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GALLERIA MELLONELLA (LEPIDOPTERA PYRALIDAE): AN EDIBLE INSECT OF NUTRACEUTICAL INTEREST

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Francardi V., Frosinini R., Pichini C., Botta M., Cito A., Dreassi E. – *Galleria mellonella* (Lepidoptera Pyralidae): an edible insect of nutraceutical interest.

The nutritive values of *G. mellonella* were until now investigated as feed for insectivore animals and notwithstanding the interesting nutritive content of the larvae and the possibility to affect n-3 fatty acid content by diets, this species is not considered of some interest for human diet. The focus of the present study is to value the effects of two diets with (D1+) and without (D1) the addition of leenseeds, on reaching n-3 fatty acid content and n-6/n-3 fatty acids ratio of interest for human health, in particular in the prevention of cardiovascular diseases. Results highlighted higher n-3 fatty acid values in *G. mellonella* larvae fed on D1 (2.0g/100g) and on D1+ (14.82g/100g) diets than reported in literature and a lower n-6/n-3 ratio in larvae from D1+ (0.859) than in those recorded from D1 (4.68). Anyway, larvae fed on both the diets showed n-6/n-3 ratio optimal for human health. The study showed the nutraceutical potential of *G. mellonella* which deserves to be more investigated in the future.

KEY WORDS: essential fatty acids; leenseeds; n-6/n-3 ratio; human health.

INTRODUCTION

There is an increasing awareness on the role of polyunsaturated acids (PUFA) as food supplements in human diet and in the prevention of different diseases. In particular linolenic fatty acids (n-3) are known to exert healthy effects in the care of cardiovascular diseases. In the last years, pharmaceutical industries focused their attention on set up diet supplements rich n-3 to integrate human western food generally more rich in linoleic acid (n-6) deriving from modern agriculture and feed supplies for animals for human consumption (SIMOLOPOULOS, 2002). Actually n-3 sources mainly derived from cereals (e.g. soy) or from animals (e.g. fish) which production involve severe environmental impact and high costs. It is so necessary and pressing to individuate alternative and environmental sustainable n-3 sources and in this trend edible insects, in particular species easy to be mass reared in confined and controlled places, may represent a valid, economic and sustainable opportunity (VAN HUIS *et al.*, 2013). Edible insects are mainly known to enter in human diet as supplements of proteins and essential aminoacids for many populations living in different parts of the world. Lipids, after protein, are the most important compounds of edible insects, which nutraceutical value was recently investigated on the mealworm beetle *Tenebrio molitor* L. (Coleoptera Tenebrionidae) and *Rhynchophorus ferrugineus* (DREASSI *et al.*, 2016; CITO *et al.*, 2017). In particular the studies evidenced interesting PUFA contents in larvae and pupae of *T. molitor* reared on diets consist of different cereal flours and the possibility to enhance n-3 content with a dietary supplement. Furthermore the n-6/n-3 ratio resulted more suitable to human consumption in larvae than in pupae.

Another insect easily mass reared is the waxmoth *Galleria mellonella* (Lepidoptera Pyralidae) recently analysed for its nutritive value in proteins, aminoacids, vitamins, minerals and fatty acids (FA) contents as feed for insectivore animals (reptiles, birds and mammals) living in zoos or kept as pets by hobbyists (reference in: FINKE *et al.*, 2002; 2015 and in: RUMPOLD & SCHLUTER., 2013). In literature among the waxmoth PUFA content, n-6 resulted the more representative while n-3 showed to be absent or at low amounts in larvae fed on different diets (LINDSAY & BARLOW, 1970; THOMPSON & BARLOW, 1972; FINKE 2002; PIERCE, *et al.*, 2004).

Nevertheless studies aimed to increase *G. mellonella* nutritional value for insectivore animals with diet supplemented with leenseeds, canola oil and fish oil, evidenced an increased level of n-3 and the presence of eicosapentaenoic acid (EPA C20:5, n-3) in the larvae that also showed a n-6/n-3 ratio lower (4) than previously reported (14) (FINKE, 2015). n-3 is essential in regulating n6/n-3 ratio so an increased level of this acid, induced by the diet, opens to the possibility to reach ratio values in the insects suitable for human health (<5) (SIMOLOPOULOS, 2002; GOMEZ CANDELA *et al.*, 2011).

Notwithstanding the interesting nutritive values of *G. mellonella* larvae derived from literature and the possibility to affect n-3 content with diets, this species is not hitherto assumed of some interest for human health. The aims of this study are so to investigate the effects of different diets on n-3 content in *G. mellonella* larvae and on their growth time, weight and survival to value the opportunity to employ this insect for human diet.

MATERIALS AND METHODS

DIETS

G. mellonella diet compositions are reported in Tab.1. The diets consisted of different cereal flours, beeswax, powdered milk and multiflowers honey. To enhance n-3, D1 was supplemented with 100 g of minced leenseeds (Dalla Buona Terra, Pedon S.P.A., Molvena (VI) Italy). D2 is a modified diet from CAMPADELLI (1973). All the diets were sterilized for 3 hours in a dry stove to prevent moulds appearing, before being use in the bioassays.

Table 1 – Diets composition.

Component	Diets composition (g)		
	D1	D2	D1+
honey	250.0	250.0	250.0
beeswax	112.5	112.5	112.5
glycerin	125.0	125.0	125.0
cornmeal	125.0		125.0
whole wheat flour	125.0	71.5	125.0
white flour	250.0		250.0
milk powder	125.0	125.0	125.0
yeast extract	62.5	62.5	62.5
rice flour		71.5	
oat flour		71.5	
rye flour		71.5	
barley flour		71.5	
soy flour		71.5	
corn starch		71.5	
cholesterol		6.0	
minced leenseeds			100.0

G. MELLONELLA REARING AND BIOASSAYS

The larvae of *G. mellonella* were obtained from the insect mass-rearing carried out in laboratory at the Research Centre of Plant Protection and Certification (CREA-DC), Florence (Italy). In the bioassays for each diet (D1, D1+ and D2) were employed 20 young larvae of about 8mm length individually put in a plastic cup with screw caps (150 ml) on which a central handmade hole was covered with a thick wire mesh heat sealed for aeration and to prevent insect escape. Each larvae were left to feed on 5 g of diet, sufficient to complete their development till the pupal stage. Bioassays were carried out in a climatic chamber at 25°C, under semi-dark condition and RH of 50-60%. The larval growth time period, in the different diets, was considered from the beginning of the bioassays to the last observation as larvae. Once a week larval weight (mg) of *G. mellonella* was recorded and the last value as larva before metamorphosis was considered in the analysis. Larval mortality was controlled twice a week.

Larvae fed on the diets that showed the shortest growth time, highest survival and mature larvae weight were selected to be analyzed in lipid composition as well as their breeding diets.

LIPID ANALYSIS

Chemicals and reagents

Supelco-37 Component FAME Mix and BF₃ 14 % methanolic solution were purchased from Sigma-Aldrich S.r.l (Milan, Italy). All solvents and reagents used for

samples preparation were from Merck (Darmstadt, Germany). Milli-Q quality water (Millipore, Milford, MA, USA) was used.

Fat content determination

Fat was extracted from insects, using mixed solvent extraction (BLIGH & DYER 1959), a method approved by the Association of Analytical Chemists (AOAC). Each sample (5 g) was exactly weighed and placed in a glass centrifuge tube with 50 mL of CHCl₃:CH₃OH mixture (2:1 v/v) and homogenized for 5 min using an IKA Labortechnik homogenizer model T25 basic (IKA WERKE GmbH & Co., Staufen, Germany).

Butylatedhydroxytoluene (BHT 100 µM) was added to the extraction mixture to inhibit lipid peroxidation.

After filtration, the sample was centrifuged at 4,000 rpm for 5 min. The CHCl₃ layer was separated and another aliquot of extraction solvent was added to residue. The organic fractions were extracted with 20 mL of KCl 0.7% solution, washed at the interface twice with the “upper phase” (obtained from CHCl₃/CH₃OH/H₂O (53:27:20 v/v) mixture) and dried. The residue was weighed for fat percentage determination and then used for fatty acid analysis.

Fatty acid analysis by GC-MS and GC-FID

The fat samples, obtained as described above, were prepared for GC analysis by converting to fatty acid methyl esters (FAME) with 14% BF₃ methanolic solution according to the method described by MORRISON & SMITH (1964). The sample (10 mg) was added with 1 mL of BF₃ methanolic solution and maintained at 90°C for 1 h and then, after cooling, extracted with 1mL of n-hexane. After centrifugation at 4,000 rpm for 5 min, the n-hexane phases were used for GC analysis.

The analysis was carried out using a gas chromatograph VARIAN 3900 with CP-8400 auto injector coupled to an ion-trap mass spectrometer (VARIAN Saturn 2000 MS–MS). A SPTM-2380 fused silica capillary column (60 m x 0.25 mm I.D., 0.2 µm film thickness) provided by Supelco (Bellefonte, PA, USA) was used. Oven temperature was programmed from 100 to 240°C at 10°C/min and then increased to 260°C at 5°C/min; finally, the oven was maintained at 260°C for 10 min. Injector temperature was set at 230°C; 1 µL aliquots were injected and the split ratio of 1:10 was used. Helium was employed as carrier gas with a flow rate of 1 mL/min. The ion-trap mass spectrometer was operated in the electron impact ionization (EI) mode with an ionization voltage of 70 eV. The analyses were performed with a filament-multiplier delay of 9 min and data acquisition was carried out in the range *m/z* 50–650 in full scan mode. The transfer line was maintained at 250°C, the manifold and trap temperatures were set at 80 and 150°C, respectively. Instrument control and mass spectrometry data were managed by a personal computer running the Saturn GC–MS WorkStation software (6.30 version).

Identification of the chromatographic peaks was made by comparison of MS spectra and retention times of the available standards, with National Institute of Standards and Technology (NIST) library and also on the basis of elution data provided in literature (SÁNCHEZ-ÁVILA *et al.*, 2009).

The GC–FID analyses were carried out using a GC–FID (Perkin Elmer Clarus 500 GC) and a split/splitless injector. Injector and detector temperatures were set at 230 and 280°C, respectively. Helium was employed as carrier

gas with a flow rate of 1 mL/min; 1 μ L aliquots were injected and the split ratio of 1:10 was used. The fused silica capillary column and the oven temperature program described for GC-MS analysis were employed. Data acquisition was carried out using Perkin Elmer TotalChrom Navigator software v 6.3.1.

Fat content and fatty acid analysis of D1 and D1+ diets

Fat was extracted from diets (8 g) and analyzed using the same methods employed in insect analysis.

STATISTICAL ANALYSIS

Data on *G. mellonella* larval growth time and weight were processed with ANOVA analysis and Tukey Test while data on larval mortality were processed with binomial test analysis with Bonferroni correction.

Quantitative analyses (FA content and FA composition) were run at least in triplicate and data expressed as means $\pm$ standard deviations (SD). A T-test was performed to assess significant differences in FA content and FA composition between larvae raised on each base diet. StatGraphics Plus Package (4.1).

RESULTS

G. MELLONELLA GROWTH DATA

Diets were selected to explore the effect on *G. mellonella* growth and fat composition. D1 is a diet set up in the Research Centre for Plant Protection and Certification of Florence (I) (CREA DC) and successfully adopted for *G. mellonella* mass rearing over the past 20 years; its composition contain only two cereal species (corn and wheat flour). D2 diet contains in addition other cereal species (rice, oat, rye, barley, soy) and cholesterol in its formulation. To compensate the poorer cereal composition of D1 diet and the absence of cholesterol, an important compound for insect metabolism and development (ROBBINS *et al.*, 1971), D1 was supplemented with minced leenseeds, known to be rich in PUFA and in particular in n-3 that also play an important role in insect physiology (DADD, 1983) to obtain D1+.

Data on *G. mellonella* mortality, growth time and mature larvae weight on the three diets are reported in Tab. 2.

Table 2 – *G. mellonella* larval mortality, growth time and mature larvae weight on the three diets.

Diets	N. of larvae	mortality (%)	Larval growth time (days)	Larval weight (mg)
D1	20	25 ^a	19.85 $\pm$ 2.20 ^a	115.65 $\pm$ 14.89 ^a
D2	20	55 ^a	33.20 $\pm$ 5.26 ^b	48.55 $\pm$ 9.13 ^b
D1+	20	60 ^a	13.30 $\pm$ 2.20 ^a	83.05 $\pm$ 17.84 ^{ab}

Notwithstanding the high larval mortality percentages in D1+ and D2, no significative difference emerged among the three diets. Diversely *G. mellonella* larvae reared on D1 and D1+ diets reached mature age in significantly shorter time (19.85 and 13.30 days, respectively) than observed in D2. The weights of mature larvae fed on D1 resulted significantly higher than those fed on D2; the larval weight from D1+ instead not differed from those

recorded in the other two diets. On the base of these results, as previously assessed, FA composition was analysed only on larvae fed on D1 and D1+ considered the best nutrition substrata for *G. mellonella*.

LIPID ANALYSIS OF D1 AND D1+ DIETS

Diet D1 analysis showed the prevalent presence of saturated fatty acids (SFA) up to C36 and the absence of n-3 (C18:3) (Fig. 1). The FA content (% w/w) of D1 (11.38 $\pm$ 2.56) and D1+ (12.09 $\pm$ 2.84) diets not resulted significantly different while, as to be expected, n-3 undetectable in D1, was present at 2% (g/100g of FA) in D1+ diet.

Fig. 1 – GC-MS profile of fatty acids methyl esters of D1 (unspecified chromatographic peaks refer to aliphatic and unsaturated hydrocarbons typical of beeswax).

FAT AND FATTY ACIDS CONTENT IN LARVAE OF G. MELLONELLA

No significative difference emerged between total FA, based on dry matter (% w/w), in larvae fed on D1 and D1+ (42.17 $\pm$ 4.52 % and 42.09 $\pm$ 4.07% respectively).

Results obtained in FA determination are reported in Tab. 3 and Fig. II. The most representative FA in *G. mellonella* larvae from both D1 and D1+ diets, resulted palmitic acid among SFA, oleic acid among monounsaturated acids (MUFA) and n-6 among PUFA. The addition of leenseeds in the rearing diet (D1+) significantly decreased palmitic acid (SFA), as well as, among MUFA, palmitoleic and heptadecenoic acids levels with a consequent lowering of total SFA and MUFA acids values in the larvae. Diversely the supplement of leenseeds increased significantly stearic acid (SFA) and, among PUFA, n-6 and n-3 values; total PUFA also resulted significantly increased and SFA/UFA ratio significantly lower in larvae fed on D1+ than on D1 diet. The supplement of leenseeds also significantly reduced n-6/n-3 ratio in *G. mellonella* larvae fed on D1+ (0.85 $\pm$ 0.069) in comparison to those from D1 (4.68 $\pm$ 0.853).

Table 3 – Fatty acids composition (g/100g of total fatty acids) of *G. mellonella* larvae fed on D1 and D1+ (mean±SD, n=3).

FA	D1		D1+	
	mean	SD	mean	SD
lauric acid C12	0.04	0.002	0.06*	0.002
myristic acid C14	0.64	0.072	0.86	0.040
pentadecylic acid C15	0.28	0.037	0.22	0.017
palmitic acid C16	35.77**	0.408	28.00	0.149
C16:1 n-5 acid	0.54	0.084	0.57	0.020
palmitoleic acid C16:1 n-7	3.79	0.402	1.51*	0.021
margaric acid C17	0.66	0.060	0.85	0.027
C16:2 n-4 acid	0.06	0.010	0.04	0.005
ni (tr 34.08 min)	0.09	0.005	0.22	0.041
heptadecenoic acid C17:1 n-6	1.66**	0.010	0.94	0.009
stearic acid C18	2.89	0.401	5.14*	0.001
oleic acid C18:1 n-9	41.46**	0.742	34.74	0.291
ni (tr 39.53 min)	0.02	0.007	0.03	0.005
α -linoleic acid C18:2 n-6	7.42	0.012	10.87*	0.529
arachidic acid C20	0.42**	0.013	0.18	0.009
ni (tr 42.70 min)	0.09*	0.005	0.04	0.003
α -linolenic acid C18:3 n-3	2.00	0.378	14.82**	0.660
eicosenoic acid C20:1 n11	0.01	0.009	0.03	0.004
C20:2 n-6 acid	0.12	0.046	0.83*	0.062
ni (tr 47.83 min)	0.07	0.010	0.06	0.000
Sum of n-3 acids	2.00	0.378	14.82**	0.660
Sum of n-6 acids	9.19	0.068	12.64*	0.458
SFA	40.70**	0.150	35.31	0.144
MUFA	47.45**	1.059	37.78	0.288
PUFA	9.59	0.446	26.56**	0.188
n-6/n-3 ratio	4.68*	0.853	0.85	0.069
SFA/UFA ratio	0.71**	0.010	0.55	0.003

T-test was performed and significant results are indicated with * $p < 0.05$ and; ** $p < 0.01$

DISCUSSION

The study of the nutraceutical value of edible insects represents a new research frontier and a challenge to more and diversely value the importance of these arthropods as human food. Lipids play major roles in the insect life and are involved in multiple metabolic functions; in the same time lipids represent also a potential source of essential fatty acids known to exert beneficial effects in human health and this motivate researches to assess the possibility of their use in the prevention of some human diseases.

FA acids composition of *G. mellonella* was mainly investigated to study their importance in the insect physiology and to assess the role of polyunsaturated fatty acids (PUFA), in particular n-3, in getting viable and normal adult emergence (DUTROVSKI, 1973; DADD, 1964; 1983). Furthermore several Authors highlighted the importance of lipid content and composition in the insect diets as factors influencing larval growth, survival and adult oogenesis (ARRESE & SOULAGES, 2010; COHEN, 2015; MIRTH & RIDDIFORD, 2007).

The analysis of FA composition of the D1 diet showed mainly the presence of saturated fatty acid (C16:0-C36:0), oleic acid, only traces of n-6 and the absence of n-3 as also observed by JIMENEZ *et al.* (2006) in a study on the composition of the beeswax. The addition of leeneseeds to the diet (D1+) not significantly increase total FA amounts in mature waxmoth larvae *vs* those fed on D1 as recently evidenced also in *T. molitor* larvae reared on diets with increasing total fat contents (DREASSI *et al.*, 2016).

The more representative fatty acids in waxmoth larvae from D1 and D1+ resulted palmitic acid, oleic and n-6 according to several Authors on larvae fed on different

Fig. II – GC-FID profile of fatty acids methyl esters of *G. mellonella* larvae fed on D1+

feeding substrata (LINDSAY & BARLOW, 1970; THOMPSON & BARLOW, 1972; DUTKY *et al.*, 1962; FINKE, 2002; 2015). Different diets may influenced FA total content in *G. mellonella*, as confirmed in the present paper, where the total FA content in *G. mellonella* larvae fed on D1 diet resulted lower ($42.17 \pm 4.52\%$) than values reported in the recent literature (from 51.40% to 60.0% in larvae reared on diets with different composition) (in: RUMPOLD *et al.*, 2013). In literature Authors reported a low (<1 g/100g of FA) or absent n-3 content in *G. mellonella* reared on different substrata (FINKE, 2002; 2015; THOMPSON & BARLOW, 1972). In the present paper, *G. mellonella* larvae fed on D1 diet evidenced higher n-3 value (2.0 g/100g of FA) than that reported by FINKE (2002) (<0.5 g/100g of FA) in larvae fed on a not specified artificial diet. Also n-3 value in larvae fed on D1+ (14.82 g/100g of FA) was higher than that recorded in larvae from a base diet (Vita-bugs®) supplemented with very rich sources of n-3 as leenseeds, canola and fish oils to increase diet nutritional value for insectivores animals (1.7 g/100g of FA) (FINKE, 2015). These supplements together with an increased content of n-3 in larvae, higher than previously reported, also lowered n-6/n-3 ratio from 14 to 4 (FINKE, 2015). Similarly the addition of leenseeds to D1 diet caused a significative increase of n-3 in the waxmoth larvae that, despite the increase of also n-6, more effectively lowered n-6/n-3 ratio from 4.68 to 0.85. Anyway both n-6/n-3 ratio of larvae fed on D1 and D1+ diets reported values suitable for human health (SIMOPOULOS 2002). This Author in fact indicated ratio varying from 1 to 4 as optimal for the prevention and management of different human diseases in particular cardiovascular ones. In addition it should also be noted that the supplement of leenseeds (D1+), at the employed dose, cause a significative crash in SFA acids content in the larvae mostly due to the significative decrease of palmitic acid (SFA) even if stearic acid resulted increased. On the other hand some FA in *G. mellonella* not resulted susceptible to be influenced by the diet as observed by THOMPSON & BARLOW (1972) that in larvae fed on diet Pabulum-based with and without the addition of stearic acid (SFA), palmitoleic acid (MUFA) and linoleic-n-6 (PUFA), highlighted a characteristic retain of these fatty acids pattern. The results obtained by FINKE (2015) and by the Authors of the present paper put in light the possibility to easily influenced n-3 content and n-6/n-3 ratio in the waxmoth larvae into values optimal for human diet.

The study so makes clear that *G. mellonella* can be an interesting source of n-3 for human diet and that the nutraceutical value of this insect species deserve to be more investigated in the future.

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