated during manure ammonia decomposition. Different manure management systems can produce different level of emission. Methane emissions are higher when manure is stored and treated in liquid systems (lagoons or ponds), while dry management system, such as drylot or solid systems, tend to favor nitrous oxide emissions. Beef and dairy cattle are the main contributor to the sector's emissions with about 5.0 gigatons CO_2-eq, which represents about 65% of sector's emissions. Pigs, poultry, buffaloes and small ruminants have much lower emissions, representing between 7% and 10% of sector's emissions (Figure 2). In particular, in pigs the 60% of the 9% of the GHG emission comes from feed and, of that the single biggest contributor is soybean meal.

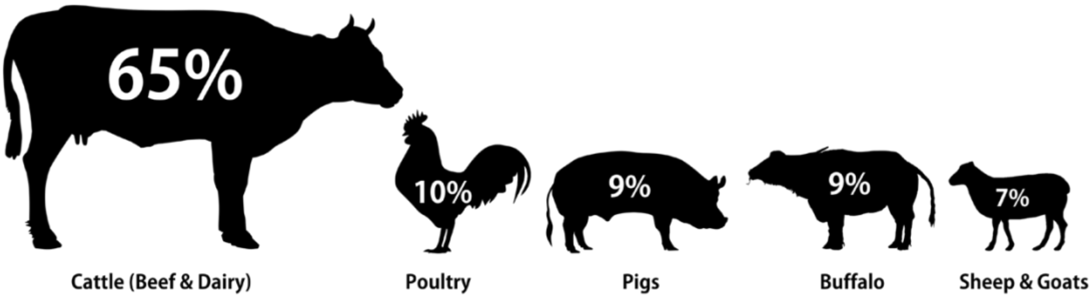

Figure 2: Global total livestock GHG emissions by different species (FAO, 2018).

It is well-known that when considering the carbon footprint of an animal product, the feed ingredient production stage represents the largest share of the GHG emissions (FEFAC, 2020). There are several emissions related also to feed production. Carbon dioxide emissions arise from expansion of feed crops and pastures into natural areas such as forests, from manufacture of fertilizers and pesticides for feed crops and from feed transportation and processing. Nitrous oxide emissions are caused by the use of nitrogenous fertilizers and by direct application of manure both in pastures and crop fields. Energy consumption occurs along the entire supply chain.

Production of fertilizers and the use of machinery for crop management, harvesting, processing and transport of feed crops generate GHG emissions, which were accounted as part of the emissions from feed production. Energy is also consumed on animal production site for ventilation, illumination, milking, cooling, etc. Finally, livestock commodities are processed, packed and transported to retail points, which involves further energy use. In Figure 3 are reported the contribution of main sources of emissions from global livestock supply chains (FAO, 2018b)

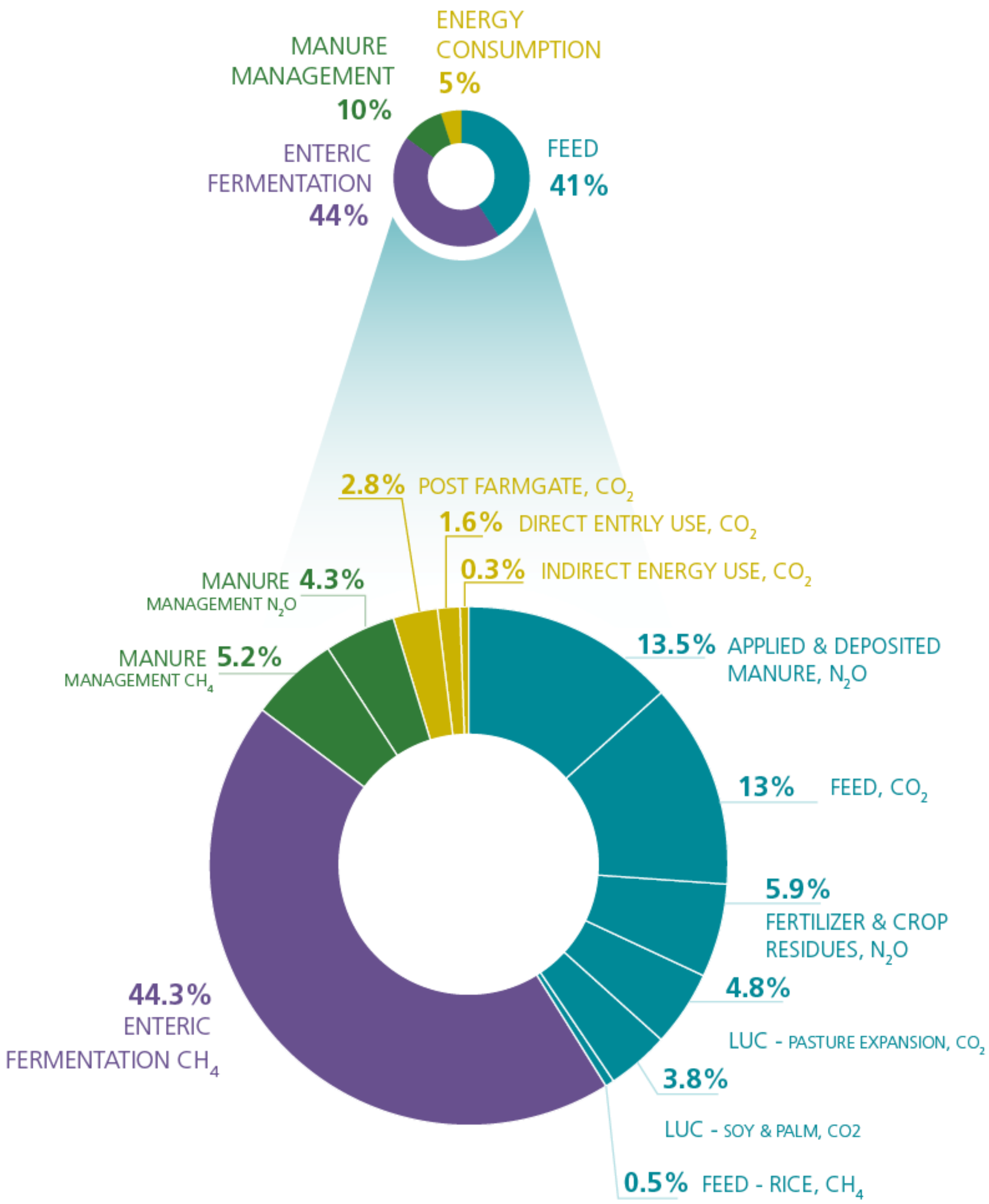

Figure 3: Global emission by different sources from livestock sector. (FAO, 2018)

Generally, animal products have a much higher water footprint than plant-based foods (Table 1) (Mekonnen and Hoekstra, 2010).

Table 1: the water footprint of some selected food products from crop and animal origin (Adapted from Mekonnen and Hoekstra, 2010).

	Litre per Kilogram	***Litre per Kilocalorie***	***Litre per gram of protein***	***Litre per gram of fat***
Sugar crops	197	0.69	0.0	0.0
Vegetables	322	1.34	26	154
Starchy roots	387	0.47	31	226
Fruits	962	2.09	180	348
Cereals	1644	0.51	21	112
Oil Crops	2364	0.81	16	11
Pulses	4055	1.19	19	180
Nuts	9063	3.63	139	47
Milk	1020	1.82	31	33
Eggs	3265	2.29	29	33
Chicken meat	4325	3.00	34	43
Butter	5553	0.72	0.0	6.4
Pig meat	5988	2.15	57	23
Sheep/goat meat	8763	4.25	63	54
Bovine meat	15415	10.19	112	153

Compared to crop products, animal products do not only require more land to obtain a certain nutritional value, but also more energy and water (Hoekstra, 2014). The water footprints of animal products can be understood from three main factors: *(i)* feed conversion efficiency of the animal, *(ii)* feed composition, *(iii)* and origin of the feed. Moreover, all the three factors are influenced by type of production system (grazing, mixed, industrial) (Mekonnen and Hoekstra, 2014). Regarding feed conversion efficiency, the more feed is required per unit of animal product, more quantity of water is necessary to produce animal feed. While regarding feed composition, the most important value is the ratio of concentrates versus roughages and percentage of valuable crop components versus crop residues in the concentrate. Finally, regarding the origin of the feed it’s important to consider that the water footprint of a specific animal product could change across different countries due to differences in climate and agricultural process, due to the place where feed components are obtained (Mekonnen and Hoekstra, 2014). Animal production requires large volumes of water for feed production, drinking water and servicing animals. By far the largest water demand in animal production is the water needed to produce animal feed. Because of the increasing demand for animal products and the growing sector of industrial farming, the demand for feedstuffs grows as well, including cereals, starchy roots, fodder crops, oilseeds and oil meals. The water footprint of meat from beef cattle (15400 m^3/ton as a global average) is much higher than the footprints of meat from sheep (10400 m^3/ton), pig (6000 m^3/ton), goat (5500 m^3/ton) or chicken (4300 m^3/ton). The global average water footprint of chicken egg is 3300 m^3/ton, while the water footprint of cow milk amounts to 1000 m^3/ton. Global animal production requires about 2422 Gm^3 of water per year (87.2% green, 6.2% blue, 6.6% grey water). One third of this volume is for the beef cattle sector; another 19% for the dairy cattle sector. Most of the total volume of water (98%) refers to the water footprint of the feed for the animals. Drinking water for the animals, service water and feed mixing water account only for 1.1%, 0.8% and 0.03%, respectively (Mekonnen and Hoekstra, 2014).

Focusing our attention on Italy, this country is among the largest agricultural producers in the European Union and this production causes significant impacts on the environment. The degradation of agricultural soils is among the most worrying in Europe: the carbon content in the soil is only 1.1% by weight, this data is below the 1.5% threshold considered at risk of desertification. At the national level, fresh water withdrawals due to agriculture amount to 6.74% of renewable water resources, but substantial volumes of "virtual water" are consumed indirectly by our country through food imports. In addition, more than 75% of fish stocks are overfished or exhausted. The 64% of annual greenhouse gas (GHG) emissions from the agricultural sector are due to animal production and 36% to plant production. The emissions are equal to approximately 2.3 Gg CO_2 eq. for each agricultural hectare, lower than other large agricultural producers such as France, Germany and the Netherlands, but higher than in Spain. The lack of a national political strategy limits the opportunities to invest in sustainable agriculture and mitigate climate change. Finally, young people represent only 5% of farmers, even if recent statistics show their rapprochement with the sector. In the future, all over the world, one of the objectives to achieve is the stabilization of greenhouse gas concentrations in the atmosphere and water/land footprint of animal production, at a level that would prevent dangerous anthropogenic interference with the climate system. Such a level should be achieved within a time frame sufficient to allow ecosystems to adapt naturally to climate change, to ensure that food and feed production is not threatened and to enable economic development to proceed in a sustainable manner (FAO, 2017).

1.2 FEED AND FOOD COMPETITION

All over the world the demand of animal products will increase between 48% and 57% and for this reason animal protein demand will increase even more (Parisi et al., 2020). Six billion tons of feed materials (DM) is consumed annually by livestock sector, including one third of global cereal production.

About food and feed competition and future food security conception, there are several studies (Godfray et al., 2010; Eisler et al., 2014; Mottet et al., 2017) that reported that about 1 billion tons of cereals are fed to livestock annually resulting in a competition for land and raw materials between human and animal nutrition. In this context, to convert losses from the food industry into ingredients for the feed industry, thereby keeping food losses in the food chain, can be considered a virtuous practice that should be implemented worldwide (Eisler et al., 2014; Pinotti et al., 2020). In addition, soybean cakes, which production can be considered as main driver of land-use, represent 4% of the global livestock feed intake. However, monogastric consume 72% of the global livestock grain intake, while grass and leaves represent more than 57% of the ruminants' intake. Animal feed and feeding are fundamental in livestock production. In animal production the highest cost (up to 70%) is represented by feed, that is economically the most important element in animal husbandry (FAO 2014a). Moreover, feed is what links livestock to land use. The classification of feed material is based on what is edible by humans (i.e., cereal grains, soybeans, pulses, banana and cassava) or not (i.e., roughages such as grass, crop residue, fodder beets, cotton seeds) (Figure 4). The feed materials are always classified as not human-edible (Mottet et al., 2017).

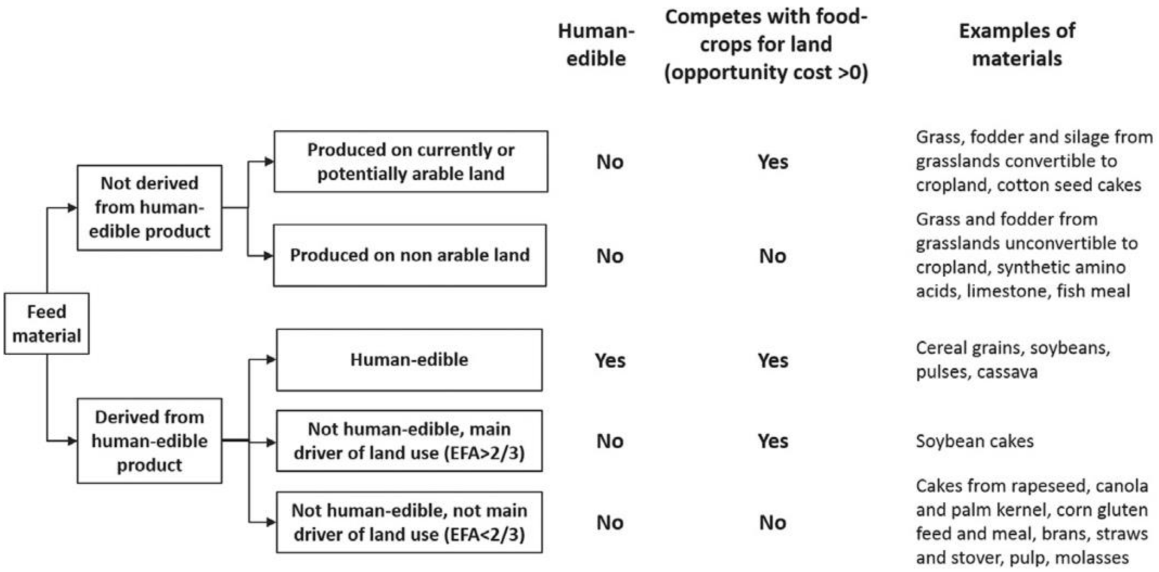

Figure 4: Feed classification (Adapted from Mottet et al., 2017).

Livestock animals use feed via grazing and indirectly via traded grain or forage. Globally, Herrero et al., (2013) reported that in 2000, livestock consumed about 4.7 billion tons of feed biomass, with ruminants consuming the bulk of feed (3.7 billion tons compared with 1 billion tons by pigs and poultry). Overall, grasses comprise some 48% (2.3 billion tons) of the biomass used by livestock, followed by grains (1.3 billion tons, 28%). Beef production is often criticized for its very huge consumption of grain, some studies reported that the quantitative vary from 6 kg to 20 kg of grain per kg of beef produced (Mottet et al., 2017; Eshel et al., 2014; Godfray et al., 2010). Diet composition and quality are determinants for productivity and feed-use efficiency of farm animals. To help create a sustainable livestock sector, high nutritional value feed from international markets is necessary to support high production farm animals (Rauw et al., 2020).

There are three negative effects that livestock sectors produced to food security: *(i)* animal feed rations contain products that can also serve as human food; *(ii)* animal feed may be produced using land suitable for human food production; *(iii)* livestock animals have the low efficiency in converting feed into human food (Mottet et al., 2017). In addition, due to the land scarcity increase and arable land decrease, the industry has relied increasingly on technological advances and new alternative resources to keep up with the demand for increased livestock production. Other changes and innovations must concern the management of waste and food surpluses. Making the food chain more efficient through waste reduction will reduce the need for new resources to be allocated for food production. In the study conducted by van Hal et al. (2019), to evaluate the impact of accounting for feed-food competition on LCA results, economic and food-based allocation were compared in an LCA of a novel egg production system that feeds only products unsuitable or undesired for human consumption. In this study was reported that compared to free range laying hens fed a conventional diet, feeding only low-opportunity-cost feedstuff (LCF) reduced global warming (GWP) potential by 48 to 58%, energy use (EU) by 21 to 37%, land use (LU) by 34 to 47% and land use ratio (LUR) by 32% in case of economic allocation. This was caused by the small environmental impact allocated to LCF due to their relatively low economic value. Moreover, using food-based allocation, the impact per kg egg was further reduced by 54% for GWP, 38% for EU, 94% for LU, and 88% for LUR. In conclusion, an LCA with economic allocation underestimates the environmental benefits of avoiding feed-food competition. Cassidy et al. (2013) reported that on a global basis, crops grown for direct human consumption represent 67% of global crop production, 55% of global calorie production, and 40% of global plant protein production. Feed crops represent 24% of global crop production by mass. However, since feed crops like maize, soybeans, and oil seed meal are dense in both calories and protein content, feed crops represent 36% of global calorie production and 53% of global plant protein production. Together crops used for industrial uses, including biofuels, make up 9% of crops by mass, 9% by calorie content, and 7% of total plant protein production.

However, in 2021, analysis conducted by FEFAC, demonstrated that practically none of the feed used to produce feed for livestock animals can be considered food grade, because, feed materials don’t meet the minimum standards required for human food production, regarding their presentation or technical characteristics. The majority of feed ingredients used in compound feed originate from crop cultivation and the 86% of the global livestock feed intake is made of materials that are currently not eaten by humans. In general, when a food produced for human consumption is sold to a feed industry, is always the result of an exceed in the production (FEFAC, 2021). Moreover, FEFAC reported that animal feed production is per definition never in competition with human consumption, from an economic point of view. That’s why, if food materials are used in feed production, will never cause a lack of food along the supply food chain (FEFAC, 2021). The use of food effectively no longer destined for human consumption to produced feed, for FEFAC, should not be automatically considered as something ‘unsustainable’, but more often that’s important to keep food grade bio-resources in the food value chain through feed for livestock animals. As reported in Figure 5, it is fundamental to make a distinction between non-food grade feed ingredients that drive (arable) land use and the ones that does not, the latter being of more traditional co-product nature (e.g., sugar beet pulp, brewers’ grains, sunflower meal). Cereals, soybean products and pulses destined to animal feed production are qualitative not suitable for human consumption, but in any case, there is an element of competition between animals and humans for land use (FEFAC, 2021)

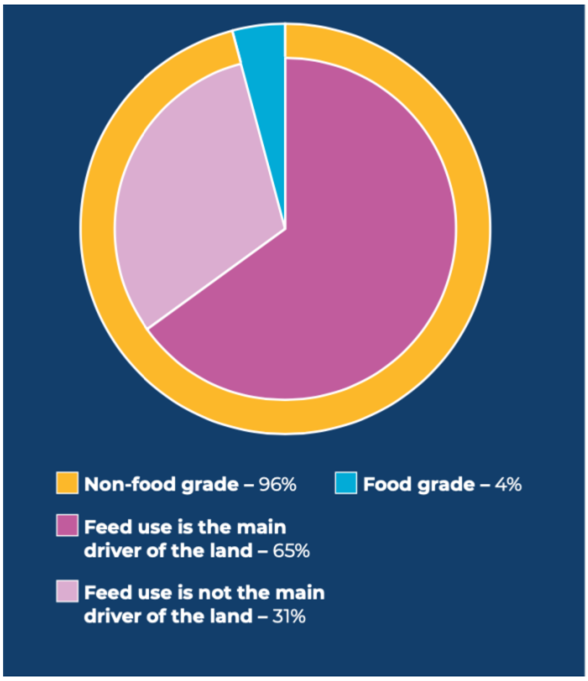

Figure 5: Feed used by compound feed manufactures (FEFAC, 2021).

From FEFAC's experience this assessment about food and feed production cannot tell the full picture in terms of sustainability, but the presence of general accusations that feed production is in direct competition with food consumption, generate insecurity. Animal feed production must to be considered part of the food production itself (FEFAC, 2021).

It has been estimated that between 30% and 50% of global food products are lost or wasted before and after reaching the consumer. In 2006, the total loss of food in EU 27 was about 90 million tons and it is estimated that in 2020 food waste will reach 126 million tons (FAO, 2011). As a result, the European Parliament recommended to the European Commission to reach the Sustainable Development Goals by 2030, in particular Goal 12 of ensuring sustainable consumption and production pattern (Union Innovation, 2014). In the European Union, especially in countries with intensive animal production, producers are not able to ensure that livestock is supplied with nutrients of regional origin, as for example protein (Guyomard et al., 2021). Regarding pig farms, it's necessary an evaluation of the availability of local feed and feedstuff co-products or alternative feed ingredients as first step to study the feasibility of the reduce farm impact (Rauw et al., 2020). In order to improve the feed efficiency, a large use of human-edible products in animal diets has been proposed.

In fact, this inefficiency can be avoided using livestock feeds that do not compete with food production, so called “low-opportunity-cost feedstuffs” (e.g., food by-products & waste and grazing resources). Reducing food loos and waste is the key to achieving sustainability and brings savings for consumers and producers and from a social point of view, the redistribution of surplus food that otherwise would be wasted, is very important. In Europe, the Commission is committed to halving per capita food waste at retail and consumer levels by 2030. Using the new methodology for measuring food waste and the data expected from Member States in 2022, it will set a baseline and propose legally binding targets to reduce food waste across the EU (European Union, 2020). In addition to quantifying level of food waste, the Commission will investigate food losses during the production, and explore ways of preventing them. Coordinating action at EU level will reinforce action at national level, and the recommendations of the EU Platform on Food Losses and Food Waste will help show the way forward for all actors (European Union, 2020).

Food waste in Italy costs over 15 billion euros, equal to about 1% of GDP. The waste of food produced each year, at the per capita level, is equal to 65 kg, of which 27.5 kg attributable to internal consumption. Considering food losses, Italy is slightly below the European average, with 2% of food lost from the post-harvest phase to industrial processing, excluding the agricultural phase. The quality of national policies against food waste is high and is realized through the National Plan for the Prevention of Food Waste which intervened to facilitate the donations of food surpluses, through a strongly participatory approach that involved numerous actors in the supply chain, increasing, according to the estimates of the Food Bank, donations of 20% in the first year of application. The definition of reduction objectives in line with the 2030 Agenda, economic incentives, the strengthening of tax deductions for the donation of surpluses and the revision of economic index on waste, could contribute to further reducing waste (Fondazione Barilla, 2019)

1.3 ALTERNATIVE FEED INGREDIENTS

Guaranteeing healthy, good and sufficient food to meet the nutritional, cultural and social needs of a growing and increasingly world population is one of the most important challenges of our century, to promote sustainable development that respects the limits of the planet.

Europe is promoting the importance of Circular Economy (CE), but this concept is fundamental all over the world. Annually, thanks to Circular Economy, the global economy would benefit about 1000 billion US dollars (Korhonen et al., 2018).

In this contest, animal products have been the basis of the diet of people with different ages, health conditions, and secular and religious beliefs and the demand of these products is set to grow (Pulina et al., 2017). Circular Economy includes the economic, environmental and social dimensions of sustainability and in this particular case, all these aspects are regarding the importance to find a strategy to develop more intensive and sustainable animal production worldwide. As reported before, feeding pigs is the most expensive aspect of pig production. Historically, feed costs have represented 65- 75% of the variable costs of pig production, but this increases drastically over the past 2 years. This has been the result of a combination of poor harvests in different parts of the world, increasing demand for feed grains from the biofuel sector, and speculative buying by funds (Kiare and Nyachoti, 2009). Moreover, feed for animals is based mainly on corn and soybean, which are adopted everywhere and, therefore, one of the most important objectives of the current research is to find alternative and suitable ingredients that can be use in animal nutrition. In the prospective of Circular Economy, animal feed is based on the exploitation of the environment and on the collection of waste coming from human food industry. Nevertheless, nutrition remains the central topic of animal husbandry.

For example, soy is the most important protein source for pigs, but recently it is crucial to be aware of the environmental impacts that the feed industry has. Reducing the amount of soy in the ration by improving genetics, health and feed regimes will significantly reduce the carbon footprint of pig feed and pig farms. Moreover, soy is associated with deforestation and conversion of natural areas, but FEFAC in 2016, introducing the new Soy Sourcing Guidelines to lead agriculture and also feed industry towards a responsible and sustainable use of soy (FEFAC, 2016). Luckily, an increasing number of animal nutrition companies are becoming aware of the sustainable impact of feed and problems regarding the large use of soy. For this reason, feed companies are trying to reduce waste of products and resources and find alternative and valid feed ingredients (Doyle, 2021).

There is an urgent need to develop alternative feeding systems based on high-yielding plants, novel feed like insects, algae, new by or co-products, aquatic plants, and fodder leaves, which can also be produced locally. As reported by Stein et al. (2007), alternative energy feed ingredients are important to try the substitution of common materials in feed for livestock animals. Using every other cereal instead of corn and wheat can be considered a minor ingredient. Sorghum, where it is raised instead of corn, can be of an equal status to these two staple energy sources. When using minor ingredients, it is best to use a mix of them, or small quantities of these minor cereals with a major cereal or other energy source, because most of these have higher content of non-starch polysaccharides that could reduce energy digestibility, cause sticky droppings, and have anti-nutritional factors. To help the use of minor cereals in livestock diets, it could be use enzymes with more or less success to limit anti-nutritional effects. Knowing the source of such ingredients is often enough for a nutritionist to determine their quality and how to best adjust their use in combination with enzymes and other additives or ingredients.

Other alternative ingredients used for feeding livestock animals, are all the co and by-products that are coming from processing and transformation of standard cereals, like corn, soy and wheat. For example, distillers dried grains with solubles (DDGS), field peas, wheat shorts and liquid co-products from the bio-fuel and food industry are all considered valid ingredients. DDGS is the most common co-product from many “ethanol plants”. Generally, it is dried to about 10% moisture content, this process ensures a long shelf life and reduces flowability issues during storage and transport (Rosentrater, 2018). At first, the majority of DDGS were used principally in cattle and weren’t used extensively in swine diets. Recently, different studies (Shurson, 2002; Stein and Shurson, 2009; Kiare and Nyachoti, 2009) reported that corn DDGS contains high levels of digestible and metabolizable energy, digestible amino acids, and available phosphorus. Stein et al. (2006) showed that the DE and ME value of corn DDGS is equal or even higher than corn (3,639 kcal DE/kg and 3,378 kcal ME/kg), but it is considered a low protein quality feed ingredient due to its low lysine content. Because of the higher nutrient value, DDGS is very well suited for pig and poultry diets, and can be a cost-effective partial replacement for corn, soybean meal, and dicalcium phosphate in swine feeding. Regarding the use of DDGS in swine diets, Whitney and Shurson (2004) conducted two experiments to study the effects of increasing levels (from 0 to 25%) of DDGS on growth performance of early-weaned pigs. Dietary treatments consisted 3-phase nursery feeding program.

All pigs were provided a commercial pelleted diet for the first 4 days post-weaning, and then they were fed with their respective experimental Phase 2 diets (for 14 days), followed by Phase 3 experimental diets (for 21 days). All the data referred to the growth performance of pigs were similar among dietary treatments regardless of dietary DDGS level fed for both experiments. In experiment 1, feed intake was unaffected by dietary treatment, but in experiment 2, increasing dietary DDGS level linearly decreased feed intake during Phase 2, and tended to decrease voluntary feed intake over the length of the experiment. These results suggest that it is important the level of inclusion in the diet, high quality corn DDGS can be included in Phase 3 diets for nursery pigs at dietary levels up to 25%, without any negative effect on growth performance. Satisfactory growth performance can also be achieved when adding up to 25% DDGS in Phase 2 diets for pigs weighing at least 7 kg in body weight. However, including high levels immediately in the post- weaning period, may negatively influence feed intake, resulting in poorer initial growth performance.

Another co-product, obtained from biodiesel production, and used for feeding pigs, is crude glycerol. Biodiesel is an alternative fuel that can be produced from vegetable oils and (or) animal fats. The oil or fat is mixed with an alcohol, generally methanol, and a catalyst (often sodium hydroxide) that causes triglycerides to separate, forming methyl esters (biodiesel) and crude glycerol (Hansen et al., 2009). Crude glycerol has been proposed as a potential beneficial energy source for pigs. It is absorbed by the gastrointestinal tract of non-ruminants and is utilized as an energy source in the gluconeogenic pathway (Tao et al., 1983) and it contains 3,021 kcal/kg of ME (Lammers et al., 2008). Recent studies have demonstrated that it can be used in nursery pig diets and growing-finishing pig diets (Seneviratne, 2009; Hansen et al., 2009). Every liter of biodiesel produced, generated 79g of crude glycerol (Thompson and He, 2006). For example, Hansen et al. (2009) studied the effect of feeding different level of inclusion of crude glycerol (0, 4, 8, 12 16%) to growing-finishing pigs on performance, plasma metabolites and meat quality at slaughter. They reported that crude glycerol could be included in finishing pig diets without any detrimental effect on growth performance and meat quality of pigs. However, blood glycerol levels became higher after prolonged feeding of this co-product, and may reduce the efficiency of glycerol when used as energy source for pigs. Furthermore, over the 8% of inclusion in diets, crude glycerol formed a firm aggregate within 24 h of mixing that presented some feeding difficulties, for this reason seems better to limit crude glycerol to less than this percentage in mash diets. Crude glycerol may play a role in the pig industry by supplying energy at a more cost-effective price than competing energy ingredients; however, a shadow-pricing exercise is necessary to ascertain whether glycerol can be economically included in current diets (Hansen et al., 2009). Some problems in the use of this co-product are related to the chemical composition, because crude glycerol contains methanol, which is poisonous at low concentrations and may cause metabolic disorders and blindness.

Tapioca (*cassava*) is produced in Southeast Asia, India and other regions and also, it is imported in other countries, like USA and UE. It is largely use like alternative ingredients in feed for monogastric animals (Mavromichalis, 2017). In India about 50 percent of the 630 million people, living in rural areas, are poor and dependent on livestock sector for their income. For this reason, the farmers coming from developing countries cannot used cereals for feeding pigs or poultry due to the high cost of cereal grains. Sustainability of livestock diets can come from the use of tapioca or cassava root meal as energy source (Tzudir et al., 2012). This ingredient is capable of providing very high yields of energy/ha. Moreover, it is low in fiber and protein, but high in soluble carbohydrate (Tzudir et al., 2012). If tapioca is chopping and sun-drying, it is completely safe for livestock feeding because the level of hydrocyanic acid (HCN) (that limits its use) will be very low (Tewe et al. 1980). The level of inclusion of tapioca in feed for pig (20-25 kg of body weight) goes from 20% to 35% (FAO, 1992). Both in the past and more recently, in the scientific literature is possible to find studies (Chou et al., 1973; Tzudir et al., 2012) in which maize has been partially replaced by tapioca in diets for post-weaning and growing pigs. Tzudir et al. (2012) showed that tapioca root meal can be included in the diet of growing cross bred pigs up to a level of 50%. Also, the inclusion of tapioca has a significant increase of the average daily gain (AGD) of the animals, a higher digestibility of dry matter (DM), organic matter (OG) and ether extract (EE) and a reduction of the severity and duration of diarrhea, compared to animals fed with a standard diet. While Chou et al. (1973) showed that replacement of maize by tapioca is possible at much higher levels (60-75%). In this study, the quality of meat was also reported and no significant difference was observed between pigs fed cassava diets and those fed maize diets.

Gou et al., (2015) showed the effects of a supplementation of candy co-product (Chocolate Candy Feed – CCF) as alternative carbohydrate source to lactose on growth performance of newly weaned pigs. Lactose in whey powder have been identified as major component that enhance appetite and weight gain of nursery pigs, but also the carbohydrates from CCF including fructose and sucrose could have the same effect. In fact, in this study, lactose was replaced for 0, 15, 30, 45% by CCF based on equal amounts of total sugars, and the experimental period was divided into 3 phases. Results showed that partially replacing dietary lactose with carbohydrates from CCF increased feed intake in phase, due to the increase in diet palatability. In phase II, pigs fed with increasing levels of dietary CCF, tended to increased blood urea N, that implied there was increased N excretion. Carbohydrates from a candy co- product can replace up to 45% of lactose without impairing growth performance, feed intake, and feed efficiency of pigs during the overall nursery period. Furthermore, partially replacing lactose with carbohydrates from CCF could cause a decrease in weight gain in later nursery phases. In addition, the price of candy co-product was 45% cheaper than the price of whey powder, and 68% cheaper compared to the price of whey permeate, commonly used in feed formulations.

Another novel feed that is recently study to feed livestock animals are seaweeds. Seaweeds are simple organisms, which are able to take advantage of sunlight and they could convert carbon dioxide into sugars and oxygen, during the photosynthesis process (Morais et al., 2020). The most common varieties of edible algae include: *Neopyropia*, *Pyropia* spp., *Undaria pinnatifida*, *Saccharina latissima*, *Palmaria palmata* and *Chondrus crispus*, these types are associated with many health benefits, such as decreasing blood pressure, preventing spills and valuable protein source (Øverland et al.,2019).

Generally, seaweeds are used unprocessed, in medicine, human diets, animals' feeds and for improvements in agricultural soil, as fertilizers (Jamal et al., 2017). The term "algae" implies more divisions of lower plants, which contain chlorophyll in cells and generally live in water, although they are quite widespread outside the aquatic environment (Kovac et al., 2013), and on the basis of dimensions they are divided into macroalgae (macroscopic algae) and microalgae (microscopic algae). Seaweeds are an important source of vitamins, minerals, proteins, polyunsaturated fatty acids, antioxidants; moreover, they are rich in potassium, sodium, calcium, magnesium and phosphorus and are a source of essential trace elements, such as iron, manganese, copper, zinc, cobalt, selenium and iodine (Gouveia et al., 2008).

Algae have a relatively high protein quality compared to cereal and soy flour. but generally, have highly variable composition, with large differences in the final content in proteins, minerals, lipids and fibre, due to different species (Makkar et al., 2016). For example, the protein content of brown algae is generally lower than in red (30-140 g/kg of dry matter in Saccharina latissima, while 80-350 g/kg in Palmaria palmata) (Berkhout, 2021).

Morover, Berkhout (2021) reported that diets integrated with seaweed were less palatable than diets integrated with fish meal. The animals fed Saccharina diet had a significantly higher water intake and urine production than the other animals. This diet also stood out regarding urine concentration of iodine, which was 300 times higher than for the fishmeal-based diet.

More than 75% of seaweed has higher proportions of total essential amino acids than wheat flour and 50% higher than soy flour and also higher than rice and corn (Li et al., 2018).

The *cyanobacteria algae* (also named blue-green algae) are the most promising organism that could isolate new active natural products, this type of algae is very interesting as ingredients used in livestock feed (Kovac et al., 2013). For example, *Ulva* is a seaweed species with bright green sheets, located in marine environments and in brackish water; most especially in estuaries. This species is one of the important types of seaweed found abundantly in many coastal areas of many countries and it is rich in minerals, protein and vitamins (Morais et al., 2020). *Ulva* seaweed could be utilized as animal feed or supplement, because its bioavailability of nutrients embedded in the polysaccharide remained elusive due to inefficient metabolism by animals. This chemical limitation generally impedes efficient use of *Ulva* seaweed as sole animal feed. Although several seaweed species contain valuable amino acids of immense nutritional efficacy; their release can be poor due to crosslinking within the polysaccharide matrix of the algae mass. In conclusion, seaweed animal feed assays occur mainly as fresh, dried or even seaweed crude seaweeds extract. Unfortunately, there is a general lack of nutritional and biochemical studies of seaweed as feeds that makes difficult the analysis of how seaweed composition affects animal welfare. Thus, more studies, regarding seaweed complete biochemical profile (macro and micronutrients, also seaweed metabolites), are needed to fully understand the impact of seaweeds in the animals (Morais et al., 2020).

However, potential of seaweeds needs to be further explored as animal feed additive or supplement, but they cannot be applied as a complete substitute of the typical animal feed. Seaweed benefic effects are generally below 10% of the total concentration in the animal feed; above that, it was demonstrated to show negative effects and even animals refused to eat the provided feed, correlated to problems of palatability (Haberecht et al., 2017).

Finally, the necessity to find alternative ingredients to replace fish and soy meal has led studies and researchers to consider insect proteins as novel feeds for animals (Henry et al., 2015; Ottoboni et al., 2018). In reality, the advantageous aspects of the use of insects as feed are many: insects have little consumption of land and water, but have a high conversion efficiency of feed into insect biomass (Eike et al., 2017). The crude protein contents of these alternate resources are high: 42 to 63% and so are the lipid contents (up to 36% oil), which could possibly be extracted and used for various applications including biodiesel production. Unsaturated fatty acid concentrations are high in housefly maggot meal, mealworm and house cricket (60-70%), while their concentrations in black soldier fly larvae are lowest (19-37%) (Chia et al., 2019). Different studies (Gasco et al., 2020; Kar et al., 2021; Bosch & Swanson, 2021) have confirmed that palatability of these alternate feeds to animals is good and they can replace 25 to 100% of soymeal or fishmeal depending on the animal species. Except silkworm meal other insect meals are deficient in methionine and lysine and their supplementation in the diet can enhance both the performance of the animals and the soymeal and fishmeal replacement rates. Most insect meals are deficient in Ca and its supplementation in the diet is also required, especially for growing animals and laying hens (FAO, 2014b; Chia et al., 2019). The levels of Ca and fatty acids in insect meals can be enhanced by manipulation of the substrate on which insects are reared. Benefits of using insects for livestock feed include high nutritional values, feed efficiency, and reproductive capacities. Insects have the ability to produce by-products; are naturally present in some livestock diets (e.g., fish, poultry, pigs) and can create additional socio-economic and environmental benefits (FAO, 2014b). A wide range of suitable insects exists, e.g., Black Soldier Fly (BSF) larvae, house fly maggots, mealworms, silkworms and locusts-grasshoppers-crickets. Several insect species are able to convert organic waste into edible biomass, of which the composition may depend on the substrate (Ottoboni et al., 2018). BSFs are considered to have the most potential for feed (Eike et al., 2017). Kar et al. (2021) reported that Black soldier fly larvae can replace soybean meal as a protein source in the feed of growing pigs. Black soldier fly larvae are potentially a more suitable and sustainable protein source as they can be grown on waste and residual streams from food production. The feeding trial was conducted with two groups of growing pigs. One group was fed a diet with regular soybean meal as a protein source, while the other was fed a diet with black soldier fly larvae as the protein source. Data from this trial were gathered on the microbiota of the small intestine and metabolites in the blood of the pigs (Kar et al., 2021). In conclusion, pigs fed insect larvae had increased levels of *Bifidobacterium* bacteria, which have been shown to have a positive effect on human and animal health. Moreover, BSF diet was able to suppress harmful bacteria, and amine metabolite profiles in blood plasma showed the ability of the black soldier fly larvae to provide functional properties that could be beneficial to pig health and performance beyond their ability to provide amino acids as building blocks for protein synthesis. Black soldier fly larvae, therefore, promote the growth of gut microbial taxa that are either indicators of a healthy gut or are recognised as beneficial microbes that have positive effects on pig health. The functional value of BSF as dietary protein source showed good effects on the small intestinal microbiome and the profile of blood plasma amine metabolites. Such functional value could ultimately improve the competitiveness and the economic perspective of insect meals as sustainable feedstuffs for pig diets compared to conventional protein sources. In addition, compared to feeding an SBM-based diet, there were no significant effects of dietary inclusion of BSF on the growth performance and on plasma cytokine and chemokine concentrations under nonchallenge conditions. As said before, growing insects requires a negligible investment of capital or land. The time-consuming part is loading the trays, switching the trays, moving the trays to where the larvae can be dried out, transferring the dried insects to the pulverising machine, and turning the insects into flour. Fortunately, each of these labor-intensive steps can easily be automated, introducing accuracy and tracking capabilities to the process along with a lower production cost.

1.4 FOCUS ON FORMER FOOD LIKE ALTERNATIVE FEED INGREDIENT

Globally, 120 -130 billion tons of natural resources are consumed every year and produce around 3.4 to 4 billion tons of municipal waste (Song et al., 2015). A bad management of these wastes causes both environmental and economic problems. In the food sector, the Food and Agriculture Organization (FAO) reported that globally, 34% of global food protein comes from livestock animals. Worldwide, will have to face the challenge of increasing the number of farms and also, at the same time, increasing the production of cereals to support the diets, both for humans and for animals (FAO 2014a). We have to consider that six billion tons of feed materials (in DM) is consumed annually by livestock animals, including one third of global cereal production. It has been estimated that between 30% and 50% of global food products are lost or wasted before and after reaching the consumer, in fact resulted that the world wastes about 1.4 billion tons of food every year (FAO 2018a). In 2006, the total loss of food in EU 27 was about 90 million tons and it is estimated that in 2020 food waste will reach 126 million tons. While, United States discards more food than any other country in the world: nearly 40 million tons. That’s estimated to be 30-40 % of the entire US food supply, and equates to 99 kg of waste per person (FUSIONS, 2016). Reducing food loos and waste is the key to achieving sustainability and brings savings for consumers and producers and from a social point of view, the redistribution of surplus food that otherwise would be wasted, is very important. reuse food loss and food waste to feed animals is a viable option that has the potential to simultaneously address waste management (landfilling), food security, and resource and environmental challenges (Luciano et al., 2020; Pinotti et al., 2021). The increasing availability of by-products from various food industries has long raised interest in animal nutrition. Besides common by-products, former food products generate large amounts of wasted, consisting mainly of unsold products (i.e., bread, croissants, biscuits, cakes, dough). Former foodstuffs have many names in different parts of the world:

dried bakery product, bakery meal, bakery waste, former food, cookie meal, bread meal (Mavromichalis, 2013). Despite the many names, and variable composition, it always describes the same source of materials, namely by-products or waste of the bakery industry, consisting primarily of wheat flour and variable quantities of sugar, salt, oils and additives (Stein et al., 2007; Mavromichalis, 2013). The foods, which are removed from the regular food chain for economic and quality reasons, can be indicated as Former Food Products (FFPs) or ex-food (Giromini et al., 2017). According to the EU Catalogue of Feed Materials (Regulation [EC] No 2017/1017) former foodstuffs are "*foodstuffs, other than catering reflux, which were manufactured for human consumption in full compliance with the EU food law but which are no longer intended for human consumption for practical or logistical reasons or due to problems of manufacturing or packaging defects or other defects and which do not present any health risks when used as feed".* From a regulatory point of view, former foodstuffs are considered a valid feed ingredient, and its use in animal nutrition would help recycling and valorizing the wasted food. In general, former foods are rich in starch because wheat flour is the main ingredient in all bakery products. Because this starch is already thermally processed (cooked), it is highly digestible, and thus, of high nutritive value. For this reason, former foods are a suitable ingredient for young pigs and starter broilers diets. The typical composition of former foods compared with two common cereals is reported in Table 1.

Table 1. Nutritional values of processing Former Food Products, Barley and Wheat (Adapted from Bouxin, 2016 and Pinotti et al., 2021)

	Former Food	**Barley**	**Wheat**
Dry matter (%)	88	88	88
Crude protein (%)	10.9	11	12.4
Crude fat (%)	9.8	2.8	2.1
Crude fiber (%)	2.2	5.5	2.7
Starch (%)	50.9	51.6	59.2
Sugar (%)	14.0	2.2	2.4
Metabolizable Energy (ME) for pig (MJ/kg)	16.48	12.95	14.43

From a nutritional point of view, former foods contain about 2.981 kcal/kg net energy (NE) (NRC, 2012), which compares very favorably with maize at 2.672 Kcal/kg NE. Accordingly, it contains 3.500 kcal/kg metabolizable energy (ME) for poultry, when maize contains 3.300 kcal/kg. Giromini et al. (2017) analyzed different types of former foods and reported that the energy values obtained ranged from 16.2 to 18.1 MJ kg^{-1} for digestible energy (DE) and from 15.9 to 17.9 MJ kg^{-1} for ME and these values were comparable with wheat control. However, if candy bars, snacks, cakes and other high-fat ingredients comprise a large part of the product mix, then former food will also be of high-fat concentration (normal levels are about 8 percent as for the above quoted energy level). Any extra fat, will increase dietary energy and must be taken into account when formulating diets. Salt is almost invariably a part of any baked product. Some contain more than others, and therefore the salt (sodium) content of bakery meal must be monitored very closely. To this end, the inclusion level of bakery meal on any formula should not exceed what is needed to meet the sodium requirements of the animal. Removing other high-salt ingredients (such as fish meal or animal plasma) and, of course, pure salt, from formulas increases the upper limit of inclusion rate for bakery meal (Mavromichalis, 2013).

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CHAPTER 2

Recycling food leftovers in feed as opportunity to increase the sustainability of livestock production

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Abstract: With the diminishing availability of farmland, climate change and the threat of declining water re- sources, livestock needs to meet the growing demand for food and feed by using fewer resources. The re- use of food losses as sustainable ingredients for feed formulations could represent a promising alter- native to cereal grains for both monogastrics and ruminants, increasing livestock sustainability and reducing the competition between animal and human nutrition. The acceptance of food leftover for feeding animals it is still far to be completely welcomed in several countries, where the outdated stereotypical image of the garbage used as feed is still existing. To implement this practice, a renewed image of food leftover as feed is needed, mainly disseminating the most recent findings about their properties, the new technologies applied for their production and their impact on the environment. This paper aims to disseminate a wide understanding of food losses and explores the potential benefits of using two main categories of food leftovers, namely former food products (FFPs) and bakery by-products (BBPs), as alternative feed ingredients in pig and ruminant nutrition. Several characteristics of those two categories of food losses are examined and compared to a standard diet, such as nutritional-related properties, safety, efficiency and environmental implications. The literature shows that both categories of food leftovers hold a significant nutritional value and are a sustainable alternative to traditional feed ingredients. They resulted as a low-risk category for animal health. In addition, when used in complete feed to replace traditional feed ingredients, neither FFPs nor BBPs do not decrease animal's growth performances. These findings valorize food losses into animal feed as a well-suited strategy to contribute to a reduced environmental and climate footprint of animal products and food waste prevention. However, a greater participation by feed/food processors and stakeholders is crucial to allow the sector to increase its contribution in the entire EU food and feed chain.

INTRODUCTION

Animal feed is the largest single cost item of livestock production, accounting for 60%e85% (FEFAC, 2018) (depending on the farm species) of the total cost inputs/year (Lawrence et al., 2008). Innovative feeding and nutrition practices have become increasingly important as livestock systems strive to become more efficient and sustainable (Luciano et al., 2020). The feed industry needs then to enhance the efficiency of livestock production by reducing GHG emissions and other factors that have a negative environ- mental impact (Audsley and Wilkinson, 2014). Livestock production needs to pay more attention to limit the use of natural resources per amount/unit of animal product, expressed as the footprint per product, such as the “water footprint”, “mineral footprint”, “land (arable or total land) footprint” (Flachowsky and Meyer, 2015). Compared to other food items, the production of animal food has a high environmental impact given that the conversion of plan biomass by animals lead to a loss of energy and proteins (Van Hal et al., 2019). The 32% and up to 68% of the yielded grains in the world and in developing countries, respectively, are being fed to livestock (Elferink et al., 2008). Feeding grains to livestock may be unsustainable due to world population growth and this leads to the research of alternative and more sustainable feed ingredients (van Zanten et al., 2015). The selection of the most appropriate raw materials and the feed formulation are two factors that can influence efficiency indicators (Pinotti et al., 2019a). There is a worldwide trend for waste reduction, including food waste reduction. This has led to an increase in the recycling and reuse of these products in the animal feed chain (Organization, 2019). Strategies and solutions, such as a “food recovery hierarchy”, are thus needed to reduce the impact of feed production on the environment by reducing the use of natural re- sources and increasing their reuse (Mourad, 2016). Food leftovers as a cereal substitution is an example, since they do typically not compete for land consumption with food production (Van Hal et al., 2019). Several products that humans cannot eat could be suitable as livestock feed, e.g., co-products, food-waste and biomasses such as plant by-products (Pinotti et al., 2020). From a circular economy perspective, feeding ex-food to livestock or using biomass to feed livestock, referred to as ‘leftover streams’, could be an effective option for using resources and reducing food losses (Fausto-Castro et al., 2020) as outlined in Figure 1.

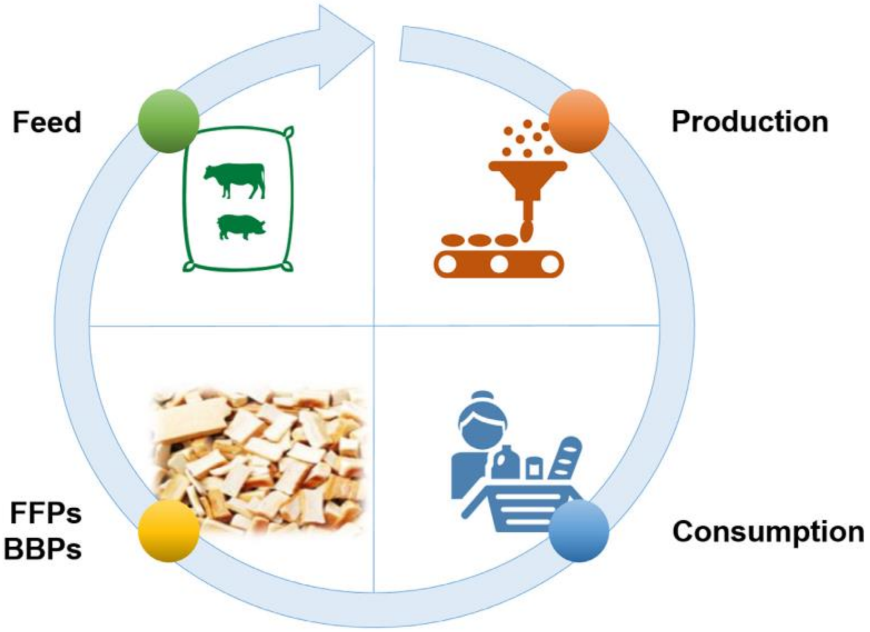

Figure 1. The role of food losses, upgraded to feed, i.e., former foodstuffs products (FFPs) and bakery by-products (BBPs) in the circular economy.

Specifically, ex-food (also known as 'former foodstuff products', FFPs), represents a sustainable and alternative energy supply for feeding animals (Pinotti et al., 2019b). There are several terms that are used to refer the different food effluents, such as food losses, food waste, and former foods products. Food waste refers to materials that remain after, or are produced during the processing, manufacture, preparation or sale of human food. This can include different types of food biomasses and edible material intended for human consumption, arising at any point in the food supply chain, such as that collected at restaurants, retail, or from household food scraps (Gustafsson et al., 2013). Food losses refer to a decrease in food quantity or quality in the early stages of the food supply chain, thus reducing the amount of food suitable for human consumption. The concept food losses are thus often related to post-harvest activities that lack systems or infrastructural capacities. Food waste, on the other hand, often refers to later stages of the food supply chain, such as retail and consumer households. Hence, the causes of food waste are often related to human behavior and take place in the later stages of the food supply chain (Gustafsson et al., 2013). Former foods products and food leftovers are food effluents that are somewhere in the middle. Specifically, food leftovers are foodstuffs that were manufactured for human consumption in full compliance with food laws, but which are no longer intended for human consumption for practical or logistical reasons or due to problems of manufacturing, packaging defects or other defects e none of which present any health risks when used as feed (Gustafsson et al., 2013; Organization, 2019). An important distinction between former foods products/food leftovers and food waste is their legal status. Former foods products can be used to feed humans or animals which does not represent a form of waste treatment; while food waste can be further processed to return nutrients to the soil, extract energy and generate heat, but cannot return to the food chain. Clearly, the animal feed chain should not be a means to dispose of degraded or contaminated foodstuffs, and that the product should have a sufficient nutritional value so that it can be considered as feed (Organization, 2019).

The evolution of livestock systems will inevitably involve a trade-off between feed security, feed safety, animal welfare, environmental sustainability and economic development (Thornton, 2010). Sustainability is not the only common denominator among many of these issues, which are often politically-sensitive (Vågsholm et al., 2020). Innovation is considered another key factor in the field of sustainable feed/food security (Pinotti and Dell'Orto, 2011). The conversion of industrial food losses into ingredients that can be employed in feed industry is regarded as a virtuous practice that should be carried out worldwide, with the aim to keep food losses -and finally nutrients-in the food chain (Georganas et al., 2020). The potential mitigation of environmental impacts due to the use of FFPs as animal feed should also be considered. Specific life cycle assessment (LCA) studies on the reuse of FFPs in animal nutrition are still limited. One study by Vandermeersch et al. (2014) clearly indicate that food losses have great potential to be con- verted into animal feed ingredients. In the same direction, Salemdeeb et al. (2017) investigated the use of food waste as animal feed. This study concluded that the use of municipal food waste for animal nutrition purposes would lead to better environmental and health impact than processing waste by composting or by anaerobic digestion (Salemdeeb et al., 2017). The use of food waste for animal nutrition is currently not allowed in the EU while the use of FFPs, which are not food waste, is already regulated by several authorities around the world and does not represent a regulation issue. Figure 2 summarizes the legislation for the use of food in feed.

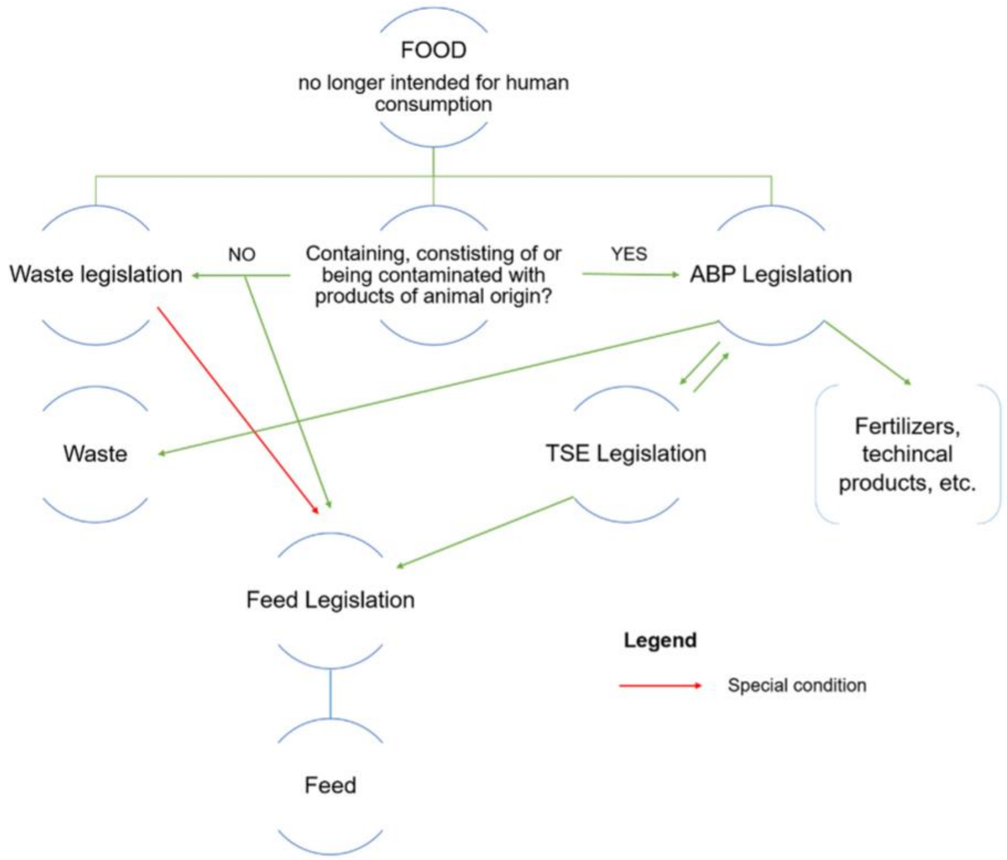

Figure 2. Flow chart from FOOD to FEED. Adapted from: European Commission Notice, 2018.

The use of FFPs as feed is still limited and, in several countries, their processing is still in a start-up phase (Luciano et al., 2020). To allow the sector to increase its contributions in livestock sustainability, it is crucial to achieve a comprehensive science-based analysis to demonstrate the feasibility, safety and sustainability implications. The gap of knowledge about nutritional properties, safe use, legal definition and good manufacturing practices represents the main factor that limits the former foodstuff processing industry to expand in Europe. The aim of this review is to fill the lack of knowledge about FFPs to promote their use in feed. The study first examines the nutritive attributes of FFPs, processing- related properties and safety-related issue. Finally, it explores resource and environmental implications.

METHOD

The method used in this review consisted of three steps: *(i)* choosing key words for the literature search, *(ii)* using different databases to identify the suitable literature *(iii)* analyzing the selected literature by extracting information. These three steps are summarized in Figure 3 and are described below.

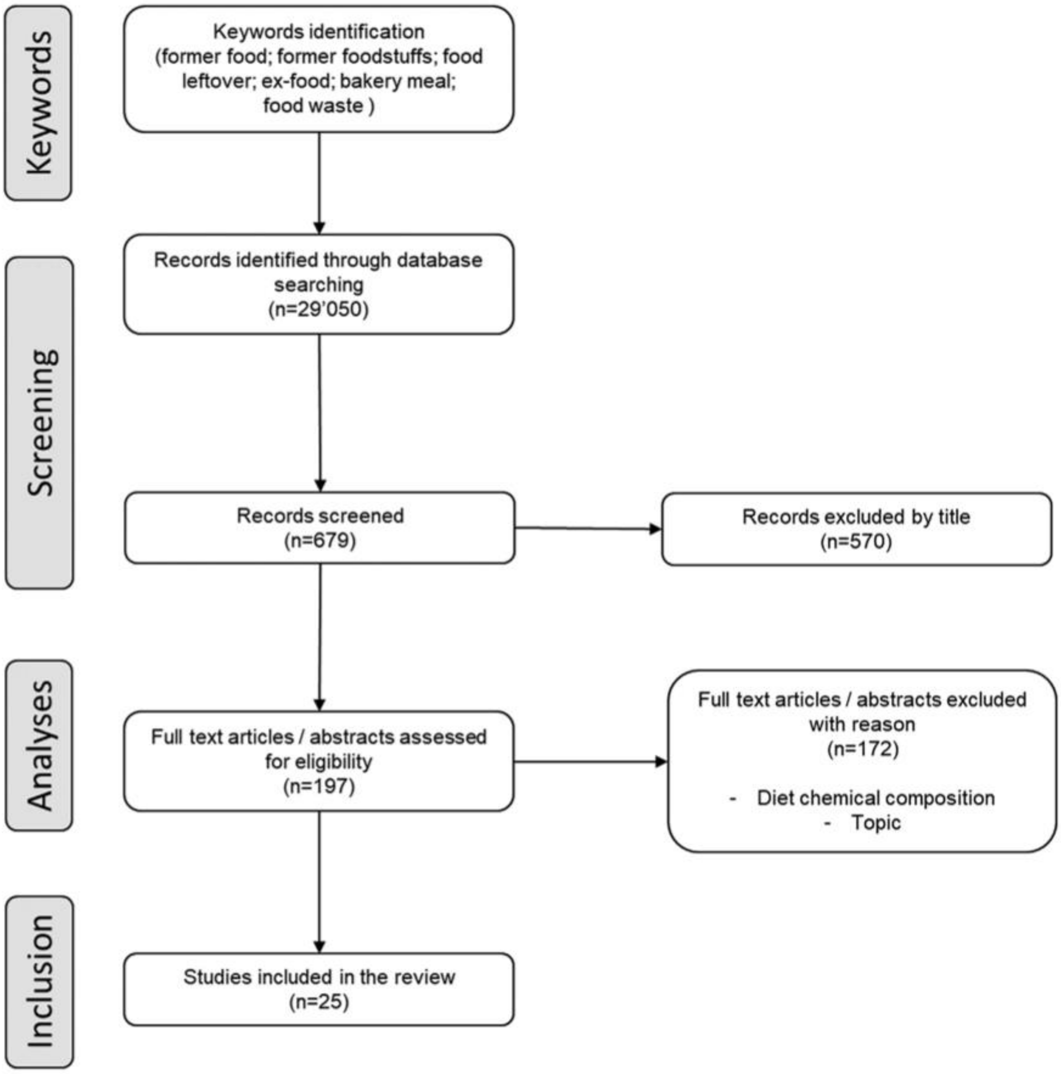

Figure 3. The selection process for the 25 studies included in this review.

CHOOSING KEY WORDS

The Official Journal of the European Union (OJ) groups and lists all the ingredients used for feeding production and in particular defines former foodstuffs as "*Foodstuffs, other than catering reflux, which were manufactured for human consumption in full compliance with the EU food law but which are no longer intended for human consumption for practical or logistical reasons or due to problems of manufacturing or packaging defects or other defects and which do not present any health risks when used as feed.*" (European Commission, 2013). This definition was updated and strengthened in 2018 by the European Commission (Figure 2), which formulated new guidelines for the employment of former foodstuff into animal nutrition.

Very often these products are identified with names other than former foodstuffs, which is the proposed name in the European Regulation and there is no single recognized definition in the scientific literature. The major difficulty in this field is how these products are defined, since different definitions can be found in the literature.

Six different key words are the most common terms in the literature and in the data bases: *(i)* former food, *(ii)* former food- stuffs, *(iii)* food leftovers, *(iv)* ex-food, *(v)* bakery meal, and *(vi)* food waste. Former food products (FFPs) represent a wide category recently introduced by the European law (European Commission, 2013), and therefore not commonly used in the literature. The term “bakery meal”, was mainly used in manuscript titles, while in the articles, they are often referred to as bakery by-products (BBPs) and bakery waste.

IDENTIFYING THE LITERATURE

We used the abovementioned key words for the literature searches in three different databases: *(i)* Scopus, *(ii)* Web Of Science, and *(iii)* Google Scholar. After the first search the found articles were checked manually principally by reading the abstract and verifying the presence of the chemical composition of the diet/ingredients used or tested in the study.

We found many articles not in line with our topic which were thus excluded. For example, in the Scopus database, we found a total of 8261 articles when searching for the word “former food”, but only three articles were selected for this review and reported in Table 1.

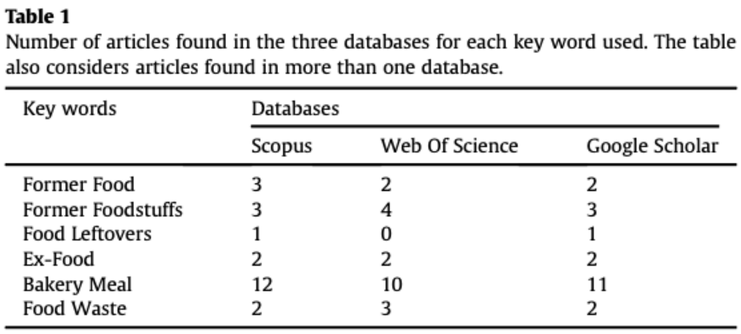

Table 1
Number of articles found in the three databases for each key word used. The table also considers articles found in more than one database.

Key words	Databases		
	Scopus	Web Of Science	Google Scholar
Former Food	3	2	2
Former Foodstuffs	3	4	3
Food Leftovers	1	0	1
Ex-Food	2	2	2
Bakery Meal	12	10	11
Food Waste	2	3	2

The research conducted in the different databases provided 25 articles on which this review was prepared.

ANALYZING LITERATURE

The 25 selected articles were chosen for this review because they reported the chemical composition of the ex-food used in animal trials. Based on the literature and the key words selected, two main categories of food leftovers were identified: former food products (FFPs) and bakery by-products (BBPs). A list of studies using the terms FFPs or BBPs are reported in Table 2.

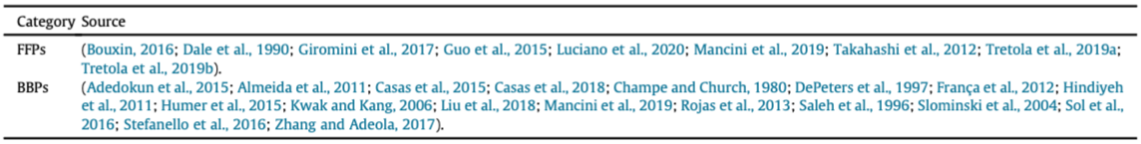

Table 2
References considered in the study. FFP = former foodstuffs products; BBP = bakery by-products.

Category	Source
FFPs	(Bouxin, 2016; Dale et al., 1990; Giromini et al., 2017; Guo et al., 2015; Luciano et al., 2020; Mancini et al., 2019; Takahashi et al., 2012; Tretola et al., 2019a; Tretola et al., 2019b).
BBPs	(Adedokun et al., 2015; Almeida et al., 2011; Casas et al., 2015; Casas et al., 2018; Champe and Church, 1980; DePeters et al., 1997; França et al., 2012; Hindiyeh et al., 2011; Humer et al., 2015; Kwak and Kang, 2006; Liu et al., 2018; Mancini et al., 2019; Rojas et al., 2013; Saleh et al., 1996; Slominski et al., 2004; Sol et al., 2016; Stefanello et al., 2016; Zhang and Adeola, 2017).

DATA ANALYSIS

In order to compare the nutrient composition of the FFPs and BBPs and the overlap and distributions between them, box plots were examined. Box plot analysis was carried out in order to calculate mean, quartiles, minimum and maximum observations and outliers of the FFPs and BBPs.

MAIN CATEGORIES AND NUTRITIONAL PROPERTIES OF FFPS AND BBPS

MAJOR CLASSES OF NUTRIENTS

In the considered literature, two main categories of ex-food have been identified: FFPs and BBPs. The starting material used for their preparation defines these two types. The leftovers originated from the food industry, where bakery products such as bread and sometimes pasta are the major source of nutrients as in the case of BBPs (Njezic et al., 2010).

Confectionary products leftovers, mainly composed by sugar- rich products like biscuits, waffle and chocolate, compose the category of FFPs. Snacks and other salty materials (chips and crackers) are usually in the first category. It can thus be speculated that there are two main types of food leftover on the market, namely salty materials (i.e., BBPs) and sweet materials (FFPs), however they are sometimes mixed together.

Both FFPs and BBPs can be used as alternative feed ingredients in farm animal diets. Former foodstuff processors start from different food leftovers and after unpacking, sorting, drying, grounding and sieving are able to obtain suitable feed ingredients. The resulting material can be used to replace some of the existing raw materials in various feed formulas. Some FFPs such as candies and dairy powders can be water dissolved and processed to obtain syrups, which can replace molasses, often used as a technological (binding) agent during the pelleting of feed (Van Raamsdonk et al., 2011). Also, sweet materials may be directly used. An example is Guo et al. (2015) who proposed that chocolate candy feed, containing of over 50% of simple sugars, could partially replace lactose in nursery pigs (Guo et al., 2015). Figure 4 shows examples of packed and unpacked food leftover before being processed.

Figure 4. Examples of packaged and unpackaged former foodstuff products ready to be processed in FFP ingredients for feed production.

An analysis of the main composition of both FFPs and BBPs reported in Table 3 and Figure 5, highlights that they have some interesting differences. Based on the latest findings (Luciano et al., 2020) and analyzing the nutritional facts reported for native products intended for human consumption, FFPs are extremely rich in carbohydrates and, depending on their origin, also in fat (Luciano et al., 2020). Among carbohydrates, simple sugars (e.g., sucrose, lactose, glucose, fructose) represent a significant quota, especially when confectionary products are considered (Guo et al., 2015). In the case of BBPs, the average nutrient concentration again indicates a high carbohydrate content [on a dry matter (DM) basis], even though in these materials' fiber fractions are also detectable.

In the studies considered in the present review, crude fiber (CF), Neutral Detergent Fiber (NDF) Acid Detergent Fiber (ADF) were always higher in BBPs than in FFPs. Bakery by-products showed on average a +40% CF content (on a DM basis), +70% NDF and +140% ADF content as shown in Table 3 and Fig. 5.

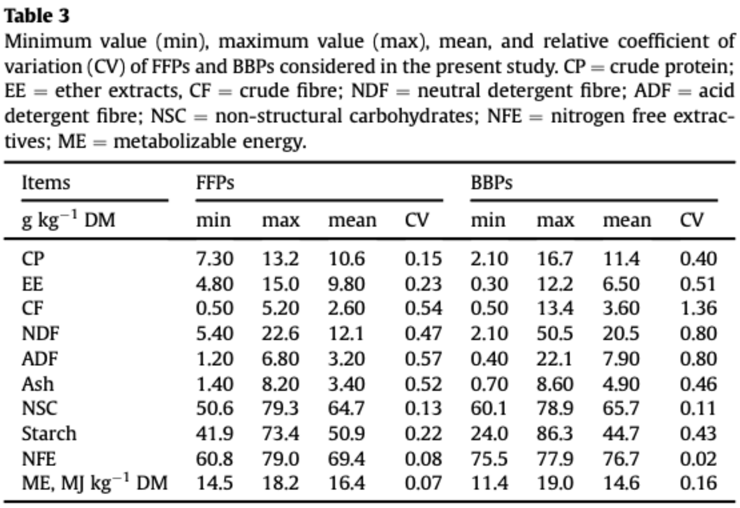

Table 3

Minimum value (min), maximum value (max), mean, and relative coefficient of variation (CV) of FFPs and BBPs considered in the present study. CP = crude protein; EE = ether extracts, CF = crude fibre; NDF = neutral detergent fibre; ADF = acid detergent fibre; NSC = non-structural carbohydrates; NFE = nitrogen free extractives; ME = metabolizable energy.

Items	FFPs				BBPs			
g kg^{-1} DM	min	max	mean	CV	min	max	mean	CV
CP	7.30	13.2	10.6	0.15	2.10	16.7	11.4	0.40
EE	4.80	15.0	9.80	0.23	0.30	12.2	6.50	0.51
CF	0.50	5.20	2.60	0.54	0.50	13.4	3.60	1.36
NDF	5.40	22.6	12.1	0.47	2.10	50.5	20.5	0.80
ADF	1.20	6.80	3.20	0.57	0.40	22.1	7.90	0.80
Ash	1.40	8.20	3.40	0.52	0.70	8.60	4.90	0.46
NSC	50.6	79.3	64.7	0.13	60.1	78.9	65.7	0.11
Starch	41.9	73.4	50.9	0.22	24.0	86.3	44.7	0.43
NFE	60.8	79.0	69.4	0.08	75.5	77.9	76.7	0.02
ME, MJ kg^{-1} DM	14.5	18.2	16.4	0.07	11.4	19.0	14.6	0.16

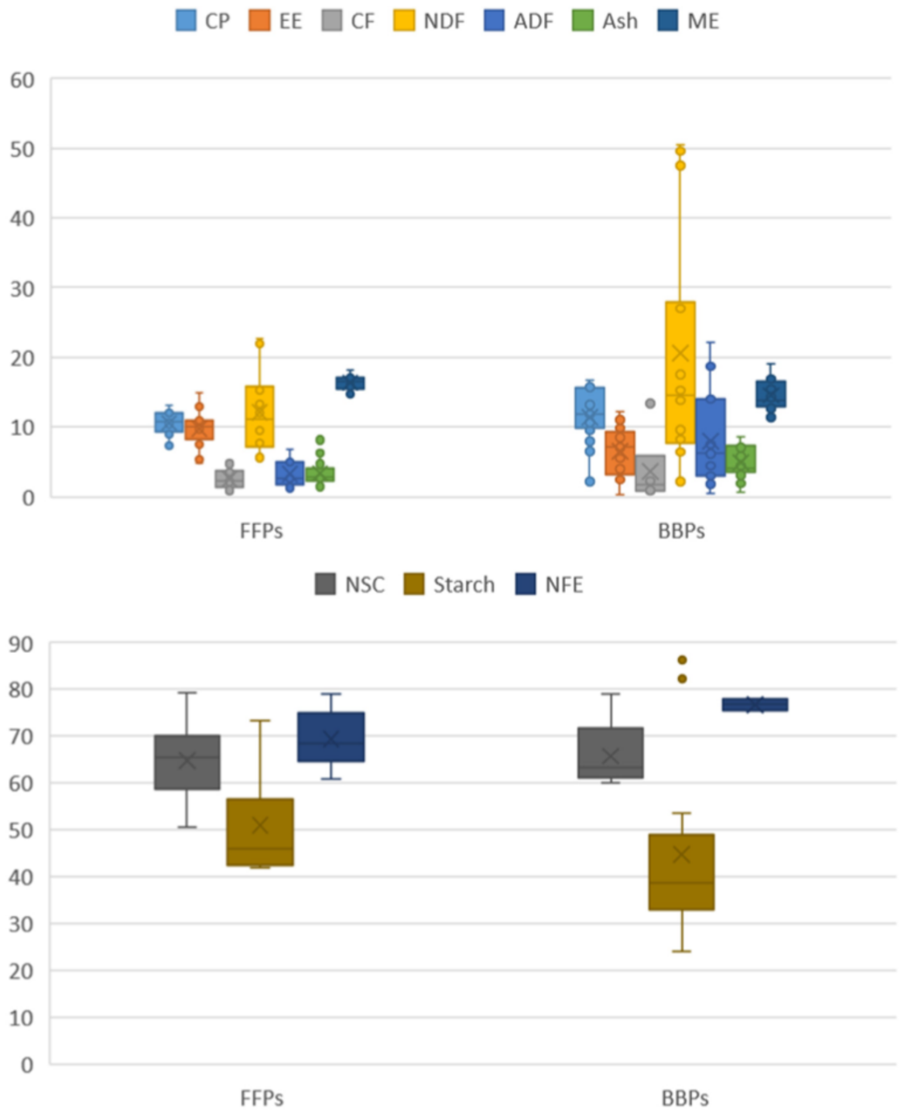

Figure 5. The various Former Foodstuffs Products (FFPs) and Bakery By-Products (BBPs) considered in the study. Each box plot reports the mean (x), median (-), minimum and maximum observations and outliers in the two classes of samples (FFPs and BBPs). Data ere expressed in g/100 g on DM for main nutrients, and in MJ/kg on DM for metabolizable energy. Abbreviations: CP 1⁄4 crude protein; EE 1⁄4 ether extract; CF 1⁄4 crude fibre; NDF 1⁄4 neutral detergent fibre; ADF 1⁄4 acid detergent fibre; ME 1⁄4 metabolizable energy; ash; are reported in the upper part. NSC 1⁄4 non-structural carbohydrate; starch; NFE 1⁄4 nitrogen free extractive; are reported in the lower part.

1.

These figures indicate that BBPs consist of a mixture of food ingredients originating from flour or whole cereal grains and with some high-fiber ingredients, such as bran or other co-products (Liu et al., 2018).

The fat content is another of the main differences between FFPs and BBPs. In Table 3 can be observed that FFPs considered in the present review showed 45% more fat than BBPs. These aspects have been extensively addressed in different studies and reviews (Giromini et al., 2017;

Luciano et al., 2020; Pinotti et al., 2019a, 2019b; 2019c) which report that FFPs have a similar nutritional composition to common cereal grains, but are generally characterized by a higher fat content that also usually affects their energy density (expressed as metabolizable energy, ME). This higher ME content in FFPs (þ12%) in comparison with BBPs, was also observed in the present study. By contrast, the protein content in both FFPs and BBPs was comparable.

In terms of the starch and Non-Structural Carbohydrate (NSC) content, the situation is more complex. While the starch content in FFPs was slightly higher (þ13%) than in BBPs, the NSC content was similar in both categories. The values recorded in Table 3 indicate that the partial contribution of different carbohydrates in defining NSC, in FFPs and BBPS, was variable. In fact, the difference between NSCdStarch in FFPs and BBPs was about 14 g/100 g and 20 g/100 g, respectively. The main reason for this is what the NSC fraction represents. NSCs are calculated by difference [100-(%NDF þ %CP þ % Fat þ Ash)], which means that NSC fraction is heterogeneous. Indeed, it is composed of different amounts of simple sugars, beta- glucans, galactans, and pectins. Combining the contents of these fractions (NSCs and starch) with the fiber fractions (CF, NDF, and ADF), in FFPs the simple sugar content would seems to be higher (Guo et al., 2015), while in BBPs, the main contributors are beta- glucans, galactans, and pectins derived from the whole grains often used in modern bakery products (Liu et al., 2018).

When compared to the feedstock, both FFPs and BBPs are characterized by a more variable nutrient profile, according to the specific materials/samples tested. An example is the starch content, which was observed to fluctuate within a range of 25%e73%, with rare outliers, as well as the digestible energy, which ranged from 11.0 to 19.0 MJ kg^{1}. The highest variability was observed for BBP, NDF and ADF contents, which ranged from 2% to 50% (on DM basis), and from 0.2% to 20%, respectively. Those data are summarized in Figure 5.

These findings are in accordance with the literature. In a study by Giromini et al. (2017), the average values of specific FFPs in terms of EE, NDF and CF contents were 10%, 5.4% and 4.5%, respectively (Giromini et al., 2017). In another study, the EE content of different bakery leftovers has been found to be around 7.5%e9.4% (DePeters et al., 1997); the NDF can also vary widely, with values of 17.9% in a bakery product analyzed by DePeters et al. (1997). A similar observation was found for the CF, which was 1.3% in bakery by- products assessed by Kwak and Kang (2006).

A further aspect is food leftovers digestibility. Both FFPs and BBPs have shown high (>80%) in vitro organic matter digestibility values (Giromini et al., 2017), but these values were obtained by testing FFPs and BBPs as single ingredients. A further step in the nutritional evaluation is to assess their digestibility (in vitro) also when these materials are used/included in pig feed. Both organic matter digestibility (Tretola et al., 2019b) and carbohydrate digestion kinetics (Ottoboni et al., 2019) were higher in diets containing FFPs (30% of inclusion) in comparison with conventional diets.

To sum up, the nutrient composition in FFPs and BBPs can be variable, which is also typical of standard/common feed ingredients. The variability in ingredients in crops is due to genetic or pedoclimatic conditions, agronomic factors, harvest and storage conditions (Gagiu et al., 2018). Both FFPs and BBPs are affected by an extra source of variability, i.e., the processing (Zijlstra, 2006). Although this great variability in FFP/BBP products can be a challenge for the feed formulation, it still offers interesting flexibility in formulating ratios according to the nutrient/energy requirements of the target animals (NRC, 1998).

The experience acquired by FFPs and BBPs processors after many years spent on the analyses of inbound products led to the possibility to predict the range in variation among different sources of products and also among the same source and different loads (Tretola et al., 2019a). It has been observed that variations between different geographical regions are relatively small in terms of the chemical composition of bakery meals (Liu et al., 2018). These findings allowed the processors to produce raw materials with very low coefficients of variation, where these average values can be used to predict concentrations of nutrients in bakery meals (Liu et al., 2018). The final feed products are produced starting from raw materials, whose nutritional data are very reliable for producers and which are assessed by analyses of final products and standards. Assuming the ability of FFPs/BBPs processors to over- come the issue of variability in FFPs/BBPs at the industrial level (during FFP preparation), a further step in the nutritional evaluation of FFPs is a better understanding of their functional/dietetic properties.

PROCESSING-RELATED PROPERTIES OF FFPS AND BBPS

Digestibility is strongly affected by feed dietary factors such as nutrient composition and feed processing (Temesgen et al., 2017). Processing is a fundamental step for FFPs and BBPs prior to their utilization in animal nutrition, because it facilitates the incorporation in animal diets (Georganas et al., 2020). Many ingredients of FFPs and BBPs, such as cereal flours, eggs, sugar and fats are usually mixed with water to form a dough or batter (Bushuk, 1986), and then subdivided into portions for the second stage of processing, i.e. cooking (Bushuk and Scanlon, 1993). Both industrial and domestic cooking can modify the chemical and physical characteristics of food (Klopfenstein, 1980), thus affecting the macro- and micro- nutrient bio-accessibility and bioavailability.

Due to their increased water absorption capacity, extruded wheat flours are an opportunity to increase bread output in bakery production. Potential issues could be the starch gelatinization, increased damage to the starch content, together with a reduction in lipid oxidation due to enzyme inactivation, an increase in soluble fiber and a reduction in thermolabile vitamins, anti-nutritional factors and microbial load (Klopfenstein, 1980).

Thermal processing can also modify ingredient’s digestibility. High-temperature treatments, in fact, can improve digestibility values by the protein denaturation of anti-nutritional factors such as the anti-tryptic activity of raw soybeans (Giuberti et al., 2014). In some cases, protein digestibility can be reduced by thermal protein aggregation (Ercolini and Fogliano, 2018). Other processing techniques such as solvent extraction or cold press, can lead to an increased variability in the values of energy content (Spragg and Mailer, 2007).

Unlike the untreated feed ingredients commonly used in live- stock production (Giuberti et al., 2014), FFPs and BBPs typically undergo to both mechanical and thermal processing (Singh et al., 2010) that affect the nutritional properties of the diet, in particular the starch fraction. Table 4 summarize the effects of various processing techniques on starch digestibility.

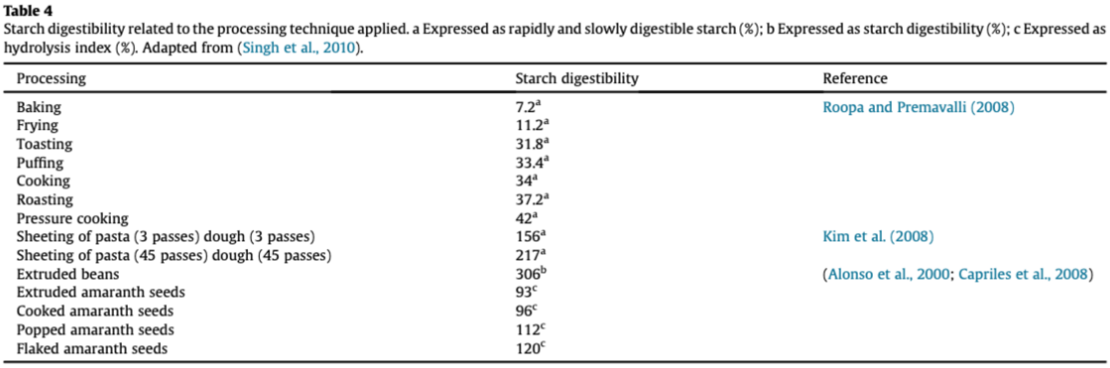

Table 4
Starch digestibility related to the processing technique applied. a Expressed as rapidly and slowly digestible starch (%); b Expressed as starch digestibility (%); c Expressed as hydrolysis index (%). Adapted from (Singh et al., 2010).

Processing	Starch digestibility	Reference
Baking	7.2[a]	Roopa and Premavalli (2008)
Frying	11.2[a]	
Toasting	31.8[a]	
Puffing	33.4[a]	
Cooking	34[a]	
Roasting	37.2[a]	
Pressure cooking	42[a]	
Sheeting of pasta (3 passes) dough (3 passes)	156[a]	Kim et al. (2008)
Sheeting of pasta (45 passes) dough (45 passes)	217[a]	
Extruded beans	306[b]	(Alonso et al., 2000; Capriles et al., 2008)
Extruded amaranth seeds	93[c]	
Cooked amaranth seeds	96[c]	
Popped amaranth seeds	112[c]	
Flaked amaranth seeds	120[c]	

The processing of FFPs and BBPs can strongly affect their nutritional characteristics and, subsequently, the resulting feed. An example is the glycemic index of processed starchy food, which can be used to classify starchy ingredients (Giuberti et al., 2012). Ottoboni et al. (2019) recently evaluated both the predicted glycemic index (pGI) and hydrolysis index (HI) of FFPs. This study revealed that in FFPs, both indexes were higher than for unprocessed corn. In terms of chemical composition, the HI and pGI of FFPs also seemed to be related to the nature and the processing of the various FFPs, with a high variability among different samples (Ottoboni et al., 2019) (Ottoboni et al., 2019). The high availability of simple sugars in FFPs represents one of the most interesting characteristics of those alternative feed ingredients, especially when used to formulate diets for young animals feeding. Several studies in humans (Holt et al., 1992; Lavin and Read, 1995; Ludwig et al., 1999) have suggested that the ingestion of high-GI meals increases hunger and promotes overeating in subsequent meals compared to low-GI meals, which is a positive effect in terms of pig nutrition. Beside the starch content, margarine, butter and partially- hydrogenated vegetable oils characterize bakery products as the main fat source. Given that bakery and pastry products are often composed of a high percentage of saturated fatty acids (Albuquerque et al., 2017), the effect of these types of fats on animal performance and product quality need to be assessed, as was done for pigs (Raj et al., 2017).

SAFETY ISSUES

Using FFPs and BBPs in animal feeding also entails safety issues to ensure a safe inclusion in animal diets. Although FFPs and BBPs have several similarities, safety issues have been mainly addressed for FFPs, probably because BBPs are usually obtained by unpacked and more stable ingredients compared to FFPs. In the FFP safety evaluation, two main categories of risks need to be considered during and after processing. In this paragraph, two main aspects related to the safe use of food leftover as feed ingredients will be considered: the microbial load of the final products and their potential contamination by scrapes of different materials arising from their packaging.

MICROBIOLOGICAL LOAD OF FOOD LEFTOVER USED AS FEED INGREDIENTS

Complying with the EU threshold levels regarding the quantity of microorganisms found in food material is crucial before put it on the market. The same goes for the use of food leftovers in feed. Tretola et al. (2017) evaluated the microbiological load in various alternative feed ingredients. Microbiological analyses showed the very high hygienic wholesomeness and safety of all the samples examined (Tretola et al., 2017). An example is given by the mean total viable count (TVC) that was 4.92 ± 0.25 Log CFU/g, in line with the EU standards. In all the samples, the microbial load was always below the threshold limit set by the Health Protection Agency (2009). The low moisture content of those products, together with the thermal processing to which both FFPs and BBPs undergo during their conversion in feed ingredient, may have contributed in achieving these standards. Based on that, we speculate that FFPs and BBPs can be considered safe from the microbiological point of view when used in animal nutrition.

CONTAMINATION DUE TO PRESUMED REMNANTS IN THE PACKAGING

When FFPs or BBPs start the conversion process into feed ingredients, not the entire packaging is removed manually before the processing but is ground together with the food. Then, most of packaging remnants are mechanically removed as described in Van Raamsdonk et al. (2011) and summarized in Figure 6.

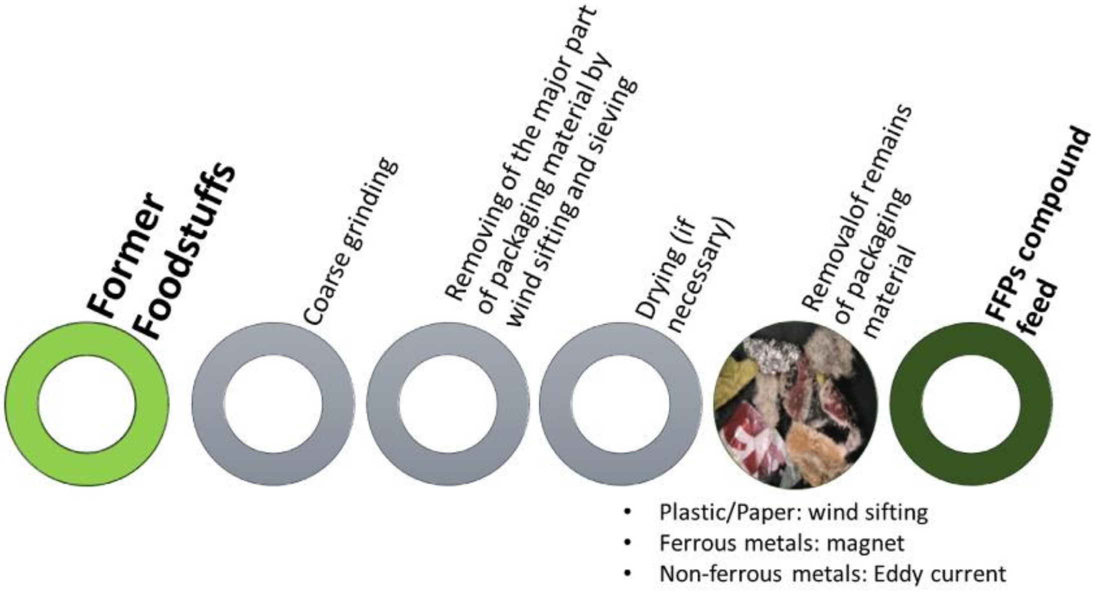

Figure 6. Steps by which packaging is removed from food during FFPs processing.

After mechanical packaging removal, packaging remnants of different sizes have been found (Tretola et al., 2017). The most common packaging materials of food products are plastics, paper, cardboard and aluminum foil (Amato et al., 2017). Packaging materials are often manufactured using adhesives with printing on the outside (Tretola et al., 2019b). Plastics are made by the polymerization of monomers and several additives may be added to obtain the physical or chemical properties of the plastics, such as fillers, polymeric additives, light stabilizers, optical brighteners, and antistatic (Van Raamsdonk et al., 2011). The contamination levels reported in different studies (see Pinotti et al. (2019a) for references), however, were always significantly below the tolerance level proposed by the feed/food authorities (European Commission, 2011), indicating that the issue of packaging remnants is limited.

FFPS AND BBPS IN PIG NUTRITION

The use of alternative feedstuffs, and especially food leftovers, is not new for the pig industry (Chen et al., 2009; FEFAC, 2005). Several studies (Almeida et al., 2011; Kwak and Kang, 2006; Rojas et al., 2013; Tretola et al., 2019a; Tretola et al., 2019b) have investigated the use of food leftover in pig diets, with special emphasis not only on pig production, but also sustainability. These studies have revealed that both FFPs and BBPs can affect pig yield in different ways. Of these, variations in diet digestibility are probably the most important: both BBPs and FFPs used as cereal substitutes have increased diet digestibility and thus improved pig efficiency (Tretola et al., 2019b). In terms of BBPs, other reported side effects are related to specific nutrients such as amino acids and minerals. Compared with corn, BBP meal has been found to have a reduced digestibility in terms of all indispensable amino acids (AA) (Stein et al., 2007), a poor source of digestible AA (Almeida et al., 2011), and inconclusive results in terms of phosphorous (Rojas et al., 2013). This mineral is essential for both humans and animals. However, for practical resound this aspect cannot be addressed in the present review. Although traditional ingredients, such as corn, can be substituted with BBP meal in pigs' diets, their use merits a specific dietetic evaluation in order to optimize both the grow performance and gut health. In terms of FFPs, the results are comparable to BBPs. Studies revealed that when FFPs are included in a diet for growing pigs this diet resulted more digestible compared to a standard diet, probably due to the partial replacement of unprocessed starch with FFPs consisting of thermal processed ingredients (Tretola et al., 2019b). Food processing and the related nutrient digestibility/availability, together with the presence of high amounts of simple sugars, may also affect animal gut health and microbiota (Knudsen et al., 2012).

Animal wellbeing and performances mainly depends by the gut health. It is thus important to investigate the effects of FFPs and BBPs, which have highly digestible starch and a high content of simple sugar on gut microbiota in piglets. Feeding post-weaning piglets with a highly digestible ex food-based diet seems to in- crease the instability and decrease both the abundance and the heterogeneity (biodiversity) of the gut bacterial population, compared to piglets fed with a standard diet (Tretola et al., 2019a).

As previously discussed, high digestibility is a characteristic of both FFPs and BBPs due to processing-related modification such as starch gelatinization and protein denaturation. The amount of un- digested nutrients reaching the large intestine of pigs fed FFPs/ BBPs-based diets is lower compared to animal fed unprocessed raw materials. This difference could lead to a different relationship between food processing/digestibility and gut microbiota, as summarized in Figure 7.

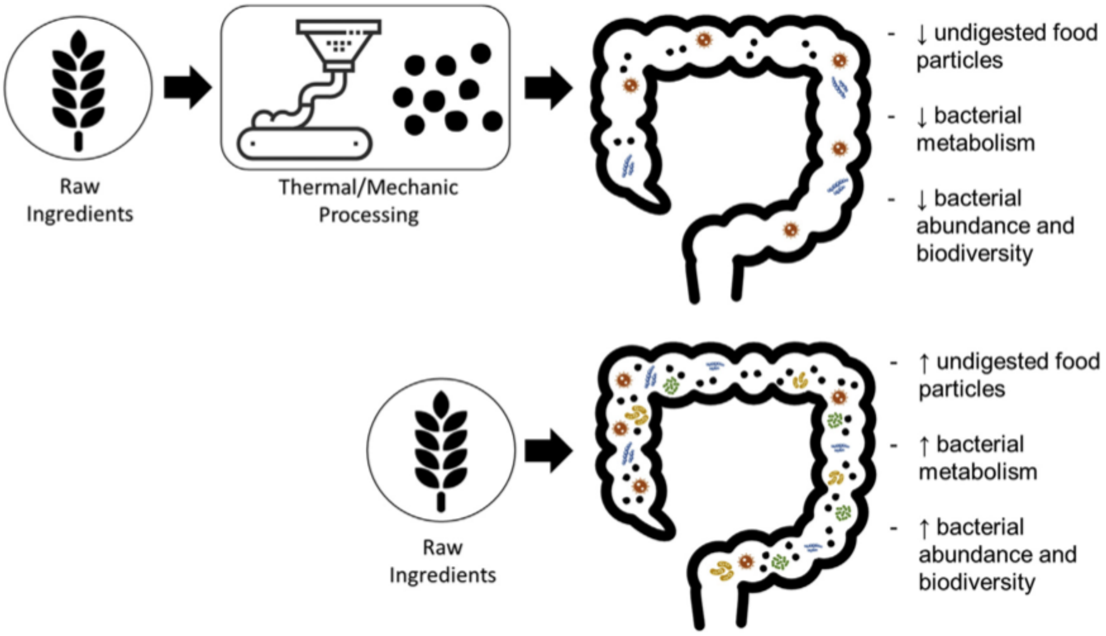

Figure 7. Potential relationship between food processing/digestibility and gut microbiota.

This means that compared to traditional diets, designing feed for pig nutrition by including FFP/BBP products with a high nutrient bio-accessibility could result in a high bioavailability of proteins, carbohydrates and lipids for the pigs. This would then lead to a higher calories' absorption for the host and a lower amount of nutrient delivery to the gut microbiota. The performance of growing or finishing pigs could therefore be affected.

A reduction in bacterial abundance and biodiversity could lead to several detrimental aspects such as decreased calories extraction from undigested feed material, but also to infections from opportunistic enteric pathogens and an immature immune system (San Yeoh and Vijay-Kumar, 2018). On the other hand, the reduction in gut bacteria abundance and diversity could also lead to a reduced competition for nutrients between bacteria and the host. Fewer bacteria mean a decreased activation of the immune system and a lower energy use to prevent the overgrowth of the bacterial population, which can cause a variety of detrimental conditions (San Yeoh and Vijay-Kumar, 2018). Based on the information mentioned in this review, it is crucial to take into account the potential effects of FFP and BBPs on intestinal microbiota when used in pig nutrition.

FFPS AND BBPS IN RUMINANT DIETS

There is a lack of information regarding the effects of FFPs/BBPs on ruminal fermentation and microbiota when included in ruminant diets. As already mentioned, FFPs/BBPs are usually rich in energy due to their high content of sugars, oils and starch. This energy sources profoundly affects the rumen fermentation. Sugars are water-soluble carbohydrates that are readily available in the rumen and are thus considered as highly-fermentable carbohydrates. Sugars in fact, ferment faster than starch or fiber in the rumen, although the rates of hydrolysis and fermentation vary greatly depending on the type of sugar and rumen environment. Despite rapid fermentation in the rumen and their potential to provide greater fermentable energy to enhance microbial protein production, feeding sugars as a starch substitute in ruminant diets may not necessarily lead to extensive acid production and low rumen pH (Oba, 2011). Factors such as the amount of high-sugar feedstuffs included, the synchrony of rumen fermentation (high- sugar diets with high soluble protein), basal diet composition and ingredients, seem to be essential in terms of the potential of this material. Especially in dairy cattle, feeding high-sugar diets often increases DM intake, butyrate concentration in the rumen, and milk fat yield (Oba, 2011).

Some of these aspects have been addressed in-vitro by (Humer et al., 2018) using BBPs in a protocol that mimics the rumen physiology. Diets that include high levels of BBPs (30-45%) have shown a better in-vitro rumen degradation of starch, while the degradation of crude protein and fiber decreased. At the same time the production of methane and the ammonia concentration decreased. The rumen fermentation was also altered towards the production of propionate at the expense of acetate and butyrate. Butyrate decreased linearly with the increasing inclusion of BBPs. As expected, these changes were associated with a different rumen microbiota. The inclusion of BBPs at up to 30% of the DM had no detrimental effects on pH, fiber degradability and ruminal micro- biota, and enhanced propionate production.

A higher inclusion level (45%) reduced rumen microbiota biodiversity, impaired ruminal fermentation and fiber degradation, thus making these inclusion levels unsuitable (Humer et al., 2018). One effect of including BBPs in the diet on the ruminal microbiota is that the higher starch digestibility in BBP diets increases the abundance of the Prevotella genus, a major propionate producer, leading to an increased proportion of propionate observed in the BBP diets (Humer et al., 2018). Another effect is the increased abundance of Megasphaera taxa due to the high content of rapidly- digestible carbohydrates in BBP diets. This taxa is a soluble sugar fermenter which is often correlated with a decreased lactic acidosis. This effect is probably correlated to the ability of Megasphaera to convert ruminal bacteria-produced lactic acid into acetic and propionic acids (Humer et al., 2018). Probably due to the high fat content and unsaturated fatty acids (e.g., oleic acid) of BBPs, compared to the conventional ingredients used in ruminant diets, the inclusion of BBPs also decreased the abundance of fibrinolytic bacteria *in-vitro* (Humer et al., 2018). Both the high fat content and high concentration of unsaturated fatty acids, in fact, have been shown to have a negative effect on the growth of such bacteria (Enjalbert et al., 2017). A recent in vivo study (Kaltenegger et al., 2020) reported that the inclusion of 15 or 30% of BBPs in mid- lactating dairy cows' feed increased energy density of the diet; these results were obtained increasing the fat and sugar content while reducing the starch and neutral detergent fiber concentration (i.e., shift in nutrient profile from glucogenic to lipogenic). The inclusion of BBPs in the diet enhanced not only DM intake (average 7%), but also milk yield (+5% in 15% BBPs and +12% in 30% BBPs compared to the control group). Under these conditions any case of clinical rumen acidosis has been observed: the time for the pH to fall below 5.8 (used as an index of rumen acidosis) was lower in cows fed BBPs compared to the control diet (-39% in 15% BBPs, -15% in 30% BBPs), suggesting that 15% BBPs diet had the lowest risk for developing rumen acidosis, followed by 30% BBPs diet and control diet. This therefore indicates that the rapid disappearance of sugar per se does not necessarily lead to an extensive fermentation acid production (Kaltenegger et al., 2020).

It has also been suggested that changes in the nutrient profile in the diet due to the inclusion of FFPs/BBPs can improve production without major detrimental effects on rumen health in dairy cows (Aljerf et al., 2018). The inclusion of FFPs/BBPs may thus represent alternative energy sources for lactating dairy cows in order to in- crease the dietary energy, with the limited risk of rumen acidosis However, the physiological mechanisms and effects of FFPs/BBPs on ruminant productivity merit further investigations.

To the best of our knowledge, only one in vivo study has tested the effects of including bakery by-products in sheep diets. In this study BBPs replaced the corn meal in different proportions (specifically 25, 50, 75 and 100%) (França et al., 2012). The authors observed no effects of BBP inclusion on the nutrient intake and digestibility, nor on the nitrogen balance, pH values or concentration of volatile fatty acids. However, due to the higher ruminal availability of energy, which allows a greater use of ammonia for microbial growth, the ammonia nitrogen concentration showed a negative correlation to the level of BBP inclusion. The authors concluded that BBPs can safely replace corn meal in concentrate rations in sheep diets (França et al., 2012).

ENVIRONMENTAL IMPLICATIONS

A sustainable livestock production is essential in the current world, in which global population is growing together with food demand. The sustainability is intended in terms of an increase in livestock productivity, a reduction in resources consumption and in GHG, not to mention an increase of animal health and food security. The livestock production sustainability is strictly correlated with sustainable agricultural development, because in order to face with an expected increase in consumption of animal-source food, several virtuous approaches can be adopted to increase crop yield, cropping intensity and a limited expansion of land use. An example is to improve resource efficiency through the adoption of agricultural practices and technologies by smallholders that currently are only the prerogative of the largest producers. These practices are the employment of feed substitutes such as by-products or food leftovers, the energy and water recycling and a more careful use of grazing land. These latter could provide a more sustainable live- stock sector, whose animal-source products contribute to the supply of high-quality proteins, thus ensuring food security (HLPE, 2016; Vågsholm et al., 2020). In this direction the development of a long-term sustainable agriculture is mandatory, and the use of food leftovers as feedstuffs should be considered, since it can reduce the competition between human and animal diets (Vågsholm et al., 2016). Although the use of food leftovers is regarded as an innovative practice in sustainable animal nutrition and circular economy, the aspect of food safety must be considered. The main risk linked to the re-entering of food leftovers in the feed-food chain is to recycle and accumulate biochemical hazards, even pathogens. For this reason, the management of food leftovers cannot be distinguished from food security and food safety. Food leftovers originate from food produced and intended for human consumption, which is usually inspected and supervised to ensure safe and contaminant-free products. Although there could be present contaminants or packaging remnants in food leftovers used in animal feeding, they are below the permitted threshold limit set by the Health Protection Agency (2009).

To our knowledge, there is a lack of studies about the assessment of the sustainability features associated with the use of food losses for livestock purposes. A number of studies using life cycle analysis (LCA) considered food waste but not food leftovers for livestock feeding (Dou et al., 2018; Tallentire et al., 2018; Van Hal et al., 2019). As stated before, there is an important legal difference between food losses and food waste, the latter forbidden by the EU law as livestock feed (Zu et al., 2016). FAO clearly clarified the differences between food losses and food waste stating that “food losses represent the decrease in quantity or quality of food in the production and distribution parts of the Food Supply Chain (FSC) mainly caused by the functioning of the food production and supply system or its institutional and legal framework" (Bellù, 2016). Contrastively, food waste is "part of the food loss which refers to the removal from the FSC of food (whether processed, semi-processed or raw) which is fit for consumption, by choice, or which has been left to spoil or expire as a result of negligence by the actor, predominantly, but not exclusively, the final consumer at the house- hold level". Food losses are then something undesired, occurred by inadequate technology, poor logistics etc. Strategies and policies to reduce food losses have to be different from those aimed to reduce food waste. Differences between food losses and food waste exists also considering their environmental implications. Taking into account LCA studies on the use of food waste for animal nutrition purposes, they need to be treated before being used, requiring additional energies and resources (Kim and Kim, 2010). Has been observed that if used as feed instead of being sent to the landfill, food waste would produce less GHG emissions. Quantitatively, 200 kg CO2-eq per ton of dry-based treated food waste, 61 kg CO2- eq per ton of wet-based treated food waste versus 1010 kg CO2-eq with landfill (Kim and Kim, 2010). The inclusion of FFPs in animal diets do not requires the same pre-treatments as in the case of food waste, therefore the values of GHG emissions related to the feed- making process would be likely lower compared to food waste. However, more studies focused on food leftovers are needed from this point of view.

Vandermeersch and co-authors (2014) compared the environ- mental footprint of "bread waste" when used to produce former foodstuff or processed for biogas production. The study realized that the conversion of the bread leftover into animal feed was the most sustainable option. Those results could be case sensitive and need to be analyzed carefully, but they clearly determine the great potential of food leftover to be converted into animal feed ingredients. The use of FFP or BBP in animal diets might represent also an opportunity for generate "new circular production system" in which smallholders are involved. The connection between farm (smallholders) and small or medium local retails indeed, can be implemented, creating conditions for keeping some food leftover in the food chain. Intuitively, in such scenarios BBPs seems to be with higher potential than other material, since well known (e.g. bread) and often ready to used. Such innovations can increase not small farm productivity but also can help smallholders, to reach the market (connection with small and medium retails), that could ameliorate the condition of small communities. Aside from the improved climate footprint, also other resources could be saved by replacing grains with FFPs. The soybean represents the world's primary plant protein, and 85% of all soybeans are cultivated for feed purposes, primarily for pigs and poultry (Organization, 2018). These protein sources require large arable lands and a huge consumption of water for their growth. At the same time, cereals comprise the largest share of global food loss and waste by caloric content (53% of the total) (Lipinski et al., 2013). This inefficiency could be moderated by replacing grains with FFPs, leading to a corresponding reduction in the use of resources correlated with grain production such as energy, fertilizer, water and land. In the U.S. 110e140 m3 water and 17 kg N fertilizer are used to produce 1 t maize grain (Kim et al., 2014). The replacement of a certain percentage of maize with the alternative energy source represented by FFPs could generate a substantial drop of the live- stock environmental impact.

The use of FFPs-based diets could also impact the cost of livestock production. Studies observed that European pork production costs V1.4 to the farmer but V1.9 of damage to the environment per kg of pork meat produced (Nguyen et al., 2012), where those environmental costs are primarily related to the processes of feed grain production (Salemdeeb et al., 2017).

Further quantitative assessment is necessary to fill the gap of information about the environmental effects on the use of food losses for livestock feeding. The knowledge on the potential environmental benefits of food leftovers could raise the interest and therefore the use of those products in animal diets, with a consequent improvement of the livestock sustainability.

CONCLUSIONS

The present review evidences that unsold or defected pasta, bread, chocolate and candies can produce distinct food leftovers products that, when mixed together, can result in uniform products/meals named former food products (FFPs) and bakery by- products (BBPs). To our knowledge, this review for the first time proposes a different definition for FFPs and BBPs, in order to facilitate their use in the livestock sector and to highlight the most important characteristics of both classes of food losses. For the first time the nutritional properties, safety issues and effects on pig and ruminant nutrition of both FFPs and BBPs are investigated by literature review. These two categories possess several similarities but also some category-specific features and once quality is assured, nutritionists can safely use them in balanced diets for monogastrics and ruminants, without impairing the productive performance (daily gain, milk yield etc.) and welfare. The exact inclusion levels however, should be verified carefully. The results discussed in this review highlight how the feed industry could give to this sector the potential to obtain the best of the nutritional and economic added value by using non-human-edible by/co-products in accordance to the circular economy principles. Both FFPs/BBPs represent an appealing opportunity to mitigate the impact of the livestock sector on the environment by converting food losses into animal protein food. Replacing traditional feed ingredients with FFPs/BBPs could also lead to a reduced competition between humans and food producing animals for raw materials such as wheat. This review also evidences that the potential of these products is not yet fully exploited as added-value products for animal nutrition. In fact, there is a lack of information on the effects of a diet containing high percentage of FFPs or BBPs on carcass composition/milk composition and rumen/gut health in growing and finisher animals, together with an assessment of the sustain- ability features associated with the use of FFPs/BBPs in animal nutrition. The idea to use food leftover for feeding animals is still far to be completely welcomed by livestock producers. A wider dissemination of the potential of those products, together with a renewed image of FFPs and BBPs far from the stereotypical image of the garbage, could increment their acceptance for a practical use as feed. Some logistical concerns should be considered for the food leftover collection and transport, since leftovers collection by former foodstuffs processor from companies located abroad would decline the sustainable potential of this practice. Life cycle cost analysis should be performed to clarify if the conversion of food leftover is cost-effective for both the livestock producers and former foodstuffs processors. Those surveys should also take into account the socio-economic effects, potential technological improvements, feedbacks from stakeholders, livestock producer and consumers, allowing the adoption of better-shaped strategy to in- crease the acceptance on the use of food leftovers in feed. This review contributes in defining an accurate picture on the nutrient profile and the safe use of FFPs and BBPs. Such information is critical for a proper inclusion of food leftover in a standardized feeding practice in the modern animal production system. The present study aimed to fill the gap of knowledge about potentials of food leftover in animal nutrition, but much remains to be done to allow the sector to increase its contribution in replacing natural resources with food losses. Hence, the livestock sector could reduce the food waste accumulation, the competition for natural resources and the environmental impact of the animal production systems.

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CHAPTER 3

Potentials and Challenges of Former Food Products (Food Leftover) as Alternative Feed Ingredients

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Simple Summary: This review focuses on the use of ex-foods, an alternative feed ingredient in farm animal diets, composed by processed and ready-to-eat food products no longer suitable for human consumption. Such foods, which are also called former food products, are usually buried in landfill sites, despite their high potential of being used as sustainable feed ingredients. In order to obtain proper balanced diets by using these alternative feed ingredients, several aspects have to be considered. In this respect, this paper aims to address the state of the art about food leftovers used in animal nutrition in general and in pig diets specifically.

Abstract: Former food products (FFPs) are foodstuffs that, even though they are nutritious and safe, have lost their value on the human consumption market for different reasons, such as production errors leading to broken or intermediate foodstuffs, surpluses caused by logistical challenges of daily delivery, or any other reason. The nutritional features of FFPs include carbohydrates, free sugars, and possibly also fats. FFPs tend to have been processed through various technological and heat treatments that impact the nutrients and the kinetics of digestion, as well as animal response and, particularly, gastro-intestinal health. This review integrates some of the most recently published works about the chemical composition, nutritional value, digestibility and glycemic index of ex-foods. In addition, a view on the relationship between the use of FFPs and safety issues and their effects on pigs' intestinal microbiota are also given.

INTRODUCTION

Nowadays agriculture, and even more so livestock production, are faced with a wide range of complex challenges. From the perspective of sustainability, livestock production has received considerable attention in recent years over the extent to which animal feed production competes for land and other resources with the production of human food. Livestock consumes a third of all cereals produced and uses about 40% of global arable land. In fact, farm animals occupy two billion ha of grasslands, of which about 700 million ha could be used to grow crops. From another perspective, 86% of the plant material fed to livestock would be inedible by humans directly, but it is instead converted into valuable food for human consumption (e.g., milk, meat), thus contributing greatly to food and nutrition security (FAO, 2018). In general, it has been estimated by the Food and Agriculture Organization of the United Nations (FAO) that about 3 kg of human-edible material, mostly grains, are needed to produce 1 kg of meat. These global figures, however, have to be considered with caution, since wide differences across species and production systems exist. While ruminants consume more dry matter per kg of protein produced compared to pigs or poultry, they require less human-edible protein, since they can rely more on grass and forages. Pigs and poultry consume less feed to produce the same amount of protein, but a far higher proportion of what they do consume could be eaten directly by humans (FAO, 2018). In livestock production systems, the cost of animal feed represents up to 85% of the farm gate value of several animal products (FEFAC, 2016). In light of this, proper feeding and nutrition strategies are becoming increasingly important as livestock systems strive to become more efficient. In this scenario, the use of alternative feed ingredients in farm animal’s diet can be an fascinating option from several standpoints, and ex-food recycling is an interesting model. By definition, “Ex-food” or “Former foodstuffs” (FFPs) means foodstuffs which were manufactured for human consumption in full compliance with the EU food law, but which are no longer intended for human consumption for practical or logistical reasons and which do not present any health risks when used as feed (Pinotti et al., 2019). It has been estimated (Bouxin, 2016) that 3–3.5 Mt of FFPs are processed in the EU. Ex-foods are already used in animal nutrition (they are in the EU’s feed catalogues), but to a limited extent (3.3%) compared to the total food waste. The potential of these products has not been fully exploited yet as feed ingredients. The target species are omnivores, such as pigs and poultry, even though their use in ruminants cannot be excluded. Examples of FFPs include various leftovers from the food industry: pasta, bread, cereals, savoury snacks, biscuits, sweets and chocolate bars. Such foods are rich in sugar, starch, oil or fat, thus giving them a high energy content (Giromini et al., 2017; Tretola et al., 2017a; Pinotti et al., 2019). Livestock systems today and in the future have to take into account not only economic development and feed security and safety, but also politically-sensitive issues such as animal welfare and environmental sustainability. Sustainable feed/food security is thus in need of innovation (Pinotti et al., 2011) and the conversion of industrial food losses into ingredients for animal feed maintains such losses in the food chain and should thus be implemented on a global basis (EFFPA, 2018). In this respect, this paper aims to address the state of the art about the use of ex-food in animal nutrition, with special emphasis on their nutritional properties and safety issue.

Former Food Products: Nutrient Content and Dietetics

Former food products or ex-food are defined in the Regulation (EC) No 68/2013 as "foodstuff other than catering reflux, which were manufactured in full compliance with EU food law but are no longer intended for human consumption for practical and logistical reasons or due to problems in manufacturing or packaging which are unlikely to cause any health risks when used as feed". These materials are dried and sorted, unpacked, ground and sieved to create new feed ingredients, which can be use as substitute of existing raw materials in various farm animal compound feeds (Giromini et al., 2017; Tretola et al., 2017a; Pinotti et al., 2019). Ex-food ingredients can be divided in two main categories: leftovers of the food industry mainly composed by bakery products (i.e., bread, pasta etc.) and leftovers of the food industry principally composed by confectionery products (e.g., chocolates, biscuits etc.). Bread and salty cakes/snacks, due to the long baking process, represent a macerated and easy to digest source of energy with high starch contents. Confectionary products that consist, for example, of chocolate, dry cakes and biscuits, waffles, and muesli products can be considered supplemental feed, available all year round and rich in simple sugars, fat and energy. In light of these features the main animal targets for FFPs are young animals, e.g., piglets, chicks and calves, due to the high amount of digestible carbohydrates, like cooked starch. Indeed, cooked starch food represent a rich source of rapidly digestible starch and rapidly available glucose, features that can strongly affect productive performances (such as feed intake) and nutrient digestibility. Moreover, thanks to the ingredient used in their preparation (e.g., butter sweet and chocolates), FFPs are often rich in fats (Giromini et al., 2017; Tretola et al., 2017a; Pinotti et al., 2019).

These properties have been studied by Giromini et al. (2017), who reported that bakery and confectionary ex-food- processed for pig nutrition have a nutrient content similar to wheat and barley grains, although with a higher energy content (Figure 1). Mean FFP's metabolizable energy (ME) content was 16.95 MJ kg^{-1} with fats and starch being the main contributors. FFPs have a lipid content of around 10%–12%, which is three to six times than reported for wheat and corn. The starch content in FFPs can be up to 50%–60% on dry matter basis (DM). Former food products have also shown high digestibility values, which in the above-mentioned study (Giromini et al., 2017) ranged from 79% up to 93% DM, depending on how the ex-food was mixed and prepared. The mean protein content in the FFPs was around 10.0%, consequently FFPs should not be considered as a valuable source of protein. These features are summarised in Figure 1.

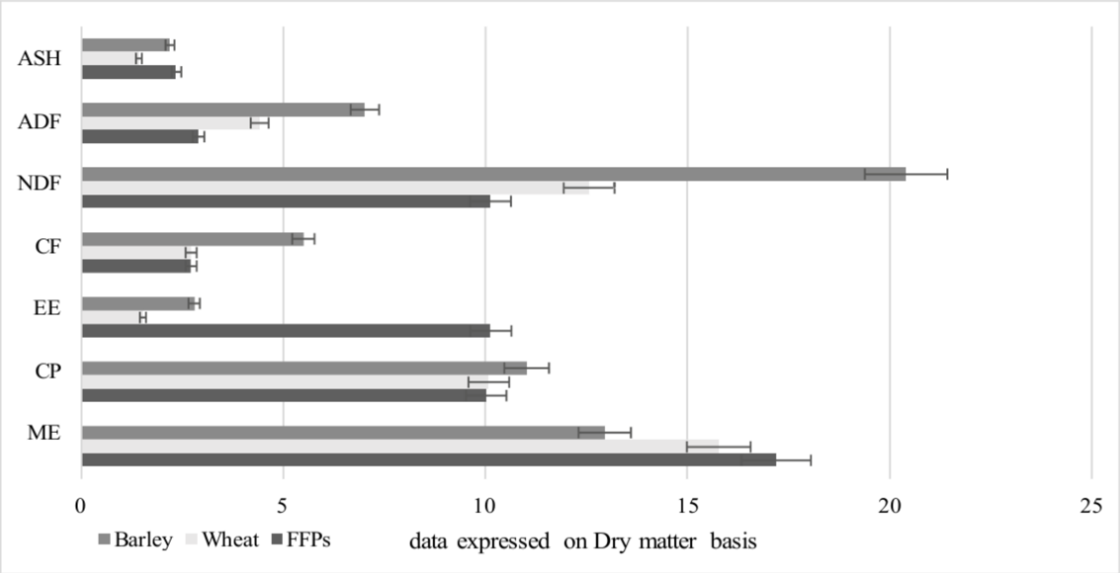

Figure 1. Nutrients—ash, acid detergent fibre (ADF), neutral detergent fiber (NDF), crude fiber (CF), fat (ether extract—EE), crude protein (CP); all expressed as % and energy content (ME, MJ kg^{-1}) of FFPs [5,7].

The free/simple sugar content of FFPs is another key quality aspect with a positive impact on the digestion kinetics of carbohydrates and which also boosts the glycaemic index (GI). In human nutrition, the GI is used to classify starchy foods based on their post-prandial glucose release into the bloodstream (Giuberti et al., 2012). In terms of livestock, the glycaemic index was initially used in equine (racing horses) nutrition in relation to disorders associated with the carbohydrate metabolism (Kronfeld et al., 2005). It was then introduced for pig nutrition by Menoyo et al. (2011) in order to classify cereals. Cereals and food preparation with a high GI tend to promote insulin production with a consequent increased feed consumption. As previously reported, FFPs are produced starting from food leftover that have been cooked and/or heat-treated during their production process in the food industry (Giromini et al., 2017; Tretola et al., 2017; Pinotti et al., 2019). As a result, these materials are characterized by a higher digestibility compared to the cereal grains commonly used in farm animal diets in general, and pig nutrition particularly (Pinotti et al., 2019). Processing techniques (e.g., thermal processing, extrusion cooking etc.) are able to affect both digestibility and absorption of digested carbohydrates (Ottoboni et al., 2019), which in turn have a major impact on the glycaemic index (Giuberti et al., 2012). These dietetics features have been investigated by Ottoboni et al. (2019), who measured hydrolysis index (HI), predicted glycemic index (pGI), and the time trend in carbohydrate digestion (k), in FFPs in comparison with common cereals. Results obtained indicated that all parameters related to carbohydrate digestion (i.e., HI, pGI and k) were always higher in ex-food compared to conventional cereals feed ingredients such as unprocessed corn (Ottoboni et al., 2019) (Figure 2). However, it is known that other constituents of the food matrix, such as proteins, lipids and fibres, play a significant role during processing which affects the physico-chemical characteristics of digesta and the final digestibility of starch (Ottoboni et al., 2019). In this respect, a further step in Ottoboni's study was to evaluate FFPs, not only as single ingredient but also when they were included in a pig formula. Data obtained on two post-weaning piglet complete diets (a cereal based vs. a FFPs diets) clearly indicated that the inclusion of FFPs (up to 30%) as a substitute for cereals (corn, wheat, de hulled barley) has produced a big impact on in vitro starch hydrolysis kinetics and digestibility. Substitution of common cereal with FFPs in piglet diets has improved starch susceptibility to enzymic digestion, thus probably optimizing their nutritional/dietetic quality (Giuberti et al., 2012; Ottobini et al., 2019). This implies a functional evaluation with special emphasis on FFPs' impact on animal welfare in general and the gastro-intestinal tract (i.e., gut health), in particular (Ottoboni et al., 2019; Pinotti et al., 2019)

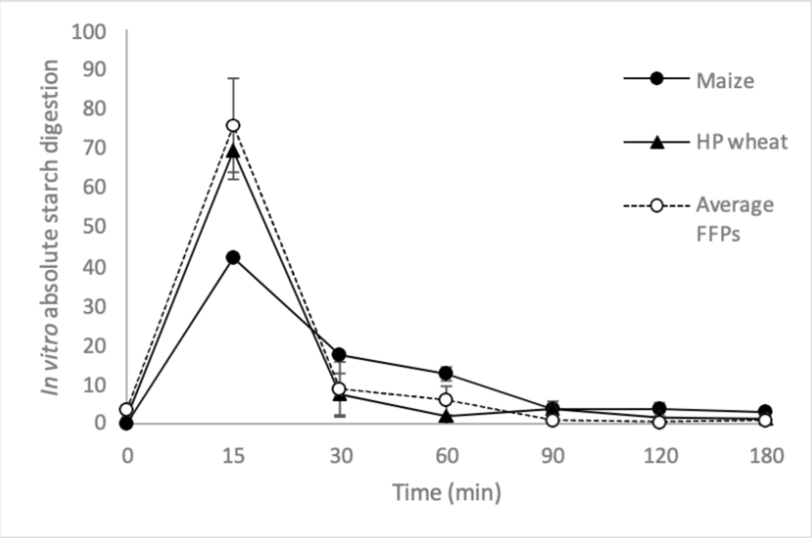

Figure 2. Absolute in vitro total carbohydrate digestion (as a fraction of total carbohydrates/min) of cereal grains (unprocessed maize and heat processed wheat) and former food products (FFPs). Adapted from [13].

The FFPs, however, might contain more than 20% of simple sugars, that can affect not only the gut transit, but also its health and ecology (Mavromichalis, 2012). Understanding what a healthy microbiota looks like and how FFPs can influence the composition of the gut microbial population, improving eubiosis and/or reducing dysbiosis, provides fundamental information to efficiently reconvert FFPs into value added products for animal nutrition. Furthermore, the diet-driven different modulation of the gut microbiota can affect the local and systemic setting of immunity (Mavromichalis, 2012; Fouhse et al., 2016; Salyers et al., 1996).

This assessment in general requires the combination of several different approaches that include in vivo studies. In this direction, recent studies have been conducted in order to investigate the effect of FFPs on growth performance (Tretola et al., 2019a) and gut microbiota in weaning pigs (Tretola et al., 2019b). In these studies, the authors evaluated the effects of substituting 30% conventional cereals for 30% FFPs in post-weaning piglet's diets (Tretola et al., 2019a-b). The results obtained indicated that both in vitro and in vivo digestibility values were higher for FFPs diets compared to the control ones.

Both average daily gain and feed intake were not affected by dietary treatment. Conversely, piglets on the FFPs diet showed a lower feed conversion rate. Therefore, it can be suggested that inclusion of FFPs -up to a level of 30% as cereal substitute- in post-weaning diets, has no detrimental effects on pig growth performance (Tretola et al., 2019a). Moreover, large intestine microbial taxa composition has shown no major modifications (Tretola et al., 2019b). Specifically, FFPs diet decreased the microbiota diversity/richness and evenness in the large intestine while minor differences have been observed in taxa composition. The main changes in the FFP group over time affected the Bacteroidetes, which increased during the first period (27% and 48% in day 0 and day 8, respectively), and decreased again to the original values (29%) in the last sampling day. Thus, FFPs led to a qualitative modification in the gut microbial community over time. Similarly, at the end of the trial FFP diet increased the amount of the *Proteobacteria* phylum and decreased the abundance of *Lactobacillus* genus, compared to the control diet (Figure 3). Even though no gastrointestinal disorders have been recorded during the trial, these differences observed at the end of the study should be considered with caution in terms of gut health. The phylum of *Proteobacteria*, in fact, includes several opportunistic pathogens often associated with gastrointestinal disorders both in animals and humans. In contrast, a decreased abundance of the bacteria belonging to the *Lactobacillus* genus, could result in a reduction of health-promoting probiotics (Tretola et al., 2019b). However, since the core microbiota composition was slightly affected, the potential impacts of FFPs on microbiota require further investigation with a wider panel of conditions and exposure time.

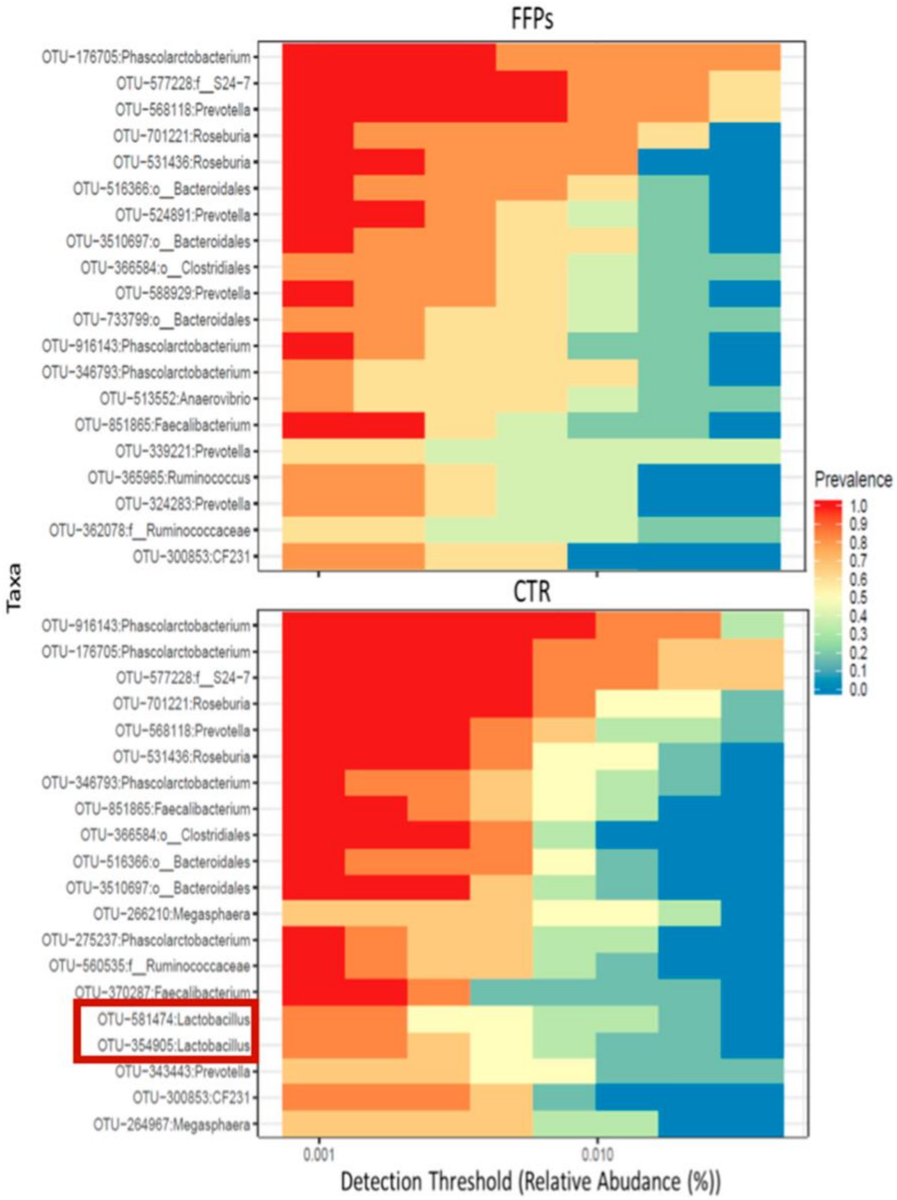

Figure 3. Large intestine microbiota with specific keystone taxa detected at the end of the experiment (D16) in piglets receiving (FFPs) or not (CTR) former foods products (30% inclusion) in their diet. Redbox evidences lactobacillus strains; adapted from Tretola et al. (2019b).

Nutritional properties of FFPs, however, are not the only plus of these materials. The use of FFPs in farm animal diets has also a big potential in terms of feed processing/manufacturing and technological quality. They are indeed energy dense ingredients often characterized by a valuable fat concentration. This can be considered a technological benefit since lipids are already embedded in the feed matrix, which means that they can be easily manipulated and processed during feed production, since there is no need for their addition. This technological feature not only facilitates feed production, but also increases the energy density of the diets. These characteristics are even more important in modern lean pig strains (average daily gain > 1kg) which have high energy requirements and impose a need for nutritious and energy dense ingredients. In summary, from the perspective of the circular economy, reprocessing FFP biomass is particularly attractive and sustainable, limiting food losses and the competition for human edible cereals.

Safety Concerns in Former Foods Products

Recycling ex-food in the feed sector involves a combination of different processes, which are related to the type of food. These processes include collection, unpacking, mixing, grinding and drying, that impact both quality and safety. In terms of safety, both microbiological load and packaging remnants are the main issues for the current regulations on feed standards (Tretola et al., 2017a; Tretola et al., 2017b; Tretola et al., 2019c).

With regards to microbiological quality, Tretola and co-workers (2017a) investigated the different FFPs. In this study the first indicator used to evaluate the general hygienic condition of feedstuff was the total viable count (TVC). The recorded values for TVC were, for all tested FFPs samples, below 5 log CFU g^{-1}. None of the samples exceeded the microbial loads of 6 log CFU g^{-1}, which is generally recognized in food as the threshold limit above which spoilage could occur (Tretola et al., 2017a). The limited microbiological load was also confirmed when different microorganisms were considered. The mean count of *Enterobacteriaceae* was also limited, confirming the low level of bacterial contamination. Both the *E. coli* and *Staphylococci* count were below the detection limit or extremely low ($\leq$2 log CFU g^{-1}), respectively. The same was for *B. cereus* and its spores, which are considered indicators of poor processing, poor quality of raw materials, or poor temperature control. In tests of FFPs, theses strains never exceed the level of 5 log CFU g^{-1}, known as the starting concentration from which toxin production may occur. Likewise, *Clostridia* were found to be countable just in a limited number of FFPs samples and in very low loads (1–1.7 log CFU g^{-1}); levels around 1 log

CFU g^{-1} are considered satisfactory and commonly levels below 4 log CFU g^{-1} are considered not of particular apprehension. Yeasts and moulds, which are among the most critical organisms for this type of feedstuff, were present in very small quantities, confirming again stability of these materials (Tretola et al., 2017a). However, the major hazard for the microbial contamination of animal feed is *Salmonella spp*. Of note, in all FFPs tested in the study (Tretola et al., 2017a), *Salmonella spp.* was never detected, matching the standard established by the main health authorities for the animal feed sector (Pinotti et al., 2019; Tretola et al., 2017a; UE Commission, 2011).

These results, however, were expected, as the tested FFPs were dry and cooked at high temperature during the production process, that probably affected their microbiological stability. A further safety issue in FFPs use and application in animal nutrition is related to the presence of packaging remnants. Packaging materials are generally not accepted as a feed ingredient in accordance with the feed standard regulations (Tretola et al., 2017a). In terms of packaging remnants, a useful example is represented by bakery co-by-products such as bread, biscuits, waffles, and breakfast cereals whose packaging must ensure the maintenance of quality during transport and storage. Food packaging vary widely based on the materials used and on how the food has been processed (Tretola et al., 2017a-b; Tretola et al., 2019c; van Raamsdonk et al., 2011). Plastic is the packaging material most commonly used in food industries. To a lesser extent, aluminium, resin, and pressed paperboard are used (van Raamsdonk et al., 2011; Tretola et al., 2017b). The main types of materials used are polyolefin such as polypropylene and polyethylene. Polypropylene can resist temperatures of up to 220 to 240 °C and tends to be made in black or clear, very rigid, crack-resistant. Polyethylene has an average melting point of 120 °C. Five other commonly used polyolefins are: *(i)* polyethylene terephthalate and its copolymers, which melt before 140 °C and are found in different colours; *(ii)* polystyrene, which has a moderate resistance to temperature and is found in a variety of colours; *(iii)* pressed paperboard, which resists in an oven for an hour at temperatures of up to 200 °C and which is manufactured in a variety of colours and patterns; *(iv)* rigid polyvinylchloride (PVC, regenerated cellulose (RC)); and finally *(v)* aluminium foil (silver or coated in colours and can withstand very high temperatures) (van Raamsdonk et al., 2011; BTSA, 2019). However, in spite of this variability of packaging materials, data available in the literature (Tretola et al., 2017a-b; Tretola et al., 2019c; van Raamsdonk et al., 2011;) indicate that packaging remnants in FFPs are usually negligible (<0.10 g/100 g).

CONCLUSIONS

Mitigating environmental impact is crucial to sustainable production in the livestock sector. This can be achieved by reducing food waste through recycling, and especially by enhancing the management of FPPs, with the added benefit of being an economic resource. As with other alternative/innovative feed ingredients (Pinotti et al., 2014; Pinotti et al., 2016; Gasco et al., 2019; Flachowsky & Meyer, 2015), exploiting FFPs in feed production fully meets the requirements of the circular economy. From the food supply industry, there are always unintentional and unavoidable food losses, which preclude foodstuffs from reaching the human food market. In this context, FFPs are seen as a potential resource rather than a waste product sent to landfill or otherwise disposed of in the natural environment. Their potential seems higher for omnivorous farm species (e.g., pigs and poultry) even though some studies, mainly on bakery products, have opened new frontiers in ruminants' nutrition (Humer et al., 2018; Oba, 2011). This will therefore save on costs and reduce the impact of livestock production on the environment.

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CHAPTER 4

Multivariate image analysis for the rapid detection of residues from packaging remnants in former foodstuff products (FFPs) – a feasibility study

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Abstract: From a circular economy perspective, feeding livestock with food leftovers or former foodstuff products (FFPs) could be an effective option aimed at exploiting food leftover resources and reducing food losses. FFPs are valuable energy sources, characterised by a beneficial starch/sugar content, and also fats. However, besides these nutritional aspects, safety is a key concern given that FFPs are generally derived from packaged food. Packaging materials, such as plastics and paper, are not accepted as a feed ingredient which means that residues should be rigorously avoided. A sensitive and objective detection method is thus essential for an accurate risk evaluation throughout the former food production chain. To this end, former food samples were collected in processing plants of two different European countries and subjected to multivariate analysis of red, green, and blue (RGB) microscopic images, in order to evaluate the possible application of this non-destructive technique for the rapid detection of residual particles from packaging materials. Multivariate Image Analysis (MIA) was performed on single images at the pixel level, which essentially consisted in an exploratory analysis of the image data by means of Principal Component Analysis, which highlighted the differences between packaging and foodstuff particles, based on their colour. The whole dataset of images was then analysed by means of a multivariate data dimensionality reduction method known as the colourgrams approach, which identified clusters of images sharing similar features and also highlighted outlier images due to the presence of packaging particles. The results obtained in this feasibility study demonstrated that MIA is a promising tool for a rapid automated method for detecting particles of packaging materials in FFPs.

INTRODUCTION

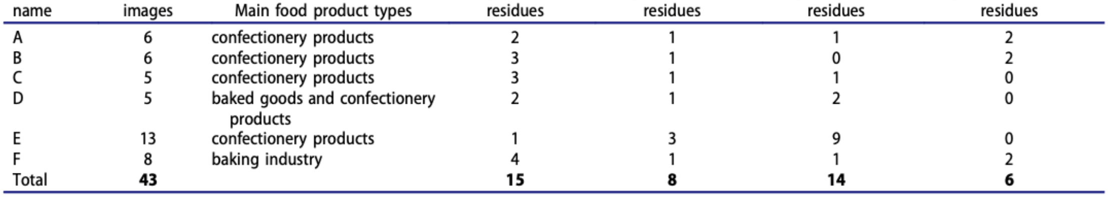
The livestock sector is inevitably going to involve trade-offs between feed security, feed safety, animal welfare, environmental sustainability and economic development. A common denominator among many of these issues, which are often politically-sensitive, is sustainability. In fact, converting losses from the food industry into ingredients for the feed industry, thereby keeping food losses in the food chain, can be considered a positive cycle that should be implemented worldwide. Food leftovers or Former Foodstuffs Products (FFPs) are animal feed ingredients consisting of processed and ready-to-eat food products, no longer suitable for human consumption due to logistical, manufacturing or packaging defects (Luciano et al., 2020). Former food products mainly consist of leftovers from the baking industry (e.g., bread, pasta) and confectionery products (e.g., chocolates, biscuits). Rejected bread, various biscuit products, high-quality baked goods and confectionary from industrial biscuit bakeries are dried and consecutively sorted, unpacked, ground and sieved to create suitable ingredients, which replace some of the existing raw materials in various animal compound feed (Pinotti et al., 2019; Ottoboni et al., 2019; Tretola et al., 2019a-b-c). Based on the nutritional facts reported for humans, FFPs are extremely rich in carbohydrates, and depending on their origin, also in fats (Giromini et al., 2017). All these factors make FFPs particularly suited to the circular economy: FFPs represent a way to convert losses from the food industry into ingredients for the feed industry (Pinotti et al., 2019). Although FFPs are nutritious and safe from a microbiological point of view (Tretola et al., 2017a-b), they may generate other safety issues, such as those related to the presence of packaging remnants. FFPs are un-packaged automatically in order to process a large amount of product. Feed processors routinely remove the packaging from FFPs mechanically in the feed plant; however, despite the removal of most of the packaging, small amounts of wrapping materials remain in the resulting feed. Consequently, a small amount of packaging remnants in the final product (feed) appears to be unavoidable (Tretola et al., 2017a-b). Typical remnant residues in FFPs include paper/ paperboard, aluminium foil, and plastics, all of which can remain as residues in the final product. In Tretola et al. (2019a) paperboard was the most detected contaminant followed by aluminium foil, and then plastic. Among these, plastics are becoming extremely important especially when small particles are considered. Microplastics are usually defined as plastic particles with a size smaller than 5 mm for their largest dimension. In general, particles with a size equal to 1–2 mm or larger can be visually detected, manually extracted and quantified based on weight. This procedure has become a daily practice in the monitoring of former foodstuffs for use in animal feeds (van Raamsdonk et al., 2020). However, irrespective of material type, packaging remnants are generally not accepted as a feed ingredient by several authorities, which prohibit the sale of feedstuffs containing packaging materials from the agri-food industry. By contrast, some national authorities have indicated that a minimum percentage of packaging remnants in FFPs is unavoidable and not risky either for animals or humans (van Raamsdonk et al., 2011; van Raamsdonk et al., 2012). From a safety point of view, in most analysed samples, the presence of these foreign mate- rials is negligible and below the maximum limit established by some control authorities/bodies (e.g., 0.12% w/w set by the German Federal Ministry of Food, Agriculture and Consumer Protection) (Tretola et al., 2017a, Tretola et al., 2019a; Pinotti et al., 2019). In terms of particles dimension, it has been established that remnants normally present in FFPs are mainly in the > 800 μm mesh fraction (van Raamsdonk et al., 2012; Tretola et al., 2017a). Quantification of particles smaller than 400 μm is generally too laborious and, according to van Raamsdonk et al. (2012), these smaller particles are excluded from the quantification, since their share in the total weight is insignificant. A sensitive and objective detection method is therefore essential for tracing and quantifying packaging remnants in FFPs. The detection and quantification of packaging remnants in bakery products using a stereo microscope was proposed by the RIKILT Institute (Wageningen) (van Raamsdonk et al., 2011; van Raamsdonk et al., 2012). Amato et al. (2017) used a similar approach to develop a sensitive gravimetric method, for routine official controls for the determination of packaging residues in feed. The two proposed methods can be summarised as follows: (1) visual selection of the undesired ingredients i.e., remnants of packaging materials; (2) weighing of the selected materials; (3) defatting; (4) dehydration; (5) final weighing; and (6) reporting of weight and percentage. In both cases however the methods appear complex, time consuming and analyst sensitive. In this respect, some of the authors of the present work (Tretola et al., 2017b) used computer sensing to visualize packaging remnants. The results showed that computer vision, when coupled with a stereomicroscope for image acquisition, acts a rapid qualitative screening approach to estimate the presence of foreign materials in food and feed, with little laborious and subjective human visual involvement (Tretola et al., 2017b). Tretola et al. (2019a) also investigated the use of an electronic nose (e-Nose) to detect these contaminants in FFPs. The results indicated that an e-Nose can be used for rapidly screening for the presence of presumed packaging remnants of aluminium, plastics and paperboard in FFPs, when the food/feed matrix is characterised by low variability (e.g., same producer, same odour print Cheli et al., 2018). The aim of this work was to assess the potential of Multivariate Image Analysis (MIA) to automatically detect packaging residues using red, green, and blue (RGB) images of FFP samples acquired with a stereomicroscope. The most common statistical tool applied in MIA is Principal Component Analysis (PCA), which highlights similarities and differences among groups of pixels based on their spectral features (i.e., on their colour for RGB images) (Esbensen & Geladi, 1989; Geladi et al., 1989; Prats-Montalbán et al., 2011). In practical applications for quality monitoring, a high number of images need to be acquired in order to calculate robust and reliable models. In this case, it is necessary to consider both within-image and between-images variability, to properly characterise each single sample and to account at the same time for the variations between the different samples (Gowen et al., 2011; Duchesne et al., 2012; Dorrepaal et al., 2016). Therefore, in order to overcome data handling issues, an image-level approach is fundamental, which is based on extracting from each image a feature vector summarising the information needed for the analysis (Ferrari et al., 2013; Kucheryavskiy 2013; Calvini et al., 2016). The colourgrams method follows this image-level approach and is specifically implemented for the analysis of RGB images (Antonelli et al., 2004). It converts each RGB image of the dataset into a one-dimensional signal, the colourgram, which summarises the colour features of the corresponding image. The colourgrams are then collected into a data matrix in which each row corresponds to the signal derived from a specific image of the dataset. The colourgrams matrix can then be analysed using common multivariate statistical methods, e.g., PCA, in order to gain an exploratory overview of the whole dataset of images, to identify clusters of images sharing similar features, and to highlight possible outliers. The colourgrams approach has been successfully applied in several case studies above all related to the analysis of food matrices by means of RGB imaging (Ulrici et al., 2012; Giraudo et al., 2018; Orlandi et al., 2018a-b). In the present study, the images of FFP samples were analysed using both the pixel-level and the image-level approaches, in order evaluate their effectiveness in detecting the presence of possible particles derived from packaging residues.

MATERIALS AND METHODS

FFPS SAMPLES AND IMAGE ACQUISITION

Six different commercial samples of FFPs, originating from two European countries, were used (Table 1).

Table 1. Summary of the FFP samples considered in this study and corresponding number of acquired images.



Sample name	Total n° of images	Main food product types	Images with no residues	Images with plastic residues	Images with paper residues	Images with aluminium residues
A	6	confectionery products	2	1	1	2
B	6	confectionery products	3	1	0	2
C	5	confectionery products	3	1	1	0
D	5	baked goods and confectionery products	2	1	2	0
E	13	confectionery products	1	3	9	0
F	8	baking industry	4	1	1	2
Total	**43**		**15**	**8**	**14**	**6**

Three samples (FFPs A, B and D) were obtained from an FFP processing plant in Country1, while 3 samples (FFPs C, E, F) were from an FFP processor in Country2. All samples were produced from different food materials, including bakery products, broken biscuits and chocolates, confectionery products (e.g., croissants, chocolate), surplus bread, rice cakes, salty snacks, and breakfast cereals. For all the FFP samples, a randomly selected aliquot of 5 g was placed in a large Petri dish (PS Ø 90, Colaver, Milan) in a manner to form a single layer. The amount of former food aliquots to be analysed was chosen based on a previous study, which verified homogenous distribution of packaging remnants in reduced amount of former food samples (Tretola et al., 2017a). Specifically, correspondence between packaging remnants levels found in 100 g of an FFP sample and relative sub-samples of 2 g was verified by Tretola et al. (2017a). In order to increase sampling representativeness, in this study sample quantity was increased to 5 g. Using a stereo microscope (OLYMPUS SZX9), each sample was investigated separately, taking utmost care in order to avoid any contamination in line with laboratory Standard Operating Procedures (SOPs) for remnants of packaging materials. For each sample, from 5 to 13 images, with or without packaging remnants, were acquired using a digital camera (CoolSNAP-Pro cf Colour or AxioCam MRc coupled with a 0.63x port) equipped with an image analysis software (Image-Pro Plus 7.0; Media Cybernetics Inc., Rockville, MD, USA). The pixel size of the acquired RGB images was equal to 1392 × 1040, corresponding to a surface area of 2.8 × 2.1 mm. Therefore, the size of a single pixel was equal to 2 × 2 μm, which is much smaller than the minimum size of 400 μm, as reported by van Raamsdonk et al. (2012).

IMAGE ANALYSIS

In order to highlight the potential of MIA to gain a preliminary evaluation at the pixel level of the colour differences between FFP matrices and particles of foreign materials derived from packaging, one image of sample A containing a plastic piece was analysed by means of PCA. The key aspect of PCA consists in representing a multivariate dataset with a low number of orthogonal variables, named principal components (PCs), which are linear combinations of the original variables (Geladi et al., 1989; Prats-Montalbán et al., 2011). The principal components are calculated so that the first PC (PC1) describes the direction of maximum variance in the data, the second PC (PC2) is orthogonal to PC1 and accounts for the maximum residual variance (i.e., the variance not described by PC1), and the same applies for the subsequent PCs. In order to apply PCA to RGB images, the three-dimensional data array composed of m pixel rows, n pixel columns and the three RGB channels, is unfolded into a bidimensional data matrix with $m \times n$ rows, corresponding to the number of pixels in the image, and three columns corresponding to the RGB values. The PCA decomposition of the unfolded RGB image (X) can be expressed as follows:

$$X = TP^t + E$$

where T is the score matrix containing the pixel coordinates in the PCs space, P is the loading matrix describing the relevance of the original variables (i.e., the R, G and B channels) in defining the PCs, and E denotes the residual matrix accounting for residual variation not included in the model. In order to recover the spatial structure of the image, the score vector of each PC can be refolded into a score image with the same spatial dimensions as the original RGB image. The same approach described for RGB images can also be applied to more complicated images, such as multispectral or hyperspectral images, which have more than three channels. In this study, PCA was applied both to the

RGB image “as is” as well as to the augmented RGB image that was obtained by considering additional colour-related channels derived from the RGB values. These additional colour parameters include light- ness (L), the relative colours (relative Red, relative Green and relative Blue), and hue (H), saturation (S), and intensity (I) obtained by converting the RGB colour space into the HSI colour space. Table 2 gives the complete list of the colour-related parameters, together with the corresponding equations.

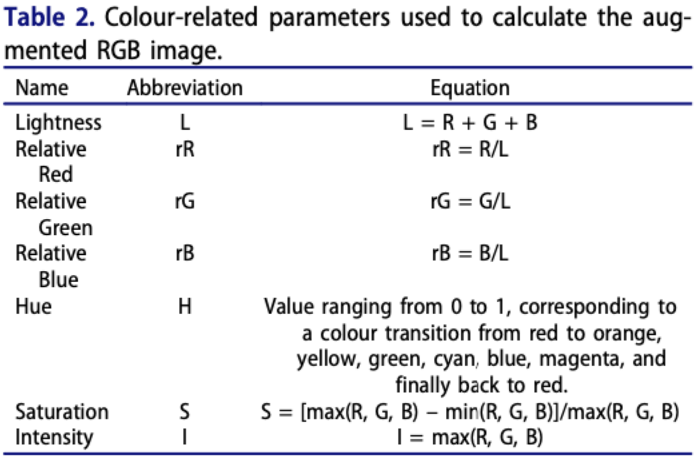


Table 2. Colour-related parameters used to calculate the augmented RGB image.

Name	Abbreviation	Equation
Lightness	L	$L = R + G + B$
Relative Red	rR	$rR = R/L$
Relative Green	rG	$rG = G/L$
Relative Blue	rB	$rB = B/L$
Hue	H	Value ranging from 0 to 1, corresponding to a colour transition from red to orange, yellow, green, cyan, blue, magenta, and finally back to red.
Saturation	S	$S = [\max(R, G, B) - \min(R, G, B)]/\max(R, G, B)$
Intensity	I	$I = \max(R, G, B)$

While in RGB images each pixel is characterised by the three R, G and B channels, in the augmented RGB image, each pixel is defined by seven parameters in addition to the RGB values, for a total of 10 channels. For both images, PCA was applied considering autoscaling as the data preprocessing method. The whole dataset of 43 images was then analysed at the image-level by converting each image into the corresponding colourgram. The first step in this conversion consists in the same unfolding procedure described for the pixel-level analysis. The unfolded RGB matrix is then expanded by adding further columns containing additional colour-related parameters. These parameters include the quantities reported in Table 2 and the score vectors obtained by analysing the RGB data matrix by means of PCA and considering three preprocessing methods (i.e., no preprocessing, mean centring and autoscaling). For all the variables, the corresponding frequency distribution curves are calculated, considering the entire range of variability of each single variable and dividing it into 256 bins. Then, for each image the corresponding colourgram is calculated by merging in sequence the frequency distribution curves of the considered colour-related parameters and by adding, at the end of the signal, the loading vectors of the PCA models, thereby obtaining a 4900-point long signal. Further details about the algorithm used to calculate the colourgrams can be found in Antonelli et al. (2004). The resulting matrix of colourgrams was then ana- lysed by means of PCA using autoscaling as a signal preprocessing method. The exploratory analysis of the dataset at the image-level helped to identify the colour-related features characterising images of FFP samples with packaging residuals. The RGB images were converted into the corresponding colourgrams using Colourgrams GUI (Calvini et al., 2020), a user-friendly interface running under MATLAB (The Mathworks, USA). Colourgrams GUI is freely downloadable from http://www.chimslab.unimore.it/downloads/. The PCA models were calculated using PLS_Toolbox (ver. 8.5, Eigenvector Research Inc., USA) and MIA Toolbox (ver. 3.0.4, Eigenvector Research Inc., USA).

RESULTS

PIXEL-LEVEL ANALYSIS

To illustrate the potential of MIA performed at the pixel-level, the image reported in Figure 1 was analysed by means of PCA. This image represents a former food matrix contaminated with a semi-opaque plastic residue.

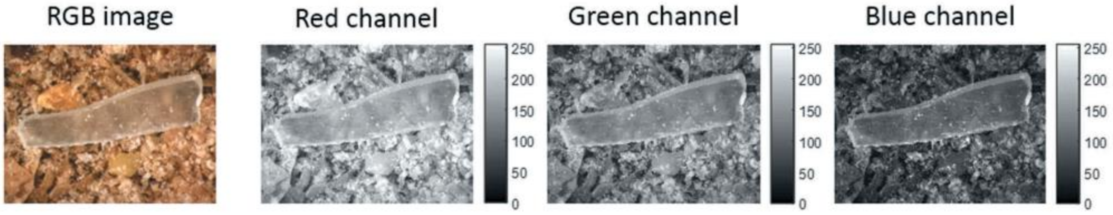


Figure 1: RGB image of a sample contaminated with a plastic residue and grey-scale images of the corresponding red, green and blue channels.

Figure 1 also shows the corresponding red, green and blue channels, reported separately from each other as grey-scale images. The PCA model was calculated considering 2 PCs, accounting for 99.76% of the total variance. In the corresponding PC1-PC2 score plot reported in Figure 2a, each object represents one pixel of the RGB image. In this plot, the objects are coloured according to the score density, i.e., a yellowish colour corresponds to an area of the PC1-PC2 score space with a high density of pixels, while blue indicates an area with a low density of pixels. There are two main clusters of pixels in the figure. A comparison of the PC1-PC2 score plot with the corresponding score images reported in Figure 2c-d reveals that the pixels with positive score values for both PC1 and PC2 are mainly ascribable to the piece of plastic. A compact way to simultaneously evaluate the information pro- vided by both PC1 and PC2 is reported in Figure 2e, which shows the composite false-colour image obtained by superimposing the PC1 and PC2 score images using the red and green channels, respectively. The PC1 and PC2 loading vectors, reported in Figure 2b, show which channels contribute most to the separation. The three R, G, and B channels have similar positive loading values on PC1, indicating that the three channels have a comparable contribution in the definition of PC1. Thus, PC1 essentially describes variations of lightness in the image. Conversely, in the PC2 loading vector, the R and B channels have a high influence on the model, while the G channel has a loading value close to zero. In addition, the R channel has a negative contribution on PC2, while the B channel has a positive contribution on PC2. Therefore, compared to the pixels of the FFP matrix, the pixels of the piece of plastic generally have higher values in the blue channel and lower values in the red channel, while the green channel does not seem to contribute much to separating the two components of the image.

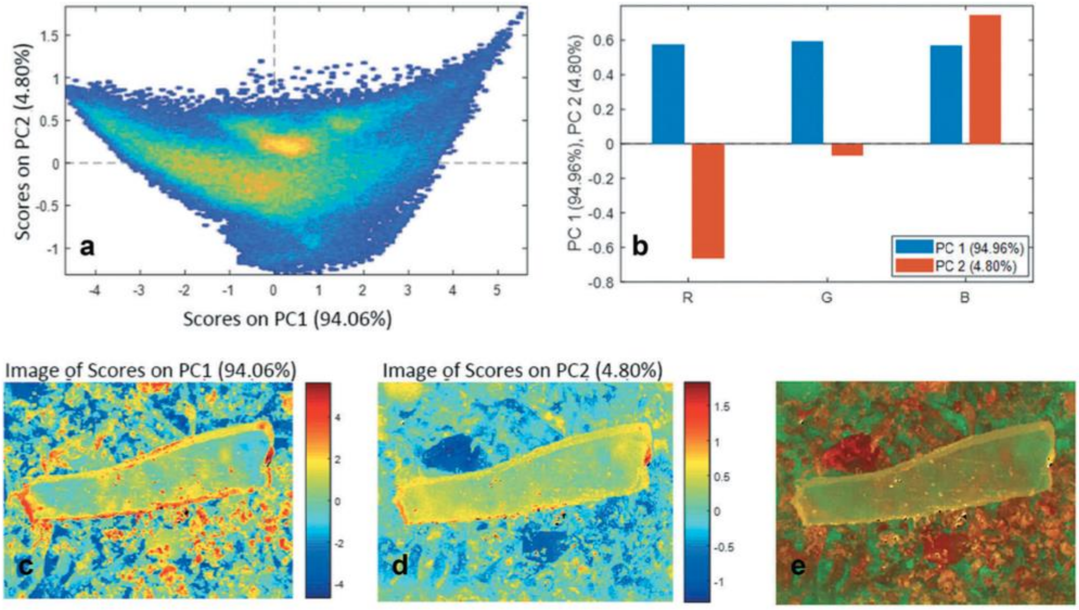


Figure 2: Results of the PCA model calculated on the RGB image of a former food sample with a plastic residue. In a: PC1 – PC2 score plot; in b: PC1 – PC2 loading plot; in c: PC1 score image; in d: PC2 score image and in e: false-colour PC1-PC2 score image.

In summary, the fact that the plastic residue has high positive scores for both PC1 and PC2, can be ascribed to its brighter and more bluish colour with respect to the FFP. To better highlight the colour-related differences between the former food and the plastic residue in this image, we also used PCA on the augmented RGB image. The optimal dimensionality of the PCA model was 3 PCs and accounted for 98.92% of the total variance. In the PC1-PC3 score space reported in Figure 3a, the pixels of the image are grouped into two main clusters. The pixel cluster lying at positive values of both the PC1 and PC3 score vectors is due to the pixels of the plastic particle. In fact, the score images of PC1 and PC3 (Figure 3c, d, respectively) highlight that the pixels of the plastic residue generally have higher scores than the pixels of the FFP. Figure 3e reports the composite false-colour image of the PC1 and PC3 scores, further confirming the differences between the former food and the plastic residue. Since the pixels of the plastic particle have high score values for both PC1 and PC3, the colour- related variables that are the most relevant for detecting the plastic fragment are those with loading coefficients of the same sign (i.e., positive or negative) on both PC1 and PC3. For example, relative blue (rB) has positive loading coefficients for both PC1 and PC3, therefore the pixels of the plastic piece have higher rB values than those of the former food matrix. Conversely, saturation (S) and relative red (rR) have negative loading coefficients on PC1 and PC3, suggesting that the pixels of the plastic residue have lower S and rR values than those of the FFP.

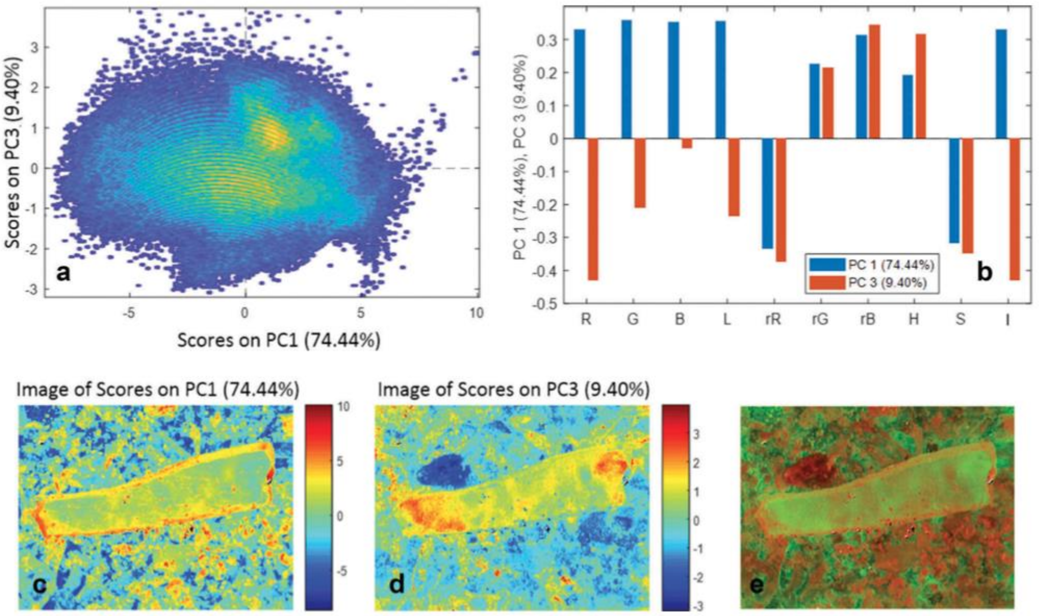


Figure 3: Results of the PCA model calculated on the augmented RGB image of a former food sample with a plastic residue. In a: PC1 – PC3 score plot; in b: PC1 – PC3 loading plot; in c: PC1 score image; in d: PC3 score image and in e: false-colour PC1-PC3 score image.

In order to confirm the results obtained by PCA, Figure 4 a, b show the grey-scale images of the rB and S parameters, respectively. The plastic residue has generally higher rB values and lower saturation values than the food matrix, as previously found by PCA. The presence of the plastic piece is also much more evident in the rB and S grey-scale images than the images of the single RGB channels reported in Figure 1. This suggests that considering additional colour-related parameters better high- lights the image features that are not clearly distinguishable from just the R, G and B values. The histograms of rB and S are reported in Figure 4c, d, respectively. In both cases, the histograms have a bimodal distribution due to the fact that the plastic fragment and the former food matrix have different rB and S values.

In order to verify whether the two peaks in the rB histogram were due above all to the differences between plastic and FFP, a reconstructed image was obtained by visualising in the original RGB domain only the pixels with rB values higher than 0.24 (i.e., the pixels whose rB values fall in the blue area highlighted in Figure 4c), while the remaining pixels are represented in black. As shown in Figure 4e, the majority of the selected pixels belong to the plastic residual. The same procedure was also carried out for the saturation parameter, and in this case only the pixels with S values lower than 0.45 (grey area in Figure 4d) are reconstructed in Figure 4f.

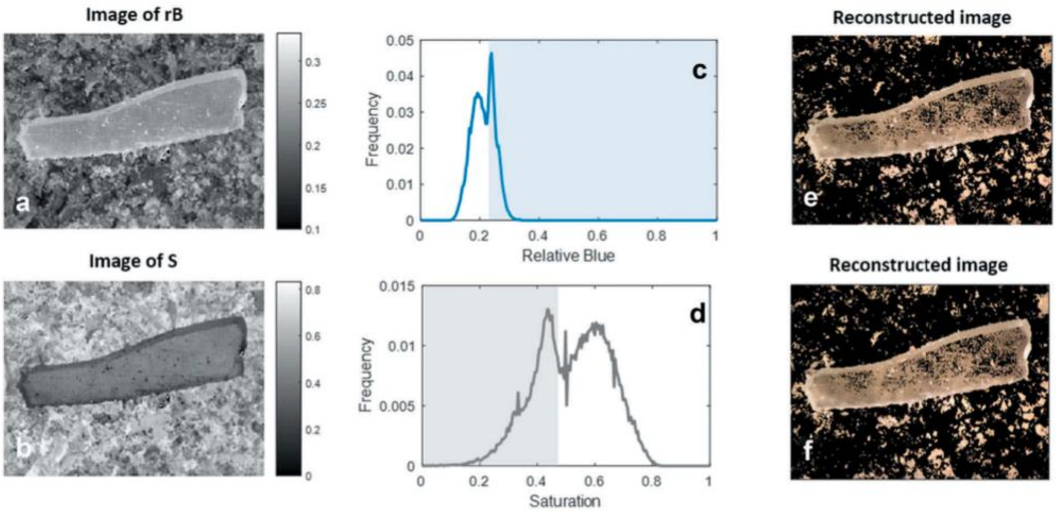


Figure 4: Gray-scale images of relative blue (a) and saturation (b); frequency distribution curves of relative blue (c) and saturation (d); images reconstructed using the selected features of relative blue (e) and saturation (f).

IMAGE-LEVEL ANALYSIS

Concerning the image-level analysis of the whole image dataset, PCA was applied to the colourgram matrix considering 3 PCs, which account for 68.67% of the total variability. In the PC1-PC2 score plot reported in Figure 5a, each object represents the colourgram of one image, and the objects are coloured according to the corresponding former food sample, while the marker indicates the presence and nature of the packaging residues. For a better interpretation of the results, the labels in the score plot indicate the names of the corresponding images, and the most relevant sample images are also reported. PC1 separates the images based on the former food type, from the images of sample E to the images of samples A and B. In fact, the former food matrices of sample E have a darker colour, while the former food matrices of samples A and B have a lighter colour. The trend observed along PC2 suggests that this principal component differentiates between the images according to the presence of white packaging residues, which are primarily due to paper and plastic. In fact, the images with higher PC2 score values included white packaging residues with higher dimensions. As shown in the PC2-PC3 score plot in Figure 5b, one image of sample F shows a particular behaviour along PC3, with a much higher score than the other images. In fact, this image contains an aluminium residual with a blue spot, which is not present in the other images with aluminium particles. Figure 5c reports the Hotelling T^2 values and Q residuals of the PCA model. The Hotelling T^2 values measure the distance of the samples from the centre of the model (i.e., the origin of the PC space), and therefore describe the variation of each sample within the model. Conversely, the Q residual values indicate how much the description of each sample by the PCA model differs from its actual values. In other words, samples with high Q residual values show anomalous features, which are not accounted for by the PCA model. Figure 5c highlights three outlier images: Image 3 has a higher Hotelling T^2 value than the corresponding 99.7% confidence limit, while Image 50 and Image 32 have Q residuals exceeding the 99.7% confidence limit. Image 32 has the highest Q residual value, while the corresponding Hotelling T^2 value falls within the 95% confidence limit, thus suggesting that this image has particular features that were not described by the PCA model. In fact, this image shows a red plastic packaging residue, while all the other images with plastic pieces show white or semi-opaque particles.

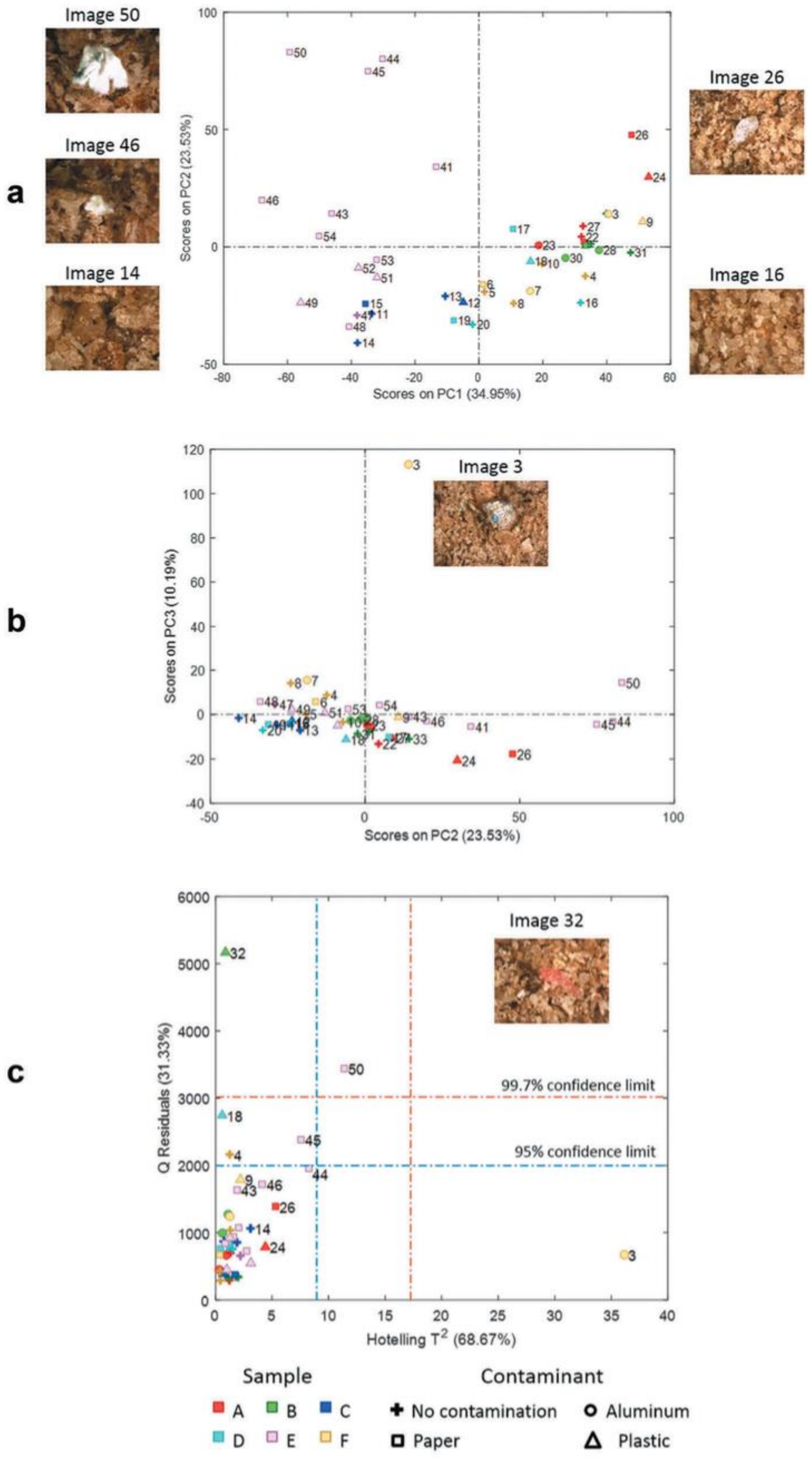


Figure 5: Results of the PCA model calculated on the colourgrams matrix. In a: PC1-PC2 score plot; in b: PC2-PC3 score plot; in c: Hotelling T2 and Q residuals plot.

In order to evaluate the colour features with the greatest influence on the Q residual value of Image 32, the corresponding contribution plot is shown in Figure 6a. The colourgram regions with the highest contributions are related to the relative green, hue and PC2 and PC3 score vectors of the PCA models calculated with the various preprocessing methods. As in Figure 4, these colour features can be visualised in the original image domain. For example, considering the rG parameter, the peak in the Q contribution plot falls within the 1341–1358 colourgram interval (highlighted in green in Figure 6a), which corresponds to rG values ranging from 0.24 and 0.30. Figure 6b reports Image 32 in the original RGB colour domain, while Figure 6c shows the same sample image in which only the pixels falling in the rG interval previously selected are displayed and the remaining pixels are represented in white. The reconstructed pixels are mainly related to the red plastic piece, confirming that the high Q value of this image is due to the presence of the red packaging residual.

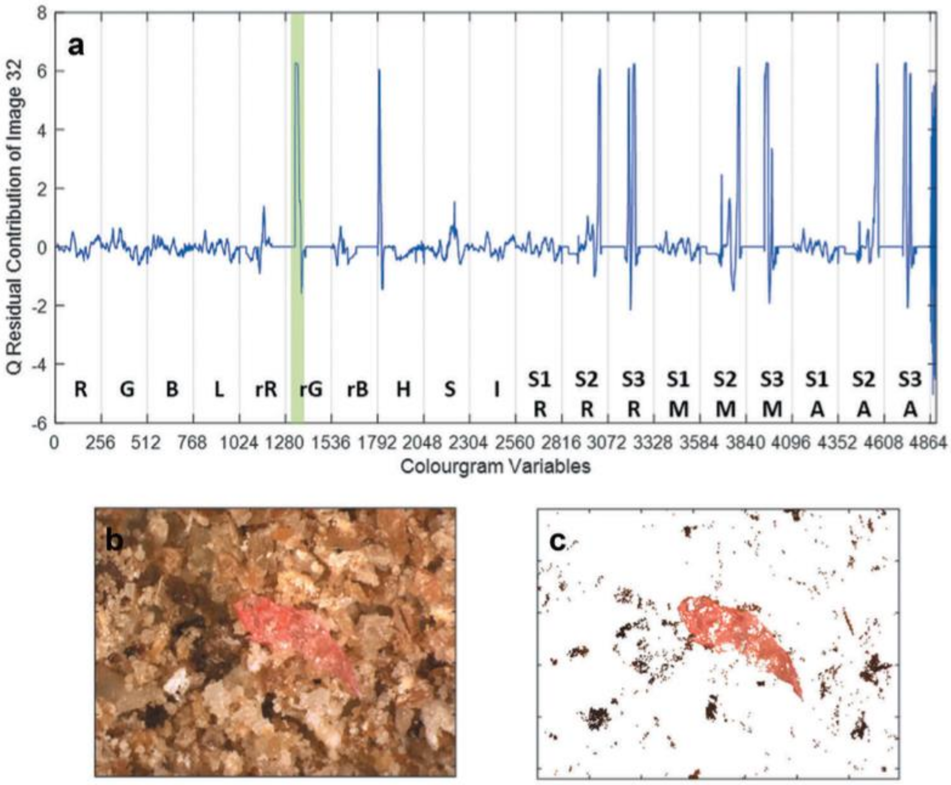


Figure 6: Q residual contribution plot of Image 32 (a), original Image 32(b), and Image 32 reconstructed using the selected features (c).

DISCUSSION

We investigated the potential of RGB imaging coupled with multivariate image analysis strategies for detecting packaging remnants in FFPs. Multivariate image analysis was conducted considering two different approaches: pixel-level analysis and image-level analysis. The pixel-level approach (Esbensen & Geladi, 1989; Prats-Montalbán et al., 2011) mainly focused on characterising the individual pixels in an image and in grouping pixels that share similar features. This approach was tested considering both simple RGB images and augmented RGB images obtained by including additional colour-related parameters in the analysis. The results suggest that including additional colour features in the analysis better highlights the differences that are not clearly visible considering the RGB values alone, in particular when the objects that need to be separated have similar colours. The image-level approach simultaneously compares all the images of the dataset, allowing from tens up to hundreds of RGB images to be analysed together (Antonelli et al., 2004; Ulrici et al., 2012; Giraudo et al., 2018; Orlandi et al., 2018a-b). The conversion of the RGB images into colourgrams (Antonelli et al., 2004), highlighted both groups of images with similar colour-related features and outlier images, e.g., those containing packaging particles. The PCA model calculated on the colourgram matrix showed that the first source of variability in the image dataset was related to the different colour of the former food matrices from different samples. This suggests that in practical scenarios, the development of specific models for each FFP type may lead to more accurate and reliable results. In this case, it will be necessary to acquire and analyse an adequate number of samples representative of each former food type. PCA also highlighted common trends in images with white residues derived from paper or plastic packaging materials and to identify images showing particular features due to the presence of aluminium or differently-coloured plastic remnants. Outlier images can be easily detected considering the Hotelling T^2 values and Q residuals, which can be used to build multivariate control charts or classification models capable of automatically detecting images with packaging remnants. However, RGB imaging only accounts for colour properties of the imaged samples, which is one limitation of this technique in the detection of packaging materials in FFPs. In fact, in some cases, the packaging residues may be too similar in colour to the FFP particles, making them difficult to identify (Tretola et al., 2017a-b). For example, paperboard is particularly difficult to differentiate from feed material, making its detection complicated and time consuming. In order to overcome this issue, the stereomicroscope could be coupled with more advanced imaging systems capable of detecting light also beyond the visible spectral region, including for example the near infrared spectral region (Gowen et al., 2011; Ferrari et al., 2013; Dale et al., 2013; Ulrici et al., 2013; Amigo et al., 2015; Calvini et al., 2016; Calvini et al., 2018; Vermeulen et al., 2017). The colour features of the samples derived from RGB images could be combined with spectral features derived from multispectral or hyperspectral images, thus leading to a more comprehensive characterisation of the differences between former food matrices and residues of packaging materials.

CONCLUSIONS

The present work is a preliminary study focused on the potential of imaging methods applied to feed and food safety. Identifying packaging remnants in former food products is important in ensuring the safety of FFPs used as feed ingredients. Generally, control procedures for the determination of packaging residues in feed are based on the visual inspection and manual selection of undesirable contaminant materials. To overcome the drawbacks of these procedures, we have explored the feasibility of using RGB imaging as a rapid and non-destructive tool for the automated detection of packaging particles in FFPs. In this paper, we have assessed the various features of this technique, together with the challenges related to the application of image analysis strategies. The preliminary results obtained in this study demonstrate the potential of the proposed approach, in particular when the colour of the undesirable packaging residues can be differentiated from the colour of the ex-food matrix. In order to develop more robust and sensitive models, we plan to increase the size of the image dataset by acquiring a higher number of images representative of each FFP type.

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Declaration of interest statement: The authors declare that they have no conflict of interest.

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CHAPTER 5

Former foodstuff products (FFPs) safety: methods for packaging remnants detection. A review.

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Abstract

Introduction. Former foodstuff products (FFPs) represent a valuable biomass in terms of conversion of food industry leftover into animal feed, circular economy and sustainability. The main concern about the use of FFPs as feed regards their safety. The problem arises during the processing of FFPs into feed, where leftovers from food industry are often ground with their packaging. Once grounded, different technologies are applied to remove the biggest parts of the packaging, while smaller particles of wrapping materials become part of the final product, leading to a potential feed contamination. Then, beyond their ascertained nutritional values, the use of FFPs in animal nutrition implies also a proper safety evaluation. Thus, packaging remnants in FFPs-derived feed need to be constantly monitored.

Literature. To assure a proper risk evaluation and risk assessment about the presence of packaging remnants in FFPs, different techniques tested for the detection of packaging in feed are summarized in this review. The stereomicroscope was one of the first method applied. However, this visual operator inspection can be time-consuming, unpredictable and non-consistent. Visual inspection, Computer vision (CV) and multivariate image analysis (MIA) methods were subsequently tested. These methods are based on the digital image acquisition of the FFPs samples, before to analyze the colour spectrum of pixels of the obtained images by a specific software. These two methods have shown to be more objective, faster and innovative compared to the stereomicroscope. The characteristic of some packaging remnants to release volatile organic compounds has been used to test the ability of the electronic nose (E-nose) to discriminate the presence or absence of packaging in feed samples.

Conclusion. Combining the different methods could be the most effective solution to evaluate these products in a safe way. All the proposed tools mostly work following a qualitative approach. Further strategies need to be tested to quantify the packaging remnants in FFPs-derived feed and to allow a proper feed safety evaluation as requested by feed industries.

Résumé

Introduction. Les anciens produits alimentaires (FFPs) représentent une biomasse précieuse en termes de conversion des restes de l'industrie alimentaire en aliments pour animaux, d'économie circulaire et de durabilité. La principale préoccupation par rapport à l'utilisation des FFPs comme aliments pour animaux concerne leur sécurité. Le problème survient lors de la transformation des FFPs en aliments pour animaux, où les restes de l'industrie alimentaire sont souvent broyés avec leur emballage. Une fois broyés, différentes technologies sont appliquées pour retirer les plus grosses parties de l'emballage, tandis que les plus petites particules de matériaux d'emballage deviennent partie intégrante du produit final, ce qui mène à une contamination potentielle des aliments pour animaux. Ensuite, au-delà de leurs valeurs nutritionnelles avérées, l'utilisation des FFPs dans l'alimentation animale implique également une évaluation adéquate de la sécurité. Ainsi, les restes d'emballage dans les aliments dérivés des FFPs doivent être constamment surveillés.

Littérature. Afin d'assurer une évaluation correcte des risques liés à la présence de restes d'emballages dans les FFPs, les différentes techniques testées pour la détection des emballages dans les aliments pour animaux sont résumées dans cette revue. Le stéréomicroscope a été l'une des premières méthodes appliquées. Cependant, cette inspection visuelle par un opérateur peut prendre beaucoup de temps, être imprévisible et manquer de régularité. Les méthodes de vision par ordinateur (CV) et d'analyse multivariée d'images (MIA) ont ensuite été testées. Ces méthodes sont basées sur l'acquisition d'images numériques d’échantillons de FFPs, avant d'analyser le spectre de couleurs des pixels des images obtenues par un logiciel spécifique. Ces deux méthodes se sont révélées être plus objectives, plus rapides et plus innovantes que le stéréomicroscope. La caractéristique de certains restes d'emballage de libérer des composés organiques volatils a été utilisée pour tester la capacité du nez électronique (e-nose) à discriminer la présence ou l'absence d'emballage dans les échantillons d'aliments pour animaux.

Conclusion. La combinaison des différentes méthodes pourrait être la solution la plus efficace pour évaluer ces produits en toute sécurité. Tous les outils proposés fonctionnent principalement selon une approche qualitative. D'autres stratégies doivent être testées pour quantifier les restes d'emballage dans les aliments dérivés des FFPs et pour permettre une évaluation correcte de la sécurité des aliments pour animaux, comme le demandent les industries de l'alimentation animale.

INTRODUCTION

Recently, the growth of global population became a key of the great demand of food productions. This trend implies increasing challenges for sustainable agriculture and livestock production (Tretola et al., 2017a; Tretola et al., 2019). In the next future, the animal protein demand will increase and sustainable livestock farming must improve food security, nutrition and healthy diets, animal health and welfare, and address climate change issues (Smarason et al., 2019). For these reasons, in the past 60 years animal diets have undergone substantial changes, especially regarding the use of alternative ingredients to limit the use of corn and other standard cereals, favoring other biomasses such as former foodstuff products (FFPs). According to the UE Regulation 68/2013, FFPs "*are foodstuffs, other than catering reflux, which were manufactured for human consumption in full compliance with the EU food law but which are no longer intended for human consumption for practical or logistical reasons or due to problems of manufacturing or packaging defects or other defects and which do not present any health risks when used as feed*" (Reg. 2017/1017 – UE). The FFPs have a high nutritional potential considering nutrients and energy contents (Giromini et al., 2017). Their nutrient composition is comparable to cereals commonly used in animal nutrition, with the exception of the fat content that is usually higher in FFPs (Pinotti et al., 2021). The main limiting factor for the use of FFPs in Europe is the lack of information about their nutritional properties and their safe use in animal's diets (Pinotti et al., 2021). From Circular Economy point of view, by using FFPs is possible to reduce food losses, since these ingredients are suitable for animal feed, especially for pigs, poultry and young animals (Tretola et al., 2019; Pinotti et al., 2021). The process to transform FFPs into animal feed ingredient not always include the elimination of the food packaging, that could be ground together with the food (Tretola et al., 2017b). With different technological processes, feed industry routinely removes the packaging from the food losses during their processing.

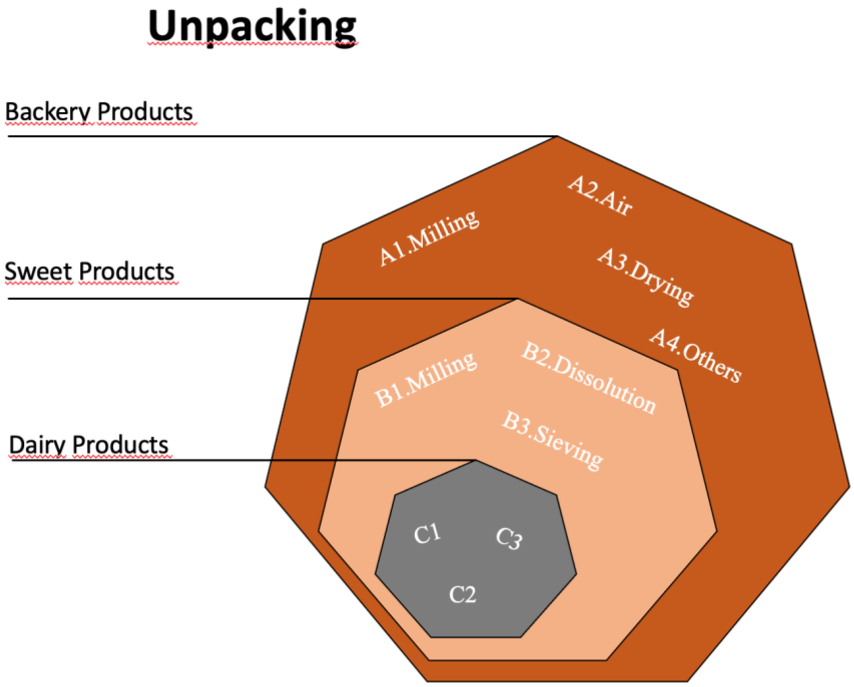

Figure 1. A1. Coarse milling; A2. Removal of most of the packing material with blown air or sieving; A3. Drying (if necessary); A4. Removal of the remaining packaging materials (plastic and paper) with blown air, ferrous metals with magnets, non-ferrous metals with eddy current separetor. B1. Coarse milling; B2. Dissolution in water; B3. Sieving.C1. Scrushing; C2. Sieving; C3. Further sieving or centrifugation if necessary.

However, it is not always possible to separate and remove small packaging remnants from food. Those packaging remnants residue in the final products and then in the animal feed. As reported elsewhere (Pinotti et al., 2019; Pinotti et al., 2021), the main concerns about safety of these products are the occurrence of food packaging remnants. Packaging remnants mainly include plastics, aluminum foils and board materials (Amato et al., 2017). Indeed, the major problem for feed safety is related to the presence of microplastics, which have a dimension between 350 micrometers and 5 millimeters (Frias et al., 2019) and that could be originated by plastic fragmentation during the processing (Rainieri et al., 2018). Even if plastic particles with a size equal or higher then 1-2mm can be easily detected by visual inspection, their detection and quantification through this method can be difficult when the remnants size is lower. In this respect, van Raamsdonk et al. (2011 - 2012), provided a method to identify the risk evaluation ad risk assessment of packaging materials in FFPs. Even if FFPs are used to improve the sustainability in animal nutrition, in the UE regulation 767/2009 there is a ban including a “*List of* *materials whose placing on the market or use for animal nutritional purposes is restricted or prohibited as referred to in Article 6. Packaging from the use of products from the agri-food industry, and parts thereof."* To interpret this ban, a correct risk assessment must be made.

A more sensitive and objective method of detection is then mandatory to trace and quantify packaging remnants in FFPs. This review wants to describe other innovative methods tested to evaluate the presence of packaging remnants in FFPs. In particular, it focuses on visual inspection (van Raamsdonk et al., 2012), stereomicroscopy associated to computer vision (CV) (Tretola et al., 2017a), multivariate image analysis (MIA) (Calvini et al., 2020a) and electronic nose (e-nose) (Tretola et al., 2019), and how they can be applied in feed and food quality and safety assessment.

LITERATURE

VISUAL INSPECTION

van Raamsdonk et al. (2011; 2012) proposed a non-chemical and semi-destructive method to detect and quantify packaging remnants in bakery products, including sweet bread and raisin bread. With eye examination, the basic principle is to detect and separate every particle that is considered by the operator not native to the sample. This method is considered laborious and subjective, because it depends by the ability of the operator to correctly recognize the packaging remnants. This method, in fact, is based on the visual selection of the "presumed" packaging remnants. Then, the collected packaging remnants are weighted, defatted, dehydrated and finally weighted again (van Raamsdonk et al., 2012; Tretola et al., 2017a-b). In these preliminary studies, van Raamsdonk et al. (2011; 2012) analyzed 243 samples, and more than 90% of them showed a level of presumed contamination, with remnants packaging material, under the level of 0.15% w/w. These particles were defined "presumed residual" because was not possible to identify the original packaging materials. The major problem for the proposed method indeed, was to characterized these remnants to be sure about their origin and nature. Some modifications appeared to be necessary, especially to the fraction of the matrix with particles smaller than 1 mm and to the cleaning of the particles of the packaging contaminants. The size of the detected remnants collected by this visual method is usually bigger than 800 µm (van Raamsonk et al., 2012; Tretola et al., 2017a). Amato et al. (2017) validated a fast and sensitive gravimetric method, fit for routine official controls, for the determination of packaging residues in feed, based on RIKILT procedure. Starting from a pelleted sample, sieving method was performed and each fraction was examined and all packaging materials were collected. Different parameters were used (specificity, limit of quantification, recovery, repeatability, reproducibility and measurement uncertainty) to perform this validated method (Amato et al., 2017). Moreover, to help the operator to perform a better visual selection of packaging remnants, stereomicroscopy it also used (Figure 2 and 3). Stereomicroscope works on low magnification observation of a sample, using light reflected from the surface of an object rather than transmitted through it. Recently, this instrument was used in food industry, for inspection and quality control (Chan et al., 1991) Even with this technology, results obtained by the visual inspection of FFPs with the use of a stereomicroscope, highly depend by the ability of the inspector to correctly recognize and quantify the different remnants (Tretola et al., 2017b). Among this material, paper, plastic and microplastics were the most common. Microplastics contamination is the most addressed in the recent years (van Raamsdonk et al., 2020), even if toxicity assays that use concentrations over 100,000 times higher than those expected in the environment have limited practical relevance. Thus, adverse effects on animal and human health of current former food concentrations can be considered neglectable (Prata et al., 2021). However, the visual inspection can be then unpredictable, time consuming, and non-consistent (Mahendran et al., 2011) and alternatives need to be found.

Figure 2. Example of image of feed sample and a piece of gray/transparent packaging material, obtain with the use of stereomicroscope and high-resolution CCD camera (CoolSNAP-Pro colour camera).

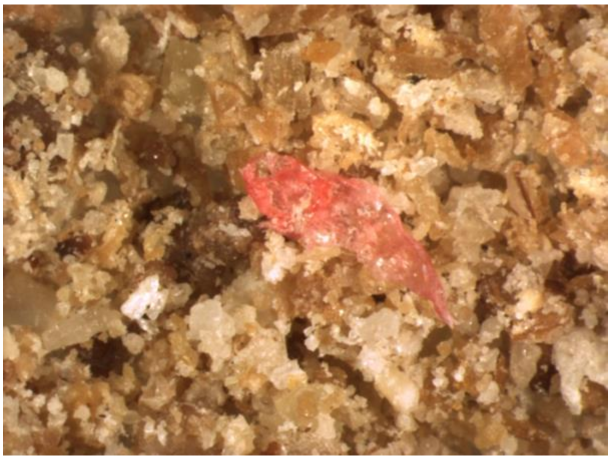

Figure 3. Example of image of feed sample and a piece of red/transparent packaging material, obtain with the use of stereomicroscope and high-resolution CCD camera (CoolSNAP-Pro colour camera).

COMPUTER VISION

The Computer Vision (CV) is an instrument composed by a light chamber with a controlled white LED light, equipped with software-controlled CMOS camera able to obtain pictures of 16 million colors. The instrument is connected to a software for system monitoring, data acquisition and multivariate statistics processing.

Tretola et al., (2017a-b) demonstrated that CV could be a well-adaptable qualitative approach for the packaging remnants detection in FFPs. A key factor in this analysis is the white lighting condition (Tretola et al., 2017b). Indeed, the efficiency of CV strongly depends on optimal illumination conditions and intensity of light (Di Rosa et al., 2017). The standard protocol applied for the investigation of packaging remnants in FFPs by using the CV can be summarized as follow: *(i)* pictures of FFPs samples are taken by the use of a high resolution CDD (charge coupled device) digital camera. *(ii)* The scanned image needs to be preprocessed before to be analyzed in order to improve the image quality and details, after that *(iii)* the picture is divided in regions related with areas of interest and then *(iv)* the system uses statistical analysis and neural networks to obtain information about feed texture and grading (Tretola et al., 2017b).

Unfortunately, given the small size of packaging remnants in FFPs, camera of the CV is not able to obtain pictures with a magnification that allow a proper image analysis. For this reason, pictures of FFPs with higher magnification needed to be obtain by the use of the stereomicroscope (Tretola et al., 2017a-b).

During image analysis, the CV captures the intensity of the light in red, green and blue spectrum, obtaining the information about color of each pixel. For each picture, the color spectrum of the sample is represented by a histogram. Starting from the color spectrum derived from sample pictures, CV is able to formulate the Statistical Quality Control chart, which include conforming and non-conforming areas. An example is reported in figure 4. Since the sample analyzed has a high variability, a training phase is required to relate the variability of the product to the sensor data recorded by the analysis system. At the end of the process, the software makes a distinction between conforming training samples and the unknown sample (Tretola et al., 2017b). Packaging remnants grounded together with FFPs could be of many different colors. For this reason, is difficult to distinguish them from the background feed (Tretola et al., 2017a). A strategy to discriminate the packaging remnants from the feed background was to evaluate the presence of a discriminant color, indicated by the software with a code, which was present only in the picture’s pixels displaying packaging material but not feed. Comparing the color codes of each pixel from several pictures of standard FFPs samples and FFPs samples from which packaging was carefully removed by using the stereomicroscope, the authors found a discriminant code that can be related to the specific presence of aluminum in feed samples (Tretola et al., 2017b). Therefore, based on the presence in the FFPs color spectrum of a discriminant color code, CVs is able to recognize the presence of packaging remnants (specifically aluminum) in pictures of contaminated FFPs samples (Figure 4).

In light of this, CV could be considered a faster qualitative screening approach, useful to simplify the human effort in visual involvement. However, this approach is possible only when pictures of FFPs samples are obtained by using the stereomicroscope and cannot be used to evaluate the presence of all kind of packaging remnants (Tretola et al., 2017b).

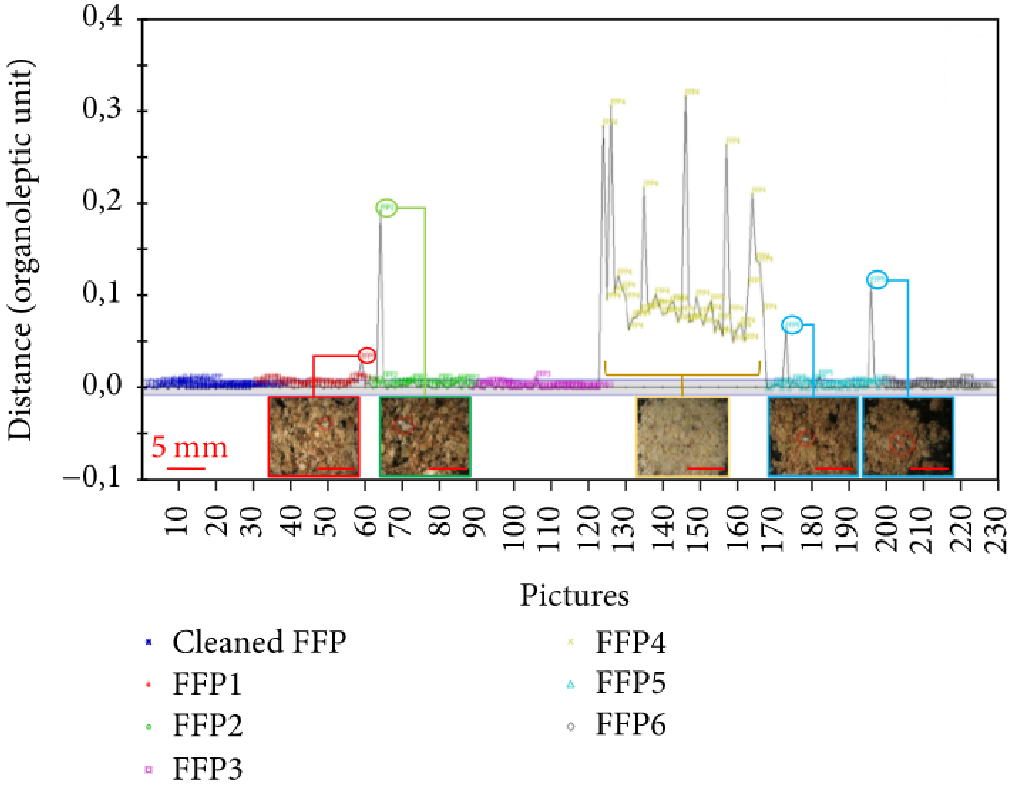

Figure 4. Statistical Quality Control Chart with conforming and non-conforming areas. The grey band correspond to the presence of the cleaned FFP sample, while symbols outside the grey range indicate sample's pictures with the packaging contamination, basing on the presence in the FFPs color spectrum of the discriminant color code (Adapted from Tretola et al., 2017a)

MULTIVARIATE IMAGE ANALYSIS (MIA)

Multivariate image analysis (MIA) is based on the application of classical multivariate statistical methods to the analysis of images (Geladi et al., 2000). This methodology can work on images with more than one channel per pixel, like e.g., the three red, green and blue (RGB) channels in color images, or the spectral channels in multispectral and hyperspectral images. The MIA can be used for different purposes, including classification, segmentation, defect detection and even prediction of quantitative parameters (Prats-Montalban et al., 2011). He et al. (2015), applied MIA to detect banned food additives because of the recent increasing attention in food safety. Calvini et al., (2020a), showed the potential of MIA to detect packaging remnants using RGB images of FFPs acquired by a stereomicroscope equipped with a digital camera. In the study of Calvini et al., (2020), six different commercial samples of FFPs coming from food companies of two different countries were considered. The MIA was applied following two different approaches, i.e., pixel-level analysis and image-level analysis. All samples included different food materials (broken biscuits, chocolates, croissants, bread, rice cakes, breakfast cereals), and contained also particles of packaging remnants, consisting of paper, plastic and aluminum. For image acquisition, aliquots of 5 g of the FFPs samples were placed in a petri dish, to form a single layer. A variable number of images (ranging from 5 to 13) was taken for each sample, considering sample aliquots both with and without packaging remnants. Firstly, each single image was analysed at the pixel-level using Principal Component Analysis (PCA), in order to highlight similarities and differences among the pixels related to the former food matrix and those related to the packaging remnants, based on their colour features. The PCA was applied both to the RGB image "as is" as well as to the augmented RGB image, that was obtained by considering additional colour-related channels derived from the RGB values. In addition, the whole dataset of images was also analysed at the image-level, considering the colourgrams approach (Calvini et al., 2020b), which is a multivariate data dimensionality reduction method that allowed to identify outlier images of former food due to the presence of packaging particles. The results suggested that including in the analysis additional colour features derived from the RGB channels allows to better highlight the differences that are not clearly visible considering the RGB values alone, in particular when the objects that need to be separated have similar colours. In practical scenarios, the development of specific models for different FFPs types may lead to more accurate and reliable results.

ELECTRONIC NOSE (E-NOSE)

Another recent, fast and objective method to search out extraneous materials in both food and feed is the electronic nose (e-nose) (Tretola et al., 2019). Figure 5 shows an example of a portable e-nose with the associated software for data analysis. E-nose through non-specific chemical detectors, simulates the olfactory system of humans and it is useful to identify and quantify simple and complexes odours and aromas, but also to discriminate between a wide range of odours (Persaud et al., 1982). These detectors interact with volatile organic compounds (VOCs) of the analyzed sample and the output is an electronic signal. This signal originates from the interaction between semi-selective sensors with VOCs and it can be considered as a fingerprint of the volatile molecules associated to the sample itself (Di Rosa et al., 2017). The system uses glass vials which contain air with accumulated VOCs derived from each analyte and through the use of a needle stacked in the cap of the vial, a gas sample is pumped to the e-nose sensors. Thanks to this procedure, the sensors are able to correctly identify the presence of packaging materials in samples characterized by the same matrix, and consequently by the same volatile organic compounds profile (Tretola et al., 2019). Basically, plastics, paper and aluminum foils release their own volatile compounds and the instrument uses VOCs profiles as markers for detecting different concentrations of packaging remnants in the analysed samples (Tretola et al., 2019). These results have shown that e-nose was able to detect the presence/absence of packaging materials in FFPs samples which had the same matrix and the same VOCs profile. Therefore, the instrument discriminated the cleaned samples from the contaminated ones when these had the same odour background (Tretola et al.,2019). In fact, the presence of presumed packaging remnants is more reliable when the feed matrix has a low variability (e.g., same batch, composed by the same ingredients, same odour prints etc.) (Cheli et al., 2008). It follows that packaging remnants can vary a lot, also because they are treated with various ink for printing and solvents that could influence the sample odour profile. In these cases, the screening ability of e-nose could be lower. At the same time, the results can be justified also by the limited quantity of presumed packaging remnants whose odour is covered by volatile compounds originating from the feed matrix (Tretola et al., 2019). In light of this, e-nose can be used as a supporting instrument to facilitate the activity at the stereomicroscope, could reduce working time and increase the objectivity of the analysis (Tretola et al., 2019).

Combining all of these informations, it can be suggested that e-nose is a modern analytical approach that has a big potential in addressing authenticity, quality and safety of food/feed and beverage (Di Rosa et al., 2017). In the field of feed safety, a previous study (Campagnoli et al., 2004) has shown that e-nose is well exploitable also to detect and recognize processed animal protein in feed, in order to ensure both animal and human safety. In terms of public health concerns and economic and safety impact its potential use in the rapid detection of mycotoxins in cereals was also reported (Cheli et al., 2018; Ottoboni et al., 2018).

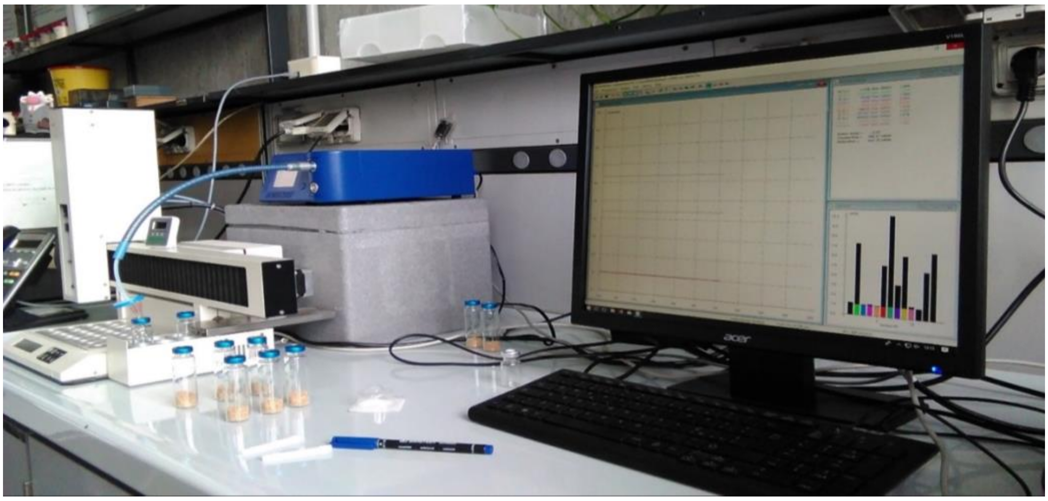

Figure 5. Portable e-nose technology used to search out extraneous materials from FFPs.

CONCLUSIONS

The increasing attention to the safety inspection of FFPs had led to use different methods that can be precise and effective to detect packaging materials. The present review reported different methods used to detect remnants in FFPs. As reported in Table 1, using stereomicroscope with a digital camera can be not useful and exhaustive. This method alone caused an underestimation of the remnants, correlated to the laborious visual analysis by the operator. Associating the stereomicroscope with other methods, such as the computer vision (CV) or multivariate image analysis (MIA), could lead to a more precise and complete analysis. The combination of stereomicroscope, digital camera and CV can estimate the contamination of FFPs samples more comprehensively in a short time with high-accuracy. Moreover, the same accuracy can be reach by the association of stereomicroscope and digital camera to MIA.

A completely different method of analysis is the E-nose, an array of electronic chemical sensors with different selectivity patterns.

All these technologies are useful for a qualitative estimation of packaging remnants in FFPs. However, food and feed safety industries require methods of analysis that can quantify and characterize these remnants in feed and further research need to be focused on this objective.

METHODS	***ADVANTAGES***	***DRAWBACKS***
Stereomicroscopy	• Quantification • Evaluation of heterogeneous distribution • Partial identification of presumed packaging remnants origin	• Underestimation • Laborious/time consuming • Operator dependent
Computer Vision	• Rapidity • Objectivity • Sensibility • Remote sample image analysis	• Artificial lighting needed for dim or dark conditions • No quantification • To be associated with stereomicroscope for a proper image acquisition • No determination of packaging remnants nature
Multivariate Image Analysis	• Performing, fast and non-invasive low-cost analysis • Easy differentiation between packaging residues colour from FFPs matrix colour • Can be used in a wide range of new applications	• Complex method which requires statisticians/experts • Can be complex as it deals with large amount of data • Problems in image acquisition. Not all the images are ideal when they are digitalized • No determination of packaging remnants nature
Electronic nose	• Great potential to discriminate experimentally cleaned samples from the standard and spiked samples	• Necessary to clarify the nature of the VOCs released by the packaging remnants • Results affected by the feed matrix • No determination of packaging remnants nature

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CHAPTER 6

Sweet vs. Salty Former Food Products in Post-Weaning Piglets: Effects on Growth, Apparent Total Tract Digestibility and Blood Metabolites

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Simple Summary: Nowadays, researchers need to find a solution to the growing demand for sustainable animal productions. Livestock animal's nutrition is the component with major impacts on environment and economy. The biggest challenge is to find alternative feed ingredients to minimize and valorize the food leftovers. Food industry leftovers, also called former food products, could be a valid alternative to grains in young pigs' nutrition. From a nutritional point of view, these ingredients are very similar to standard cereals, like corn. The results from this study suggest that a partial substitution of standard ingredients with two different sources of former food products in the diets of post-weaned pigs is possible, without any negative effects on growth performance and health of animals.

Abstract: Former food products (FFPs) have a great potential to replace conventional feed ingredients. This study aimed to investigate the possibility to partially replace standard ingredients with two different types of FFPs: bakery (FFPs-B) or confectionary (FFPs-C) FFPs and their effects on growth performances, feed digestibility and metabolic status in post-weaning piglets. Thirty-six post-weaning piglets were randomly assigned to three experimental diets (n = 12 per diet) for 42 days: a standard diet (CTR), a diet where 30% of standard ingredients were replaced by confectionary FFPs (FFPs-C) and a diet where 30% of standard ingredients were replaced by bakery FFPs (FFPs-B). Individual body weight and fecal dry matter were measured weekly. Feed intake (FI) was determined daily. Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated. Fecal samples were collected daily for three days/week to determine apparent total tract digestibility of dry matter (ATTD). At day 0, 21 and 42, blood samples were collected from all the piglets. No significant differences ($p > 0.05$) between groups were found in growth performances and metabolic profile. However, ATTD in FFPs-B group was lower ($p < 0.05$) compared to the CTR group at the end of the experiment. This study confirmed the possibility to formulate homogeneous diets integrated with 30% of both categories of FFPs. Further investigations are needed to clarify the effects of bakery former food products on the digestibility of the diet.

INTRODUCTION

The loss of natural resources within the food cycle is a global problem. An estimated 1.3 billion tons of food are wasted or lost every year (Gustavsson et al., 2011). This amount represents one-third of all that is produced for human consumption (Gustavsson et al., 2011). The 2030 Agenda for Sustainable Development reflects the increased global awareness of the problem. One target of the FAO Sustainable Development Goals calls for halving per capita global food waste at retail and consumer levels by 2030, as well as reducing food losses along the production and supply chains (FAO, 2019). Fighting food waste and increasing the sustainability starting from food production could be a valid contribution, but it is certainly one of the biggest challenges we are called to participate in (Gasco et al., 2020). In this scenario, animal nutrition researchers are focusing their attention on the use of food losses in animal diets as a valid alternative to standard ingredients like corn or wheat (Pinotti et al., 2021). Indeed, food losses from these food industries are converted into ingredients for the feed industry and are re-entered in the food chain with a circular economy vision (Pinotti et al., 2021). Food losses, also called former food products (FFPs), ex-food, food leftover or bakery meal, have been demonstrated to represent valid and authorized (Pinotti et al., 2021; UE Commision, 2017; EFFPA, 2019) ingredients from a nutritional point of view for both monogastrics and ruminants (Luciano et al., 2020). The practice to replace cereals or other standard ingredients with FFPs in animal diets is increasing thanks also to the rising knowledge about their properties. In this sense, the inclusion of FFPs in feed and in the livestock supply chain is a good compromise for improved livestock sustainability (Luciano et al., 2020). For a better investigation of the FFPs properties, these products have been classified into two main categories: bread, pasta and salty snacks from bakery production (FFPs-B) and chocolates, biscuits and sweet snacks from confectionary production (FFPs-C) (Luciano et al., 2020). Recent studies analyzed FFPs for their nutritional, functional, chemical composition and digestibility features (Pinotti et al., 2021; Ottoboni et al., 2019; Tretola et al., 2019; Gao et al., 2019). They have been defined as a “fortified version of common cereals'', because of their higher metabolizable energy, fat and starch content on dry matter basis, compared with traditional feedstuffs. In vitro studies on carbohydrate digestibility showed that, compared to common cereals, FFPs are characterized by a higher digestibility potential due to the presence of readily available simple sugars and processed starch (Pinotti et al., 2021; Casas et al., 2018). The high digestibility of FFPs is probably related to the industrial processes they undergo such as heat and mechanical treatments. The processing led to a starch gelatinization which facilitates the enzymatic hydrolysis of starch increasing the product digestibility. On one hand, the high digestibility of FFPs can be considered an advantage, especially for young animals characterized by a lower ability to digest nutrients compared to older animals (Tretola et al., 2019). On the other hand, gut health could be affected by the lower amount of undigested particles that can reach the large intestine as substrate for bacterial growth. The high predicted glycemic index (pGI) observed when FFPs were included in a complete diet for monogastric animals (Ottoboni et al., 2019) could result in a potential more rapid return to a state of hunger of the animal, improving the feed intake. Concerns could be related to the nutritional diarrheas due to the high concentration of simple sugars, which may disturb the osmotic balance across the enteric epithelium, causing excessive water secretion and loose stools (Gao et al., 2019). Therefore, more information about digestibility, pGI and other properties of diets including FFPs is needed to improve the formulation of more accurate and balanced diets for young pigs that must adapt their digestive enzymes to new alternative dietary components (Casas et al., 2018). Filling the lack of knowledge about the effects of FFPs on animal performances and health is essential to increase their use in animal nutrition for a new and sustainable livestock nutrition. A recent study on post-weaning piglets demonstrated that FFPs can partially substitute conventional ingredients without detrimental effects on apparent total tract digestibility (ATTD), growth performance or hematological parameters in the short period (16 days) (Tretola et al., 2019). Another study tested co- products from rice milling industry in diets for pig weanling, resulting in an improved growth performance of the animals fed experimental diets (Casas et al., 2018). In a similar trial, co-products including rice hulls, rice bran and broken rice were included in diets for nursery pigs with no damaging effects on growth performance (Casas et al., 2015). To our knowledge, no studies investigated the effects of high inclusion (more than 30%) of sweet and salty FFPs in piglets' diets during the entire post-weaning period. Based on all the above-mentioned information, the objective of this study was to partially replace common ingredients (30% on dry matter basis) with FFPs in pig diets and to evaluate the growth performance, ATTD and blood metabolites in piglets at the early (21 days) and late (42 days) post-weaning period using the same diet.

MATERIALS AND METHODS

The protocol for this experiment was reviewed and approved by the Animal Care and Use Committee for Livestock of the University of Milan, OPBA (Organismo Preposto al Benessere Animale) and received the authorization from the Italian Ministry of Health (N° 405/2019-PR). Moreover, the principles of the 3R (Replacement, Reduction and Refinement) were applied to the trial authorized by the Italian Ministry of Health. The trial was conducted at the Experimental Animal Research and Application Center in Lodi (LO), at the University of Milan.

Animals and Experimental Design

Thirty-six post-weaning female piglets (Large White × Landrace pigs −28 days of life, 6.5 ± 1.1 kg) were selected from a breeding farm in the north of Italy. Animals were housed in individual pens in the same room and same environmental conditions, with controlled temperature and air speed. Each pig was able to interact with other subjects according to the regulations on animal welfare. According to a European Directive (EC Directive 2008/120/EC), environmental enrichment was provided in the form of small plastic balls for kids, completely safe and resistant for piglets.

The piglets were randomly grouped to obtain similar conditions of initial body weight in piglets fed with the standard post-weaning diet (CTR), salty (bakery) former food products (FFPs-B) and sweet (confectionary) former food products (FFPs-C) as described below. All pigs always had *ad libitum* access to water. After 7 days of adaptation, pigs received the three experimental diets *ad libitum* for 42 days. At the end of the experiment, six pigs per group were slaughtered to collect samples used in parallel investigations.

Experimental Diets

The chemical composition of the three diets was analyzed according to the Association of Official Analytical Chemists (AOAC) and the European Commission N° 152/2009. Piglets belonging to the CTR group were fed a standard diet for post- weaning piglets. Piglets belonging to the FFPs-B and FFPs-C groups were fed diets in which the 30% of conventional ingredients were replaced by bakery and confectionary FFPs, respectively. Table 1 resumes the chemical composition of the pure FFPs-B and FFPs-C products used to formulate the complete experimental diets. The nutrient composition of the three experimental diets met NRC (2012) requirements and were iso- energetic (14.0 MJ/kg DM) and iso-nitrogenous (19.0% DM).

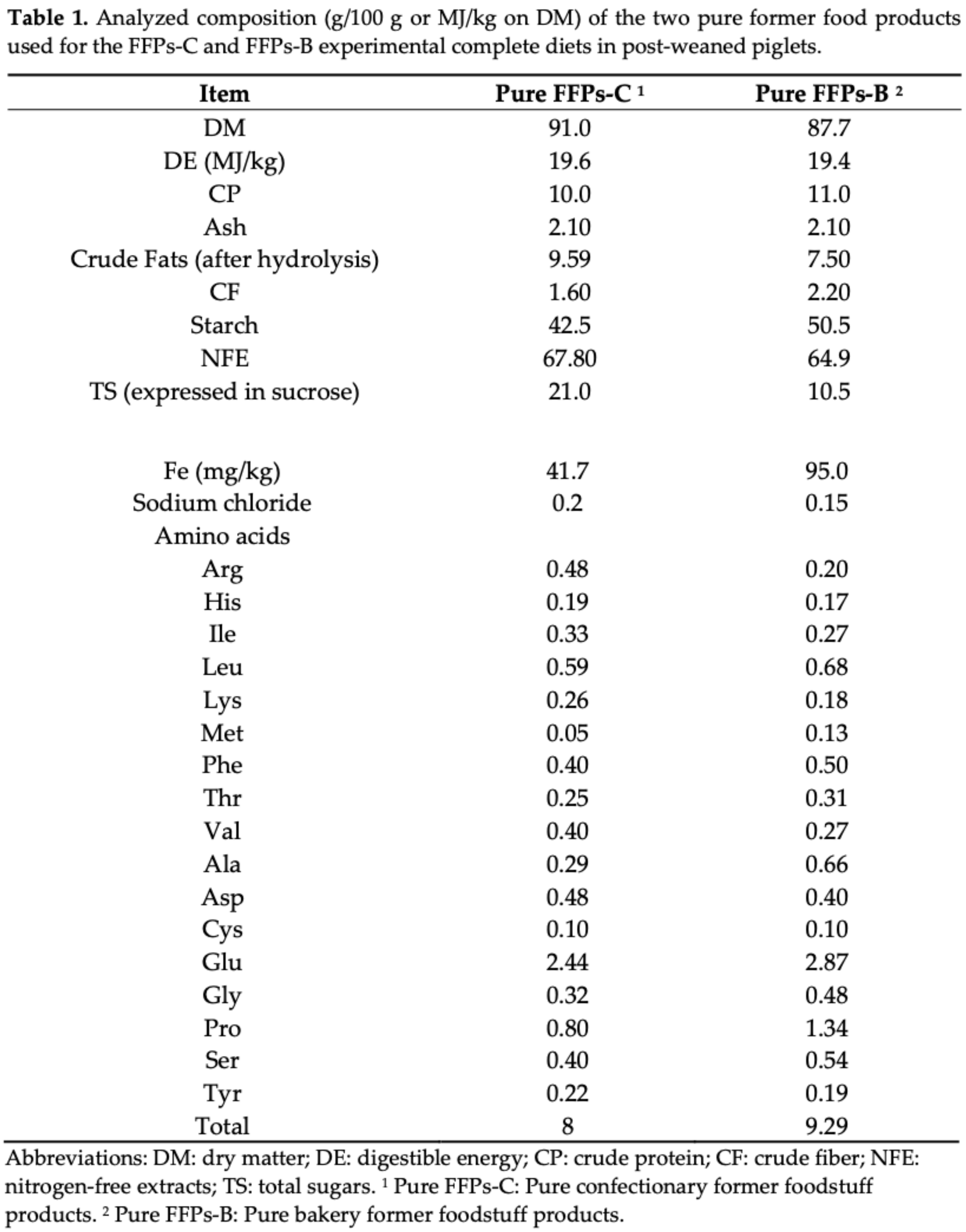

Table 1. Analyzed composition (g/100 g or MJ/kg on DM) of the two pure former food products used for the FFPs-C and FFPs-B experimental complete diets in post-weaned piglets.

Item	Pure FFPs-C [1]	Pure FFPs-B [2]
DM	91.0	87.7
DE (MJ/kg)	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	67.80	64.9
TS (expressed in sucrose)	21.0	10.5
Fe (mg/kg)	41.7	95.0
Sodium chloride	0.2	0.15
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: DM: dry matter; DE: digestible energy; CP: crude protein; CF: crude fiber; NFE: nitrogen-free extracts; TS: total sugars. [1] Pure FFPs-C: Pure confectionary former foodstuff products. [2] Pure FFPs-B: Pure bakery former foodstuff products.

The FFPs and the complete diets were provided mixed in mash form and prepared by two FFP processing companies based in the north of Italy. Table 2 reports the ingredients composition of the three complete diets used in this trial.

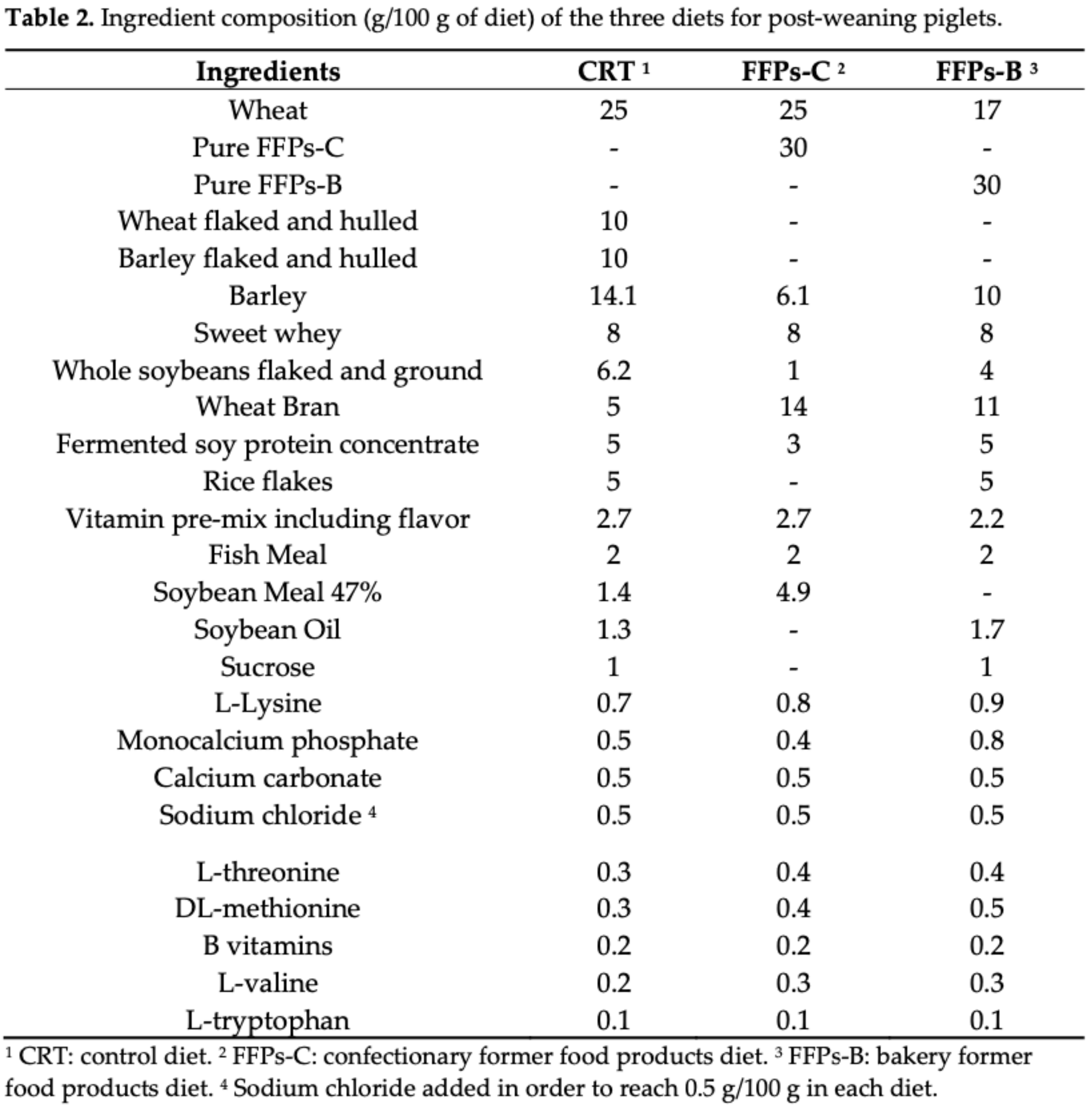

Table 2. Ingredient composition (g/100 g of diet) of the three diets for post-weaning piglets.

Ingredients	CRT [1]	FFPs-C [2]	FFPs-B [3]
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
Wheat Bran	5	14	11
Fermented soy protein concentrate	5	3	5
Rice flakes	5	-	5
Vitamin pre-mix including flavor	2.7	2.7	2.2
Fish Meal	2	2	2
Soybean Meal 47%	1.4	4.9	-
Soybean Oil	1.3	-	1.7
Sucrose	1	-	1
L-Lysine	0.7	0.8	0.9
Monocalcium phosphate	0.5	0.4	0.8
Calcium carbonate	0.5	0.5	0.5
Sodium chloride [4]	0.5	0.5	0.5
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	0.2	0.3	0.3
L-tryptophan	0.1	0.1	0.1

[1] CRT: control diet. [2] FFPs-C: confectionary former food products diet. [3] FFPs-B: bakery former food products diet. [4] Sodium chloride added in order to reach 0.5 g/100 g in each diet.

The analyzed chemical composition of the three experimental diets is reported in Table 3.

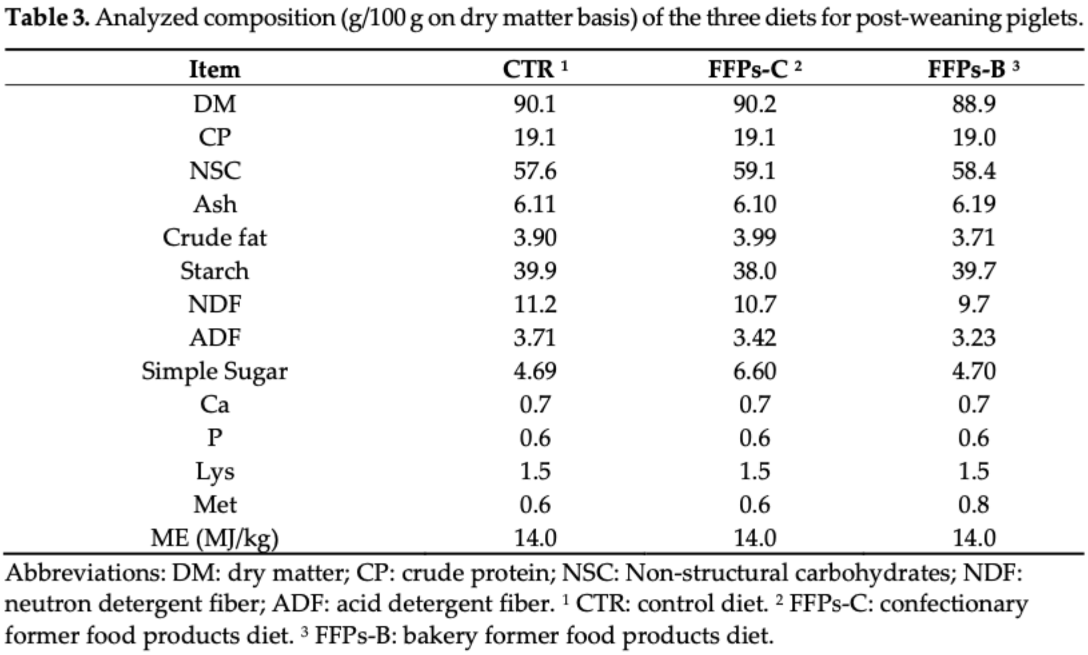

Table 3. Analyzed composition (g/100 g on dry matter basis) of the three diets for post-weaning piglets.

Item	CTR [1]	FFPs-C [2]	FFPs-B [3]
DM	90.1	90.2	88.9
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	9.7
ADF	3.71	3.42	3.23
Simple Sugar	4.69	6.60	4.70
Ca	0.7	0.7	0.7
P	0.6	0.6	0.6
Lys	1.5	1.5	1.5
Met	0.6	0.6	0.8
ME (MJ/kg)	14.0	14.0	14.0

Abbreviations: DM: dry matter; CP: crude protein; NSC: Non-structural carbohydrates; NDF: neutron detergent fiber; ADF: acid detergent fiber. [1] CTR: control diet. [2] FFPs-C: confectionary former food products diet. [3] FFPs-B: bakery former food products diet.

Growth Performance

Individual pig body weight (BW) and fecal dry matter were measured weekly, while feed intake (dFI) was recorded daily. In addition, average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated. These measurements were carried out to evaluate the growth performance of the animals in relation to the different experimental diets.

Apparent Total Tract Digestibility (ATTD) of Dry Matter

For the determination of the apparent total tract digestibility of dry matter (ATTD of DM), fresh fecal samples were collected daily before the morning feeding for three consecutive days for each week.

The ATTD of DM was determined using the acid-insoluble ashes (AIA) method (Kavanagh et al., 2001). Acid-insoluble ash (AIA) is considered a neutral marker for determining digestibility of feed in pigs and seems more suitable than metal elements like iron or chromium (Prawirodigdo et al., 2021). The amount of natural occurring AIA in the feed was verified in all experimental diets in order to avoid the inclusion metal elements as indigestible markers. The amount of natural occurring AIA was above 4 $\text{g}\cdot\text{kg}^{-1}$ feed in all experimental diets, which is considered more than adequate for the estimation of ATTD in pig (Kavanagh et al., 2001; Prawirodigdo et al., 2021); accordingly, non-inclusion of supplemental AIA sources was performed. After being collected, the feces were stored in plastic containers and frozen at −20 °C until the time of analysis. The samples collected in the three consecutive days within the week were analyzed as a single pool. The feces were weighed in a 50 mL crucible and then dried in a ventilated oven at 80 °C for 48 h, cooled in a desiccator to room temperature, reweighed and then incinerated in a muffle at 450 °C. The ash was placed in a beaker to which 100 mL of HCl 4N was added. The compound was boiled for 5 min on a stove-top. Then, the hydrolyzed compound was filtered, and the filter was rinsed to remove the acid with hot distilled water (85–100 °C). The filter and the ash content were placed in the original crucibles and then they were incinerated in a muffle at 450 °C. Finally, the crucibles with the contents were cooled in a desiccator at room temperature, weighted with the ash content (Wf) and weighted again after being emptied (We). The percentage of insoluble acid ash was calculated using the equation

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

where Wf = weight of the crucible with the ashes, We = weight of the empty crucibles and Ws = weight of the dry matter sample.

The ATTD of DM was calculated according to the indirect digestibility method [16], as follows:

$$\text{ATTD (g/100 g DM)} = (1 - A/B) \times 100$$

where A and B were the AIA concentrations in the feed and feces, respectively.

Blood Samples and Metabolic Profile

During the trial, all piglets were fasted over the night before blood collection at day 0, 21 and 42. In total, 54 blood samples were collected from jugular vein. Blood was collected using Vacutainer EDTA tubes and immediately centrifuged at 14,000 rpm for ten minutes at room temperature to obtain plasma. The plasma was then collected and frozen at −80 °C in the presence of protease inhibitors, for further determine the following metabolites: Total proteins, Albumin, Globulin, Albumin/globulins (A/G), Urea, Alanine aminotransferase (ALT-GPT), Aspartate aminotransferase (AST-GOT), Alkaline phosphatase (ALP), Total bilirubin, Glucose, triglycerides, amylase, total cholesterol, calcium, phosphorus and magnesium. Next, blood samples were sent to external laboratory to be analyzed. These parameters were measured through a standard enzymatic colorimetric analysis using a multiparameter autoanalyzer for clinical chemistry (Instrumentation Laboratory Company, Lexington, MA, USA).

Statistical Analysis

Data were analyzed using IBM SPSS Statistics version 27 (SPSS, Chicago, IL). Single pig was considered the experimental unit. Data were tested for normality with the Shapiro–Wilk test before statistical analysis and boxplot analysis was conducted in order to detect and delete outliers. Growth performance data (BW, ADFI, ADG and FCR), ATTD of dry matter and plasma biochemical values were analyzed using one-way analysis of variance (ANOVA) in order to compare means. The REPEATED statement was used for variables measured over time (BW, ADFI, ADG, FCR, ATTD and plasma metabolites). Differences with p values < 0.05 were considered to be significant. The analysis was performed using the following model:

$$yij = \mu j + \varepsilon ij$$

where yij is the observation (values), μj is the mean of the observations for the j-th group (sample) and εij represents the within-sample random variability. Differences with p values < 0.05 were considered significant. Data are presented as means ± Standard error mean.

RESULTS

Growth Performance and Apparent Total Tract Digestibility of Dry Matter

All animals remained in good health throughout the experiment and there were no morbidity or mortality issues. No effect between experimental group and period was observed for all the growth performance parameters. Accordingly, data were presented as week 1, week 5 and the overall mean of experimental period. The results of the present study regarding pigs' growth performance showed that there were no significant differences in BW between groups ($p > 0.05$) (Table 4). Body weight measured at the piglets' arrival and at the end of the trial did not differ between diets (Table 4). The body weight of the pigs increased regardless of the diet treatments, without showing any statistically significant differences between groups at the same time point (Table 4). The ADFI was not affected ($p > 0.05$) by any dietary treatments (Table 4). In addition, the experimental diets did not influence ($p > 0.05$) the ADG and feed conversion ratio (FCR) (Table 4) either. The results revealed that initial ATTD of DM values did not differ ($p > 0.05$) between the CTR and the two experimental diets. However, the final values of the ATTD of DM showed that final ATTD of DM of CTR diet is similar to the one of FPPs-B diet, but the ATTD of DM of FFPs-C diet is lower compared to the final ATTD of DM of the CTR and FPPs-B diets ($p < 0.05$).

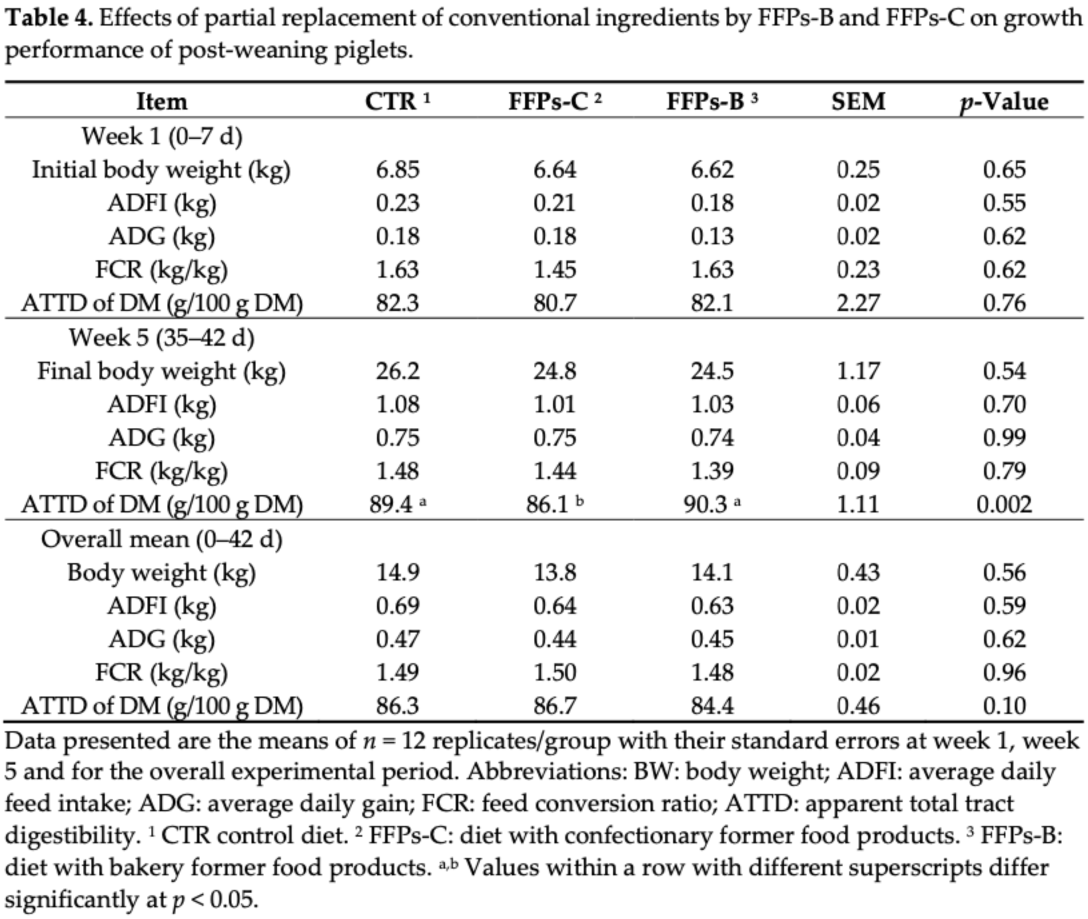

Table 4. Effects of partial replacement of conventional ingredients by FFPs-B and FFPs-C on growth performance of post-weaning piglets.

Item	CTR [1]	FFPs-C [2]	FFPs-B [3]	SEM	*p*-Value
Week 1 (0–7 d)					
Initial body weight (kg)	6.85	6.64	6.62	0.25	0.65
ADFI (kg)	0.23	0.21	0.18	0.02	0.55
ADG (kg)	0.18	0.18	0.13	0.02	0.62
FCR (kg/kg)	1.63	1.45	1.63	0.23	0.62
ATTD of DM (g/100 g DM)	82.3	80.7	82.1	2.27	0.76
Week 5 (35–42 d)					
Final body weight (kg)	26.2	24.8	24.5	1.17	0.54
ADFI (kg)	1.08	1.01	1.03	0.06	0.70
ADG (kg)	0.75	0.75	0.74	0.04	0.99
FCR (kg/kg)	1.48	1.44	1.39	0.09	0.79
ATTD of DM (g/100 g DM)	89.4^{a}	86.1^{b}	90.3^{a}	1.11	0.002
Overall mean (0–42 d)					
Body weight (kg)	14.9	13.8	14.1	0.43	0.56
ADFI (kg)	0.69	0.64	0.63	0.02	0.59
ADG (kg)	0.47	0.44	0.45	0.01	0.62
FCR (kg/kg)	1.49	1.50	1.48	0.02	0.96
ATTD of DM (g/100 g DM)	86.3	86.7	84.4	0.46	0.10

Data presented are the means of $n = 12$ replicates/group with their standard errors at week 1, week 5 and for the overall experimental period. Abbreviations: BW: body weight; ADFI: average daily feed intake; ADG: average daily gain; FCR: feed conversion ratio; ATTD: apparent total tract digestibility. [1] CTR control diet. [2] FFPs-C: diet with confectionary former food products. [3] FFPs-B: diet with bakery former food products. [a,b] Values within a row with different superscripts differ significantly at $p < 0.05$.

Blood Samples and Metabolic Profile

All the metabolites analyzed in the plasma of pigs are reported in Table 5. No significant effects of the diets ($p > 0.05$) were found in the analyzed hematological parameters between groups over the entire experiment (data not shown). Accordingly, the overall mean values were reported (Table 5).

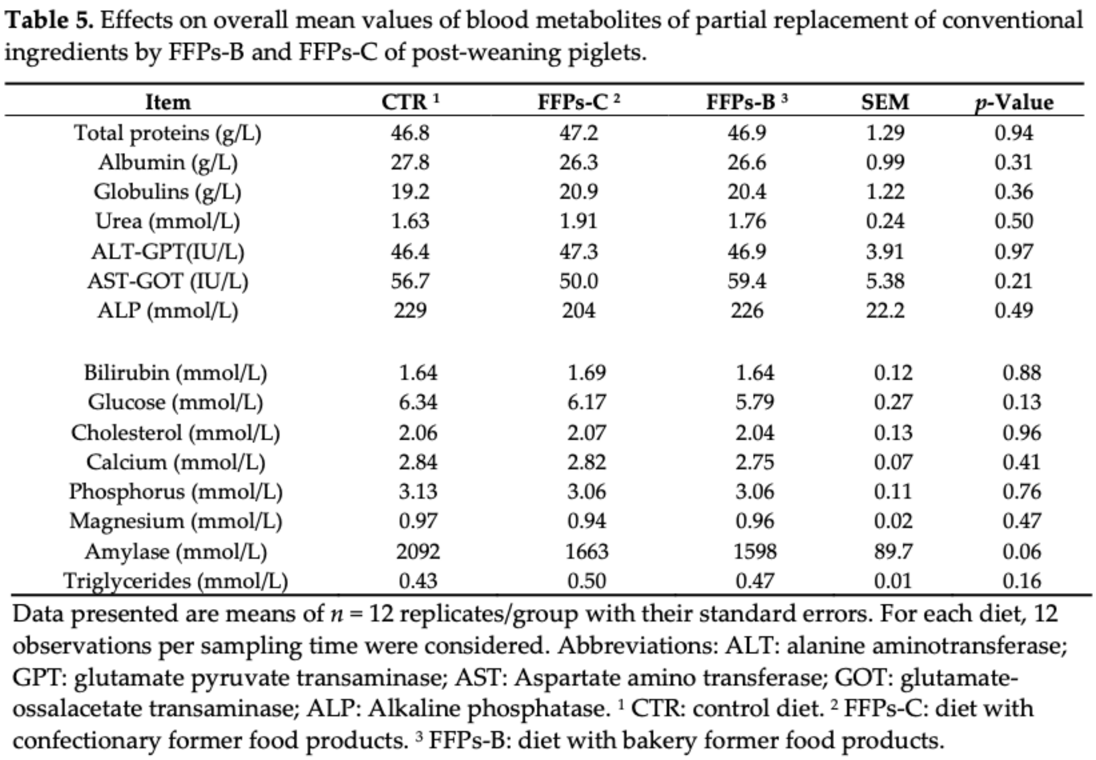

Table 5. Effects on overall mean values of blood metabolites of partial replacement of conventional ingredients by FFPs-B and FFPs-C of post-weaning piglets.

Item	CTR [1]	FFPs-C [2]	FFPs-B [3]	SEM	*p*-Value
Total proteins (g/L)	46.8	47.2	46.9	1.29	0.94
Albumin (g/L)	27.8	26.3	26.6	0.99	0.31
Globulins (g/L)	19.2	20.9	20.4	1.22	0.36
Urea (mmol/L)	1.63	1.91	1.76	0.24	0.50
ALT-GPT(IU/L)	46.4	47.3	46.9	3.91	0.97
AST-GOT (IU/L)	56.7	50.0	59.4	5.38	0.21
ALP (mmol/L)	229	204	226	22.2	0.49
Bilirubin (mmol/L)	1.64	1.69	1.64	0.12	0.88
Glucose (mmol/L)	6.34	6.17	5.79	0.27	0.13
Cholesterol (mmol/L)	2.06	2.07	2.04	0.13	0.96
Calcium (mmol/L)	2.84	2.82	2.75	0.07	0.41
Phosphorus (mmol/L)	3.13	3.06	3.06	0.11	0.76
Magnesium (mmol/L)	0.97	0.94	0.96	0.02	0.47
Amylase (mmol/L)	2092	1663	1598	89.7	0.06
Triglycerides (mmol/L)	0.43	0.50	0.47	0.01	0.16

Data presented are means of *n* = 12 replicates/group with their standard errors. For each diet, 12 observations per sampling time were considered. Abbreviations: ALT: alanine aminotransferase; GPT: glutamate pyruvate transaminase; AST: Aspartate amino transferase; GOT: glutamate-ossalacetate transaminase; ALP: Alkaline phosphatase. [1] CTR: control diet. [2] FFPs-C: diet with confectionary former food products. [3] FFPs-B: diet with bakery former food products.

DISCUSSION

FFPs Use in Post-Weaning Pig Diets and Composition

The use of FFPs is still limited due to the lack of knowledge regarding the effects of those products on growth performance and animal wellbeing (Pinotti et al., 2021). One concern could be the difficulty to formulate a standard feed due to the variability of ingredients used for the FFPs production (Luciano et al.,2020). However, in USA, a study conducted on 46 sources of bakery meal collected from the 6-state area reported that the differences among geographical regions in the chemical composition of bakery meals were small and only the concentration of ash and some other nutrients differ between the different area (Liu et al., 2018). Former food producers can predict the variations between the different products' storage and guarantee a final product with a standardized composition, due to different process of homogenization (Luciano et al., 2020). This study showed that the 30% inclusion of two different types of FFPs in the diets of post-weaning piglets is possible. Based on the nutritional facts reported for humans, FFPs are rich in carbohydrates and fat, depending on their origin. For this reason, FFPs are commonly used in young animals, especially post-weaning pigs and calves (Luciano et al., 2020). Similarities between the chemical composition of common cereals and different FFPs have been already demonstrated (Pinotti et al., 2021; EFFPA, 2019; Luciano et al., 2020; Ottoboni et al., 2019), while a higher glycemic index potential in FFPs compared to corn and heat processed wheat has been observed in vitro (Ottoboni et al., 2019). As reported in Kaltenegger et al. (2020), FFPs have a high nutritional value for animal feed because of their high energy content. In this study, the ME of two types of FFPs diets was ~14.0 MJ/kg. These values are in line with the literature (Rojas et al., 2013), where the digestible energy in bakery meal diet was 14.0 MJ/kg was similar compared to the standard diet with corn that was 14.2 MJ/kg. The high energy content of the FFPs products make them a valuable opportunity to replace other energy-rich ingredients traditionally used for feed formulation, with positive effects on the circularity of the food production. Regarding the chemical composition and nutritional values, the FFPs-C and FFPs-B diets are comparable with other studies (Luciano et al., 2020; Guo et al., 2015). One of the main concerns about this material is its homogeneity and stability in composition over the time. It is correct that a wide range of different ingredients for the production of the final product could be used. An example is the effect of the seasonality, where at some times of the year (e.g., Easter, Christmas, etc.) a large amount of sugary products could be available, instead of other products. However, FFPs processors are able to assure a final product with no significant variations in the chemical composition. This result is obtained by stocking different ingredients singularly, analyzing their chemical composition and, based on the results, and mixing them in a way to obtain a final product with similar characteristics all over the year (Pinotti et al., 2021). In the current study, the two FFP-based diets were characterized by comparable amount of starch and NDF contents in respect of the CTR diet. Has been showed that the starch and fiber content of different categories of FFPs can vary based on the main sources of ingredients used for their production (Pinotti et al., 2021). As expected, a difference between the three diets is the content of simple sugar, higher in FFPs-C compared to FFPs-B and CTR. In the previous study of Tretola et al. (2019), only one source of FFPs containing both bakery and confectionary products was tested. Because of the intestinal health implication related to the sugar content in the pig's diet, a categorization of the FFPs based on their ingredients composition (e.g., sugary vs. salty) could result in an easier diet formulation.

Growth Performance

During weaning, piglets are exposed to a wide range of stressors such as the change of environment, transition from a liquid to a solid diet and immature digestive system that may could lead to a depression in growth rate (Tretola et al., 2019; Guo et al., 2015; Luciano et al., 2021). The inclusion of processed food such as FFPs could represent an additional stressful factor for the weaned piglets on one hand or a source of more readily available nutrients for the still immature intestine on the other hand. In this study, the use of FFPs in post-weaning diet did not affect the growth performance of the piglets. In particular, body weight, ADFI, ADG and FCR were similar between the three groups. Those results confirm what has been already observed in a similar study, where no detrimental effect on growth performance have been observed in pigs fed FFPs-based diet (Tretola et al., 2019). Similarly, Rojas et al. (2013) did not find any differences in feed intake between diets composed by bakery meal and standard diet with corn (473 g DM/d and 481 g DM/d, respectively). Another study investigated the effect of candy co-products as an alternative source of carbohydrates and lactose in newly weaned pigs (Guo et al., 2015). The results showed that candy co-products could replace up to the 45% of dietary lactose without compromising growth performance, feed intake and dietary efficiency. In particular, ADFI, ADG and gain to feed ration did not have any statistical change if whey permeate was substituted with candy coproduct (Guo et al., 2015). More recently, Luciano et al. (2021) have investigated the effect of bakery meal as corn substate (substitution rate from 25% up to 100%). Results indicated that for the overall 5-wk nursery period, increasing concentrations of bakery meal above 30% (i.e., corn substitution rate of 50%) tended to reduce average daily gain and reduced gain to feed ratio of pigs, whereas blood indicators of energy and protein utilization were not affected. Specifically, there was no effect of increasing concentrations of bakery meal on growth permeance of pigs from day 1–14. However, ADG of pigs from day 15–35 and for the overall experimental period tended to decrease as the concentration of bakery meal increased in the diets above 30% on dry matter basis. The G:F from day 15–35 and for the overall experimental period linearly decreased as bakery meal inclusion increased in the diets. However, no differences among dietary treatments were observed from day 15–35 or for the overall experimental period for feed intake and the final body weight on day 35. These results in Luciano et al. (2021) are in line with the present study, confirming that both FFP-C and FFP-B do not represent any issue to formulate balanced and homogeneous diets for piglets during weaning up to 30% of inclusion. Considering that the weaning is the most critical stage of the pig's life, the lack of detrimental effects in piglets should also support the use of those ingredients in finishing pig's diets, when the feed intake is higher and the potential mitigation of the environmental impact increased.

Apparent Total Tract Digestibility (ATTD) of Dry Matter

Confectionary and bakery products used for the production of FFPs-C and FFPs-B, respectively, are subject to numerous technological processes that can improve their digestibility. While the ATTD of DM of piglets fed FFPs-B was similar to that of the CTR group, FFPs-C decreased the ATTD of DM, in contrast with our expectations. In previous experience, the partial replacement of cereals with FFPs resulted in an increased ATTD, with ATTD values of 83% and 78% for FFPs and CTR diets, respectively. Food processing, in fact, often results in small food particles which have a greater surface in contact with digestive enzymes compared to coarser ones, leading to a greater digestion rate (Klopfenstein et al., 2018). Heat is another factor that influences feed digestibility, where high-temperature treatments can improve digestibility values by the protein denaturation of some anti-nutritional elements such as the anti-tryptic factor of raw soybeans (Guiberti et al., 2014). Extrusion significantly increases the digestibility of starch for both humans and animals (Altan et al., 2009). It is widely known that piglets benefit from the consumption of easily digestible carbohydrates until their digestive system is fully able to use the starch. Thus, it is not clear why the FFPs-C resulted in a decreased ATTD of DM. One hypothesis could be the balance between simple sugars and starch content of the diet. The ratio between simple sugars/starch was 0.17 for FFPs-C diet, while it was ~0.11 for the other two diets. In this context, the role of fiber needs to be addressed too (Noblet et al., 2001). The rates of sugar absorption depend on the form in which they are consumed and also on the effects of individual food matrices on gastric emptying (Southgate et al., 1995). A second hypothesis could be associated to the processing of bakery products itself. The fast intestinal transit of the small processed food particles through the intestinal tract can negatively affect their digestibility due to a reduced contact time with digestive enzymes (Klopfenstein et al., 2018). Therefore, the right balance between a particle's size and its transit speed through the gastrointestinal tract is essential to obtain the highest digestibility value. We could also speculate that sugary ingredients used in FFPs-C, such as chocolate products, were richer in tannins compared to the ingredients used in CTR and FFPs-B. Even if the content of tannins has not been quantified in this study, it is known that the content of tannins in cocoa products is higher compared to cereals (Hellstrom et al., 2009). Tannins are polyphenolic biomolecules that can interact with and precipitate macromolecules, such as proteins, gelatins, polysaccharides and alkaloids (Girard & Bee, 2020). Therefore, the interaction of tannins from cocoa products with nutrients and digestive enzymes could have led to a reduced digestion rate in FFP-C group.

Blood Metabolic Profile

Diet has a measurable and significant effect on blood components (Etim et al., 2014). The analysis of hematological parameters represents a readily available assessment of the health status of animals during a feeding test and at the same time can be used as an appropriate measure of nutritional status (Olabanji et al., 2010). In the current study, no differences in blood metabolites between the two FFPs diets compared with CTR diet were found. This result is in line with our previous experience, where FFPs did not significantly affected the selected blood parameters, but increased glucose and decreased urea concentration compared to the CTR group (Tretola et al., 2019). The high potential glycemic index of FFPs due to the high content of simple sugar and processing-related characteristics of the starch has been already described (Tretola et al., 2019). However, in the present study no differences in the glycaemia were observed between the three experimental diets. These findings could be related to several aspects and characteristics of FFPs. First, only the FFPs-B diet had a higher sugar content compared to the CTR diet. However, its starch content was lower. These differences in the chemical composition could have led to a balanced contribution to the glycemic index, resulting in similar values. Another explanation could be associated to the easily available sugars in the FFPs diets. As described by Ottoboni et al. (2019), the highest amount of sugar content by FFPs is released from the matrix within the first 15 min after digestion, while in the following time points the glucose release is similar to the standard cereals. In this study, blood samples for the serum metabolites investigation were collected after 8 h of fasting, when tissues probably already metabolized the blood glucose, restoring the glycemia to baseline values independently by the diet. Post-prandial blood sampling in further research could confirm the potential of FFPs to increase pig glycemia and therefore potentially increase the hanger status of the animals, even if the similar feed intake observed between groups suggest a lack of significant effects in this respect. The results obtained in this study suggest that the introduction of 30% FFPs into the feed mixture for weaned piglets does not affect the metabolic profile of the animal, under the condition that the nutrients and metabolizable energy in the mixture are properly balanced and cover the animal requirements.

CONCLUSION

The results obtained in this study suggest that 30% of FFPs can be included in post-weaning pig diets as alternative ingredients to improve sustainability in the livestock sector. Moreover, the two types of bakery or confectionary FFPs are completely comparable to the blend of FFPs used in previous study and conventional diet. However, further investigations are necessary to clarify the reason why FFPs-C decreased the ATTD of DM and to evaluate the effects of FFPs obtained by confectionary or bakery companies on other parameters like carcass composition and gut health.

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Institutional Review Board Statement: The study was approved by the Animal Care and Use Committee for Livestock of the University of Milan, OPBA (Organismo Preposto al Benessere Animale - protocol OPBA code 21-2019, approved 8th April 2019) and received the authorization from the Italian Ministry of Health (N° 405/2019-PR). Moreover, the principles of the 3R (Replacement, Reduction and Refinement) were applied to the trial authorized by the Italian Ministry of Health. The trial was conducted at the Experimental Animal Research and Application Center in Lodi (LO), at the University of Milan.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare no conflicts of interest.

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CHAPTER 7

Sugary vs salty food industry leftovers in post-weaning piglets: effects on gut microbiota and intestinal volatile fatty acids production.

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Abstract: The awareness about the need to reduce the waste of natural resources and to improve the sustainability of food production is significantly growing. This study investigated the effects of two categories of food industry leftovers, also called former foodstuff products (FFPs), on pig gut microbiota and intestinal volatile fatty acids (VFAs) production. Thirty-six post-weaning (28 days old) female piglets (Large White x Landrace, 6.5 ± 1.1 kg) were randomly divided in three groups to receive a conventional diet (CTR), and diets in which cereals were partially replaced (30% w/w) by sugary confectionary products (FFPs-C) or salty bakery products (FFPs-B), respectively. After 42 days of dietary treatments, feces were collected from the rectal ampulla, snap-frozen and used for the 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. Both the FFPS-C and FFPS-B diets had no effect on the abundance and the biodiversity of the microbial community. Only few taxa, considered as biomarker of healthy gut, increased with FFPS-C and FFPS-B compared to CTR. The experimental diets did not affect the production of the VFAs in the feces. This study demonstrated that there are no risks on gut health and gut microbiota with the inclusion at 30% (w/w) of both categories of FFPs diets, which can thus be used as a safe and sustainable feed ingredient in growing pigs.

IMPLICATIONS

The re-use of losses from the food-industry to replace cereal grains in feed represents a promising strategy for a sustainable food. Because of the limited information about their effects on animal health and performance, their acceptance for feeding animals it is still far to be completely welcomed. This study increases the knowledge about the effects of sugary and salty food losses, obtained respectively by the confectionary and bakery industry, on gut health in growing pigs. These findings valorise industrial food losses into animal feed to contribute to a reduced environmental and climate footprint of animal products and food waste prevention.

INTRODUCTION

Food can be lost or wasted in different steps of the manufacturing chain. The United Nations of Food and Agriculture Organization (FAO) defines food loss as food unintentionally lost due to technical limitations such as improper handling, storage, transportation, packaging or inefficient marketing systems at the early stages of the food supply chain (McGuire, 2015). Worldwide, the amount of produced and not- eaten food is around 1.3 billion tons per year (McGuire, 2015) and is around 102.5 million tons per year in the European Union (Girotto et al., 2015). The 2030 Agenda for Sustainable Development proposed several actions to mitigate the loss of food. Those actions include alternative use of food loss such as use as animal feed, biogas production, composting and, at the lowest priority, landfill and incineration (FAO, 2019). At the same time, by 2050 the demand for food is expected to increase significantly, while the urbanization limits the availability of the natural resources (McMichael et al., 2007). Interestingly, the recovery of food loss as animal feed addresses both food reduction and food security challenges. For a better investigation of their properties, food losses also called former foodstuff products (FFPs) can be distinguished into two main categories: sugary confectionary FFPs (FFPs-C), which include chocolates, biscuits and sweet snacks from confectionary production, and salty FFPs from bakery production (FFPs-B) such as bread, pasta (Luciano et al., 2020). Until now, 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 conducted to explore the effects of FFPs on animal health and performance, on both post-weaning piglets (Tretola et al., 2019c) 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, they exert no detrimental effects on growth performance when used at specific percentage of inclusion. To our knowledge, the impact of cereal's partial replacement with FFPs on gut bacterial community has not been deeply examined yet, despite the peculiar properties of these ingredients. The higher digestibility and simple sugar content of FFPs compared to traditional and un-processed cereals could influence the structure and biodiversity of the host gut bacteria (Pinotti et al., 2021). Moreover, their nature to be highly processed and often simple sugar-enriched increase the risk of osmotic diarrhoea (Pinotti et al., 2021). A pilot study on post-weaning piglets demonstrated that FFPs-based diets decreased the intestinal bacteria richness and evenness, with only slight effects on the taxa composition (Tretola et al., 2019a). This study was conducted without discriminating FFPs-C and FFPs-B and only during the first 16 days after weaning, in contrary to the present study. Thus, there is a lack of information about these effects on a longer period such as the growing phase. The present study intends to increase the knowledge about the impact of both FFPs-C and FFPs-B included in growing pig's diet on the large intestinal microbial community composition and biodiversity, together with faecal volatile fatty acids production.

MATERIALS AND METHODS

FORMER FOODSTUFF PRODUCTS INGREDIENTS

Two different FFPs products have been provided by an Italian FFP-processing plan to partially replace traditional feed ingredients in the two experimental diets. The two products differed for the source of food used for their production. Specifically, sugary FFPs-C were mainly composed of chocolate, biscuits and sweet snacks, while bread, pasta and salty snacks were used for salty FFPs-B products. The chemical composition of the two FFPs products used to further formulate complete experimental diets is reported in Table 1.

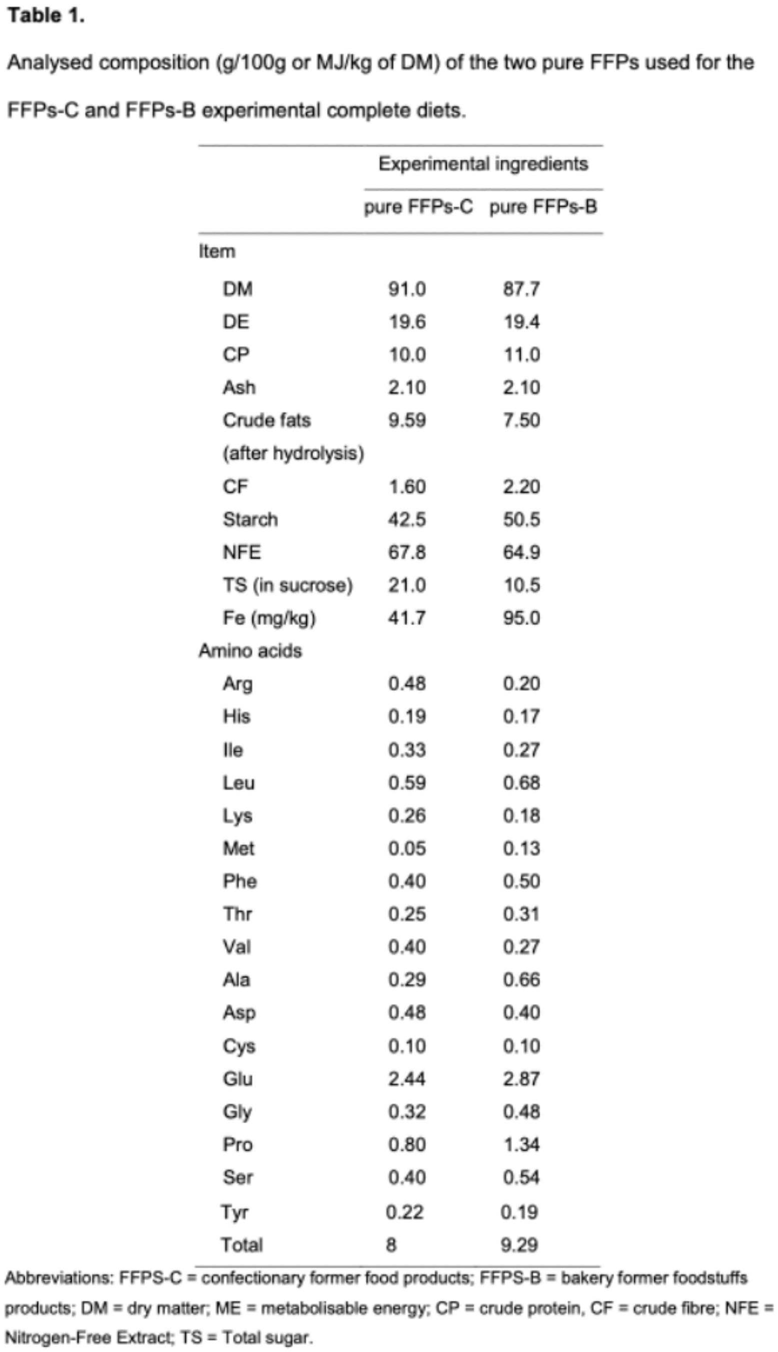


Table 1.

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

	Experimental ingredients	
	pure FFPs-C	pure FFPs-B
Item		
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; DM = dry matter; ME = metabolisable energy; CP = crude protein, CF = crude fibre; NFE = Nitrogen-Free Extract; TS = Total sugar.

EXPERIMENTAL DIETS

The nutrient composition of the three experimental diets met the NRC (2012) requirements and were iso-energetic (14.0 MJ/kg DM) and iso-nitrogenous (19% DM). The FFPs and the complete diets were already mixed and prepared by two FFP-processing companies based in the North of Italy. Table 2 reports the ingredients composition of the three complete diets used in this trial.

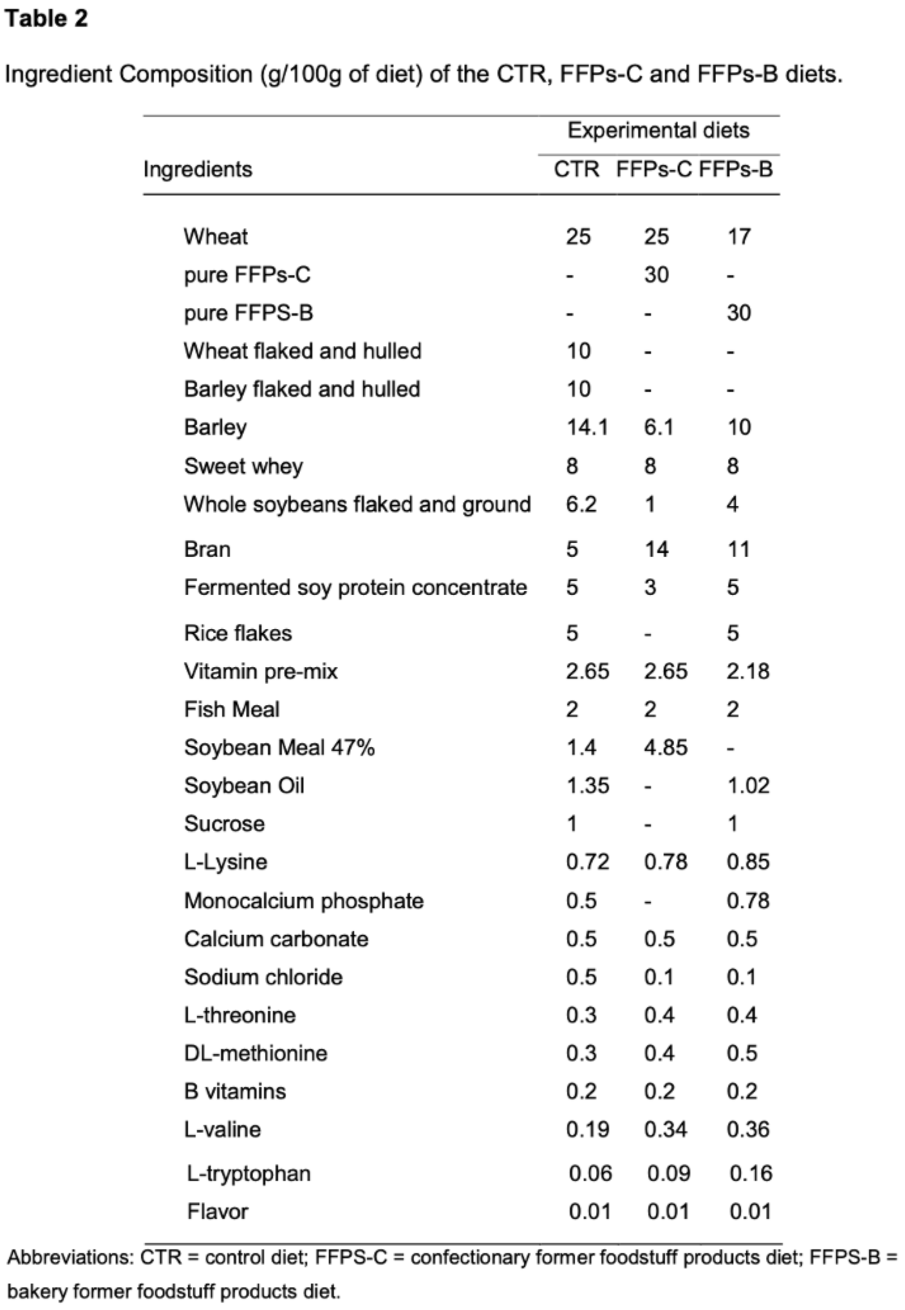


Table 2

Ingredient Composition (g/100g of diet) of the CTR, FFPs-C and FFPs-B diets.

	Experimental diets		
Ingredients	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 pre-mix	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
Flavor	0.01	0.01	0.01

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

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 in accordance with the Italian ethical regulation (DL 26/2014, protocol 711/-PR) and authorized by the Italian Health Ministry. The principles of the 3Rs (Replacement, Reduction and Refinement) were applied to the trial.

Thirty-six post-weaning female piglets (Large White x Landrace pigs - 28 days of life, 6.5 ± 1.1 kg) were used. Animals were housed in individual pens in the same room and environmental controlled conditions. The interaction between pigs was possible and plastic environmental enrichments provided, in accordance with the animal welfare regulation and the European Directive (EC Directive 2008/120/EC). The access to fresh water was always possible and an adaptation period of seven days was used to allow piglets to acclimatize to the new conditions. Piglets were then randomly assigned to a standard post weaning diet (CTR), a sugary confectionary FFPs based diet (FFPs-C) or a salty bakery FFPs based diet (FFPs-B) for 42 days. Individual pig body weight (BW) was measured weekly while feed intake daily (dFI). Individual dFI has been condensed in week mean and used for statistical analysis. In addition, average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated. These measurements were carried out to evaluate the growth performance of the animals in relation to the different experimental diets.

SAMPLES COLLECTION, DNA EXTRACTION AND SEQUENCING

The fecal samples were collected from rectal ampulla after 42 days of experimental diets feeding. 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 manufacturers' procedure and quantified with Nanodrop ND2000. Variable regions V3 and V4 of the 16S rRNA were amplified by PCR with universal primers for prokaryotic (341F/802R: CCTACGGGNGGCWGCAG / GACTACHVGGGTATCTAATCC, respectively). The DNA quality assessment and the next generation sequencing (NGS) of the extracted amplicons were both performed by BMR Genomics (Pavia, Italy) to obtain raw paired-end reads 2 × 300 bp.

INTESTINAL VOLATILE FATTY ACIDS QUANTIFICATION

Reagents, materials, solutions

All the standards (purity >99%), acetic, propionic, butyric, valeric acids were provided by Sigma and were used to prepare calibration solutions for quantification (linear response) and identification. A 10% perchloric acid solution in water was prepared in laboratory and used for the extraction of digesta samples. Headspace solid-phase microextraction (HS-SPME) was performed by using a 75 μm Carboxen / polydimethylsiloxane fibre (CAR/PDMS).

Samples collection

Digesta collection has been done at the slaughterhouse on the pigs that have been sacrificed at the end of the trial (i.e., 42 days of experiment). From all animals in each group, approximatively 50g of intestinal digesta have been collected and store at -20 °C to prevent degradation of the samples.

Procedure

Volatile fatty acids were determined by simultaneous HS-SPME GC-MS analysis described by Fiori et al. (2018) with some modifications. Briefly, about 1.0 g of digesta samples were homogenized after the addition of 5 mL of 10% perchloric acid solution in water and centrifuged at 15,000 g for 5 min at 4 °C. 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 HS-SPME extraction as follow. Calibration curves were prepared adding the IS to scalar amounts of the acids in diluted samples or water (for external standardization). VFAs extraction conditions were: 75 μm CAR/PDMS fibre, 10 min of equilibration time, temperature 70 °C, and 30 min of extraction time. The analytes were desorbed into the gas chromatograph (GC) injector port at 250 °C for 10 min, including fibre cleaning.

STATISTICAL ANALYSIS

Data on growth performance, ADG and feed intake and volatile fatty acids (VFA) were analyzed using IBM SPSS Statistics version 27 (SPSS, Chicago, IL). Data were tested for normality with the Shapiro–Wilk test before statistical analysis. Those data (BW, ADFI, ADG and FCR), were analyzed using one-way analysis of variance (ANOVA) for repeated measurements in order to compare means. Differences with P values <.05 were considered significant. All microbiota data analyses were performed in R v2.5.0 (Boston, MA, USA). Alpha diversity was estimated using the Richness (Observed and Chao1), Simpson and Shannon indices. Beta diversity was calculated using the weighted and unweighted Unifrac distance methods on the basis of rarefied OTU abundance counts per sample. Additionally, the variance (PERMANOVA) and similarities (ANOSIM) of the tested groups were analyzed. The packages used were phyloseq v1.26.1 and vegan v2.5–5. For the linear discriminant analysis effect size (LEfSe) to determine statistical differences in taxa abundance between groups the following conditions were used: the alpha value for the non-parametric factorial Kruskal–Wallis sum-rank test among the classes was < 0.05 and the threshold on the logarithmic linear discriminant analysis score for the discriminative features was > 3.0 (Segata et al., 2011). The “microbiome” library was used to estimate the common core microbiota, with a detection threshold of 0.001 and prevalence in 80/100 samples. Multivariate analysis by linear models was conducted using MaAsLin (Morgan et al., 2012) to test for associations of microbial abundances (at all taxonomic levels from domain to genus) with faecal VFA content. Default settings were used for this analysis.

RESULTS

The three diets did not evidence any effect on live animals in term of body weight and connected variables. Growth performance have been reported in Figure 1. No differences were found in ADG, ADFI and FCR (data not shown).

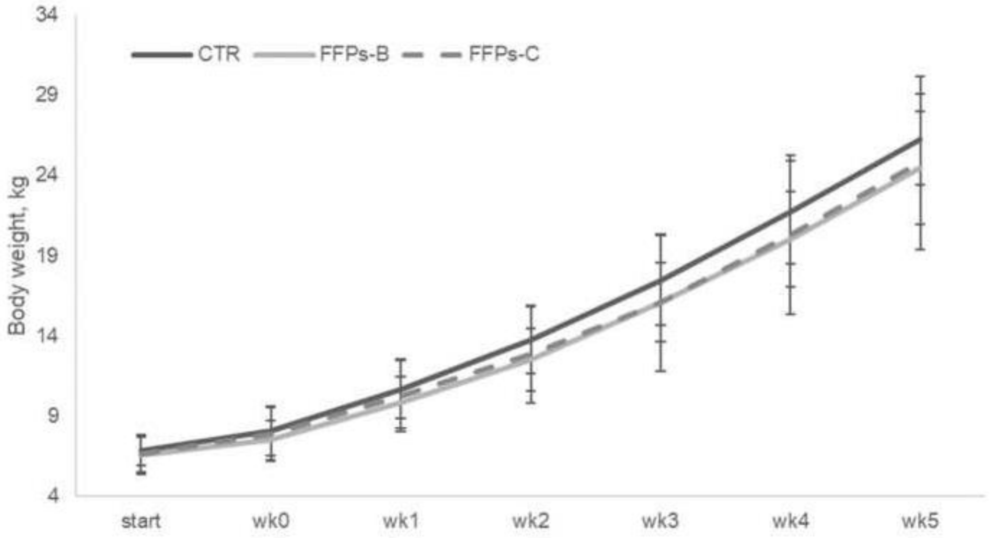


Figure 1. Pig body weight (kg). Data are presented as means by group and by dietary treatment ± standard deviation. CTR – standard post weaning diet group; FFPs-B – salty (bakery) FFPs based diet group; FFPs-C – sugary (confectionary) FFPs based diet group.

EXPERIMENTAL DIETS COMPOSITION

The analysed chemical composition of the three experimental diets was similar and presented in table 3.

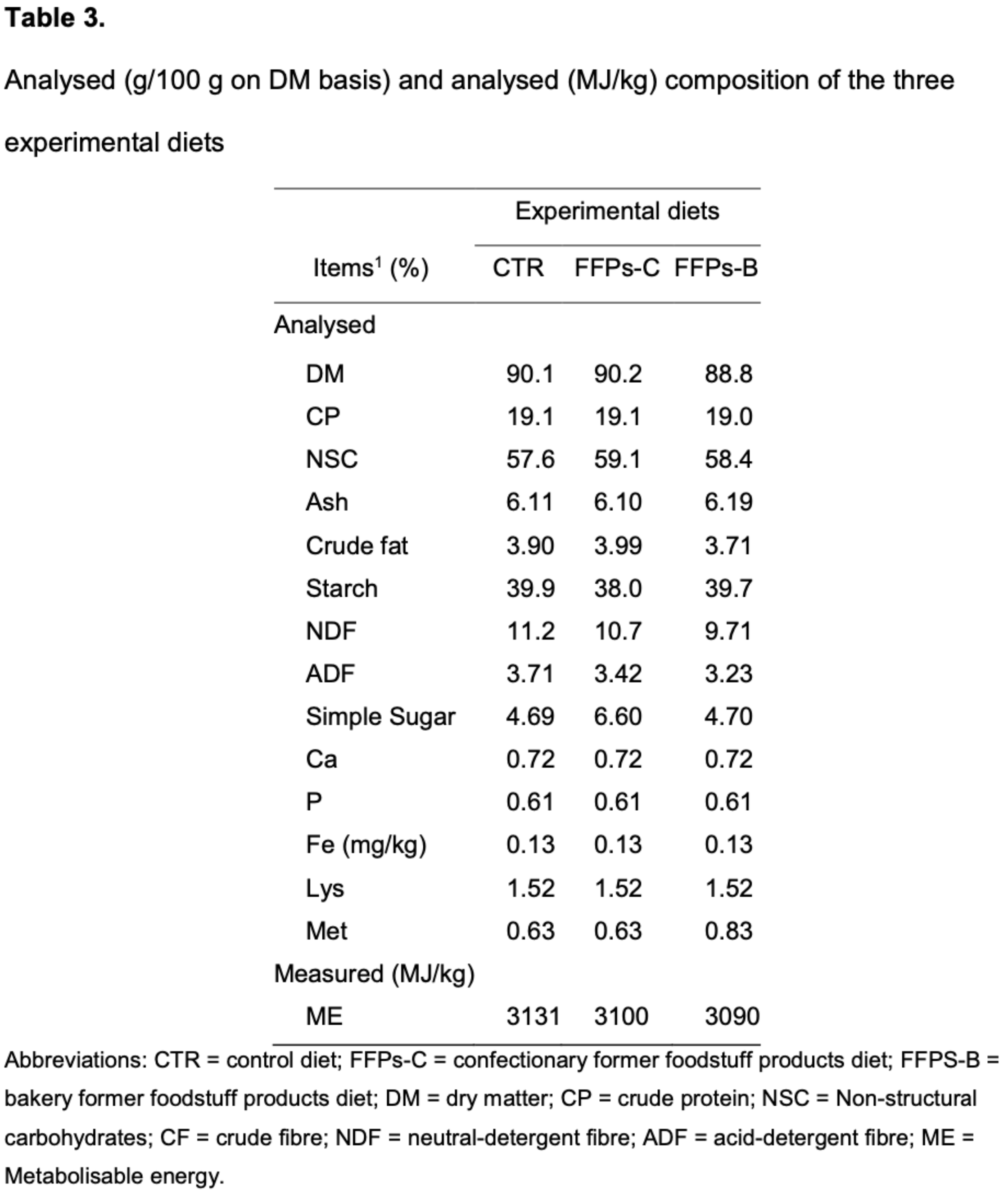


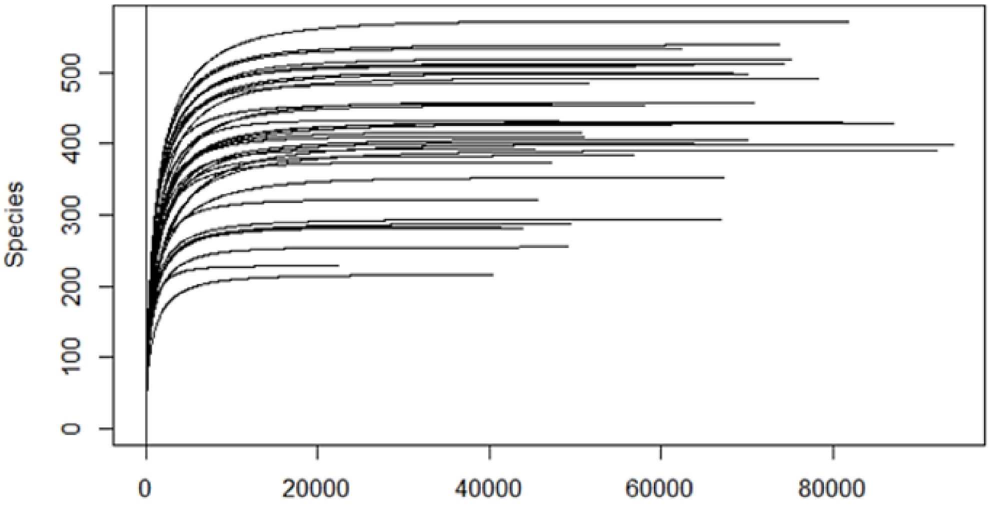
Table 3.

Analysed (g/100 g on DM basis) and analysed (MJ/kg) composition of the three experimental diets

Items[1] (%)	Experimental diets		
	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
Measured (MJ/kg)			
ME	3131	3100	3090

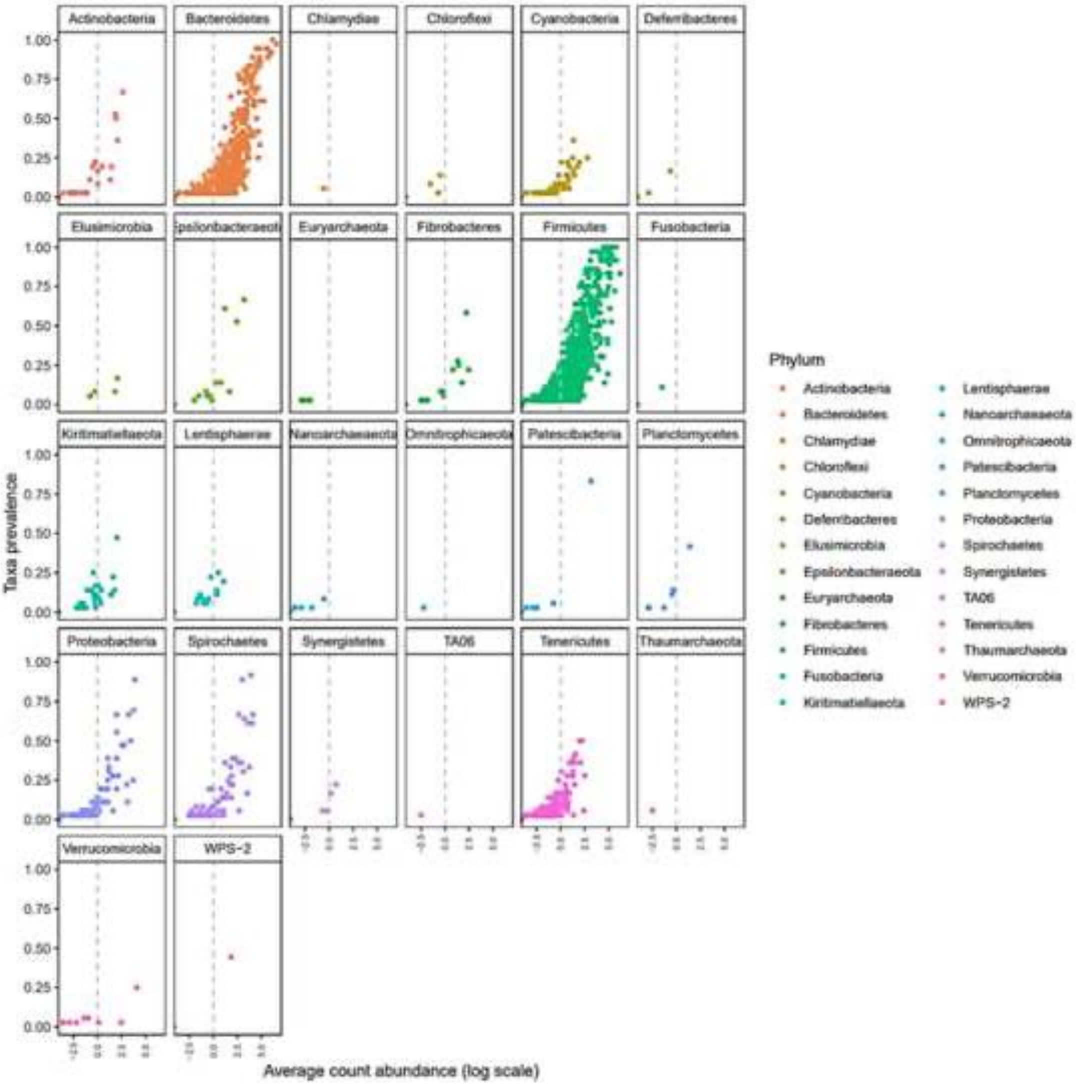
Abbreviations: CTR = control diet; FFPs-C = confectionary former foodstuff products diet; FFPS-B = bakery former foodstuff products diet; DM = dry matter; CP = crude protein; NSC = Non-structural carbohydrates; CF = crude fibre; NDF = neutral-detergent fibre; ADF = acid-detergent fibre; ME = Metabolisable energy.

The diets resulted to be iso-nitrogenous and iso-energetics. The FFPs-B diet had a lower content of NDF compared to CTR and FFPs-C. As expected, the content of simple sugar was higher in FFPs-C diet compared to CTR and FFPs-B. Another slight difference was in the NSC content, which was higher in FFPs-C diets, followed by FFPs-B and CTR diets. Gut microbiota characterization A total of 4’435’844 sequences, 3’021 taxa by seven taxonomic ranks were obtained thought the next generation sequencing of 16S rRNA gene present in collected faeces, with a sparsity value of 0.87. The minimum number of sequences obtained in a sample was 22’385 and this value was used to obtain an equal sample sum for downstream analysis of alpha diversity. The supplementary figure 1 reports the rarefaction curve and indicates that the sequencing depth was high enough for a correct data analysis.



Supplementary figure 1. Rarefaction curve of obtained sequences at different depths of next generation sequencing. On the x and y-axis, the number of sequences and species, respectively.

The most representative phyla were Firmicutes, Bacteroidetes, Proteobacteria, Spirochaeta and Tenericutes (Supplementary figure 2).



Supplementary figure 2. Composition plots at phylum level of pig gut bacterial community, independently of the diets.

Diets did not affect the gut microbial community at family level. In all the pigs, the most representative families were Prevotellaceae, Ruminococcaceae, Lachnospiraceae, Veillonellaceae and Lactobacillaceae, as showed in Figure 2.

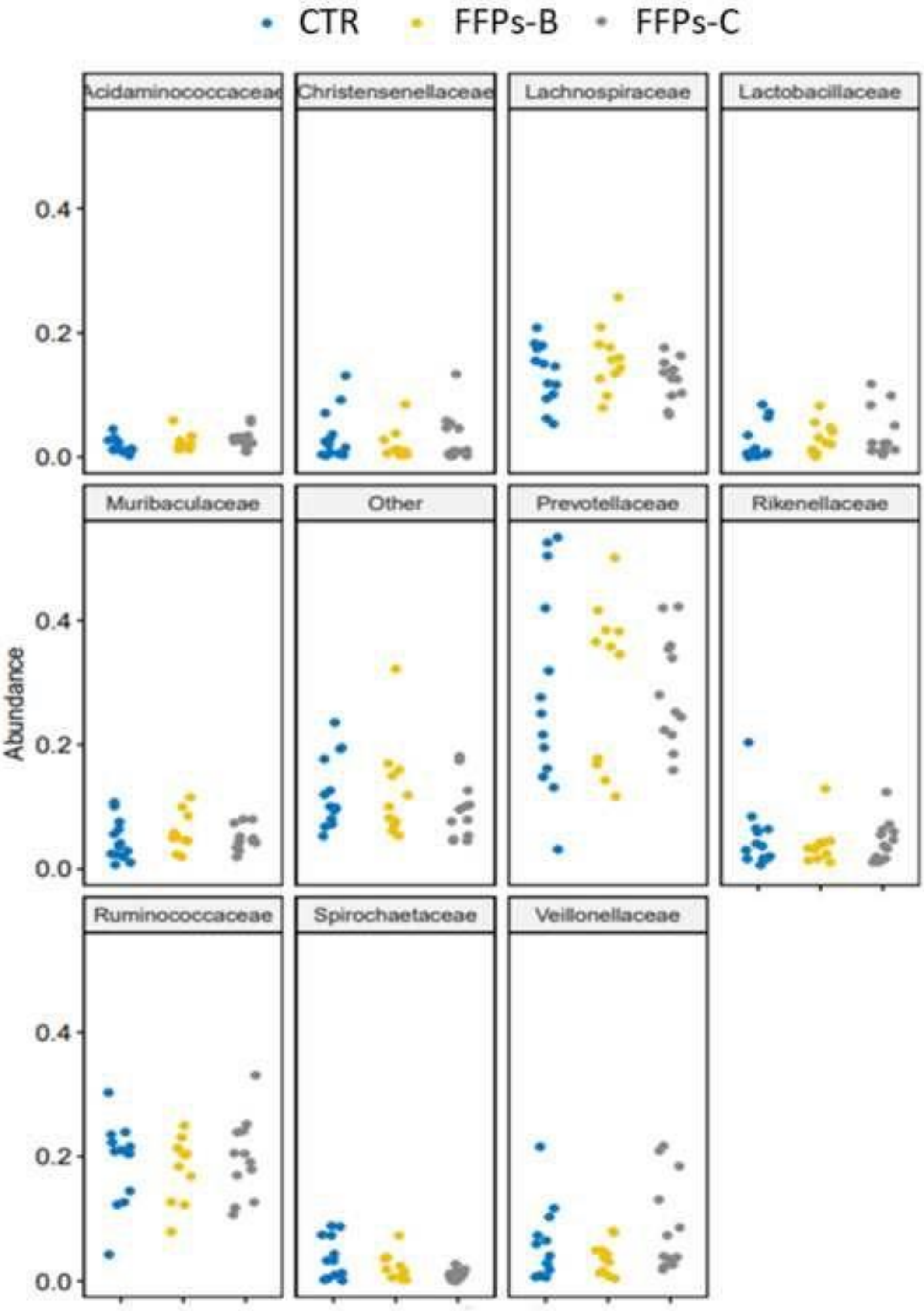
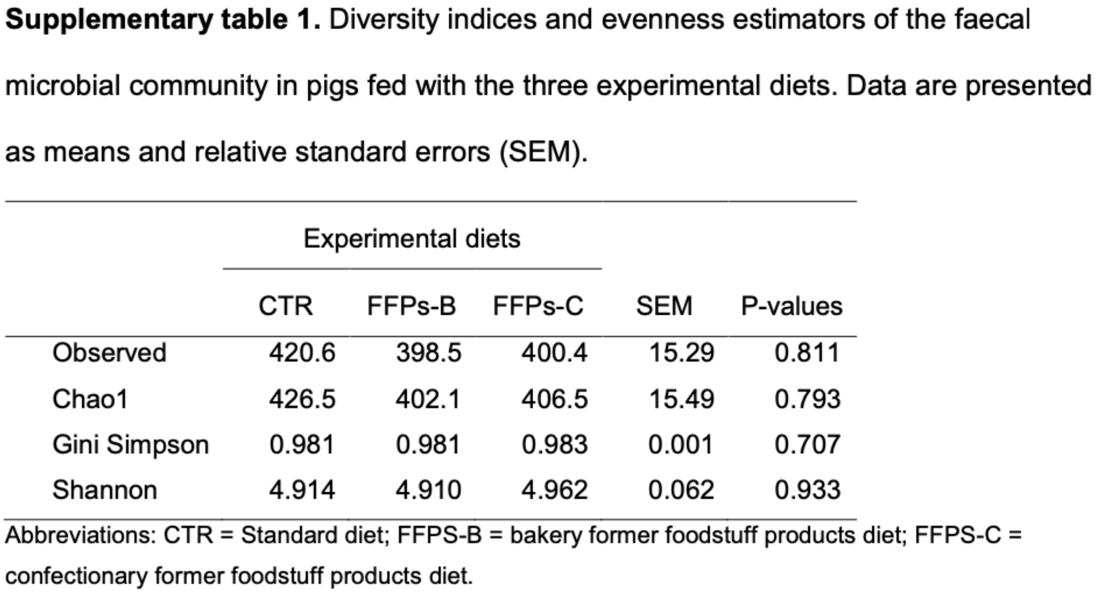


Figure 2. The most representative families of gut microbiota in piglets fed standard diet (CTR), bakery (FFPs-B) and confectionary (FFPs-C) former foodstuff products diets for 42 days after weaning.

No significant differences (P > 0.05) in the analysed alpha diversity indexes have been observed between groups. All the results are resumed in supplementary table 1.



Supplementary table 1. Diversity indices and evenness estimators of the faecal microbial community in pigs fed with the three experimental diets. Data are presented as means and relative standard errors (SEM).

	Experimental diets				
	CTR	FFPs-B	FFPs-C	SEM	P-values
Observed	420.6	398.5	400.4	15.29	0.811
Chao1	426.5	402.1	406.5	15.49	0.793
Gini Simpson	0.981	0.981	0.983	0.001	0.707
Shannon	4.914	4.910	4.962	0.062	0.933

Abbreviations: CTR = Standard diet; FFPS-B = bakery former foodstuff products diet; FFPS-C = confectionary former foodstuff products diet.

No differences (P > 0.05) in the phylogenetic diversity were found between groups (data not showed). No differences were observed in both Unweighted (PERMANOVA, P = 0.16, data not showed) and Weighted beta diversity between groups (PERMANOVA, P = 0.23), where axis 1 and 2 explained the 92.6% and 3.5% of the differences, respectively (Figure 3 A). Different bacteria as potential biomarkers between the three groups have been identified through the LefSe analysis in the end of the experiment. As shown in Figure 3 B and C, coprostanoligenes group and U29_B03 taxa was more abundant in FFPs-C then in FFPs-B and CTR.

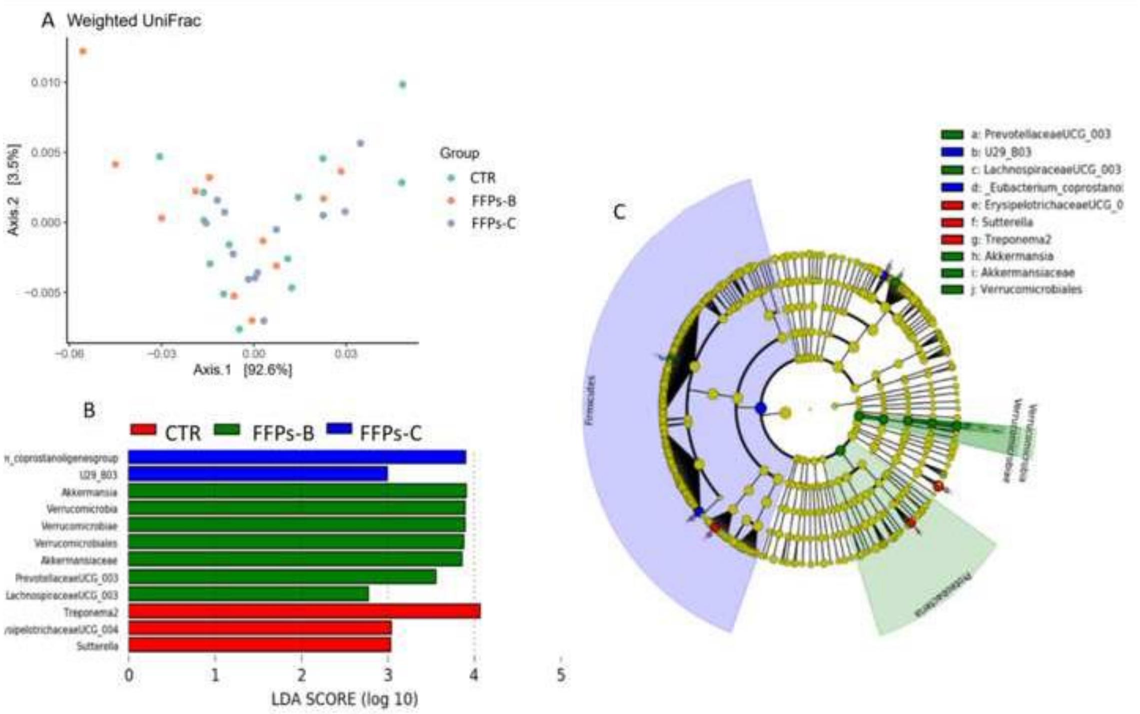


Figure 3. A) Alpha diversity indexes in gut microbiota of piglets fed standard diet (CTR), bakery (FFPs-B) and confectionary (FFPs-C) former foodstuff products diets for 42 days after weaning. B, C) The most differentially abundant taxa found in stool samples of piglets fed with a standard diet (CTR, in red), a bakery (FFPs-B, in green) or a confectionary (FFPs-C, in blue) FFPs-based diet for 42 days after weaning. A) Linear discriminant analysis (LDA) coupled with effect size measurements (LEfSe); B) Cladogram showing the distribution of the most differentially abundant taxa on the phylogenetic tree.

The abundance of bacteria belonging to the phylum of Verrucomicrobia was higher in piglets fed FFPs-B diet compared the other two groups, while bacteria belonging to the Treponema and Sutterella genera, together with members of the erysipelotrichaceae UCG_004 family were more abundant in the CTR group then in the other two categories of piglets. Volatile fatty acids content in the intestine and correlations with gut microbiota. The volatile fatty acids acetate, proprionate, butyrate and valerate have been quantified in intestinal content of pigs belonging to the three experimental groups. No significant differences (P > 0.05) were found between dietary treatments. Results are reported in table 4.

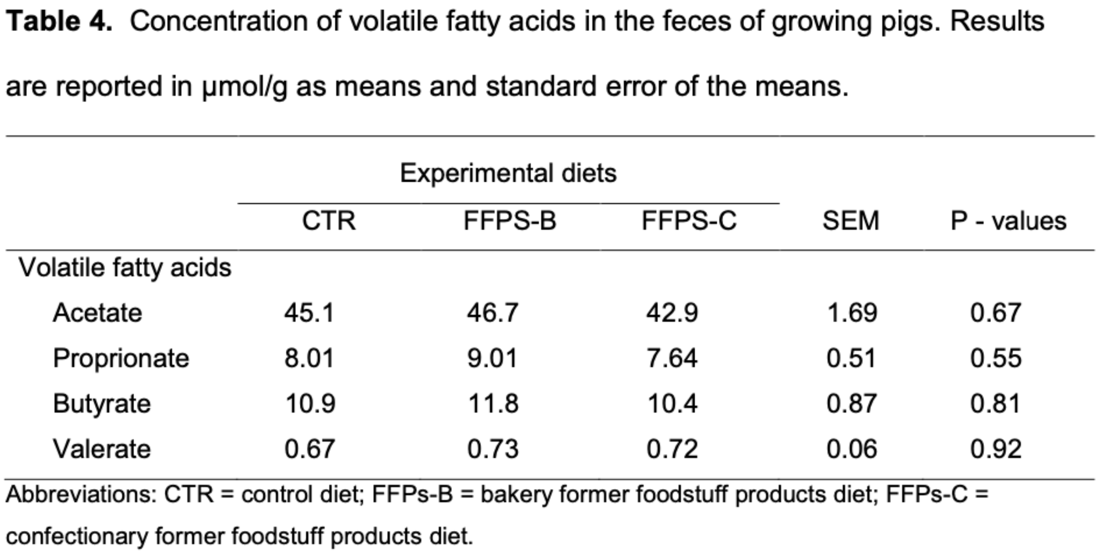


Table 4. Concentration of volatile fatty acids in the feces of growing pigs. Results are reported in μmol/g as means and standard error of the means.

	Experimental diets				
	CTR	FFPS-B	FFPS-C	SEM	P - values
Volatile fatty acids					
Acetate	45.1	46.7	42.9	1.69	0.67
Proprionate	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.

As reported in figure 4, the multivariate analysis by linear models found that the OTU corresponding to the genus Ruminococcaceae UCG-008, belonging to the family Ruminococcaceae, was positively correlated ($p < 0.01$) with acetate concentration.

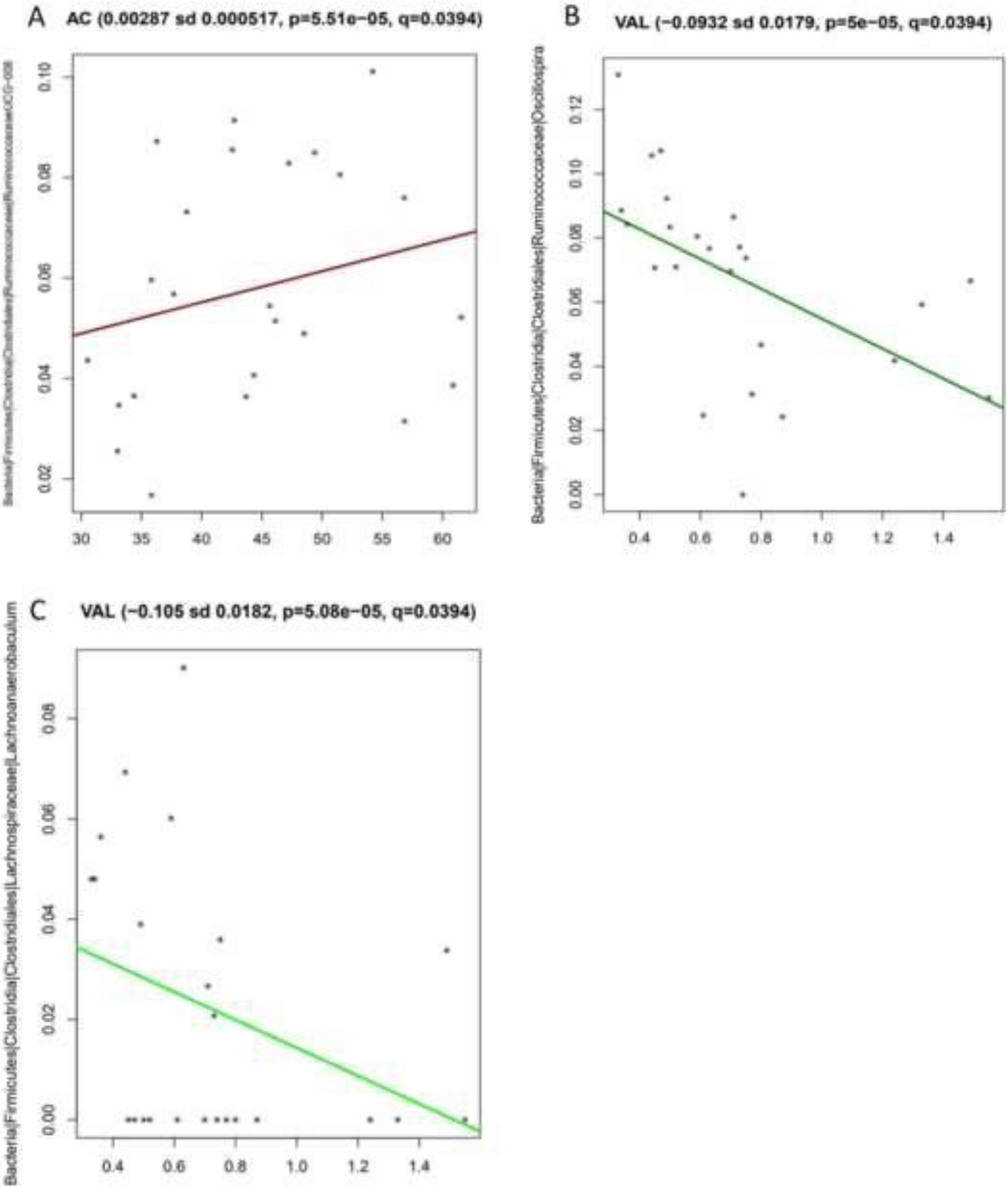


Figure 4. Correlations between specific gut bacteria OTUs and faecal acetate (A) and valerate (B, C), independently of the experimental diet.

Contrastingly, the genera Oscillospira and Lachnoanaerobaculum, belonging to the Ruminococcaceae and Lachnospiraceae families, respectively, showed a negative correlation ($P < 0.01$) with the intestinal content concentration of valerate.

DISCUSSION

DIETARY INCLUSION OF SUGARY AND SALTY FFPS

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). Growth performance highly depends on the gut health and gut microbiota, which can be definitely affected by the diet (Fouhse et al., 2016). Even if iso-energetic and iso-nitrogenous, the three diets of the present study differed for the ingredients used in their formulation. In the FFPs-C and FFPs-B diets, part of un-processed ingredients such as wheat and barley were replaced by highly processed FFPs. The diets also slightly differed in 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 FFPs-C diet, no signs of liquid feces were observed during the trial in none of the three groups. In a pilot study performed in post-weaning piglets, the inclusion of FFPs increased the ATTD of the diet compared to the standard one (Tretola et al., 2019c). It was speculated that the differences in the ATTD were due to the nature of the FFPs ingredients. The un-processed grains used for the standard feed formulation were partially replaced by the high-processed and high-digestible FFPs originally produced for human consumption (Tretola et al., 2019c). The way food is processed is a key factor determining the amount and type of material reaching the gut bacteria and influencing their growth and the production of microbiota metabolites (Ercolini and Fogliano, 2018). Diet digestibility and dietary fibres are in fact key dietary components for the gut health. In the present study, despite the partial replacement of the grains with FFPs, the difference in the fibre content between the diets was low due to the higher amount of bran included in FFPs-C and FFPs-B diets. We hypothesized that, by balancing the amount of fibre and consequently the ATTD, it is possible to avoid the potential adverse effects of FFPs diets on gut microbial community due to the high ATTD that we observed in our previous study on post- weaning piglets (Tretola et al., 2019a).

EFFECTS OF FFPS ON GUT MICROBIAL COMMUNITY COMPOSITION

The interaction between gut bacteria and the host strongly affects host metabolic function and well-being (Fouhse et al., 2016). Dysbiosis are often associated to inflammatory, metabolic and/or neurological disorders (Carding et al., 2015). The high-throughput sequencing used in this study is considered nowadays as one of the best technology to evaluate the composition and potential dysbiosis in the gut microbial community (Malla et al., 2019). According to the literature, the phyla of Firmicutes, Bacteroidetes, Proteobacteria, Spirochaeta and Tenericutes represented the largest proportion of the bacterial population (Isaacson and Kim, 2012). It is well known that the gut microbiota structure is susceptible to changes in the diet. For example, bacterial community depends on the amount and type of dietary fibres that 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 different sources of carbohydrates (Guo et al., 2015; Tretola et al., 2019a). Despite the different nature of FFPs-C and FFPs-B ingredients compared to the traditional ingredients, no differences at family level have been found between the three dietary treatments. The abundance and biodiversity of the gut microbiota were not affected by the different diets neither and no clusters can be observed by the beta diversity analysis, indicating that those diets had no major effects on microbial community in feces. These results are in contrast with findings obtained in the previous study on post- weaning piglets fed a FFPs diet (Tretola et al., 2019a). The study found that when FFPs were included in the diet to replace the 30% of traditional ingredients, the bacterial abundance and biodiversity decreased (Tretola et al., 2019a). Compared to the diets previously used in post-weaning piglets, in this study the experimental diets were more similar in their chemical compositions. Accordingly, also the ATTD values of FFPs diets were not improved, as found by Pinotti et al. (2020). These results confirm that diets including both FFPs-C and FFPs-B need to be carefully formulated to correctly feed the gut microbiota and avoid major effects on the bacterial community. As showed by the LefSe analysis, only minor differences can be 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 can only be 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 taxa represents a cholesterol-reducing bacteria (Freier et al., 1994) and it is known to ferment simple sugar such as fructose, glucose and mannose (Freier et al., 1994). U29-B03, member of Bacteroidetes phylum, was more abundant in the sugary FFPs-C group compared to the salty FFPs-B and CTR groups. However, no exhaustive information about the taxa can be found in literature. They are mainly found in ruminants and in environments undergoing complex carbon degradation (Hongoh et al., 2005). Compared to the CTR and FFPs-C diets, 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 as biomarkers for 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 is also able to produce acetate and proprionate within the mucus layer, easily available to the host absorption (Belzer and De Vos, 2012). Its abundance in FFPs-B diet is therefore promising concerning the effects of this FFPs category on gut health. Prevotellaceae UCG-003 belongs to the Prevotella genus. Prevotella are known to play a key role in the metabolisms of carbohydrate (such as sugar, starch and xylan) and they can effectively grow at a low pH (Adeyemi et al., 2020). Thus, the increased relative abundance of Prevotellaceae UCG-003 in the feces of pigs fed FFPS-B diet is probably due to increased fermentation of NSC, which are more abundant in FFPs-B diet then in CTR. It is not clear why this taxa did not increased in FFPs-C group, which has the highest NSC content. A hypothesis could be that a different cross-feeding relationship between bacteria has been established in the FFPs-C and FFPs-B groups, which resulted in the growth of different taxa specialized in the carbohydrates fermentation. The excreted products from one strain in fact, may be the preferred energy source for another strain and this complex cross-feeding relationship can be particularly complex in environments such as the lower gut of pigs (Smith et al., 2019). The Lachnospiraceae family belong to the core of gut microbiota. It is usually associated with healthy gut and is known to be the main 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. The 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 Erysipelotrichi class, belonging to the Firmicutes phylum (Kaakoush, 2015). Their increased abundance in the GI tract has been associated with detrimental effects to the host health (Kaakoush, 2015). The ability of Erysipelotrichi class to improve cholesterol and lipid metabolism in the GI tract has been also reported (Parmentier-Decrucq et al., 2009). No information about functional roles for the UCG-004 subtype have been reported in the literature. In addition, the Sutterella, higher in 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). Summarizing, 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 able to ferment carbohydrates and produce VFAs and decreased the abundance of potential pathogenic bacteria compared to the CTR group.

IMPACT OF FFPS DIET ON VOLATILE FATTY ACIDS PRODUCTION

The main sources for the production of VFAs by intestinal bacteria fermentation are carbohydrates (Ríos-Covián et al., 2016). Despite the differences in the carbohydrates content of the three diets, no differences have been observed in intestinal VFAs between the groups. According to the literature (Ríos-Covián et al.,2016), acetate was the most abundant VFA produced, followed by butyrate, propionate and valerate. The health benefits of the VFAs are well known, since they lead to a reduced luminal pH resulting in the inhibition of pathogenic microorganisms and an increased nutrient absorption (Macfarlane and Macfarlane, 2012). An example is the protection of Bifidobacteria from enteropathogenic infection through the production of acetate, which improves intestinal defence mediated by epithelial cells (Fukuda et al., 2011). Higher propionate production in large intestine has been positively correlated to feed efficiency and reduced inflammatory response in pigs (Gardiner et al., 2020). Butyrate increases the mucin production, which results in an improved tight-junctions integrity (Peng et al., 2009). Other VFAs such as valerate, contribute to the ATP generation and influence cell metabolism in enterocytes when butyrate concentrations become low (Gardiner et al., 2020). Thus, the production of VFAs is essential to maintain a proper gut barrier function. According to the similarities in the VFAs concentration measured in the intestinal content, we can assume that no risks of deteriorated gut barrier functions can be associated to the use of FFPs-C or FFPs-B in growing pig's diets. The results on the VFAs production are in accordance with the lack of significant differences in the microbiota composition between the three groups. It is known that diets can affect the production of VFAs by modulating the gut microbiota composition. For example, high fibre-low fat diets are characterized by the presence of higher amounts of intestinal short chain fatty acids than diets with reduced fibre content (De Filippo et al., 2010). As discussed above, the differences in the NSC, simple sugar and NDF contents between the FFPs-C, FFPs-B and CTR diets were not enough impact the gut microbiota. Even if FFPS-B and FFPs-C increased the abundance of some short- chain fatty acids producing bacteria compared to the standard diets, these differences did not affect 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 differently expressed in the three dietary treatments. This supports the hypothesis that FFPs-C and FFPs-B can be used in growing pigs' diets without harmful effects on gut microbiota and the related VFAs intestinal production and gut integrity.

CONCLUSION

Confectionary and bakery losses can be used as ingredients for the formulation of FFPs-C and FFPs-B, respectively. No significant differences have been observed between FFPs-C, FFPs-B and standard diets on the gut microbiota composition and intestinal concentration of VFAs. Minor modifications in specific bacterial taxa suggest potential beneficial effects of FFPs-C and FFPs-B against the growth of potential pathogenic bacteria. Based on the past and the present findings, can be concluded that industrial leftovers cannot be considered as waste but as a valid alternative to common cereal grains for sustainable and safe diets in pig nutrition.

Ethics approval: The in vivo trial was in accordance with the Italian ethical regulation (DL 26/2014, protocol 711/-PR) and authorized by the Italian Health Ministry. The principles of the 3Rs (Replacement, Reduction and Refinement) were applied to the trial.

Data and model availability statement: No data were deposited in an official repository.

Declaration of interest: The authors are unaware of any potential conflict of interest

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CHAPTER 8

Standardized total tract digestibility of phosphorus in bakery meal fed to pigs and effects of bakery meal on growth performance of weanling pigs

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Abstract: Two experiments were conducted to test the hypotheses that microbial phytase improves the standardized total tract digestibility (STTD) of phosphorus (P) in bakery meal and that corn may be replaced by bakery meal in diets for weanling pigs without negative effects on growth per- formance. Two sources of bakery meal were used in experiment 1 and one of these sources was also used in experiment 2. In experiment 1, eighty weanling barrows (initial body weight: 14.25 ± 1.91 kg) were allotted to a randomized complete block design with 10 diets and 8 replicate pigs per diet. Two basal diets based on each source of bakery meal (i.e., bakery meal 1 and bakery meal 2) were formulated without addition of microbial phytase. Eight additional diets were formulated by adding 500, 1000, 1500, or 3000 units of microbial phytase to each of the 2 basal diets. Pigs were housed individually in metabolism crates and feces were collected quantitatively for 4 d after 5 d of adaptation. Results indicated that greater increases in apparent total tract digestibility and STTD of P were observed in bakery meal 1 compared with bakery meal 2 when phytase was added to diets (interaction, quadratic, $P < 0.05$). In the second experiment, 160 newly weaned pigs (initial body weight: 7.17 ± 0.94 kg) were randomly allotted to 5 treatments with 8 pens per treatment and 4 pigs per pen. A 2-phase feeding program was used with d 1–14 being phase 1 and d 15–35 being phase 2. A control diet, containing primarily corn, soybean meal, and no bakery meal was formulated in each phase. Four additional diets in each phase were formulated by replacing 250, 500, 750, or 1000 g/kg of corn in the control diet with bakery meal. Results indicated that for the overall 5-wk nursery period, increasing concentrations of bakery meal tended (linear, $P = 0.064$) to reduce average daily gain and reduced (linear, $P < 0.01$) gain to feed ratio of pigs, whereas blood indicators of energy and protein utilization were not affected. In conclusion, digestible P in bakery meal may be increased by including microbial phytase in the diets, but a full replacement of corn with bakery meal in diets for weanling pigs may reduce growth performance.

INTRODUCTION

Approximately one-third of all food produced in the world is lost or wasted before being consumed by humans (Kummu et al., 2012), but some of this wasted food may be re-used in diets for animals (Jinno et al., 2018; Shurson, 2020). The large use of grains in the feeding of livestock may not be sustainable due to the growth in global population and competition between the production of food and feed (Pinotti et al., 2021). However, if more food-based coproducts can be recycled as animal feed, the usage of grain can be reduced, and the negative impact of un-consumed food on the environment may be reduced (Jinno et al., 2018). Food leftovers such as bakery meal are produced by collecting and mixing unconsumed human foods and consists of a mixture of bread, breakfast cereals, cookies, and other foods that were not used for their intended purpose (Slominski et al., 2004; Liu et al., 2018; Pinotti et al., 2019; Luciano et al., 2020). The collected food products are sorted, unpacked, ground, sieved, and sometimes dried to create feed ingredients that may replace cereal grains in the feeding of animals (Ottoboni et al., 2019). More than 500,000 tons of bakery meal is produced annually in the United States (Liu et al., 2018), whereas about 90,000 tons of ex-food (also termed former foodstuff) are processed in the EU (Luciano et al., 2020). In both cases, however, these quantities represent only a limited part of all wasted human food (Jinno et al., 2018) indicating that more of these ingredients may be used in animal feeding in the future. One of the challenges with using bakery meal in animal feeding is that chemical and nutritional composition may vary depending on the raw materials that are available for production (Slominski et al., 2004). However, results of a recent survey of the nutritional composition of bakery meal sold in the United States indicated that bakery meals sold in the United States have a consistent composition regardless of where in the country it is produced (Liu et al., 2018). It therefore appears that producers of bakery meal are able to blend different product streams to produce a final product with a constant nutrient profile. The digestibility of CP and amino acids (AA) in bakery meal has been reported (Almeida et al., 2011; Casas et al., 2015, 2018) and data for digestible energy, metabolizable energy, and the standardized total tract digestibility (STTD) of phosphorus (P) in bakery meal are also available (Rojas et al., 2013; Luciano et al., 2020). Most P in plant-based feed ingredients is bound to phytate, but pigs and poultry do not synthesize adequate amounts of endogenous phytase to liberate the P in phytate; therefore, P digestibility in plant ingredients is relatively low when fed to pigs (Liao et al., 2005). Use of microbial phytase in diets for pigs improves P absorption and utilization by hydrolyzing phytate within the gastrointestinal tract of pigs (Pallauf et al., 1994). However, to our knowledge, data for effects of increasing levels of microbial phytase on STTD of P in bakery meal have not been reported. Although data on growth performance of weanling pigs fed diets containing 300 g/kg bakery meal have been reported (Tretola et al., 2019a, 2019b), data for greater inclusion of bakery meal in diets for weanling pigs are not available. Due to differences in nutrient composition between bakery meal and corn, protein utilization of pigs fed diets containing bakery meal instead of corn may be different, but data to demonstrate this are limited. Therefore, the objectives of this work were to test the hypotheses that inclusion of graded levels of microbial phytase in diets based on bakery meal improves the STTD of P and that replacing corn with bakery meal will not influence growth performance of weanling pigs if diets are balanced for digestible nutrients.

MATERIALS AND METHODS

Protocols for 2 experiments were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois Urbana-Champaign. Pigs that were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN, USA) were used in both experiments. Two sources of bakery meal (bakery meal 1 and bakery meal 2; Quincy Farm Products; Quincy, IL, USA) were used (Table 1).

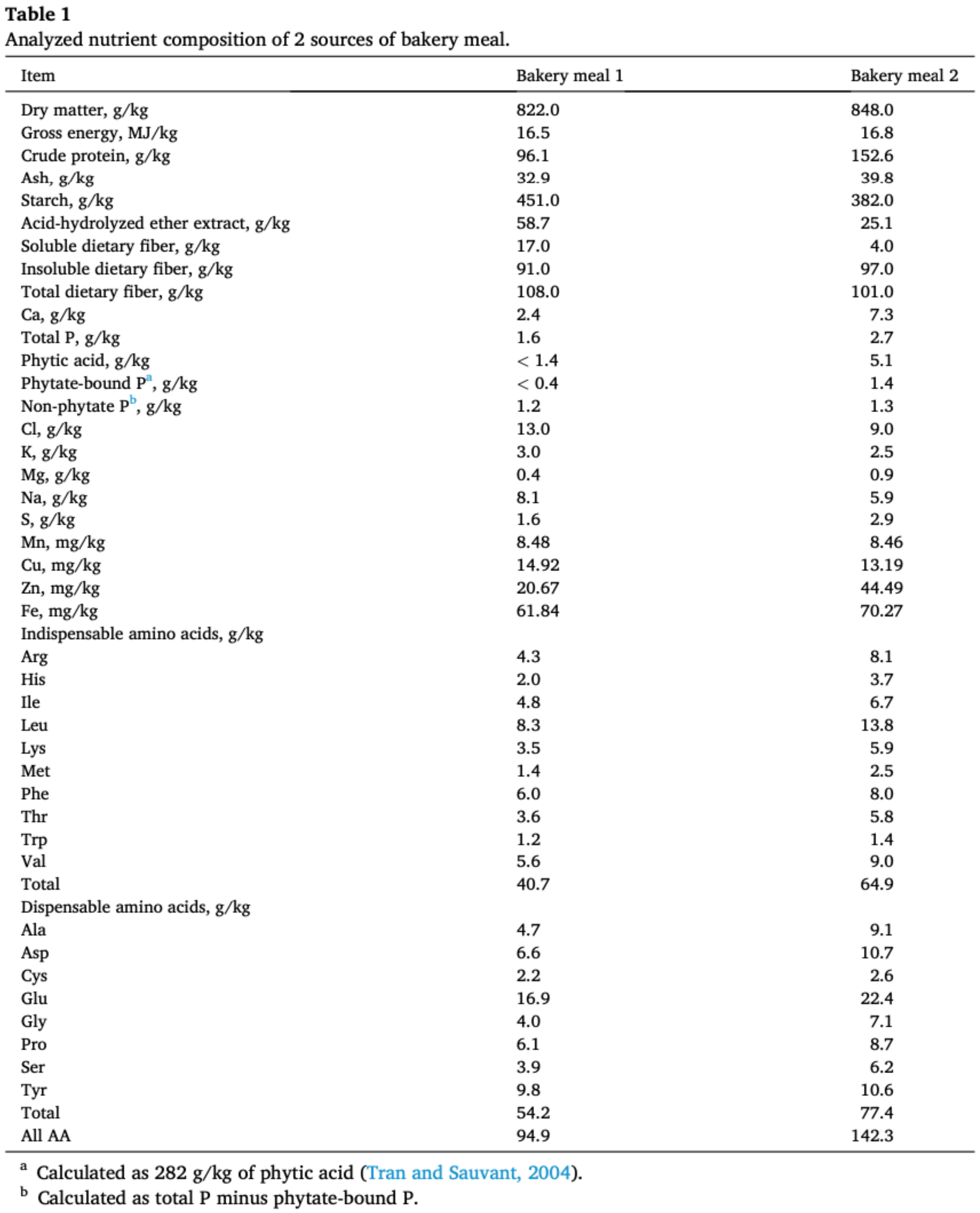

Table 1
Analyzed nutrient composition of 2 sources of bakery meal.

Item	Bakery meal 1	Bakery meal 2
Dry matter, g/kg	822.0	848.0
Gross energy, MJ/kg	16.5	16.8
Crude protein, g/kg	96.1	152.6
Ash, g/kg	32.9	39.8
Starch, g/kg	451.0	382.0
Acid-hydrolyzed ether extract, g/kg	58.7	25.1
Soluble dietary fiber, g/kg	17.0	4.0
Insoluble dietary fiber, g/kg	91.0	97.0
Total dietary fiber, g/kg	108.0	101.0
Ca, g/kg	2.4	7.3
Total P, g/kg	1.6	2.7
Phytic acid, g/kg	< 1.4	5.1
Phytate-bound P[a], g/kg	< 0.4	1.4
Non-phytate P[b], g/kg	1.2	1.3
Cl, g/kg	13.0	9.0
K, g/kg	3.0	2.5
Mg, g/kg	0.4	0.9
Na, g/kg	8.1	5.9
S, g/kg	1.6	2.9
Mn, mg/kg	8.48	8.46
Cu, mg/kg	14.92	13.19
Zn, mg/kg	20.67	44.49
Fe, mg/kg	61.84	70.27
Indispensable amino acids, g/kg		
Arg	4.3	8.1
His	2.0	3.7
Ile	4.8	6.7
Leu	8.3	13.8
Lys	3.5	5.9
Met	1.4	2.5
Phe	6.0	8.0
Thr	3.6	5.8
Trp	1.2	1.4
Val	5.6	9.0
Total	40.7	64.9
Dispensable amino acids, g/kg		
Ala	4.7	9.1
Asp	6.6	10.7
Cys	2.2	2.6
Glu	16.9	22.4
Gly	4.0	7.1
Pro	6.1	8.7
Ser	3.9	6.2
Tyr	9.8	10.6
Total	54.2	77.4
All AA	94.9	142.3

[a] Calculated as 282 g/kg of phytic acid (Tran and Sauvant, 2004).

[b] Calculated as total P minus phytate-bound P.

Animals, treatments, and experimental procedure

Experiment 1: phosphorus digestibility

Eighty barrows (initial body weight: 14.25 ± 1.91 kg) were allotted to a randomized complete block design with 2 blocks, 10 diets, 4 pigs per diet in each block for a total of 8 replicate pigs per diet. Pigs were weaned 2 weeks apart and weaning group was used as the blocking factor. Two basal diets based on each source of bakery meal without microbial phytase were formulated (Tables 2 and 3).

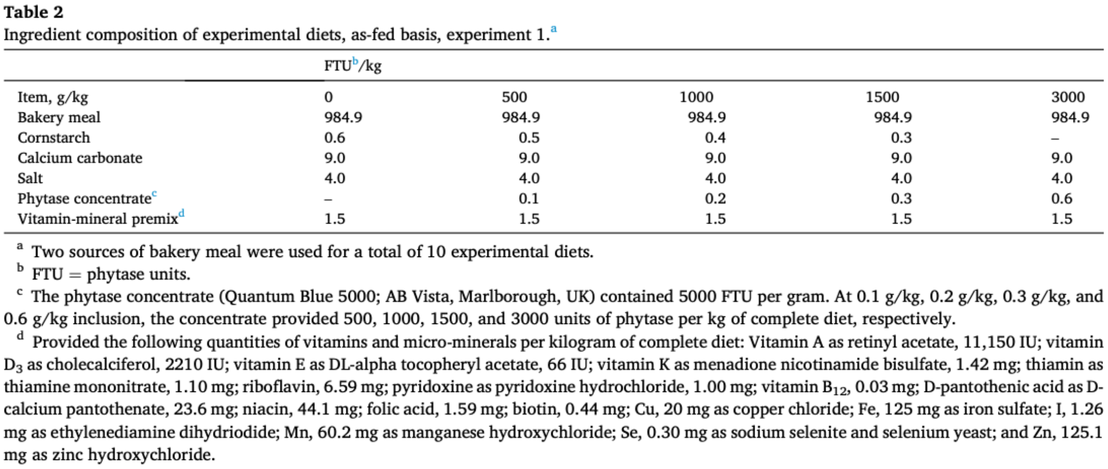

Table 2
Ingredient composition of experimental diets, as-fed basis, experiment 1.[a]

	FTU[b]/kg				
Item, g/kg	0	500	1000	1500	3000
Bakery meal	984.9	984.9	984.9	984.9	984.9
Cornstarch	0.6	0.5	0.4	0.3	–
Calcium carbonate	9.0	9.0	9.0	9.0	9.0
Salt	4.0	4.0	4.0	4.0	4.0
Phytase concentrate[c]	–	0.1	0.2	0.3	0.6
Vitamin-mineral premix[d]	1.5	1.5	1.5	1.5	1.5

[a] Two sources of bakery meal were used for a total of 10 experimental diets.

[b] FTU = phytase units.

[c] The phytase concentrate (Quantum Blue 5000; AB Vista, Marlborough, UK) contained 5000 FTU per gram. At 0.1 g/kg, 0.2 g/kg, 0.3 g/kg, and 0.6 g/kg inclusion, the concentrate provided 500, 1000, 1500, and 3000 units of phytase per kg of complete diet, respectively.

[d] Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as cholecalciferol, 2210 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

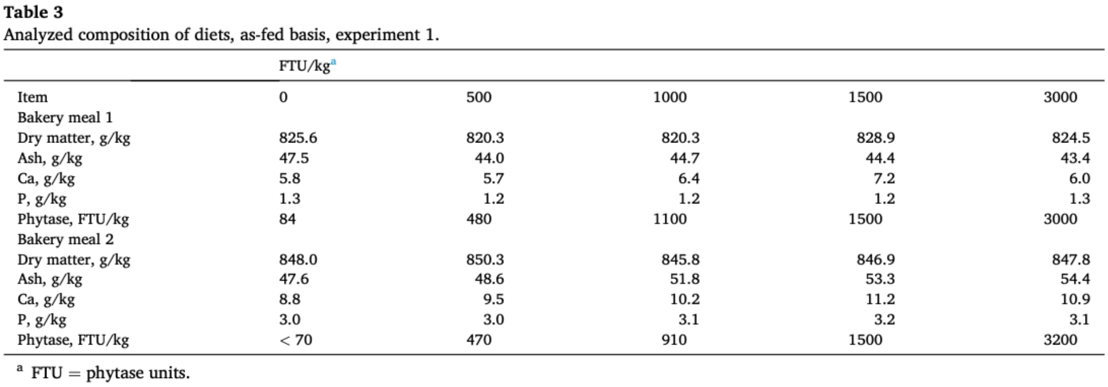

Table 3
Analyzed composition of diets, as-fed basis, experiment 1.

	FTU/kg[a]				
Item	0	500	1000	1500	3000
Bakery meal 1					
Dry matter, g/kg	825.6	820.3	820.3	828.9	824.5
Ash, g/kg	47.5	44.0	44.7	44.4	43.4
Ca, g/kg	5.8	5.7	6.4	7.2	6.0
P, g/kg	1.3	1.2	1.2	1.2	1.3
Phytase, FTU/kg	84	480	1100	1500	3000
Bakery meal 2					
Dry matter, g/kg	848.0	850.3	845.8	846.9	847.8
Ash, g/kg	47.6	48.6	51.8	53.3	54.4
Ca, g/kg	8.8	9.5	10.2	11.2	10.9
P, g/kg	3.0	3.0	3.1	3.2	3.1
Phytase, FTU/kg	< 70	470	910	1500	3200

[a] FTU = phytase units.

Eight additional diets that were similar to the 2 basal diets were formulated with the exception that 500, 1000, 1500, or 3000 units of microbial phytase (Quantum Blue 5 G, AB Vista, Marlborough, UK) were added to each diet. Other than P, vitamins and minerals were included in all diets to meet or exceed the estimated nutrient requirements for weanling pigs (NRC, 2012). Pigs were housed individually in metabolism crates that were equipped with a self-feeder, a nipple waterer, a slatted floor, and a screen under the slatted floor that allowed for total collection of feces. Pigs were fed 3.2 times the metabolizable energy requirement for maintenance (i.e., 0.824 MJ per kg body weight$^{0.60}$; NRC, 2012), which was provided each day in 2 equal meals at 0730 and 1530 h. Throughout the experiment, pigs had free access to water. Feed consumption was recorded daily and diets were fed for 12 days. The initial 5 days were considered the adaptation period to the diet, whereas feces were collected during the following 4 days according to standard procedures using the marker-to-marker approach (Adeola, 2001). Chromic oxide (at approximately 3 g/kg) was used as the marker. Fecal samples were stored at − 20 °C immediately after collection.

Experiment 2: growth performance

A total of 160 newly weaned pigs (initial body weight: 7.17 ± 0.94 kg) were allotted to 1 of 5 dietary treatments in a randomized complete block design with body weight as the block. A 2-phase feeding program was used with day 1–14 as phase 1 and day 15–35 as phase 2. There were 4 pigs per pen and 8 replicate pens per treatment. A total of 10 diets were formulated (Tables 4 and 5), and all diets in phases 1 and 2 were formulated to meet nutrient requirements for weanling pigs (NRC, 2012).

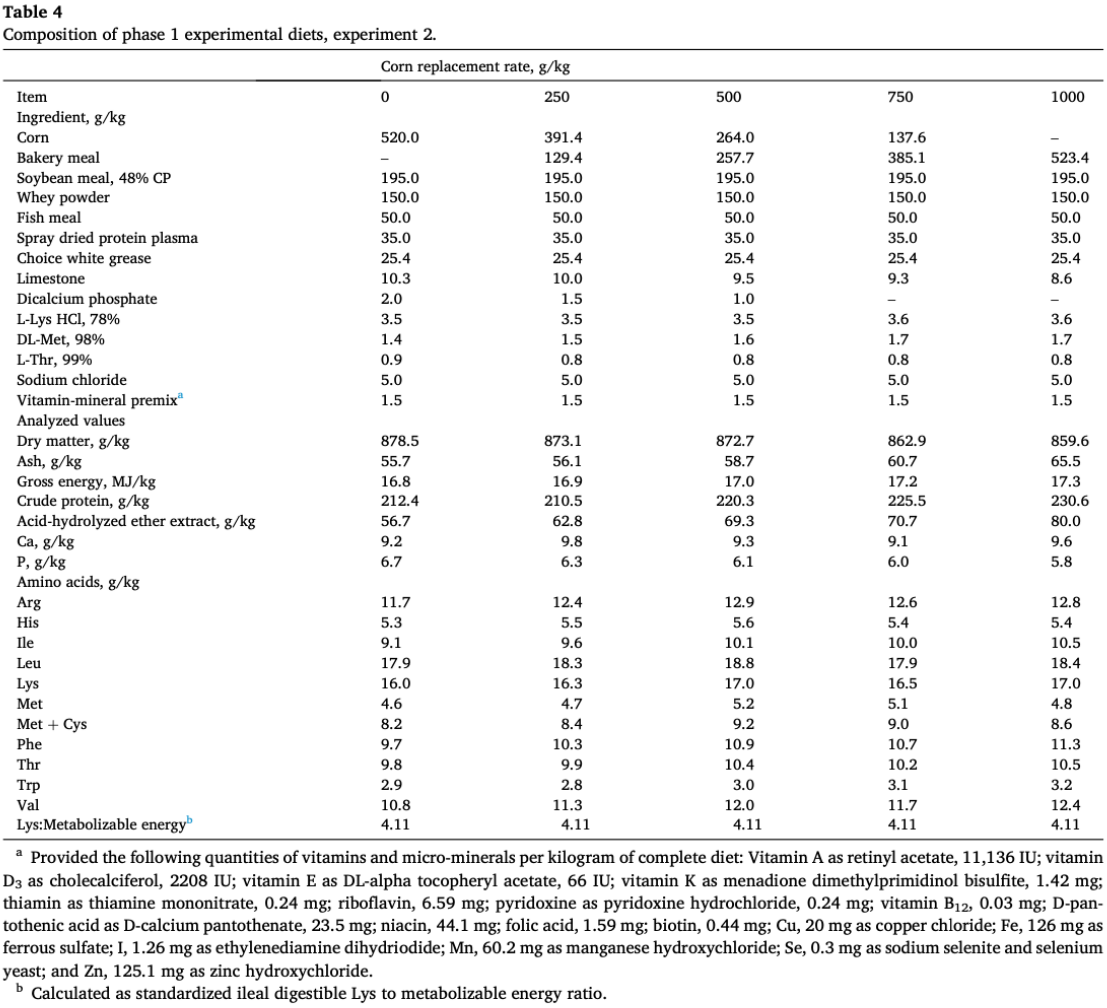

Table 4
Composition of phase 1 experimental diets, experiment 2.

	Corn replacement rate, g/kg				
Item	0	250	500	750	1000
Ingredient, g/kg					
Corn	520.0	391.4	264.0	137.6	–
Bakery meal	–	129.4	257.7	385.1	523.4
Soybean meal, 48% CP	195.0	195.0	195.0	195.0	195.0
Whey powder	150.0	150.0	150.0	150.0	150.0
Fish meal	50.0	50.0	50.0	50.0	50.0
Spray dried protein plasma	35.0	35.0	35.0	35.0	35.0
Choice white grease	25.4	25.4	25.4	25.4	25.4
Limestone	10.3	10.0	9.5	9.3	8.6
Dicalcium phosphate	2.0	1.5	1.0	–	–
L-Lys HCl, 78%	3.5	3.5	3.5	3.6	3.6
DL-Met, 98%	1.4	1.5	1.6	1.7	1.7
L-Thr, 99%	0.9	0.8	0.8	0.8	0.8
Sodium chloride	5.0	5.0	5.0	5.0	5.0
Vitamin-mineral premix[a]	1.5	1.5	1.5	1.5	1.5
Analyzed values					
Dry matter, g/kg	878.5	873.1	872.7	862.9	859.6
Ash, g/kg	55.7	56.1	58.7	60.7	65.5
Gross energy, MJ/kg	16.8	16.9	17.0	17.2	17.3
Crude protein, g/kg	212.4	210.5	220.3	225.5	230.6
Acid-hydrolyzed ether extract, g/kg	56.7	62.8	69.3	70.7	80.0
Ca, g/kg	9.2	9.8	9.3	9.1	9.6
P, g/kg	6.7	6.3	6.1	6.0	5.8
Amino acids, g/kg					
Arg	11.7	12.4	12.9	12.6	12.8
His	5.3	5.5	5.6	5.4	5.4
Ile	9.1	9.6	10.1	10.0	10.5
Leu	17.9	18.3	18.8	17.9	18.4
Lys	16.0	16.3	17.0	16.5	17.0
Met	4.6	4.7	5.2	5.1	4.8
Met + Cys	8.2	8.4	9.2	9.0	8.6
Phe	9.7	10.3	10.9	10.7	11.3
Thr	9.8	9.9	10.4	10.2	10.5
Trp	2.9	2.8	3.0	3.1	3.2
Val	10.8	11.3	12.0	11.7	12.4
Lys:Metabolizable energy[b]	4.11	4.11	4.11	4.11	4.11

[a] Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

[b] Calculated as standardized ileal digestible Lys to metabolizable energy ratio.

Table 5
Composition of phase 2 experimental diets, experiment 2.

	Corn replacement rate, g/kg				
Item	0	250	500	750	1000
Ingredient, g/kg					
Corn	542.1	406.9	271.9	135.8	–
Bakery meal	–	135.5	271.1	407.6	543.5
Soybean meal, 48% CP	305.0	305.0	305.0	305.0	305.0
Whey powder	100.0	100.0	100.0	100.0	100.0
Choice white grease	22.0	22.0	22.0	22.0	22.0
Limestone	9.5	9.5	9.2	9.0	8.8
Dicalcium phosphate	8.6	8.3	8.0	7.8	7.8
L-Lys HCl, 78%	3.8	3.8	3.8	3.8	3.8
DL-Met, 98%	1.5	1.5	1.5	1.6	1.7
L-Thr, 99%	1.0	1.0	1.0	0.9	0.9
Sodium chloride	5.0	5.0	5.0	5.0	5.0
Vitamin-mineral premix[a]	1.5	1.5	1.5	1.5	1.5
Analyzed values					
Dry matter, g/kg	877.5	872.7	864.7	860.5	853.6
Ash, g/kg	51.0	51.0	53.9	53.2	61.0
Gross energy, MJ/kg	16.5	16.7	16.8	16.8	17.0
Crude protein, g/kg	200.9	205.9	202.8	213.3	219.9
Acid-hydrolyzed ether extract, g/kg	54.0	54.5	58.9	63.2	68.5
Ca, g/kg	7.5	7.7	7.8	8.0	8.2
P, g/kg	6.0	6.0	6.0	6.0	6.1
Amino acids, g/kg					
Arg	12.8	13.0	13.3	13.5	13.8
His	5.4	5.8	6.1	6.5	6.8
Ile	8.7	8.9	9.1	9.3	9.6
Leu	17.4	17.2	17.1	17.0	16.9
Lys	14.2	14.4	14.6	14.8	15.0
Met	4.6	4.6	4.6	4.7	4.8
Met + Cys	7.1	7.1	7.1	7.1	7.1
Phe	9.8	9.9	10.1	10.2	10.4
Thr	8.9	9.0	9.2	9.2	9.3
Trp	2.5	2.6	2.7	2.8	2.9
Val	9.5	9.6	9.8	10.0	10.2
Lys:Metabolizable energy[b]	3.82	3.82	3.82	3.82	3.82

[a] Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

[b] Calculated as standardized ileal digestible Lys to metabolizable energy ratio.

In each phase, a control diet containing primarily corn and soybean meal and no bakery meal was formulated, and within each phase, 4 additional diets were formulated by replacing 250, 500, 750, or 1000 g/kg of the corn in the control diet with bakery meal (bakery meal 1; Quincy Farm Products; Quincy, IL, USA). All diets were calculated to have a similar standardized ileal digestible Lys to metabolizable energy ratio. Individual pig weights were recorded at the beginning of the experiment, on day 14, and on day 35. Feed additions were recorded daily and the weight of feed left in the feeder was recorded on day 14 and 35. Diarrhea scores were assessed visually per pen every other day using a score from 1 to 5 (1 = normal feces; 2 = moist feces; 3 = mild diarrhea; 4 = severe diarrhea; and 5 = watery diarrhea). Diarrhea frequency was obtained by totaling the number of pen days with diarrhea scores greater than or equal to 3 divided by the total number of pen days multiplied by 100, with pen days referring to the number of pens multiplied by the number of days assessing diarrhea scores. At the end of each phase, a blood sample was collected from one pig per pen via vena puncture. Samples were collected in vacutainers with heparin to yield blood plasma and these samples were stored at − 20 °C until analyzed.

Sample analyses

Experiment 1: phosphorus digestibility

At the conclusion of the experiment, fecal samples were thawed and mixed within pig and diet, and then dried at 50 °C in a forced air-drying oven and ground through a 1-mm screen in a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ, USA). After wet ash sample preparation (Method 975.03; AOAC International, 2007), fecal samples, ingredients, and diets were analyzed for P by inductively coupled plasma spectroscopy (Method 985.01; AOAC International, 2007) and for dry matter by oven drying at 135 °C for 2 h (Method 930.15; AOAC International, 2007). Diets and ingredients were also analyzed for Ca and ash (Method 942.05; AOAC International, 2007), and the 2 sources of bakery meal were analyzed for insoluble dietary fiber and soluble dietary fiber (Method 991.43; AOAC International, 2007) using the ANKOM^{TDF} Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Total dietary fiber was calculated as the sum of IDF and SDF. Nitrogen was also analyzed in ingredients using the combustion procedure (Method 990.03; AOAC International, 2007) on an FP628 protein analyzer (Leco Corporation, St. Joseph, MI, USA). Aspartic acid was used as a calibration standard and crude protein was calculated as the concentration of analyzed nitrogen multiplied by 6.25. In- gredients were analyzed for phytic acid (Ellis et al., 1977), and all diets were analyzed for phytase activity (Method 2000.12; AOAC International, 2007; Eurofins Scientific Inc., Des Moines, IA, USA). Using benzoic acid as internal standard, ingredient samples were analyzed for gross energy using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA), and AA were analyzed on a Hitachi Amino Acid Analyzer (Model L8880, Hotachi High Tecnologies America Inc., Pleasanton, CA, USA). The con- centration of acid-hydrolyzed ether extract in ingredients was determined by acid hydrolysis using 3*N* HCl (Sanderson, 1986) followed by crude fat extraction with petroleum ether (method 2003.06, AOAC International, 2007). Bakery meal samples were also analyzed for Mg, Cu, Fe, Mn, and Zn as explained for the analysis of P, for Na and K using flame emission photometry (Hald and Mason, 1958), for Cl using manual titration (Gilliam, 1971), and for S using a gravimetric method (Wu and Mousavi, 2017).

Experiment 2: growth performance

All diet samples were ground through a 1-mm screen in a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ, USA) prior to chemical analysis. Diets were analyzed for dry matter, ash, gross energy, CP, AA, Ca, and P as indicated for experiment 1. Blood samples were analyzed for blood urea nitrogen (BUN), total protein, and albumin using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA, USA).

Calculation and statistical analyses

Experiment 1: phosphorus digestibility

The apparent total tract digestibility (ATTD) of P in each diet was calculated (NRC, 2012) by subtracting the amount of P output in feces from P intake and this was then divided by P intake. By correcting values for ATTD of P in each diet for the basal endogenous losses of P (i.e., 190 mg per kg dry matter intake; NRC, 2012), the STTD of P in each diet was calculated. Because bakery meal was the only source of P in the diets, values for ATTD and STTD of P in each diet also represented the ATTD and STTD of P in the 2 sources of bakery meal that were used in the experiment. Data were analyzed using the Mixed Procedure of SAS with the pig as the experimental unit (SAS Institute Inc., Cary, NC, USA). Homogeneity of the variances was confirmed using the UNIVARIATE procedure in SAS. Outliers were identified and removed as values deviated from the treatment mean by more than 3 times the interquartile range. Treatment means were calculated using the least squares means statement in SAS. Orthogonal contrasts for a 2 × 5 factorial arrangement of treatments were used to determine linear and quadratic effects of phytase, the main effect of bakery meal, and bakery meal × phytase interactions. Contrast statements were used with coefficients for unequally spaced treatments being generated using the interactive matrix language procedure in SAS. Block and replicate within block were considered random effects. Statistical significance was considered at $P < 0.05$.

Experiment 2: growth performance

Data were summarized to calculate average daily feed intake (ADFI), average daily gain (ADG), and gain to feed ratio (G:F) within each pen and treatment group. Data were summarized from day 1–14, day 15–35, and for the entire experiment. Data were analyzed using the Mixed Procedure of SAS with the pen as the experimental unit. Homogeneity of variances was confirmed and data were tested for outliers as explained for experiment 1. The model included bakery meal inclusion rate as the fixed effect, whereas block was the random effect. Least squares means were calculated, and linear and quadratic effects of increasing levels of bakery meal on growth performance and diarrhea scores were determined as explained for experiment 1. The frequency procedure of SAS was used to analyze frequency of diarrhea with diet as the fixed effect. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

RESULTS

Phosphorus digestibility

The concentration of P in bakery meal 1 and bakery meal 2 was 1.6 and 2.7 g/kg, respectively. Due to increased concentration of P in diets containing bakery meal 2, P intake of pigs fed diets containing bakery meal 2 was greater ($P < 0.01$) compared with that of pigs fed the bakery meal 1 diets (Table 6). Greater reduction in P in feces and fecal P output was observed in pigs fed diets with bakery meal 2 compared with that of pigs fed the bakery meal 1 diets upon phytase supplementation (linear and quadratic interaction, $P < 0.01$). Phosphorus absorption of pigs fed diets with bakery meal 2 increased more than that of pigs fed the bakery meal 1 diets as the concentration of phytase increased (quadratic interaction, $P < 0.05$). As a result, greater increases in coefficients of ATTD and STTD of P in bakery meal 2 was observed as phytase supplementation increased (quadratic interaction, $P < 0.01$). However, due to increased concentration of phytate in bakery meal 2, coefficients for ATTD and STTD of P in bakery meal 1 were greater compared with that of bakery meal 2 ($P < 0.01$).

Table 6
Coefficients of apparent total tract digestibility (ATTD), and standardized total tract digestibility (STTD) of P in 2 sources of bakery meal fed to growing pigs, experiment 1.[a]

Item	ADFI, g/d	P intake, g/d	P in feces, g/kg	P output, g/d	P absorption, g/d	ATTD of P	Basal EPL[b], mg/d	STTD of P[c]
Bakery meal 1								
0 FTU[d]/kg	758	1.21	4.65	0.38	0.84	0.688	120	0.787
500 FTU/kg	756	1.21	3.75	0.31	0.90	0.744	118	0.841
1000 FTU/kg	763	1.20	3.58	0.29	0.89	0.742	119	0.839
1500 FTU/kg	806	1.29	3.38	0.31	0.98	0.765	134	0.869
3000 FTU/kg	801	1.28	3.62	0.32	0.96	0.746	134	0.851
Bakery meal 2								
0 FTU/kg	758	2.05	9.13	0.98	1.06	0.520	122	0.580
500 FTU/kg	730	1.96	6.06	0.64	1.32	0.670	118	0.730
1000 FTU/kg	731	1.98	5.95	0.71	1.26	0.652	117	0.711
1500 FTU/kg	755	2.04	5.24	0.56	1.48	0.723	122	0.783
3000 FTU/kg	746	2.03	5.23	0.62	1.46	0.696	120	0.756
SEM	50.0	0.106	0.214	0.042	0.114	0.026	8.0	0.026
P-values								
Bakery meal	0.092	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.073	< 0.001
Phytase, Linear	0.268	0.487	< 0.001	< 0.001	< 0.001	< 0.001	0.035	< 0.001
Phytase, Quadratic	0.905	0.920	< 0.001	< 0.001	0.014	< 0.001	0.947	< 0.001
Bakery meal × Phytase, Linear	0.303	0.437	< 0.001	< 0.001	0.031	0.009	0.038	0.016
Bakery meal × Phytase, Quadratic	0.728	0.769	< 0.001	< 0.001	0.019	0.043	0.662	0.042

[a] Data are least squares means of 6–8 observations per treatment.
[b] EPL = endogenous P loss. This value was estimated to be at 190 mg/kg dry matter intake. The daily basal endogenous P loss (mg/d) for each diet was calculated by multiplying the endogenous P loss (mg/kg dry matter intake) by the daily dry matter intake of each diet (Almeida and Stein, 2010).
[c] Values for STTD were calculated by correcting values for ATTD for basal endogenous losses (NRC, 2012).
[d] FTU = phytase units.

Growth performance

There was no effect of increasing concentrations of bakery meal on final body weight, ADG, ADFI, or G:F of pigs from day 1–14 (Table 7). However, ADG of pigs from day 15–35 and for the overall experimental period tended to decrease ($P < 0.10$) as the concentration of bakery meal increased in the diets. The G:F from day 15–35 and for the overall experimental period linearly decreased ($P < 0.01$) as bakery meal inclusion increased in the diets. However, no differences among dietary treatments were observed from day 15–35 or for the overall experimental period for ADFI and the final body weight on day 35 was not different among treatments.

Table 7
Growth performance of pigs fed diets containing increasing levels of bakery meal, experiment 2.[a]

	Corn replacement rate, g/kg						*P*-value	
Item	0	250	500	750	1000	SEM	Linear	Quadratic
Day 1–14								
Initial body weight, kg	7.16	7.18	7.11	7.21	7.13	0.331	0.529	0.815
ADG[b], kg	0.17	0.17	0.18	0.17	0.19	0.011	0.878	0.619
ADFI[b], kg	0.23	0.24	0.24	0.24	0.24	0.014	0.761	0.739
G:F[b]	0.74	0.71	0.76	0.71	0.79	0.034	0.937	0.336
Final body weight, kg	9.54	9.53	9.62	9.53	9.75	0.357	0.777	0.654
Day 15–35								
ADG, kg	0.61	0.60	0.59	0.58	0.53	0.021	0.055	0.150
ADFI, kg	0.88	0.87	0.96	0.93	1.03	0.050	0.126	0.582
G:F	0.70	0.70	0.63	0.63	0.53	0.026	0.003	0.204
Final body weight, kg	22.29	22.20	22.10	21.69	20.84	0.738	0.149	0.316
Day 1–35								
ADG, kg	0.43	0.43	0.42	0.41	0.39	0.013	0.064	0.090
ADFI, kg	0.62	0.61	0.67	0.65	0.71	0.031	0.129	0.714
G:F	0.70	0.70	0.64	0.64	0.54	0.024	0.002	0.147

[a] Data are least squares means of 8 observations for all treatments.
[b] ADG = average daily gain; ADFI = average daily feed intake; G:F = gain to feed ratio.

Increasing concentrations of bakery meal in diets did not affect fecal scores or diarrhea frequency of pigs (Table 8).

Table 8
Diarrhea score and frequency of diarrhea for pigs fed diets containing increasing levels of bakery meal, experiment 2.[a]

	Corn replacement rate, g/kg						*P*-value	
Item	0	250	500	750	1000	SEM	Linear	Quadratic
Diarrhea score[b]								
Day 1–14	1.27	1.27	1.34	1.20	1.34	0.124	0.798	0.878
Day 15–35	1.66	1.58	1.49	1.61	1.54	0.086	0.582	0.407
Day 1–35	1.50	1.49	1.43	1.44	1.46	0.084	0.636	0.572
Frequency of diarrhea								
Day 1–14								
Pen days[c]	56	56	56	56	56			
Frequency[d]	0.00	3.57	7.14	1.79	3.57	–	0.282	
Day 15–35								
Pen days	80	80	80	80	80			
Frequency	7.50	3.75	8.75	10.00	6.25	–	0.600	
Day 1–35								
Pen days	136	136	136	136	136			
Frequency	4.41	3.68	8.09	6.62	5.15	–	0.520	

[a] Data are least squares means of 8 observations for all treatments.
[b] Diarrhea score = 1, normal feces; 2, moist feces; 3, mild diarrhea; 4, severe diarrhea; 5, watery diarrhea.
[c] Pen days = number of pens × the number of days assessing diarrhea scores.
[d] Frequency = (number of pen days with diarrhea scores $\geq$ 3/pen days) × 100.

Likewise, bakery meal did not affect the concentration of BUN or plasma concentrations of total protein and albumin (Table 9).

Table 9
Blood characteristics for pigs fed diets containing increasing levels of bakery meal, experiment 2.[a]

	Corn replacement rate, g/kg						*P*-value	
Item	0	250	500	750	1000	SEM	Linear	Quadratic
Day 14								
BUN[b], mg/dL	7.63	7.75	6.38	8.88	9.25	0.849	0.155	0.156
Total protein, g/dL	4.78	4.75	4.51	4.69	4.78	0.161	0.644	0.145
Albumin, g/dL	2.66	2.74	2.68	2.74	2.74	0.086	0.535	0.934
Day 35								
BUN, mg/dL	8.75	9.00	8.00	9.00	10.13	0.672	0.537	0.145
Total protein, g/dL	5.39	5.30	5.29	5.30	5.46	0.199	0.911	0.485
Albumin, g/dL	3.13	3.26	3.19	3.33	3.33	0.122	0.231	0.891

[a] Data are least squares means of 8 observations for all treatments.
[b] BUN = blood urea nitrogen.

DISCUSSION

Bakery meal is a high-energy ingredient containing approximately 16.7 MJ of metabolizable energy per kg dry matter (Luciano et al., 2020) due to its high concentrations of starch and fat and low concentration of fiber (Liu et al., 2018; Pinotti et al., 2019). The concentration of protein in bakery meal is low and the digestibility of Lys is sometimes very low due to excessive heating of the in- gredients used in manufacturing bakery meal (Almeida et al., 2011; Casas et al., 2015, 2018). Nevertheless, bakery meal may sub- stitute cereal grains in diets for pigs because the chemical composition is close to that of wheat and barley (Pinotti et al., 2019; Luciano et al., 2020). Indeed, by balancing diets for concentrations of digestible nutrients, it is possible to partially substitute traditional sources of energy and crude protein (CP) in animal diets with bakery meal (Pinotti et al., 2014).

Phosphorus digestibility

Phosphorus needs to be included in diets for pigs because it is the second most abundant mineral in the body (Viveros et al., 2002). The majority of body P is located in bones and teeth, but P is also important in soft tissue, and is involved in many physiological functions in pigs (Almeida and Stein, 2012). Corn, which is one of the major ingredients in pig diets, contains approximately 2.6 g/kg of P (NRC, 2012), but there may be slightly more P in bakery meal (Casas et al., 2018). Therefore, bakery meals can be considered a corn substitute that will provide not only energy and starch (Liu et al., 2018; Luciano et al., 2020), but also minerals (Liu et al., 2018) to diets. However, P in animal manure may result in environmental pollution (Gerritse and Zugec, 1977) and it is, therefore, important that P nutrition is managed to avoid excessive P excretion from pigs. In cereal grains and grain co-products, oilseed coproducts, and other plant protein sources, more than 50% of P is often bound to phytic acid (Iyayi et al., 2013) in the form of myoinositol phosphate (Nasi, 1990). It is, therefore, common practice to add phytase to diets for pigs (Dersjant-Li et al., 2018) because phytase may release some of the phytate-bound P in the diet, and thereby reduce the need for feed phosphates in the diet (Dersjant-Li et al., 2018). The concentration of P in bakery meal is somewhat variable as was also illustrated for the 2 sources used in this experiment. The observation that microbial phytase increased the STTD of P in bakery meal is in agreement with Rojas et al. (2013) who observed an increase in the STTD of P in bakery meal if 500 phytase units (FTU) was used. The values for STTD of P in bakery meal 2 without phytase and in the diet with 500 FTU of phytase were in very good agreement with previous values (Rojas et al., 2013). The reason microbial phytase was less effective in increasing the STTD of P in bakery meal 1 than in bakery meal 2 is that bakery meal 1 had a low concentration of phytate, and therefore, a low concentration of phytate-bound P. As a consequence, the STTD of P in bakery meal 1 without phytase was greater than in bakery meal 2 without phytase. Corn co-products and soybean meal with a low amount of phytate-bound P, and therefore a high STTD of P without phytase, also have a lower response to microbial phytase than co-products with more phytate-bound P (Almeida and Stein, 2012; Rojas and Stein, 2012). The difference between bakery meal 1 and 2 in con- centration of phytate and the STTD of P is likely a consequence of the different product mixes that may be used in the production of bakery meal. Ingredients with high concentrations of P and phytate (e.g., bran and canola co-products) are often included in bakery meal (Liu et al., 2018), and differences in inclusion rates of these ingredients may explain the differences between the 2 sources of bakery meal used. However, because the product mixes included in the 2 sources of bakery meal used is unknown, we are unable to conclude that differences in phytate concentration were due to different product mixes.

Growth performance

All animals remained in good health throughout the experiment. The reason ADG and G:F were reduced from day 15–35 and for the overall experiment as greater quantities of bakery meal were used is unclear. Bakery meal may contain bran and cereal co-products (Liu et al., 2018), and these ingredients may have reduced digestibility of energy and AA as bakery meal increased in the diets with a subsequent reduction in G:F of pigs. The observed reduction in G:F with bakery meal inclusion is in contrast with data indicating that pig growth performance was not affected when weanling pigs were fed diets with bakery meal at 300 g/kg (Tretola et al., 2019a). Because bakery meal contains some cooked or baked materials characterized by a greater nutrient digestibility than conventional ingredients, newly weaned pigs were expected to have increased utilization of nutrients from bakery meal compared with corn. The present data, however, indicate that a complete substitution of corn for bakery meal may not be beneficial for pigs after the initial 2 weeks post-weaning and this observation is in agreement with results of other experiments (Tretola et al., 2019b). The lack of differences in fecal scores of pigs indicates that replacing corn with bakery meal does not elicit a detrimental change in the microbiota profile in the intestinal tract of pigs, and therefore, does not influence intestinal health of pigs. Blood urea nitrogen is an indicator of AA utilization efficiency (Coma et al., 1995), whereas albumin binds and transports AA in the blood (Quinlan et al., 2005). Therefore, the observation that no differences were observed in concentrations of BUN or albumin indicates that absorption and utilization of dietary protein and AA were not affected by replacing corn with bakery meal.

CONCLUSION

Results of the experiments demonstrated that it is possible to include bakery meal in pig diets, although the nutritional composition may vary among sources of bakery meal. By adding phytase to pig diets containing bakery meal, P digestibility may be improved, which can contribute to a reduction of P in manure. Overall gain to feed ratio of pigs was reduced when corn was replaced by bakery meal in the diets; therefore, a complete substitution of corn for bakery meal may not be beneficial for pigs after the initial 2 weeks post- weaning. However, it appears that bakery meal does not influence nutrient metabolism and fecal scores of pigs.

CRediT authorship contribution statement: AL and HHS conceptualized the experiments. AL conducted the animal part of the experiments and summarized data. AL and CDE analyzed data. CDE, LP, and HHS contributed with data interpretation. AL wrote the first draft of the manuscript. CDE, LP, and HHS edited the final version of the manuscript. HHS supervised the project.

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CHAPTER 9

Natural resources for pig production: a global assessment

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Abstract: Population plus with the rise in incomes in many countries have led to the ever-increasing consumption of animal source foods. Pig meat is currently the most consumed meat globally, slightly exceeding the consumption of poultry meat. Global analysis has revealed the correlation between food of animal origin and pressure on the environment, as well as the feed-food debate. However, several gaps have been found in the global detailed assessment of natural resources, not recently updated, specifically associated with the pig feeding sector. Moreover, it is also unclear and not quantified the specific role of international feed trade. In this analysis, we focus on the country-scale internal and external natural resources (i.e., land and water) consumption in the pig feeding sector in 2018. Using country- and production system-specific diets, crop-specific yields, and an agro- hydrological model, we find that in 2018, 87 Mha of agricultural land and 402 km3 of total water (both green and blue) were consumed globally to produce pig feed. Furthermore, the consumption of resources tends to decrease thanks to the international feed trade, especially in China and EU-27, but not without significant environmental impacts. Therefore, both direct and indirect effects contribute to the impact of pig meat production. Our results highlight that innovative and sustainable supplies for feeding animals should thus be investigated along with ways to make the food system more resilient.

INTRODUCTION

Since the global population is increasing, the rapid growth in the consumption of food of animal origin, particularly from monogastric livestock (i.e., pork and poultry), is also increasing (Delgado, 2003; Speedy, 2003; Whitnall & Pitts, 2019; Alexandratos & Bruinsma, 2012; FAO, 2018a). Meat and animal products play an important role in global food security, giving a significant contribution to both protein and calories supply, as well as micronutrients, in human diets (Henchion et al., 2017; FAOSTAT, 2018). However, the production of animal source foods is often also related to food security and food safety issues (Adesogan et al., 2020; Mottet et al., 2017). Since the 1960s, pig meat has become the most produced and consumed meat, though only slightly more than poultry meat in recent years, and it has become an essential source of nutrition for many people around the world (Szűcs & Vida, 2017). The global per capita consumption of pig meat increased from 8.0 kg in 1961 to 15.6 kg in 2018, almost doubling its value (FAOSTAT, 2018). One reason for this rapid growth lies in the production in China, which currently amounts to half of all the pigs raised in the world. This is the result of a set of policies and trade agreements aimed at liberalizing and industrializing Chinese agriculture (Schneider, 2011). On the one hand, this has succeeded in reducing hunger, but not without severe implications for the environment, public health, smallholder farmers, and also food safety (Wu et al., 2020; Schneider 2011).

Livestock production systems demand high energy inputs (Makkar & Ankers, 2014), but also huge amounts of land and water resources to produce feed crops (Karlsson & Röös, 2019; Gerbens-Leenes et al., 2013; Howard et al., 2019), and these natural resources are becoming increasingly scarce in different areas worldwide (FAO, 2018b). The surge in feed production observed in the last years (IFIF, 2018) and the increase in the use of food-grain for the production of high-value animal protein (FAO, 2020) as well, might be exacerbating the competition for arable land and freshwater for primary food (Karlsson & Röös, 2019; Di Paola et al., 2017; Makkar & Ankers, 2014; Schader et al., 2015). Specifically, being pigs monogastric, they are more efficient feed converters but they require higher amounts of food-competing feed compared to ruminants (Mottet et al., 2017). Although cereals made up only 13% of the world’s feed demand in the livestock sector in 2010, cereals for feed accounted for about one-third of all cereal production and, consequently, one-third of the agricultural land devoted to cereals crops (Mottet et al., 2017; FAO, 2013; FAO & Steinfeld, 2006). Added to this, one-third of the global agricultural water demand is devoted to the livestock sector (Ran et al., 2016; Mekonnen & Hoekstra, 2012).

Several authors have, therefore, studied the link between resource use and livestock production (Ran et al., 2016; Gerbens-Leenes et al., 2013; Hoekstra, 2012, van Zanten et al., 2016), with global estimates of food-feed competition (Mottet et al., 2017). However, there are still gaps in information concerning how pigs, with their predominant role in global meat production and the high content of concentrated feed in their diets, contribute to this consumption of natural resources, both directly and indirectly. Mottet et al. (2017) carried out a detailed global analysis on animal feed supply by animal categories, including pigs, and on the land needed to produce such feed. As regards global water consumption related to the production of pig meat, there only to be the country-based study by Mekonnen & Hoekstra (2012), who estimated both the green and blue water footprint for the average period 1996-2005. However, it is mentioned that there was still no available dataset on feed composition by animal category, production system, and country, and therefore results were based on assumptions and combinations of outdated data (Seré & Steinfeld, 1996; Hendy et al., 1995; Wheeler et al., 1981). Some other authors have estimated water and/or land resources associated with pig production in specific regions or countries such as the EU (Sporchia et al., 2021) or the US (Thoma et al., 2015), but often failing to assess the diversity of animal diets between countries and production systems.

In addition, to the best of our knowledge, there are no comprehensive studies in the literature that investigates the role played by international trade in animal feed on the consumption of resources for pig production, even on long distances turning into *virtual* land and water trade. Being the change in dietary supply and the increasing demand for animal products promoted by the development of international trade (Sans & Combris, 2015), this is a key aspect in the research on resource-use in the livestock sector, as is clear, for example, from the now recognized link between soybean production and deforestation in the tropics with the soybean imports from China to produce animal feed (Fuchs, 2020; Taherzadeh & Caro, 2019; Please, 2015; Dou et al., 2020).

This study aims to fill these gaps, assessing for each country both the internal and external natural resources (i.e., land and water) consumption in the pig feeding sector in 2018. To this goal, refers to the amount and type of feed reported in the most up-to-date country and production system-based pig diets (FAO, 2018b), to crop-specific and country-specific yield data (FAOSTAT, 2018), and it uses the spatially distributed crop hydrological model WATNEEDS (Chiarelli et al., 2020), parametrized with the most up-to-date climate data available. It also analyses the international feed crop trade and the resulting *virtual* natural resources consumption, which may bring to light several hidden environmental impacts on the partner countries.

METHODS

The impacts of pig meat production on natural resources were assessed under two different scales. An initial country-scale analysis at a global level on the natural resources used to produce pig feed in 2018 was performed, assuming a domestic production for all the feed crops needed. Then, a detailed analysis focused on the three major producers (China, EU- 27 countries, and the United States) was done: an estimate of the extent of the impact of imports and exports in the sector was added to the generic quantification of natural resources, introducing the concept of *virtual* international trade of natural resources.

PIG FEED DEMAND: DIETS, PRODUCTION SYSTEMS, AND HERD PARAMETERS

The pressure on natural resources and the feed-food competition caused by pig meat consumption and production can be assessed by selecting from the literature conventional country diets typical of different production systems. In this study, the composition of pig diets is based on the Global Livestock Environmental Assessment Model (GLEAM, version 2.0) developed by FAO (FAO, 2018b). GLEAM feed rations were compared with the FAOSTAT Food Balance Sheets (FBS) reporting a country's feed use (FAOSTAT, 2018), and some adjustments were made to cereal rations while maintaining balanced diets. The diets are country-specific for the major producers (China, EU-27 countries, and the United States) and region-specific for the ten global macro-regions. Pig production is differentiated into the backyard, intermediate and industrial systems in GLEAM (Gilbert et al., 2015), and the same subdivision was maintained in our study.

The pig diet is composed of energy sources (70%), of which cereals make up the largest share (grains from wheat, maize, barley, millet, rice, and sorghum); cassava, and sugarcane tops, as well as other energy crop by-products that are sometimes added. Almost all the remaining items in the diet regard the protein intake, with oilseeds playing the greatest role (cakes made from soybean, rape, cotton, and palm kernels), in addition to legumes if produced locally. Swill and scavenging (particularly used in backyard systems) and other secondary ingredients such as fishmeal as well as supplements (amino acids, minerals) are added to complete and balance the diet. Daily dry matter intake was taken from Mottet et al. (2017) studies.

Crops are used as animal feed in various forms: whole crops, crop residues, and by-products. To assess the land and water resources needed to feed pigs at global scale, crop residues and by-products (such as those from grain industries) were not converted into natural resources because they are produced primarily for food or other uses, while the feed fraction is from waste processing. However, among oilseed crops, soybean is the main protein source among feeds: Soyatech (2003) reports that *'About 85% of the world's soybeans are processed annually into soybean cake and oil, of which approximately 97% of the meal is further processed into animal feed*'. Although soybean cake is derived from an edible product, it can therefore be considered as the main driver of soybean production and was thus included in the analysis. Fishmeal and complements were not considered due to their limited use in pig diets (due to legal restrictions in some parts of the world).

The demand for pig feed in 2018 was estimated according to the number of animals slaughtered in each production system within the country/region's borders, the associated diet, and considering a feeding life cycle of 160 days (final weight of 110-120 kg/animal) for each animal, as they are not fed in the first 40-50 days before weaning. In Italy a 270-day life cycle after weaning was considered (final weight of 160-170 kg/animal) because of the heavy pig production, accounting for more than 90% of the pig meat production in the country (Bava et al., 2017).

INTERNATIONAL TRADE IN PIG FEED

A country's pig feed demand is almost never completely met by domestic production. The demand was therefore split into the animal feed from local crop production and imported feed. The same approach by Govoni et al. (2021) for chicken feed production was used. In the specific case of the largest producer countries, the import share of each feed crop was sub-divided into the different partner countries, according to the FAO Detailed Trade Matrix (DTM) (FAOSTAT, 2018). Before using FAO DTM, Kastner et al. (2011) data treatment approach was applied to identify crop producers and final consumers, avoiding double-counting of re-exports. In the case of regional analyses, local and imported feed was not divided, thus the total quantities of land and water demand result from the assumption that feed production is totally satisfied by domestic crop production.

LAND AND VIRTUAL LAND TRADE ASSOCIATED WITH PIG FEED

Once the shares of local and imported feed demand from each partner country were obtained for each crop, these quantities were converted into the fertile land to be cultivated to produce them. This land comes under the definition of cropland area, which includes all the arable land and land under permanent crops. The fertile land is estimated through crop- specific and country-specific crop yields from FAO for 2018 (FAOSTAT, 2018). This led to both the local and *virtual* imported land demand (Govoni et al., 2021).

WATER AND VIRTUAL WATER TRADE ASSOCIATED WITH PIG FEED

After calculating the land required to produce feed crops, the water needed for plant growth needs to be quantified. The crop-specific hydrological model WATNEEDS was used (Chiarelli et al., 2020), which is spatially distributed and physically-based, and differentiates between the demand for blue water (BW) and green water (GW), where *the BW footprint refers to the volume of surface and groundwater consumed (evaporated) as a result of the production of a good* (crop) *and the GW footprint refers to the rainwater consumed* (Hoekstra et al., 2011). The model solves a vertical soil water balance equation at a resolution of 5 arc-min (approximately 10 km), using as input irrigated and rainfed global maps of cultivated areas, taken from the MIRCA dataset (Portmann et al., 2010). Irrigated crops have both water components (BW plus GW), while those that are rainfed only have GW. For the average of the years from 2013 to 2018 the model inputs include potential reference evapotranspiration (ETo) (University of East Anglia's Climate Research Unit Time Series version 4.01 dataset; Harris et al., 2014) and daily precipitation (Climate Hazards Group InfraRed Precipitation with Station v. 2.0 CHIRPS dataset, Funk et al., 2015; National Oceanic and Atmospheric Administration's Climate Prediction Center Global Unified Gauge-Based Analysis of Daily Precipitation CPC dataset; Chen et al., 2008).

RESULTS

GLOBAL PIGMEAT PRODUCTION

In 2018, a total of 1.5 billion of pigs were bred globally, requiring more than 300 Mton of feed crops. This represents an annual agricultural area at least equal to the size of France and the United Kingdom together (87.4 Mha), under the assumption of domestic feed production. This land in turn required 39.7 km^3 of BW and 362.4 km^3 of GW to grow plants (Table S1, Figure 1). The latter takes into account only the feed crops present in the pig diet, and which are grown mainly for feed purposes, thus excluding all by-products (see Methods).

Cereals represent about 70% of the composition of a pig's diet. However, cereals account for only 50% of the diet's land and water demand, thanks to the high agricultural yields obtained in many countries (i.e., the United States, EU countries). On the other hand, oilseed crops, which make up 30% of a pig's diet, cover 50% of the demand for natural resources due to lower agricultural yields. Cereals require 40.5 Mha of agricultural land, 18.3 Mha of maize, 9.1 Mha of wheat, and 13.1 Mha of other cereals; at the same time, 45.8 Mha of oilseed crop (90% for soybean) and 1 Mha for legumes are consumed. This demand is not evenly divided among the global macro-regions due to the uneven distribution of pigs and agricultural yields. More than half of the land required is in East and Southeast Asia (45.7 Mha), mainly due to demand in China. Regions such as the Near East and North Africa, Oceania and South Asia require less than 1 Mha of land (Table S1, Figure 1). The latter is due to the extremely low pork consumption, mainly related to religious customs. Similarly, the water demand reflects the distribution of land between regions, especially as regards GW. However, BW is also influenced by the distribution of irrigation infrastructure, concentrated in East and Southeast Asia, South Asia, Western Europe, and North America (Figure 1).

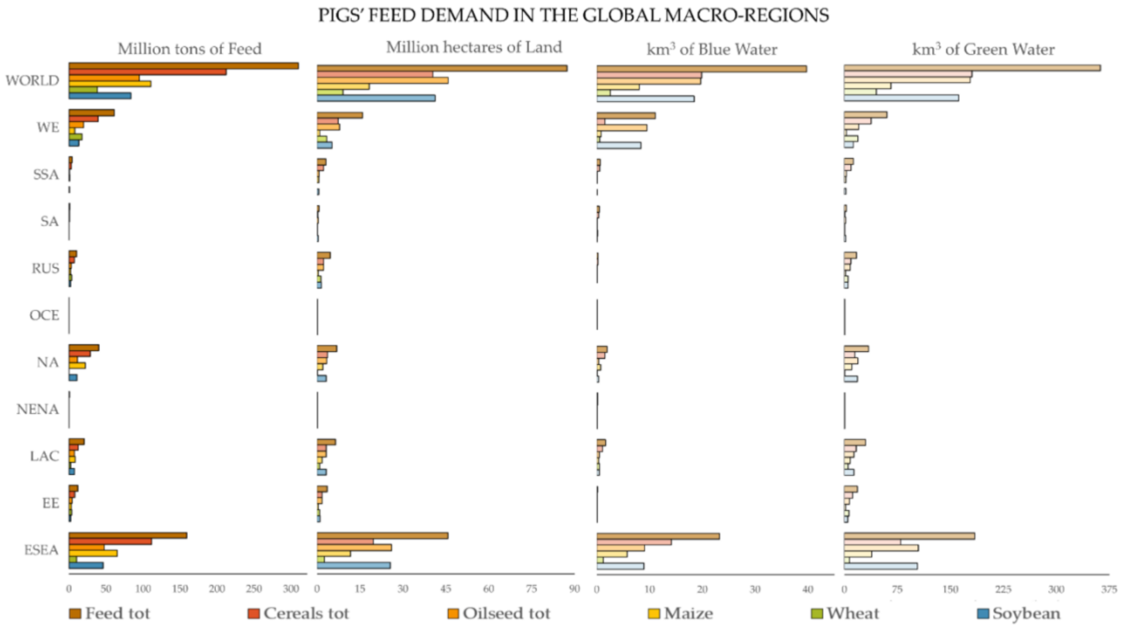


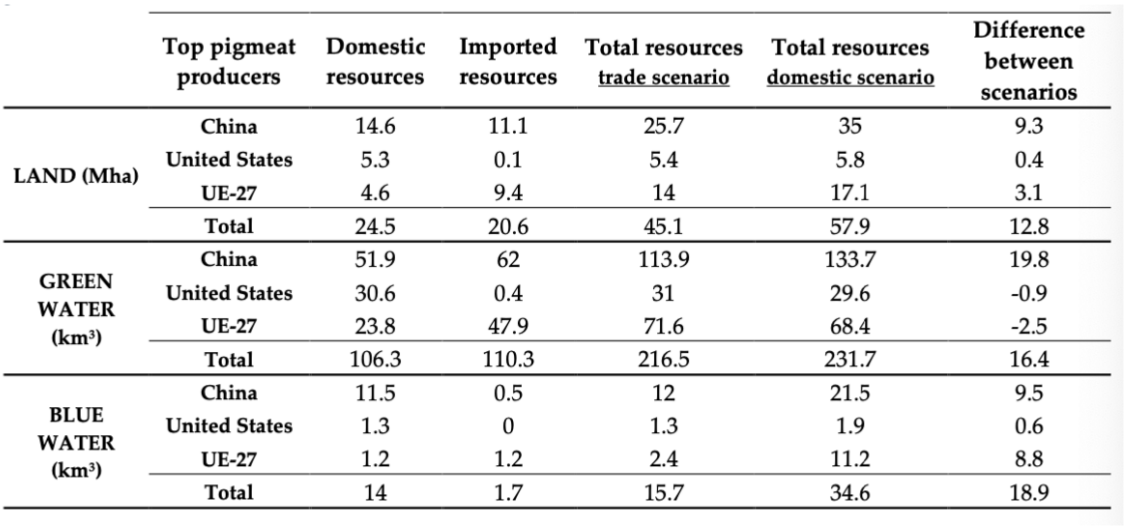
Figure 1. Amounts of feed and natural resources associated with pig production in the ten global macro-regions, under the assumption of domestic crop production. Regions: NA (North America), RUS (Russian Federation), WE (Western Europe), EE (Eastern Europe), NENA (Near East and North Africa), ESEA (East and Southeast Asia), OCE (Oceania), SA (South Asia), LAC (Latin America and the Caribbean) and SSA (Sub-Saharan Africa).

TOP PIGMEAT PRODUCER COUNTRIES: CHINA, UE-27, AND THE UNITED STATES

Unlike with other livestock, the pig meat sector is strongly dominated by China, which hosts about half of the world's pigs. European countries (EU-27) own 17% and the United States own 8% of the world's pigs. The remaining production is subdivided among all the other countries in negligible proportions (FAOSTAT, 2018). Therefore, China, EU-27, and the United States bred more than 70% of the pigs in 2018. However, estimating the natural resources required by these three countries to meet their pig feed demand, the land used includes 57.9 Mha, under the assumption of domestic feed production, and 45.1 Mha considering the feed trade. Either way, the results represent less than 70% of the total land required by all countries breeding pigs (87.4 Mha). On the other hand, water consumption by these countries, and in particular BW, is estimated to be 34.6 km^3 if crops are locally grown, meaning almost 90% of the total demand (39.7 km^3), value falling to 15.7 km^3 with the trade (Table 1).

Concerning land consumption, China, EU-27, and the United States need 25.7 Mha, 14 Mha, and 5.4 Mha of agricultural land in the trade scenario, respectively. This figure is roughly the same as that obtained in the global analysis for the United States (5.8 Mha), where the domestic production is not just an assumption and feed imports account only for 1% of the demand, while the new estimated land is lower than before for the other two countries (Table 1). China's land demand decreased by 9.3 Mha and the EU-27's by 3.1 Mha, considering the more realistic scenario of international feed trade. Trade, in fact, is necessary to fill the lack or the scarcity in the country's production of a crop that is not able to meet its demand, however trade may also replace domestic production when a locally unproductive crop is imported from a country with higher yields. This effect does not always occur in the same way in terms of water use. Generally, there is some correlation between the demand for GW and the total land consumption needed for a crop, and between the demand for BW and the extension of the area equipped for irrigation. However, if trade leads to a decrease in the demand for land, this does not always imply an associated decrease in GW. In fact, in China, with a 27% saving in agricultural land (from 35 Mha to 25.7 Mha, Table 1), the GW decreases by 15% (from 133.7 km^3 to 113.9 km^3), while the BW by 44% (from 21.5 km^3 to 12 km^3). This can be explained by considering that in China, most crops are irrigated, and particularly soybean, which is the most traded (32 Mton imported and 5 Mton domestic). Crops therefore require a significant amount of BW if produced locally, with a lower demand for GW. Instead in Brazil, from where China imports the majority of soybean (75% of its soybean needs), irrigation is negligible, and the GW provides for almost all the crop water requirement. The situation in EU-27 is similar to that in China: BW savings are significantly higher than GW savings, since there is even a slight increase in GW when moving from local production to imports (11.2 km^3 to 2.4 km^3 and 69.1 km^3 to 71.6 km^3, respectively BW and GW) (Table 1). North and South America, in particular Brazil, the United States and Argentina, are net exporters of feed crops, with soybean covering at least 60% of the feed traded (Table S2, Table S3, Table S4). China is the largest net importer, to which more than 50% of the feed is directed. The EU-27 countries are oilseed importers from Brazil and the United States, but at the same time they are exporters of cereals (mainly wheat) (Figure 2). Spain and Germany are the biggest pig meat producers in the region and thus are the biggest importers in Europe of both land and water through feed trade.

Table 1. Use of natural resources by the three largest pig producers (China, United States and EU-27) in terms of land, green and blue water.



	Top pigmeat producers	Domestic resources	Imported resources	Total resources trade scenario	Total resources domestic scenario	Difference between scenarios
LAND (Mha)	China	14.6	11.1	25.7	35	9.3
	United States	5.3	0.1	5.4	5.8	0.4
	UE-27	4.6	9.4	14	17.1	3.1
	Total	24.5	20.6	45.1	57.9	12.8
GREEN WATER (km^3)	China	51.9	62	113.9	133.7	19.8
	United States	30.6	0.4	31	29.6	-0.9
	UE-27	23.8	47.9	71.6	68.4	-2.5
	Total	106.3	110.3	216.5	231.7	16.4
BLUE WATER (km^3)	China	11.5	0.5	12	21.5	9.5
	United States	1.3	0	1.3	1.9	0.6
	UE-27	1.2	1.2	2.4	11.2	8.8
	Total	14	1.7	15.7	34.6	18.9

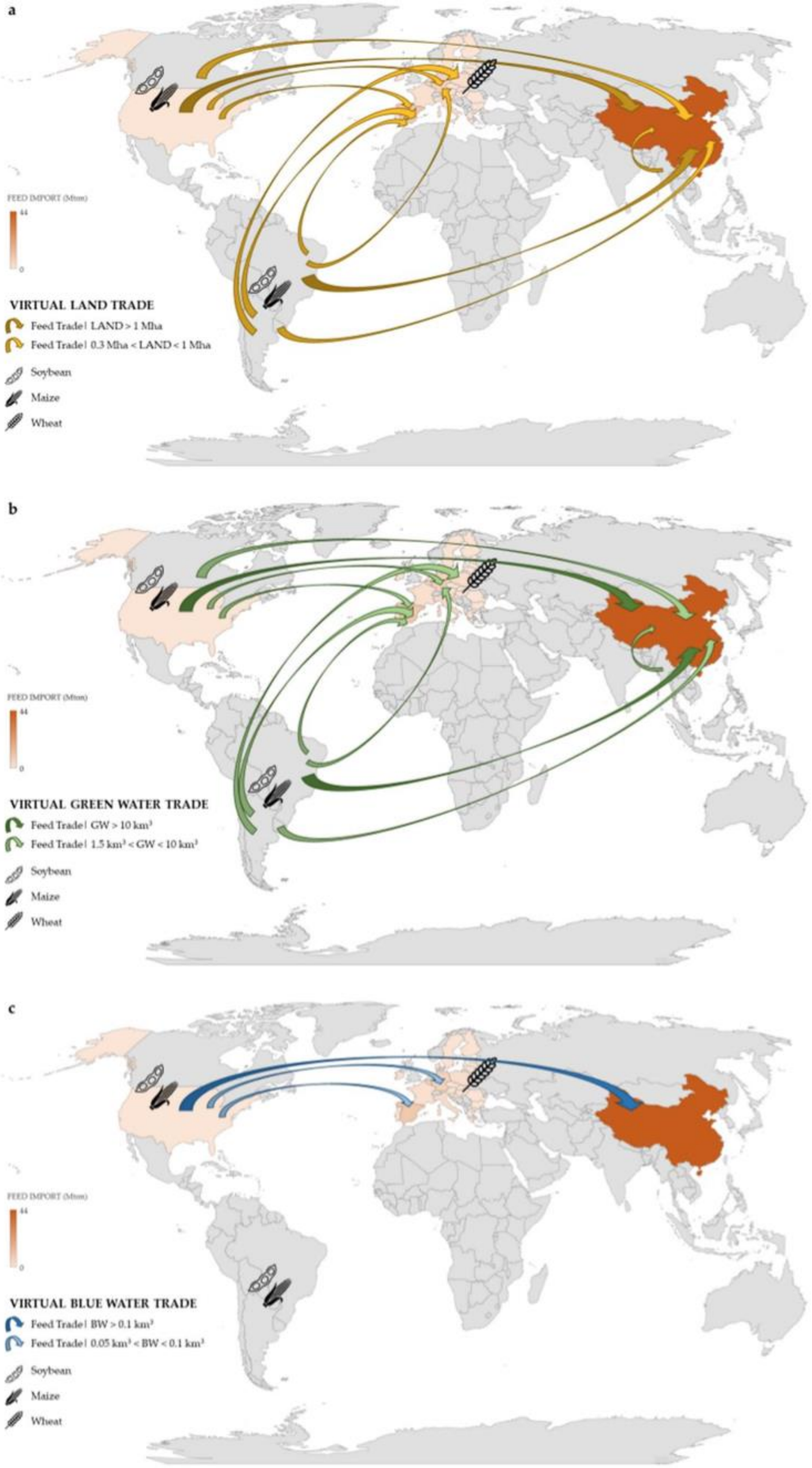


Figure 2. Virtual trade of natural resources associated with pig feed crops in China, EU-27, and the United States. **a**. Virtual Land Trade, **b**. Virtual Green Water Trade, **c**. Virtual Blue Water Trade.

DISCUSSION

In 2018, pig farming required the use of 87.4 Mha of land, 362.4 km^3 of GW, and 39.7 km^3 of BW globally. The required feed input and, in turn, the related resources used originated partly from the countries' own crop production and the remainder from international feed trade. The pressure on natural resources caused by the pig meat sector has several drivers and implications, such as the feed-food competition, the effects of international trade, and sustainability issues.

FEED-FOOD DEBATE AND COMPARISON WITH OTHER STUDIES

Pigs are monogastric animals, and like chickens can only digest simple carbohydrates and struggle to digest fibers, which comprise only in small amounts in the diets of these animals. As a result, monogastrics play a greater role in the debate regarding the consumption of digestible ingredients even by humans (Mottet et al., 2017). This debate arises from the recent awareness of the scarcity of the land and water resources that are needed to continue to increase food production, given the further increase in the global population. The lack of these resources is exacerbated by the increase in intensive animal husbandry, which mainly consumes cereal grains and requires a third of the world's production of such grains (Di Paola et al., 2017; Govoni et al., 2021). For intensive animal husbandry, maize and wheat are the main ingredients since they are the main source of energy in pig diets, as with broilers and layers (Figure 1, Table S1). Wheat-based diets are confirmed to be on-trend in Europe, Australia and New Zealand, and maize-based diets in the United States and Asia (Akter et al., 2017; Govoni et al., 2021) (Table S1). As for the estimated croplands, the results can be compared with those of Mottet et al. (2017). The latter authors calculated 45 Mha of land for cereals and 39 Mha for oilseed crops. The difference between our 40.5 Mha of cereals and their 45 Mha seems to be related to our use of more up-to-date (and greater) agricultural yields (FAOSTAT, 2018). In the case of oilseed crops, however, we calculated that 45.8 Mha are needed, slightly more than their calculation. This can be explained by trade. Mottet et al. (2017) included a global trade matrix in their calculations on soybean and palm oil cakes. Our global oilseed estimate (45.8 Mha) was obtained under the hypothesis of domestic production. However, as confirmed by our results in the detailed trade scenario, trade leads to a decrease in the hectares used for oilseed crops in many countries (China and Europe).

This difference is not relevant in the case of cereals calculations as they are less traded than oilseeds and more produced and consumed locally. Focusing on the EU-27 results, Sporchia et al. (2021) estimated that total EU pig meat production in 2017 led to a total resource use of 14.5 Mha of land, which is only 4% higher than our estimate (14 Mha). Their estimated water, on the other hand, was 28% lower 60 than ours in the case of GW (51.9 km^3 versus our 71.6 km^3), and higher in terms of BW (3.9 km^3 and 2.4 km^3). These differences seem to be attributed to a lower soybean content in the diet adopted in their analysis. In fact, this difference implies a lower need to import oilseed crops (a land-demanding crop) from South America, involving more local use of BW than GW compared to a trade scenario. However, water is more difficult to compare due to the different methods used and the different periods to which the climate data refer (average 1996-2005 in their study and 2013-2018 in ours).

INTERNATIONAL FEED TRADE: SAVING OR DESTROYING THE ENVIRONMENT?

The inclusion of trade in the livestock feed production sector could lead to domestic feed savings, and/or natural resources savings (Govoni et al., 2021). This last is the case when crops are exported from countries with higher agricultural yields and/or higher water-use efficiency, to countries where the domestic crop cultivation would be less profitable and/or more resource-demanding (Zhang et al., 2016; Qiang et al., 2020; D’Odorico et al., 2014). China and EU-27 are the largest feed importers, mainly of oilseed crops, resulting in savings thanks to international trade. These savings mean being able to access to 9.3 Mha and 3.1 Mha of agricultural land and 29.3 km^3 and 6.3 km^3 of water (GW plus BW) which can be used for other purposes in China and EU-27, respectively (Table 1).

However, this gain also has negative effects on the environment. The expansion of cropland areas and tree plantations in the tropics, where the main countries that export agricultural products are located, takes place at the expense of forests and other land cover classes (Pendrill et al., 2019; Balogh & Jámbor, 2020). Pendrill et al. (2019) estimated that in China and Europe, but also in many other developed countries, emissions from deforestation embodied in imports equal or even exceed emissions from the domestic agricultural sector. In addition, the assessment of water efficiency through GW and BW savings may not take into account the unsustainability of certain water abstractions and local water scarcity situations (Dalin & Rodríguez-Iturbe, 2016; Fader et al., 2011). Land-use changes, biodiversity losses, soil erosion, changes in nitrogen and carbon cycles, greenhouse gas emissions and water scarcity and pollution are just some of the effects caused by international trade which subsequently create water and food security issues (Rulli et al., 2019).

Further analyses could therefore consider the use of resources, not only from the point of view of the producer country (where the animal is reared and fed), but also from the view of the consumer country, which is the real receiver of the production and where the supply chain ends. In this case the live pig trade is negligible, amounting to less than 3% of the global herd. However, the processed pig meat and derivative products are significant, covering 14% of the production (FAOSTAT, 2018). The latter share changes dramatically from country to country. China consumes its entire pig meat production domestically, unlike the United States and the EU-27 where exports cover 22% and 45% of the production, respectively. In EU-27, however, exports are partially offset by a share of pig meat imports from non-EU countries (41% of domestic consumption). (FAOSTAT, 2018).

Transfers of emissions and natural resources through international trade are not negligible and should be considered by countries in addition to that those within their own borders.

UNEQUAL FEED AND RESOURCES USE: RELIGION, VEGETARIANISM AND POVERTY

Religion has a significant impact on food patterns and may even impose restrictions on individual dietary choices. Several religions, therefore, take a stand on the possibility for their followers to consume meat or certain types of meat (Hong, 2013). Since meat and all animal source foods have a strong environmental impact, the correlation between religion and diets may therefore have implications on the use of natural resources in some countries (Westhoek et al., 2016). In this context, the ban on pig meat consumption in some countries, and therefore on production, results in less pressure on natural resources. This happens in all predominantly Muslim countries (i.e., with a 70% Muslim population), located above all in the Near East and North Africa and South Asia (Iran, Pakistan, Bangladesh) (Table S1, Figure 1). Jews, like Muslims, are forbidden to eat pork, which is why even in Israel (>75% Jews), the consumption of this type of meat compared to the total meat is less than 2% (FAOSTAT, 2018). Christianity does not put tight restrictions on dietary habits; however, the Eritrean and the Ethiopian Orthodox Church do not permit pork consumption (Seleshe et al., 2014). In other countries, religious traditions (Hinduism, Buddhism and others) are intertwined with the spread of vegetarianism, as in India (Arora et al., 2020). Most Indians are not vegetarians (only 39% define themselves as vegetarians), however more than 80% do follow at least some limitations on meat in their diet and thus India is one of the least meat- consuming countries (Corichi, 2021; FAOSTAT, 2018). In fact, India is among the not predominantly Muslim countries with the lowest per capita rate of natural resources consumption in the production of meat. A different situation arises in Africa, in particular in the Sub-Saharan region. Here the consumption of pig meat is negligible in the diet of most countries due to poverty. In some countries such as Mali, Sudan, Niger, Djibouti, the religious restriction is relevant due to the strong Muslim component. However, the greatest weight is low incomes, to the extent that not only pork but meat consumption in general is very low (Szűcs & Vida, 2017). These countries, in fact, are still faced with severe burdens of undernutrition and malnutrition associated with the low consumption of animal source and other protein-rich foods (Willett et al., 2019). A greater weight is given in these countries to poultry meat from chickens bred in backyard systems, which are mainly subsistence driven or oriented at local markets (Govoni et al., 2021). Although difficult to estimate, although "bushmeat" consumption is widespread in Central Africa (Ziegler, 2010), it is often not included in the reported country meat consumption.

PIG FEED SUSTAINABILITY AND ALTERNATIVE SOLUTIONS

As regards sustainability, the production of animal source foods has attracted considerable attention in recent years due to the importance of mitigating environmental impacts and finding solutions to the growing consumption of natural resources and the increasing competition of the sector with the production of human food. Several innovative strategies have recently been proposed to stem the problem. Among these, the use of alternative ingredients as animal feed stands out due to its environmental benefits (Luciano et al., 2020; zu Ermgassen et al., 2016; Pinotti et al., 2021; Luciano et al., 2021). These ingredients include mainly ex-food from the food supply industry, unintentional and unavoidable food losses prevented from reaching the human food market for practical or logistical reasons, i.e., former foodstuffs. Exploiting former foodstuffs in the feed production chain can not only save resources but also ensure the effective disposal of these products, thus fully meeting the needs of the circular economy (Kummu et al., 2012). The target species of these kinds of solutions are omnivores, e.g., pigs and poultry, although recent studies did not exclude the possibility of their use also in ruminant diets. In this context, zu Ermgassen et al. (2016) estimated a potential land saving of 21.5% in EU pork production with the use of swill feeding in pig diets. While former foodstuffs could be used specially to replace cereals as the energy source, insects are one of the most promising alternatives as protein source in animal nutrition (Gasco et al., 2020; Pinotti et al., 2019). Insects have always been included in the natural diet of many farmed animals. Their nutritional value however, varies considerably depending on the species and the rearing substrate (Pinotti & Ottoboni, 2021). Although still in its early stages, industrial insect production is booming worldwide, with an estimated annual growth of more than 24% over the next decade (Wood, 2019). The use of all these kinds of alternative ingredients however, needs to be in full compliance with the countries’ food laws in order to ensure their safety when used as feed (Pinotti et al., 2021; Pinotti & Dell’Orto, 2011).

CONCLUSIONS

This study has revealed that in 2018, in order to satisfy demand for pig feed of China, the EU-27 and the United States, a total resource use of 25.7 Mha, 14 Mha, and 5.4 Mha of agricultural land, respectively, was required, for a final global estimate of 87.4 Mha. These results are accompanied by a total water consumption of 39.7 km^3 of blue water and 362.4 km^3 of green water to grow feed crops, of which the three largest producers mentioned consume the largest share, with China at the top (12 km^3 and 113.9 km^3 of blue and green water, respectively). Pig feed is made up of more than 70% of cereals, whose demand is generally met by the countries' own production, consuming resources domestically. On the other hand, the protein source that makes up the remaining part of the diet and that is represented by oilseed crops, including mostly soybean and related by-products, appears to be highly traded, causing a *virtual* trade of resources between countries even across large distances. Exceptions are countries such as the United States, which is fully able to domestically meet its demand for pig feed. The international trade in animal feed is still highly controversial from the point of view of sustainability due to its dual environmental impact. It represents a potential resource-saving strategy for countries where there are still unproductive crops with a high resource-use inefficiency, but at the same time it is one of the main drivers of global issues such as land- use change, biodiversity losses, food insecurity, and water scarcity. The soy supply issue has also been addressed by the feed industry which now promotes responsible sourcing practices for soybean (FEFAC, 2021). The objective is to contribute to the mainstream market transformation of responsible soybean products used in compound feed produced in some parts of the world (e.g., in the EU by 2025). Such kinds of policies aim to stimulate a responsible expansion of soybean. It is common knowledge that deforestation is concentrated in certain South American biomes, which require a targeted supply chain action to effectively delink the soybean sourcing from deforestation. This latter issue is the most important and most discussed in all soybean standards and why the feed industry has decided to focus on soy sustainability (IFIF, 2021). The relentless research in the field of animal nutrition remains essential in order to make the livestock sector more sustainable and to stem the growing pressure on natural resources, in a world that is expected to be increasingly populated in the coming decades.

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CHAPTER 10

REVIEW: Reduce, Reuse, Recycle for Food Waste: A Second Life for Fresh-Cut Leafy Salad Crops in Animal Diets

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Simple Summary: There is a historical link between co/by-products and animal feed, however innovative options are now available. Adopting the principles of the circular economy guarantees further progress for the food–feed chain. By-products and biomasses, such as former foodstuffs or plant by-products (PBPs) from the food processing industries, could be recycled as feedstuff for farms. This review focuses on the biomass derived from the processing of vegetables, and in particular on fresh-cut leafy salad crops as potential ruminant feedstuff. The chemical composition of this class of PBPs makes them comparable to other traditional feeds, such as fresh forage, and suggests that they could be considered for ruminant nutrition. Although at a very early stage, the potential of this new biomass seems high. These products can be used to reduce the environmental impact of both the food and livestock sectors.

Abstract: The world's population is growing rapidly, which means that the environmental impact of food production needs to be reduced and that food should be considered as something precious and not wasted. Moreover, an urgent challenge facing the planet is the competition between the food produced for humans and the feed for animals. There are various solutions such as the use of plant/vegetable by-products (PBPs) and former foodstuffs, which are the co/by-products of processing industries, or the food losses generated by the food production chain for human consumption. This paper reviews the by-co-products derived from the transformation of fresh-cut leafy salad crops. A preliminary nutritional evaluation of these materials is thus proposed. Based on their composition and nutritional features, in some cases similar to fresh forage and grasses, this biomass seems to be a suitable feedstuff for selected farm animals, such as ruminants. In conclusion, although the present data are not exhaustive and further studies are needed to weigh up the possible advantages and disadvantages of these materials, fresh-cut leafy salad crops represent a potential unconventional feed ingredient that could

help in exploiting the circular economy in livestock production, thereby improving sustainability.

INTRODUCTION

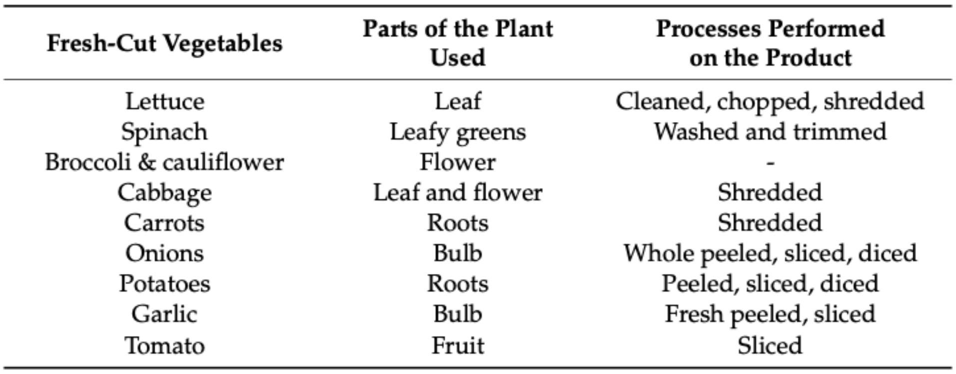
Food waste is abundant: approximately one third of food produced and intended for human consumption is lost or wasted, which translates into approximately 1.3 billion tons per year on a global level (FAO, 2011). The disposal of food waste poses a large environmental problem with several implications in terms of the sustainability and profitability of the food system. In order to reduce these negative impacts, the 3R slogan "Reduce, Reuse, Recycle" should be adopted in order to redesign the management of food leftovers and food waste (Memon, 2010; Sakai et al., 2011). In the European Union, the Waste Framework Directive 2008/98/EC proposed the following waste management hierarchy: prevention, processing for reuse, recycling, energy recovery and disposal (UE Commission, 2008). In this scenario, several authors (Makkar and Ankers, 2014; Pinotti et al., 2019; Georganas et al., 2020) have suggested that the use of less food-competing foodstuffs in animal diets is a potential strategy for reducing food–feed competition and mitigating the environmental impact of livestock. This approach is particularly pertinent when coupled with other strategies such as improvements in livestock productivity (Schader et al., 2015; Roos et al., 2016; Van Zanten et al., 2015). Plant by-products include a wide range of secondary residues generated from the industrial processing of plants into commercially valuable products (Meridas et al., 2012). These products are obtained from agro-industrial processes such as distillery and biofuel production, oilseed processing, fruit and vegetable processing, sugar production, root and tuber processing, and herb, spice and tree processing (Salami et al., 2019). These co/by-products are considered safe and are widely accepted as animal feed. At the food manufacturing level, there are always unintentional and unavoidable food losses that prevent foodstuffs from reaching the human consumption market. Former foodstuff products (FFPs) are a significant example, which have been proposed as animal feed. FFPs are foodstuffs that are manufactured for human consumption, but which are no longer intended for human consumption, despite maintaining important nutritional features (Giromini et al. 2017; Tretola et al., 2017a-b; Pinotti et al., 2019; Luciano et al., 2020). Plant co/by-products

(PBPs) such as fresh-cut leafy salad crops are potentially another category of former foodstuffs. The present paper addresses the potential of these fresh-cut leafy salad crops (also called salad crops), as a feed ingredient for sustainable ruminant diets.

FROM FRESH AND CUT VEGETABLES TO SALAD CROPS: CATEGORIZATION, MARKET, NUTRITIONAL FACTS AND PROCESSING

PBPs, are a wide category that includes several types of materials (Salami et al., 2019). The main ready-to-eat fresh-cut vegetables and fruits are: arugula and radicchio, parsley, mixed herbs, chard, chicory, puntarelle, rocket in bunches, loose rocket, celery hearts, escarole hearts, courgette flowers, carrots, broccoli, spinach, peeled and sliced potatoes, onion cubes, sliced champignon mushrooms, sliced peaches, mangoes, melons, and oranges (PRFP). The vegetable products offered to the consumer are based on one or more varieties (mixed salads), which are ready for raw consumption or for cooking (spinach, herbs, vegetable side dishes, legumes). As shown in Table 1, the processing differs considerably according to the type and parts of the vegetable used. In the case of salads, washing, chopping and shredding are the most common processes, while some types of vegetables may also be peeled or cut into slices (slices, rounds or cubes) before being offered to consumers. These latter two treatments are more common for fruits (citrus fruits, pears, apples, pineapples, carrots).

Table 1. Fresh-cut vegetable products: parts of the plants and processes performed on the products.



Fresh-Cut Vegetables	Parts of the Plant Used	Processes Performed on the Product
Lettuce	Leaf	Cleaned, chopped, shredded
Spinach	Leafy greens	Washed and trimmed
Broccoli & cauliflower	Flower	-
Cabbage	Leaf and flower	Shredded
Carrots	Roots	Shredded
Onions	Bulb	Whole peeled, sliced, diced
Potatoes	Roots	Peeled, sliced, diced
Garlic	Bulb	Fresh peeled, sliced
Tomato	Fruit	Sliced

The fresh-cut vegetable market, however, is primarily represented by salad crops, including mixed crunchy salads, while single-variety products, such as lettuce, valerian and arugula together represent one third of total sales of fresh-cut fruit and vegetable products. Figure 1 shows the production rate of fresh-cut vegetable and fruit products in Italy.

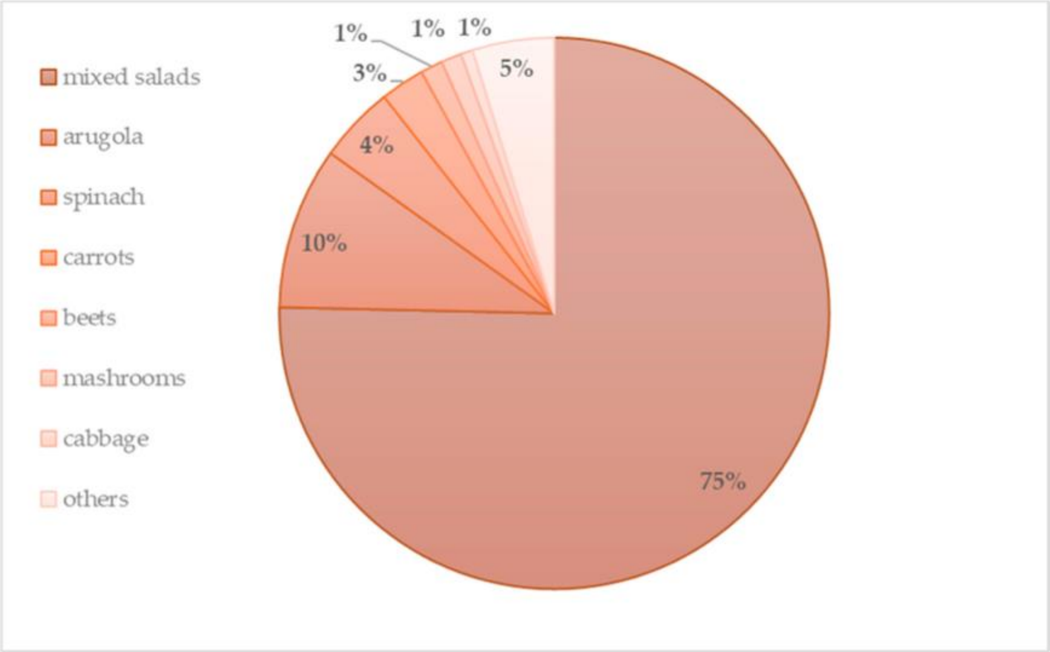


Figure 1: Production of fresh-cut vegetable products in Italy by percentage (Italia Fruit News).

Salad crops derive from conventional/organic or integrated cultivation systems, and include those all those varieties of ready-to-eat fresh vegetables, which during post-harvesting processing are selected, sorter, husked, cut and washed. They are then packed in envelopes or in sealed food trays, and, after passing through the cold chain, are sold on the fruit and vegetable market, ready for raw consumption or for cooking (Beaulieu et al., 2004). The salad crop market is increasing. In supermarkets, the space assigned to these products has expanded greatly in order to meet new preparation and presentation styles that are practical both for the consumer and modern distribution. The market share of salad crops is estimated at around 8% of the total fruit and vegetable market in France and Great Britain (PFRP). There is a similar situation in Italy, where salad crops now represent approximately 10% of the turnover of fruit and vegetable sales. With about 90,000 tonnes, Italy is ranked as the second largest producer of salad crops in the main European markets, immediately after Great Britain. This scenario is also supported by the per capita consumption of salad crops, which has grown in several European countries in the last 10 years (Figure 2). From this expanding market perspective, the higher the economic and productive importance of salad crop products, the higher the food wastage derived from them (Caldeira et al., 2019).

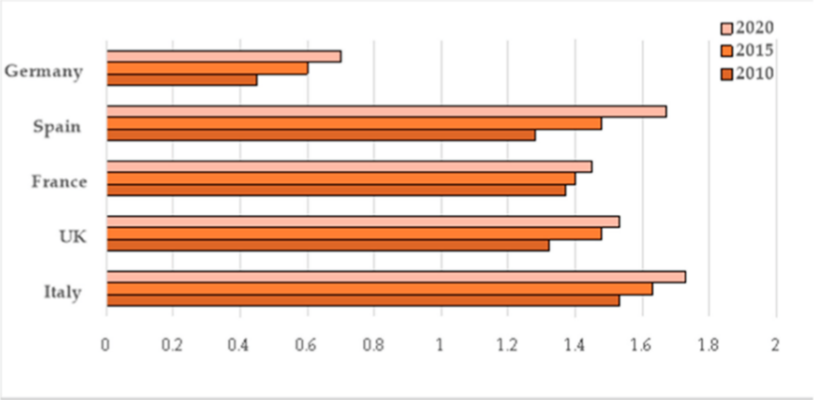


Figure 2: Per capita consumption of salad crops (data expressed as kg/year) (Italia Fruit News)

Most Representative Species of Salad Crops and Their Nutritional Facts

Although the generic name of "salad" indicates a group of leafy vegetables consumed mainly as raw material, salads are divided into three botanical families: chicory (which includes radicchio), endives, and lettuce. The following species are the most commonly used in the production of salad crops:

1. Lettuce (*Lactuca sativa*)
2. Arugula (*Eruca sativa*)
3. Endives (*Cicorium sativa*)
4. Valerian (*Valerianella locusta*)

Lettuce (Lactuca sativa) is the most important leafy vegetable crop worldwide, and Spain and Italy are the largest producers in Europe (FAOSTAT, 2018). Lettuce is an important component of the modern Western diet as it is consumed in large amounts and contains compounds that are thought to be beneficial to health, particularly flavonoids (Steinmetz et al., 1991; Gao and Mazza, 1994; Henriques et al., 2000). Lettuce has traditionally been sold as a whole head. However, there has been an increase in the proportion of fresh-cut, bagged leaf production because of the increased consumption of convenience foods both in catering and at home. Table 2 shows the gross composition of the most consumed salad crops.

Table 2. Chemical composition of most consumed salad crops (g/100 g of fresh material).

Salad Crops	Water Content	Fiber	Protein	Carbohydrate	Fat	Ash	MJ/100 g
Lettuce	94.61	2.1	1.23	3.29	0.3	0.58	17
Arugula	91.71	1.6	2.58	3.65	0.66	1.4	25
Endives	93.79	3.1	1.25	3.35	0.2	1.41	17
Valerian	92.8	1	2	3.6	0.4	1.2	21

The way fresh-cut salad crops are presented to consumers is another way of classifying the raw materials used in their production: *(i)* whole-head salad (e.g., iceberg salad), which are vegetables that form a tight cabbage-like head with the leaves branching from a single stalk; *(ii)* baby salads, also termed baby leaf (e.g., rocket salad), usually harvested at the young leaf stage. The small leaves are supplied intact, which differentiates this product from common cut salads. One of the advantages of baby-leaf salads is that, given the smaller cut surface of the leaves, their color does not fade; various types of baby-leaf salads are available on the market.

Salad Crops: The Production Process

Before being traded and consumed, fresh vegetables undergo a series of technological processes, all strictly based on not compromising the freshness and naturalness of these products. Conserving the organoleptic properties depends on the processing procedures, preservation techniques, and the time required for the product to reach the dealer, beginning at the processing plant. The preservation of salad crops is based on the combined action of different treatments, which are all designed to prevent bacterial contamination and delay the appearance of alterations and spoilage.

When processing a whole-head salad, cutting operations are required. Cutting damages the plant tissues, which consequently reduces their quality during storage. Moreover, in the case of whole-head salads, the percentage of usable product is significantly lower (due to the preliminary removal of the external leaves and core) than in baby salad processing, for which the whole leaf is harvested and processed (Martinez-Sanchez et al., 2012). Whole-head salad processing is thus responsible for a huge amount of waste.

The total wasted salad can be calculated as the sum of the waste generated during preliminary cleaning, the three washing stages, and the waste generated at the optical selector step. Data indicates that up to 41% of salad is wasted during typical fresh-cut iceberg salad processing because of the removal of the external leaves and core, accounting for nearly all the total waste production (Plazzotta et al., 2017).

A generic example of the steps that salad crops undergo before being marketed is represented in Figure 3. It is clear that waste production occurs at several levels of the production chain.

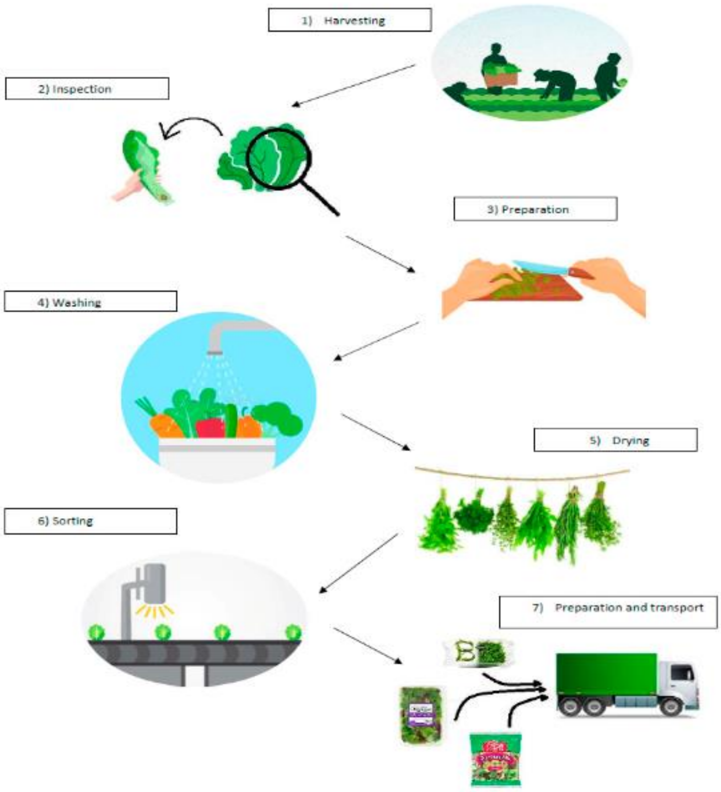


Figure 3. Salad crop production process.

The main salad crop production steps can be summarized as follows: selection (choice of variety); cleaning and washing (water moves through mechanical or air agitators); cutting (when needed); re-washing and drying (in order to guarantee a limited microbiological load); packaging (needed to keep the characteristics of freshness for the shelf life of the product); labelling; retailing; and transportation.

SALAD CROPS AS ANIMAL FEED INGREDIENTS

A feasibility study was carried out at the University of Milan in conjunction with a salad processing plant, in order to evaluate the chemical and nutritional properties of different kinds of salad crops as animal feed sources. Several samples of salad crop leftovers were collected in Autumn 2019. Samples were analyzed in relation to dry matter (DM), crude protein (CP), crude oils and fats (EE), ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). Specifically, the DM, EE, CP and ash analyses were performed in compliance with Commission Regulation N◦ 152/2009 (UE Commission, 2009). Neutral detergent fiber, ADF and ADL analyses were performed in accordance with the methods 2002.04 and 973.18 for NDF and ADF-ADL, respectively (Mertens, 2002). The energy content was estimated using the equations proposed by Weiss (1998), and data reported by CRPA (Pacchioli & Fattori, 2014).

Comparison of Macronutrients

Table 3 compares the nutritional value of fresh forage (grasses and legumes) and salad crops. The results show that these materials are similar to fresh forage or pasture used in conventional ruminant feed. One of the main differences is the water content: the dry matter (DM) concentration ranges from 6% in salad crops to 23% or 25% in forage (Pacchioli & Fattori, 2014). This indicates that salad crops are extremely wet, which is usually related to the age of the grass. Salads contain a lot of water, which provides high palatability and high bulkiness. Bulkiness is an important feature in terms of their mechanical action on rumen walls, and it can be adapted according to the biomass available from the salad crop processing plant. For example, a salad variety with a fast fiber clearance and degradation rate could reduce its residence time in the rumen, ensuring more space for extra material to be ingested, and this would have a positive impact on increasing DMI, especially in early lactation in dairy cows (Taweel, 2007).

Table 3. Comparison between nutritional values of fresh forage and salad crops.

Item [1]	Salad Crops	Fresh Forage ** (Grasses)	Fresh Forage ** (Legumes)
Dry matter (DM), %	5.80	25.0	23
CP, % DM	21.2	8.80	18.9
EE, % DM	2.85	1.55	2.8
NDF, % DM	36.0	58.3	40
ADF, % DM	23.8	39.0	31
ADL, % DM	7.40	5.39	7
Ash, % DM	18.5	9.94	9.7
NEL, MJ/kg *	3.70	3.90	4.18

[1] crude protein (CP), crude oils and fats (EE), ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL); * The Net Energy Lactation (NEL) was estimated using the equation from (Weiss, 1998); ** (Pacchioli & Fattori, 2014).

However, the main components of these materials are crude proteins (CP) and fibers (NDF, ADF, etc.). The crude protein content is very high and comparable to lucerne grass. The overall mean protein content in salads is about 21% DM. This value is in line with Bakshi et al. (2016), who have reported that vegetable waste has about 20% CP, high moisture and high palatability. These data, however, should be interpreted with caution since different protein fractions were not reported. Usually, young grasses are characterized by a high proportion of soluble nitrogen: in most feedstuffs, a large fraction of soluble nitrogen is in the form of non-protein nitrogen.

As seen from the present analyses (Table 3), the protein content in salad crops is high, but it is not clear whether this content derives from proteins or from non-protein nitrogen. These foodstuffs contain high concentrations of soluble nitrogen, which, if not properly balanced, can have a negative effect on the diet. In fact, high soluble proteins, not accompanied with an adequate carbohydrate intake, can cause lameness or meteorism in animals. At an early stage of development, both grass and salads are rich in water and proteins, due to the intense metabolic activity of the tissues (Macdonald et al., 2000).

Nevertheless, it would also be interesting to know the amino acidic profile of salads and the degradability and digestibility rates of proteins, and whether there are bypass proteins. In terms of amino acids content, it has been reported (Gent, 2005) that glutamate and/or glutamine are the predominant amino acids in the leaves of all salad crops species. However, how these amino acids are distributed among the different proteins fractions is unknown, even though a high incidence of soluble protein is expected. This fraction has several dietetic implications that need to be addressed. One recent study (De Evan et al., 2019) reported high rates of in vitro digestibility and rumen fermentation kinetics of selected vegetables. Specifically, de Evan et al. (2019) reported that the rumen degradability of vegetable proteins at 12 h of in situ incubation was greater than 91.5% for all tested materials, while the in vitro intestinal digestibility of proteins ranged from 61.4 to 90.2%. Thus, when exposed to an artificial rumen environment, proteins and sugars from the tested vegetables were rapidly and extensively fermented. Furthermore, the same materials, when mixed with conventional ingredients, also had a reduced in vitro methane/total volatile fatty acid ratio.

As seen from the same analysis (data reported in Table 3), salad crops are also rich in fiber, as demonstrated by the NDF content that reached 36% DM, i.e., not far from the value reported in legume forage (40% DM), but lower than forage grasses (58.2% DM). The ADF content is about 24% DM, however this is lower than fresh forage (legumes and grasses) probably due to the early growth stage of these baby leaves.

These results indicate a different potential in the ruminal degradability of salad crop fiber, which introduces a further issue for salad crops, i.e., digestibility. Digestibility is influenced by the relationship between the leaves/stems: in very young herbs, as in baby leaf salad crops, the stems appear more digestible than the leaves, but later in the phenological stage, the digestibility of the leaf fraction decreases very slowly, while that of the stems decreases rapidly (Macdonald et al., 2000). These features suggest that salad crops are a potential source of highly degradable fiber. A comparison with sugar beet pulp (excellent fiber source) would thus be useful here (De Evan et al., 2019). For instance, salad crops have a lower NDF content (36.0% DM) than beet pulp (48% DM). In addition, while the ADF content is the same in salad crops and sugar beet pulp (on average 23–24% on a DM basis), the ADL fraction is higher in salad crops (around 7% on a DM basis) than in sugar beet pulp (less than 3% on a DM basis). An important focus should be the evaluation of the physically active NDF. All these data are necessary to evaluate the possible levels of inclusion and the possible changes in rumen microflora and related fermentations.

Further, as reported in Table 3, the ash content is very high in salad crops (18.5% DM), even twice as much as in common forage (9.5% DM grasses, 9.7% DM legumes). The high value of ash content could correspond to contamination during harvesting by soil remaining on the leaves and other vegetable parts. Usually, the forage ash content comes from both internal and external sources. Internal sources include minerals that accumulate in the leaves and stems of forage plants, but this is probably not the case for young materials such as salad crops. External sources include soil and sand that are deposited on the surface of the forage. An average internal ash content for grasses, as reported in Table 3, is around 9% DM. Values above this represent external sources and are negatively associated with forage quality and animal performance because the other nutrients are replaced by ash. Although a high ash content is an intrinsic feature of this type of biomass, salad crops are a leftover and thus they contain a higher percentage of soils and contaminated leaves than salads that pass through all the processing steps and reach the market, because the majority of salad crops are subject to the cleaning and washing involved in the production chain.

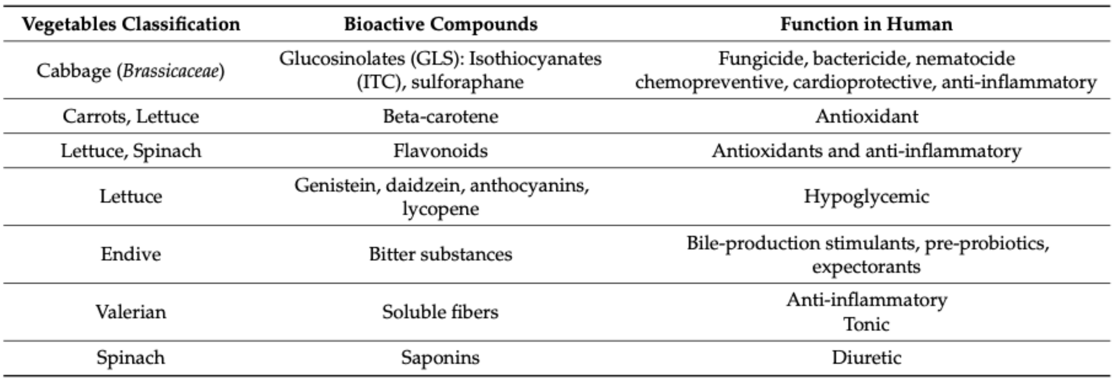
Although the results reported in Table 3 should be interpreted with caution since they are case sensitive, i.e., they thus represent a few examples of the different ex-foods that are potentially available for the feed stock used for feeding ruminants, the key features in this respect are the nitrogen and fiber contents. Some varieties from the same botanical families of salad crops have already been proposed as ingredients in ruminant diets. For instance, Marino et al. (2010) investigated the potential nutritional value of vegetables and fruits recovered from a supermarket. Even though they observed a similar protein content for leafy vegetables, the NDF and EE contents were different. In their study (Marino et al., 2010), the NDF content in leafy vegetables was about 40%, slightly higher than in our study, while the fat content was almost twice the amount we found. Both these aspects also affected the estimated energy content, which, as expected, reached about 8 MJ/kg−1 DM [37].

García-Rodríguez and collaborators (Pino et al., 1996) recently assessed the nutritive value of 26 agro-industrial by-products (sugar beet, asparagus, different citrus pulps, lettuce, etc.) in terms of chemical composition, in vitro digestibility, and rumen fermentation kinetics. The results showed differences in chemical composition, in vitro digestibility and fermentation kinetics, even in the same by-product processed in different ways (e.g., dehydrated/ensiled sugar beet pulp, tops and leaves). Taken together, these results indicate that composition variability seems to be one of the main limitations in defining a possible scale-up for the use of these biomasses (Pino et al., 1996). The salad crops here analyzed, however, have shown some similarities with brassicas forages: both contain high levels of easily fermentable carbohydrates, which can improve DM digestibility and ruminal fermentation. Furthermore, these compositional features have been associated with a reduction in the acetate-to-propionate ratio and energy losses in the rumen (mainly methane emissions), which in turn improved feed efficiency (Sun et al., 2016). This is in line with other studies (Couvreur et al., 2006), in which the progressive inclusion of fresh grass in lactating dairy cows' diets linearly increased milk yield (+0.21 kg/d per 10% proportion of fresh grass in the diet). In terms of composition, fat yield was unchanged, while fat content was slightly reduced. A side effect was also on milk fat globule size, which was decreased when the proportion of grass reached 30% in the diet. This latter also affects the technological quality of milk fat and of the resulting butter. Even though the nutrient fractions responsible of these effects are unknown, a combination of fiber and protein portions in the rumen seems to be the best option. Intuitively, there are no studies on the use of salad crops in dairy cows' diets and their effects on milk production and product quality, but their features are promising.

Micronutrient Content in Vegetables

Salad crops are a major source of micronutrients, such as vitamins C, B complex (thiamin, riboflavin, B6, niacin, folate), A, E, as well as minerals and polyphenols, carotenoids, and glucosinolates (Plazzotta et al., 2017). The main bioactive compounds in salad crops are summarized in Table 4.

Table 4. Major bioactive compounds found in vegetables (Plazzotta et al., 2017).



Vegetables Classification	Bioactive Compounds	Function in Human
Cabbage (*Brassicaceae*)	Glucosinolates (GLS): Isothiocyanates (ITC), sulforaphane	Fungicide, bactericide, nematocide chemopreventive, cardioprotective, anti-inflammatory
Carrots, Lettuce	Beta-carotene	Antioxidant
Lettuce, Spinach	Flavonoids	Antioxidants and anti-inflammatory
Lettuce	Genistein, daidzein, anthocyanins, lycopene	Hypoglycemic
Endive	Bitter substances	Bile-production stimulants, pre-probiotics, expectorants
Valerian	Soluble fibers	Anti-inflammatory Tonic
Spinach	Saponins	Diuretic

Despite this long list (Table 4), there are few studies in the literature regarding possible effects of this novel ingredient on animal performance, yet it is likely that they can improve some aspects of animal performance, such as productivity and reproductive parameters, and also reduce methane and nitric atmosphere emissions (Bakshi, 2016; Oskoueian et al., 2013). As proved in vitro by Oskoueian et al. (2013), selected bioactive compounds such as naringin and quercetin (both flavonoids), at the concentration of 4.5% of the substrate (dry matter basis), were able to suppress methane production without any negative effect on rumen microbial fermentation and total populations of protozoa. Accordingly, methanogens were significantly suppressed by adding these compounds. The presence of some bioactive compounds, such as flavonoids, could improve not only the rumen functions but potentially also animal wellbeing and product quality. Fresh herbs could modify the acidic composition of lipids in milk and thus improve the quality of ruminant products. These qualitative aspects can also concern meat production, with enhancements to the intramuscular lipid fatty acids profile, reducing the fraction of saturated fat. However, there is little evidence on these aspects and further investigation in controlled studies is needed to define the real potential of salad crops.

Nitrate Monitoring

Under certain soil and environment conditions, plants can accumulate nitrates. Virtually all plants have the capability of accumulating nitrates. The amount of nitrate in plant tissues is affected by several factors like: plant species, stage of maturity, part of the plant. Above these “plant factors”, other things/practices can affect the uptake and accumulation of nitrate by plants, namely nitrogen fertilization, herbicide application, drought, cloudy or cold weather, etc. However, nitrate concentrations are usually higher in young plants and decrease as plants mature. It is known that several vegetables, including salad varieties, have the potential to accumulate nitrate under specific growing intensive conditions (Nitrate Poisoning of Livestock). In the rumen, ingested nitrate is broken down to nitrite and then undergoes further degradation to ammonia, which is used to form microbial proteins. The reduction of nitrate to nitrite occurs much more rapidly in the rumen than the reduction of nitrite to ammonia. Consequently, when ruminants consume plants high in nitrate, excess nitrite formed in the rumen enters the bloodstream where it converts blood hemoglobin to methemoglobin, which, when excessive, may induce nitrate poisoning. Plants containing more than 1% nitrate (10,000 ppm) have to be managed with caution, and nitrate consumption in amounts of as little as 0.05% of the animal's weight can be dangerous. Forages containing more than 1% nitrate can be fed if diluted with nitrate-free plant material (Allison & Wenzel, 2019). However, recent surveys (Nitrate Monitoring in Spinach and Lettuce) conducted on lettuce, rocket, spinach and other leafy green vegetables, have evidenced a nitrate concentration between 2800 to 4130 mg/kg, which is very far away from the risk limits.

SALAD CROPS: FUTURE PERSPECTIVE AS A FEED FOR RUMINANTS

The farm management of salad crops entails risks associated with the high-water content and possible undesirable fermentations. Firstly, the ways in which salad crops can be fed to animals should be evaluated. Salad crops can be used fresh and mixed in the diet with total mixed ration (TMR), dried or in silage. Drying and ensiling are attractive means to preserve vegetable waste and by-products, although dehydration seems to be the least effective solution in terms of energy inputs and cost. In general, convectional drying processes, commonly used for forages, such as open solar drying, have some drawbacks in terms of quality, capacity, accuracy and process efficiency. On the other hand, fossil-fuelled dependent drying systems presented other drawbacks, indeed such technologies are often uneconomical and unsustainable from an environmental point of view. However, as reported elsewhere, when food leftovers or waste are considered as animal feed, their dry matter content is a key issue. In this respect, the most exhaustive example comes from Vandermeersch et al. (2014) study, which has made a direct comparison between food leftover processing and biogas production of 'bread waste'. In that study, it was pointed out that valorizing food waste to animal feed seems to be the better option, especially for those fractions of food waste with low water content (such as bread waste). Thus, since salad crops are very wet materials, their management imposes that they be considered "as is" for conversion into animal feed ingredients. These aspects are also linked to the energy, water and food (EWF) nexus, which refers to the interdependencies that inherently exist between these resources. An alternative solution is ensiling these materials. Silage includes multiple harvest, transport, and storage operations, while preservation is guaranteed by fermentation. In order to guarantee high quality silage, by maintaining economic profitability, all of these need to be coordinated, and the number of equipment components needs to be adjusted according to the processing capacity. In this respect, however, it is known that forages that have excess moisture (>70%) can get unintended fermentations (e.g., clostridial fermentation). This aspect (DM content) is the most important for fresh salad crops that are extremely rich in water. Wilting high-moisture forage to at least 35% DM is a good practice that reduces dangerous fermentations. Wilting usually results in good silage, particularly when sugar concentration is low and buffering capacity against pH decline is high (Factors Affecting Silage Quality). However, the use of ensiling in salad crop fields has rarely been studied in the literature, which limits an adequate evaluation of this process. Consequently, using fresh salad crops in the diet with a TMR is the most common and easiest way to incorporate them into animal feed. This, however, may be affected by the distance between the producing plant and the potential users: salad crops should be used/fed fresh in the surrounding areas of the vegetable processing plants (Bakshi et al., 2016).

More than a quarter of all salad crops are estimated to be processed into prepackage salads. Interest in ready-to-eat salads is still increasing worldwide and has an enormous potential for further growth. Italy has the highest per capita consumption of fresh-cut salads in Europe and produced 110,000 tons of fresh-cut vegetables in 2015, with a value near to € 750 million (Gullino et al., 2019). The increase in the fresh-cut vegetables market implies the potential production of high amounts of food leftovers that could be converted to animal feed ingredients (Caldeira et al., 2019). The salad crops market combines the advantages of ready-to-eat foodstuffs, convenience and innovation, with healthy eating. The innovation represented by this sector involves the technologies adopted in growing, processing, and marketing. Agronomists, microbiologists, chemists and food engineers are providing new solutions to enhance quality and safety attributes. Like all fresh-cut products, salad crops require substantial capital investment in plants and machinery. For this reason, in Europe, dairy processing plants often also specialize in ready-to-eat salads, since both products require daily delivery (Gullino et al., 2019). This link between the two sectors should be exploited in the development of an integrated system in which the surplus of salad production returns to the food chain by being introduced into the diet of dairy cattle. An integrated system of this kind could help make the use of salad crops in the diets of farm animals economically sustainable. Indeed, the main problem regarding salad surpluses is the high-water content, which makes any handling and processing economically uncompetitive. By contrast, the re-use of these biomasses (salad crops leftovers) in ruminant diets is a sustainable solution that saves not only nutrients, but also a huge amount of water, from waste.

In order to correctly manage salad crops and consider them as a possible animal feed resource for a sustainable ruminant diet, it is essential to investigate how they are processed. Companies need to ensure that all the processing of fresh-cut vegetables (including salad crops), from the selection of the product to the transport and sale, takes place within 24 h. Usually, the process takes 6–7 h for the product to pass from harvesting to the completion of the processing phase, and the remaining 17–18 h are needed to reach the retailers. The leftover biomass is generated in the processing plant and is stored in transport semitrailers until their departure to the final destination. In this scenario, pH levels could be determined in order to assess the freshness of the leftovers: according to our analysis, the most appropriate value is around 6 or neutral (Pinotti, unpublished results). In addition, supplementation with salad crops in the diet should be introduced gradually in order to evaluate the effects on animals. From a nutritional point of view, there may be differences depending not only on the agronomic/cultivation factors (Gent, 2005), but also on the steps performed after harvesting and during processing. A key example is cutting, which subjects the salads to more alterations, due to the stimulation of ethylene production, which increases respiration and senescence, and exposure of the cutting surface to microbial enzymes and potential spoilage. Furthermore, as highlighted by the high value of ash, these products may have large traces of soil derived from field harvesting. A decrease in nutritional values is also expected when plant tissues are wounded, and in vivo data indicate that fresh-cut lettuce contains fewer antioxidants than the fresh product (Serafini et al., 2002). However, little information is available concerning the effects on nutritional components, particularly antioxidant constituents, in fresh-cut products during handling, storage and senescence.

In summary, salad crops are potentially suitable ingredients for the feed stock supply for feeding ruminants, as demonstrated by their nutritional features as well as the high biomass content derived from the processing plants.

CONCLUSIONS

This article summarizes the features and potential of salad crops as new ingredients in ruminant diets. These types of feed are an example of the application of the principles of the circular economy and, given their nutritional value, are a potential alternative to conventional feeds such as forage used within the conventional ruminant diet. In fact, in terms of animal nutrition, these feeds have many benefits—especially for ruminants—because of their high fiber and protein contents. Their nutritional features, however, can also be considered for supplements and feed specialty formulation. Fresh salad crops, indeed, are recognised to be important for human diet due to their abundance in micronutrients like minerals (e.g., potassium, calcium and phosphorus) as well as vitamins (mainly A, C and E). Salads also contain bioactive phytochemicals such as carotenoids, polyphenols, glucosinolates and CLA. The main classes of polyphenols are caffeic acid derivatives, flavonols and anthocyanins, which play an important role as antioxidants (Plazzotta et al., 2017; Llorach et al., 2008). The processing steps of salad crops (reviewed in Paragraph 2.2) influence the micronutrient content of salads and, although some studies have been carried out (Martinez-Sanchez et al., 2012; Degl'innocenti et al., 2008), further research is needed to elucidate the variation of micronutrients content in fresh-cut products and their potential as animal feed. These features, in combination with their palatability, can be exploited to prepare new formulations that can be used as supplements in specific phases (e.g., early lactation, early dry period).

In addition, the high content of available biomass, due to the increasing market of fresh-cut products, ensures that these leftovers can be continuously implemented in the feed industry. Key to their successful use is how they are managed, from the waste origin to administration on the farm. Their nutritional characteristics need to be better understood in order to be able to use them correctly and to prevent the risks associated with their use. We believe that future work should first investigate how salad crops are processed as animal feed. The focus should then be on the nutritional and functional role of specific nutrients, which could positively affect the animal's performance, but also affect their digestibility, firstly in terms of proteins (it is not clear whether the nitrogen content derives from proteins or from non-protein nitrogen) and then the ash content (a high ash content is an intrinsic feature of this type of biomass, due to soil contamination).

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CHAPTER 11

GENERAL DISCUSSION

11.1 INTRODUCTION

The livestock sector has a profound impact on agriculture, and therefore on the environment, due to the large demand for raw materials (Tullo et al., 2019; Pinotti et al., 2021). The 32% of the cereals produced worldwide are used to produce feed for livestock animals, while in developing countries the percentage is even higher, in fact 68% of the cereals produced in these areas are fed to farm animals (Elferink et al., 2008). The already high demand is likely to increase further as population growth continues over the coming decades. A large number of ingredients are required to feed animals and the most commonly used ones today, such as soy and corn, require a huge number of resources defined as the footprint per product, such as the "water footprint", "mineral footprint", and "land (arable or total land) footprint" (Flachowsky & Meyer, 2015).

The production of feed ingredients limits the farmland available for human food production with ever-increasing competition from arable land (Luciano et al., 2020), which is worsened by energy policies that increase competition between food, feed, fuel, and fiber.

It is not only the competition between food and feed that changes the demand for natural resources, but also the production of energy. The intrinsic links between food, water and energy systems (the so-called Nexus) could provide synergistic strategies aimed at the resilient security of food, water and energy, i.e., the circular economy (Chiarelli et al., 2018). One way to reduce the environmental impact and pressure from the livestock sector is to search for alternative or recycled ingredients. In recent years, there has been a trend to reduce food waste by increasing the recycling/recovery of these products and putting them on the market (FAO & WHO, 2019). Food losses, food waste, and former food products are all terms that refer to the various food effluents (Pinotti et al., 2021). Food lost along the entire food supply chain, i.e., food that could have been used for human consumption, is not always identified in the same way (Gustavsson et al., 2013). In fact, a distinction is often made between "*food losses*" and "*food waste*".

Food losses refer to a decrease in food quantity or quality in the early stages of the food supply chain, thus reducing the amount of suitable food available for human consumption (Pinotti et al., 2021). The concept of food losses is thus often related to post-harvest activities that lack systems or infrastructural capacities (Parfitt et al., 2010). On the other hand, according to Gustavsson et al. (2013), the term *food waste* is often used for food losses that occur at the end of the supply chain (retail and final consumption), where most losses are caused by wasteful consumer behavior. Strategies and solutions, such as a "food recovery hierarchy", are therefore needed to recover the different types of food waste and introduce it into feed production by reducing the use of natural resources and increasing their reuse (Mourad, 2016). These goals can be achieved by converting food losses into ingredients for the feed industry (Pinotti et al., 2021).

11.2 FORMER FOODS PRODUCTS: IMPLICATIONS AND EFFECTS IN DIFFERENT SECTOR

Former foods products, also named food leftovers, bakery meal or FPPs, are food effluents that lie somewhere in the middle of the food chain (Figure 1).

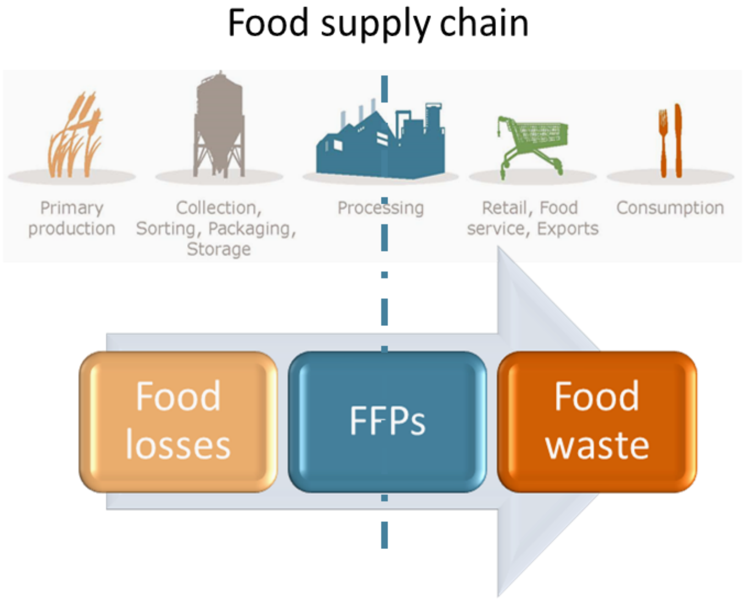

Figure 1. Former food products in the food supply chain.

In particular, former foods are food products that are no longer intended for human consumption for practical or logistical reasons or due to manufacturing problems, packaging defects or other defects. However, none of these problems present health risks when used as feed (FAO and WHO, 2019; Giromini et al., 2020; Gustavsson et al., 2013; Pinotti et al., 2021). Former foods represent one of these innovative sources of feed materials, where food industry losses are transformed into ingredients for the feed industry, thus keeping food losses within the food chain.

The EFFPA estimates that about five million tons of FFPs are available in the EU, while only 3% of this biomass is reused today in animal feed formulas (EFFPA, 2021). These data indicate that the biomass potentially available for the feed sector is very big. In fact, it is estimated that the introduction of three and a half million tons of FFPs in feed instead of traditional ingredients leads to a saving of about 350,000-400,000 hectares of wheat (EFFPA, 2021). Spiker et al. (2017) estimated that food waste accounts for 23% of arable land, 24% of freshwater resources used for crop production, and an amount of food per capita of roughly 625 kcal/cap/day, including large quantities of nutrients, micronutrients, and minerals (Spiker et al., 2017). Re-using food waste therefore also reduces its environmental impact.

11.3 SUSTAINABILITY CONCERN

The largest contribution to the water footprint (WF) of all animal products occurs in the feed production. In fact, growing feed, covers about 98% of the total animal WF (Mekonnen and Hoekstra, 2012) and roughly 21% of the total freshwater supply is intended for the livestock production (Gerbens-Leenes et al., 2013; Mekonnen and Hoekstra, 2012). Livestock sector has also exacerbated the competition for arable land for food: 26% of the Earth's surface land is utilized for livestock grazing, and one third of croplands for livestock feed production, mainly through cereals crops (FAO, 2013; Karlsson and Röös, 2019; Mottet et al., 2017). Feed ratio and ingredients included in animal diets are very different, depending on animal categories, production systems and from country to country; thus, the land and water demand associated with meat and animal-derived food may differ greatly (Mottet et al., 2017). Furthermore, an increasingly important role is played by the production of biofuels, which is expected to grow with a corresponding impact on the production and consumption of food and feed crops (D'Odorico et al., 2018; Muscat et al., 2020; Rulli et al., 2016).

As a consequence, the competition between food and non-food uses of biomass has increased the interdependence in the feed-food-energy nexus, with the risk of negative impacts on food security as well as on the access to land and water resources (D'Odorico et al., 2018; FAO, 2017). Thus, this century needs new sustainable strategies aimed at improving the use of land and water for food production. Since the livestock system is based on animal nutrition, a crucial point regards animal diets employing less and less resources, with a view to sustainable livestock production (FAO, 2018). From the analysis of different strategies of water saving for increasing food production (i.e. waste reduction, dietary shifts, crop water management, and improved crop distribution), what has come to light is that remarkable water saving can be obtained from waste reduction (Kummu et al., 2012).

In this context, the use of former foods, represents an active and promising area of feed research, both in terms of assessing alternative feed ingredients and food waste reprocessing (Luciano et al., 2020; Pinotti et al., 2019). The use of FFPs in animal feeding, therefore, is intended to have positive impacts on the reduction of the current pressure on the agricultural sector's consumption of natural resources.

Furthermore, consumers often have little awareness that animal products require a large amount of land and water. In addition, feed is often cultivated and harvested in completely different and distant areas from where the final product is consumed (Mekonnen and Hoekstra, 2012). On this point, FFPs are also part of a potential strategy to reduce the dependence of animal-producing countries on international feed trade. In some cases, in fact, it may also be possible to meet the internal feed demand through the use of FFPs, so as to reduce the virtual land and water flows resulting from international feed trade and the consequent environmental effects such as land use change, deforestation, water scarcity and biodiversity loss (Fader et al., 2011; Rulli et al., 2019; Seekell et al., 2017; Wang et al., 2018).

Former foods, after all, represent a way of transforming surplus from the food industry into ingredients for the feed industry, thereby reducing overall food losses in the food chain and natural resources consumption.

11.4 NUTRIENT COMPOSITION OF FORMER FOOD PRODUCTS

Former food products mainly consist of leftovers from confectionery products (i.e., chocolates, biscuits, cake, sweet snacks) and the baking industry (i.e., bread, pasta, chips, salty snacks), as well as other high-quality baked products (Pinotti et al., 2021).

Former foods are generally extremely rich in carbohydrates, free sugars, and depending on their origin, also in fats (Luciano et al., 2020). The FFP starch content reaches an average of 50-60% on a dry matter basis (DM) (Luciano et al., 2020). Former foods have also shown high digestibility values, ranging from 79% to 93% DM, according to the ex-food mixture used in their preparation (Luciano et al., 2020).

Due to their high carbohydrate/free sugar content, former foods are energy ingredients for feed. In fact, they cannot be considered as useful protein sources given their low protein content of 10% (Giromini et al., 2017; Luciano et al., 2020). However, these results are case sensitive and thus represent just a few examples of different ex-foods found on the feed market.

The composition of FFPs varies from product to product, and some compositional characteristics (for example the content in free sugars) should be studied with caution.

Some studies (Luciano et al., 2020; Pinotti et al.,2021; Luciano et al., 2021a, Luciano et al., 2021b) (Figure 3) have reported that it is possible to substitute partially traditional sources of energy with former foods using a balanced combination of by-products without any major changes in the diet composition. Animal responses, in the tested phases (mainly the weaning period), were comparable and without substantial detrimental effects on animal performance (Luciano et al., 2021a; Luciano et al., 2021b), but it’s important to consider the level of inclusion in the diets. For this reason, Luciano et al., (2021a) showed that the best level of substitution is under the 50%, over this percentage seems to be not suitable for the growth performance of animals.

In general, the positive response observed in piglets has mainly been attributed to the food processing. In fact, former foods typically undergo both mechanical and thermal processes, unlike the untreated feed ingredients commonly used in livestock production (Giuberti et al., 2012). These processes affect the nutritional properties of the diet, in particular the starch fraction, and digestibility in general (Giromini et al., 2017).

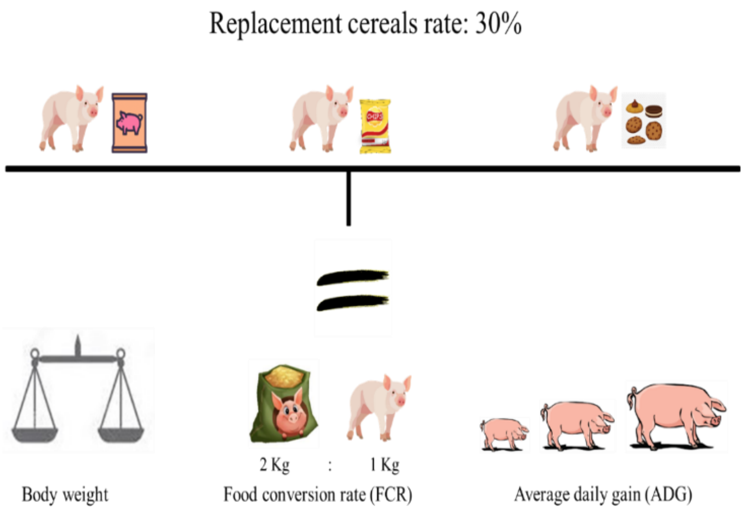

Figure 3. Effects of cereal substitution with FFPs.

Another interesting aspect of former foods is their free/simple sugar content, which affects not only the digestion kinetics of carbohydrates, but also the glycemic index potential. The glycemic index was originally introduced in human nutrition to classify starchy foods based on their post-prandial glucose release in the bloodstream (Giuberti et al., 2012). In the livestock sector, the glycemic index was traditionally used in equine nutrition (Kronfeld et al., 2005), specifically concerning disorders associated with carbohydrate metabolism in racing horses. However, Menoyo et al., (2011) also introduced this concept in pig nutrition, classifying cereals according to their glycemic index. In addition, cereals with a high glycemic index led to increased insulin production over time and a subsequent increased feed intake.

Former foods are often heat-treated, depending on the origin of the raw materials (food leftover) used (Luciano et al., 2020). As a result, they are more digestible than the cereal grains commonly used in pig nutrition. Given that starch processing can modulate the kinetics of starch digestion (Ottoboni, et al., 2019) and the glycemic index (Giuberti et al., 2012), a higher Hydrolysis Index (HI) and predicted Glycemic Index (pGI) have been observed in FFPs compared to unprocessed corn (Ottoboni et al., 2019). Since carbohydrates represent the main energy source of a pig's diet, for a balanced diet it is important to consider the variability of HI and pGI in the different FFPs. An extensive functional evaluation is essential for the safe use of FFPs, and particularly for assessing their impact on animal welfare and gut health.

In livestock production, not only sustainability and cost reduction are important, but also animal welfare (Godyń et al., 2019). This requires a functional evaluation of former foods, particularly the impact on the gastro-intestinal tract welfare (i.e., gut health). It is widely recognized, that the well-being of animal gut system is essential for optimal health and production rates (Fouhse et al., 2016). The gut of piglets is very complex. Gut health and the complex interactions between microbiota and gut maturation are essential to maintain a healthy gut environment (Kim & Isaacson, 2015). Gut health, maturation and microbiota are influenced by the host and a wide spectrum of environmental factors with feeding strategies and husbandry practices being the most significant factors (Tretola et al., 2019a). Understanding how a healthy gut works and looks therefore helps to improve animal welfare and increase/improve production (Wang et al., 2019). The intestinal environment is healthy when the right interactions take place between the microbiota and the intestine itself (Tremaroli et al., 2012). The health of the intestine depends on a wide variety of factors, with the breeding practices and feeding of the animal being the most important. Also evaluating FFPs from this point of view and not only from a chemical point of view can therefore help to increasingly integrate them into the animal diet (Tretola et al., 2019b).

Studying how ex-food influences the composition of the intestinal microbial population, improving eubiosis and / or reducing dysbiosis, provides fundamental information for converting ex-food into value-added products for animal nutrition (Lalles et al., 2007; Tretola et al., 2019b). In this respect, *in vivo* trials investigating the effects of FFPs on growth performance (Tretola, et al., 2019c) and gut microbiota were recently performed (Tretola, et al., 2019d). In these studies, the authors evaluated the effects of substituting 30% conventional cereals with 30% FFPs in post-weaning piglet diets (Tretola, et al., 2019b). The results revealed that both *in vitro* and *in vivo* digestibility values were higher for FFP experimental diets compared to the control diets. Average daily gain, feed intake and general feed efficiency were not affected by dietary treatment.

Regarding the gut health and gut microbiota, the partial replacement of common cereal grains with FFPs in post-weaning piglet’s diets slightly affected the bacterial community (Tretola et al., 2019a-c). The Next Generation Sequencing analysis of the 16S rRNA gene revealed that FFP based diet decreased the abundance and the evenness of gut bacteria. Compared to the piglets fed a standard diet, FFPs increased the abundance of bacteria belonging to the Proteobacteria phylum and decreased the abundance of Lactobacillus genus. A decreased abundance and evenness of gut bacteria is often related to a decreased resilience of the gut ecosystem to gastrointestinal perturbations (McCann 2000) and to an increased probability of pathogen colonization in the gut (Dillon et al., 2005). Furthermore, in other host species, high abundances of Proteobacteria and low abundance of Lactobacillus have been associated with dysbiosis in the hosts with metabolic or inflammatory disorders (Park et al., 2015; Banna et al., 2017). 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 but further investigations are necessary for clarifying their impact on gut health and the microbiota ecosystem. To confirm their safe use as alternative to cereal grains, future studies need to be focused on the use of FFPs in pig’s diet for longer period, such as growing and finishing.

generally, former foods undergo mechanical and thermal processing, unlike the feed ingredients commonly used in animal production. Depending on the type, there may also be additional stages such as mixing, grinding, and drying (Luciano et al., 2020). Not only are nutritional properties affected, but the safety must also be guaranteed, the main problems being the bacterial load and the presence of packaging remnants (Amato et al., 2017; Tretola et al, 2017a; Tretola et al, 2017b). Of course, the marketing of feed ingredients containing packaging residues is prohibited, however the bacterial load must also be contained below legal levels to ensure animal well-being and health (Pinotti et al., 2021). There are several microorganisms found within FFPs such as bacteria, molds, and yeasts. The main hazard related to the use of these products as feed ingredients is the presence of pathogenic organisms such as *Salmonella* spp. However, none of the FFP samples analyzed in the study presented a detectable *Salmonella* ssp. load (Tretola et al., 2017a). In fact, FFPs are dried and cooked during the production process which should help to ensure the microbiological safety of these products. In the same study (Tretola et al., 2017a), microbiological quality was also confirmed through the total viable count (TVC) for other species of bacteria. Recorded values for TVC were below 5 log CFU g^{-1} for all the FFP samples tested, with a microbial load value of 6 log CFU g^{-1} which is recognized as the threshold limit above which spoilage could occur. In Tretola's study, both the *E. coli* and *Staphylococci* count were below the detection limit or extremely low ($\leq$ 2 log CFU g^{-1}), respectively. The same was true for *B. cereus* and its spores, which are considered indicators of poor processing, poor quality of raw materials, or poor temperature control. Yeasts and molds, which are among the most critical organisms for this type of feedstuff, were present in very small quantities, again confirming the stability of these materials.

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CHAPTER 12

GENERAL CONCLUSION

Nowadays, reducing food losses and waste is important, especially in a world where the number of hungry people has been on the rise since 2014, and tons of food are lost and / or wasted every day. According to the United Nations' Committee on World Food Security, *food security* is defined as “all people, at all times, have physical, social, and economic access to sufficient, safe, and nutritious food that meets their food preferences and dietary needs for an active and healthy life”. Furthermore, achieving food security requires food production and distribution systems that are resilient to disruption. Moreover, when food is lost or wasted, all the resources that were used to produce this food - including water, land, energy, labour and capital – go to waste. In addition, the disposal of food loss and waste in landfills, leads to greenhouse gas emissions, contributing to climate change. Food loss and waste can also negatively impact food security and food availability, and contribute to increasing the cost of food.

Limiting environmental impact is crucial to sustainable production especially in the livestock sector.

For this reason, the use of former food products as alternative feedstuffs to convert food losses into animal protein food, represent a promising opportunity to mitigate the impact of the livestock industry on the environment, but also to reduce the competition between humans and pigs for raw materials such as corn, soy and wheat.

This thesis demonstrated that unsold or defected pasta, bread, chocolate, cake, and candies can produce distinct food leftovers products that, when mixed together, can result in uniform product named former food products. However, it's important to consider the level of substitution of corn with bakery meal, because in the first *in vivo* trial reported in Chapter 8, the ADG and G:F were reduced from day 15–35 and for the overall experiment as greater quantities of bakery meal were used; in fact, in the study resulted that a total substitution of corn with bakery meal may not be suitable for pigs. But, in the second *in vivo* trial showed in Chapter 8, P digestibility was improved in diets with bakery meal where phytase was added; this can contribute to a reduction of P in manure. Moreover, it seems that bakery meal does not influence nutrient metabolism and fecal scores of pigs. While, in Chapter 6, the percentage of substitution of common ingredients with former foods wasn't over the 30%, the two types of former foods (sweet and salty) were completely comparable to standard diet, and there wasn't any difference in growth performance or metabolic profiles of animals. The only difference was in the sweet diet, where ATTD of DM decreased: considering the immaturity of gastrointestinal tract and different rate of nutrient absorption of young pigs, further investigations are needed to explain this change. Both studies have suggested that the percentage of inclusion that goes from 27% to 30% of former food in weaning pig diets, do not affect growth performance and health of animals, proposing former food as valuable alternative feed ingredient.

In terms of safety, farmers, nutritionists, industries, and governments are obliged to pay serious attention to animal feedstuff production, considering that quality and safety of feed are essential prerequisites for human food safety and quality. This is particularly true for former food products in terms of packaging material residuals, which need to comply with laws on the constituents of feed. For this reason, using RGB imaging as a rapid and non-destructive tool for the automated detection of packaging particles in former food products, is important in ensuring the safety of former foods used as feed ingredients. By recognizing that former foodstuffs that are not suitable for human consumption are a resource and not a waste product, our food industry can reduce the amount of waste sent to landfill or deposed-of every year, thus saving costs, and reducing the environmental impact of the food production chain. These goals can be achieved by a comprehensive assessment of ex-food.

“We don't need to go faster. We have all the time in the world.”

James Bond – No Time to Die