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Review

Will Yellow Mealworm Become a Source of Safe Proteins for Europe?

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Received: 27 April 2020; Accepted: 16 June 2020; Published: 17 June 2020

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Abstract: Continued population growth could lead to protein deficiency in the human diet. To counteract this risk, attempts are being made to identify new edible sources of protein. The aim of this paper was to review the existing literature and to analyse the current state of yellow mealworm (*Tenebrio molitor*) rearing for food and feed, especially in Europe. The yellow mealworm is the most widely bred and traded insect species in Europe that has high feed conversion ratio; 3.4 to 6.1 kg of feed ingested per kg of harvested larvae. Mealworms could compete with livestock due to their high protein and fat content and low environmental impact. Mealworms have been extensively researched as a source of feed for animals, including poultry, fish, pets and birds. Its nutrient content depends on the processing method, where thermal processing is least desirable. Mealworms are characterised by a high and variable microbial load which has to be reduced before consumption. The antibiotics, pesticides and other substances should also be analysed to ensure that mealworms are a safe protein source for human consumption. The nutritional benefits of mealworms have to be communicated to European consumers who are generally averse to eating insects.

Keywords: *Tenebrio molitor*; food and feed; safety aspects; nutritional value; insects rearing; consumer attitudes

1. Introduction

According to the Food and Agriculture Organization (FAO), the number of undernourished people in the world increased from 783.7 million in 2014 to 804.2 million in 2016 and 820.8 million in 2017 [1]. Despite the above, approximately one-third of globally produced food is lost or wasted in different stages of the supply chain [2]. It is estimated that the global population will reach around 9.7 billion by 2050, and the demand for food will more than double [3].

In view of climate change and the limited availability of food resources, edible insects offer a highly nutritional alternative for feed and food production [1,4,5]. The kingdom Insecta is one of the largest taxonomic classes containing more than 1 million of known [6] but underexploited species [7].

The postulate to replace popular sources of livestock feed, such as grains, soymeal or fishmeal, with alternative sources was made to reduce the area under agricultural crops and to improve the efficiency with which plant proteins are converted by animals with reduced losses of nutrients and other valuable components [8,9]. The use of insects as feed and food can help reduce the present and future risk of hunger in the world [10]. According to some authors, insects represent mankind's "last great hope of saving the planet" from hunger and poverty [11].

The interest in insects as sources of feed and food continues to increase, and considerable research has been done into edible insects [12–14] due to their promising characteristics [13,15,16]. The consumption of insects, also referred to as entomophagy, has been known since the ancient times [17], and it exists in the contemporary food culture, mostly in the tropics [18]. According to estimates, 1900 to 2111 insect species in various developmental stages are consumed by around 2 billion people worldwide [15,17,19]. Around 679 insect species are consumed in the Americas, and the main insect-consuming countries are found in Central and South America (549 species in Mexico alone) [12]. Insects are also widely consumed in Africa (524 species), Asia (349 species) and Australia (152 species). In contrast, only 41 insect species are used as food in Europe [20].

Animal proteins are superior to plant proteins [21]. Michaelsen et al. [22] demonstrated that even if consumed in small amounts, insects can contribute to the recommended intake of protein and micronutrients in the diets of malnourished children.

According to estimates, fish feeds account for around 50% of production costs in aquaculture. The prices of some feed ingredients rose from 20% to 92% between June 2007 and June 2008 [23], which increased the prices of fish and fish products, including fish meal for livestock production.

Insect farming is not only a source of high quality protein [24–26], but it also delivers environmental benefits [21]. Insect production decreases pollution [14,27,28] and energy consumption [29,30], and it contributes to food security [15,31].

The yellow mealworm (*Tenebrio molitor* L. 1758—Coleoptera: Tenebrionidae) is the most widely bred and traded insect species in Europe [15,32,33] due to its rapid growth, minimum breeding requirements and ease of handling [34,35]. This omnivorous species is a common pest in flour, grains and food [35,36] and it consumes animal products such as feathers or meat [36]. Insects are sold alive, dried or in powdered and canned form for livestock, pets and fish [37]. The life cycle of yellow mealworms ranges between 280 and 630 days [8], depending on ambient temperature, photoperiod, relative humidity and other factors [38].

Insects can convert organic side-streams [39,40], which delivers economic profits by reducing the amount of generated waste and minimizing environmental pollution [14]. Insects bioconvert waste into high-quality products [39], and they can be used as safe feedstock with the minimal involvement of other resources [8,36]. Yellow mealworm can successfully replace fish or soymeal for livestock or fisheries [8,41,42]. The assimilation of plant proteins by carnivorous fish species can be problematic, but the meal and oil from *Tenebrio molitor* larvae can be an effective and nutrient-dense feed resource. Mealworms can also be used to supplement the diets of poultry and other domestic birds [41].

Insects are widely used as feed and food in Asia, Africa and the Americas, but entomophagy is not widespread in Europe. However, insects have attracted a growing interest as sources of food and feed in the past decade, as demonstrated by this review. A noticeable interest in this nutritional source has been reported since 2015 when insects became regarded as a novel food in the European Union [43]. Therefore, the aim of this paper was to analyse the progress and directions of research on yellow mealworm, one of the most common reared insect for feed and food in Europe.

2. Materials and Methods/Data Collection and Selection

The reviewed articles are original research studies, reviews and observational studies published in English. This review focuses mostly on European papers to evaluate the present state of insect farming and prospects for the production of selected species of edible insects. Scopus and Science Direct data bases were searched to select articles published between 2012 and 2020 based on the following key words: yellow mealworm, Tenebrio, *Tenebrio molitor*, mealworm and mealworms. These keywords were used separately to identify original papers and review articles for the current review.

The reviewed papers were divided into several categories based on different types of mealworm utilisation as well as fields of research, including ecology, immunity, influence of various substances on mealworms as model organisms, microbial load, processing methods, and separation of various components and nutrients. The exclusion criteria for the selected articles included duplication of data, such as review papers or focus on other mealworm species such as lesser/buffalo mealworm (*Alphitobius diaperinus*) or giant mealworm (*Zophobas morio*). The articles were collected and sorted according to the country of origin, the number of articles per country, the year of publication, and the most important keywords.

3. Results and Discussion

3.1. Progress in Research on Yellow Mealworms in Europe

A total of 291 articles by European researchers were chosen from the analysed databases. After a thorough analysis, 201 articles were included in the review (Figure 1). The selected papers were classified based on the year of publication. The analysis demonstrated that the number of research studies into mealworms had increased considerably since 2015, which could be attributed to the growing interest in mealworms in the scientific community. Other contributing factors include the opinion of the European Food Safety Authority (EFSA) of 8 October, 2015, on the risk profile related to the production and consumption of insects as food and feed, Regulation 2283/2015 of the European Commission on novel foods, and Regulation 2017/893 of the European Commission authorising the production of animal protein from insects, which came into effect in 2017 [43,44]. Regulation 2283/2015 lays down rules for the placing of novel foods on the market within the EU and replaces older regulations (e.g., No 258/97). The above regulation defines novel foods as any food that was not used for human consumption to a significant degree within the Union before 15 May 1997. The regulation expands the existing categories of food and, most importantly, classifies "whole insects and their parts" as novel foods. Further progress was achieved two years later when Regulation 2017/893 authorised the use proteins derived from the black soldier fly (*Hermetia illucens*), common housefly (*Musca domestica*), lesser mealworm (*Alphitobius diaperinus*), house cricket (*Acheta domesticus*), banded cricket (*Gryllodes sigillatus*), field cricket (*Gryllus assimilis*) and yellow mealworm (*Tenebrio molitor*). However, the above insect species can be fed only with plant-based materials or with a limited number of materials of animal origin.

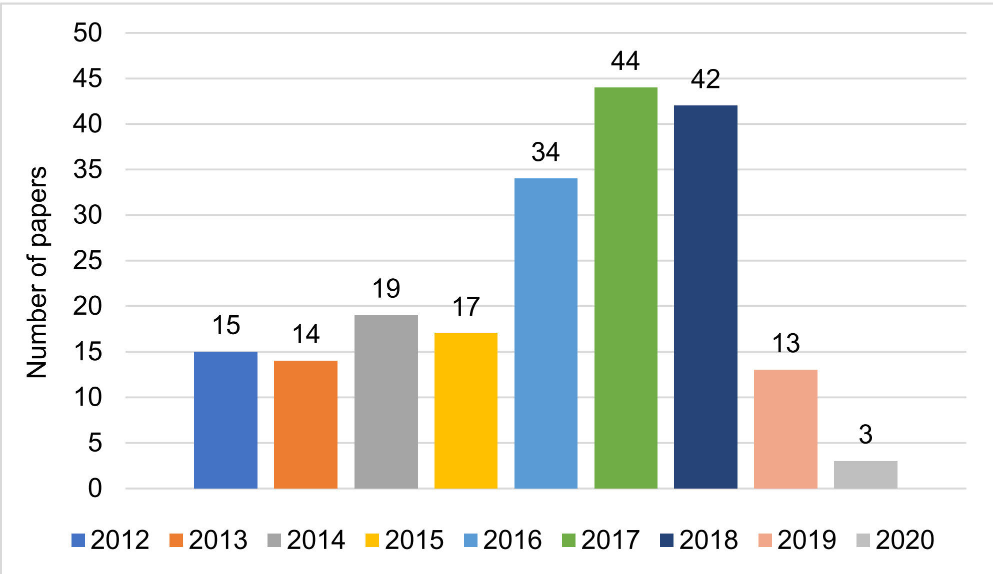

Figure 1. Number of research papers on mealworms published between 2012 and 2020 in Europe.

The reviewed articles were sorted based on the affiliation of the first author and his/her country of origin. If first author had more affiliations from different countries, the paper was regarded as originating from the first affiliation country. The number of research papers on mealworms published in different European countries between 2012 and 2020 is presented in Figure 2. The highest number of papers was published in Italy (35 papers between 2012 and 2020), followed by the Netherlands (30 papers), Belgium (21), UK (18), Poland (18), Germany (15), Czech Republic (12) and France (12). Some articles addressed food neophobia as an obstacle to the consumption of mealworms as a source of protein. The mealworm beetle was used as a model organism in some immunoecological studies, and its behaviour was investigated at different levels in various experiments. Most of the reviewed articles explored the use of mealworms as feed for livestock [8,37,41,45–47], poultry [48–55], fish [42,56–61], small birds (barbary partridge, quail, pheasants) [62–66] and pets [67–73]. Other studies analysed the extraction of mealworm proteins, oil and other components for medical and other purposes.

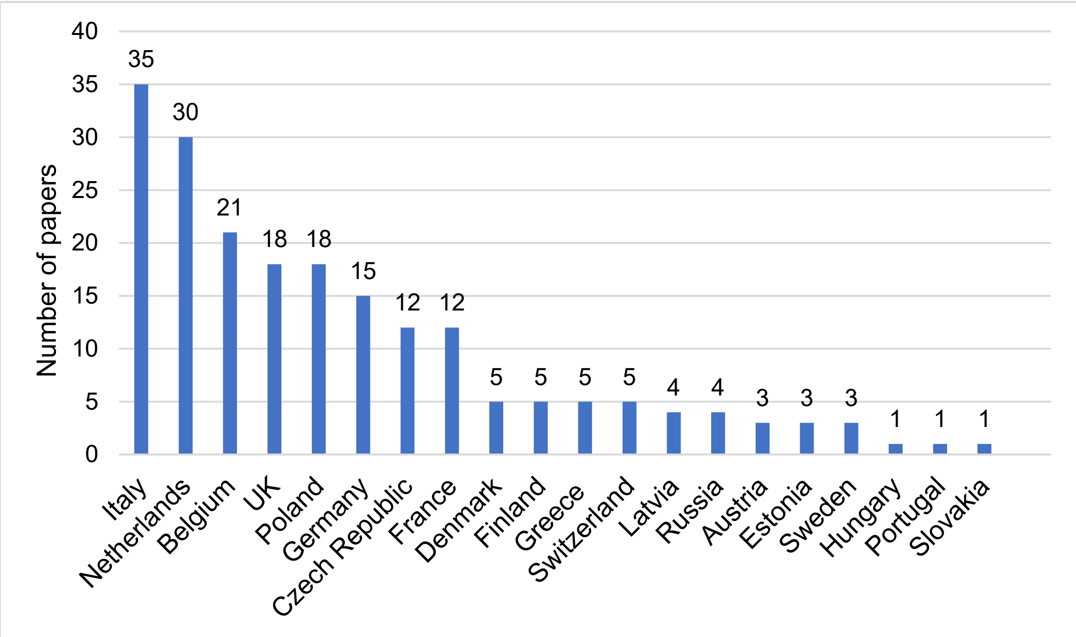

Figure 2. The number of research papers on the yellow mealworm published in different European countries between 2012 and 2020.

3.2. *Tenebrio Molitor Development*

Yellow mealworms can convert low-energy feeds, substrates and biomass into products with high nutrient content [36,74]. Research has shown that mealworms are able to transform organic by-products from brewing, baking, bioethanol production and potato processing into nutritious feed for livestock and other components [40].

The optimal rearing conditions for *T. molitor* larvae that guarantee successful mass production have been described by numerous studies. Mealworms thrive at a temperature of 25–28 °C and relative humidity (RH) of ≥70% (the optimal RH range is 60–75%). Optimal growth is achieved when mealworm diets are abundant in yeasts (5–10%), carbohydrates (80–85%) and B-complex vitamins [35]. The composition of mealworm diets is crucial for obtaining high-quality larvae characterised by a high feed conversion ratio (FCR) and high body weight. Various diets have been analysed to determine their impact on the growth and nutritional value of larvae. The protein and starch content of some of the tested diets affected the growth, survival and chemical composition of mealworms. Van Broekhoven et al. [40] observed that high protein and high starch (HPHS) diets considerably accelerated larval development (79 days). High protein low starch (HPLS) diets were also found to improve larval survival rates. The feed conversion ratio ranged from 2.62 to 6.05 on a fresh weight basis. The crude protein content of mealworms was highly similar in all tested diets (between 45.1–48.6% dry matter (DM)). The crude fat content of mealworms ranged from 18.9% DM in insects fed LPLS (low protein, low starch) diets to 27.6% DM in insects fed HPLS (high protein, low starch) diets [40].

Mealworm larvae are able to synthesise fat even if they are fed low-fat diets. Therefore, the final fat content of larvae fed low-fat diets is highly similar to that of larvae whose diets contain more fat. In a study where mealworms were fed diets with a fat content of 0.46–9.34% DM, the crude fat content of mealworm larvae was determined at 39.75–48.31% DM in the first generation and 34.42–48.17% DM in the second generation [75]. Yellow mealworms are less heat-adapted insects [76], and rearing temperature influences their fat content. The fat content of mealworms reached 47.4% at 31 °C, and it decreased to 30.2% at 37 °C and to 16% at 39 °C [76]. Adámková et al. [77] reported that fat content was maximised at a temperature of 23 °C (24.56%). An analysis of the effects of rearing temperature on the protein content of mealworms revealed that this parameter increases with a rise in temperature [76].

Several studies have analysed the concentrations of digestive peptidases and specific serine peptidases that play an important role in the regulation of digestive processes, as well as their location within the gut of mealworm larvae and adaptation of specific digestive enzymes to convert feed more efficiently [78,79].

Selected European studies investigating the effect of environmental factors, such as temperature and feed quality, on mealworm growth, and studies analysing mealworm physiology and immunity are presented in Table 1.

Table 1. Selected European studies on mealworm growth, physiology and immunity.

Research Topic	Topic
Mealworm development	Review of the optimal rearing conditions [35]. Rearing of mealworms for use in various by-products (beer brewing, baking/cookies, potato processing and bioethanol production) [40]. Possibility of modifying the fat and fatty acid composition of mealworm by feeding different substrates (ground oat flour, corn flour, wheat flour, chickpea flour, bread and beer yeasts) [76]. Different temperature rates influence larval metabolism, growth rate, efficiency and macronutrient composition [76]. The influence of processing temperature on the fat content of mealworm [77] An analysis of the mealworm digestive system (role of peptidases in digestion) [78]. Isolation and characterization of proline-specific serine peptidase from the anterior midgut of mealworm larvae [79].
Physiology	Use of mealworm beetle as a model species (reproductive characteristics, survival and three components of innate immunity) [80]. Mealworm beetle as a biological model for analysing the effect of lifetime dietary supplementation with astaxanthin (antioxidant) when exposed to early life inflammation [81]. Immune responses of mealworm beetle to the microbial activity of *Staphylococcus aureus* [82,83]. Correlation between cuticle melanism, immune defence and life-history traits [84]. Impacts of adult density, reproduction period and age on the fecundity of mealworm beetles [85]. Influence of inbreeding on the attractiveness of sexual signalling in TM beetle [86]. The applicability of X-ray microcomputed tomography (μCT) based methods for investigating the insect tracheal system at different stages of development [87,88]. Analysis of hiding behaviour and anti-predator responses of TM beetle exposed to a predator [89–91].
Immunity	Testing the mealworm beetle terminal investment hypothesis [92,93]. Correlations between melanism, immune defence by beetle traits at different temperatures and in different sexes [84]. Morphofunctional organization of extrachromosomal nuclear structures in insects [94]. Trans-generational immune priming [95,96]. RNAseq analysis of the temporal dynamics of insect immune responses (TM was the model insect) [97]. Antimicrobial/antifungal immune responses [98–100]. Endogenous egg and beetle immune responses [101]. Senescence in immune priming and attractiveness of beetles [102]. Influence of immune challenge [93,103].

3.3. Mealworm Physiology and Immunity

The mealworm beetle has been used as a model species in many immunoecological [80–82] and genetic studies [94]. Dhinaut et al. [95,96] studied the mechanism by which maternal immune effectors are transferred to the egg and found that egg immunity is largely dependent on maternal immunity. *Tenebrio molitor* larvae have also been researched to determine their resistance to bacterial infections and the expression of immune response genes [97,98]. Many immune genes are induced in both adults and eggs. A strong innate immunity response was reported in the eggs laid by mealworm beetles [101]. Another study revealed that the immune system is more effective in young than in older males [102]. Immune investment in males was also highly dependent on food availability. When food is limited, the male invests more in its attractiveness for females than in its immune response and survival. Males with abundant access to food invest more in their survival and do not decrease their lifespan [92].

Prokkola et al. [84] studied the immune defences, melanism (colour), body size and individual development of male and female mealworms exposed to different temperatures. A positive correlation between the above traits was noted at the lowest temperature (18 °C). In another study, the immune response of mealworm beetles was induced with nylon monofilament implants, and cuticle darkness was assessed. Females had darker cuticle than males, which suggests that females invest more in immune defence than males [103].

Berggreen et al. [85] conducted two experiments to determine the influence of beetle density and the length of the reproductive period on fecundity. The production of larvae was maximised by the highest beetle density (0.84 beetles cm^{-2}) and the longest reproductive period (6 days). Despite the above, the authors recommended a shorter reproductive period (2–4 days) due to considerable differences in the size of larvae obtained during a long reproductive period.

Nielsen and Holman [93] studied the production of pheromones in immune-challenged mealworm beetles and found that investment in pheromones can compromise survival in males. Males that were not affected by the immune challenge were more attractive to females, which resulted in a higher mating rate.

Pölkki et al. [86] tested the preferences for odours of inbreed and outbreed (control) mealworm beetles of both sexes. Females were more attracted to the odours produced by outbred males then those produced by inbred males, which suggests that inbreeding can reduce the attractiveness of male sexual signalling. Males did not discriminate between the odours produced by both female batches, which could suggest that quality of females is irrelevant for males or that odour is not an indicator of female quality.

The respiratory tract of mealworm beetles was investigated by Iwan et al. [87] and Raś et al. [88] to analyse the relationship of the tracheal system and other tissues. Moreover, [87] developed the first three-dimensional visualisation of the respiratory tract in the diverse order Coleoptera.

Maistrou et al. [99] analysed an antifungal peptide protecting *T. molitor* beetles against infections by the entomopathogenic fungus *Beauveria bassiana* and the progression of the infection in the host. They concluded that Tenecin 3 exerted direct antifungal effects on *B. bassiana* and protected beetles against the infection. Krams et al. [100] studied antifungal protection mechanisms in mealworm beetles. They found that survival rates were higher in beetles that were immune-challenged with nylon implants after fungal exposure than in beetles exposed to the fungal pathogen only. The implants were regarded as broad spectrum "immune priming" which enabled beetles to fight not only the same intruder but also other parasites.

3.4. Sustainability of Mealworm Rearing

Insect rearing offers a viable solution to global problems such as environmental degradation, waste management, food loss, hunger and deforestation [28]. The life cycle of mealworms for human consumption was assessed in Finland by Joensuu and Silvenius [104] who investigated the global warming potential (GWP) of industrial-scale mealworm farming. They found that crop production and direct heating energy were responsible for around 95% of total GWP. Despite the above, the environmental impact of mealworm farming was still lower relative to pork, lamb and beef production. Thévenot et al. [45] performed a cradle-to-gate life cycle assessment of *T. molitor* and found that the environmental impact of 1 kg of insect meal in five impact categories (cumulative energy, climate change, land use, acidification, and eutrophication potential) was higher in comparison with soybean or fish meal. The authors concluded that the use of mealworm meal as animal feed did not decrease the environmental impacts of livestock.

The water footprint of mealworm meal was estimated at 23 dm^3 of water per gram of mealworm protein, and it was lower than that of chicken protein (34 dm^3 g^{-1}), pork protein (57 dm^3 g^{-1}), and beef protein (112 dm^3 g^{-1}) [105]. Oonincx and De Boer [27] compared the environmental impacts of mealworm production and the production of other sources of protein such as milk, pork, chicken and beef. The energy inputs associated with mealworm production were higher than for milk and chicken and similar to pork and beef. Despite the above, mealworms produced far less greenhouse gases and required far less land, which suggests that they are a more sustainable source of protein than other animal products.

3.5. Mealworm Uses in Europe

3.5.1. Mealworms as the Sustainable Food of the Future

The nutrient content of *Tenebrio molitor* larvae determines the extent to which mealworms can be regarded as a novel food for combating global malnutrition [10]. Edible insects are a valuable source of protein, and their lipid, fat, mineral and vitamin content varies across growth stages, and is influenced by processing [31,106].

Despite numerous studies emphasizing the nutritional benefits of mealworms [26,107], some researchers are of the opinion that the protein content of insects has been overestimated due to the applied methods of protein extraction, and that some proteins originate from the fibre in insect cuticles that cannot be digested by humans or livestock [108].

Fresh mealworm larvae are abundant in protein (17.2%), and they can easily compete with raw livestock meat (Table 2). Lean beef and veal are livestock meats with the highest protein content (22.3% and 21.3%, respectively), but the quantity of feed required to produce the same amount of protein is much higher in livestock (10 kg and 5.4 kg of feed per kg of live weight in beef and pork, respectively) than in mealworms (3.4–6.1 kg of feed per kg of live weight), subject to feed type. Fish have a lower feed conversion ratio than mealworms, but insects' ability to convert low-quality feed offers a potential solution to the depletion of marine fishery resources. Mealworm larvae have high fat content (21.3% in raw and 42.48% in powdered mealworms), which could be a certain disadvantage. In the reviewed studies, mealworms were compared only with the lean cuts of other meat types, where fat content ranged from 0.6% in fish to 22.7% in lamb on a fresh matter basis (Table 2). Mealworm larvae contain mostly unsaturated fatty acids that deliver health benefits for humans. The types of fat in mealworm larvae will be discussed in subsequent parts of the article.

The protein content of mealworms varies subject to feed and rearing conditions. According to Marono et al. [113] protein content was highest (51–59%) in commercial mealworm samples, and differences were observed between processing methods. In a study by Kulma et al. [114], the crude protein content of mealworm larvae was determined at 63% DM, and it was much higher than that reported by other authors [24,75]. The energy content of mealworm larvae was estimated at 444 kcal per 100 g [24].

Table 2. Nutritional value of conventional meat and yellow mealworm (*Tenebrio molitor*) (on a fresh matter basis).

Source	Water (%)	Protein (%)	Fat (%)	Ash (%)	Feed Conversion Ratio (kg Feed per kg of Live Weight)	References
Beef (lean)	75.0	22.3	1.8	1.2	10.0	[109,110]
Veal (lean)	76.4	21.3	0.8	1.2	-	[109]
Pork (lean)	75.1	22.8	1.2	1.0	5.4	[109,110]
Lamb, raw (unspecified part)	60		22.7	1	-	[111]
Goat, lean, raw	74.6	19.5	4.3	1	-	[111]
Chicken	75.0	22.8	0.9	1.2	2.5	[109,110]
Fish (cod fillet, raw)	81.6	17	0.6	1.2	1.5 (for carp)	[110,111]
Mealworm (fresh larvae)	56.27	17.92	21.93	1.55	3.4–6.1	[74,112]
Mealworm (powdered larvae)	2.43	44.72	42.48	3.69		

The nutritional composition of mealworms in different life stages and processed forms, including raw/fresh, freeze-dried and fried, is presented in Table 3. Protein content ranged from 17% [74] to 63% [115], depending on factors such as moisture content. Fat content was lower (18.23%) in the study by Zielińska et al. [24] and higher in the work of Caparros Megido et al. [116] (40.9% in raw mealworms, 64.9% in fried mealworms). The ash content of mealworms ranged from 1.55% to 4.9%.

Table 3. The nutritional composition of mealworms in the reviewed studies.

Life Stage/Processed Form	Protein Content (%)	Fat Content (%)	Ash Content (%)	Dry Matter Content (%)	Comments	References
Raw mealworms	43	40.9	3.4	85	Dry matter basis	[116]
Mealworm larvae	52.35	18.23	4.74		Dry matter basis	[24]
Fresh larvae	17.92	21.93	1.55		Dry matter basis (different types of diet)	[74]
Fresh larvae	44.1–53.6	22.6–34.5		30.2–41.5	Dry matter basis (different types of diet)	[74]
Freeze-dried larvae	51.5	32.9	4.9	96.1	Dry matter basis. Larvae were not starved before the experiment	[117]
Fresh larvae	63.93	n.d.	4.37	37.55	Mean value of 3 repetitions	[115]
Fried * mealworms	43	64.9	2.2	63.5	Dry matter basis	[116]

n.d. = not determined, * frozen mealworms were pan-fried for 1 min in 15 mL of olive oil (preheated for 1 min, and dried on a paper towel).

The mean amino acid content of mealworms determined in two studies [34,118] is presented in Figure 3. Mealworm larvae contain nearly all types of amino acids, especially essential amino acids that cannot be synthesised by the human body and have to be obtained from food. On average, mealworm larvae contained 364.1 g kg^{-1} DM of non-essential amino acids and 198.6 g kg^{-1} DM of essential amino acids. Mealworms were also abundant in four purine compounds (9.12 g kg^{-1} DM) that play an important role in the diets of hyperuricemia and gout patient where high-protein and high-purine foods have to be replaced with low-purine products [118].

Mealworms are also a source of fatty acids that play an important role in the human diet. The concentrations of different fatty acids, including saturated fatty acids (SFAs), monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs) in mealworms are presented in Table 4. Myristic, palmitic and stearic acids increasing cholesterol levels in the blood. Lauric acid contributes to the formation of immune cells, palmitoleic acid promotes insulin sensitivity, arachidonic acid reduces inflammation, and alpha-linolenic acid is essential for the proper functioning of the central nervous system.

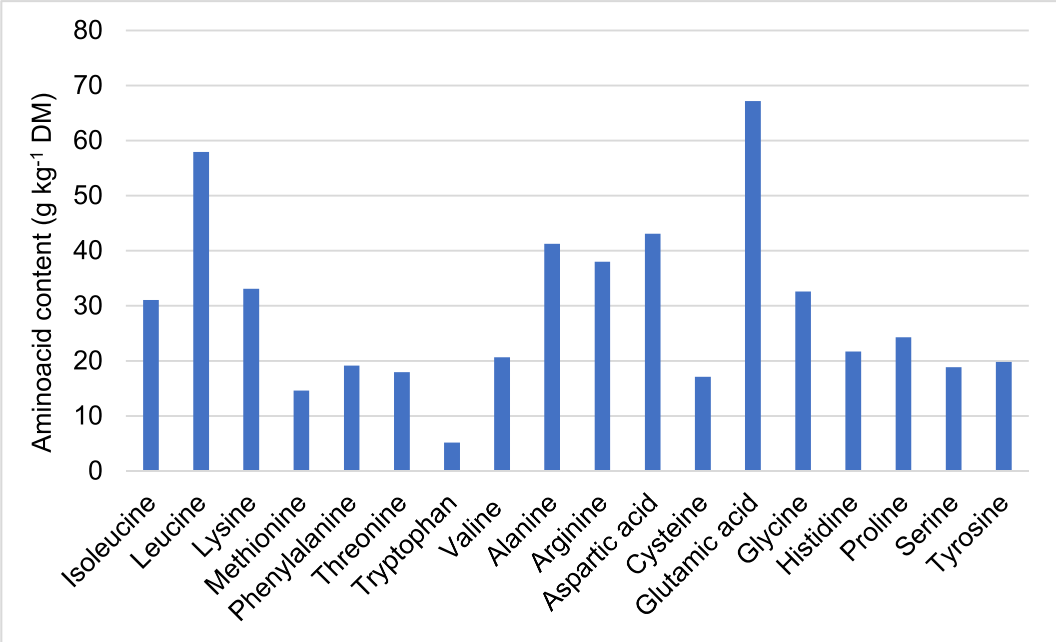

Figure 3. Content of essential and non-essential amino acid in mealworm larvae (g kg^{-1} DM). Source: [34] and [118].

Mealworm larvae can be recommended for the contemporary human diet due to low levels of SFAs, high PUFA content, and a low n-6/n-3 PUFA ratio [41,75].

In a study by Adámková et al. [119], the total content of SFAs in mealworm fat was determined at 29.7%. Mealworms were more abundant in total MUFAs (38.4%) than in total PUFAs (31.8%). The concentrations of SFAs (25.32%) and PUFAs (31.37%) were lower, but the content of MUFAs was higher (43.27%) in the mealworms analysed by Zielińska et al. [24]. The fat content of *T. molitor* was estimated at 32% on a dry matter basis by Paul et al. [120]. According to the authors, mealworm diets should be supplemented with products rich in n-3 fatty acids to reduce the n-6/n-3 ratio. In a study by Zielińska et al. [24], the n-6/n-3 PUFA ratio was determined at only 18.44.

Table 4. Main fatty acid composition of mealworms.

Fatty Acid	C:D	% of Total Fatty Acids
SFAs		
Lauric acid	C12:0	0.3–0.38
Myristic acid	C14:0	3.19–5.5
Palmitic acid	C16:0	15.5–21.33
Stearic acid	C18:0	2.72–7.92
Arachidic acid	C20:0	0.16
MUFAs and PUFAs		
Palmitoleic acid	C16:1	1.4–2.88
Oleic	C18:1	35.83–49.5
Linoleic acid	C18:2	16.3–25.4
Linolenic acid	C18:3	0.2–0.8

C:D—total amount of (C)arbon atoms of the fatty acid, and the number of (D)ouble (unsaturated) bonds; SFA—saturated fatty acids; MUFA—Monounsaturated fatty acids, PUFA—Poliunsaturated fatty acids. Source: [40,74,120].

Francardi et al. [121] analysed linseed flour as a potential feed source for reducing the n-6/n-3 PUFA ratio of mealworm. Linseed oil is known to promote cardiovascular health. The n-6/n-3 PUFA ratio was significantly lower (4.05–6.38) in mealworms fed linseed-enriched diets than in mealworms that were not administered linseed flour (34.68–40.53).

Fatty acids influence blood cholesterol levels and play a role in cardiovascular disease. In studies analysing sterol (cholesterol and phytosterol) levels in *T. molitor* larvae, the concentration of free cholesterol ranged from 0.41 to 0.59 g 100 g^{-1} in lipids extracted by industrial (Soxhlet) and laboratory (Folch) methods [122,123]. In humans and animals, sterols play an important role in the formation of cell walls, hormone synthesis, and the production of antibodies. Phytosterols are sterols of plant origin which can decrease cholesterol absorption in the intestinal tract. Mealworms are abundant in phytosterols, and they could be helpful in reducing cholesterol absorption [123].

Mealworms contain peptides with anti-inflammatory and antioxidant properties, which can reduce oxidative stress in human cells. The highest levels of antioxidant activity were noted in hydrolysates obtained from raw mealworms [24].

The elemental composition of mealworms differs in various stages of growth. Third-instar larvae (6.8 ± 1.9 mm) are characterised by the highest content of Ca (736 mg kg^{-1}), Fe (250 mg kg^{-1}), K (14.6 g kg^{-1}), Mn (18.9 mg kg^{-1}), Na (2.1 mg kg^{-1}), S (9.1 g kg^{-1}), Sr (8.8 mg kg^{-1}) and Zn (171 mg kg^{-1}) [124]. The highest P level (65 g kg^{-1}) was reported in eight-instar larvae (7.8 ± 1.3 mm), whereas last-instar larvae (20.4 ± 1.2 mm) were most abundant in Mg (2.0 g kg^{-1}). The concentration of Cu (14.4 mg kg^{-1}) was higher in first instar and much higher in pupae or adults [124]. Therefore, the decision to add mealworms to feed should be made based on the desired outcomes of supplementation. Fresh and powdered *T. molitor* also differ in mineral content. Powdered mealworms are more abundant in minerals than fresh larvae [112].

Latunde-Dada et al. [125] demonstrated that mealworms can be a more available source of soluble minerals than beef. Mineral solubility was always higher in mealworm larvae, even in larval samples that were less abundant in minerals than sirloin.

Mealworm larvae are also a rich source of vitamin D. Oonincx et al. [126] found that mealworms synthesise vitamin D under exposure to ultraviolet (UVb) light. Prolonged UVBb exposure increased vitamin D_2 and vitamin D_3 synthesis up to a threshold level of 6400 UI kg^{-1}. The study also demonstrated that vitamin D synthesis increased in response to higher UVb irradiance.

3.5.2. Nutrient Extraction

Various methods for extracting mealworm proteins, lipids and other nutrients have been proposed in the literature. Purschke et al. [127] analysed protein recovery in response to different extraction parameters, including solubilisation pH and centrifugation speed time. They found that centrifugation speed and solubilisation pH were the key parameters that influenced protein content and total protein recovery, respectively. Bußler et al. [128] extracted around 20% of crude fat and 53.8% of crude protein from insect meal. Defatting reduced the content of crude fat and crude protein to 68%. Protein solubility was optimised by modifying the pH and temperature of the extraction solvent. Yi et al. [129] found that higher extraction pH and the application of NaCl increased the recovery of soluble protein from mealworm larvae from 23% to 100%.

Proteins were extracted using different concentrations of the NaOH solution. The total protein content of the extract was around 75% at an extraction rate of 70%. The alkaline extraction method is easy to use and produces a satisfactory yield. Extraction parameters (temperature, sample concentration, use of enzymes or salts, low pH) can be modified to obtain mealworm protein extracts suitable for various food applications [117].

Sipponen et al. [7] studied the dry fractionation technology for upgrading yellow mealworm larvae by extraction with super-critical carbon dioxide (SC-CO_2), followed by separation to fine and coarse fraction by air classification, and its effect on crude protein and lipid content. The total lipid content of the crude mealworm extract was 35.6% on a dry matter basis. Defatting increased crude protein content from 40 to 56%. Proteins were then partially fractionated by air classification to protein-enriched fractions containing less chitin. Significant differences were noted between the fine and coarse fraction, where the fine fraction of mealworms had a more meat-like flavour than the coarse fraction.

Mealworm components (proteins, oil, fatty acids, minerals) can be incorporated in the diet as supplements or additives to eliminate the discomfort associated with the consumption of whole insects. A number of processes have been developed to add insect protein extracts to emulsion formulations. Mealworm proteins show considerable promise as food emulsifiers [130,131] and gelling agents [132].

Deproteinated (98.5%) and demineralised (97.9%) solid mealworm fractions can be used to extract chitin after microbial fermentation. Chitosan, a by-product of chitin, has a wide range of applications. The relevant process requires optimization [133].

Mealworm larvae contain high-quality lipids. The chemical characteristics and content of mealworm lipids differ subject to the applied extraction method, including aqueous extraction, Soxhlet extraction and Folch extraction. Lipid content was higher in the Folch extraction method (12.9 g per 100 g of fresh insects) and lower in the aqueous method (7.8 g per 100 g of fresh insects). The n-6/n-3 ratio of the extracts was determined at 27:1 [122].

Purschke et al. [134] described different parameters for oil extraction from mealworm larvae and the properties of the resulting oil. Maximal defatting (95%) of *Tenebrio molitor* larvae was achieved at 400/250 bar, 45 °C, in 105 min. The extracted oil contained 72% of unsaturated fatty acids, and oleic acid accounted for 42% of total fatty acid methyl esters.

3.5.3. Mealworm as Feed

Mealworm larvae are a highly nutritious substrate that can be used in the production of feed for pigs, poultry, fish and pets. The inclusion of mealworms in livestock diets has generally produced satisfactory results, but the optimal dietary inclusion rates have to be developed in some cases. Research studies investigating the use of mealworms as feed are presented in Table 5.

Table 5. Research into the use of mealworms as feed.

Livestock/Product	References
livestock feed	[8,41,45]
pigs	[46,47]
rabbits	[136,137]
poultry	[47–52,54,55,135,138,139]
fish	[42,56–59,61,140]
reptiles, amphibians	[141–144]
other animals	[62,63]—Barbary partridge; [145]—tit bird; [146]—passerine birds; [65]—Japanese quail; [64,73,147]—bats; [68,148]—dog; [149]—passerine birds; [69]—golden hamsters; [150]—spiders; [151]—red billed chough; [152]—European robin (*Erithacus rubecula*), great tit (*Parus major*), European black bird (*Turdus merula*); [153–158]—European starlings (*Sturnus vulgaris*); [70–72]—house sparrows; [66]—pheasants; [67,68]—cats and dogs

Mealworm larvae have higher protein and fat content than other edible insects. The use of insects as a sustainable source of proteins for pig and poultry diets is technically feasible [47].

Raw mealworms and larvae cooked at 150 or 200 °C were fed to pigs. Mealworm larvae were more readily digestible than crickets. Thermal treatment appears to exert a negative effect on insect digestibility in pigs; therefore, insect species and the thermal processing method should be carefully selected when incorporating insect-based protein sources in porcine diets [46].

Growth performance and daily feed intake were similar in broiler chickens fed mealworm larvae and typical feeds. Analyses of chicken organs revealed no significant morphometric or histological changes. Biasato et al. [135] concluded that 75 g of mealworms can be safely added per kg of regular poultry feed. The proportions of mealworms can be increased in female broilers for enhanced growth performance. Despite the above, mealworms can decrease feed efficiency [48,49]. In broiler diets, the complete replacement of soybean oil with mealworm oil obtained by super-critical CO_2 extraction did not exert adverse effects on growth performance. Diets enhanced with mealworm oil also increased the fatty acid content of chicken breasts, thus increasing their appeal for consumers [55].

Mealworm larvae were fed to small chicks in behavioural tests [52,138], and they were incorporated in the diets of laying hens [139]. Mealworms can be successfully added to poultry diets, but the relatively high cost of insect meal can limit the large-scale use of insects in poultry nutrition [54].

Yellow mealworms are also a sustainable substitute for fishmeal or soybean meal in fish diets. The demand for fish from aquaculture has increased since the 1990s due to overfishing concerns [42,57]. Fresh and dried mealworm larvae are an acceptable alternative protein source for most commercial species of fish, and they can replace 25%, 50% or even 80% of fish proteins in aquaculture diets.

If a suitable rate of fishmeal substitution is chosen, it may have the same effect on growth as regular feed, and in some cases, it may even improve the growth performance and composition of the fish, depending on the species [42,56,57]. Partial replacement of fishmeal with mealworm larvae meal (25%) over a period of 6 weeks stimulated immune and anti-inflammatory systems in small fish species (European sea bass, *Dicentrachus labrax*). The above could be attributed to the presence of chitin or low concentrations of n-3 and n-6 PUFAs ratio that may alter immune system function in mealworms [58]. In another experiment, diets containing 25% and 50% of yellow mealworms (corresponding to 35% and 67% of fishmeal replacement) were fed to rainbow trout (*Oncorhynchus mykiss*) for 90 days. The experimental diets led to a greater increase in the antioxidant activity of intestinal enzymes and a greater decrease in lipid peroxidation than the control diet [59]. These findings confirm that partial replacement of fishmeal with yellow mealworms delivers positive effects in aquaculture. An experiment with a similar design was conducted by Iaconisi et al. [140] who observed no differences in the proximate composition of raw and cooked fillets from the experimental and control fish fed diets with and without mealworm larvae meal, respectively, excluding their fatty acid profile.

In other research 50% replacement of fishmeal with insect meal (*T. molitor*) did not improve the growth performance of common catfish (*Ameiurus melas*) [61], and only minor differences were noted between the experimental and control fish. These findings suggest that the dietary inclusion rates of mealworms should be modified or that other sources of feed should be identified to improve fish growth [61]. Similar results were reported in juvenile European sea bass whose diets contained 0%, 25% and 50% of TM meal on an as-fed basis. The final body weight and weight gains were lower in the group fed diets containing 50% of mealworm meal relative to the control batch. The only significant positive change induced by the dietary inclusion of mealworms was observed in the fatty acid profile, where the content of C18:2 n−6 increased by 91% and 173% relative to the control batch. The content of the remaining fatty acids was not altered [56].

Blackspot sea bream (*Pagellus bogarevo*) is widely consumed in the Mediterranean Region. The use of mealworm larvae meal in fish diets influenced selected fish traits, such as skin or fillet hue (colour). The skin of fish whose diets were supplemented with 50% of insect meal was characterised by a greater contribution of redness relative to control fish. The colour of fish fillets differed between epaxial and hypaxial muscles. The dietary inclusion level of mealworm larvae meal did not influence the proximate composition of fish fillets, and the only differences were noted in the fatty acid profile. The use of mealworm larvae meal in the analysed species seems to be encouraging since it had no adverse effect on the growth performance of fish, but fillet quality should be taken into consideration [60]. Iaconisi et al. [140] reported similar results in raw and cooked fillets of rainbow trout.

In recent study, by Motte et al. [159], fishmeal was replaced with defatted mealworm larvae meal in diet of Pacific white shrimp (*Litopenaeus vannemei*). The best result replacing rate was achieved with 50% mealworm proportion. The authors observed the improvement of the growth parameters and higher efficiency of feed conversion rate. It was also found that the use of insects as feed improved the survival rate and resistance to immunosuppressive factors [159].

Mealworm oil can replace the soybean oil (1.5% of diet) used in raising rabbits. Thus, Italian researchers [136,137] investigated a replacement of a soybean oil in rabbit diets in a proportion of

50% and 100%. In the first study rabbits raised with insect oil did not show any differences regarding growth performance, apparent digestibility, gut mucosa traits and rabbit health from those raised on soybean oil [136]. Moreover, the meat quality features (proximate composition, lipid peroxidation, and fatty acid profile), and the consumer acceptance of the rabbit meat were not affected [137].

In some studies, mealworm larvae were fed to reptiles, mammals and arthropods, including Shangcheng stout salamanders [141], bearded dragons (*Pogona vitticeps*) [144], green lizards (*Lacerta viridis*) [142], leopard geckos (*Eublepharis maculaius*) [143], golden hamsters [69], bats [64,73,147,148], and spiders [150]. Mealworms were also administered to passerine birds [146,149], barbary partridges [62], tit birds [145], European robins (*Erithacus rubecula*), great tits (*Parus major*), European black birds (*Turdus merula*) [152], Japanese quails [65], European starlings (*Sturnus vulgaris*) [153–158], red-billed choughs [151], house sparrows [70–72] and pheasants [66]. Several researchers analysed mealworm larvae as an alternative source of proteins and amino acids for dogs and cats [67,68]. Mealworm larvae were characterised by very high digestibility in vitro (91.5%) [67]. A study investigating different insect species also demonstrated that unprocessed dried mealworms were more often favoured by male than female dogs. These data suggest that mealworms have an attractive aroma for dogs and that this feature can be used to enhance the appeal of commercial canine feeds [160].

3.6. Safety Aspects of Mealworm as Food

The safety of mealworms as food or feed has to be researched before the relevant products are introduced to the European market. The preservation, sterilisation, processing and conservation techniques for reducing the microbial load and retaining the nutritional value of mealworms have been extensively analysed. Research studies investigating various aspects of mealworm safety (toxins, pesticides, antibiotics, heavy metals) are presented in Table 6. These safety concerns are discussed in subsequent subchapters.

Table 6. Research studies investigating various aspects of mealworm safety.

Topic	References
Antibiotic resistance	[82,161–163]
Allergies	[107,164–172]
Heavy metals	[77,173–176]
Pesticides	[175,177–181]
Hazardous substances, chemicals, toxins, mycotoxins and other compounds	[175,182–185]

3.6.1. Conservation Techniques

Research studies have emphasised the importance of the optimal techniques for the preparation and thermal processing of mealworms intended for human consumption. Even if mealworms larvae are already used in Europe as a novel food, there is insufficient information for consumers about the processing and cooking methods that should be applied to preserve the nutritional and energy value of mealworms and to reduce their microbial load. Grabowski and Klein [186] studied different types of marketed insects and various insect processing methods. They found that the microbial load of mealworm larvae was most effectively reduced by boiling. Powdered and dried mealworm samples were characterised by higher microbial counts relative to other processed insects [186].

Research conducted in Europe has demonstrated that different post-harvest techniques and pre-treatments, such as blanching, freezing and drying (oven drying, fluidised bed drying, freeze drying) influence the physicochemical properties of mealworm larvae, including colour and the size of ground particles [187,188]. Drying also influences nutritional quality and lipid oxidation in mealworms, i.e. parameters that are most sensitive to processing and preservation. Kröncke et al. [189,190] analysed the influence of drying methods on the nutritional value of mealworms [189,190] and observed that some processes (microwave drying, fluidised bed drying and drying with vacuum) decreased protein solubility. Vacuum and microwave drying were the most effective alternatives to conventional freeze drying [189]. The processing of mealworms at high temperatures can have an influence on their colour and can influence some sensory properties. Krönke et al. [190] found that rack oven drying caused pronounced darkening compared to vacuum drying and freeze drying.

Several preservation and processing methods can be combined to reduce microbial load and increase the safety of mealworms as feed or food. The most popular methods for reducing total microbial counts are freeze-drying with sterilization [191], as well as blanching with drying which decrease the number of vegetative cells, but is not highly effective in eliminating endospores that are most resistant to processing [192]. Drying appears to reduce microbiological load more effectively (by 10^8 to 10^3) than freezing (10^8 to 10^7) [193].

Boiling (100 ± 0.5 °C for 1 min) and vacuum cooking (74 ± 0.5 °C for 60 min) were most effective in reducing microbial load and preserving high levels of protein (43.9% and 42.6%, respectively) and PUFAs (40% and 30.6%, respectively, on a dry matter basis). According to Caparros Megido et al. [116], incomplete cooking of mealworms under home conditions changed the fatty acid composition of mealworm larvae, decreased the content of SFAs and increased crude protein digestibility.

Some researchers analysed the most popular insect cooking techniques. Borremans et al. [194] investigated the microbial load of blanched mealworms marinated in red wine and soy sauce can influence microbial load. They demonstrated that marination can extend the shelf life of blanched mealworms for at least 7 days. Another proposed conservation technique was to subject the mealworm larvae paste to the fermentation process with commercial meat starter cultures (*Lactococcus lactis*, *Lactobacillus curvatus*, *L. farciminis*, *L. plantarum*, *L. sakei*, and *Pediococcus acidilactici*). The results showed that all tested starters fermented the mealworm paste and inhibited the development of *Bacillus*, *Salmonella* and *Clostridium* species. *L. farciminis* was the most promising. Its application also increased content of aspartic and glutamic acids [195].

Lenaerts et al. [115] studied microwave drying as an alternative to the energy-demanding freeze drying technique. Only minor differences in proximate composition were reported in mealworms processed with the use of the compared techniques, and microwave drying induced a greater decrease in vitamin B_{12} content (0.85 µg per 100 g in freeze-dried vs. 0.31 µg per 100 g in microwave-dried samples). Lipid oxidation was also lower in microwave-dried than in freeze-dried mealworms.

Thermal processing can compromise the nutrient content and properties of mealworm larvae. According to Melis et al. [196] the low-temperature long-time (LTLT) method is less recommended than the high-temperature and short-time (HTST) approach. The degradation of some compounds, such as sugars, is inevitable in both drying processes. Poelaert et al. [197] reported that the nutritional value of insects is most effectively preserved by cooking at a temperature of 200 °C for 10 min.

Long-term storage of mealworms can also influence the qualitative and nutritional properties of pasta obtained from whole non-defatted mealworm larvae even in the presence of preservatives. According to research of De Smet et al. [198] the best temperature for long-term storage was below −20 °C, in which no significant changes in nutritional properties were observed, with very small deviations only for the fat content [198].

3.6.2. Microbial Load of Mealworm Larvae

Despite the nutritional benefits of mealworm for livestock and humans, insects intended for consumption have to be analysed for microbiological load and the risk of disease transmission. The infections caused by protozoa, fungi, bacteria, viruses and other pathogens can generate considerable losses for insect producers. Studies analysing the microbial load of mealworm samples from different countries and producers, in mealworms stored under different conditions and processed with the use of various methods are presented in Table 7.

Table 7. Microbial load of mealworm larvae from different breeders and countries.

Samples	Microbial Species	References
Insect samples analysed by the Mendel University in Brno. Year of insect breeding: 2012 (killed by freezing), 2015 (killed by boiling water, dried at 103 °C for 12 h, homogenised and stored at room temperature until analysis in January 2017) and 2016 (killed by freezing).	Freshly killed: total microbial counts (2.2×10^8 cfu g^{-1}), enterobacteria (1.910^8 cfu g^{-1}), Lactic acid bacteria (7.2×10^7 cfu g^{-1}), yeasts and moulds (8.9×10^3 10^7 cfu g^{-1}). Frozen: total microbial count (TMC) (3.4×10^7 cfu g^{-1}), enterobacteria (4.2×10^6 cfu g^{-1}), lactic acid bacteria (2.4×10^5 10^7 cfu g^{-1}), Yeasts and Moulds (3.3×10^3 cfu g^{-1}). Dried: TMC (max. 6.6×10^3 cfu g^{-1}), enterobacteria (<10 cfu g^{-1}), lactic acid bacteria (up to 3.8×10^3 cfu g^{-1}), yeasts and moulds (up to 1.7×10^4 cfu g^{-1}).	[193]
Fully grown, non-starved live larvae from a breeder in Belgium	*Enterobacteriaceae*, bacterial endospores, lactic acid bacteria, sulphite reducing clostridia.	[194]
Flour prepared from *T. molitor* that were purchased from a local breeder (Germany)	7.72 cfu g^{-1} DM: microbial load at the beginning of processing with cold atmospheric pressure plasma.	[203]
Live mealworms were bought from a local company (Sixlegs SA, Belgium). Freeze-dried mealworms were also supplied by a local company (BugsInMugs, Belgium)	Total aerobic count (TAC) in untreated freshly killed mealworms (8.58 log cfu g^{-1}) and freeze-dried mealworms (4.47 log cfu g^{-1}), yeast and mould counts (4.70 log cfu g^{-1}). Blanching (1 min) and sterilisation reduced TAC by around 50% in freshly killed mealworm and decreased yeast and mould counts by less than 1 log cfu g^{-1}.	[191]
Fresh mealworms reared by the Laboratory of Functional Entomology (Liège University, Belgium)	Total aerobic count.	[116]
Pre-packed, shelf-stable insects purchased online from various commercial suppliers (10 samples)	Total aerobic spore count Log10 (<5 cfu g^{-1}), *Bacillus cereus* *Kocuria rhizofila*, *Macrococcus* spp., and other.	[204]
Whole dried mealworm larvae from a company located in The Netherlands.	*Enterobacteriaceae*, total mesophilic aerobes, lactic acid bacteria, *Clostriudium perfrimgens spores*, yeasts, Moulds (<2.00 log cfu g^{-1} of all enumerated microbiological parameters). No *Salmonella* spp., and *Listeria monocytogenes* in 25 g of samples.	[201]
Insect samples (reared in Europe or Asia) were obtained from Germany and the Netherlands between 2015 and 2016. Different processing methods were analysed	Aerobic bacterial count: *Enterobacteriaceae* (1/3 samples), *E. coli* (3/3 samples), coagulase-positive staphylococci (3/3 samples) (mealworms were dried, powdered and cooked). Dried mealworms were colonised by *Listeria ivanivii*, *Penicillium* spp., *Mucor* spp.	[186]
Live mealworm larvae supplied by Van de Ven, the Netherlands, were processed with the use of different methods and stored for different periods of time	*Enterobacteriaceae*, Bacterial spores.	[202]
30 samples were purchased online from European (Belgium and the Netherlands) and Asian suppliers	*Staphilococcus* spp., *Exiguobacterium* sp., *Eikenella corrodens*, *Eikenella* sp., *Bacillus* sp. were identified in more than 82% of the samples.	[163]

Table 7. *Cont.*

Samples	Microbial Species	References
Samples of processed (dried) edible insects were purchased online from two suppliers in the Netherlands and one supplier in Belgium.	*Enterobacteriaceae* (<log 1 cfu g^{-1}), total mesophilic aerobes (up to log 4.8 cfu g^{-1}), sulphite-reducing clostridia (up to log 4 cfu g^{-1}), *Staphylococcus aureus* (<log 1 cfu g^{-1}), *Bacillus cereus* (<log 1 cfu g^{-1}), lactic acid bacteria (up to log 2.8 cfu g^{-1}), yeasts (up to log 2.4 cfu g^{-1}—in samples from one Dutch supplier; <log 1 cfu g^{-1} in the remaining samples), moulds (up to log 2.3 cfu g^{-1}—in samples from one Dutch supplier; <log 1 cfu g^{-1} in the remaining samples).	[199]
Second generation, last instar mealworms purchased from a local pet store (Italy). Larval frass was also analysed.	*Enterobacteriaceae* (*Xenorhabdus* spp., *Enterobacter* spp. and *Pantoea* spp.), Lactic acid bacteria (*Lactococcus garviae*, *Enterococcus: E. faecium*, *E. gallinarum*, *E. mundtii*), mesophilic aerobes, spore-forming bacteria. *Psychrobacillus* spp., *Serratia* spp., *Erwinia* spp., *Aeromonas* spp., *Burkholderia* spp., *Klebsiella* spp. and other.	[200]
Live mealworm larvae were purchased from Futtertier-Shop.de (Germany)	Microbial surface load.	[205]
Samples were obtained from a supplier of organic insects (Belgium)	*Enterobacteriaceae*, lactic acid bacteria, bacterial endospores, yeasts and moulds. (*Propionibacterium* sp., *Lactobacillus* sp., *Streptococcus* sp., *Haemophilus* sp., *Enterobacteriaceae bacterium*, *Pseudomonas* sp., *Staphylococcus* sp., *Acidovarax* sp., *Varibaculum* sp., *Clostridium* sp. and other.	[206]
Samples were obtained from a supplier of organic insects (Belgium). The insects were used to prepare a minced meat-like product	Enterobacteriaceae, lactic acid bacteria, yeasts and moulds. (*Serratia* sp., *Erwinia* sp., *Rickettsiealla* sp., *Spiroplasma* sp., *Pseudomonas* sp., *Enterobacter* sp., *Hafnia* sp./*Citrobacter* sp., Propionibacterium sp.).	[207]
Samples were purchased from Belgian and Dutch suppliers	*Spiroplasma* sp., *Erwinia oleae*, *Eneterobacteriaceae* sp., *Buttiauxella agrestis*, *Pseudomonas deceptionensis*, *Lactococcus* sp., *Citrobacter koseri*, *Brevibacillus* sp., *Enterococcus* sp., *Clostridia* sp.	[208]
Samples were purchased from four suppliers of edible insects in Belgium and the Netherlands, and were prepared according to the method described by Stoops et al. (2016).	Lactic acid bacteria, *Enterobacteriaceae*, aerobic bacterial endospores, psychrotrophic aerobic counts, Yeasts and moulds.	[209]
Last instar larvae were purchased from an industrial rearing company in Belgium	Lactic acid bacteria, *Enterobacteriaceae*, yeasts and moulds, aerobic bacterial endospores.	[192]
Mealworm larvae were supplied by an industrial rearing company in Belgium	*Enterobacteriaceae*, aerobic psychotropic count, yeasts and moulds, aerobic bacterial endospores *Erwinia* sp., *Gammaproteobacteria* sp., *Lactococcus* sp., *Enterococcus* sp., *Cronobacter* sp., *Enterobacteriaceae* sp., *Weissella* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Lactobacillus* sp., *Pseudomonas* sp., *Pediococcus* sp., and other.	[210]

Some mealworm samples were colonised by a wide range of bacterial species, including *Enterobacter* sp. (ludwigii, cloacae, hormaechei, etc.), *Klebsiella* sp., *Staphylococcus aureus*, *Clostridium thermopalmarium*, *Vibrio* sp. and *Escherichia coli* [163,199–201]. Osimani et al. [199] were the first researchers to report the presence of *Agrococcus*, *Loktanella* and *Rufibacter* in 8 samples of ready-to-eat insects supplied by different producers, including two Dutch companies and one Belgian company (four samples from each country).

In some cases, *Enterobacteriaceae* can be effectively eliminated by simple processing methods (boiling or roasting for 10 min). However, these methods do not eliminate spore-forming bacteria that survive in cooked insects [202]. Bußler et al. [203] found that longer exposure to cold atmospheric pressure plasma (CAPP) during postharvest processing considerably reduced microbial concentrations.

Antibiotics could be used during insect rearing to minimise the adverse effects of microbial load on mealworm health and reduce the contamination of target consumer groups (livestock and humans). Mealworm larvae harbour genes that encode resistance to tetracycline, erythromycin and vancomycin [161]. Some types of feed could contribute to the expression of antibiotic resistance genes, and edible insects could be natural carriers of antibiotic resistance if feed is not a source of antibiotic resistance genes (wheat meal) [162]. The effects of antibiotics on mealworms have not been studied extensively, and antibiotics should be used with caution in insect farming [161,162].

3.6.3. Contaminants

Mealworms can grow on various types of substrates, and therefore they could pose a risk to agricultural crops. Insects that act as pests are eliminated with the use of insecticides. Athanassiou et al. [177] studied the effects of different doses (0.025 and 0.1 mg cm^{-2}) and combinations of insecticides (alpha-cypermethrin and thiamethoxam) on mealworms exposed to these chemicals for 1, 3 or 7 days. They found that *T. molitor* was susceptible to both tested insecticides, but alpha-cypermethrin was more effective than thiamethoxam. A lower dose of thiamethoxam (0.025 mg cm^{-2}) eliminated 38.9% of small larvae. The mortality rate associated with the higher dose ranged from 88.9% to 95.6%. Mortality was low in large larvae.

Attempts have also been made to limit mealworm reproduction by injecting females with the gonadoinhibitory peptide Neb-colloostatin. The peptide strongly inhibited oocyte development by promoting apoptosis, a mechanism of programmed cell death [211]. This method effectively limited reproductive activity in pests. Similar tests were performed on mealworm beetles to test their humoral response to different substances. These studies analysed the antibacterial activity of mealworm haemolymph against the tested compounds [212,213].

The effects of other insecticides on mealworms were also studied, and the highest mortality rate was reported for a neonicotinoid insecticide [179]. Some researchers have also attempted to find alternative solutions to synthetic pesticides. The optimal compound should effectively eliminate pests without exerting toxic effects on livestock. The effectiveness of insecticides depends on extraction methods, doses and mode of action on the organs and tissues of pests [181]. The extract of *Solanum nigrum* fruits exerted only sublethal effects on mealworms by disrupting their development and metabolism. When combined with other pest management strategies, these extracts could offer a cheap, effective and environmentally-friendly solution to crop protection [180,181].

Two fungal species collected in Antarctica, *Geomyces* sp. and *Mortierella* sp., were tested for their ability to eliminate mealworms. These cold-tolerant fungi possess insecticidal properties, and they could be used to control pests in the temperate climate. A suspension containing *Mortierrella signyensis* (10 µL) was most effective in eliminating mealworms (67.5% mortality rate vs. 7.5% in control) [183].

The use of combined magnetic fields can influence hatching time of pupae. The exposure of pupae to weak combined magnetic fields decreased the time of transition from pupae to imagines (adult beetle). In some studies, combined magnetic fields did not exert a noticeable effect on hatching times [184].

Grain-based feeds such as maize, wheat and oats can be used to breed high-quality larvae that are safe for consumption. Crops are usually protected against pests with chemical or biological agents, which can lead to the accumulation of selected compounds in mealworms. In a study by

Houbraken et al. [178], mealworms intended for human consumption were exposed to different agricultural residues. Agricultural residues often contain pesticides which can be accumulated in larvae. Larval contamination decreased significantly after 24 h of starvation, depending on the type of pesticide.

Poma et al. [175] investigated the influence of hazardous substances such as pesticides, heavy metals, DDT and dioxins on mealworms and other edible insect species. The concentrations of these chemicals were lower in mealworms than in widely consumed animal products, such as eggs, fish and meat. Mlček et al. [174] analysed Cu, Zn, Cd and Pb levels in mealworms fed wheat bran, oat bran and soy flour. The study revealed high and potentially dangerous Cd concentrations in mealworms (157–186 mg kg^{-1}).

Mealworm larvae were exposed to different concentrations of heavy metals, including As, Pb and Cd, in feed. The tested heavy metal contamination exerted varied effects on mealworm survival rates and the live weight of larvae relative to control. Contaminated feed did not exert a significant influence on larval development time. The live weigh of larvae increased in response to higher concentrations of Pb (3.4 g) and Cd (3.3 g), whereas the reverse was noted for As (maximum allowable level of 2.0 g per 2 ML). Mealworm larvae administered feed with three different concentrations of As accumulated heavy metals. Mealworm excreta also contained less As than larvae, which suggests that As is bioaccumulated by mealworm larvae. Lead was the least bioaccumulated metal (Bioaccumulation factor around 0.05) [176].

Food and feed contaminated with mycotoxins can affect human and animal health. Aflatoxin B1(AFB1) produced by fungi of the genus *Aspergillus* genus is one of the most dangerous mycotoxins. In a study by Bosch et al. [182], the concentration of AFB1 in mealworms administered 0.023 mg AFB1 per kg of feed was 20 times lower than the legal limit, which suggests that mealworms are able to excrete or catabolise AFB1 after ingestion. Similar results were obtained by Van Broekhoven et al. [185] in a study of deoxynivalenol (DON), a mycotoxin produced by the genus *Fusarium*. Mealworm larvae excreted DON with faeces, and DON levels in larvae were below the detection limit, including in larvae that were administered feed with a high concentration of the mycotoxin (up to 8000 μg kg^{-1}). The yellow mealworm larvae are able to excrete another type of mycotoxin and its metabolites, such as Zearalelone (ZEN) [214]. At least 24 h of mealworm starvation, before larvae harvesting, should be applied for the compound depletion from the larval body. The remaining traces of ZEN and its metabolites can be negligible after this treatment.

3.7. Consumer Attitudes towards Edible Insects and Safety Concerns

Extensive research has been done into consumer attitudes towards edible insects ever since these food sources had been introduced officially into the European Union market and approved for sale in Belgium and the Netherlands in 2014 [215]. These novel foods can be incorporated into familiar products to foster more positive consumer attitudes. The taste and appropriateness of the modified product can be less appealing in comparison with the original product that the consumers already know [216].

In the Netherlands, the acceptance of edible insects was studied by presenting consumers with different types of food, including products containing visible and invisible mealworms as well as foods without the addition of insects [217]. Mealworm visibility influenced consumer preferences, and acceptability was higher when mealworms were invisible. Only 28.8% of the participants had tasted mealworms before the study. The awareness that mealworms can be used as food was high. Only 8.4% of the respondents were not aware that mealworms are edible. Male participants were more inclined to try novel foods than female respondents [217,218]. Young people were more willing to try, but not buy novel foods. The consumers' educational attainment was not clearly correlated with the acceptability of novel foods [217]. In a sensory study performed on 135 volunteers, younger people (mean age—33.0 years) were more willing to try novel foods, and females accounted for 80% of these respondents. Seventy-nine tasters were unwilling to try novel foods (mean age—59.9 years), and 65.8% of them were women. The percentage of willing participants was higher among those who have completed tertiary education (90%). The results of the study were influenced by experimental conditions because the survey was conducted among the students and employees of the Wageningen University [216]. Willingness was the key factor in the respondents' decision to consume and buy mealworms [216,218,219].

Caparros Megido et al. [32] studied the perceptions of entomophagy among Belgian consumers who were served insects, crickets and mealworms in different form (baked, boiled, crushed, flavoured with a pinch of vanilla or paprika, or dunked in chocolate). The respondents had the greatest preference for crispy mealworms with chocolate and paprika and for naturally baked mealworms. A similar study was conducted by Caparros Megido et al. [220] who investigated consumer attitudes towards insect-based burgers. The taste of mealworm burgers was rated between that of fully meat and fully vegetable burgers.

Food products and ingredients containing mealworm larvae may not be suitable for consumers suffering from allergies. Consumers who are allergic to dust mites and crustaceans could have an allergic reaction to foods that contain mealworm proteins [164,169–171]. For example, a Dutch study tested the sera of 19 patients who were allergic to crustaceans and house dust mite. Nearly all of the tested yellow mealworm samples (raw, lyophilized, boiled and fried) elicited at least one allergic response, excluding in two patients [169]. Another study demonstrated that even when thermally processed, fresh mealworms induced skin reactions [165] or other allergy symptoms (oral allergy, urticaria, nausea, dyspnoea) [166,168] in most consumers who were allergic to shrimp. In a study of people allergic to shrimp, 87% had a probable cross-reactivity to mealworms [167]. Nebbia et al. [172] reported that two out of ten male employees processing yellow mealworm flour displayed the first symptoms of allergy within the first few weeks of employment. These symptoms were intensified by sifting larvae from mealworm faeces. Interestingly, despite the fact that the employees had previously consumed various insects, none of them were allergic to other insect species (black soldier fly, crickets, wax moth). The authors concluded that the employees had probably developed an allergy to mealworm through contact with faeces. A recent report [221] presents the case of food anaphylaxis in a person who consumed an appetizer with mealworm for the first time in his life. Following the tests, it was found that he had an allergy to dust mites and *Tenebrio molitor* flour but no negative reaction to shrimp [221].

4. Conclusions

The interest in mealworms as a source of food and feed has been increasing steadily in Europe due to the high nutritional value of mealworms, ease of production and lower environmental impact in comparison with livestock. Our analysis shows that number of research studies on *Tenebrio molitor* increased in recent years, especially since 2015, when European Union defined insects as novel food.

Mealworms are able to convert low-energy feeds into food products with a high value and they can mitigate the environmental pressure exerted by livestock. Larvae can deliver almost all types of essential amino acids that cannot be synthesised by the human body. *Tenebrio molitor* larvae can be successfully added to feed of many species of fish, poultry or even rabbits. Recent research showed that commercial feeds partially replaced with insects may improve livestock growth, but also can have positive impact on fatty acid profile.

Many analysed studies referred to the safety of mealworm use, both as feed and food. Microbial load played a major role in the further use in mealworm implementation as feed for various livestock rearing; insects from different producers and countries may have various microbial count. Therefore, it is necessary to adopt preservation methods and standards to ensure that insect-based products are safe for consumption. Moreover, new regulations concerning contaminations included in biomass used for mealworm farming, e.g., pesticides, heavy metals or other toxins should be introduced as well. Mealworm breeders and food and feed producers should also develop new methods, standards and labelling to ensure that insect-based products are safe for consumption, especially for people with allergy to dust mite and crustaceans.

Last but not least, European consumers have to be educated about edible insects as a rich source of protein. Consumers who are unwilling to eat whole insects could still benefit from food where typical livestock proteins have been replaced with mealworm proteins. Educational measures are also needed to increase consumer awareness and acceptance of insects as nutritionally valuable components of the daily diet.

Author Contributions: All authors have read and agree to the published version of the manuscript. Conceptualization, M.K. and M.J.S.; methodology, M.K. and A.B.; validation, M.K. and M.J.S.; formal analysis, M.K. and M.J.S.; investigation, A.B., M.K. and D.P.; resources, M.K. and M.J.S.; data curation, A.B. and M.K.; writing—original draft preparation, A.B., M.K., M.J.S. and D.P.; writing—review and editing, A.B. and M.K.; visualization, A.B. and M.K.; supervision, M.K. and S.C.; funding acquisition, M.J.S. and M.K.

Funding: This paper is the result of a study carried out at the University of Warmia and Mazury in Olsztyn, Faculty of Environmental Management and Agriculture, Department of Plant Breeding and Seed Production, topic number 20.610.008–300. Project was financially supported by Minister of Science and Higher Education in the range of the program entitled "Regional Initiative of Excellence" for the years 2019–2022, Project No. 010/RID/2018/19, amount of funding 12.000.000 PLN. Anna Bordiean and Dumitru Peni have received scholarships under the Interdisciplinary Doctoral Programme in Bioeconomy (POWR.03.02.00-00-I034/16-00) funded by the European Social Fund.

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

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