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Review

The Superworm, *Zophobas morio* (Coleoptera:Tenebrionidae): A 'Sleeping Giant' in Nutrient Sources

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Abstract

The aim of this review is to compile up-to-date information on the superworm, *Zophobas morio* (F.), regarding its biology and ecology, but also its further potential for use as a nutrient source for food and feed. We illustrate certain basic characteristics of the morphology and bio-ecology of this species, which is marginally considered as a 'pest' in durable amylaceous commodities. More recent data show that *Z. morio* can be a valuable nutrient and antimicrobial source that could be utilized further in insect-based feed and food production. The inclusion of this species in aquafeed has provided promising results in a wide range of feeding trials, both in terms of fish development and health. Additional data illustrate its potential for use in poultry, indicating that this species provides comparable results with those of other insect species that are used in feed. Moreover, *Z. morio* can be a viable waste management agent. This review aims to summarize the available data and underline data gaps for future research, toward the potential of the utilization of *Z. morio* for human food and animal feed. Based on the data presented, *Z. morio* appears to be a well-promising insect-based protein source, which potential still remains to be unfold.

Key words: alternative nutrient source, insect farming, nutritional value, sustainability, Tenebrionidae

In a steadily increasing world population that is projected to get near to 10 billion by 2050, the demand for animal protein will continue to grow over the years to come (Boland et al. 2013, Searchinger et al. 2018, FAO 2019). Indicatively, the per capita meat consumption is expected to increase >1 kg retail weight equivalent by 2027 at a global level (OECD-FAO 2018), while this increase will be more vigorous in developing countries where the per capita consumption of animal protein will rise by 22% by 2030 and 25% by 2050 (FAO/WHO 2017). However, the capabilities to increase animal protein production through the further intensification of the traditional livestock production systems are rather marginal, as this would trade-off with adverse environmental impacts, i.e., effect on climate change through greenhouse gas emissions, extensive land use for livestock farming and deforestation, as well as extensive water usage and pollution (Steinfeld et al. 2006). Additionally, the animal feed industry is seeking for new protein sources to reduce the dependence and reliance of livestock production on soybean meal and fishmeal, which are the main ingredients for animal feed. Therefore, there is an urgent need for alternative animal proteins both for human consumption and feed production.

Several insect species have been identified during the last decade as an alternative protein source to be included in human food and animal feed as they have numerous advantages (Van Huis 2013, Gasco et al. 2019). Namely, they are highly nutritious (Rumpold and Schlüter 2013) efficient feed converters (Oonincx et al. 2015, Halloran et al. 2016), they can be easily reared on organic side-streams and agricultural wastes being aligned with circular economy strategies (Gasco et al. 2020), whereas their production has a low environmental footprint (Van Huis and Oonincx 2017). Apart from the aforementioned advantages, several species have specific physiological traits, such as high reproduction rate, short life cycle, rapid growth, as well as ease in handling and manipulation, which favor their commercialization. Currently, a number of insect species are commercially produced in large-scale industrial facilities (Van Huis 2019). Although insect consumption is still not common in the western society, the increasing willingness to adopt insect-based foods has been recently detected in several western countries (Schlup and Brunner 2018, Orsi et al. 2019). Moreover, the majority of the population seems to not mind consuming meat products originating from livestock that were fed by insects (Kulma et al. 2020a). Therefore, new EU regulations have been released in order to pave the way for these products. According to the EU Regulation 2017/893, in force since July 2017, seven insect species are so far allowed to be used in EU as ingredient in aquafeeds [Commission Regulation (EU) 2017/893]. Regarding the production and consumption of insects as food in EU level, the 'Novel Food' legislation [Commission Regulation (EU) 2015/2283] regulates also the dietary inclusion of insects in human diets, whereas more recently the approval of larvae of the yellow mealworm, *Tenebrio* *molitor* L. (Coleoptera: Tenebrionidae), for human consumption by the European Food Safety Authority (EFSA 2021) is expected to be a breakthrough in the promotion of insect-derived food products.

Among the most common mass-reared insect species, the black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae) (Tomberlin and Van Huis 2020), *T. molitor* (Ribeiro et al. 2018), and the lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) (Rumbos et al. 2019), have attracted most of the scientific and commercial interest. However, the list of insects which have shown potential for exploitation as a nutrient source and merit further investigation is extensive. One insect species with great potential as food and feed, which has been overlooked by researchers and insect producers is the superworm or giant mealworm, *Zophobas morio* (F., 1776) (Coleoptera: Tenebrionidae). It is a large neotropical beetle species, belonging to the darkling beetles family, which is commonly reared as feed for birds, reptiles and fish. *Zophobas* species are reported by Ramos-Elorduy (2009) to be eaten by several ethnic groups in Mexico, whereas *Z. morio* is reported as one of the two main insect species, together with the field cricket, *Gryllus assimilis* (F.) (Orthoptera: Gryllidae), bred in captivity in Brazil, intended only for animal feed (Araújo et al. 2019). The first research records on *Z. morio* date back to the 1970s and 1980s (Tschinkel and Willson 1971; Tschinkel and van Belle 1976; Tschinkel, 1978, 1981, 1993). However, since then a considerable amount of research on *Z. morio* has been conducted (Kim et al. 2015, Van Broekhoven 2015, Van Broekhoven et al. 2015, Harsányi et al. 2020). Indicatively, the number of research articles on *Z. morio* published each year has exponentially increased during the last decade (Fig. 1), with the main focus of the recent studies being the potential of *Z. morio* as a nutrient source for livestock animal feed and aquaculture. Recently, the complete mitochondrial genome of *Z. morio* was sequenced (Bai et al. 2019), signifying the increased interest of researchers for this species. In this framework, this review aimed to present collectively significant information on *Z. morio*, in order to better highlight its potential as a nutrient source for food and feed and provide a useful tool for researchers working with this promising beetle species.

Taxonomy, Systematic Position, and Distribution

The taxonomy and classification of *Z. morio* [formerly *Tenebrio morio* (F., 1778); *Helops morio* (F., 1777)] has been a matter of controversy and confusion (Tschinkel 1984; Ferrer 2006, 2011). Currently, *Z. morio* is identified as conspecific with *Zophobas atratus* (F., 1775) [formerly *Tenebrio atratus* (F., 1775); *Zophobas rugipes* (Kirsch, 1866) (Park et al. 2013, Soldati and Touroult 2014, Bousquet et al. 2018) and as such they will be considered in this review.

Zophobas morio belongs to the large beetle family of Tenebrionidae, which contains many stored-product insect species, such as *T. molitor* and *A. diaperinus*, but also the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), and the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Although listed among storage insects, *Z. morio* has been found in association with only one stored commodity, i.e., wheat flour (Hagstrum and Subramanyam 2009), indicating its negligible importance as secondary storage insect pest. In nature, it has been reported in association with fruit bat guano and organic litter (Tschinkel and Willson 1971). It traces its origins in the tropical regions of Central and South America (Marcuzzi 1984, Tschinkel 1984, Hagstrum and Subramanyam 2009); however, it has nowadays been also introduced to other regions in Europe and Asia (Yuan et al. 2012, Fursov and Cherney 2018).

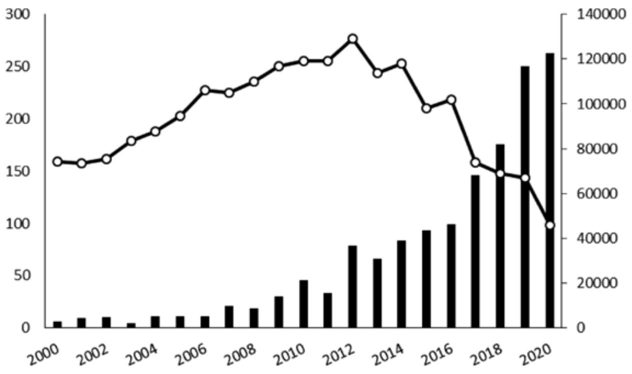

Fig. 1. Number of published articles per annum indexed by Google Scholar matching the search queries '*Zophobas morio*' (bars, left axis) and 'insect' (line, right axis), shown per year of publication (2000–2020) (Date of Google Scholar search: 27 January 2020).

Identification, Key Characteristics, and Biology

Eggs

The eggs of *Z. morio* are oval with rounded edges, white and ~1.7 mm in length and 0.7 mm in width (Fig. 2A; Fursov and Cherney 2018). Each female can lay a high number of eggs (up to 2,200) during its lifespan, with the number of eggs being negatively correlated with female maternal age and positively correlated with adult density (Tschinkel 1993).

Larvae

The larvae are yellow with dark brown anterior and posterior ends (Fig. 2B; Fursov and Cherney 2018). They have a cylindrical, strongly sclerotized exoskeleton, conically narrowed from the base of the seventh to the ninth abdominal segment. They can get up to 55-mm long (Friederich and Volland 2004). They hatch after 8 d at 25°C (Kim et al. 2015). The number and duration of larval instars is density-dependent, i.e., it varies depending on whether larvae are maintained under isolated or grouped conditions. For instance, the isolation of newly hatched larvae considerably prolongs their development time in comparison to early instar larvae kept under grouped conditions (Quennedey et al. 1995). If kept isolated, larvae pupate after 11–18 instars, whereas the largest percentage of pupation occurs after 16 or 17 molts (Quennedey et al. 1995, Kim et al. 2015). One of the most interesting characteristics of this species is that its larvae fail to pupate under crowded conditions, although larval molts continue to occur until death (Tschinkel and Willson 1971, Quennedey et al. 1995). For instance, Tschinkel and Willson (1971) demonstrated that the rate of pupation was retarded with the increase of larval density. This phenomenon does not seem to be pheromone-mediated, or to be caused by auditory or visual stimuli and is rather attributed to the mechanical stimulation resulting from inter-larval contacts (Tschinkel and Willson 1971). In terms of commercial production, the larval requirement for isolated conditions for pupation, can significantly affect the industrialization of *Z. morio* production and impact its commercial production efficiency. Pupation inhibition induced by crowding conditions has been described also for other tenebrionid species, e.g., *Tribolium freemani* Hinton (Coleoptera: Tenebrionidae) (Nakakita 1982, Kotaki et al. 1993) and has been proposed to be a defense mechanism against cannibalism that is often observed among larvae and pupae (Tschinkel and Willson 1971, Ichikawa and Kurauchi 2009). It is suggested that different hormones, such as ecdysteroids and juvenile hormones, play a role in this larval developmental variability (Quennedey et al. 1995, Aribi et al. 1997). Approximately 6 d after isolation at 25°C, larvae get immobilized as prepupa in a c-shaped posture, which marks the initiation of the metamorphosis process. The prepupae do not walk, but respond to tactile stimuli by flicking their body, whereas it takes them seven more days to become pupae (Quennedey et al. 1995).

Pupa

The pupae (Fig. 2C) are mostly quiescent, however, when tactile stimulated, they have the ability to rotate their abdominal segments in a circular motion (Ichikawa and Kurauchi 2009, Ichikawa et al. 2012b) or exhibit other physical responses (Ichikawa et al. 2012a, Ichikawa and Sakamoto 2013). All these reactions are considered to be an effective pupal defense mechanism against predator attacks and larval cannibalistic behaviors and are triggered by the stimulation of different types of mechanoreceptive sensilla on the pupal body surface (Kurauchi et al. 2011). The duration of the pupal stage is 13–15 d at 25°C, depending on the pupal weight (adults emerging faster from small pupae) and temperature (faster adult eclosion at 29°C) (Quennedey et al. 1995). Similarly to other tenebrionid species, such as *T. molitor* (Bhattacharya et al. 1970) and *A. diaperinus* (Esquivel et al. 2012), individuals can easily be sexed at this life stage by noting two distinct projecting pygopods at the ninth abdominal segment of the female pupae close to the urogomphus, which are absent from the male ones (Fursov and Cherney 2018).

Adults

They are large (38- to 57-mm body length) with elongated body and filiform antennae (Fig. 2D). The surface of the elytra is punctuated with nine rows of bristle-bearing punctures. Adults can live up to 6 mo (Fursov and Cherney 2018).

Rearing

Information on the dietary requirements of *Z. morio* is available in several studies. Larvae are commonly produced on wheat bran alone (Quennedey et al. 1995, Aribi et al. 1997) or supplemented with various cereal grains (e.g., oat) or other related amylaceous commodities (Maciel-Vergara et al. 2018). A moisture source, e.g., fruit peelings (Quennedey et al. 1995), carrots (Van Broekhoven et al. 2015), or other organic materials with high water content, is

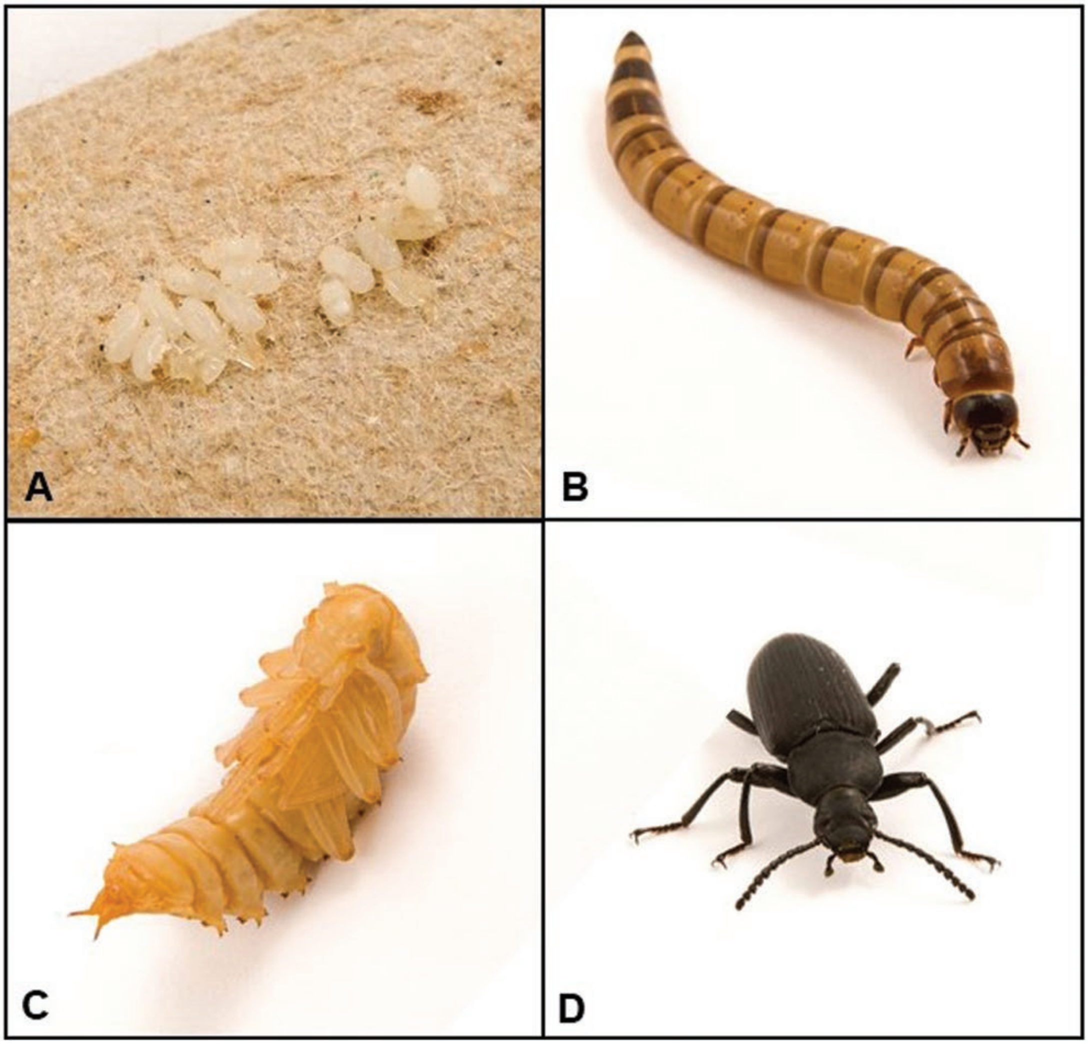

Fig. 2. Life stages of the superworm, *Zophobas morio*, from egg to adult: (A) eggs, (B) late-instar larva, (C) pupa, and (D) adult.

provided to larvae and adults in order to cover their water needs, as larvae deprived of water exhibit strong cannibalistic behavior (Ichikawa and Kurauchi 2009). Lately, there has been an increasing interest in the valorization of organic side-streams for the rearing of *Z. morio* (Van Broekhoven 2015, Van Broekhoven et al. 2015, Harsányi et al. 2020). For instance, Van Broekhoven et al. (2015) reported that *Z. morio* larvae could grow successfully on most diets tested composed of spent grains and beer yeast, bread and cookie remains, potato steam peelings, and maize distillers' dried grains. In contrast, the dietary inclusion of vegetable and garden waste, as well as of cattle and horse manure resulted to reduced growth compared with chicken feed used as control diet (Harsányi et al. 2020).

Regarding rearing conditions, temperatures ranging between 25 and 28°C and an average relative humidity of 60–70% are commonly used for *Z. morio* production. Although high growth rates have been reported for *Z. morio* larvae (Zaelor and Kitthawee 2018), these are reduced under crowding conditions (VandenBrooks et al. 2020). Cannibalism occurs often in *Z. morio* (Tschinkel 1981, Ichikawa and Kurauchi 2009), negatively affecting biomass production and yield, although it does not appear to be density-dependent (Zaelor and Kitthawee 2018). Moreover, when talking about commercial large-scale production, care should be taken to avoid disease outbreaks that could totally devastate the insect cultures. Information about diseases of this species is limited, however, there are few reports that start providing insight into the occurrence of infectious microbial agents of *Z. morio* (Liu et al. 2012, Bakonyi et al. 2015, Maciel-Vergara et al. 2018, Tokarev et al. 2019). The cannibalistic behavior of *Z. morio* larvae seems to further enhance the transmission of microbial agents, offering additional entry routes to pathogens (Maciel-Vergara et al. 2018). Regarding mass production methods for *Z. morio*, available information on the efficient, cost-effective and sustainable large-scale production of this species is rather limited, in contrast to other insect species, such as *T. molitor* and *H. illucens*, whose industrial production has attracted already considerable attention. Still, these methods are not expected to be sufficiently different than those that are currently in use for the relative *T. molitor*.

Nutritional Value

Studies of the nutritional profile of *Z. morio* larvae have shown its high nutritional value (Barker et al. 1998; Finke 2002, 2007, 2015; Barroso et al. 2014; Bosch et al. 2014; Adámková et al. 2016, 2017; Araújo et al. 2019). Larvae are rich in nitrogen, as their total nitrogen content ranges between 6.2 and 8.6% (Table 1), whereas it is not affected by their age (Kulma et al. 2020b). Not surprisingly, a higher total nitrogen content (10.8%) was estimated for defatted flour from *Z. morio* larvae (Botella-Martínez et al. 2020). We avoid intentionally in this review to refer to protein content, since no protein-to-nitrogen conversion factor (Kp) has been proposed for *Z. morio* individuals, as is the case for other related species, such as *T. molitor* and *A. diaperinus* (Janssen et al. 2017, Boulos et al. 2020). Therefore, the Jones' default nitrogen-to-protein conversion factor of 6.25 that all studies have used so far to convert nitrogen content to protein may overestimates the body protein content due to the chitin nitrogen of *Z. morio* individuals. For larvae, the chitin content has been reported to be 3.9–6% (Adámková et al. 2017, Soon et al. 2018, Shin et al. 2019, Benzertiha et al. 2020, Kulma et al. 2020b). A full physicochemical characterization of chitin, as well as its derivative chitosan, from *Z. morio* larvae is provided by Soon et al. (2018). The amino acid profile of *Z. morio* larvae contains relatively high amounts of all essential amino acids, with the exception of methionine (Table 2). Regarding fat content, *Z. morio* larvae have a high proportion of lipids, ranging between 35.0 and 43.6% (Table 1), considerably higher that other insect species also considered as nutrient source (Barroso et al. 2014). According to their fatty acid profile, *Z. morio* larvae have high saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) content, with palmitic and oleic acid being the most abundant ones, respectively (Table 3). Among polyunsaturated fatty acids (PUFA), the omega-6 linoleic acid is abundantly found in *Z. morio* larvae (Table 3; Barroso et al. 2014). Apart from their high nitrogen and lipid content, *Z. morio* larvae contain several minerals (Table 4), as well as vitamins (Finke 2002, 2015).

In contrast to larvae, only one study has investigated the nutrient profile of *Z. morio* adults (Oonincx and Dierenfeld 2012). Compared with larvae, adults have a higher nitrogen content (10.9%), which could be attributed mainly to the higher proportion of chitin of the well-sclerotized adults (Finke 2007); however, no information is available so far on the chitin content of adults. In contrast, adult fat content is considerably lower (14.3%) in comparison to larvae, following the general trend according to which larvae are more rich in fat than adults (Kouřimská and Adámková 2016). The limited interest on the exploitation of *Z. morio* adults as nutrient source may be attributed to specific traits of these beetles that render their consumption unfavorable. For instance, similarly to other tenebrionid beetles such as *T. molitor* (Attygalle et al. 1991), *Z. morio* adults produce, as a chemical defense mechanism, various volatile secretions through their abdominal glands, mainly quinones (Tschinkel 1969, Hill and Tschinkel 1985), which have an undesirable odor and taste (Belluco et al. 2013). Additionally, time-to-harvest is more increased for adult beetles compared with larvae, requiring more feed, energy and space to rear them up to

Table 1. Proximate composition of *Zophobas morio* larvae and adults

	Larvae[a]	Adults[b]
Dry matter (% as fed)	35.2–42.1	38.2
Total nitrogen (% DM)	6.2–8.6	10.9
Crude fat (% DM)	35.0–43.6	14.3
Ash (% DM)	2.4–8.2	6.2
Neutral detergent fiber (NDF) (% DM)	9.3–13.0	50.1
Acid detergent fiber (ADF) (% DM)	6.3–6.5	32.1
Energy (kcal/100 g DM)	559.2–575.5	n.r.

(n.r.) not reported

[a] Values show the range of mean values from published sources (Barker et al. 1998; Finke 2002, 2007, 2015; Yi et al. 2013; Barroso et al. 2014; Bosch et al. 2014; Adámková et al. 2016, 2017; Araújo et al. 2019).

[b] Source: Oonincx and Dierenfeld (2012).

Table 2. Amino acid content (% DM) of *Zophobas morio* larvae

Arginine	2.2–3.5	Valine	2.4–3.4
Histidine	1.4–2.3	Alanine	3.4–4.0
Leucine	3.4–4.5	Aspartic acid	3.8–4.7
Lysine	2.4–2.9	Glycine	2.3–3.0
Isoleucine	2.2–2.4	Serine	2.2–2.7
Phenylalanine	1.6–2.2	Proline	2.6–3.7
Methionine	0.5–1.0	Cystine	0.4–0.5
Threonine	1.9–2.0	Glutamic acid	5.7–6.6
Tryptophan	0.4–0.5	Tyrosine	3.3–3.9

Values show the range of mean values from published sources (Finke 2002, 2007, 2015; Bosch et al. 2014).

Table 3. Fatty acid composition (% DM and % of total fatty acids) of *Zophobas morio* larvae

Fatty acid	% DM	% of Total fatty acids
Palmitic (C16:0)	9.7–12.5	29.1–32.4
Palmitoleic (C16:1)	0.2–0.4	1.0–3.2
Stearic (C18:0)	3.0–3.1	6.4–8.8
Oleic (C18:1)	11.6–15.7	31.1–38.0
Linoleic (C18:2)	7.1–7.8	15.6–23.4
Saturated (SFA)	–	38.8–44.6
Monounsaturated (MUFA)	–	32.1–42.4
Polyunsaturated (PUFA)	–	15.7–24.0
Omega-6	–	16.5–24.0

Values show the range of mean values from published sources (Finke 2002, 2015; Barroso et al. 2014; Adámková et al. 2016, 2017; Kierończyk et al. 2018; Araújo et al. 2019).

Table 4. Mineral composition (mg/100g DM) of *Zophobas morio* larvae and adults

Mineral	Larvae[A]	Adults[b]
Calcium	31.9–70.8	60.0
Phosphorus	562.9–564.9	710.0
Magnesium	39.2–118.3	150.0
Sodium	104.1–112.8	180.0
Potassium	750.6–773.0	970.0
Chloride	361.1–440.5	n.r.
Iron	2.3–5.4	9.2
Zinc	2.5–8.2	8.3
Copper	0.5–1.0	1.5
Manganese	0.5–1.0	2.2
Aluminum	4.6	n.r.

(n.r.) not reported.

[a] Values show the range of mean values from published sources (Finke 2002, 2015; Araújo et al. 2019).

[b] Source: Oonincx and Dierenfeld (2012).

their adulthood (Liu and Zhao 2019), and generally increasing production cost. Therefore, the potential of *Z. morio* adults as nutrient source is limited in comparison to larvae. However, adults could be utilized for other applications, e.g., chitin extraction.

The variability observed among the results of the different studies regarding the body composition of *Z. morio* individuals may be due to the various diets used for its rearing (Payne et al. 2016, Oonincx and Finke 2020). Several studies with other insect species have shown that diet is a major determinant with regard to the insect body nutrient composition (Oonincx and van der Poel 2010, Danieli et al. 2019). Although data on the diet impact on *Z. morio* composition is limited, Latney et al. (2017) showed variations in the calcium and phosphorus content, as well as the metabolizable energy of *Z. morio* larvae, fed on four commercially available diets with different nutritive profiles, indicating the potential to manipulate their body composition by adjusting the feed based on the end-user requirements.

Utilization as a Nutrient Source

Fish

Although not listed in EU Regulation 2017/893 and therefore not being officially authorized for inclusion in aquafeeds in EU, a number of studies have evaluated the effect of the partial replacement of fishmeal with *Z. morio* meal in fish feeding trials (Table 5), as its nutrient profile easily meets the basic fish nutrient requirements. Particularly, apart from being a high protein source, *Z. morio* meal contains adequate quantities of the most limiting amino acids in fish diets, i.e., lysine, threonine, and arginine, with the exception of methionine, in which it is deficient (Table 2). In a recent study, 15 and 30% dietary inclusion of full-fat *Z. morio* larvae meal in replacement of soybean meal and soybean oil did not impact the survival and growth performance of Nile tilapia [(*Oreochromis niloticus* (L.)] juveniles (Alves et al. 2020). However, in the same study, the fish body composition was altered by insect meal inclusion, as fish fed on the diets containing 30% *Z. morio* meal had higher moisture and lipid contents and lower ash and protein contents, as compared with fish that were fed on the control diet. Moreover, the inclusion of *Z. morio* in aquafeeds may positively influence several innate immunity parameters (Alves et al. 2020). Regarding the digestibility of *Z. morio* meal, Fontes et al. (2019) assessed the nutrient and energy apparent digestibility coefficients (ADCs) from a *Z. morio*-based diet at a 20% inclusion level for Nile tilapia. According to their findings, the *Z. morio* meal-based diet, together with the *T. molitor* meal-based diets, showed higher ADCs for energy and dry matter compared with the other diets tested containing insect meals of the speckled cockroach, *Nauphoeta cinerea* (Olivier) (Blattoidea: Blaberidae), the hissing cockroach, *Gromphadorhina portentosa* (Schaum) (Blattodea: Blaberidae), and *G. assimilis*. Specifically, *T. molitor* and *Z. morio*-based diets showed dry matter ADC close to the reported values for fishmeal and soybean meal for Nile tilapia (Fontes et al. 2019). At a 30% inclusion level, the *Z. morio*-based diet digestibility of dry matter, protein and lipid was significantly lower than fishmeal digestibility for Nile tilapia juveniles (Jabir et al. 2012a). When higher replacement levels or total fishmeal replacement were evaluated in diets for Nile tilapia juveniles, feed utilization, and body composition was not affected by up to 25% replacement; however, higher replacement exerted an adverse effect on growth parameters, and this effect was attributed by the authors to the deficiency of these diets with reduced fishmeal levels to one or more essential amino acids present in fishmeal (Jabir et al. 2012b). To further improve the suitability of *Z. morio* meal diets, Jabir et al. (2012c) supplemented a Nile tilapia diet in which 50% of fishmeal was replaced by *Z. morio* meal with various levels of mushroom stalk meal (10, 15, and 20%) as a prebiotic and reported that 10% mushroom stalk meal inclusion positively affected growth performance and particularly weight gain. Similar conclusions were also drawn for the suitability of hydrolyzed *Z. morio* meal for sea trout (*Salmo trutta* m. *trutta* L.) fingerlings, as 10% hydrolyzed *Z. morio* meal inclusion ensured high survival rates and satisfactory growth performance and feed utilization parameters (Mikołajczak et al. 2020). Recently, feeding trials with the gilthead sea bream (*Sparus aurata*) have shown that *Z. morio* meal, even as full-fat or defatted, can be included in its diet at high inclusion levels replacing fishmeal protein up to 30% without any negative effects on fish growth performance and feed utilization (Asimaki et al. 2020, Karapanagiotidis 2020, personal communication).

Poultry and Pigs

Besides aquafeeds, the inclusion of *Z. morio* meal or oil in poultry diets has lately attracted considerable interest (Table 6). For instance, Benzertiha et al. (2020) studied the effect of the supplementation of broiler chicken diets with small amounts (0.3%) of *Z. morio* larvae full-fat meal, added ‘on top’ of a complete diet or calculated into diets, on the growth performance and selected

Table 5. Studies on the inclusion of *Zophobas morio* larvae meal (ZM) in fish diets

Animal species	Life stage	Duration	% dietary inclusion	Main outcome	Reference
Oreochromis niloticus (Nile tilapia)	Fingerlings	56 d	30%	Reduced digestibility of dry matter, protein and lipid of ZM-based diets compared to fishmeal digestibility	Jabir et al. (2012a)
Oreochromis niloticus (Nile tilapia)	Fingerlings	56 d	7.5, 15, 22.5, and 30% (25, 50, 75, and 100% replacement of fishmeal)	Up to 25% of fishmeal can be replaced by ZM without any adverse effect on feed utilization and body composition	Jabir et al. (2012b)
Oreochromis niloticus (Nile tilapia)	Fingerlings	56 d	15% (50% fishmeal replacement) + 10, 15 and 20% of mushroom stalk meal	Diet amendment with mushroom stalk meal improved fish growth performance and survival	Jabir et al. (2012c)
Oreochromis niloticus (Nile tilapia)	Fingerlings	56 d	20%	ZM showed dry matter ADC close to the values reported for fishmeal and soybean meal for Nile tilapia	Fontes et al. (2019)
Oreochromis niloticus (Nile tilapia)	Fingerlings	84 d	15 and 30% (50 and 100% replacement of soybean meal	No negative effects on fish growth performance. Lipid content increase and protein content decrease in fish fed the 30% ZM-based diet	Alves et al. (2020)
Salmo trutta m. *trutta* (sea trout)	Fingerling	56 d	10% of hydrolyzed ZM (44% fishmeal replacement)	No adverse impacts on growth performance, feed utilization or gut histomorphology	Mikołajczak et al. (2020)
Sparus aurata (sea bream)	Fingerlings	100 d	5 and 10% (4.4 and 9.4% fishmeal replacement)	High survival rates. No adverse effect on feed intake, final weights, specific growth rates and FCR	Asimaki et al. (2020)

blood, and immune system traits of the birds. The authors reported a positive effect on the body weight gain and feed intake, moreover, there was a positive effect on the level of plasma immunoglobulins, i.e., IgY and IgM. In a similar study, Benzertiha et al. (2019) evaluated the effect of *Z. morio* full-fat meal added in small amounts (0.2 and 0.3%) to a complete diet on the coefficients of apparent ileal digestibility, pancreatic enzyme activity, short-chain fatty acid concentrations, bacterial enzymes, and microbiota community in the cecal digesta of broiler chickens and they did not report any negative effects on the nutrient ileal digestibility coefficients or the activity of pancreatic enzymes. In the same study, dietary inclusion of *Z. morio* full-fat meal was capable of improving the health status of the birds by reducing pathogenic bacterial concentrations, such as those of the *Bacteroides–Prevotella* cluster and *Clostridium perfringens*. In addition, this small amount of supplementation stimulated the gastrointestinal tract microbiota to produce enzymes, especially glycolytic enzymes. Similarly, the addition of small amount of *Z. morio* meal (0.2 and 0.3%) in broiler diets has a prebiotic effect, as it increases the relative abundance of probiotic and commensal bacteria such as Actinobacteria in the cecal microbiome that act protectively against infections with pathogenic bacteria (Józefiak et al. 2020). When soybean oil, the most commonly used energy source ingredient in poultry diets, was totally replaced by oil obtained using super-critical CO_2 extraction from *Z. morio* larvae, no adverse impact on the growth performance of broiler chicken and nutrient digestibility was noted (Kierończyk et al. 2018).

Regarding pigs, only one study has evaluated to date the effect of *Z. morio*-based diets on these livestock animals. Briefly, Liu et al. (2020) studied the effect of the supplementation of weanling piglet diets with 5% *Z. morio* powder, and reported improved amino acid transportation in the intestine of pigs fed on the *Z. morio* containing diets compared with the control corn–soybean basal diet.

Food

Additionally to the feed applications, the incorporation of *Z. morio* larvae in food products has recently attracted scientific interest. Scholliers et al. (2019) explored the formulation of batter containing larvae from three insect species, i.e., *T. molitor*, *A. diaperinus* and *Z. morio*, and concluded that based on the quality characteristics of the tested insect-based products *Z. morio* larvae show more potential for food applications. Similarly, Scholliers et al. (2020a, b) studied the properties of hybrid meat products containing *Z. morio* larvae to provide valuable insight into the composition and processing of insect:meat applications and paving the way for the inclusion of *Z. morio* larvae in insect-based food products.

Other Applications

Apart from an alternative nutrient source, *Z. morio* has been recently shown to be capable of eating, biodegrading and mineralizing various types of plastics, as polystyrene or polyethylene (Miao and Zhang 2010, Choi et al. 2020, Kim et al. 2020a, Li et al. 2020, Peng et al. 2020, Xu et al. 2020, Yang et al. 2020). For instance, Yang et al. (2020) demonstrated that *Z. morio* larvae could exclusively be fed on styrofoam at a four-fold higher rate than the other plastic eating tenebrionid *T. molitor*, and could ingest long-chain plastic molecules and depolymerize them into low molecular-weight degraded compounds. Furthermore, it is suggested that the larval gut microbiota contributes to plastic degradation, as the plastic-degrading capability of the larvae was inhibited when gut microbiota was suppressed by antibiotic treatment (Peng et al. 2020, Yang et al. 2020). In an effort to screen the plastic-degrading microbes of the larval gut microbiota, several bacterial strains (e.g., *Pseudomonas*) have been isolated and is believed that are associated with the plastic-degrading ability of *Z. morio* larvae (Kim et al. 2020a, Li et al. 2020, Xu et al. 2020). These new findings are of high importance for plastic waste

Table 6. Studies on the inclusion of *Zophobas morio* larvae meal (ZM) in poultry diets

Animal species	Life stage	Duration	% Dietary inclusion	Main outcome	Reference
Broiler chicken (Ross 308)	1-d old	28 d	5% replacement of soybean oil by ZM oil	Similar or better growth performance results compared to the soybean diet	Kierończyk et al. (2018)
Broiler chicken (Ross 308)	1-d old	35 d	0.2 and 0.3% amendment with ZM meal	Body weight gain and feed intake increase in dietary groups supplemented with ZM	Benzertiha et al. (2019)
Broiler chicken (Ross 308)	1-d old	35 d	0.2 and 0.3% amendment with ZM meal	Improved growth performance and changes in selected immune system traits	Benzertiha et al. (2020)
Broiler chicken (Ross 308)	1-d old	35 d	0.2 and 0.3% amendment with ZM meal	Commensal and probiotic microbiome composition modulated in the cecum. Increase of the relative abundance of positive bacteria	Józefiak et al. (2020)
Broiler chicken (Ross 308)	1-d old	7d	0.4% of a fermented with *Lactobacillus plantarum* and *Saccharomyces cerevisiae* mixture of 30% ZM larvae, 35% DDGS[a], and 35% defatted rice bran	Increase of average daily gain and Immunoglobulin G and A levels, reduction of FCR, mortality and cecal *E. coli* and *Salmonella* spp. contents	Islam and Yang (2017)

[a] Distiller's dried grains with solubles.

management and could offer a reliable solution to the problem of plastic accumulation, which represents a global issue of major environmental importance.

The spectrum of *Z. morio* applications is continuously growing. Recently, Du et al. (2020) showed that *Z. morio* hemolymph can effectively protect bovine mammary epithelial cells against bacterial infections and proposed *Z. morio* hemolymph as an efficient, alternative therapeutic candidate for bovine mastitis, the most prevalent disease affecting the dairy industry worldwide. Additionally, this species is often used as a model organism. Due to the fact that it is easily reared and is rich in fat, Gołębiowski et al. (2020) used *Z. morio* larvae to study the effect of the entomopathogenic fungus *Metarhizium flavoviride* (Gams and Rozsypal) (Hypocreales: Clavicipitaceae) on the fat body lipid composition of insects, and reported qualitative and quantitative changes in the profiles of lipids in larvae of *Z. morio* due to the fungal infection. Previously, this species was used to study lipid metabolism and the endocrinological system of insects (Gołębiowski et al. 2014), offering a valuable model organism to study various aspects of insect physiology.

Future Research and Challenges

Based on the above, the utilization of *Z. morio* as an alternative nutrient and protein source holds promises for the future. However, an essential prerequisite for the successful exploitation of this species as food and feed is the adjustment of the legislative framework that regulates the use of insects in food and feed applications. Within EU, a first step could be the inclusion of *Z. morio* in the list of insect species that are allowed by EU Regulation 2017/893 to be included in aquafeeds, whereas the approval of the insect use in poultry and swine diets, including *Z. morio* in the permitted species, would further boost its utilization.

The route to the better exploitation of *Z. morio* comprehend several challenges. Apart of constituting solely a protein and nutrient source, the challenge is to illustrate the functional properties of *Z. morio*-derived diets. Zielińska et al. (2017) suggested that together with other insect species, *Z. morio* larvae are a valuable, largely unexploited source of antimicrobial peptides with antiradical activity, therefore, their consumption could potentially have an immune-triggering and health promoting effect. Similarly, Islam and Yang (2016) proposed the use of probiotics from *Z. morio* larvae as alternatives to antibiotics in broiler chicken. However, further research is warranted to illuminate and unfold this potential. The prebiotic effect of other *Z. morio*-derived substances which are known to have antimicrobial activity, e.g., chitin, could be on the focus of future research toward this direction.

Safety issues should also been addressed with regard to the use and exploitation of *Z. morio*, in order to ensure food and feed safety. Studies on the potential microbiological risks that have to be considered should be prioritized. Grabowski and Klein (2016) evaluated the microbiological quality of *Z. morio* larvae processed with four different drying techniques in order to identify the methods that can ensure food quality and safety. Similarly, an evaluation of the safety of freeze-dried skimmed powder of *Z. morio* larvae revealed no adverse effect in oral toxicity tests in rats at doses up to 5,000 mg/kg/d (Kim et al. 2020b). Such safety data should be examined into more detail and be adequately provided, in order to illustrate potential risks that may endanger human and animal health. The same counts for potential *Z. morio*-related allergic responses that could be generated through consumption or inhalation of airborne insect-derived material (Freye et al. 1996). However, the safety concerns related to *Z. morio* applications are not expected to be higher than the ones described for other insect species currently commercially exploited (Van der Fels-Klerx et al. 2018).

To conclude, the so far available data classify *Z. morio* as a promising insect-based nutrient provider with great potential and future perspectives. Its comparative evaluation together with other insect species has shown that *Z. morio* can adequately offer an alternative to the species commonly used for this purpose so far (Van Broekhoven et al. 2015, Adámková et al. 2016, Araújo et al. 2019). Apart from its utilization in food and feed, it seems that this species can also be an effective waste management agent. Further research is needed to fully unfold the potential applications of this species and optimize its farming systems at an industrial scale. Considering that its relative *T. molitor* is already included in the list of 'EU-authorized' insect species for use in aquafeeds, future research should also focus on potential risk assessment aspects that can be further utilized by regulatory and legislative authorities, toward the practical exploitation of this species for the applications mentioned in this work. *Zophobas morio* has the full potential of playing a significant role in the future of insects as food and feed and research on this species should emphasize on highlighting the advantages of its use and shading light on unexplored aspects that need to be considered.

Author Contributions

Both authors contributed equally to the conceptualization and writing of this review paper.

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