The lesser mealworm for food security on Mars

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For food security on Mars; the lesser mealworm

Running title: the lesser mealworm for Mars

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Keywords

Lesser mealworm; Mars; Space; Food; Food security

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Abstract

When humans are going to travel to and live on Mars, it will be beneficial if fresh food can be grown, including insects as the lesser mealworm as a high-quality protein source. It contains high amounts of essential amino acids and causes rapid protein digestion and amino acid absorption and promotes muscle protein synthesis. Therefore, the aim of this study was to determine whether it is possible to rear the lesser mealworm on crops that were grown on Mars regolith simulant. The first step consisted of a pilot experiment with larvae fed on fifteen different feeds consisting of vegetables and their byproducts to determine whether the larvae could survive on this. The second step included growing carrots, potatoes, rye, sweet corn, green bean, green pea and garden cress plants on Mars simulated regolith and Earth potting soil as a control. In the third step, a feed mix of these Martian crops and Earth control was prepared and fed to the larvae. Five hundred larvae were kept per tray and were provided with 88.49 + 1.29 grams of feed. Six trays of both treatments were kept for twelve days. The larvae in the pilot experiment largely survived but showed a weight loss of 23.4% on average. During the Mars experiment, 30 larvae were weighed individually at start and finish. They showed significant growth both on Mars feed (+10.77%, $p = 0.0050$), and Earth feed (+10.03%, $p = 0.0011$). The totality of living larvae per tray were also weighed at the start and end of the experiment. The final weight was divided by the amount of remaining live larvae to determine the average weight difference per larva. No significant weight increase was found for the of larvae that were fed on Mars feed ($p = 0.08786$). Yet, a significant weight increase was found for the larvae that were fed on Earth feed (+8.63%, $p = 0.01393$). The total weight of larvae showed a decrease for both treatments. A significant decrease in total weight was found for both the Mars larvae (-8.76%, $p = 0.001147$) and the Earth larvae (-6.14%, $p = 0.00586$). No significant difference was found between the Mars and Earth trays ($p = 0.1616$). The protein content of the Mars feed was 18.0% and 19.9% of the Earth

feed. The larvae fed on Mars feed consisted for 12.8% of protein, while the larvae fed on Earth feed consisted for 13.2% of protein. The lesser mealworm larvae have converted largely inedible biomass for humans to a high-quality protein. Therefore, the lesser mealworm can be a valuable food source to support astronaut nutrition and enhance the circularity of a bioregenerative life support system on Mars. This innovation for space research serves as an example to improve sustainability and circularity of natural resources on Earth as well.

INTRODUCTION

In the last centuries, many developments have taken place regarding space exploration. The developments have accelerated due to the privatization of space travel and the wish to return to the Moon and explore Mars. An important part of these developments focusses on the production of food in space (Cannon & Britt, 2019; Katayama et al., 2008).

Astronauts operate under extreme conditions, like zero gravity, raised radiation levels, and altered circadian rhythms. This stresses the human physiology considerably (Garrett-Bakelman et al., 2019). Additionally, protocols for space travel prescribes two hours of strength training per day to minimize the impact of zero gravity on bone density and muscle functioning, which strains the body even more. Moreover, space travel has caused large shifts in gene regulation and metabolic functions of astronauts (Garrett-Bakelman et al., 2019). Because of the extreme conditions the astronauts operate in, an optimal diet is essential to maintain bodily functioning, maintain muscle mass and help prevent health complications (Garrett-Bakelman et al., 2019; Hu et al., 2010; Katayama et al., 2008; Kok & van Huis, 2021).

When it comes to future Mars missions, space travel agencies are mainly focused on developing preserved food. Bringing preserved food to space has also been the standard up to now (Brown et al., 2021). However, for multiple reasons it would be beneficial if fresh food can be grown on Mars, cultivated on the regolith that is available (Cannon & Britt, 2019; Fu et al., 2018; Wamelink et al., 2014). Supplying all food to Mars from Earth is an especially vulnerable supply chain. It is an advantage to grow food in a self-sustained manner, due to the vulnerability of the food chain. Additionally, growing fresh food on Mars reduces the load that must be sent from Earth to Mars, which is expensive and causes extra emission (Cannon & Britt, 2019). Lastly, consuming fresh food has a positive effect on human psychological and mental well-being (Pandith et al., 2022).

Several food crops were successfully grown on Martian simulated regolith (since actual Martian regolith is not available on Earth yet). The crops include: garden cress, rocket, tomato, radish, rye, quinoa, chives, peas and leek (Wamelink et al., 2014, 2019). The chemical composition of these Mars crops showed minimal differences compared to the control groups on Earth soil and they are safe for human consumption (Wamelink et al., 2014, 2019), meaning that the toxic heavy metals that are present in the Mars regolith simulant are not passed onto the crops. Although several crops can be grown on Mars simulated regolith, a high-quality protein source for human nutrition is still lacking. Meat, eggs, and milk are typically regarded as high-quality protein sources because they meet the essential amino acid requirements and their high digestibility. However, bringing cattle or poultry into space is for now not an option. Insects have been abundantly suggested as a protein source to enhance space diets (Cannon & Britt, 2019; Katayama et al., 2008, 2009; Kok & van Huis, 2021; Kok, 1983). A recent double-blind randomized trial concluded that the lesser mealworm can also be considered a high-quality protein source (Hermans et al., 2021). It contains high amounts of essential amino acids. Additionally, consuming a meal-sized amount (30g) of lesser mealworm protein causes rapid amino acid absorption and protein digestion and a considerable increase in muscle protein synthesis (Hermans et al., 2021). Therefore, the lesser mealworm is an interesting high-quality source of protein that can contribute to astronaut nutrition during deep space missions.

Additional advantages of using the lesser mealworms for space missions is that it can be added as a valuable component of a bioregenerative life support system (BLSS). Organic waste that is inedible to humans can be broken down by the larva, resulting in manure that can be used to fertilize soil or regolith to grow more crops (Soetemans et al., 2020).

Aside from having useful applications for space travel, the findings are also useful for terrestrial applications. In the current commercial lesser mealworm farms, the lesser mealworms are fed very high-quality feeds. It is not clear whether this insect species can also grow on side streams and biomass that is inedible to humans. If this is the case this will have a positive effect on insect farms on Earth as well, since the feed for the lesser mealworm can then be more sustainable and cheaper.

In theory, the lesser mealworm is a suitable food source of high-quality animal protein to bring into space. To confirm whether this is the case in reality as well, it must be tested whether the larva can live and grow on crops that were already successfully grown on simulated regolith. Therefore, the aim of this study is to determine whether it is possible to rear the lesser mealworm on crops that were grown on Mars regolith simulant.

MATERIALS & METHODS

The experiment was carried out in three steps.

Step 1. Pilot experiment: feeding larvae on individual plant components

<u>Larvae</u>

In the first step, a pilot experiment was performed to determine whether the larvae can survive on various crops for feed. The lesser mealworm larvae were received from Ynsect Nederland. Fifteen different feeds were tested. The species and condition of feed are shown in Table 1. The experiment was carried out in threefold, resulting in 45 trays of larvae. Each tray contained 10.19 ± 0.12 grams of larvae. At the day of harvest fresh weight of the larvae was weighed per tray. The used trays were of transparent plastic material and 15x9.5x8.5 cm in size (0.65 L).

Table 1. Overview of feeds for lesser mealworm larvae and the way they were applied.

Feed	***Latin name***	***Condition of feed***	***Obtained from***
Potato without peel	*Solanum tuberosum*	Fresh	Private home garden
Potato peel		Fresh	
Sweet corn seeds	*Zea mays*	Peeled from cobs	Local farmer's market Wageningen
Sweet corn cob + leaf		Fresh, packaged	
Green pea	*Pisum sativum*	Frozen	Jumbo Supermarket Wageningen
Carrot	*Daucus carota*	Fresh	Local farmer's market Wageningen
Carrot leaf		Fresh	
Rye seeds	*Secale cereale*	Fresh	Welkoop Wageningen
Rye leaf		Fresh	Private home garden
Garden cress stems + leaf	*Lepidum sativum*	Fresh, whole plant	Jumbo Supermarket Wageningen
Arugola	*Eruca sativa*	Fresh in bag	Local farmer's market Wageningen
Basil	*Ocimum basilicum*	Fresh, whole plant	Jumbo Supermarket Wageningen
Chives	*Allium schoenoprasum*	Fresh, whole plant	Jumbo Supermarket Wageningen
Broad bean	*Vicia faba*	Frozen	Jumbo Supermarket Wageningen
Green bean	*Phaseolus vulgaris*	Fresh	Jumbo Supermarket Wageningen

<u>Feed</u>

Each tray contained 20.5 ± 0.5 grams of feed, except for feed no. 9: rye leaf and feed no. 12: basil leaf. Due to lack of availability, these treys contained 10.3 ± 0.47 grams of feed. The larvae were 21 days old on arrival and were previously fed on Ynsect's standard feed, consisting of various grains, grain by-products and oilseed by-products. The pilot feed was retrieved from the local store and market (Table 2). The feed was supplied as fresh feed. The trays were randomly placed in an open circuit climate respiration chamber (OCCRC) for seven days at a temperature of 28.0 °C ± 0.09 °C, a relative humidity of 60.0 ± 1.19 and a CO_2 rate of 1501.2 ± 130.15. Table 2 shows the abbreviation and number per feed, and Figure 1 shows the random placement of the trays in the OCCRC.

Table 2. Abbreviations, numbers, and color codes of the fifteen pilot feeds.

Feed	***Abbrev.***	***Number***	***Color code***
Potato without peel	Po	1, 2, 3	
Potato peel	Pp	4, 5, 6	
Corn seeds	Cs	7, 8, 9	
Corn cob + leaf	Cc	10, 11, 12	
Green peas	Pe	13, 14, 15	
Carrot	Ca	16, 17, 18	
Carrot leaf	Cl	19, 20, 21	
Rye seeds	Rs	22, 23, 24	
Rye leaf	Rl	25, 26, 27	
Garden cress	Gc	28, 29, 30	
Rocket	Ro	31, 32, 33	
Basil	Ba	34, 35, 36	
Chives	Ch	37, 38, 39	
Broad bean	Bb	40, 41, 42	
Green bean	Gb	43, 44, 45	

The dry matter content of each feed was determined by placing a sample in an oven at 70 °C for 72 hours, drying the feed out. The sample was weighed before and after drying.

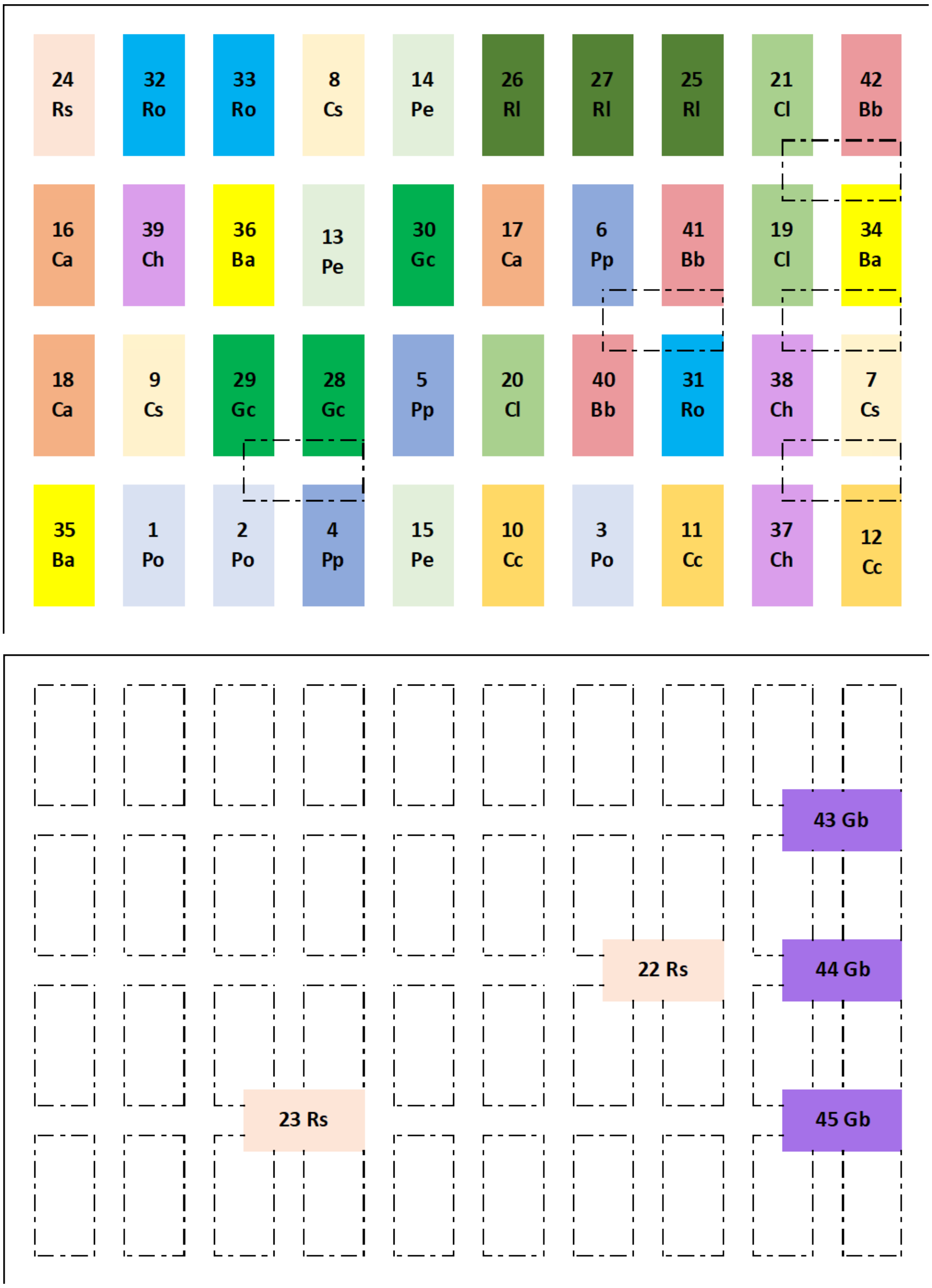

Figure 1. Randomized setup of larvae trays in OCCRC – first layer and second layer. The trays of the second layer are shown in dotted line in the figure of the first layer and vise versa. Numbers, abbriviations and colour codes can be found in Table 1.

Step 2. Growing Martian crops.

<u>Crop growth on Mars regolith simulant</u>

In the second step seven different crops were grown on Mars regolith simulant and control Earth potting soil. Table 3 shows the species, variety, and distributor where the seeds were obtained. This step was performed in twofold, growing crops on Martian regolith simulant and on Earth potting soil. For the Martian regolith, 50%v/v MMS1 soil (Peters et al., 2008) was mixed with 50%v/v potting soil to get an optimal growth (Wamelink et al., 2019). The control Earth soil consisted of 100% potting soil.

The crops were sown and planted in the different pots (Table 4). The experiment started on 7 October 2022 and was harvested on 22 November 2022. The crops grew for forty-six days.

T-tests were performed to determine the significance of the results. Additionally, samples were measured for their protein quantity.

Table 3. Crops grown on Mars simulated regolith and Earth control.

Latin name	***Crop***	***Variety***	***Distributor***
Daucus Carota	Carrots	Amsterdam Bak 2 - Adam	Horti tops
Solanum tuberosum	Potatoes	Agria	HZPC
Secale cereale	Rye	Snelle Lente Rogge	AgruniekRijnvallei
Zea mays	Sweet corn	Tasty Sweet F1	Horti tops
Phaseolus vulgaris	Green bean	Modesto	Horti tops
Pisum sativum	Green pea	Wonder van Amerika	van Binsbergen
Lepidum sativum	Garden cress	Gewone	Horti tops

Table 4. Usage of pot type per crop and number of replicas (n) per species. Number of replicas for both Mars Regolith simulant and Earth potting soil.

Crop	***n***	***Volume (L)***	***Diameter (cm)***	***Height (cm)***
Sweet corn	9	0.2	7	8
Garden cress	4	0.45	10.5	8
Rye	8	0.46	10.5	8

Green pea	8	0.47	10.5	8
Green bean	4	0.48	10.5	8
Carrot	2	4	17	17
Potato	2	5	23	17

Greenhouse

The crops were placed in a greenhouse. The setpoint for daytime temperature (16h) is 20 °C , and 18 °C for nighttime temperature (8h). The average temperature during the duration of these 46 days was 19.94 °C ± 1.72 °C. The relative humidity was 69.84% ± 9.39%. During this period, the greenhouse lighting was used to extend the daytime period to sixteen hours. Lamps yielding 80 $\mu mol/m^2/s$ (HS2000 from Hortilux Schréder) were used when the sunlight intensity was below 150 $watt/m^2$. The lights were turned off for the remaining eight-hour nighttime period.

After forty-six days, the crops were harvested. A feed mix was prepared based on the availability of components. The composition of the feeds based on the contribution of each crop is shown in Table 5.

Table 5. Contribution of each crop to the Mars and Earth feed mix.

Added	***Mars feed (%)***	***Earth feed (%)***
Potato leaf	23.96%	23.93%
Garden cress	22.90%	22.87%
Potato	13.93%	13.91%
Carrot leaf	10.95%	10.94%
Potato stalks	8.26%	8.25%
Green bean leaf	8.21%	8.20%
Rye leaf	5.67%	5.75%
Green pea leaf	3.60%	3.60%
Green pea pods	1.73%	1.75%
Sweet corn leaf	0.79%	0.81%
TOTAL	100.00%	100.00%

Step 3. Feeding larvae on Mars feed

Larvae

The experiment was carried out in sixfold; six trays of larvae on Mars feed and six trays of larvae on Earth feed. Five hundred larvae were counted and added per tray. Thirty larvae per tray were weighed individually, and the larvae per tray were weighed altogether as well. The average total weight of the five hundred larvae per tray was 9.16 ± 0.30 grams. The used trays were of transparent plastic material and 15x19.5x11 cm in size (2 L). Figure 2 shows the randomized setup of the trays with larvae in the OCCRC.

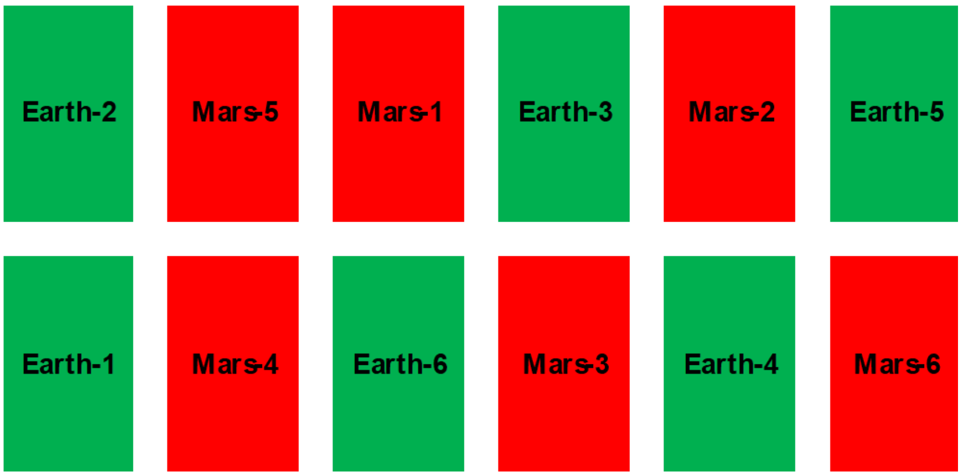

Figure 2. Randomized setup of Mars and Earth larvae trays in open circuit climate respiration chamber (OCCRC).

Feed

In the final step of the experiment larvae were fed on the Mars and Earth feed that was prepared in the previous step of the experiment. The total availability of feed was added to the trays. On average 88.49 ± 1.29 grams of feed per tray. On the last three days, the feed was carefully sprayed with a water spray to keep the feed moist. Video 1 shows the inside of a tray. Larvae, Mars feed and frass is visible.

Video 1. Tray containing larvae, Mars feed and frass. Filmed on day 3 of the experiment (25 November 2022) in the OCCRC.

<u>Open Circuit Climate Respiration Chamber</u>

The trays were kept in the same OCCRC as that of the pilot experiment. The temperature setpoint was 28 °C, the average temperature for the duration of the experiment was 28 °C ± 0.10 °C. The relative humidity was 60 ± 1.29 (setpoint: 60%).

<u>Harvest</u>

The experiment started on 23 November 2022, and ended on 5 December 2022. After twelve days, the larvae were harvested. They were handpicked from the feed residues, counted, and weighed. Thirty larvae per tray were weighed individually and the total mass per tray was weighed as well.

<u>Analyses</u>

After harvesting the larvae, a sample of the Mars and Earth feed, and a sample of the Mars and Earth larvae was sent to an external lab for a protein content analysis. The samples were sent to Eurofins Food Testing Netherlands B.V., Heerenveen. Kjeldahl analyses were performed on both the Mars samples combined, and Earth samples combined. The nitrogen content was determined. A nitrogen-to-protein conversion factor of 4.76 was used to definitively determine the protein content of the Mars and Earth larvae (Janssen et al., 2017).

Additionally, the weight difference of the larvae from before and after the experiment was determined in three different ways. Firstly, thirty larvae were weighed individually at the start and finish of the experiment to get insight in the weight differences between the larvae. Secondly, the individual average weight per larvae per tray was determined by weighing the totality of larvae divided by the number of larvae at the start and finish of the experiment. Lastly, the total weight of all larvae together per tray was determined to see how much the total weight of larvae per tray increased or decreased.

RESULTS & DISCUSSION

Pilot experiment: feeding larvae on individual plant components

After harvesting, the larvae of the pilot experiment showed a weight loss of 23.4% (Table 6). It is unclear whether the larvae reduced in size or in number, since the larvae were not counted or weighed individually.

Table 6 also shows the average weight decrease of the larvae per feed type, and the p-value. The various feeds are arranged from lowest to highest decrease in percentage weight. The table shows that the larvae decreased weight on each of the pilot feeds. Feed number 7: Carrot leaf, 9: Rye leaf, 13: Chives, and 11: Rocket showed some outliers. Due to the large variance in these weight decreases, no significant differences were found for feed 9: Rye leaf, 13: Chives, and 11: Rocket. Each showed one outlier, also giving rise to an unequal variance. Therefore, for these four the outlier was excluded, and the T-test was performed again for unequal variance. For carrot leaf the weight decrease was only found significant when assuming unequal variances. In the last column, the weight decrease for these four feeds is shown excluding the outliers. The overall average weight decrease for all feeds is 23.34%, and 16.41% excluding the outliers.

The raw data including the start weight, final weight and percentage decrease of all trays can be seen in Appendix A. The average weight decrease per feed is shown in Appendix B.

Table 6. Average weight decrease of larvae per feed type sorted from least to most, including p-value of t-test.

		Significance			
No.	***Feed***	***Assuming equal veriances***	***Assuming unequal variances***	***Weight decrease total***	***Weight decrease without outliers***
3	Cs	p<0.05		-4.11%	
14	Bb	p<0.01		-8.37%	
8	Rs	p<0.05		-11.49%	
4	Cc	p<0.000		-12.85%	
6	Ca	p<0.000		-13.34%	
1	Po	p<0.000		-13.68%	
5	Pe	p<0.000		-14.06%	
2	Pp	p<0.000		-14.18%	
12	Ba	p<0.000		-20.79%	
15	Gb	p<0.000		-22.93%	
7	Cl	n.s.	p<0.01	-31.35%	-10.09%
9	Rl	n.s.	n.s.	-39.79%	-15.29%
13	Ch	p<0.05	n.s.	-42.78%	-14.41%
10	Gc	p<0.01		-45.65%	
11	Ro	p<0.05	n.s.	-54.73%	-24.95%
Total		**p<0.000**	**p<0.000**	**-23.34%**	**-16.41%**

For abbreviations, see Table 2.

Table 7 shows whether the differences in larvae weight between the various feeds were significant. For example, it shows that the yield of feed 3: Corn seeds and feed 14: Broad bean show significant differences with ten and seven other feeds respectively. However, feed 7: Carrot leaf and feed 9: Rye leaf showed no significant differences with other feeds. Furthermore, the table shows that the differences between the larvae weight on the various feeds were often not significant. A table with all P-values is included in Appendix C.

Table 7. Lesser mealworm weight loss results of t-tests performed on pair wise differences in weight loss between the feeds. (p<0.05; ** p<0.01, *** p<0.001). For abbreviations see Table 2.*

No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Feed	**Po**	**Pp**	**Cs**	**Cc**	**Pe**	**Ca**	**Cl**	**Rs**	**Rl**	**Gc**	**Ar**	**Ba**	**Ch**	**Bb**	**Gb**
1	**Po**															
2	**Pp**	n.s.														
3	**Cs**	**	***													
4	**Cc**	n.s.	n.s.	**												
5	**Pe**	n.s.	n.s.	***	n.s.											
6	**Ca**	n.s.	n.s.	**	n.s.	n.s.										
7	**Cl**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.									
8	**Rs**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.								
9	**Rl**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.							
10	**Gc**	*	*	**	*	*	*	n.s.	*	n.s.						
11	**Ar**	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.					
12	**Ba**	*	**	***	**	**	**	n.s.	*	n.s.	*	n.s.				
13	**Ch**	n.s.	n.s.	*	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.			
14	**Bb**	*	***	*	**	***	*	n.s.	n.s.	n.s.	**	*	***	n.s.		
15	**Gb**	**	***	**	***	***	***	n.s.	*	n.s.	*	n.s.	n.s.	n.s.	***	

Discussion: possible explanations for larvae weight reduction on individual plant components

No research was previously performed on whether the lesser mealworm would be able to live on individual plant components. The pilot experiment showed that the larvae were in fact able to live on various individual plant components. However, even though the larvae were able to live on these ingredients, they decreased on average 23.4% in weight. Corn seeds (-4.11% weight decrease) and broad bean (-8.37% weight decrease) performed best, presumably because of their higher protein content and lower moisture content. Corn contains 24% dry matter and 3.27% protein on wet basis (USDA, 2019c). Broad bean contains 28.5% dry matter and 7.6% protein on wet basis (USDA, 2019b). Garden cress (-45.65% weight decrease) and rocket (-54.73% weight decrease) performed worst, presumably because of their lower nutrient content and higher moisture content. Garden cress contains 10.6% dry matter and 2.6% protein (USDA, 2019d), and rocket contains 8.3% dry matter and 2.58% protein (USDA, 2019a).

The net weight reduction of the larvae in the pilot experiment may be due to a variety of reasons. One possible explanation is the monotonous diet. The larvae were only fed on one specific ingredient, which is not optimal to maintain population health. Studies of feeding the lesser mealworm on a single component feed were not performed. However, it was performed for another insect species that is edible to humans. A study on the development of *Hermetia illucens* larvae on organic byproducts showed that the larvae fed on orange byproducts took 19 days to complete the larval stage and reach the mature stage, instead of 13 days on a standard diet; a 46.2% delay (Scieuzo et al., 2022). Even though it concerns a different species, it is an indication that feeding the lesser mealworm on merely one ingredient has impacted the population health and consequently growth in a negative manner.

Another reason for the weight reduction could be a low dry matter content of the ingredients that were added to the larvae; 2.8 ± 1.8 grams dry weight on average, with the exception of rye grains (18.4 grams). A low dry matter content means a low availability of nutrients per eaten volume food, resulting in a lower growth rate. In current large-scale lesser mealworm farming for food, the larvae are fed on a feed mix similar to chicken feed: a very dry feed mix consisting of various grains and seeds. This is very nutrient dense feed but not optimally economic or sustainable (Halloran et al., 2018).

An optimal feed composition for the lesser mealworm in commercial farming is still being developed. It is currently unclear what an optimal feed composition for the lesser mealworm looks like, taking sustainability and economic efficiency into consideration (Halloran et al., 2018).

Feeding larvae on Mars feed

Table 8 shows the average weight per tray and per treatment based on the average weight of 30 weighed larvae per tray for Mars and Earth. The raw data including the start weight and final weight of 30 weighed larvae per tray is included in Appendix D. The live larvae showed a significant average growth both on Mars feed (+10.77%, p = 0.0050), and Earth feed (+10.03%, p = 0,0011). No significant difference was found between the different treatments (p=0.9664).

Table 8. Average weight increase/decrease based on the average weight of 30 individually weighed larvae per tray. Given are the results per tray and the average per 'soil type'.

	Start weight		Final weight		Δ Weight	
	Average (mg)	St. dev. (mg)	Average (mg)	St. dev. (mg)	Average (mg)	Percentage (%)
Mars-1	19.9	6.3	24.4	6.1	4.5	+22.41%
Mars-2	18.5	6.3	22.0	4.6	3.5	+18.84%
Mars-3	21.1	5.7	21.3	6.6	0.3	+1.28%
Mars-4	18.9	5.6	22.2	6.3	3.3	+17.48%
Mars-5	22.3	15.7	19.8	5.6	-2.5	-11.37%
Mars-6	20.4	6.4	24.4	5.1	4.1	+19.90%
Mars Average	**20.2**	**8.5**	**22.4**	**5.9**	**2.2**	+10.77%
Earth-1	21.3	5.5	24.3	5.2	3.0	+13.89%
Earth-2	21.8	7.5	23.4	7.6	1.7	+7.64%
Earth-3	20.6	5.9	22.0	4.9	1.4	+7.00%
Earth-4	21.2	5.8	27.1	5.2	5.9	+27.68%
Earth-5	20.5	5.4	20.2	5.8	-0.3	-1.38%
Earth-6	21.0	6.6	22.0	5.6	1.0	+4.82%
Earth Average	**21.1**	**6.1**	**23.2**	**6.1**	**2.1**	+10.03%

Table 9 shows the decrease of the number of larvae per tray. There was a significant average decrease of 12.57% (p <0.000) larvae in the Mars trays and a significant average decrease of 13.23% (p = 0.0016) of larvae in the Earth trays. The average total decrease is 12.90% There was no significant difference between the Earth and Mars trays (p = 0.0858).

Table 9. Number of larvae per tray for the Mars experiment. Given are the results per tray and the average per 'soil type'.

Tray number	Start weight (mg)	Start number of larvae	Final number of larvae	Number decrease	Percentage decrease
Mars-1	9.28	500	408	-92	-18.40%
Mars-2	9.80	500	473	-27	-5.40%
Mars-3	9.11	500	429	-71	-14.20%
Mars-4	9.47	500	440	-60	-12.00%
Mars-5	8.92	500	453	-47	-9.40%
Mars-6	9.15	500	420	-80	-16.00%
Mars Average			**437**	**-63**	**-12.57%**
Mars St. Dev.			**23**	**23**	**4.69%**
Earth-1	9.10	500	428	-72	-14.40%
Earth-2	9.27	500	427	-73	-14.60%
Earth-3	8.75	500	374	-126	-25.20%
Earth-4	8.78	500	492	-8	-1.60%
Earth-5	8.95	500	437	-63	-12.60%
Earth-6	9.34	500	445	-55	-11.00%
Earth Average			**434**	**-66**	**-13.23%**
Earth St. Dev.			**38**	**38**	**7.58%**
TOTAL Average	**9.16**		**435.5**	**-64.5**	**-12.90%**
TOTAL St. Dev.	**0.30**		**30**	**30**	**6.02%**

The living larvae per tray were also weighed at the start and end of the experiment as a lump sum. The final weight was divided by the final number of larvae. Based on this data no significant weight increase was found for the larvae that were fed on Mars feed ($p = 0.0879$) after twelve days. Yet, there was a significant weight increase for the larvae that were fed on Earth feed (+8.63%, $p = 0.0139$). However, no significant difference was found between final weight on Mars and Earth feed as lump sum ($p = 0.0322$).

The total weight of larvae showed a decrease. A significant decrease in total weight was found for both the Mars larvae (-8.76%, $p = 0.0011$) and the Earth larvae (-6.14%, $p = 0.0059$). No significant difference was found between the Mars and Earth trays (p = 0.1616). Appendix E shows the total start weight, total final weight, and weight difference per tray.

The protein content of the Mars feed was 18.0%. The protein content of the Earth feed was 19.9%. There is a substantial difference between the two, although this cannot be statistically underpinned. The larvae fed on Mars feed consisted for 12.8% of protein, while the larvae fed on Earth feed consisted for 13.2% of protein. Hence, the Earth feed and larvae that were fed on it showed a higher protein content than the Mars feed and larvae, however this cannot be statistically underpinned.

Discussion: possible explanation for population reduction

The weight of the live larvae individually increased significantly both on Mars regolith simulant and Earth control, based on the thirty larvae that were individually weighed, and the total weight of the larvae divided by the number of (live) larvae per tray. However, the number of larvae and total weight per tray decreased. Since no dead larvae were found, it is assumed that the larvae ate each other, which is not uncommon for the lesser mealworm (Dunford & Kaufman, 2006). Larvae are especially vulnerable when they molt; their skin is soft at that moment, hence they can easily be eaten by other larvae (Ichikawa & Kurauchi, 2009). Based on this experiment, it is hypothesized that, when feed is administered that consists of a higher nutrient content and lower moisture content, the mortality rate will decrease, and total weight of the larvae will increase. Due to the short growing time of the Mars crops, a higher quality feed was not available for this experiment.

When combining the results of the Earth and Mars trays, an average decrease in number of larvae of 12.90% was found. This is an acceptable loss and comparable to losses in commercial rearing of the lesser mealworm compared to commercial farming. However, the mortality rate is expected to be lower if higher quality feed is provided.

CONCLUDING REMARKS

The aim of this study was to determine whether it is possible to rear the lesser mealworm on crops that were grown on Mars regolith simulant. The experiment was successful, the results showed that this was indeed possible. The larvae were able to live on the Mars feed that was grown on the Mars regolith simulant.

The lesser mealworm can contribute to human nutrition during deep space missions because it is a high-quality protein source: it contains high amounts of essential amino acids, causes rapid amino acid absorption and protein digestion and increases muscle protein synthesis. Additionally, it can be a valuable component of a bioregenerative life support system (BLSS) because it is able to convert organic biowaste to fertilizer that can be used again on the regolith.

In this experiment, the larvae gained weight individually, however they reduced in number and so in total weight. Taking into account these results, there are two possible applications to consider when adding the lesser mealworm as a component to a BLSS on Mars. In the first possible scenario, the larvae can be fed mainly on organic waste streams. This is similar to the feed that was administered in this experiment. With this application, the yield of larvae might be lower, but all food parts of the plants are saved for humans to eat. In the other possible scenario, the larvae are fed a higher quality feed, containing more grains and seeds. The yield of larvae can presumably become higher this way, but the larvae will consume some food parts of the growing crops that are edible to humans, meaning that they will take away from the total availability of food for humans. Depending on the desired function of the lesser mealworm as a component in a BLSS, these options should be considered.

From literature, utilizing insects as food for space missions was already suggested in various papers. In this experiment, it has been brought into fruition. It has been shown that the lesser mealworm can be a valuable addition as a component in a bioregenerative life support system to support astronaut nutrition and enhance the circularity of the BLSS on Mars. As ever, innovation for space research serves as an example to improve sustainability and circularity of natural resources on Earth as well.

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Reflection report

Research internship

Lotte Bohlander - Food for Mars and Moon

Project 'Food for Mars and Moon'

▲ Lotte Bohlander (links) kwam met het idee meelwormen te gaan kweken op Mars. FOTO'S HERMAN STÖVER

Komt de meelworm in het dieet van de Marsbewoner?

Een onderzoeksteam van de Wageningen Universiteit bekijkt welk voedsel we buiten onze aardse atmosfeer kunnen gaan kweken.

Job van Gasselt
Wageningen

pelschillen en wortelblad te eten kregen, bleek recent dat de meelwormen de niet-essentiële bestanddelen van de groenteresten heel efficiënt omzetten in voor de mens essentiële eiwitten. „Sommige meelwormen die we kweekten, bevatten tot wel 22 procent proteïne", vertelt Bohlander. „Dat is bijna evenveel als kip."

De komende weken wordt onderzocht of de meelwormen ook de groenten eten die zijn gekweekt op de nagemaakte Marsbodem. Wamelink: „Als ze daar ook zo van groeien, verwacht ik

Haar eigen fietspad

Wageningse geëerd met Annemiek van Vleutenpad. ▶ P2/3

Rijnstate zet 3D-bril in

Arts kijkt als het ware door lichaam heen.

▶ P4/5

'Verdrinking Pool was ongeluk'

De politie van Veenendaal gaat ervan uit dat de dakloze Pool, wiens levenloze lichaam begin september werd gevonden in het Omleidingskanaal, door een ongeluk is overleden.

Host supervisor: Wieger Wamelink | University supervisor: Catriona Lakemond
Examiner: Vincenzo Fogliano | Date: 6 March 2023
Master: Food Quality Management | Chair group: Food Quality & Design

- *Scientific reflection*

At the start of the experiment, holes were cut into the lids to aerate the containers. After one day, it became too humid inside the containers because the water was not evaporating enough. To prevent the larvae from drowning, within the first day, the lids were taken off and left off for the rest of the experiment. In some of the trays (carrot leaf, rye leaf, chives, garden cress) the feed turned soggy. This caused the larvae to drown, since they are unable to stop moisture absorption. This is what caused the outliers which were previously referred to. In future research, lids should be kept off from the beginning to prevent such a phenomenon.

Due to the limited growth period of the plants, the feed for the Mars experiment mainly consisted of stems and leaves. Larvae are able to grow better on feed consisting of various seeds and legumes; due to the high protein content and lower moisture content. For future research, it is advised that the Mars and Earth crops have a longer growing time, so there will be more seeds and legumes, and of better quality, available from harvest for a more optimal larvae feed.

In this experiment, the feed for the larvae was administered once, at the beginning of the experiment. The warm environment in the OCCRC caused the feed to dry out. For future research, it is advised that the feed for the larvae is administered in phases, so the feed remains as fresh as possible. As an alternative, dried and grinded food could be administered with one source of moisture, e.g. carrot.

From a food perspective, the logical thing to do is to provide higher quality feed for the larvae, as mentioned above. In this way, the larvae can increase in weight and there is more food for humans to consume. However, the larvae could also be used in a different application. Since it was concluded that the larvae are able to live on side streams, this could have an important contribution from an ecological perspective. The larvae could be used to degrade biowaste, like leaves and stems, and produce faeces. This can then be used as fertilizer. Meanwhile, some food for humans is grown as well. In essence, depending on the desired application, either a high-quality feed, organic by-products, or even a mix of the two can be provided as feed to the larvae.

The results of this experiment form a good starting point for this line of research. It is expected that, if this line of research is continued, the lesser mealworm will prove to be a useful element in the development of closed life support systems to be used on Mars. *Academic skills*

During this internship I have further developed my academic skills. With Wieger's supervision, I was able to design a research project and carry it out from start to finish. I learned about designing an experimental setup concerning various crops, how to sow, harvest and check on them in the meantime. Having a good harvest was crucial for this experiment. I learned about working with insects, and how to handle them. I learned about working in a climate chamber with the insects and how to rear and harvest them. I managed and ran my own project, so it was also important to learn how to involve other experts and collaborate with them. Expertise and supply on the insects needed to be arranged. No specific assignment was arranged when I started my internship. It was just my idea that we worked off from. Designing and executing the whole idea was challenging, but I learned a lot along the way. Additionally, I learned a great deal about the space industry and the current research in that field. This was also due to the MELiSSA conference that Wieger and I attended in

Toulouse in November. Here, I was able to attend many presentations about research for space and terrestrial application and talk to very interesting people in the field. For example, during this project I had the opportunity to discuss our project with former astronaut André Kuipers and Christophe Lasseur (ECLSS R&D coordinator & head of MELiSSA project @ ESA). Lastly, very important academic skills that I learned were the working on the scientific paper. As I am writing this reflection, the draft was sent out to the co-authors, who will review the document and provide me with notes. I am hopeful, that after finishing this document, it will be of sufficient quality to submit it to the Journal of Insects as Food and Feed.

- *Personal development*

During the course of this internship, I have developed myself in several ways. An important aspect I had to develop myself in prioritizing the project over other activities that were not related to my project, but that were relevant to Food for Mars and Moon. My supervisor sometimes asked if I'd like to help with other tasks for 'Food for Mars and Moon'. For example, we once discussed making a video addressing the relevance of the 'Food for Mars and Moon' research project for terrestrial applications. However, I was learning to be more assertive because there wasn't enough time to combine my research project with other activities. This was important because there was only a short timeframe to complete a lot of work. I still find it difficult sometimes to say no, but I think that I've gotten better at it. Also, during my thesis I sometimes stumbled upon decision paralysis; getting stuck in my mind on which steps to take and therefore becoming paralyzed. Loss of motivation sometimes also played part in this. During this project I became better at overcoming this. I was taking the necessary steps to start working again using several tools that I have learned along the way, like the 5-second-rule by Mel Robbins, and getting up and coming to the office early to keep the focus high, especially while writing the paper. I noticed the periods of 'paralysis' became shorter, which I am proud of.

Another one of my characteristics is impatience. In my opinion this is a positive quality 80% of the time. It is this characteristic combined with my drive that made it possible to finish all these experiments in this short time. I also know that sometimes this can be a negative quality in collaborating with others because they might feel ambushed. This can sometimes create friction with others. But I have learned that if I try to plan things in advance a bit more and communicate well, friction with others because of impatience will be less of a problem.

Additionally, some good qualities which made this project a success are my entrepreneurial spirit, my drive, perseverance and enthusiasm. This project also made the national news on the radio at some point, I think mainly due to these qualities. I was interviewed by Astrid Kersseboom on the NPO Radio 1 news on 22 November 2022. We discussed how insects for food is currently a very relevant research topic concerning food innovation and sustainability. This includes insects for food for space and terrestrial applications.

- *The general and personal learning goals*

The personal learning goals from the learning agreement are the following:

1. Learn how to write a scientific article about the experiment so that it is of sufficient quality to be published.
2. Finding a balance between executing tasks properly and having work done in a reasonable time.

I feel like I have achieved both goals. The idea of the whole internship was to perform an experiment that a paper could be written about. This is exactly what I did. In the first 3.5 months or so, I carried out the experiment. In the last weeks, I focused mainly on writing the paper. For both components I have had a very steep learning curve. I went from knowing nothing in the beginning, to trying to accomplish every step that was necessary in this process, and I think I did. I carried out an experiment that was not simple. It consisted of three steps with very little room for mistakes or delay. I managed to finish everything. It was a very steep learning curve. Even though the idea in the beginning was to finish in January, this was unreasonable. Now, I finished in six months, which is I think a very reasonable duration for an internship, and even short taking into account everything that was accomplished. In my opinion, I took the speed that I had in the last months of my thesis and applied it here as well.

- *Contribution of internship to your career*

When it comes to the contribution of this internship to my career, I have the following thoughts. I am very happy that I was able to do an internship with a very academic focus. Comparing myself to other students from Food Quality Management, I noticed that I have taken a bit of a different path. I noticed that most people have a somewhat technical background with degrees in for example Food Science, Biology or Food Technology. I, however, have a bachelor's degree in Entrepreneurship and Retail Management from a University of Applied sciences. This is much more focused on developing practical skills and much less on being academic. That is why I wanted my master's degree to be focused on developing my academic skills, which was also the focus of this internship. In the near future, I see myself going back to commerce related work in the field of food. I am very happy though, that I pushed myself to hone my academic skills and do and internship like this (and take several courses) that were maybe not the expected route for me to take considering my background, because I think that understanding the field of food related research will be a great asset for me in the future.

- *Goals for further development*

Since I am expecting to take my next step towards commerce, my goals for further development lay in a different field. It will not be focused on improving academic skills, but rather skills of negotiating, collaborating and sales. This is what I want the next step in my life to be focused on.

Over all, I am very proud of the work that I did in this project and grateful for all the things that I have learned, and I am very excited about the future.