

A Review of the Scientific Literature for Optimal Conditions for Mass Rearing *Tenebrio molitor* (Coleoptera: Tenebrionidae)

Author(s): Nuno Ribeiro, Manuela Abelho, and Rui Costa

Source: Journal of Entomological Science, 53(4):434-454.

Published By: Georgia Entomological Society

<https://doi.org/10.18474/JES17-67.1>

URL: <http://www.bioone.org/doi/full/10.18474/JES17-67.1>

BioOne (www.bioone.org) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

A Review of the Scientific Literature for Optimal Conditions for Mass Rearing *Tenebrio molitor* (Coleoptera: Tenebrionidae)¹

Nuno Ribeiro², Manuela Abelho³, and Rui Costa²

Polytechnic Institute of Coimbra, Coimbra College of Agriculture, Bencanta, 3045-601, Coimbra, Portugal

J. Entomol. Sci. 53(4): 434–454 (October 2018)

Abstract Several insect species are mass produced for animal feeds, fish bait, or human consumption. The yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), is one of the more-popular insects considered for this use. To make *T. molitor* competitive with more-traditional protein sources, mass production must be optimized. Herein, we review the scientific literature on the range and optimal environmental, physical, and dietary conditions for the rearing *T. molitor*. Our review identified that: (a) the optimum temperature for *T. molitor* growth is 25–28°C; (b) *T. molitor* larval growth rate is greatest at $\geq 70\%$ relative humidity (RH) with an optimum range between 60–75% RH, and; (c) optimal growth is achieved with diets containing 5–10% yeast, 80–85% carbohydrate, and the addition of B-complex vitamins.

Key Words *Tenebrio molitor*, temperature, relative humidity, diet, rearing

The rapid growth of the world population has forced consideration of nontraditional food sources to achieve food security, preserve natural resources, and provide for sustainable development. Since van Huis et al. (2013) promoted mass production of insects for food and feed, there has been a growing movement worldwide, and several “start-up” companies have been established for mass production of insects as an alternative source of protein.

Only a few insect species have been commercially mass produced for a protein source, either in an industrial or a “cottage” setup. These have been the black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), the common housefly, *Musca domestica* L. (Diptera: Muscidae), silkworms, *Bombyx mori* L. (Lepidoptera: Bombycidae), several species of termites (Isoptera), several species of grasshoppers and locusts (Orthoptera), and the yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae).

Tenebrio molitor is relatively easy to culture. Small producers have been rearing it in Europe and elsewhere, either for animal feed, fish bait, or human consumption. They generally culture the insects in trays with a substrate where the different stages develop separately. This process is highly dependent on manual labor, as the feeding remains and waste materials must be separated from the insects by

¹Received 27 May 2017; accepted for publication 30 January 2018.

²Polytechnic Institute of Coimbra, ESAC, CERNAS, Bencanta, 3045-601 Coimbra, Portugal.

³Corresponding author (email: abelho@esac.pt). Jointly appointed to Coimbra College of Agriculture (Polytechnic Institute of Coimbra) and the Centre for Functional Ecology (Department of Life Sciences, University of Coimbra).

sieving the contents of the trays. Although the process can be mechanized (Cortes Ortiz et al. 2016; Onincx and de Boer 2012), the use of insects for feed or food will only be a reality if production costs are competitive when compared to production of other, more-traditional protein sources (e.g., soybean, fishmeal). Optimization of production costs may be achieved by using low-cost food sources, automated rearing techniques, and optimal growth conditions.

To our knowledge, no single paper contains all the information related to the optimal conditions for the rearing *T. molitor*. Our objective here was to review and compile the studies identifying the range and optimal environmental, physical, and dietary conditions for rearing *T. molitor*. We have divided our review of the pertinent literature into four areas of: (1) biology and life cycle; (2) environmental conditions; (3) diet components, and; (4) nutritive value and use.

Biology and Life Cycle

Tenebrio molitor feeds primarily on farinaceous materials such as flour and meal. It is considered a pest where these are produced and stored. Females produce an average of 250–500 eggs which they oviposit singly or in small clusters attached to the substrate or the walls and floor of the containers in which they are oviposited (Cotton 1927; Ghaly and Alkoaik 2009; Hardouin and Mahoux 2003; Hill 2002; Manojlovic 1987; Spencer and Spencer 2006;). Eggs hatch in 4 d at 26–30°C (Cotton 1927; Siemianowska et al. 2013) but may take up to 34 d to hatch at 15°C (Kim et al. 2015). Duration of the larval stage varies from 57 d in controlled conditions (Weaver and McFarlane 1990) to 629 d in ambient natural conditions (Cotton 1927), with an average duration of 112–203.3 d (Damborsky et al. 2000; Martin et al. 1976). Larvae undergo several molts, varying from a minimum of 9 (Cotton 1927; Hill 2002) to a maximum of 23 (Ludwig 1956), with an average of 11–19 instars (Damborsky et al. 2000; Ludwig 1956). The number and the duration of the larval instars obviously influences the total duration of the life cycle of *T. molitor*.

As larvae approach pupation, they enter a short period of latency in which they appear as a “C” shape before turning into a pupa. The pupal stage lasts 6 (Cotton 1927; Ghaly and Alkoaik 2009) to 20 d (Hill 2002). Adults first emerge as whitish-colored beetles with soft exoskeletons, which gradually harden and darken. Mating and oviposition begin in about 3 d after emergence (Manojlovic 1987), and the adult stage lasts 16 to 173 d (Damborsky et al. 2000) with an average of 31.8 (Urs and Hopkins 1973a) to 62 d (Damborsky et al. 2000). The entire life cycle occurs in the same habitat or vicinity and, with optimal conditions, lasts 75 (Spencer and Spencer 2006) to 90 d (Hardouin and Mahoux 2003) (Table 1).

Environmental Conditions

Temperature and relative humidity (RH). Temperatures commonly used to rear mealworms are 25–28°C (Kim et al. 2015; Koo et al. 2013; Ludwig 1956; Punzo 1975; Punzo and Mutchmor 1980; Spencer and Spencer 2006.), with an absolute minimum of 10°C (Punzo and Mutchmor 1980) and a maximum of 35°C (Martin et al. 1976; Punzo and Mutchmor 1980). Temperatures <17°C inhibit embryonic development (Koo et al. 2013) and temperatures >30°C increase death rates (Koo

Table 1. Biological and life cycle parameters of *Tenebrio molitor*. Minimum, maximum, and average values for the number of eggs, length, lifespan, duration of the life cycle, and number of larval instars. A dash (—) indicates no data.

	Minimum	Average	Maximum
Number of eggs	77 (Cotton 1927) 250 (Ghaly and Alkoaik 2009)	250 (Spencer and Spencer 2006) 276 (Cotton 1927) 280 (Hardouin and Mahoux 2003) 414 (Manojlovic 1987) 400–500 (Ghaly and Alkoaik 2009)	500 (Hill 2002) 576 (Cotton 1927) 1,000 (Ghaly and Alkoaik 2009)
Length (mm)			
Larvae	20 (Ghaly and Alkoaik 2009) 28 (Hardouin and Mahoux 2003)	16 (Barker et al. 1998) 28 (Hill 2002)	25 (Ghaly and Alkoaik 2009) 31.6 (Park et al. 2014) 32 (Hardouin and Mahoux 2003)
Adult	12 (Hill 2002) 15.5 (Kim et al. 2015)	—	16 (Hill 2002; Kim et al. 2015)
Body mass (mg)			
Larvae	75 (Martin et al. 1976) 130 (Makkar et al. 2014) 140 (Tschinkel and Willson 1971)	111 (Martin et al. 1976) 120 (Connat et al. 1991) 126 (Finke 2002) 191 220 (Kim et al. 2016)	134 (Ramos-Elorduy et al. 2002) 145 (Martin et al. 1976) 160 (Makkar et al. 2014) 182.7 (Ghaly and Alkoaik 2009) 190 (Tschinkel and Willson 1971) 220 (Kim et al. 2016)
Adult	—	136 (Finke 2002)	—

Table 1. Continued.

	Minimum	Average	Maximum
Duration (d)			
Lifespan (complete cycle)	75 (Spencer and Spencer 2006)	80–83.7 (Park et al. 2012)	90 (Spencer and Spencer 2006)
	90 (Hardouin and Mahoux 2003)	189 (Urs and Hopkins 1973a)	120 (Hardouin and Mahoux 2003)
	181 (Urs and Hopkins 1973a)	294 (Damborsky et al. 2000)	196 (Urs and Hopkins 1973a)
	280 (Hill 2002)		630 (Hill 2002)
Egg stage	4 at 26–31°C (Cotton 1927)	7 (Hardouin and Mahoux 2003; Li et al. 2016b)	10 (Spencer and Spencer 2006)
	4–6 (Siemianowska et al. 2013)	7.55 at 25°C (Manojlovic 1987)	12 (Hill 2002)
	5 at 35°C (Kim et al. 2015)	7–8 (Park et al. 2014)	15 (Damborsky et al. 2000)
	7 (Damborsky et al. 2000)	9.2 (Damborsky et al. 2000)	19 at 18–21°C (Cotton 1927)
	10 (Hill 2002)	12.6 at 20°C (Manojlovic 1987)	34 at 15°C (Kim et al. 2015)
		15 (Ghaly and Alkoaik 2009)	
Larval stage	57 (Weaver and McFarlane 1990)	112 (Martin et al. 1976)	202.5 (Urs and Hopkins 1973a)
	87.7 (Urs and Hopkins 1973a)	120 at 25°C (Ludwig 1956)	216 (Kim et al. 2015; Morales- Ramos et al. 2015)
	110.8 at 30°C (Koo et al. 2013)	151.15 (Urs and Hopkins 1973a)	
	120 (Hill 2002)	173 at 30°C (Ludwig 1956)	240 (Ghaly and Alkoaik 2009)
	127 (Kim et al. 2015)	180 (Cotton 1927)	244 at 17°C (Koo et al. 2013)
	129 (Morales- Ramos et al. 2015)	203.3 (Damborsky et al. 2000)	540 (Hill 2002)
	143 (Morales- Ramos et al. 2010)	241.9 (Park et al. 2014)	629 in nature (Cotton 1927)
	180 (Ghaly and Alkoaik 2009)		
	280 in nature (Cotton 1927)		

Table 1. Continued.

	Minimum	Average	Maximum
Pupal stage	6 at 27°C (Cotton 1927)	5–6 (Siemianowska et al. 2013)	10.1 (Urs and Hopkins 1973a)
	6 at 28°C (Ghaly and Alkoaik 2009)	7 (Hardouin and Mahoux 2003)	18 at 18°C (Cotton 1927; Ghaly and Alkoaik 2009)
	6.51 at 27.5°C (Kim et al. 2015)	7.3 (Morales-Ramos et al. 2010)	20 (Hill 2002; Kim et al. 2015)
	7.7 (Urs and Hopkins 1973a)	7.6 (Damborsky et al. 2000)	
		8.9 (Urs and Hopkins 1973a)	
	9 (Hill 2002)		
Adult	16 (Damborsky et al. 2000)	31.8 (Urs and Hopkins 1973a)	60 (Hill 2002)
	25 (Urs and Hopkins 1973a)	61.5 (Cotton 1927)	94 (Cotton 1927)
	30 (Hill 2002)	62.05 (Damborsky et al. 2000)	96 (Ghaly and Alkoaik 2009)
	37 (Ghaly and Alkoaik 2009)		105 (Urs and Hopkins 1973a)
	38 (Cotton 1927)		173 (Damborsky et al. 2000)
Number of larval instars	9 (Cotton 1927; Hill 2002)	11 (Damborsky et al. 2000)	15 at 25°C (Ludwig 1956)
	11 at 25°C (Ludwig 1956)	13 (Koo et al. 2013)	15 (Morales-Ramos et al. 2010)
	12.2 (Urs and Hopkins 1973a)	13.2 at 25°C (Ludwig 1956)	
	14 (Morales-Ramos et al. 2015)	14 (Morales-Ramos et al. 2010)	16.1 (Urs and Hopkins 1973a)
	15 at 30°C (Ludwig 1956)	14.15 (Urs and Hopkins 1973a)	18 (Morales-Ramos et al. 2015)
		15 (Hardouin and Mahoux 2003)	20 (Cotton 1927; Hill 2002; Park et al. 2014)
		15–17 (Park et al. 2014)	
		17–19 (Cotton 1927)	23 at 30°C (Ludwig 1956)
	19.1 at 30°C (Ludwig 1956)		

Table 2. Minimum, maximum, and optimal temperature (°C) conditions used on the rearing of eggs, larvae, pupae, and adults of mealworms.

	Minimum	Optimal	Maximum
Eggs	10 (Punzo and Mutchmor 1980) 15 (Kim et al. 2015) 17 (Koo et al. 2013)	23–27 (Manojlovic 1987) 25 (Murray 1960, 1968; Punzo and Mutchmor 1980) 25–27 (Siemianowska et al. 2013)	30 (Manojlovic 1987) 35 (Kim et al. 2015; Punzo and Mutchmor 1980)
Larvae	10 (Punzo and Mutchmor 1980) 17 (Koo et al. 2013) 20 (Martin et al. 1976)	25 (Ludwig 1956; Murray 1960, 1968; Punzo 1975; Punzo and Mutchmor 1980) 27–28 (Kim et al. 2015; Koo et al. 2013; Spencer and Spencer 2006)	30 (Koo et al. 2013; Ludwig 1956) 35 (Martin et al. 1976; Punzo and Mutchmor 1980)
Pupae	10 (Punzo and Mutchmor 1980) 18 (Cotton 1927) 21 (Hardouin and Mahoux 2003)	25 (Murray 1960, 1968; Punzo 1975) 27 (Cotton 1927) 27.5 (Kim et al. 2015) 28 (Ghaly and Alkoaik 2009) 27–33 (Hardouin and Mahoux 2003)	35 (Punzo and Mutchmor 1980)
Adult	10 (Punzo and Mutchmor 1980) 14–16 (Dick 1937)	25 (Murray 1960, 1968; Punzo 1975)	35 (Punzo and Mutchmor 1980)

et al. 2013; Ludwig 1956). The minimal and maximum lethal temperatures for exposure periods of 24 h are 7–8°C (Mutchmor and Richards 1961) and 40–44°C (Altman and Dittmer 1973; Martin et al. 1976), respectively. There are no significant differences in the temperature requirements for the different stages of development of this species (Table 2).

Mealworm response to RH varies, although their resilience depends largely on temperature (Table 3). In all studies focused on the influence of RH on the development of *T. molitor*, optimum values varied from 60% (Manojlovic 1987) to 75% RH (Punzo 1975; Punzo and Mutchmor 1980).

Temperature combined with RH affects both the number and the length of the instars and also influences the water absorption capacity of the different stages of the life cycle. The pupal stage is the most resilient to extreme conditions of

Table 3. Minimum, maximum, and optimal relative humidity (%) conditions on the rearing of eggs, larvae, pupae, and adults of mealworms.

	Minimum	Optimal	Maximum
Eggs	12 (Punzo 1975; Punzo and Mutchmor 1980)	60–75 (Manojlovic 1987) 70 (Murray 1960, 1968) 75 (Punzo and Mutchmor 1980)	98 (Punzo 1975; Punzo and Mutchmor 1980)
Larvae	12 (Punzo 1975; Punzo and Mutchmor 1980) 30 (Fraenkel 1950)	75 (Punzo 1975; Punzo and Mutchmor 1980) 60–70 (Spencer and Spencer 2006) 70 (Fraenkel 1950; Fraenkel and Blewett 1944; Hardouin and Mahoux 2003; Martin et al. 1976; Murray 1960, 1968)	98 (Punzo 1975; Punzo and Mutchmor 1980)
Pupae	12 (Punzo 1975; Punzo and Mutchmor 1980)	70 (Murray 1960, 1968) 75 (Punzo 1975; Punzo and Mutchmor 1980)	98 (Punzo 1975; Punzo and Mutchmor 1980)
Adult	12 (Punzo 1975; Punzo and Mutchmor 1980) 20 (Dick 1937; Hardouin and Mahoux 2003)	70 (Murray 1960, 1968) 75 (Punzo 1975; Punzo and Mutchmor 1980) 90–100 (Hardouin and Mahoux 2003)	98 (Punzo 1975; Punzo and Mutchmor 1980)

temperature and RH while the egg and early larval stages are the most sensitive (Punzo and Mutchmor 1980).

Oviposition does not occur at temperatures below 14°C with an RH of 65%, but even at the optimal temperature of 27°C in low humidity (20%), oviposition is significantly reduced due to the loss of body moisture by evaporation and subsequent death (Dick 1937). Female adult activity is also reported to be greatest at 90–100% RH (Hardouin and Mahoux 2003).

At 10°C (Punzo 1975; Punzo and Mutchmor 1980) and 12.5°C (Kim et al. 2015), water absorption in eggs is reduced and embryological development halts. Whatever the temperature, extremely dry conditions of about 12% RH potentiate water losses from the eggs, culminating in death due to desiccation (Punzo 1975).

The growth rate of *T. molitor* larvae is highly dependent on moisture, with higher growth rates at 90–100% RH (Hardouin and Mahoux 2003) and even at 70% RH, but the rate slows at 30% RH and hardly occurs at 13% (Fraenkel 1950). During times of extremely dry conditions, mealworm larvae may cease feeding and become inactive until RH rises to more-favorable levels (Urs and Hopkins 1973). In

Table 4. Physical conditions and population density used on the rearing of mealworms.

Population Density (Larvae/dm ²)	Tray Dimensions (cm)	Area (dm ²)	Volume (dm ³)	References
8.4				Morales-Ramos et al. 2012
50–150	11 × 18 × 3	1.98	0.6	Connat et al. 1991*
80–145	16 × 21.5 × 7	3.44	2.4	Park et al. 2012
48	48 × 49.5 × 10.5	23.76	25	Park et al. 2014
2	30 × 15	4.50		Spang 2013
30	17.5 × 9.3 × 6.3	1.63	1	Oonincx et al. 2015
30	40 × 30 × 25	12	30	Alves et al. 2016
40–60	40 × 17 × 11	6.80	7.5	Rho and Lee 2016
30	10 (diameter) × 28	0.79	2.2	Dick 1937
3	60 × 45 × 15	27	40.5	Ghaly and Alkoaik 2009
375–750		6		Martin et al. 1976
1–20	10 (diameter)	0.79		Tschinkel and Willson 1971

* The population density was the object of study.

general, the higher the RH, the higher the growth rate, but high levels of moisture favor the growth colony contaminants (e.g., fungi, other microorganisms, mites) (Fraenkel 1950).

Larval instar stages are shorter, and the numbers of instars are greater at 30°C than at 25°C, resulting in a longer total larval developmental period at 30° than at 25°C (Ludwig 1956). Temperatures below 10°C and above 35°C clearly constitute stress conditions for this species, although the minimal lethal temperature is 7°C and the maximum lethal temperature is 44°C (Punzo and Mutchmor 1980).

Tenebrio molitor development and survival are affected by the interaction of temperature and moisture. The effect of temperature is potentiated at extreme moisture conditions, and the effect of moisture is also more critical at extreme temperatures (Punzo and Mutchmor 1980). For example, at optimal temperatures of 25–27.5°C, *T. molitor* displays no stress even under extreme humidity conditions and long exposure periods. Moreover, while at 25°C the decrease in moisture has no significant effects on adults, larvae, or pupae, a temperature of 10°C increases mortality (Punzo and Mutchmor 1980).

Population density. Our search found that several densities of larvae have been used in experiments, depending on the objective of the study. These have ranged from 1 larva/dm² (i.e., a single larva in a 10-cm diameter Petri dish) to a maximum of 750 larva/dm² (Table 4). Morales-Ramos et al. (2012) studied the effect of density on adult population survival and production, obtaining an optimal density of 8.4 adults/dm² for mass production purposes.

Population density influences the number and the duration of the larval molts, with higher population densities resulting in fewer larval instars and smaller-sized penultimate larvae (Connat et al. 1991; Morales-Ramos et al. 2012; Morales-Ramos and Rojas 2015; Tschinkel and Willson 1971; Weaver and McFarlane 1990). Early studies have noted inhibition of pupation and cannibalism associated with crowding in tenebrionid beetles (Tschinkel and Willson 1971) as well as the occurrence of incomplete transformations and slower growth rates due to a reduced feeding opportunity induced by conspecific competition (Weaver and McFarlane 1990). Although high population density leads to a reduction in food consumption, the lower growth rates are also a consequence of the decrease in the efficiencies of both ingested and digested food conversion (Morales-Ramos and Rojas 2015). Overcrowding also reduces female progeny and reproductive output, with rearing of two insects per chamber being the most productive in terms of number of progeny produced per female (Morales-Ramos et al. 2012). Furthermore, overpopulation also affects the prepupal stage, with isolated larvae exhibiting accelerated larval-pupal apolyses and a reduced number of larval instars (Connat et al. 1991). Moreover, the metabolism of larvae in overcrowded populations results in a substantial increase in temperature that can easily be lethal.

Photoperiod. Mealworms are negatively phototropic and phototactic (Balfour and Carmichael 1928; Cloudsley-Thompson 1953), with adults and larger larvae positioning below the surface of the substrate during daylight and coming to surface in darkness. Although photoperiod influences growth and development of mealworms (Tyshchenko and Ba 1986), the response to photoperiod tends to disappear under constant conditions, when *T. molitor* becomes arrhythmic (Cloudsley-Thompson 1953). Recent studies show that larval development was optimal in long-day conditions, with lower development times observed in photoperiods of 14L:10D (Kim et al. 2015). Photoperiod also influences eclosion rates, with higher rates in long-day conditions, i.e., 45.5% at 14L:10D versus 24.2% at 10L:14D (Kim et al. 2015) and pupation with 100% pupation induced by a 12L:12D regime at 25°C. Under other photoperiod regimes, long-day result in more-protracted delays than did shorter-day conditions. However, at 30°C the photoperiodic response was reversed, with an inhibition of pupation under 12L:12D and enhancement under 18L:6D (Tyshchenko and Ba 1986) (Table 5).

Oxygen. Low oxygen concentrations increase larval mortality and hypertrophy of tracheae and other respiratory exchange organs (Greenberg and Ar 1996; Loudon 1988, 1989), and hypoxia conditions (10–10.5% O₂) inhibit growth rate, lead to the development of abnormalities, and to a higher proportion of females in mealworm populations (Loudon 1988). Although development time is similar for both normoxia (21% O₂) and hyperoxia (40% O₂) conditions, hyperoxia induces a lower number of instars, thus resulting in lower final larval biomass than with normoxia (Greenberg and Ar 1996).

Dietary Components and Conditions

Mealworms have the ability to select foods in order to balance their diet ratio/intake according to their nutritional needs (Morales-Ramos et al. 2011; Rho and Lee 2014, 2016; Urrejola et al. 2011). *Tenebrio molitor* can be fed only with bran, which

Table 5. Photoperiod regimes used in the rearing of *Tenebrio molitor*.

Light:Dark (h)	References
12:12	Allen et al. 2012; Gerber and Sabourin 1984; Greenberg and Ar 1996; Kim et al. 2015; Lardies et al. 2014; Lv et al. 2014; Martin et al. 1976; Menezes et al. 2014; Oonincx et al. 2015; Rho and Lee 2014, 2016; Tang et al. 2012; Urrejola et al. 2011; van Broekhoven et al. 2015
Darkness	Morales-Ramos et al. 2013; Oonincx et al. 2010; Punzo 1975; Punzo and Mutchmor 1980
14:10	Kim et al. 2015; Koo et al. 2013; Morales-Ramos et al. 2010; Ravzanaadii et al. 2012
10:14	Alves et al. 2016; Kim et al. 2015; Rho and Lee 2016; Weaver and McFarlane 1990
16:8	Tindwa et al. 2015; Urs and Hopkins 1973a

contains all the necessary nutrients but not in optimal proportions, making diet supplementation beneficial (Morales-Ramos et al. 2010) at different developmental stages. A diet composed of bran and a water source (e.g., fresh vegetables such as carrot, apple, potato, or cabbage) and/or a protein source (e.g., beer yeast, casein, or soy protein) is the most common diet composition in mealworm industry and laboratory rearing facilities (Table 6).

Nutrient intake plays a central role in *T. molitor* life cycle, influencing development time (Davis 1970a; Fraenkel 1950; Martin and Hare 1942; Morales-Ramos et al. 2010, 2013; Rho and Lee 2016; Urrejola et al. 2011; van Broekhoven et al. 2015), fertility (Gerber and Sabourin 1984; Manojlovic 1987; Morales-Ramos et al. 2013; Urrejola et al. 2011), number of instars (Morales-Ramos et al. 2010), survival rate (Morales-Ramos et al. 2010; van Broekhoven et al. 2015), the intensity and period of oviposition (Manojlovic 1987; Morales-Ramos et al. 2013), and progeny production by increasing the number of eggs and decreasing adult mortality (Gerber and Sabourin 1984).

Water intake. Although *T. molitor* can survive under extremely dry conditions for extended periods of time, being able to obtain water from both the food ingested (even substances with low water content) and the atmosphere (Fraenkel and Blewett 1944), larvae grow faster in humid conditions higher than 70% RH. However, the development of mites, fungi, and other microorganisms enhanced by these high-humidity levels are undesirable to the mass rearing of this insect. Mealworms reared on dry substrates exhibit higher growth rates when there is any source of water (Mellandby and French 1958; Oonincx et al. 2015; Urs and Hopkins 1973b). In fact, on low-moisture substrates when metabolic water per unit of food is low (24–35 g water/100 g food), development may halt if there is no drinking water. When suffering from water deprivation, *T. molitor* larvae ingest less food, and the conversion rate of ingested food into body mass decreases (Murray 1968). The

Table 6. Diets and protein sources used on the rearing of mealworm.

Diet	Reference
Bran/flour + water source (vegetable or water)	Allen et al. 2012; Baek et al. 2015; Dick 2008; Greenberg and Ar 1996; Houbraken et al. 2016; Li et al. 2015; Ludwig 1956; Lv et al. 2014; Damborsky et al. 2000; Morales-Ramos et al. 2012; Punzo 1975; Ravzanaadii et al. 2012; Siemianowska et al. 2013; Spang 2013; Tschinkel and Willson 1971; Urs and Hopkins 1973b; Vinokurov et al. 2006; Xuegui et al. 2010;
Bran/flour + water source (vegetable or water) + protein source	Connat et al. 1991; Ghaly and Alkoaik 2009; Kim et al. 2015; Lardies et al. 2014; Li et al. 2016a; Manojlovic 1987; Murray 1968; Oonincx et al. 2010; Ramos-Elorduy et al. 1997; Tang et al. 2012; Weaver and McFarlane 1990
Varied artificial diet	Alves et al. 2016; Davis 1970a, 1974, 1978; Davis and Sosulski 1973a; Fraenkel 1950; Gerber 1975; Hardouin and Mahoux 2003; John et al. 1978; Martin and Hare 1941; Martin et al. 1976; Menezes et al. 2014; Morales-Ramos et al. 2010, 2013; Murray 1960; Oonincx et al. 2015; Rho and Lee 2014, 2015; Tindwa et al. 2015; Urrejola et al. 2011; van Broekhoven et al. 2016
Protein source	
Beer yeast	Gerber 1975; Ghaly and Alkoaik 2009; John et al. 1978; Lardies et al. 2014; Oonincx et al. 2010, 2015; Ramos-Elorduy et al. 1997; Tang et al. 2012; Tindwa et al. 2015; Urrejola et al. 2011; van Broekhoven et al. 2015; Weaver and McFarlane 1990
Casein	Davis 1970b, 1978; Davis and Leclercq 1969; Fraenkel 1950; Murray 1960; Rho and Lee 2014
Dried yeast	Connat et al. 1991; Murray 1960, 1968
Albumin	Morales-Ramos et al. 2010, 2013; Murray 1960; Rho and Lee 2014

Table 6. Continued.

Diet	Reference
Soy	Davis and Sosulski 1973b; Hardouin and Mahoux 2003; Manojlovic 1987; Morales-Ramos et al. 2013
Dry potato	Morales-Ramos et al. 2016
Lactalbumin	Davis 1970b; Davis and Leclercq 1969
Lactalbumin hydrolysate	Davis 1970b; Davis and Leclercq 1969
Aminoacid mixture	Davis 1974; John et al. 1978
Bird feed	Menezes et al. 2014
Bocaiuva (<i>Acrocomia aculeata</i>)	Alves et al. 2016
Cookie crumbs	Oonincx et al. 2015; van Broekhoven et al. 2015
Beef (blood, muscle, liver)	Martin and Hare 1941

addition of a source of water, such as carrots, results in the increase of survival rates ($\geq 80\%$) and reduces the development time from 145–151 d to 91–95 d (Oonincx et al. 2015).

The reported effects of water intake on biomass composition are contradictory. Urs and Hopkins (1973) observed that the availability of water increases the concentrations of total lipids while Oonincx et al. (2015) reported that the supplementation of the diets with a water source increases the moisture content but not the total fatty acids content. Both studies found no influence of water on mealworm fatty acid profile.

Protein. Dietary protein and amino acids greatly influence *T. molitor* life cycle, with direct benefits for larval development time, survival, and weight gain. Supplementation of diets with protein, even in small percentages, has been shown to increase the developmental rate of larvae. Diets supplemented with high protein content (33–39% dry mass) reduce the time for pupation from 191–227 d to 116–144 d at 28°C and 70% RH (Oonincx et al. 2015) and the time to 50% pupation from 95–168 to 79–95 d at 28°C and 65% RH (van Broekhoven et al. 2015), when compared with diets supplemented with low protein content (12% dry mass). Likewise, supplementation with protein increases survival rates from 84–88% to 88–92% (van Broekhoven et al. 2015) and from 19–52% to 67–79% (Oonincx et al. 2015).

The growth rate is enhanced by protein, with a weight gain per larva of 2.3–2.9 mg on a protein-free diet to 45.5–55.6 mg on a diet supplemented with 10 parts of yeast and 90 parts of whole ground wheat during a period of 4 wk (John et al. 1979). Supplementation with casein enhances growth rates from 4.08 g/g of larva with 3% casein to 6.16 g/g of larva with 20% casein (Davis 1970a). The benefits of adding protein to the diet are visible in pupae, with weight gains enhanced from 123 mg/g of

food with no supplement to 238 mg/g of food supplemented with protein (Morales-Ramos et al. 2013) and pupal weight from 117–145 mg with a low-protein diet (5% yeast) to 146–161 mg with a rich-protein (40% yeast) diet (van Broekhoven et al. 2015).

Fertility is also highly influenced by the presence of protein in mealworm diet, with increases in average female fecundity from 3 eggs/d in diets without protein increased to 6–7 eggs/d in diets supplemented with protein (Morales-Ramos et al. 2013). Urrejola et al. (2011) also obtained an increase in female fecundity from 5 to 12 eggs/d in diets with yeast at 20% when compared to diets with 2% yeast addition (w/w). Additionally, the period of maximum oviposition occurs earlier in females reared with soybean flour (9th–12th day) than in females reared only with wheat (12th–15th day).

Mealworms seemingly have a highly constrained body protein content with 2–3-fold increases in crude protein content (11.9–39.1% dry mass) of diets resulting in similar body protein composition (van Broekhoven et al. 2015). Yeast, at concentrations of 5–10%, is considered the best protein source acting as a feeding stimulant (Fraenkel 1950; Martin and Hare 1942). Other protein sources that provide optimal effects are 2–32% casein (Davis and Leclercq 1969) and at a lower level, lactalbumin (Davis and Leclercq 1969; Fraenkel 1950; Leclercq 1948). Zein, gliadin, and enzymatic hydrolysates from casein or lactalbumin have residual effects when compared to casein and lactalbumin (Fraenkel 1950). Although soybean is a rich protein source, it contains a potent trypsin inhibitor that negatively influences the larval growth (Birk et al. 1962).

The amino acid contents in the mealworm larval tissues are 8.9–9.9% alanine, 7.9–8.7% aspartic acid, 7.7–8.0% leucine, 6.5–6.8% phenylalanine, 6.5–6.7% valine, 4.6–7.5% proline, 4.6–5.9% arginine, 4.5% isoleucine, 3.9–4.0% threonine, 2.8–2.9% histidine, 1.8–1.9% cysteine, 1.5–1.6% methionine, and 0.7–0.8% tryptophan (John et al. 1979). The ideal diet should contain similar levels of amino acids to those found in larval tissues, with the exception of phenylalanine, which should be provided at 50% of the concentration in body mass, and of the limiting amino acids, threonine and tryptophan, which should be provided at twice the concentration found in larvae tissues.

Fats. Mealworm fat composition is rather constant when fed different diets rich in oleic, linoleic, and palmitic acids (Oonincx et al. 2015). While palmitic and oleic acids remain almost stable, independent of diet, possibly due to the synthesis of fatty acids *ad novo* by mealworms, linoleic acid may have to be supplied to diets (van Broekhoven et al. 2015). Increased ingestion of polyunsaturated C18 fatty acids lowers the proportion of C18 monounsaturated fatty acids in larval tissues (van Broekhoven et al. 2015).

The addition of lipid to dietary regimes is beneficial at low concentrations while high quantities are unfavorable and potentially pernicious (Morales-Ramos et al. 2013). While cholesterol is a necessary diet ingredient, a fat concentration above 1% does not benefit mealworm life cycle parameters (Fraenkel 1950) and becomes an inhibitory factor at concentrations >3% (Martin and Hare 1942). Moreover, high-fat foods promote the potential agglomeration of the substrate, resulting in lower aeration and movement of mealworms, thus negatively interfering with respiration (Alves et al. 2016). In addition, it has been proven that supplementation with 20%

lipid content increases the susceptibility to parasitism by nematodes (Shapiro-Ilan et al. 2008).

Carbohydrates. *Tenebrio molitor* growth is almost nil in the absence of carbohydrates. The optimal range is 80–85% (Fraenkel 1950). Although Fraenkel (1950) reported no significant differences in the growth of mealworms using glucose or starch, Davis (1974) observed less growth with starch, sucrose, or lactose than with glucose in diets containing amino acid mixtures. Similarly, bacteriological dextrin as a carbohydrate source induced weight gain of almost twice that achieved with glucose (Davis 1974).

The mealworm life cycle is highly influenced by the dietary protein-to-carbohydrate ratio (Martin and Hare 1942; Rho and Lee 2016; Urrejola et al. 2011). Rho and Lee (2016) reported an optimal protein-to-carbohydrate ratio of 1:1 for lifespan and lifetime reproductive success while Martin and Hare (1942) observed maximum growth at a minimum of 50% carbohydrate and a minimum of 15% (or more than 25%) protein in the diet.

The ingestion of diet consisting of several combinations of organic waste material (vegetables), yeast, and *Tenebrio* excreta results in body protein contents 2-fold higher and body fat content 5- to 6-fold higher than that of food, with a significant reduction in the values of crude fiber and carbohydrates (Ramos-Elorduy et al. 2002). Moreover, mealworms fed on low protein:carbohydrate ratios (0:42 and 7:35) diets have higher body lipid content (Rho and Lee 2014).

Vitamins. *Tenebrio molitor* shows no growth in the absence of vitamins of the B-complex (carnitine, thiamine, riboflavin, nicotinic acid, pyridoxine, or pantothenic acid) while there is growth, albeit slow, with the lack of biotin or pteroylglutamic acid (Hardouin and Mahoux 2003; Leclercq 1948; Martin and Hare 1942). On the contrary, the addition of vitamins A, C, D, E, and K to the diet provides no beneficial effect (Fraenkel 1950; Martin and Hare 1942). After mealworms reach half- or full-grown size, no addition of vitamins is required for the mealworms to complete larval development and to pupate (Leclercq 1948).

Nutritive Value and Use *Tenebrio molitor*

Tenebrio molitor larvae have been used for several feed and food purposes, from fish bait and feed for animals in captivity (Ghaly and Alkoaik 2009; Martin et al. 1976) to human consumption in many cultures (van Huis et al. 2013). For human consumption, mealworm larvae can be used fresh as a whole (Ghaly and Alkoaik 2009; Zhao et al. 2016) or as flour (Aguilar-Miranda et al. 2002). For animal feed, the flour can be used as a supplement in pig and poultry diet (Bovera and Piccolo 2015; Jin et al. 2016; Józefiak et al. 2016; Ramos-Elorduy et al. 2002; van Huis 2013; Veldkamp et al. 2012) and in aquaculture (Barroso et al. 2014; Belforti M. et al. 2014; Gasco et al. 2016; Henry et al. 2015; Ng et al. 2001).

Tenebrio molitor larvae, as in the majority of insects, are rich in protein with content between 43.3–66.8% dry mass (Ghaly and Alkoaik 2009; Jin et al. 2016). The amino acid profile shows the presence of almost all essential amino acids necessary for human consumption: 1.39–4.8% isoleucine, 2.81–8.65% leucine, 1.6–6.6% lysine, 0.64–7.6% methionine + cysteine, 3.99–13.05% phenylalanine + tyrosine, 0.93–4.43% threonine, 3.14–7.61% valine, 1.61–3.64% histidine, and 0–

1.8% tryptophan (Aguilar-Miranda et al. 2002; Barroso et al. 2014; Davis and Sosulski 1974; Ghaly and Alkoaik 2009; Jin et al. 2016; Jones et al. 1972; Ramos-Elorduy et al. 2002; Zielińska et al. 2015). Larvae also have a fat content between 17–42.48% dry mass (Adámková et al. 2016; Siemianowska et al. 2013). In relation to total fatty acids, there is a predominance of palmitic acid (9.33–23.7%), oleic acid (36.5–52.94%), and linoleic acid (3.8–33.58%) (Adámková et al. 2016; Aguilar-Miranda et al. 2002; Alves et al. 2016; Finke 2002; Jin et al. 2016; Jones et al. 1972; Martin et al. 1976; Zhao et al. 2016). While rich in protein and fat, the mealworm larvae are poor in calcium content, which can be problematic when exclusively feeding upon this insect (Klasing et al. 2000). The values of mealworm mineral composition range at 0.32–0.75 mg/g Ca, 1.45–3.4 mg/g Mg, 5.37–13.45 mg/g P, 6.7–13.8 mg/g K, 0.025–1.76 mg/g Na, 0.032–0.13 mg/g Fe, 0.012–0.04 mg/g Cu, and 0.082–0.145 mg/g Zn in a dry mass (Barker et al. 1998; Jones et al. 1972; Martin et al. 1976; Siemianowska et al. 2013; Simon et al. 2013; Zielińska et al. 2015).

Conclusions

Tenebrio molitor are a viable source of nutrition that may be used as a food or as a feed source or supplement. Its life cycle and relative ease of rearing make it amenable to mass production for such uses. Herein, we have reviewed available scientific literature to delineate the optimal environmental and dietary conditions for such mass production. The *T. molitor* studied lived its entire life cycle in the same diet and rearing system. We found that there are optimal temperature (25–28°C) and humidity levels (60–70% RH) for optimal growth and developmental time of *T. molitor*. Overpopulation in the rearing arena can inhibit pupation and incite cannibalistic behavior (Tschinkel and Willson 1971) and, thus, should be avoided. And, although mealworms can survive feeding exclusively on wheat bran, supplemental sources of protein (i.e., yeast, casein), fats (<1%), carbohydrates (80 to 85%), and B-complex vitamins improves growth rate and production of the colony.

Currently, there is little, if any, information on the microbiological (including zoonoses) or chemical risks resulting from the use and consumption of insects for food or feed. Environmental impacts are comparable to other animal production systems and can be decreased by the reuse of insect farming products and by-products (substrate and manure) (European Food Safety Authority 2015).

Acknowledgment

The authors wish to thank Fundação para a Ciência e Tecnologia for financial support under UID/AMB/00681/2013.

References Cited

Adámková, A., L. Kourimská, M. Borkovcová, M. Kulma and J. Miček. 2016. Nutritional values of edible Coleoptera (*Tenebrio molitor*, *Zophobas morio* and *Alphitobius diaperinus*) reared in the Czech Republic. *Potravinárstvo* 10: 663–671. doi: 10.5219/609.

- Aguilar-Miranda, E.D., M.G. Lopez, C. Escamilla-Santana and A.P. Barba de la Rosa. 2002.** Characteristics of maize flour tortilla supplemented with ground *Tenebrio molitor* larvae. *J. Agric. Food Chem.* 50: 192–195. doi: 10.1021/jf010691y.
- Allen, J.L., S. Clusella-Trullas and S.L. Chown. 2012.** The effects of acclimation and rates of temperature change on critical thermal limits in *Tenebrio molitor* (Tenebrionidae) and *Cyrtobagous salviniae* (Curculionidae). *J. Insect Physiol.* 58: 669–678. doi: 10.1016/j.jinsphys.2012.01.016.
- Altman, P.L. and D.S. Dittmer. 1973.** *Biology Data Book.* Federation of American Societies for Experimental Biology, Bethesda, MD. 1,432 pp.
- Alves, A.V., E.J. Sanjinez-Argandona, A.M. Linzmeier, C.A.L. Cardoso and M.L.R. Macedo. 2016.** Food value of mealworm grown on *Acrocomia aculeata* pulp flour. *PLoS One* 11: e0151275. doi: 10.1371/journal.pone.0151275.
- Baek, S., A.E. Perez, R.M. Turcotte, J.B. White, F. Adedipe and Y.-L. Park. 2015.** Response of *Tenebrio molitor* (Coleoptera: Tenebrionidae) adults to potato: Implications for monitoring and sampling. *J. Stored Prod. Res.* 60: 5–10. doi: 10.1016/j.jspr.2014.11.002.
- Balfour, C. and L. Carmichael. 1928.** The light reactions of the mealworm (*Tenebrio molitor* Linn). *Am. J. Psychol.* 40: 576–584.
- Barker, D., M.P. Fitzpatrick and E.S. Dierenfeld. 1998.** Nutrient composition of selected whole invertebrates. *Zoo Biol.* 17: 123–134. doi: 10.1002/(SICI)1098-2361(1998)17:2<123::AID-ZOO7>3.0.CO;2-B.
- Barroso, F.G., C. de Haro, M.J. Sánchez-Muros, E. Venegas, A. Martínez-Sánchez and C. Pérez-Bañón. 2014.** The potential of various insect species for use as food for fish. *Aquaculture* 422–423: 193–201. doi: 10.1016/j.aquaculture.2013.12.024.
- Belforti M., C. Lussiana, V. Malfatto, L. Rotolo, I. Zoccarato and L. Gasco. 2014.** Two rearing substrates on *Tenebrio molitor* meal composition: Issues on aquaculture and biodiesel production. *Insects to Feed the World Conference 2014.*
- Birk, Y., I. Harpaz, I. Ishaaya and A. Bondi. 1962.** Studies on the proteolytic activity of the beetles *Tenebrio* and *Tribolium*. *J. Insect Physiol.* 8: 417–429. doi: https://doi.org/10.1016/0022-1910(62)90075-6.
- Bovera, F. and G.M. Piccolo. 2015.** Yellow mealworm larvae (*Tenebrio molitor*, L.) as a possible alternative to soybean meal in broiler diets. *Br. Poult. Sci.* 56: 569–575.
- Cloudsley-Thompson, J.L. 1953.** Studies in diurnal rhythms. IV. Photoperiodism and geotaxis in *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). *Proc. R. Entomol. Soc. London A* 28: 117–131.
- Connat, J.L., J.P. Delbecque, I. Glietho and J. Delachambre. 1991.** The onset of metamorphosis in *Tenebrio molitor* larvae (Insecta, Coleoptera) under grouped, isolated and starved conditions. *J. Insect Physiol.* 37(9): 653–657, 659–662. doi: 10.1016/0022-1910(91)90042-X.
- Cortes Ortiz, J.A., A.T. Ruiz, J.A. Morales-Ramos, M. Thomas, M.G. Rojas, J.K. Tomberlin, L. Yi, R. Han, L. Giroud and R.L. Jullien. 2016.** Insect mass production technologies, Pp. 153–201. *In* Dossey, A.T., J. Morales-Ramos, and M.G. Rojas (eds.), *Insects as Sustainable Food Ingredients.* Elsevier Academic Press, Amsterdam, the Netherlands.
- Cotton, R.T. 1927.** Notes on the biology of the mealworms *Tenebrio molitor* L. and *T. obscurus* Fab. *Ann. Entomol. Soc. Am.* 20: 81–86. doi: 10.1093/aesa/20.1.81.
- Damborsky, M., P.S.T. Bar and M.E. Oscherov. 2000.** Ciclo de vida de *Tenebrio molitor* (Coleoptera, Tenebrionidae) en condiciones experimentales. *Comun. Cient. y Tecnol. UNNE* 6: 35–38.
- Davis, G. 1970a.** Protein nutrition of “*Tenebrio molitor*” L. XII. Effects of dietary casein concentration and of dietary cellulose on larvae of race F. *Arch. Int. Physiol. Biochim.* 78: 37–41. doi: http://dx.doi.org/10.3109/13813457009075180.
- Davis, G. 1970b.** Protein nutrition of “*Tenebrio molitor*” L. XIII. Consideration of some dietary factors of casein, lactalbumin, and lactalbumin hydrolysate. *Arch. Int. Physiol. Biochim.* 78: 467–473. doi: 10.3109/13813457009075197.

- Davis, G. 1974.** Protein nutrition of *Tenebrio molitor* L.: XVII. Improved amino acid mixture and interaction with dietary carbohydrate. Arch. Int. Physiol. Biochem. 82: 631–637. doi: 10.3109/13813457409072315.
- Davis, G. 1978.** Growth response of larvae of *Tenebrio molitor* L. to concentrations of dietary amino acids. J. Stored Prod. Res. 14: 69–71. doi: 10.1016/0022-474X(78)90022-X.
- Davis, G. and J. Leclercq. 1969.** Protein nutrition of “*Tenebrio molitor*” L. IX. Replacement caseins for the reference diet and a comparison of the nutritional values of various lactalbumins and lactalbumin hydrolysates. Arch. Int. Physiol. Biochim. 77: 687–693. doi: 10.3109/13813456909059780.
- Davis, G. and F.W. Sosulski. 1973a.** Improvement of basic diet for use in determining the nutritional value of proteins with larvae of *Tenebrio molitor* L. Arch. Int. Physiol. Biochim. 81: 495–500. doi: 10.3109/13813457309073399.
- Davis, G. and F.W. Sosulski. 1973b.** Protein nutrition of *Tenebrio molitor* L. XVI. Effects of dietary protein concentration on larvae of race F. Arch. Int. Physiol. Biochim. 81: 661–665. doi: 10.3109/13813457309074470.
- Davis, G. and F.W. Sosulski. 1974.** Nutritional quality of oilseed protein isolates as determined with larvae of the yellow mealworm, *Tenebrio molitor* L. J. Nutr. 104: 1172–1177.
- Dick, J. 1937.** Oviposition in certain Coleoptera. Ann. Appl. Biol. 24: 762–796. doi: 10.1111/j.1744-7348.1937.tb05055.x.
- European Food Safety Authority. 2015.** Risk profile related to production and consumption of insects as food and feed EFSA Scientific Committee. EFSA J. 13: 4257–4317. doi: 10.2903/j.efsa.2015.4257.
- Finke, M.D. 2002.** Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zool. Biol. 21: 269–285. doi: 10.1002/zoo.10031.
- Fraenkel, G. 1950.** The nutrition of the mealworm, *Tenebrio molitor* L. (Tenebrionidae, Coleoptera). Physiol. Zool. 23: 92–108. doi: 10.1017/CBO9781107415324.004.
- Fraenkel, G. and M. Blewett. 1944.** The utilisation of metabolic water in insects. Bull. Entomol. Res. 35: 127. doi: 10.1017/S0007485300017351.
- Gasco, L., M. Henry, G. Piccolo, S. Marono, F. Gai, M. Renna, C. Lussiana, E. Antonopoulou, P. Mola and S. Chatzifotis. 2016.** *Tenebrio molitor* meal in diets for European sea bass (*Dicentrarchus labrax* L.) juveniles: Growth performance, whole body composition and in vivo apparent digestibility. Anim. Feed Sci. Technol. 220: 34–45. doi: 10.1016/j.anifeedsci.2016.07.003.
- Gerber, G.H. 1975.** Reproductive behaviour and physiology of *Tenebrio molitor* (Coleoptera: Tenebrionidae): II. Egg development and oviposition in young females and the effects of mating. Can. Entomol. 107: 551–559. doi: 10.4039/Ent107551-5.
- Gerber, G.H. and D.U. Sabourin. 1984.** Oviposition site selection in *Tenebrio molitor* (Coleoptera: Tenebrionidae). Can. Entomol. 116: 27–39. doi: 10.4039/Ent11627-1.
- Ghaly, A.E.E. and F.N.N. Alkokaik. 2009.** The yellow mealworm as a novel source of protein. Am. J. Agric. Biol. Sci. 4: 319–331. doi: 10.3844/ajabssp.2009.319.331.
- Greenberg, S. and A. Ar. 1996.** Effects of chronic hypoxia, normoxia and hyperoxia on larval development in the beetle *Tenebrio molitor*. J. Insect Physiol. 42: 991–996. doi: 10.1016/S0022-1910(96)00071-6.
- Hardouin, J. and G. Mahoux. 2003.** Zootechnie d’insectes—Elevage et utilisation au bénéfice de l’homme et de certains animaux. Bull. Semest. d’Information sur le Mini-Elevage. http://www.fastonline.org/CD3WD_40/LSTOCK/001/ITProv_May_2005/h4338fZootehnied'insectes/begin.htm. Accessed 14 March 2016.
- Henry, M., L. Gasco, G. Piccolo and E. Fountoulaki. 2015.** Review on the use of insects in the diet of farmed fish: Past and future. Anim. Feed Sci. Technol. 203: 1–22.
- Hill, D.S. 2002.** Pests: Class Insecta. Pp. 135–315. *In* Pests of Stored Foodstuffs and Their Control. Springer, Dordrecht, the Netherlands. doi: https://doi.org/10.1007/0-306-48131-6_14.

- Houbraken, M., T. Spranghers, P. De Clercq, M. Cooreman-Algoed, T. Couchement, G. De Clercq, S. Verbeke and P. Spanoghe. 2016. Pesticide contamination of *Tenebrio molitor* (Coleoptera: Tenebrionidae) for human consumption. *Food Chem.* 201: 264–269. doi: 10.1016/j.foodchem.2016.01.097.
- Jin, X.H., P.S. Heo, J.S. Hong, N.J. Kim and Y.Y. Kim. 2016. Supplementation of dried mealworm (*Tenebrio molitor* larva) on growth performance, nutrient digestibility and blood profiles in weaning pigs. *Asian-Australasian J. Anim. Sci.* 29: 979–986. doi: 10.5713/ajas.15.0535.
- John, A.M., G.R. Davis and F.W. Sosulski. 1978. Protein nutrition of *Tenebrio molitor* L. XIX. Growth response to levels of dietary protein and of an amino acid mixture. *Arch. Int. Physiol. Biochim.* 86: 761–770. doi: 10.3109/13813457809069528.
- John, A.M., G.R. Davis and F.W. Sosulski. 1979. Protein nutrition of *Tenebrio molitor* L. XX. Growth response of larvae to graded levels of amino acids. *Arch. Int. Physiol. Biochim.* 87: 997–1004. doi: 10.3109/13813457909070548.
- Jones, L.D., R.W. Cooper and R.S. Harding. 1972. Composition of mealworm *Tenebrio molitor* larvae. *J. Zoo Anim. Med.* 3: 34. doi: 10.2307/20094161.
- Józefiak, D., A. Józefiak, B. Kierończyk, M. Rawski, S. Świątkiewicz, J. Długosz and R.M. Engberg. 2016. Insects—A natural nutrient source for poultry—A review. *Ann. Anim. Sci.* 16:297–313.
- Kim, S.Y., J. Bin Park, Y.B. Lee, H.J. Yoon, K.Y. Lee and N.J. Kim. 2015. Growth characteristics of mealworm *Tenebrio molitor*. *J. Sericultural Entomol. Sci.* 53: 1–5. doi: 10.7852/jses.2015.53.1.1.
- Kim, S.Y., H.G. Kim, K.Y. Lee, H.J. Yoon and N.J. Kim. 2016. Effects of Brewer's spent grain (BSG) on larval growth of mealworms, *Tenebrio molitor* (Coleoptera: Tenebrionidae). *Int. J. Ind. Entomol.* 32: 41–48. doi: 10.7852/ijie.2016.32.1.41.
- Klasing, K.C., P. Thacker, M.A. Lopez and C.C. Calvert. 2000. Increasing the calcium content of mealworms (*Tenebrio molitor*) to improve their nutritional value for bone mineralization of growing chicks. *J. Zoo Wildl. Med.* 31: 512–517. doi: 10.1638/1042-7260(2000)031[0512:ITCCOM]2.0.CO;2.
- Koo, H., S. Kim, H. Oh, J. Kim, D. Choi, D. Kim and I. Kim. 2013. Temperature-dependent development model of larvae of mealworm beetle, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). *Korean J. Appl. Entomol.* 52: 387–394. doi: 10.5656/KSAE.2013.11.0.066.
- Lardies, M.A., M.B. Arias, M.J. Poupin and L.D. Bacigalupe. 2014. Heritability of hsp70 expression in the beetle *Tenebrio molitor*: Ontogenetic and environmental effects. *J. Insect Physiol.* 67: 70–75. doi: 10.1016/j.jinsphys.2014.06.005.
- Leclercq, J. 1948. Sur les besoins nutritifs de la larve de *Tenebrio molitor* L. *Biochim. Biophys. Acta* 2: 2–5. doi: 10.1016/0006-3002(48)90046-8.
- Li, L., M. Stasiak, L. Li, B. Xie, Y. Fu, D. Gidzinski, M. Dixon and H. Liu. 2016a. Rearing *Tenebrio molitor* in BLSS: Dietary fiber affects larval growth, development, and respiration characteristics. *Acta Astronaut.* 118: 130–136. doi: 10.1016/j.actaastro.2015.10.003.
- Li, L., B. Xie, C. Dong, D. Hu, M. Wang, G. Liu and H. Liu. 2015. Rearing *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) in the “Lunar Palace 1” during a 105-day multi-crew closed integrative BLSS experiment. *Life Sci. Sp. Res.* 7: 9–14. doi: 10.1016/j.lssr.2015.08.002.
- Li, L., B. Xie, C. Dong, M. Wang and H. Liu. 2016b. Can closed artificial ecosystem have an impact on insect microbial community? A case study of yellow mealworm (*Tenebrio molitor* L.). *Ecol. Eng.* 86: 183–189. doi: 10.1016/j.ecoleng.2015.09.015.
- Loudon, C. 1988. Development of *Tenebrio molitor* in low oxygen levels. *J. Insect Physiol.* 34: 97–103. doi: 10.1016/0022-1910(88)90160-6.
- Loudon, C. 1989. Tracheal hypertrophy in mealworms: Design and plasticity in oxygen supply systems. *J. Exp. Biol.* 147: 217–235.
- Ludwig, D. 1956. Effects of temperature and parental age on the life cycle of the mealworm, *Tenebrio molitor* Linnaeus (Coleoptera, Tenebrionidae). *Ann. Entomol. Soc. Am.* 49: 12–15. doi: 10.1093/aesa/49.1.12.

- Lv, X., C. Liu, Y. Li, Y. Gao, H. Wang, J. Li and B. Guo. 2014.** Stereoselectivity in bioaccumulation and excretion of epoxiconazole by mealworm beetle (*Tenebrio molitor*) larvae. *Ecotoxicol. Environ. Saf.* 107C: 71–76. doi: 10.1016/j.ecoenv.2014.02.013.
- Makkar, H.P.S., G.H. Tran, V. Heuzé and P. Ankers. 2014.** State-of-the-art on use of insects as animal feed. *Anim. Feed Sci. Technol.* 197: 1–33. doi: 10.1016/j.anifeeds.2014.07.008.
- Manojlovic, B. 1987.** A contribution of the study of the influence of the feeding of imagos and of climatic factors on the dynamics of oviposition and on the embryonal development of yellow mealworm *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). *Zaštita bilja* 38: 337–348. doi: 10.7852/jses.2012.50.2.126.
- Martin, H.E. and L. Hare. 1942.** The nutritive requirements of *Tenebrio molitor* larvae. *Biol. Bull.* 83: 428–437. doi: 10.2307/1538240.
- Martin, R.D., J.P.W. Rivers and U.M. Cowgill. 1976.** Culturing mealworms as food for animals in captivity. *Int. Zoo Yearb.* 16: 63–70. doi: 10.1111/j.1748-1090.1976.tb00130.x.
- Mellandby, K. and R.A. French. 1958.** The importance of drinking water to larval insects. *Entomol. Exp. Appl.* 1: 116–124. doi: 10.1111/j.1570-7458.1958.tb00014.x.
- Menezes, C.W.G., S.S. Camilo, A.J. Fonseca, S.L. Assis Júnior, D.F. Bispo and M.A. Soares. 2014.** A dieta alimentar da presa *Tenebrio molitor* (Coleoptera: Tenebrionidae) pode afetar o desenvolvimento do predador *Podisus nigrispinus* (Heteroptera: Pentatomidae). *Arq. Inst. Biol.* (Sao. Paulo). 81: 250–256. doi: 10.1590/1808-1657001212012.
- Morales-Ramos, J.A. and M.G. Rojas. 2015.** Effect of larval density on food utilization efficiency of *Tenebrio molitor* (Coleoptera: Tenebrionidae). *J. Econ. Entomol.* 108: 2259–2267. doi: 10.1093/jee/tov208.
- Morales-Ramos, J.A., S. Kay, M.G. Rojas, D.I. Shapiro-Ilan and W.L. Tedders. 2015.** Morphometric analysis of instar variation in *Tenebrio molitor* (Coleoptera: Tenebrionidae). *Ann. Entomol. Soc. Am.* 108: 146–159. doi: 10.1093/aesa/sau049.
- Morales-Ramos, J.A., M.G. Rojas, S. Kay, D.I. Shapiro-Ilan and W.L. Tedders. 2012.** Impact of adult weight, density, and age on reproduction of *Tenebrio molitor* (Coleoptera: Tenebrionidae). *J. Entomol. Sci.* 47: 208–220. doi: 10.18474/0749-8004-47.3.208.
- Morales-Ramos, J.A., M.G. Rojas, D.I. Shapiro-Ilan and W.L. Tedders. 2010.** Developmental plasticity in *Tenebrio molitor* (Coleoptera: Tenebrionidae): Analysis of instar variation in number and development time under different diets. *J. Entomol. Sci.* 45: 75–90. doi: 10.18474/0749-8004-45.2.75.
- Morales-Ramos, J.A., M.G. Rojas, D.I. Shapiro-Ilan and W.L. Tedders. 2011.** Self-selection of two diet components by *Tenebrio molitor* (Coleoptera: Tenebrionidae) larvae and its impact on fitness. *Environ. Entomol.* 40: 1285–94. doi: 10.1603/EN10239.
- Morales-Ramos, J.A., M.G. Rojas, D.I. Shapiro-Ilan and W.L. Tedders. 2013.** Use of nutrient self-selection as a diet refining tool in *Tenebrio molitor* (Coleoptera: Tenebrionidae). *J. Entomol. Sci.* 48: 206–221. doi: 10.18474/0749-8004-48.3.206.
- Morales-Ramos, J.A., M.G. Rojas, K.S. Shelby and T.A. Coudron. 2016.** Nutritional value of pupae versus larvae of *Tenebrio molitor* (Coleoptera: Tenebrionidae) as food for rearing *Podisus maculiventris* (Heteroptera: Pentatomidae). *J. Econ. Entomol.* 109: 564–571. doi: 10.1093/jee/tov338.
- Murray, D.R.P. 1960.** The stimulus to feeding in larvae of *Tenebrio molitor* L. *J. Insect Physiol.* 4: 80–91. doi: 10.1016/0022-1910(60)90069-X.
- Murray, D.R.P. 1968.** The importance of water in the normal growth of larvae of *Tenebrio molitor*. *Entomol. Exp. Appl.* 11: 149–168. doi: 10.1017/CBO9781107415324.004.
- Mutchmor, J.A. and A.G. Richards. 1961.** Low temperature tolerance of insects in relation to the influence of temperature on muscle apyrase activity. *J. Insect Physiol.* 7: 141–158. doi: 10.1016/0022-1910(61)90051-8.
- Ng, W.-K. Liew, F.-L. L.-P. Ang and K.-W. Wong. 2001.** Potential of mealworm (*Tenebrio molitor*) as an alternative protein source in practical diets for African catfish, *Clarias gariepinus*. *Aquac. Res.* 32: 273–280. doi: 10.1046/j.1355-557x.2001.00024.x.

- Ooninx, D.G.A.B. and I.J.M. de Boer. 2012.** Environmental impact of the production of mealworms as a protein source for humans—A life cycle assessment. *PLoS One* 7: e51145. doi: 10.1371/journal.pone.0051145.
- Ooninx, D.G.A.B., S. Van Broekhoven, A. Van Huis and J.J.A. Van Loon. 2015.** Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. *PLoS One* 10: e0144601. doi: 10.1371/journal.pone.0144601.
- Ooninx, D.G.A.B., J. van Itterbeeck, M.J.W. Heetkamp, H. van den Brand, J.J. a van Loon and A. van Huis. 2010.** An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. *PLoS One* 5: e14445. doi: 10.1371/journal.pone.0014445.
- Park, J.B., W.H. Choi, S.H. Kim, H.J. Jin, Y.S. Han and N.J. Kim. 2014.** Developmental characteristics of *Tenebrio molitor* larvae (Coleoptera: Tenebrionidae) in different instars. *Int. J. Ind. Entomol.* 28: 5–9. doi: <http://dx.doi.org/10.7852/ijie.2014.28.1.5>.
- Park, Y., Y. Choi, Y. Lee, S. Lee, J. Lee and S. Kang. 2012.** Fecundity, Life span, developmental periods and pupal weight of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). *J. Seric Entomol. Sci.* 50: 126–132. doi: 10.7852/jses.2012.50.2.126.
- Punzo, F. 1975.** Effects of temperature, moisture and thermal acclimation on the biology of *Tenebrio molitor* (Coleoptera: Tenebrionidae). Ph.D. Dissertation, Iowa State University, Ames.
- Punzo, F. and J.A. Mutchmor. 1980.** Effects of temperature, relative humidity and period of exposure on the survival capacity of *Tenebrio molitor* (Coleoptera: Tenebrionidae). *J. Kansas Entomol. Soc.* 53: 260–270. doi: 10.2307/25084029.
- Ramos-Elorduy, J., E.A. González, A.R. Hernández and J.M. Pino. 2002.** Use of *Tenebrio molitor* (Coleoptera: Tenebrionidae) to recycle organic wastes and as feed for broiler chickens. *J. Econ. Entomol.* 95: 214–20. doi: 10.1603/0022-0493-95.1.214.
- Ramos-Elorduy, J., J.M.P. Moreno, E.E. Prado, M.A. Perez, J.L. Otero, O. Ladron De Guevara, J.M. Pino Moreno, E. Escamilla Prado, M. Alvarado Perez, J. Lagunez Otero and O. Ladron De Guevara. 1997.** Nutritional value of edible insects from the state of Oaxaca, Mexico. *J. Food Compos. Anal.* 10: 142–157. doi: 10.1006/jfca.1997.0530.
- Ravzanaadii, N., S.-H. Kim, W.H. Choi, S.-J. Hong and N.J. Kim. 2012.** Nutritional value of mealworm, *Tenebrio molitor* as food source. *Int. J. Indust. Entomol* 25: 93–98. doi: 10.1016/j.jinsphys.2014.10.001.
- Rho, M.S. and K.P. Lee. 2014.** Geometric analysis of nutrient balancing in the mealworm beetle, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). *J. Insect Physiol.* 71: 37–45. doi: 10.1016/j.jinsphys.2014.10.001.
- Rho, M.S. and K.P. Lee. 2015.** Nutrient-specific food selection buffers the effect of nutritional imbalance in the mealworm beetle, *Tenebrio molitor* (Coleoptera: Tenebrionidae). *Eur. J. Entomol.* 112: 251–258. doi: 10.14411/eje.2015.030.
- Rho, M.S. and K.P. Lee. 2016.** Balanced intake of protein and carbohydrate maximizes lifetime reproductive success in the mealworm beetle, *Tenebrio molitor* (Coleoptera: Tenebrionidae). *J. Insect Physiol.* 91–92: 93–99. doi: 10.1016/j.jinsphys.2016.07.002.
- Shapiro-Ilan, D., M.G. Rojas, J.A. Morales-Ramos, E.E. Lewis and W.L. Tedders. 2008.** Effects of host nutrition on virulence and fitness of entomopathogenic nematodes: Lipid- and protein-based supplements in *Tenebrio molitor* diets. *J. Nematol.* 40: 13–9.
- Siemianowska, E., A. Kosewska, M. Aljewicz, K.A. Skibniewska, L. Polak-Juszczak, A. Jarocki and M. Jędras. 2013.** Larvae of mealworm (*Tenebrio molitor* L.) as European novel food. *Agric. Sci.* 4: 287–291. doi: 10.4236/as.2013.46041.
- Simon, E., E. Baranyai, M. Braun, I. Fábíán and B. Tóthmérész. 2013.** Elemental concentration in mealworm beetle (*Tenebrio molitor* L.) during metamorphosis. *Biol. Trace Elem. Res.* 154: 81–87. doi: 10.1007/s12011-013-9700-1.
- Spang, B. 2013.** Insects as food: Assessing the food conversion efficiency of the mealworm (*Tenebrio molitor*). M.S. Thesis, The Evergreen State College, Olympia, WA.

- Spencer, W. and J. Spencer. 2006.** Management guideline manual for invertebrate live food species. EAZA Terr. Invertebr. TAG. 1–54.
- Tang, Q., Y. Dai and B. Zhou. 2012.** Regulatory effects of *Tenebrio molitor* Linnaeus on immunological function in mice. African J. Biotechnol. 11: 8348–8352. doi: 10.5897/AJB12.340.
- Tindwa, H., Y.H. Jo, B.B. Patnaik, Y.S. Lee, S.S. Kang and Y.S. Han. 2015.** Molecular cloning and characterization of autophagy-related gene TmATG8 in *Listeria*-invaded hemocytes of *Tenebrio molitor*. Dev. Comp. Immunol. 51: 88–98. doi: 10.1016/j.dci.2015.02.017.
- Tschinkel, W.R. and C.D. Willson. 1971.** Inhibition of pupation due to crowding in some tenebrionid beetles. J. Exp. Zool. 176: 137–145. doi: 10.1002/jez.1401760203.
- Tyshchenko, V.P. and A.S. Ba. 1986.** Photoperiodic regulation of larval growth and pupation of *Tenebrio molitor* L. (Coleoptera, Tenebrionidae). Entomol. Rev. 65: 35–46.
- Urrejola, S., R. Nespolo and M.A. Lardies. 2011.** Diet-induced developmental plasticity in life histories and energy metabolism in a beetle. Rev. Chil. Hist. Nat. 84: 523–533. doi: 10.4067/S0716-078X2011000400005.
- Urs, K.C.D. and T.L. Hopkins. 1973a.** Effect of moisture on the lipid content and composition of two strains of *Tenebrio molitor* L. (Coleoptera, Tenebrionidae). J. Stored Prod. Res. 8: 299–305. doi: 10.1016/0022-474X(73)90046-5.
- Urs, K.C.D. and T.L. Hopkins. 1973b.** Effect of moisture on growth rate and development of two strains of *Tenebrio molitor* L. (Coleoptera, Tenebrionidae). J. Stored Prod. Res. 8: 291–297. doi: 10.1016/0022-474X(73)90045-3.
- van Broekhoven, S., S. Bastiaan-Net, N.W. de Jong and H.J. Wichers. 2016.** Influence of processing and in vitro digestion on the allergic cross-reactivity of three mealworm species. Food Chem. 196: 1075–1083. doi: 10.1016/j.foodchem.2015.10.033.
- van Broekhoven, S., D.G.A.B. Oonincx, A. van Huis and J.J.A. van Loon. 2015.** Growth performance and feed conversion efficiency of three edible mealworm species (Coleoptera: Tenebrionidae) on diets composed of organic by-products. J. Insect Physiol. 73: 1–10. doi: 10.1016/j.jinsphys.2014.12.005.
- van Huis, A. 2013.** Potential of insects as food and feed in assuring food security. Annu. Rev. Entomol. 58: 563–586. doi: 10.1146/annurev-ento-120811-153704.
- van Huis, A., J. van Itterbeek, H. Klunder and P. Vantomme. 2013.** Edible insects. Future prospects for food and feed security. Food and Agriculture Organization of The United Nations, Rome.
- Veldkamp, T., G. van Duinkerken, A. van Huis, C.M.M. Lakemond, E. Ottevanger, G. Bosh and M.A.J.S. van Boekel. 2012.** Insects as a sustainable feed ingredient in pig and poultry diets—A feasibility study. Wageningen UR Livest. Res. Report 63: 62. doi: ISSN 1570 - 8616.
- Vinokurov, K.S., E.N. Elpidina, B. Oppert, S. Prabhakar, D.P. Zhuzhikov, Y.E. Dunaevsky and M.A. Belozersky. 2006.** Diversity of digestive proteinases in *Tenebrio molitor* (Coleoptera: Tenebrionidae) larvae. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 145: 126–37. doi: 10.1016/j.cbpb.2006.05.005.
- Weaver, D.K. and J.E. McFarlane. 1990.** The effect of larval density on growth and development of *Tenebrio molitor*. J. Insect Physiol. 36: 531–536. doi: 10.1016/0022-1910(90)90105-O.
- Xuegui, W., Z. Xiaowei, L. Xiaoyu, Y. Jianming, J. Surong and Z. Min. 2010.** Study on the biological characters of *Tenebrio molitor* L. Chin. Agr. Sci. Bull. 230–233.
- Zhao, X., J.L. Vázquez-Gutiérrez, D.P. Johansson, R. Landberg and M. Langton. 2016.** Yellow mealworm protein for food purposes—Extraction and functional properties. PLoS One 11: 1–17. doi: 10.1371/journal.pone.0147791.
- Zielińska, E., B. Baraniak, M. Karaś, K. Rybczyńska and A. Jakubczyk. 2015.** Selected species of edible insects as a source of nutrient composition. Food Res Int. 77(3): 460–466. doi: 10.1016/j.foodres.2015.09.008.