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Review

Alternative and Sustainable Protein Sources in Pig Diet: A Review

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Simple Summary: Nutritional and anti-nutritional factors of Spirulina, insect larvae such as *Tenebrio molitor* and *Hermetia illucens*, rapeseed meal, and grain legumes such as faba beans, peas, lupins and chickpea are reviewed, in order to re-evaluate the use of these potential protein ingredients in pig diets. The effects on pig performance, digestion, immune system, physicochemical and sensorial characteristics of pork are updated. Limits of their use to be accounted for in pig diet formulation are revisited, together with the possible treatments to improve their nutritional value.

Abstract: The search for alternative protein sources to soy-bean meal (SBM) in animal feeding is a strategic objective to reduce production costs and contribute to a sustainable animal production. Spirulina, due to the high protein content, has emerged as a potential cost-effective, sustainable, viable and high nutritional value food resource for many animal species. Insect larvae (*Tenebrio molitor* and *Hermetia illucens*) are also considered potential alternatives to SBM, given their high edible percentage of almost 100%, as well as a protein value higher than that of vegetable proteins. Rapeseed meal and grain legumes, such as faba beans, peas, lupins and chickpea can also be used as locally producible protein ingredients. This study reviews the nutritional value of these potential alternatives to SBM in pig diets, effects on animal performance, digestion, immune system, physicochemical and sensorial characteristics of meat, including processed meat products of pork. Limits on their use in pig feeding are also reviewed to indicate gaps to be filled in future research on the supplementation level of these potential alternative protein sources into pig diets.

Keywords: pigs; insect larvae; Spirulina; rapeseed meal; grain legumes; nutritional value

1. Introduction

Pork consumption has been predicted to increase by 105% between 2010 and 2050 [1], due to the increase in the world population [2,3]. The main protein ingredients in diets for monogastrics (chickens and pigs) are currently fish meal, processed animal proteins, milk by-products, soybean meal (SBM), rapeseed meal and canola meal [3,4,5]. Average SBM content in compound feeds for pig farming accounts for 28.8% [6]. However, it is widely noted that in recent years, genetically modified soy imported into Europe has increased and its price has fluctuated significantly [2,3]. Therefore, the scientific community is currently intensifying its efforts in finding alternative protein sources to soya bean in animal diets in order to meet the demand for high-quality and sustainable proteins [7]. A new food resource should ideally be characterized by high nutritional value and feed conversion efficiency, but also be able to provide high quality animal products, using land and water efficiently [8]. The high economic and environmental costs associated with the production and long-distance transportation of feedstuffs such as cereals and soybeans, mainly produced in North and South America, and their direct competition with human consumption, have important repercussions on the sustainability of food and livestock production [9,10]. Furthermore, the search for alternative, long-term and low-cost protein and energy sources for use in farm animal diets has intensified, becoming a strategic objective after the COVID-19 pandemic [11].

In recent years, the use of blue-green algae has also become widespread as biologically active food supplements in both human and animal nutrition [17,18,19,20]. They are used in human and animal nutrition, in cosmetics and in the production of valuable substances (e.g. fatty acids, pigments) [21]. It has been reported that microalgae, due to the high protein content, carbohydrates and fats, would be comparable or even superior to conventional feedstuffs, such as soybean meal [22]. Spirulina (*Athrospira sp.*) has emerged as a potential cost-effective, sustainable, viable and high nutritional value food resource for many animal species [7]. It has been reported that the direct intake of *Spirulina* can improve growth performance and meat quality in ruminants, chickens, pigs and rabbits [7,11,23].

Edible insects (entomophagy) are receiving attention as an alternative sustainable nutritional source for both humans and animals [2], thanks to the advantage that may be produced without the use of arable land and are rich in proteins [12]. Indeed, insect larvae would use less water, land and resources for their production when compared to conventional plant-based ingredients used for feed [12,13]. In general, insects have an advantageous food conversion ratio and would be able to convert low-value organic waste into high-value protein, that could replace soybean meal and fishmeal, available in increasingly limited quantities [14]. According to Hong et al. (2020) [2] the use of edible insects in animal feed is expected to increase in the future. As a result, many companies, such as in France and China, have undertaken large-scale production of insects, both for food and feed, around the world. Based on these predictions, the price and use of insects in diets will be achievable and expand, respectively. Insects have several advantages as the percentage of edible part is almost 100% [2,15], their nutritional value is much higher than that of plants in terms of proteins, essential amino acids, vitamins and minerals [2]. Makkar et al. (2014) [14] reported that insects may have an essential amino acid composition that matches the requirements of growing pigs and broiler chickens. Indeed, insects, in general, contain high quantities of lysine, threonine and methionine, the main limiting essential amino acids (AAs) in low-protein diets based on cereals and legumes for pigs and poultry. It has been reported that insects, as well as being an alternative protein source, are also rich in fat, especially full-fat black soldier fly larvae (*Hermetia illucens* L.) (BSFL) and can also be a source of net energy (NE) in pig diets [12,16].

Rapeseed meal, legume grains (including chickpea, pea, lupin, faba bean) and other plant seeds can be a source of both energy and protein in animal diets [24-27]. Rapeseed meal (RSM) can represent an alternative to SBM in monogastric diets as it is an abundant and economical by-product of oil and biofuel production [28-30]. China, India, Canada, Europe and Australia are the countries where rapeseed is mainly grown [28]. Legume grains are under cultivation in a considerable area worldwide and are an excellent low-fat source of protein, dietary fibre, starch, micronutrients and phytochemicals [25-27,31,32]. Rapeseed meal (RSM) and legume seeds have been proposed as possible alternative protein sources as high protein ingredients that can be produced in Europe, contributing to more sustainable pork production [5,33,34]. Germany, France and Finland are adopting a common strategy to encourage extensive cultivation and production of legumes as excellent potential protein sources, to reduce the cost of diets while maintaining production efficiency [35].

This review focuses on the nutritional values of these potential protein sources (micro algae, insect larvae, rapeseed meal and grain legumes) and on their effects on growth performance, carcass yield, meat quality, digestion and immunity when used in pig diets.

2. Microalgae

2.1. Nutritional value

Among microalgae, spirulina (*Arthrospira platensis*) is the most used in the production of food, both for humans and livestock [9]. *Spirulina platensis* and the freshwater microalgae *Chlorella vulgaris* are often indicated in human nutrition for their beneficial actions on intestinal health, such as antioxidant, anti-inflammatory and antimicrobial activities [36,37].

Spirulina is an edible blue-green filamentous, spiral-shaped microalgae of the cyanobacteria phylum and is therefore an autotrophic prokaryote [9,38]. It is naturally present in the alkaline lakes of Mexico and Africa, where it has historically been a source of food for the ancient inhabitants of those places [7].

Several authors have been dealing with the nutritional properties of spirulina for some time [39-42].

Spirulina has a high nutritional value, in particular a high protein content (from 50% to 70% of dry weight) and an interesting lipid content (from 5% to 14% of dry weight) [37,43,44]. It has a unique composition due to its balanced content of amino acids, vitamins, minerals, pigments, carotenoids, chlorophyll and essential polyunsaturated fatty acids, such as γ-linoleic acid (GLA), which has beneficial health effects [7,23,38]. It also contains high quantities of phycocyanin (27%) [17].

Commercial production of spirulina may occur using a nutrient-rich liquid medium with high efficiency in land use [7]. Spirulina has been reported to outperform many other traditional types of animal feed, such as wheat, corn, barley, and soy, in terms of protein production per unit of land [45,46]. Furthermore, desalinated waste water [47] and animal feces can be actively used for its production in order to improve the replication medium. This has been reported with faeces from pigs [48] and cattle [49], with results demonstrating the safety of administering Spirulina to livestock [50].

However, microalgae cell wall carbohydrates are very difficult to digest and utilize by monogastrics, such as pigs [9]. The cell wall of microalgae has a complex structure and composition that has not been explored in depth, although the presence of a polymeric matrix has been reported, made up of cellulose and a further trilaminar sheath in which there is a substance resistant to enzymatic degradation, the algaenan [51,52].

It has been hypothesized that Spirulina, integrated with active carbohydrate enzymes (CAZymes) capable of degrading the complex polysaccharides of the cell wall, can be usefully used to facilitate the access of digestive enzymes to the cell content, improving nutrient digestibility [9]. Among the enzymes used there is lysozyme, a CAZime capable of degrading the peptidoglycan constituent of the cell walls of prokaryotes, thus making proteins and pigments accessible to the enzymes of the digestive system [9,53].

2.2. The use of Spirulina in pig diet

Several studies focuse on the use of Spirulina in piglet diets as a supplement [17,19,54]. First results on the use of spirulina in pig diets are contrasting [7]. As already argued by Holman and Malau-Aduli (2013) [7] the different results obtained in growth of pigs fed diets supplemented or not with spirulina would be attributable to the different experimental procedures, i.e. to the genetics and state of health of the animals, the composition of the diet, the level and the form of inclusion of spirulina in the diet, pelleted or not [19].

In pigs, Spirulina and Chlorella have been studied as an alternative to antibiotic use to ensure gut health after weaning, limiting intestinal damage caused by immune and inflammatory responses to dietary transition and oxidative stress [55,56]. Studies highlighted a potential effect of Spirulina and Chlorella supplementation on intestinal development, prevention of mild digestive disorders with reduction of sick animals [17,54]. However, results are not conclusive and the authors see the need for further investigations to determine the mechanism of action of Spirulina and Chlorella on the health and physiology of the intestine. Furthermore, research has addressed the enrichment of microalgae biomass with microelements through biosorption and bioaccumulation, in order to produce dietary supplements for animals, as done for humans [57]. In the study by Saeid et al., (2013) [57] it is observed the effect of the use of the microalgae *Spirulina maxima* enriched with copper (Sm-Cu, as a replacement for inorganic salts) on performance, metabolical and physiological values of fattening pigs. The study reports a lowering of LDL cholesterol (by 23%) and total cholesterol (by 10.5%) in blood serum, as well as an increase in the parameter a* in the meat from pigs fed Sm-Cu diets.

Simkus et al. (2013) [58] observed the growth performance and meat quality of Landrace and Yorkshire fattening pig crossbreds fed daily and individually with 2 g of fresh blue algae biomass Spirulina platensis at 75% moisture mixed with forage. The average daily weight gain of pigs fed fresh Spirulina biomass was 9.26% higher, 100 kg of weight being reached in 7.37 days faster and the

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food energy consumed per 1 kg increase of weight was 1.28 MJ lower than that of pigs in the control group. The carcass weight of the experimental pigs was 2.02% higher and the amount of intramuscular fat 0.33% lower compared to the control group. Color, pH, cooking loss and tenderness were not affected by dietary treatments.

The effects of Spirulina supplementation on the performance, carcass and meat quality of pigs have recently been studied if administered not directly but to their gestating and lactating mothers until weaning [38]. In particular, Lugarà et al (2022) [38] studied the long-term influence of maternal spirulina dietary supplementation (no supplementation and 20 g of spirulina per day in tablets) and the energy density of the diet (control and high-energy density, HED), throughout gestation and lactation until weaning, on growth performance, carcass characteristics, meat quality and fatty acid profile, in both male and female pig. Daily weight gain during the entire fattening period (4 months) was reduced (-7.4%) in males from mothers who had received spirulina supplementation compared to males from sows who had not received supplementation, even if slaughter weight did not differ between the two groups. Daily gain, slaughter weight and fattening period were not influenced by maternal spirulina supplementation in females. No significant differences were found regarding feed conversion rate, feed intake and carcass characteristics in pigs of both sexes from mothers with and without dietary spirulina supplementation. Dietary treatments did not influence the physicochemical quality of the meat. Dietary spirulina supplementation by mothers tended to improve the polyunsaturated fatty acid (PUFA)/saturated FA (SFA) ratio in the intramuscular fat, without any influence on the n–6/n–3 FA ratio. The results of this study highlight a sex-specific response of offspring growth following maternal spirulina supplementation.

Neveda et al. (2014) [17] evaluated the supplementation of 0.15% and 0.2% spirulina in the diets of piglets (from 12.2–12.5 to 30.9–33.9 kg live weight) and observed an increase in growth performance together with a decrease in the feed conversion ratio. In contrast, Grinstead et al. (2000) [19] found minimal improvement in growth performance in piglets weaned on 0.2%, 0.5% and 2% Spirulina for 28 days.

However, these different studies concern the use of microalgae in pig diets as supplements and not as feed ingredients.

The incorporation of microalgae Spirulina as a feedstuff together with the supplementation of two exogenous enzymes is considered for the first time by Martins et. al (2020) [9]. Authors evaluated the effect of the use Spirulina (10%) in post-weaning piglet diets (slaughtered at weights lower than the standard weight of 100 kg in order to obtain the spit-roasted pig), supplemented or not with two exogenous enzymes (a commercial mixture of enzymes that degrade carbohydrates and lysozyme), on growth performance, digestibility and meat quality in piglets. The use of 10% Spirulina in the piglets' diet negatively affected growth performance, with a 9.1% decrease in final weight, a 14.2% decrease in average daily weight gain (ADG) and an 11.0% increase of the feed conversion ratio (FCR), compared to the control group. An increase in the viscosity of the digesta and a lower apparent digestibility of crude proteins were observed as a result of their resistance to piglets' endogenous proteolytic enzymes. Indeed, the authors of the study, in agreement with what was reported by Evans et al. (2015) in chickens [59] argue that the strong increase in viscosity of piglet digesta who had consumed the microalgae was likely the result of the gelation of poorly digestible proteins of spirulina. Furthermore, lysozyme increased the apparent digestibility of crude fat and acidic detergent fiber, compared to the *Spirulina* supplemented group and the control group, respectively. In the experimental conditions of these Authors, the use of exogenous peptidases in the piglet diets did not improve digestibility of *Spirulina* proteins nor does it prevent their gelation. Results showed that lysozyme, unlike the commercial mixture of carbohydrate-degrading enzymes, was effective in degrading *Spirulina* cell wall in the intestine of piglets. Meat quality was not negatively affected by the addition of *Spirulina* in the piglets' diet, either alone or associated with enzymes. Contrary to what has been reported by other authors on the antioxidant activity of *Spirulina* [37,43], no protective effect against meat lipid oxidation was observed during 7-day storage period.

Interestingly it has been reported an improvement in sperm quality (11% increase in volume, 5% increase in motility and vitality after conservation) in wild boars that had received a dietary Spirulina extract compared to those fed unsupplemented diet [7]. This would be of interest for revaluation of this species breeding [60].

3. Insect larvae

3.1. Nutritional value of insect larvae

Yellow mealworm (*Tenebrio molitor*), black soldier fly (BSF) (*Hermetia illucens*) and common housefly (*Musca domestica*) are the insect species that could potentially be produced on a large scale for use as protein ingredients in animal diets [2,3].

Tenebrio molitor larvae, also known as mealworm or yellow mealworm, have good nutritional value, thanks to their protein and fat contents [61-63], their digestibility [64,65], flavor [66] and functionality, given by chitin and antimicrobial peptides (AMPs) [2,67]. Hong et al. (2020) [2] recently reviewed studies regarding *Tenebrio molitor* larvae as an alternative protein source in monogastric diets. Crude protein content (dry matter basis) of *T. molitor* larvae averages 52.4% [2] ranging from 47.0 [67] to 60.2% [68] on dry matter basis, being higher than that of conventional SBM (49.4%) [69], although lower than that of fishmeal (67.5%) [69]. The amino acid profile is of high quality, to be considered a highly sustainable protein source alternative to SBM or fishmeal [2]. Fiber is found in their cuticles and varies from 4.19% [70] to 22.35% [68]. Crude fat content (dry matter basis) averages 30.8% [2] ranging from 19.1% [68] to 37.7% [62] and would vary depending on whether it has been defatted or not (Figure 1) [2]. Regarding fatty acid composition of *Tenebrio molitor* larvae (DM basis) it has been reported that the percentages of SFA and UFA range from 22.2% [71] to 23.3% [68] and from 77.7% [71] to 79.0% [72], respectively. Essential polyunsaturated fatty acids (PUFA), from the ω-3 and ω-6 series are also detected [2]. However, *T. molitor* larvae can undergo not only the defatting treatment but also hydrolysis before grinding (Figure 1) [2].

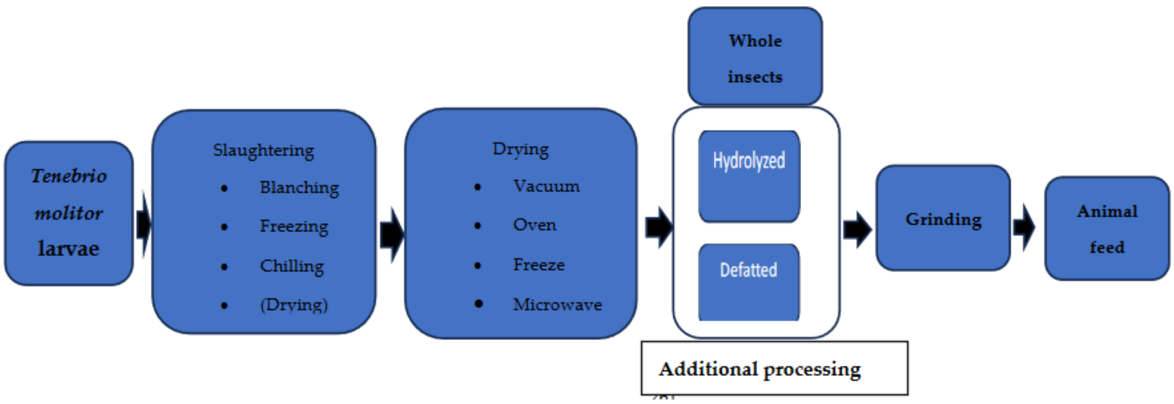

Figure 1. Schematic representation of processes that *Tenebrio molitor* larvae undergo before animal consumption [2].

T. molitor larvae contain various minerals such as calcium, phosphorus, sodium, potassium, magnesium, iron, zinc and copper. Among these, the most represented are iron (63.0-100.0 mg/kg) and zinc (102.0-117.4 mg/kg) [68,71]. It is reported that *Tenebrio molitor* larvae are easy to raise and feed, as they have a stable protein content, independent of their diet [2]. Thanks to these characteristics they have been industrially produced as feed for pets, zoo animals and livestock [2].

Lu et al. (2022) [3] recently reviewed studies regarding the composition of black soldier fly larvae (*Hermetia illucens* L., BSFL) and its potential use as an alternative protein source in animal diets. BSFL are also reported as a good source of proteins (216-655 g/kg of crude protein on dry matter basis for defatted BSFL) and essential amino acids, being also rich in other nutrients such as fats (298-515.3 g/kg of crude fat on dry matter basis) and minerals (27-132 g/kg of ash on dry matter basis for full-fat BSFL) [3]. Moreover, BSFL are a potentially source of antimicrobial peptides (AMPs) that are produced as a reaction against invading pathogens, with a broad-spectrum action on both Gram-

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positive and Gram-negative bacteria [73]. It is known that BSFL have a high content of saturated fatty acids (SFA, 362-782.9 g/kg on dry matter basis) while that of PUFA is usually low [74]. BSFL have a high concentration of the medium-chain fatty acid lauric acid C12:0 (75-575.6 g/kg on dry matter basis), a natural antimicrobial that acts in particular against Gram-positive bacteria [74]. However, the substrate on which larvae are reared significantly influences their fatty acid composition, i.e. the overall synthesis of fatty acids from the ω-3 and ω-6 series [74]. The calcium-phosphorus ratio varies depending on whether the BSFL meal is defatted or not and this requires attention in the formulation of pig diets, in order to ensure appropriate calcium-phosphorus ratios in the complete feed and avoid antagonisms among minerals [75]. Rigorous quality controls of BSFLM are required by suppliers [12].

BSFL can be easily grown and spread on any nutrient substrate, which can be represented by vegetation residues, manure, animal waste, food scraps, agricultural by-products or straw [12,16,76], and their composition is influenced by the growth substrate [16]. The ability of BSFL to grow on organic waste substrates makes them more sustainable protein sources for preparing pig diets. It is reported that 1 kg of BSFL biomass can be obtained per 2 kg of growth substrate [14], thus reducing the organic matter discharged to landfills [12]. In Spranghers et al.'s study (2016) [16], BSFL were grown on four different substrates such as chicken feed, vegetable waste, biogas digestate and restaurant waste, with the aim of evaluating their influence on the amino acid, fatty acid and mineral composition. Prepupae protein content ranged between 399 and 431 g/kg (on dry matter basis) among experimental groups, with minimal differences found in the amino acid composition, due to the growth substrate. Conversely, prepupae reared on biogas digestate showed lower ether extract (EE) and higher ash (218 and 197 g/kg dry matter (DM), respectively) contents than those reared on vegetable waste (371 and 96 g/kg DM, respectively), chicken feed (336 and 100 g/kg DM, respectively) and restaurant waste (386 and 27 g/kg DM, respectively). Fatty acid composition was characterized by high contents of C12:0 in all experimental groups. According to the authors of this study, BSFL can represent an interesting alternative protein source in animal feeding, due to high quality standards of prepupae raised on different substrates. However, the growth substrate would influence the EE and ash contents. Calcium concentration in the BSFL meal was also highly variable and dependent on larvae growth substrate [16]. Conversely, phosphorus concentration in BSFL meal was less affected by substrate on which they were grown [12,16].

However, Joanas et al. (2017) [77] observed that the nitrogen-to-protein conversion factor in insects is less than 6.25 and should instead be 4.74, 4.75, or 5.41, due to the presence of non-protein and indigestible nitrogen in the chitin of the exoskeleton [2,77]. Since the fibrous fraction of larvae is found in chitin, the major component polymer of the larval exoskeleton which is not digestible by the endogenous enzymes of monogastrics [78], even the N encapsulated in chitin would not be digestible in the pig intestine. Other studies reported that insects contain non-protein nitrogen, such as chitin, nucleic acids, ammonia, nitrite, etc., which would lead to overestimation of the protein content of insects [79,80]. Furtheremore, the different methods used to defat (Figure 1) [2] and dry insect larvae would be responsible for differences in the standardized ileal digestibility of the AAs [81]. Indeed, it has been reported that the use of cold pressing to defat insect larvae minimally affects its apparent ileal digestibility in broilers [82]. Huang et al. (2018) [83] reported that in vitro digestibility of AAs was higher when the method used for drying BSFL meal was the conventional one (60 °C until constant weight), compared to that with microwave irradiation.

Other scientific evidence concerns the chitin contained in the cuticle of insects. Even though it is an indigestible fiber, it has been shown that it can have positive effects on immunity [84,85]. Species and developmental stages influence the composition and quantity of chitin in insects [2]. Larvae have the lowest chitin content compared to other forms of development [2]. Chitin is a linear polymer of β-(1-4) N-acetyl-D-glucosamine units, which is found in a complex structure with cuticular proteins, lipids and other substances [86]. Chitin can perform a bacteriostatic function and indeed, in a study conducted on piglets, it was highlighted that the use of chitin derivatives (such as chitosan) in diets are potentially capable of reducing or inhibiting the growth of pathogenic microorganisms, that cause post-weaning diarrhea [87].

3.2. Safety issues

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Safety issues limit the use of insect larvae as a feed ingredient in animal feeding. The Food and Agriculture Organization (FAO) of the United Nations establishes safety requirements for BSFL to be used in diets of livestock and pets [88]. Currently, food safety regulations constitute the main obstacles to the large-scale use of BSFL in animal feed [13]. There are also restrictive indications on the growth substrate of BSFL larvae and meat, manure, "restaurant waste" and "other waste" are explicitly prohibited [89].

Indeed, although insects are a potential source of high-quality and quantitative proteins, their use in animal feed faces safety problems, as they could convey toxic substances produced by their defensive glands. [90,91]. Benzoquinone is among the toxic substances found in *T. molitor*, which can interfere with cellular respiration, triggering kidney damage, as well as being carcinogenic in humans and animals [2]. Benzoquinone, being continuously accumulated in *T. molitor*, increases its concentration with age [2]. As reported by Hong et al. (2020) [2] so far anyway, it has not been clearly established how much benzoquinone remains in *T. molitor* larvae after cleaning, drying, heating and grinding processes, and what are the tolerance limits of benzoquinone in monogastric animals. Therefore, as indicated by Hong et al. [2] it is essential to indicate a control method to monitor residual quantity of benzoquinone in products based on *T. molitor* larvae, in order to establish the level of toxicity. Currently, there are no standardized and unified production and processing procedures but small-scale equipment with low yield and efficiency [3].

Moreover, it has been reported that insects can express antibiotic resistance genes [92], which would mean that they can be contaminated with pathogenic microorganisms or contain mycotoxins, resulting from contaminated growth substrates [2]. However, it has been observed [93] that monitoring the presence of pathogenic microorganisms (*Escherichia coli* and *Salmonella* spp.) in the growth substrate as well as in the larvae would be an effective prevention against the survival of such pathogens, both in larvae and in adults of *T. molitor*. Furthermore, several authors have reported that *T. molitor* larvae fed diets contaminated by different types of mycotoxins grew normally without any accumulation in their body, being able to degrade the mycotoxins [94,95]. As suggested by Hong et al. [2] it would be necessary to investigate the mechanism of resistance to mycotoxins in *T. molitor*. Another problem regarding the food safety of insects is that of the accumulation of heavy metals, deriving both from the environment and from the growth substrate [2]. A check in this regard by means of X-ray fluorescence spectrometry would also be necessary [2].

However, the introduction of BSFL meal into the diets of salmonids, trout, tilapia and poultry (considering chickens, ducks, turkeys and geese) has been approved and regulated by the American Association of Feed Control Officials [96].

3.3. Edible insects in studies of swine

As already highlighted by authors who reviewed the literature on the topic, studies on pigs are more limited than those on chickens, since feed consumption is greater in pigs than in chickens, and given high production cost and low availability of *T. molitor* and *H. illucens* larvae.

H. illucens larvae used as an ingredient in complete chicken diets affected their growth performance, nutrient digestibility, and blood analysis [97,98]. Other studies have indicated a clear effect of dietary use of *H. illucens* larvae on the intestinal microbiota [97,99] and on the microbial metabolites found in the cecal digesta of laying hens or broilers [97,99,100]. It was observed that insect meals derived from *Tenebrio molitor* and *Hermetia illucens* are useful in providing apparently metabolizable energy and digestible amino acids in broiler diets [63].

BSFL (whole or partially or completely defatted) has been used in pig diets without compromising growth performance, feed intake, digestive utilization of nutrients or intestinal morphological characteristics [12,74,100,101,102].

Hong et al. (2020) [2], in reviewing the use of *T. molitor* larvae in diets fed monogastric animals concluded that up to 6% in weaning pig's diet and 10% in that of growing pigs, could be used as a protein source, without negative effects or with improved growth performances, as well as AA digestibilities, compared to conventional protein sources.

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Yu et al. (2019) [100] studied the effects of using *Hermetia illucens* larvae meal in the diet of crossbred fattening pigs (Duroc × Landrace × Large White), with inclusion levels of 4 (group H1) and 8% (group H2) compared to a of control without larvae, on growth performance, the microbiota, metabolites and intestinal barrier genes expression in the colon. The H1 diet increased the average daily gain of pigs (0.89, 0.98 and 0.86 kg/d in control, H1, and H2 group, respectively) and decreased the feed conversion ratio (F:G) (3.21, 2.85 and 3.23 in control, H1 and H2 group, respectively) compared with control and H2 diets. However, there were no difference in the average daily feed intake between the control group (2.83 kg/d), H1 group (2.77 kg/d), and H2 group (2.87 kg/d). H1 and H2 diets affected colon microbial population increasing the numbers of *Lactobacillus* and different butyrate-producing bacteria (*Pseudobutyrivibrio*, *Roseburia*, and *Faecalibacterium*), and decreasing the abundance of *Streptococcus*. Moreover, diets including *H. illucens* larvae increased the number of *Clostridium* cluster XIVa bacteria. Microbial fermentation metabolites were also influenced by dietary treatments with concentrations of total short chain fatty acids, butyrate and isobutyrate greater in the H1 group than in the control group and concentrations of protein fermentation products, that is total amines like cadaverine, tryptamine, phenol, p-cresol, and skatole lower in the H1 diet compared with the control group. H2 diet also showed increased concentrations of butyrate and decreased concentrations of phenol, p-cresol, and skatole compared with control group. Changes in bacterial composition and in their metabolites were associated with changes in gene expression in the colonic mucosa. Regarding the immune status of the intestinal mucosa, pigs in the H1 group showed a more reduced expression of TLR-4 and proinflammatory cytokines (IFN-γ) compared to pigs in the control group and upregulated anti-inflammatory cytokine (IL-10) and intestinal barrier genes (ZO- 1, occludin and mucin-1). In the pigs of the H2 group there was an increased expression of ZO-1 compared to the control group. According to the results of this study, the inclusion of *Hermetia illucens* larvae in pig diet can improve the immune status of the intestinal mucosa of pigs, through an alteration of the bacterial composition and its metabolites. The findings would provide a new perspective on the use of this insect's larvae as a sustainable protein source rich in nutritional ingredients for pigs.

Yu et al. (2019) [103] also evaluated the effects of including different percentages of *Hermetia illucens* larval meal (0, 4 and 8%; named as groups HI0, HI4 and HI8, respectively) in the diet of crossbred female finishing pigs (Duroc × Landrace × Large White) on their growth performance, carcass traits and meat quality, including fatty acid composition. Effects of administration of *Hermetia illucens* larvae on relative mRNA expression of genes related to lipid metabolism and to myosin heavy-chain (MyHC) in *longissimus thoracis* (LT) muscle of the finishing pigs were also evaluated. The HI4 group showed higher final body weight and average daily gain as well as lower feed/gain ratio compared to the HI0 and HI8 groups. There were no significant differences for average daily feed intake among groups. Varying dietary *H. illucens* larvae meal inclusion did not affect the 45 min and 24 h pH values, the 45 min and 24 h L*, a* and b* parameters, drip loss, and shear force. Groups HI4 and HI8 had greater loin-area, marbling scores, and inosine monophosphate (IMP) content in the (LT) compared to group HI0. The HI4 group had a higher intramuscular fat content compared to the HI0 group. In addition, HI4 group showed greater intramuscular fat content in the LT muscle than HI0 group. Although dietary treatments influenced the concentrations of several individual fatty acids in the LT muscle of pigs, the total saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) fatty acids, n-6 PUFA/n-3 PUFA ratio did not differ significantly between the three experimental groups. The expression level of fatty acid synthase (FAS) mRNA was significantly increased in the HI4 and HI8 groups compared to the HI0 group. Furthermore, the mRNA expression degree of acetyl CoA carboxylase α (ACCα) and lipoprotein lipase (LPL) was also increased in the HI4 group than in the HI0 group. As regard genes related to the LT muscle fiber composition there was an increased mRNA expression level of myosin heavy chain (MyHC)- IIa with HI4 diet than with HI0. According to this study, the inclusion of H. illucens larvae in the diet has a positive influence on growth performance and meat quality, and the authors argue that the underlying mechanism could be related to the alteration of the lipogenic potential induced by *H. illucens* larvae.

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Altman et al. (2019) [76] conducted a very interesting and multifaceted study in which they studied the physico-chemical and sensory traits of pork from barrows ((Pietrain × (Large White × Landrace)) fed diets containing *Spirulina* (*Arthrospira platensis*) or black soldier fly (*Hermetia illucens*) partly-defatted larval meal as alternative protein ingredients to soybean meal. Pork quality was evaluated under highly oxygenated modified atmosphere industrial packaging conditions. Diets were administered during three growth periods (25-50, 51-75 and >75 kg) and two of the three were experimental diets as, both in the first and second growth periods, 50 (replicate 1) or 75 % (replicate 2) of the soybean meal was replaced with Spirulina or larval meal, while in the last fattening period 100% of the soybean meal was replaced. The third diet was administered to the control group animals and was a typical diet in which the primary protein source was soybean meal. The diets, as a consequence of the replacement of soybean meal, they differed markedly in the integration of essential amino acids. Overall, dietary protein source rarely affected physico-chemical parameters of pork (Table 1) [76], even when packaged in standardized industrial conditions.

Table 1. Effect of replacement soybean meal with either Spirulina or partly-defatted *Hermetia illucens* larval meal in barrows diet (Pietrain × (Large White × Landrace)) on meat physical and chemical characteristics [76].

Parameter	C (n = 16)	HI (n = 15)	SP (n = 16)
Carcass weight (kg)[1]	95.08 (1.17)	97.99 (1.21)	93.11 (1.17)
Lean meat yield (%)[1]	59.52 (0.45)	58.77 (0.46)	59.05 (0.45)
GM pH_{45min}	6.08 (0.05)	6.24 (0.05)	5.97 (0.05)
LTL pH_{45min}	5.91 (0.08)	6.05 (0.09)	6.04 (0.09)
LTL pH_{u}	5.41 (0.02)	5.40 (0.03)	5.43 (0.03)
Cooking loss (%)	32.4 (0.30)	31.3 (0.30)	32.4 (0.30)
Instrumental tenderness (N)	10.78 (0.27)	10.49 (0.30)	10.51 (0.29)
Protein (%)	23.10 (0.12)	23.05 (0.13)	22.93 (0.13)
Intramuscular fat (%)	2.96 (0.19)	3.27 (0.21)	3.04 (0.20)
Water content (%)	72.60 (0.16)	72.02 (0.18)	72.44 (0.17)
Backfat L*	79.09[a] (0.27)	78.17[b] (0.30)	78.72[ab] (0.29)
Backfat a*	4.09 (0.18)	4.26 (0.19)	4.02 (0.19)
Backfat b*	4.85 (0.20)	5.15 (0.22)	5.52 (0.21)
Lean colour L*	63.18 (0.89)	61.62 (0.97)	62.96 (0.93)
Lean colour a*	2.74 (0.23)	3.56 (0.25)	2.94 (0.24)
Lean colour b*	13.84 (0.30)	13.77 (0.33)	13.71 (0.32)
TBARS (μg/g)	0.357 (0.043)	0.399 (0.047)	0.470 (0.047)[2]

[a-b] different superscript letters indicate statistical differences between groups at $p < 0.05$; the absence of letters indicates no significant difference.[1] Calculated without considering carcass weight as a covariate. [2] n = 15.

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The experimental diets provided products that hardly differed for the sensory aspects from those from the control group and indeed the differences found were interpreted as sensory improvements, such as the more intense smell and greater juiciness. The two alternative protein sources influenced the fatty acid composition of backfat, showing a higher polyunsaturated fatty acid content compared to using soybean meal as the primary protein source. Furthermore, lauric acid (C12:0) content of backfat was higher (five times) in the group fed *Hermetia illucens* and authors suggest that this fatty acid may be a biomarker for pork from animals fed this alternative protein source.

Spranghers et al. (2018) [74] conducted two studies to evaluate the influence of different amounts of prepared fat from BSF prepupae on intestinal microbiota of pigs, simulating digestion in the small intestine of piglets by means of the *in vitro* technique, but also by conducting an *in vivo* test in weaned piglets. An incubation medium was prepared containing a synthetic diet, a microbial inoculum from a donor piglet, and 0.20, 0.50, 1.00, and 1.50 g/100 mL of BSF fat medium. At the end of the incubations (37 °C for 4 h), several aliquots of medium were taken and coliforms, D-streptococci, lactobacilli and total anaerobic bacteria were counted. Weaned piglets (fifty-six, males and females, weaned on 21 days of age; 6.178 ± 0.562 kg) were fed diets, containing whole (4 and 8%) and defatted (5.4%) BSF prepupae, compared with a control diet containing soybean as a protein and fat source. The experiment lasted 15 days. Average daily gain (ADG), average daily feed intake (ADFI) and feed to gain ratio (F:G) were registered. After slaughter, the digesta and sections of the intestine were collected. Total ileal and fecal apparent nutrient digestibilities (gross energy, dry matter (DM), CP and ether extract (EE) were calculated. The C12:0 content in the BSF prepupae was 57.9 g/100 g of ether extract. From the *in vitro* digestibility studies it emerged that at the inclusion level of 1.00 g/100 mL (corresponding to 0.58 g C12:0/100 mL), the growth of lactobacilli was suppressed, with a more marked effect against D-streptococci. The highest inclusion level of prepupal fat (1.50 g fat/100 mL corresponding to 0.87 g C12: 0/100 mL), resulted in approximately 2-fold log reductions in D-streptococci. From the *in vivo* trial, piglets fed diets containing BSF showed log reductions of only 0.5-fold for D-streptococci in their intestines. There were no differences between the experimental groups regarding ADG, ADFI and F:G. Apparent fecal digestibility of nutrients did not differ between groups. Ileal protein digestibility of the 8% full-fat BSF-containing diet was lower (67.4%) than that of the control (69.7%), while the observed values for the 4% full-fat BSF and defatted BSF diets were greater (73.3%). Authors conclude that considerable amounts of full-fat or defatted BSF (up to 8%) may be used in piglet diets to replace soybean products, without having negative effects on growth performance.

Chia et al. (2021) [102] studied the effects of substituting fish meal (FM) with full-fat black soldier fly larval meal (BSFLM), according to replacement rates (w/w) of 25 (D25), 50 (D50), 75 (D75) or 100% (D100), in the diets of pigs (hybrid Large White and Landrace) slaughtered at weights of around 100 kg. Dietary treatment influenced growth performance with greater average daily gains in D50, D75 and D100 groups compared to D0 one. Final body weights were greater in D50 and D100 pig groups, than in D0 and D25 ones, while feed conversion ratios were lower in D50, D75 and D100 finisher pigs, compared to D0 and D25 groups. Carcass yield of pigs fed diets containing BSFLM with FM replacement rates of 50, 75 or 100% was greater than for pigs from the control group, consuming 100% FM as protein source. Crude protein content of the different tissues analyzed was high, varying (on dry matter basis) between 65 (in the heart) and 93% (in the lung) among all dietary treatments. Furthermore, the different tissues of pigs fed diets containing 50-100% BSFLM had a higher crude fat content than those of pigs fed diets prepared with 0-25% replacement rate of FM with BSFLM. According to the authors, the improvement in growth performance of pigs fed with increasing levels of BSFLM as a replacement for FM in the diet would be indicative of improved palatability of the diet together with sufficient consumption of digestible nutrients. The reduction in the feed conversion ratio with the greater levels of replacement of FM with BSFLM would open up the prospect of a reduction in feed costs for pig production.

Crosbie et al. (2020) [12] conducted two experiments on growing barrows (Yorkshire × Landrace × Duroc; 25.1 kg BW ± 0.41 kg) in order to determine the standardized ileal digestibility (SID) of amino acids (AAs) (Exp. 1) and net energy (Exp. 2) of two samples of black soldier fly larvae meal (BSFLM), one full-fat (FF; 42.5% crude protein, CP, as-fed) and one defatted (DF; 40.8% CP, as-fed). For this purpose, two corn starch-based diets were formulated containing FF (50%, as-fed basis) or DF (36.5%, as-fed basis) BSFLM as unique dietary AA sources. The study showed that SID of CP (i.e., 80.6%; average for FF and DF BSFLM) and Lysine (i.e., 88%; average for FF and DF BSFLM) did not vary between FF and DF BSFLM. SIDs of some AAs (i.e. Arg, Val, Ala and Pro) were or tended to be lower for the FF than for DF BSFLM while there was the contrary for the SID of Met. According to the authors this was most likely attributable to the relative major concentration of NDF-N in FF BSFLM. Furthermore, the authors report that the differences in the SID of the AAs would be due to the methods applied to defat and/or dry the BSFLM. Digestible Energy (4.927 vs. 3,941 ± 75 kcal/kg), the metabolisable energy (4,569 vs. 3,396 ± 102 kcal/kg) and the calculated net energy (3,477 vs. 2,640 ± 30 kcal/kg; 3,479 vs. 2,287 ± 28 kcal/kg, using the Noblet or Blok equations, respectively) were higher for FF than for DF BSFLM. Furthermore, the apparent total tract digestibility of neutral detergent fiber and acidic detergent fiber was higher for FF than for DF BSFLM. The study therefore revealed that both FF and DF BSFLMD had high SID values for the majority of AAs, although FF BSFLM provided greater net energy for growing pigs. According to the authors of the study, both FF and DF BSFLM would be potential alternative protein sources in the formulation of the diets of growing pigs.

Ipema et al. (2021) [104], evaluated the opportunity of providing live BSFL larvae as an environmental enrichment to benefit pig welfare, as tested in broiler chickens.

4. Rapeseed meal and legume grains

4.1. Nutritional value

Rapeseed meal (RSM) is a by-product from oil and biofuel production [29]. RSM, with a crude protein content which varies between 33.7 and 35.6% (as fed) [105] , can represent an opportunity to diversify the ingredients of pig diets using home-produced feed [29]. However, the high content of fiber and anti-nutritional factors such as glucosinolates, sinapin, tannins and erucic acid have limited the use of rapeseed meal in pig diets over time [106,107]. Glucosinolates are sulfur-containing glycosidic compounds whose decomposition would produce products capable of reducing feed intake, altering the production of thyroid hormones, [107] and consequently influencing metabolism and animal performance. However, production of hormones by the thyroid gland would be inhibited depending on the nature and concentration of degradation products from RSM glucosinolates [107]. It was observed an increase in weight of the thyroid gland in pigs fed RSM, despite a level of total glucosinolates lower than the recommended limit of 2.1 mmol/kg [29]. Phenolic compounds such as tannins in RSM can also reduce protein digestibility and interfere with protein metabolism [108]. Higher thyroid gland weight but normal thyroid hormone concentration was also observed in pigs fed 6-10% RSM in the growing-finishing phase [109]. However, traditional rapeseed varieties have been selected in order to decrease levels of erucic acid in the oil and glucosinolates in the non-oily part of the seed, as well as to obtain an improved AA profile [105]. Indeed, due to progress in plant breeding, rapeseed seeds containing low levels of erucic acid (< 2%) in the oil and glucosinolates (< 15 μmol/g) in defatted meal have recently been placed on the market, called canola in North America, "double-zero" or "double-low" rapeseed or 00 rapeseed in Europe [105].

Grain legume seeds, such as faba beans, peas, lupins, chickpea, contain protein, soluble and insoluble fiber, slowly digested starch, micro- and macronutrients, vitamins and numerous bioactive phytochemicals, such as flavonoids and other antioxidants [31]. CP content of common grain legumes varies from 20 to 30%, with the highest content in yellow lupin (324-381 g/kg dry matter), but they are low in sulphur-containing amino acids (methionine and cystine) and, in addition, of tryptophan when compared to SBM [31]. Moreover, the use of legumes in animal diets has been limited due to the presence of secondary plant metabolites, so-called anti-nutritional factors (ANFs). ANFs includes condensed tannins (proanthocyanidins, non-hydrolysable), protease inhibitors (trypsin and chymotrypsin inhibitors in most legume species), alkaloids (toxic amines mainly contained in lupins), lectins (phytohaemagglutinins, glycoprotein compounds mainly in common beans), pyrimidine glycosides (vicine and convicine in faba beans) and saponins (glycosides contained in many plants [31]. These secondary plant compounds reduce palatability (tannins and alkaloids), nutrient digestibilities (tannins, protease inhibitors, lectins) or may have toxic (alkaloid) effects [24,31]. Furthermore, negative effects in pigs, such as excessive fermentation, flatulence and diarrhea, may be caused by galactosides contained in high percentages in some grain legumes [31]. Moreover, in many legumes such as lupins, non-starch polysaccharides (NSP) are present in considerable quantities, which can have negative effects on pigs by reducing the passage rate of the digesta and feed intake and growth performance [31]. The high neutral detergent fiber content of legume seed hulls has also been reported to reduce nutrient digestibility in pigs [24,108]. Authors also suggested that the complex structure and conformation of legume seed proteins would be, at least partially, responsible for their reduced digestibility, as it would make them more resistant to proteolysis [24,31]. However, advances in plant breeding have enabled the commercial release of cultivars with improved nutritional values, as well as lower contents of secondary metabolites [31]. Moreover, the use of different processing methods can reduce or eliminate ANFs, such as physical treatments (e.g. decortication, soaking), thermal treatments (e.g. extrusion, cooking) or biological methods (e.g. germination, enzyme integration) [31,110].

4.2. The use of rapeseed meal and legume grains in pig diets

Batterham et al. (1993) [111] investigated the tolerance of pigs in the growth phase between 20 and 50 kg to trypsin and chymotrypsin inhibitors contained in chickpeas (Cicer arietinum) and pigeon peas (Cajanus cajan). Gradually increasing levels of meals rich in these inhibitors were added to diets of growing pigs, in order to evaluate their influence on growth performance and internal organs. Diets containing either 250, 500 or 750 750 g kg-1 of Opal chickpea, dehulled Tyson chickpea, or dehulled pigeonpea meals were fed to pigs and compared to a wheat-soyabean meal control diet. Pigs consumed the two chickpea meals showed growth performance comparable to that of the pigs fed the control diet based on soybean meal. Conversely, pigeonpea meal-based diet decreased growth rate and feed intake, while increased feed conversion ratio. Moreover, chickpea meals had no influence on organ weights at the inclusion levels used, while pigeonpea meal influenced liver and pancreas weights, suggesting the presence of other antinutritional factors. Authors concluded that growing pigs would have a dietary tolerance of at least 4.7 and 4.5 mg/g of trypsin and chymotrypsin inhibitors, respectively, amounts that would occur with the inclusion of 750 g kg-1 of chickpea meals in the diet. According to the authors, it would not be likely that these threshold levels would be exceeded in common diets that include most legume seeds. According to the results of the study, dehulled pigeon pea meal contains other anti-nutritional factors for growing pigs.

Contrary to Batterham et al. (1993) it has been reported a lower maximum dietary tolerance level for fattening pigs of approximately 0.5 mg trypsin inhibitor/g [31].

Jansmann et al. (1993) [112] evaluated the effects of diets prepared with different field bean cultivars (300 g/kg) in piglets. Colored-flowered field bean cultivars (1.0-2.3 g condensed tannin/kg diet) showed significantly lower ileal digestibility of CP and most AAs than those without white-flowered tannin. Flis et al. (1999) [113] did not observe negative effects on growth performance of pigs (25-63 kg body weight) fed a faba bean diet having 0.59 g/kg of condensed tannins, compared to pigs fed diets having values less than 0.07 g /kg diet.

Mustafa et al. (2000) [24] conducted a study in which they compared different diets for crossbred pigs, based on barley and wheat, supplemented with SBM (control), different types of chickpeas (Kabuli and Desi, 300 g/kg) or field peas (300/g kg). There were lower dry matter and gross energy digestibility coefficients in pigs fed the Desi (Indian origin) chickpea-supplemented dieds than in pigs fed the Kabuli (Mediterranean origin) chickpea-supplemented diets. However, dry matter and gross energy digestibilities for both chickpea-supplemented diets (i.e., Desi and Kabuli types) were comparable to those of soybean- and pea-supplemented diets. Indeed, the two varieties of chickpeas differed in their nutritional composition, with the Kabuli type containing less fibre, more starch and fat compared to the Desi type. Authors hypothesized that the lower growth performance (i.e. daily gain and feed conversion) of growing pigs fed diets supplemented with chickpeas compared to those fed SBM or peas could be related to the higher fiber contents of the chickpea-based diets. Indeed,

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according to the authors, inclusion levels of the two types of chickpeas in pigs' diets were not such as to exceed the tolerance levels of trypsin and chymotrypsin inhibitors indicated in the work of Batterham et al. (1993) [111]. Crude protein digestibility did not differ for both diets containing the two types of chickpeas and those supplemented with peas, but was 9.6% lower than that of the SBM-supplemented diet. However, during the finishing period and throughout the experiment, dietary treatments did not affect pig performance, and indeed there were no significant differences in carcass traits among pigs fed diets supplemented with SBM, peas or chickpeas.

Several studies have reported that grain legume processing effectively improves starch and protein digestibility in pigs, thanks, at least in part, to a reduction in secondary plant metabolites. The inclusion of hulled lupins in pig diets improves their nutritional value by increasing feed intake and feed conversion ratio compared to whole seeds [114]. The extrusion of peas at a temperature of approximately 115 ◦C increases apparent (AID) and standardized ileal digestibilities (SID) of CP and Aas, as well as starch digestibility and energy supply in growing pigs [115]. In the same manner, improved AID and SID of CP and most AAs are reported when extruded peas (135 ◦C) are fed to weaned piglets [116]. Furthermore, the inclusion of extruded peas (130 ◦C for 30 seconds) in diets of growing- finishing pigs increase growth and feed conversion ratio compared to untreated peas [117]. A lupine-based diet supplemented with the enzyme -galactosidase increases digestibility of -galactosides, AAs, energy supply, N retention and growth performance in growing pigs [118].

Christodoulou et al. (2006) [119] evaluated the effect of replacement soybean meal with extruded chickpeas in the grower and finisher pig diets on meat quality. There were small differences in meat chemical composition among experimental treatments, i.e. control (soybean-based diet) and experimental groups fed diets containing 100, 200 and 300 kg/t of extruded chickpeas. Similarly, meat fatty acid composition, pH and color did not differ between treatments, although the control group showed a lower cooking loss. As regards sensory evaluation, slightly greater scores for tenderness and juiciness were attributed for the control group, than the chickpea treatments. The authors concluded that the replacement of soybean meal with extruded chickpeas, up to inclusion levels of 300 kg/t in isoenergetic and isoprotein diets for growing-finishing pigs, does not affect meat quality.

Jezierny et al. (2010) [31] in their review on grain legumes as a protein source in pig nutrition reported that faba beans may be used in growing and finishing pig diets up to 150 and 250 g/kg respectively, preferring white-flowered cultivars for their low tannin content. An inclusion level of up to 400 g/kg of peas in diets it is recommended for growing and finishing pigs, preferring, even for peas, the white-flowered cultivars due to the low tannin content compared to the flowered ones. The recommended inclusion level for lupins in diets for growing and finishing pigs is 200 g/kg, but only up to 150 g/kg for L. albus in diets for growing pigs (30–60 kg body weight).

A recent work by Grabež et al. (2020) [5] address the effect of replacing SBM with rapeseed meal and faba beans (RSM/FB) on growth performance, carcass quality, metabolite status and meat quality in Norwegian crossbred ([Landrace x Yorkshire] x Duroc) growing-finishing pigs. Pigs fed the RSM/FB diet did not show different growth performance compared to pigs fed SBM diet during the entire test period, except for a higher feed conversion ratio (F:G, kg/kg) during the finishing phase (2.44 vs 2.33 for RSM/FB and SBM fed pig diets respectively). According to the authors, the higher F:G in the finishing period could be related to anti-nutritional factors present in both RSM and FB. Both RSM and FB are characterized by a higher fiber content than SBM and also contain several anti-nutritional factors responsible for reduced feed use. However, carcass traits were not affected by diet [5], in agreement with other authors who had examined the effect of administering legume and rapeseed cakes to pigs [33,34,120]. However, Grabez et al. 2020 [5] reported that pigs fed RSM/FB as a replacement for SBM exhibited darker meat, that is lower L* (lightness) and b* (yellowness) parameters. However, darker meat, i.e. with lightness values (L*) lower than 48, would have higher visual ratings, without compromising meat quality [5,120]. Regarding meat fatty acid composition, RSM/FB affected the contents of individual fatty acids but not the total of SFA, MUFA and PUFA. Previously, it has been reported that the complete replacement of SBM with RSM/FB together with a reduced use of tallow in a balanced diet for digestible energy, does not influence total SFA and MUFA contents of pork muscle, although it tends to reduce PUFA [121]. Replacing SBM with RSM/FB in

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growing-finishing pigs affected metabolites extracted from pre rigor Longissimus thoracis muscle and meat after a chilled storage period (7 days at 4°C). Metabolite profile of pre rigor muscle showed reduced levels of β-alanine and glucose when feeding RSM/FB. Moreover, the reduction of oxidized metabolites (glycine and pyroglutamic acid) in the muscle of the RSM/FB pigs would indicate less oxidative stress in the muscle. Furthermore, the decrease in pyroglutamic acid would suggest efficient synthesis of glutathione and an improvement in the cell's defense mechanisms [5]. Results would be in agreement with those of Skugor et al. (2019) [29] who highlighted an increased regulation of genes active against oxidation and reactive oxygen species (ROS) in RSM-fed pigs. Grabez et al. [5] concluded that aromatic characteristics of pork from pigs fed the RSM/FB diet were desirable, concentrations of free amino acids and metabolites characterized by a sweet flavor were increased, while at the same time the reheated flavor was reduced. The results of this study therefore show a desirable quality of pork obtained from a diet containing rapeseed meal and faba beans.

Previous studies [33,34] observed that pigs fed a diet containing RSM and field beans as a replacement for SBM provided less tasty but more tender pork than pigs fed SBM-based diet.

A meta-analysis conducted by Hansen et al. (2020) [122] showed that up to 30% rapeseed meal in growing-finishing pig diets did not compromise growth performance compared to a SBM based diet, when added to a nutritionally balanced diet.

Skugor et al. (2019) [29] studied the effects of including 20% RSM in diets for growing finishing pigs, as a replacement for SBM, on growth performance carcass and meat quality characteristics. Furthermore, authors aimed to investigate diet influence on gene expression in Longissimus dorsi muscle and to identify major responsibles of phenotipic differences. Inclusion of 20% commercial expeller pressed RSM in pig diet reduced growth performance and dressing percentage of growing-finishing pigs compared to SBM control diet. However, meat quality characteristics were not affected by dietary treatment. According to the authors changes in gene expression of skeletal muscle from pigs fed RSM diet were likely due to an increased amount of fiber and polyunsaturated fatty acids, as well as bioactive compounds, such as glucosinolates. In accordance with lower growth performance, it was observed the negative action of growth regulators (IER5, KLF10, BTG2, KLF11, RETREG1, PRUNE2) in pigs of the RSM group. Furthermore, increased expression of different muscle genes (PDK4, UCP3, ESRRG and ESRRB) implicated in glucose and lipid metabolism, and mitochondrial function in pigs fed RSM were detected, suggesting a lower availability of energy and nutrients in RSM-fed pigs and intervention of well-known metabolic controllers to ensure energy homeostasis. Regarding genes regulating protein metabolism several genes were implicated in more pronounced proteolysis (ABTB1, OTUD1, PADI2, SPP1) and reduced protein synthesis (THBS1, HSF4, AP1S2) in muscle tissue of RSM pigs. Moreover, authors observed increased expression of genes regulating lipolysis, fatty acid oxidation (greater levels of NR4A3, PDK4 and FGF21, and a drop in adropin, ELOVL6 and CIDEC/FSP27) and oxidative stress (GPX1, GPX2, and TXNIP) in muscle of pigs fed RSM. The study of gene expressions deepens knowledge of the molecular mechanisms underlying phenotypic observations.

Chen et al. (2018) [30] carried out a 3-week study in young pigs (17.8 ± 2.7 kg initial BW) to compare a soybean meal-based control diet with a rapeseed-based diet (200 g/kg as fed basis). The metabolic effects of the two diets were examined by analyzing digesta, liver and serum samples from these animals. Reduced apparent ileal digestibility of AAs was observed in pigs consumed RBM diet. However, although the RSM diet had a higher fiber content, microbial fermentation products in digesta were not affected, i.e. short-chain fatty acids and secondary bile acids. Increased contents of oxidized metabolites (e.g. oxidized glutathione) and aldehydes and decreased levels of ascorbic acid and lipids containing docosahexaenoic acid were found in the liver and serum of RSM-fed pigs, highlighting an alteration of the redox balance in these young pigs. Contrary to what was observed by Grabež et al. [5], Chen et al. [30] reported that the metabolic markers of oxidative stress (pyroglutamic acid and butanal) were more expressed in the liver of pigs fed RSM diet. Processing interventions are recommended in order to increase the use of rapeseed feed ingredients in pig diets.

Zmudzińska et al. (2020) [4] evaluated the effect of total dietary replacement of SBM with legume grains (pea and yellow lupin) and RSM on growth performance and meat quality of both sexes

DanBred hybrid piglets. There was no interaction between sex and diet. Replacing SBM with legume grains and RSM in growing-finishing pig diet reduced final body weights. Indeed, dietary treatment decreased daily weight gain in the period between 35-83 days and throughout the whole rearing period (0-83 days). The experimental factors did not influence most of the meat quality characteristics. Pigs fed legume grains and RSM showed lower fatness than those fed SBM. The authors concluded that including peas, yellow lupins and RSM in pig diets does not affect meat quality, although growth performance may be impaired.

He et al. (2023) [28] conducted a study with the aim of determining the effects of replacing SBM with different plant protein sources (rapeseed meal, cottonseed meal and sunflower seed meal) on growth, apparent nutrient digestibility, serum parameters, including free amino acids, intestinal microbiota of growing pigs (Duroc × Landrace × Yorkshire) weighing 50 to 75 kg. The threemeal mixture was added in one of the three experimental groups (corn– soybean–various meal group, CSM) in a 1:1:1 ratio and as a partial replacement of 10.99% of SBM compared to the control group (corn– soybean meal group, CON). Pigs in the CM group (corn -various meal group, CM) received a diet in which the threemeal mixture (7.69% rapeseed meal, 7.69% cottonseed meal, 7.68% sunflower seed meal) totally replaced SBM. Dietary treatments did not influence average daily gain, average daily feed intake or feed-to-gain ratio of growing pigs weighing between 50 and 75 kg. Same results were obtained for crude protein, crude fat or gross energy of the three experimental diets. As regards serum parameters the CM group showed increased values of alanine aminotransferase (ALT) and triglyceride (TG), but reduced urea values. Serum free amino acids were not affected by dietary treatment. Regarding composition and diversity of fecal microbiota the CM group showed a decrease in Euryachaeota abundance at the phylum level compared to the CON group, suggesting improved intestinal crude fiber-digesting bacterial flora. Results would be indicative of useful use of miscellaneous meals (rapeseed meal, cottonseed meal, and sunflower seed meal) as potential alternative feed ingredients to SBM in swine diets.

5. Conclusions

Microalgae are currently relatively expensive to produce on a large scale compared to other animal feeds. Research focuses on their use as diet supplements, primarily to influence gut health. When used as protein ingredients in post-weaning piglet diets, at an inclusion level of up to 10%, growth performances are decreased, even if meat quality is not negatively affected. This decrease in pig growth performance is linked to the gelation and therefore low digestibility of Spirulina proteins in the gut, due to the resistance of their cell wall carbohydrates to digestion. Furthermore, other factors that contribute to the limited use of Spirulina in animal feed are palatability, dried powdery form and odor.

From the literature consulted, it emerges that insect larvae, i.e. *Hermetia illucens* and *Tenebrio molitor*, can be used in growing pig diets up to an inclusion level of 10%, without negative effects on growth performance and nutrient digestibility. Results therefore confirm insect larvae as potential sources of sustainable protein in pig feeding. However, for their large-scale use, the problems of safety, high non-competitive production costs, as well as acceptability by consumers should be addressed. Studies, similarly to those on laying hens or broiler chickens, are exploring their potential added value (as a feed additive and not an alternative protein source) in pig feeding, i.e. that of regulating intestinal microbiota and microbial metabolism products, probably through the effects of chitin, lauric acid and/or antimicrobial peptides, especially during the post-weaning period.

Locally produced protein sources, such as rapeseed meal and grain legumes, used in combination with cereals and taking care not to exceed the anti-nutritional factor threshold values, can be included in diets of growing-finishing pigs, without any negative effect on growth performance and meat quality. The use of these ingredients can contribute to greater sustainability of pig farming in Europe, as well as a reduction in feeding costs. Furtheremore, the organic farming sector, i.e. in general GMO free, would particularly benefit from these alternative protein sources, given that the majority of soybean imported from American is genetically modified.

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