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Faculty of Natural Resources and Agricultural Sciences

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Mjölmask *Tenebrio molitor*, en potentiell källa till matlipider

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Abstract

The yellow mealworm *Tenebrio molitor* is considered as a sustainable alternative to animal-derived source of protein. The population of the world increases and is believed to be over 9 billion by 2050, and the demand of protein will increase. Among edible insects, the mealworm is the one most commonly reared in Europe. After protein, the second largest part of the mealworm is lipids, which amounts to about 33% of dry matter. With this large amount of lipids, the mealworm could be a potential source of food lipids. In this work, the aim is to review the current knowledge about the lipid content and fatty acid composition in mealworms.

The most common fatty acids in mealworms are palmitic acid, oleic acid and linoleic acid. The mealworm can synthesize both linoleic acid (LA) and alpha linolenic acid (ALA), which are essential fatty acids for humans. Ratios between polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) (PUFA/SFA) and ratios between n-6/n-3 are used as index of healthy diet. The PUFA/SFA ratio in the mealworm has been found to be within the recommended values. Mealworms are rich in unsaturated fatty acids (UFAs) (monounsaturated fatty acids and polyunsaturated fatty acids), but the ratio of n-6/n-3 varied greatly. It would be desirable with higher content of ALA (n-3) to get a better n-6/n-3 ratio. It has been shown that it is possible to manipulate the content of lipids and the composition of fatty acids in the mealworm through the feed. However, the composition of the feed and the content of the mealworms do not necessarily match, so there is a physiological regulation of the composition of FAs. Other conditions such as temperature, humidity and development stage are also factors that have an impact on the lipids and FAs. In the reviewed literature, the results vary and all conditions are not always known. The conclusion is that more research is needed before the mealworm can be promoted as a new source of food lipids. The reason could be that the mealworm primarily has been highlighted as a source of protein, which may have overshadowed the mealworm as a potential source of food lipids.

Keywords: mealworm, Tenebrio molitor, lipids, fatty acids, edible insects, future food

Sammanfattning

Mjölmasken *Tenebrio molitor* framhålls som ett hållbart alternativ till animaliskt protein. När jordens befolkning ökar och antas vara över 9 miljarder år 2050 kommer efterfrågan på protein att öka. Bland ätbara insekter är mjölmasken den vanligaste som föds upp i Europa. Efter protein är den näst största delen av mjölmasken lipider, som uppgår till cirka 33% torrsubstans. Med denna stora mängd lipider är mjölmasken intressant att utforska som en potentiell källa till mat lipider. Målet med detta arbete att granska den nuvarande kunskapen om lipidinnehåll och fettsyrasammansättning i mjölmask. De vanligaste fettsyrorna i mjölmaskar är palmitinsyra, oljesyra och linolsyra. Mjölmasken kan syntetisera både linolsyra (LA) och alfa-linolensyra (ALA), vilka är essentiella fettsyror för människan. Förhållandet mellan fleromättade fettsyror (PUFA) och mättade fettsyror (SFA) (PUFA/SFA) och förhållandet mellan n-6/n-3 används som index för hälsosam kost. PUFA/SFA-förhållandet i mjölmask har visat sig ligga inom de rekommenderade värdena. Mjölmaskar är rika på omättade fettsyror (UFA) (enkelomättade fettsyror och fleromättade fettsyror). Förhållandet mellan n-6/n-3 har visat sig variera väldigt mycket. Det skulle vara önskvärt med ett högre innehåll av ALA (n-3) för att få ett bättre förhållande mellan n-6/n-3. Det har visat sig att det är möjligt att manipulera innehållet av lipider och sammansättningen av fettsyror i mjölmask genom fodret. Fodrets sammansättning och mjölmaskens innehåll följs däremot inte alltid åt. Således finns det en fysiologisk reglering av lipidinnehållet och fettsyrornas sammansättning i mjölmasken. Förhållanden som temperatur, fuktighet och utvecklingsstadiet är också faktorer som påverkar sammansättningen av lipider och fettsyror. I den genomgångna litteraturen är resultaten varierande och alla förhållanden är inte alltid redogjorda för. Slutsatsen är att det behövs mer forskning innan mjölmasken kan framhållas som en ny källa till mat lipider. Mjölmasken har främst blivit uppmärksammad som en källa till protein, vilket kan ha överskuggat dess potential som källa till matlipider.

Nyckelord: mjölmask, Tenebrio molitor, lipider, fettsyror, ätbara insekter, framtidens mat

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Abbreviations

AA	Arachidonic acid
ALA	Alpha-linolenic acid
CLA	Conjugated linoleic acid(s)
DGLA	Dihomo-gamma-linoleic acid
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
EPA	Eicosapentaenoic acid
FA	Fatty acid(s)
GLA	Gamma-linoleic acid
LA	Linoleic acid
MUFA	Monounsaturated fatty acid(s)
PUFA	Polyunsaturated fatty acid(s)
SFA	Saturated fatty acid(s)
TAG	Triacylglycerol(s)
UFA	Unsaturated fatty acid(s)

1 Introduction

Food lipids supply us with energy, fat soluble vitamins, and essential fatty acids (FAs). Food lipids also enhance the sensory characteristics of the food (Paul *et al.* 2017). In this work, the yellow mealworm *Tenebrio molitor*, a beetle in the family *Tenebrionidae*, (henceforth mealworm) will be studied.

This study focuses on the mealworm as a potential source of food lipids and examines what is known about the content of lipids and the composition of FAs in mealworms.

Since the human population is expecting to reach about 9.1 billion by 2050 (FAO 2009) there are challenging questions concerning food production in more sustainable ways. The demand for animal-derived protein is expected to increase and to meet this demand, the required production of meat has to rise with 72% over the next 35 years (Dunkel & Payne 2016). More than two-thirds of all agricultural land is used by livestock (van Huis 2016) and when problems concerning land-use, water-use, emissions of greenhouse gases (GHGs), and feed conversion – all of which are strongly connected to livestock – are considered, an option to observe and even promote is edible insects (FAO 2013, Gahukar 2016, van Huis 2013, Dunkel & Payne 2016).

In many parts of the world people eat insects, but especially in urban and Western societies it is rare, and even seen as disgusting or culturally inappropriate (Nowak *et al.* 2014). Among edible insects the mealworm is growing in popularity, and it is also the most commonly reared in Europe (Paul *et al.* 2017). The reasons for why mealworm is a favourable option in Western countries are that the species is endemic, suitable for rearing on a large scale, and the availability of experts on farming mealworms (the pet industry has reared mealworms for a long time) (FAO 2013).

Primarily, insects are highlighted as an alternative protein source. Most species have large quantities of good quality protein. Lysine, methionine, and leucine are essential amino acids that are limited in sources of plant origin, but are present in animal-derived protein. The amino acid profiles are taxon-related (Downs *et al.*

2016). The mealworm is rich in isoleucine, leucine, and lysine (Ravzanaadii *et al.* 2012). Compared with beef, the mealworm has a significantly higher amount of amino acids such as isoleucine, leucine, valine, tyrosine, and alanine (Sun-Waterhouse *et al.* 2016). Especially the potential to be a source of protein has caught the public eye when the demand of meat is increasing (Broekhoven 2015, van Huis 2016). In mealworms, the content of protein is shown to be stable even if the feed differs 2-3 fold in protein content. After protein, the second largest portion of the mealworm is lipids, around 33%. Due to the high content of lipids, mealworms can be seen as a novel source of food lipid (Paul *et al.* 2017).

1.1 A future alternative

Worldwide there are about 1500-2000 insects and other invertebrates that are eaten by humans, especially in Central and South America, Asia, and Australia (Sun-Waterhouse *et al*. 2016). Approximately 2 billion people commonly use insects within their food (Makkar *et al.* 2014).

Most of the insects are harvested in nature but in the future, we may see another scenario. Mini-livestock could be an option to replace conventional livestock (small-sized organisms, mainly insects, which can be reared and consumed by humans are called mini-livestock). It is indeed the same idea as for conventional livestock (Abbasi *et al.* 2016). Mini-livestock can also include small animals reared for feed (van Huis 2013). Among all human activities livestock is one of the most ecologically harmful (Abbasi *et al*. 2016).

Supposing that the demand of insects increases dramatically in the future, then production techniques (for mass-rearing) have to be developed. To succeed with commercial farming of insects, new procedures also must be developed. The new challenge to scale-up the production of insects is something for industries specialized within the field (van Huis 2013).

1.2 Objectives

If the mealworm should be an option for human consumption, knowledge about the nutritional composition is fundamental. The focus of this work is to review the current knowledge of the nutritional content of lipids and the composition of FAs in mealworms and the feed they have got. Our choices of what we eat could for example be built on health aspects, ethical issues, and environmental issues. In order to promote mealworms as a source of food lipids, knowledge is of the highest importance.

1.3 Method

This work is a literature study. In addition to books, databases listed at the SLU library have been used (Web of science, Scopus) and the library's search tool Primo. The papers examined for this study are published between 2013 and 2017. I have searched keywords such as *mealworms, Tenebrio molitor, lipids, fatty acid, edible insects, future food,* and *sustainable food.*

2 Background

The background will contain a description of lipids and fatty acids. It will be valuable to have knowledge about lipids and FAs as a help when interpreting the results of analyses that have been done on mealworms. It is also a help to understand what constitutes a healthy diet when it comes to food lipids.

There could be several interesting ethical issues about edible insects as well, but these are not within the scope of this work.

2.1 Lipids and fatty acids

Lipids have many vital functions except providing us with energy. Our cell membranes are built of lipids. Lipids work as precursors to different biological molecules, and are also protectors of internal organs. We need lipids as insulation to keep the body temperature (Undeland 2005). Lipids are also linked to several health concerns about the consumption of lipids. Additional problems are an imbalance between n-3 and n-6 intake, and a shortage of fatty vitamins A, D and E (Gurr *et al*. 2016). Undeland (2005) also mentions shortage of K vitamin.

2.1.1 What are lipids and fatty acids?

A definition of lipids as compounds that are soluble in organic solvents is not specific enough. A more satisfying definition is one that includes fatty acids and their derivatives (esters or amides), but also compounds that are related to fatty acid and their derivatives through biosynthetic pathways (prostanoids, aliphatic ethers, and alcohols), or by functions (cholesterols and tocopherols) (Christie & Han 2010).

> "Lipids are fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds." (Christie & Han 2010).

Next is to define FAs as compounds that are synthesised in nature from units of malonyl coenzyme A (Christie & Han 2010). FAs are molecules containing a long hydrocarbon chain with a carboxylate group in the end. FAs are mainly stored as triacylglycerols (TAGs) in adipocytes. FAs are important as building blocks in membranes and are also necessary when proteins are covalently attached to them. FAs are needed as precursors of hormones and intracellular messengers (Berg *et al*. 2015).

Two main aspects of food lipids are the total amount of lipids, and the content and composition of FAs. The first is referred to as the quantity and the latter as the quality (Gurr *et al*. 2016). Most of our dietary lipids are TAGs, representing about 90%. Further, about 35-45% of all dietary energy is formed by TAGs. The quantity is closely related to body weight. All natural lipids contain saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs). The combination and thereby the quality is variable depending on the source (Gurr *et al*. 2016).

2.1.2 Fatty acids

The chemical structure of FAs is a hydrocarbon chain with a methyl group in one end and a carboxylic group in the other end. The properties of a FA are dependent on the length of the hydrocarbon chain and on the degree of saturation (Berg *et al*. 2015). FAs are divided into different groups. SFAs contain no double bonds. MUFAs have one double bond. PUFAs have two or more double bonds. Sometimes MUFAs and PUFAs together are just called unsaturated FAs (UFAs). UFAs can form isomers, positional or geometric and therefore the nomenclature is complex. In positional isomers, the double bonds are disposed in different positions within the hydrocarbon chain. In Figure 1, one SFA (decanoic acid) and two isomers of UFAs are shown. In order to name these two isomers, the number of carbon are counted from the carboxyl carbon to the double bonds which are located between C6-7 and C7-8 i.e. trans-6-deceonic acid and trans-7-deceonic acid (Gurr *et al*. 2016).

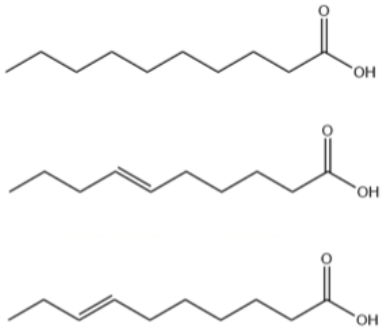

Figure 1. The SFA is decanoic acid, in the middle *trans*-6-decenoic acid, and at the bottom *trans*-7-decenoic acid (the figure is created with Chemdraw Professional 15.0).

Geometric isomers occur when the configuration at the double bonds are either in *cis* or *trans* (also referred as Z or E). In Figure 2, the difference between *cis* and *trans* is shown. In nature, *cis* configuration is the most common. All possibilities with isomers will give FAs different properties (Gurr *et al*. 2016).

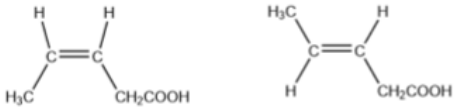

Figure 2. To the left a *cis* configuration, and to the right a *trans* configuration (the figure is created with Chemdraw Professional 15.0).

Conjugated linoleic acid (CLA) is a collective name of FAs with 18 carbon atoms and two double bonds without any methylene group in between (Undeland 2005). Often FAs are named as n-3 or n-6 (omega), which is another system. The omega-carbon is the last one, namely the carbon in the methyl group. Thus, when naming FAs as an omega-3 FA, the double bond is between C3-4 (counting from the methyl end) (Gurr *et al*. 2016).

2.1.3 Essential fatty acids

Essential FAs cannot be synthesized in our body and so must be ingested in our diet. Linoleic acid (LA) (cis, cis-9,12 octadecadienoic acid or 18:2n-6) and alpha-linolenic acid (ALA) (all cis-9,12,15 octadecatrienoic or 18:3n-3) are essential FAs (Gurr *et al*. 2016). In Figure 3, the chemical structures of the two essential FAs are shown. From LA, arachidonic acid (AA) is formed. AA, a 20:4 FA, is a

major precursor of eicosanoid hormones. Prostaglandins, prostacyclins, thromboxanes, and leukotrienes are all eicosanoids (Berg *et al*. 2015).

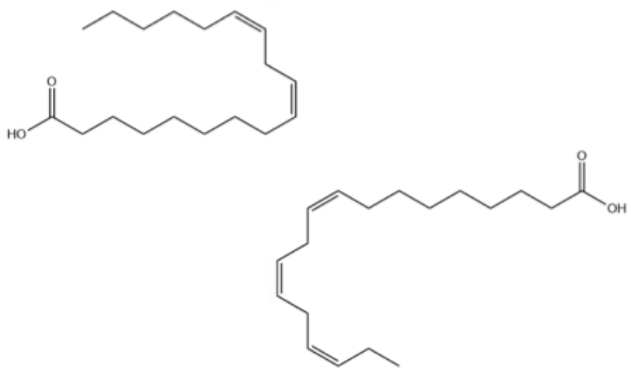

Figure 3. The chemical structure of the two essential FAs. Top left is linoleic acid (LA) and the bottom right is right alpha-linolenic acid (ALA) (the figure is created with Chemdraw Professional 15.0).

From ALA the body can form the two elongated FAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which in turn also produce different eicosanoids (Gurr *et al*. 2016).

2.1.4 EPA and DHA

Even if humans are able to synthesize EPA and DHA, the amounts are often limited. Both EPA and DHA are important n-3 FAs. EPA and DHA are long-chained PUFAs. To highlight their importance, EPA and DHA are necessary as precursors of signalling molecules. Also, a major part of eye and brain tissue contains DHA (Gurr *et al.* 2016). The most common sources of these two long-chained FAs are fish and shellfish (Gurr *et al.* 2016). Unfortunately, fish is associated with other problems such as overfishing and accumulation of hazardous substances.

2.2 Health aspects and recommendations

In a healthy diet the recommended daily intake of lipids should not exceed 30% of the total energy (%E). Of these 30%E the share of SFA should not exceed 10%E (Undeland 2005). In industrialized countries, the total intake of lipids can be higher than the recommended value, around 35-45%E. So, there is a link between the quantity and bodyweight (Gurr *et al.* 2016). For adults, the recommendations of n-6 (LA) range between 2.5-3%E and of n-3 (ALA) between 0.5-2%E (FAO 2010).

When it comes to healthy diets, the ratio n-6/n-3 is a commonly used index. Another widely used index is the ratio PUFA/SFA (P/S).

2.2.1 The n-6/n-3 ratio

The ratio between n-6 and n-3 is widely used as an index of a healthy diet. Our ancient ancestors, who lived as hunter-gatherers, had a n-6/n-3 ratio of 1 in their diet (Gurr *et al.* 2016). Today's diets in Western countries have a ratio that is much too high. As an example, the diets in UK and US have a ratio of 10-20. A ratio of 4 is recommended within a healthy diet (Gurr *et al*. 2016). The reason of why a good balance between n-6 and n-3 is important to maintain, is that these PUFAs are competing for the same enzymes (Δ-6-desaturase and Δ-5-desaturase) in the metabolic conversion of LA and ALA to AA or EPA and DHA, respectively (see Figure 4). The more n-6 in the diet, the less n-3 products are formed (Gurr *et al.* 2016). Instead of using n-6/n-3 ratio, recommendations of a daily intake expressed as percent of energy (%E) or g/day sometimes are preferred. All n-6 FAs do not have the same effects and the same is true for different n-3 FAs. Therefore, it could be better to give recommendations of each FA. As regards the ratio, n-6/n-3 takes no account of which n-6 or n-3 FAs and therefore could be misleading (Gurr *et al.* 2016).

Figure 4. In the n-6 and n-3 series, Δ-6-desaturase and Δ-5-desaturase are needed for desaturation. The competing for the same enzymes occur if the n-6/n-3 ratio is not balanced. The figure is slightly modified after Christie & Han (2010).

The products from the n-6 and n-3 series sometimes have the opposite effect and therefore an imbalance could have an impact on several diseases (Undeland 2005).

2.2.2 The PUFA/SFA (P/S) ratio

The PUFA/SFA ratio is a useful index of a healthy diet and the recommended ratio should be close to 1 (Paul *et al.* 2017). This ratio is used and signals if there is a need of replacing SFAs with PUFAs.

2.2.3 Health aspects

The expected result of replacing SFAs with PUFAs in our diet would be beneficial to our health. One result is less circulating lipids such as cholesterol and TAGs, which reduces the risk of cardiovascular diseases (Gurr *et al.* 2016). The n-3 FAs have several positive effects on the health. Cardiovascular diseases, diabetes, cancer, and inflammatory responses can be affected in a positive way (Undeland 2005). For intake of ALA, there is convincing evidence for lower risk of coronary heart disease (CHD) (FAO 2010). The PUFA/SFA (P/S) ratio, a high value ≥3 could promote tumour formation, and a low value of ≤0.33 could instead be atherogenic (Paul *et al*. 2017). The ratio between n-6/n-3 is important because n-6 and n-3 FAs may have the opposite effects when it comes to inflammatory responses. While n-6 FAs potentially increase, n-3 could potentially reduce the inflammatory responses (Gurr *et al*. 2016). A high value of n-6/n-3 ratio may be linked to cancer and coronary heart disease (Paul *et al*. 2017).

2.3 Environmental aspects of insect production

The advantages of edible insects are several when it comes to environmental concerns and sustainability. Sustainable development is defined as

> "development that meets the needs of the present without compromising the ability of future generations to meet their own needs" (World Commission on Environment and Development, 1987).

The production of insects is more sustainable and with smaller ecological footprint compared with livestock (Dossey *et al*. 2016). As an example, the amount of protein that could be produced from 1 ha of land from mealworm had required 10 ha for beef (Gahukar 2016).

Feed conversion ratio (FCR) is a measure of an animal´s efficiency converting feed mass to body mass. One reason for the efficiency is that insects are poikilothermic so no metabolic energy has to be invested in maintaining a constant body temperature (van Huis 2013).

Insects emit lower GHGs and lower ammonia emissions compared to livestock (FAO 2013). Water-use is also an aspect to consider. The use of water when rearing insects is much lesser compared to conventional livestock (Gahukar *et al*. 2016).

3 The yellow mealworm *Tenebrio molitor*

The yellow mealworm *T. molitor* is a species of a darkling beetle. The life cycle is the development stages from egg to the adult (darkling beetle). In Figure 5, a simple picture of the life cycle is shown. The length of a life cycle is highly variable, from 280 to 630 days. The larva stage is the most variable in time, from 3 to 18 months. The temperature has an impact on the large variation in time. As an example, the pupa stage is 7-9 days at 25 °C but can be as long as 20 days at lower temperatures (Makkar *et al*. 2014).

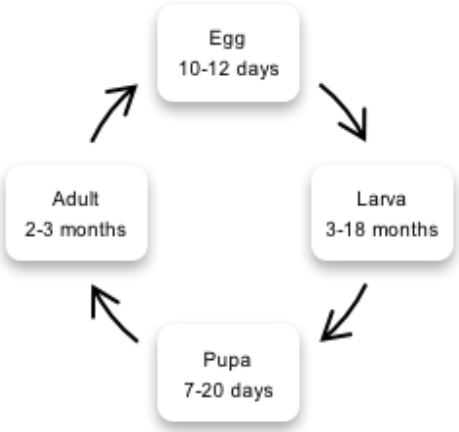

Figure 5. The life cycle of the yellow mealworm Tenebrio molitor. The time variation of each stage is taken from Makkar *et al*. (2014).

Another factor that will have an impact on development time is the feed. It is possible to feed mealworms only with wheat bran, but supplements such as vegetables (potatoes, carrots and cabbage) shorten the development time and improve larval survival, efficiency of food conversion, and adult fecundity (Cortes Ortiz *et al*. 2016).

The larvae (not adults) are able to use water dissolved in the air. Therefore, it is possible to rear mealworms without providing any water at a humidity of 75% or more. The disadvantages are high costs to maintain such high humidity and that it affects the growth of the mealworms (spending metabolic energy when absorbing water vapour) (Cortes Ortiz *et al.* 2016).

Mealworms are a protein source with potential and Nowak *et al.* (2014) also claim that the larvae are a source of micronutrients such as calcium, zinc, and magnesium. In general, insects are not a source of calcium because they do not have an internal skeleton, but it could be manipulated by the feed (Nowak *et al*. 2014).

3.1 Mealworms as a novel source of lipids?

Could mealworms be used as a source of food lipids? This is a relatively new question due to the rising popularity of rearing and consuming mealworms as food (Paul *et al*. 2017). The mealworm can synthesize LA and ALA (essential FAs for human) *de novo*. EPA and DHA are not likely to be found in mealworms, and occur only if they are supplied by the feed (Dreassi *et al*. 2016).

Several studies have shown high values of n-6/n-3 ratio so to be able to offer wholesome mealworms experiments have been made in order to manipulate the content of lipids and the composition of FAs.

3.1.1 Different feed and rearing conditions

In a study by Broekhoven *et al.* (2015) (A in Table 1) the mealworms were obtained from the rearing company Kreca (Ermelo, The Netherlands) and they were maintained in constant temperature at 28 ˚C, at humidity of 65% RH, and with a 12 h photoperiod. The mealworms were reared on a diet containing mixtures of spent grains, brewer's yeast, bread remains, cookie remains, and maize distillers' dried grains with solubles. Carrots were used as a source of moisture. Before harvest the larvae were degutted (starved) for 24 h and then frozen to death at -20 ˚C. The mixture of feed was classified as follows: high protein and high starch (HPHS), high protein and low starch (HPLS), and low protein and high starch (LPHS). The diets classified as high starch were based on cookie remains and caused high larvae mortality. Therefore, the cookie remains in the high starch diets were changed to potato steam peelings.

In the study made by Paul *et al.* (2017) (B in Table 1) the mealworms were reared on a diet containing wheat flour, wheat bran and brewer's yeast. Before the mealworms were frozen to death in -20 ˚C, they had been fasting overnight (degut-

ting), approximately 15 h. Unfortunately, no information about the rearing conditions like temperature, photoperiod, and humidity is presented in Paul *et al.* (2017). The only information is that the mealworms were reared in controlled environments, and were obtained from the Functional and Evolutionary Entomology Laboratory (University of Liege-Gembloux Agro-Bio Tech).

In a study made by Siemianowska *et al.* (2013) (C in Table 1) the mealworms were reared on a diet containing oat flakes and supplement of vegetables as a source of moisture. They were kept in 25 ˚C and were obtained from an insect culture at the Chair of Phytopatology and Entomology, University of Warmia & Mazury in Olsztyn. Instead of freezing the mealworms to death, they were boiled for three minutes and then dried in 60 ˚C. Another batch of mealworms was put in 4 ˚C, thereby making the larvae sleep (the authors' choice of words). The rearing conditions are not further described, so there is no information about either the photoperiod or the humidity.

Dreassi *et al.* (2017) (D in Table 1) made a study with the main goal to investigate if it is possible to change the lipid content and the FA composition within the mealworms when they are fed on different diets, and also to find out the best rearing conditions to improve the content of lipids. In this study, six different diets were used to feed the larvae that were obtained from the commercial supplier *La Voliera*. The larvae were kept in 27±1 ˚C with a humidity of 40-50%. The mealworms were frozen to death at -50 ˚C for 24 hours and then freeze-dried.

In Table 1, a summary of the different conditions – if the mealworms were degutted or gut-loaded, and harvest method – in the different studies is shown.

Table 1. *A summary of the different rearing conditions used, including information about degutting vs gut-loading, and the harvest method.*

Conditions	Temperature (˚C)	Humidity (% RH)	Photoperiod (h)	Degutting vs gut-loading	Harvest method
A	28	65	12	Degutting 24 h	Frozen to death -20 ˚C
B	-	-	-	Degutting 15 h	Frozen to death -20 ˚C
C	25˚C	-	-	½ Gut-loading ½ -	½ Boiled and dried in 60 ˚C ½ 4 ˚C (sleep)
D	27±1 ˚C	40-50	-	Gut-loading	Frozen to death -50 ˚C

A Broekhoven *et al*. (2015). B Paul *et al*. (2017). C Siemianowska *et al.* (2013) (there is no information given about the time that passed when the mealworms fall in sleep, and therefore it is not possible to decide if the mealworms were degutted or not). D Dreassi *et al*. (2017).

In Table 2, the six different diets that were used as feed in the study by Dreassi *et al*. (2017) are shown.

Table 2. *The different diets that were used as feed in Dreassi et al. (2017).*

Diet (D)	Content
D1	100% wheat flour bread
D2	100% oat flour
D3	25% wheat flour, 25% oat flour, 25% corn flour, and 25% chickpea flour
D4	50% oat flour, and 50 % wheat flour
D5	5% beer yeast, 47.5% wheat flour, and 47.5% oat flour
D6	0.5% beer yeast, 33.17% wheat flour, 33.17% oat flour, and 33.17% corn flour

Degutting is when the mealworms are fasting before harvesting, and the time varied from 15 h to 24 h in the different studies. The opposite is when the mealworms are gut-loaded, which means that they have food in their gut when they are harvested. In the study by Siemianowska *et al*. (2013) (C) one batch of mealworms were placed in 4 ˚C and the time to fall in sleep is not presented. In this case, it is not possible to determine if the mealworms were degutting (fasting) or not. The total time of each experiment varied: in the study by Broekhoven *et al.* (2015) (A) the larvae were taken when 50% pupation had been reached. The time until 50% pupation was 79±3.2 days for the HPHS, 95±3.6 days for the HPLS and 168±11.5 days for the LPHS diet. Paul *et al.* (2017) (B) have not specified any time for the experiment. In the study, by Siemianowska *et al*. (2013) (C) the larvae were taken when they were three months old and 25-30 mm in length, but the start time is not presented, so the length of the experiment is not stated. Dreassi *et al*. (2017) (D) had a similar strategy: the larvae were taken when they reached a length of 25-30 mm (they were 15-20 mm at the start).

3.1.2 The lipid content and FA compositions in the diets and the mealworms fed on the diets

In Table 3, the composition of FAs in the diets is shown. These results are only available in two of four papers. In the paper by Broekhoven *et al.* (2015) (A) the total amount of identified FAs in the diets were 99.04% (A1), 99.74% (A2), and 91.47% (A3) of all detected FAs. The LPHS diet (A3) had the lowest percentage of identified FAs. The LPHS diet (A3) had a lower total amount of PUFAs compared with the other two diets (A1 and A2) 41.81% (A3) compared to 51.01% (A1) and 54.15% (A2). There were no big differences within the study by

Broekhoven *et al.* (2015) (A) when comparing the total amount of SFAs and MUFAs in the three diets.

In the study by Dreassi *et al* (2017) (D) 100% of the FAs in all of the diets were identified. In diet D1, the share of SFAs has a value of 29.17%, which is more than any of the other diets. After D1, the closest is diet D5, which contained 22.59% of SFAs.

All diets but D2 have a distribution of FAs (in %) as follows: the smallest part is SFA, the second part is MUFAs and the biggest part is PUFAs. In D2, the part (in %) of MUFAs seems to be bigger than PUFAs.

Unidentified FAs are calculated and presented as UI in Table 3.

Table 3. *The FAs in the diets. The values are presented in % of all detected FAs.*

FAs	Type of FA	HPHS A1	HPLS A2	LPHS A3	D1	D2	D3	D4	D5	D6
C10:0		-	-	-	0.14	-	0.02	-	0.23	0.26
C12:0		0.09	-	0.41	0.40	-	0.14	0.06	0.12	0.22
C13:0		-	-	-	0.03	-	-	-	-	-
C14:0		0.41	0.28	1.21	2.15	0.29	0.39	0.40	0.58	0.34
C15:0		0.16	-	-	0.14	-	0.08	0.03	0.03	-
C16:0		13.16	14.90	14.04	22.58	18.56	16.18	19.55	19.40	19.31
C17:0		0.32	0.20	1.33	0.11	-	0.06	0.03	0.04	0.12
C18:0		2.35	2.09	2.90	3.47	1.88	2.12	1.84	1.96	1.98
C20:0		0.27	0.33	0.57	0.17	0.15	0.43	0.15	0.14	0.14
C22:0		0.18	-	0.60	-	0.07	0.21	0.07	0.07	-
C24:0		0.24	0.24	0.54	-	-	-	-	-	-
SFA		17.18	18.04	21.60	29.17	20.95	19.63	22.13	22.59	22.37
C14:1	n-5	0.08	-	-	0.14[1]	-	-	-	-	-
C16:1	n-5	-	-	-	0.48	0.25	0.26	0.26	0.68	0.70
C16:1	n-7	3.28	1.79	3.31	1.38	0.16	0.16	0.20	0.17	-
C18:1	n-9	26.20	24.48	22.88	29.74	40.49	31.55	35.84	35.72	34.68
C18:1	n-7	0.67	0.65	1.45	-	-	-	-	-	-
C20:1	n-9	0.26	0.39	0.42	0.31	0.54	0.46	0.54	0.51	0.48
C24:1	n-9	0.36	0.24	-	-	-	-	-	-	-
MUFA		30.85	27.55	28.06	32.04	41.44	32.44	36.83	37.08	35.86
C14:2	n-3	-	-	-	0.05	-	-	-	-	0.12
C16:2	n-4	0.27	-	1.31	-	-	-	-	-	-
C16:3	n-4	0.48	0.26	0.72	-	-	-	-	-	-
C18:2	n-6	47.28	51.03	32.02	36.98	36.54	46.07	39.64	38.93	40.28
C18:3	n-3	2.67	2.86	4.32	1.67	1.07	1.81	1.34	1.35	1.37
C18:4	n-3	0.23	-	2.34	-	-	-	-	-	-
C20:2	n-6	0.08	-	0.68	0.09	-	0.05	0.05	0.05	-
C20:4	n-3	-	-	0.42	-	-	-	-	-	-
PUFA		51.01	54.15	41.81	38.79	37.61	47.93	41.04	40.33	41.78
UI		0.96	0.26	8.53	0.00	0.00	0.00	0.00	0.00	0.00

A Broekhoven *et al.* (2015). D Dreassi *et al.* (2017). – indicate that the FA is not detected, 1 The value 0.14 is the sum of the two isomers 14:1n-5 acid (cis) (0.10%) and 14:1n-5 myristoleic acid (trans) (0.04%). UI is the calculated value without any regard to eventually SD-values of unidentified FAs.

In Table 4, the n-6/n-3 ratio, the PUFA/SFA ratio, and the total content of lipids within the feed are shown. Unfortunately, there was no information about the diets from Paul *et al*. (2017) (B) and Siemianowska *et al*. (2013) (C). The diet LPHS (A3) had a low n-6/n-3 ratio of 5 and when it comes to the total fat content

(% of dry weight), the diet (D1) is the one that stands out with a very low value of 0.46%.

Table 4. *The value of n-6/n-3 ratio, PUFA/SFA ratio, and the total content of lipids in the diets that the mealworms were fed on.*

Diets	n-6/n-3 ratio	PUFA/SFA ratio[1]	Total fat content (% of dry weight)
HPHS A1	16	2.97	5.5
HPLS A2	18	3.00	5.8
LPHS A3	5	1.94	2.3
D1	21.55	1.33	0.46
D2	34.27	1.80	5.02
D3	25.51	2.44	6.23
D4	29.54	1.85	7.34
D5	28.79	1.79	7.92
D6	26.91	1.87	9.34

A Broekhoven *et al*. (2015) (the feed were supplied with carrots contained crude fat 2.1 %DM which not is included in the values). D Dreassi *et al.* (2017). 1 PUFA/SFA ratios are only approximately values, just calculating without any regard to eventually SD-values

In Table 5, the composition of FAs in the mealworms are shown. The study by Broekhoven *et al*. (2015) (A) had more than 18% unidentified FAs in mealworms fed on the HPLS diet (A2) and 5.7% unidentified FAs in mealworms fed on LPHS diet (A3). In the study by Paul *et al*. (2017) (B) 5.5% of the FAs was unidentified. All the others were close to 100% of identified FAs. In the study by Siemianowska *et al*. (2013) (C) a FA found in the mealworms is referred to as sapienic acid but this is a very rare FA, only found in human sebum (Prouty & Pappas 2015).

In Table 5, the FA called sapienic acid is added as C16:1n-7 but it could also be C16:1n-5. The part of unidentified FAs is calculated and presented as UI in Table 5.

Interestingly, in all mealworms, irrespective of diets, the share of MUFAs in % was the largest part. The second largest part was the SFAs and the smallest part was the PUFAs.

Table 5. *The FAs in the mealworms. The values are presented in %.*

FAs	Type of FA	HPHS A1	HPLS A2	LPHS A3	B	C1	C2	D1	D2	D3	D4	D5	D6
C10:0		-	-	-	-	-	-	0.03	0.03	0.05	0.03	0.03	0.03
C12:0		0.38	-	-	-	0.36	0.36	0.69	0.66	0.78	0.72	0.79	0.78
C13:0		-	-	-	-	-	-	0.06	0.08	0.09	0.08	0.10	0.09
C14:0		3.19	2.20	2.79	4.45	4.26	4.22	6.08	4.90	6.98	6.82	6.99	7.21
C15:0		0.19	-	-	-	-	-	0.08	0.09	0.09	0.08	0.09	0.08
C16:0		16.96	16.13	16.67	21.33	23.02	21.53	19.40	20.17	19.43	20.84	20.09	20.52
C17:0		0.34	0.49	-	-	-	-	0.05	0.08	0.07	0.06	0.07	0.06
C18:0		2.72	2.64	-	7.92	6.89	6.89	3.07	3.08	3.30	3.37	2.82	3.20
C20:0		0.16	-	-	-	0.52	0.46	0.15	0.13	0.10	0.10	0.09	0.10
SFA		23.94	21.46	19.46	33.70	35.05	33.46	29.61	29.22	30.90	32.09	31.07	32.07
C14:1	n-5	-	-	-	-	-	-	0.32[3]	0.33[3]	0.44[3]	0.41[3]	0.51[3]	0.46[3]
C16:1	n-5	-	-	-	-	-	-	1.23	0.97	1.46	1.38	1.60	1.66
C16:1	n-7	2.88	2.67	1.56	1.97[1]	1,40[2]	1.86[2]	2.45	1.75	1.73	1.83	2.10	1.94
C17:1	n-6	-	-	-	-	-	-	0.08	0.10	0.06	0.05	0.07	0.06
C18:1	n-7	0.26	0.40	0.20	-	-	-	-	-	-	-	-	-
C18:1	n-9	48.68	39.78	57.63	35.83	50.05	51.74	45.88	43.36	44.46	44.92	43.66	46.21
C20:1	n-9	-	-	-	-	-	-	0.04	0.09	0.04	0.04	0.05	0.04
MUFA		51.82	42.85	59.39	37.80	51.45	53.60	50.00	46.60	48.20	48.62	47.99	50.37
C14:2	n-3	-	-	-	-	-	-	0.21	0.17	0.21	0.19	0.25	0.22
C16:2	n-4	-	-	-	-	-	-	0.27	0.31	0.27	0.26	0.32	0.24
C16:3	n-4	0.37	-	-	-	-	-	-	-	-	-	-	-
C18:2	n-6	20.99	31.25	15.45	22.83	10.97	12.09	19.02	22.39	19.68	18.56	19.87	16.63
C18:3	n-3	0.67	1.29	-	0.11	0.10	0.12	0.33	0.55	0.28	0.28	0.32	0.23
C20:2	n-6	0.10	0.34	-	-	-	-	0.06	0.05	0.02	0.02	0.02	0.02
C20:5	n-3	-	0.21	-	-	0.69	0.74	-	-	-	-	-	-
C22:2	n-6	-	0.24	-	-	-	-	-	-	-	-	-	-
PUFA		22.13	17.53	15.45	22.94	11.76	12.95	19.89	23.47	20.45	19.31	20.78	17.33
UI		2.11	18.16	5.70	5.56	1.74	0.00	0.50	0.71	0.45	0.00	0.16	0.23

A Broekhoven *et al*. (2015). B Paul *et al*. (2017). C Siemianowska *et al.* (2013) (C1 boiled and dried) (C2 fresh). D Dreassi *et al.* (2017). – indicate that the FA is not detected, 1 no information whether it is n-5 or n-7. 2 referred as sapienic acid (C16:1n-10), 3 the sum of the two isomers 14:1n-5 acid (cis) (D1 0.31%, D2 0.32 %, D3 0.43%, D4 0.40%, D5 0.50%, and D6 0.45%) and 14:1n-5 myristoleic acid (trans) (0.01% in D1-D6), UI is the calculated value without any regard to eventually SD-values of unidentified FAs.

In Table 6, the n-6/n-3 ratio, the PUFA/SFA ratio, and the total content of lipids in the mealworms from the various studies are shown. In the study by

Broekhoven *et al*. (2015) (A) no n-3 FAs were detected in the mealworms fed on the diet A3, therefore no n-6/n-3 ratio could be calculated.

In the study by Paul *et al.* (2017) (B) a very high value of the n-6/n-3 ratio (204.15) was reported, but none of the other values PUFA/SFA and the total content of lipids (% DM) are extreme.

Siemianowska *et al*. (2013) (C) reported a very low value (6.76) of the n-3/n-6 ratio. According to an assumed calculation error in the paper by Siemianowska *et al*. (2013) new calculations have been made and used in Table 6. The sum of n-3 FAs is calculated to 1.86±0.136 % but the two n-3 FAs that should be summed are 0.12±0.027 and 0.74±0.109, therefore the sum probably is miscalculated and ought to be 0.86±0.136 %. In addition, the SFA C20:0 is counting as a n-6 FA, but according to the FA analyses, the only n-6 FA is C18:2 so the n-6/n-3 ratios included in Table 6 were, in the present study, calculated with values from Table 5, 10.97/(0.10+0.69) for C1 and 12.09/(0.12+0.74) for C2. The lowest n-6/n-3 ratios (13.89 and 14.06), are found in the study by Siemianowska *et al.* (2013) (C). In the same study by Siemianowska *et al.* (2013) (C) the lowest values of the PUFA/SFA ratios were calculated to 0.34 and 0.39 respectively. In the paper written by Siemianowska *et al*. (2013) (C) the sum of PUFAs and the sum of SFAs were presented separately. When calculating the ratio, the values were low but within the recommended values.

In the paper by Dreassi *et al*. (2017) (D) values of the FAs composition are reported as means of two generations of mealworms. Therefore, to have the best values to compare with the other studies (A, B, and C), the values of generation one (G1) (from the supplementary material in the paper by Dreassi *et al*. (2017)) have been used.

Table 6. *The value of n-6/n-3 ratio, PUFA/SFA ratio, and the total content of lipids in the mealworms in the four different studies (A-D)*

Mealworm	n-6/n-3 ratio	PUFA/SFA ratio[1]	Total content of lipid (% DM)
Mealworm HPHS A1	32	0.92	26.3
Mealworm HPLS A2	21	0.82	27.6
Mealworm LPHS A3	-	0.79	18.9
Mealworm B	204.15	0.68	31.67±1.60
Mealworm C1	13.89[2]	0.34	42.48±0.808
Mealworm C2	14.06[2]	0.39	21.93±0.577[3]
Mealworm D1	35.62	0.67	45.00 ± 3.57
Mealworm D2	31.54	0.80	39.75 ± 6.08
Mealworm D3	40.54	0.66	44.62 ± 5.36
Mealworm D4	39.05	0.60	48.31 ± 3.81
Mealworm D5	34.69	0.67	45.00 ± 7.00
Mealworm D6	37.06	0.54	44.91 ± 2.51

A Broekhoven *et al.* (2015). B Paul *et al.* (2017). C Siemianowska *et al.* (2013) (C1 boiled and dried) (C2 sleeping in 4˚C). D Dreassi *et al.* (2017). 1 PUFA/SFA ratios are only approximately values, just calculating without any regard to eventually SD-values. 2 corrected value. 3 this value is on fresh mealworms, not % DM.

The result of the total content of lipids in the feed was between 0.46% and 9.34% based on dry matter. In the larvae, the total content of lipids ranged between 18.9% and 45.00% based on dry matter. Both in the mealworms and the diets the most common FAs were palmitic acid (C16:0), oleic acid (C18:1n-9), and LA (C18:2n-6). The PUFA/SFA ratio ranged between 1.33 and 3.00 in the diets and between 0.34 and 0.92 in the mealworms. Among the diets, the n-6/n-3 ratio varied between 5 and 34.27. Finally, the n-6/n-3 ratio ranged between 13.89 and 204.15 in the mealworms. In the study by Broekhoven *et al*. (2015) (A) there is a change in the order of values for the n-6/n-3 ratio in the mealworms compared to the feed.

4 Discussion

The most common FAs in mealworms are palmitic acid, oleic acid, and LA, which together account for around 84% of all FAs. These results are what also have been reported by for example Makkar *et al*. (2014) and Sun-Waterhouse *et al.* (2016). There are some difficulties with the naming of the FAs in the report written by Siemianowska *et al.* (2013) because the name they used for IUPAC names do not correspond to the other names (structure). In nature cis configuration is more common than trans configuration so it is strange that Siemianowska *et al*. (2013) reported a large amount of elaidic acid but not oleic acid. These two FAs are isomers. Oleic acid is the most common MUFA in animals and plants. It is also found in microorganisms, whereas elaidic acid is found in ruminant fats and in hydrogenated margarines (Gurr *et al.* 2016). The elaidic acid is an exception, but otherwise the results were expected.

The total amount of SFA, MUFA, and PUFA in the mealworms did not follow the same trends as in the diets. The most prevalent FAs in the diets showed to be the most common FAs in the mealworms. As regards both LA (C18:2n-6) and ALA (C18:3n-3) (see Table 3 and Table 5) they always had a lower value in the mealworms than in the diets. The consequence of this was that even if the diets had more PUFAs than MUFAs, the mealworms always contained more MUFAs than PUFAs.

According to the study made by Dreassi *et al*. (2017) (D) the content of lipids in the mealworms do not match the diets. Even if there are differences in the diets the mealworms contain almost the same amount of lipids. In the study made by Broekhoven *et al.* (2015) (A) the content of lipids is almost the same in the diets, but in the mealworms there are differences. The researchers concluded that if the mealworms are fed on a diet with a low nutritional quality, the larvae will use fat reserves for energy (Broekhoven *et al*. 2015). This statement was not confirmed by Dreassi *et al*. (2017) (D).

When it comes to the n-6/n-3 ratio, all diets but one had a lower value than the mealworms. Of the known values, D2 was the only exception and only in genera-

tion one (G1). The researchers reported results from analyses of two generations of mealworms (G1 and G2) (Dreassi *et al.* 2017). Paul *et al*. (2017) write that it is suggested that n-3 FAs supplementation in the feed could improve the n-6/n-3 ratio. In the study (A) by Broekhoven *et al*. (2015) diet A3 had the lowest n-6/n-3 ratio, a value of 5, but in the mealworms no n-3 FAs were detected. Despite the highest percentage of n-3 FAs (7.08%) in the feed there were no n-3 FAs detected in the mealworms. In the same study by Broekhoven *et al.* (2015) (A) the n-6/n-3 ratio with the highest value in the feed (18 in diet HPLS (A2)) resulted in the lowest n-6/n-3 ratio of 21 in the mealworms (Table 4 and Table 6).

In the study by Dreassi *et al.* (2017) (D) the lowest value of n-6/n-3 ratio in the diet did not result in the lowest n-6/n-3 ratio in the mealworms. The lowest value of n-6/n-3 ratio in the mealworms was achieved by diet D2, which had the highest value of n-6/n-3 ratio. None of the two studies (Broekhoven *et al.* 2015 and Dreassi *et al*. 2017) confirmed the suggestion of supplementation of n-3 FAs that Paul *et al*. (2017) reported. In addition, there was a difference between the study by Broekhoven *et al*. (2015) and Dreassi *et al*. (2017) namely, in the first mentioned study the mealworms were degutted, which not was the case in the second. The result is not what could be expected, especially not with the mealworms that were not degutted. The absolutely lowest n-6/n-3 ratios (13.89 and 14.06) are calculated from corrected values in the paper by Siemianowska *et al*. (2013) but unfortunately the n-6/n-3 ratios in the feed are not known.

Besides the diet, other factors such as habitat and environment have an impact on the FA composition. Interestingly, in the study by Siemianowska *et al*. (2013) the mealworms had the same feed and the same rearing conditions until the time of harvest. The only difference was the method of killing the mealworms. In addition, there was a difference in the FA composition of the mealworms. The difference between "fall to sleep" at 4 ˚C and boiled for 3 minutes plus dried resulted in slightly more UFAs and less SFAs in the batch mentioned first. Almost no differences in n-6/n-3 ratio were seen after correction of the values. Unfortunately, it is not possible to evaluate if the batch that was put in 4 ˚C was degutted or gutloaded. What impact did the low temperature actually have on the UFAs and SFAs? Or could the boiling have changed these FAs?

Another factor is the development stage; the insects are able to biosynthesize different FAs depending on the stage of life cycle (Paul *et al*. 2017). Due to the feed the mealworms could have slowed down the development time and therefore results depend on how the experimental time is handled. Allowing the experiments to continue until 50% pupating may ensure a better method if one wants to compare results between batches of mealworms.

This study focused on the two commonly used ratios PUFA/SFA and n-6/n-3. Both are used as an index of a healthy diet. Both ratios are needed because if only

the PUFA/SFA ratio is used, the whole picture of PUFAs is not precise. It could be of major importance for example if the PUFAs are n-6 FAs or n-3 FAs, which is not obvious from this ratio. Because of this, the interpretation of the PUFA/SFA ratio is fuzzy and even confusing. A value close to 1 therefore cannot guarantee a healthy diet.

In summary, it has been shown that it is possible to manipulate the content of lipids and the composition of FAs in mealworms by feed. The results are highly variable so to understand the impact of other factors that could be of importance there is a need of more research. In order to promote mealworms as a source of lipids for consumption, knowledge is of the highest importance. The aim of this work was to review the current knowledge of the content of lipids and the composition of FAs in feed and mealworm, and as a conclusion there is still much work to do. The lack of knowledge concerning lipids is probably because mealworms are primarily seen as an alternative source of protein.

There are reasons to consider several environmental aspects in order to promote mealworms as food. If mealworm-meat offers the same nutritional compositional values as conventional livestock, there are environmental issues that become decisive and therefore promoting mealworms as a source of food lipids could be the right path for the future.

4.1 Future studies

- Find the optimal feed to get the best content of lipids and FA composition (from a human perspective) that at the same time yields suitable growth rate.
- What other factors such as temperature and photoperiod have an impact on the lipids and FAs.
- When is the right time to harvest the larvae to get the most favourable composition of lipids and FAs.

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