Azolla as a component of the space diet during habitation on Mars

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Abstract

We evaluate a candidate diet and specify its space agricultural requirements for habitation on Mars. Rice, soybean, sweet potato and a green-yellow vegetable have been selected as the basic vegetarian menu. The addition of silkworm pupa, loach, and Azolla to that basic menu was found to meet human nutritional requirements. Co-culture of rice, Azolla, and loach is proposed for developing bio-regenerative life support capability with high efficiency of the usage of habitation and agriculture area. Agriculture designed under the severe constraints of limited materials resources in space would make a positive contribution toward solving the food shortages and environmental problems facing humans on Earth, and may provide an effective sustainable solution for our civilization.

Keywords: Space agriculture; Space diet

1. Introduction

Mars is a sister planet of Earth. Mars might be the only planet, other than Earth, where either extant or extinct living organisms could be found. This expectation drives us to plan a manned mission to Mars for astrobiology exploration. For habitation on extraterrestrial planets, such as Mars, bio-regenerative space agriculture [1–3] is required to support human life. By recycling bio-materials, the launch mass from Earth and landing mass on Mars could be reduced for missions with large crews and long operational periods. At the same time, design of space diet is important for making manned activities in outer space more creative and productive [4–6].

We evaluated a candidate menu designed for space agriculture. A combination of rice (Oryza sativa), soybean (Glycine max), sweet potato (Ipomoea batatas), green-yellow vegetable, silkworm (Bombyx mori) pupa [7–9], loach (Misgurnus anguillicaudatus) [10], and Azolla was examined to determine whether it is an appropriate diet that fulfills human nutritional requirements. The potential use of Azolla (an aquatic fern) and its life support capability in space has been studied [11,12]. Rice, Azolla, and loach will be co-cultured in rice paddies in order to enhance productivity...
under limited available resources [13–15]. Symbiotic cyanobacteria in Azolla can quickly fix nitrogen when there is a shortage of nitrogen fertilizer. Azolla is an effective green manure, and also suppresses the growth of weeds by covering the water surface [16,17]. Co-culture of fish is further advantageous, by converting Azolla to animal meat, and upgrading biomass to edible food. Action of fish or bird in rice paddies brings other positive effects on rice production as well. In this study, we examined the feasibility of adding Azolla to the human space habitation diet, and the nutritional value of the Azolla menu.

2. Outline of space agriculture

Space agriculture is engineered to support life by synthesizing an artificial ecosystem, which consists of plants, microorganisms and animals. Materials are recycled in a closed loop among those members. We define the requirements for space agriculture to initiate our design of life support system. For the Mars habitation system, we assume a population size of 100 people for a duration of 20 years. One hundred is the typical number of people on an Antarctic science expedition team. Although the crew members may not be fixed for the full 20-year period, due to change-out of crews at certain intervals, the crew size present at any time should remain fixed. Team of 100 crew members will be organized with scientist, facility engineer, medical staff, farmer, and so on.

Our space agriculture concept employs plants to (per person per day) regenerate 100 kg of water, revitalize 0.5 kg of oxygen from carbon dioxide, and produce 2 kg of food materials from human metabolic waste and inedible biomass. Composted inorganic components of waste are fed to plants in order to achieve the higher degree of closure in the materials recycle. However, we do not intend to close the loop 100%. We will conduct a trade-off to find the optimum closure level. Solar light is the energy source to drive space agricultural production. On-site planetary resources, such as bio-elements in Martian regolith, a dilute atmosphere, and subsurface water, are utilized for expanding space agriculture on Mars.

There are three loops of materials circulation in space agriculture. Waste water is irrigated to the plant farming yard. Based on the ratio between photosynthetic production and water respiration of ordinary plant species, 100 kg of water would be recovered from water vapor evaporated from plant leaves, during the production of food materials. The psychological problem of drinking recycled water could be eased by “natural” filtering of water through plants. Stoichiometry of the photosynthetic reaction produces oxygen at an amount equivalent to food biomass. Cultivation of trees is a core concept of our space agriculture. Tree leaves support sericulture and produce excess oxygen. Wooden lumber is a byproduct used to furnish the interior of the habitation module. Although the Martian atmosphere is diffuse, it contains carbon dioxide with trace nitrogen. A pressurized greenhouse dome creates living environment with air inside at 10 kPa partial pressure of oxygen, verified as the lowest pressure required for driving the life cycle of plants, balanced by nitrogen and minor H₂O and CO₂ to keep total pressure at 20 kPa [3]. We should note that the day length of Mars is close to 24 h, even though solar light intensity is about half.

3. Nutritional requirements

Next to water and oxygen demands, human nutritional requirements are factors that must be considered when designing space agriculture. As a nutritional reference, we chose the Standard of Foods Intake distributed by the Ministry of Health, Labour and Welfare of Japan [18]. In addition to the target level of energy intake and each nutrient, the allowance level is defined for many items. Considering that the levels depend on age, sex and physiological state, we took the standard for an adult under normal activity levels. Items analyzed in this study are energy, proteins, fat, carbohydrates, dietary fiber, vitamins, minerals, trace elements, and electrolytes. The detailed items are the same as those listed by Katayama et al. [8].

The ratio between proteins, fat, and carbohydrate (PFC ratio), for energy intake, was evaluated. The amount of essential amino acids was examined, in terms of balance of nutrients. The amino acid score is an index to examine if amino acids composition is adequate. Excess uptake of certain amino acids causes a heavier load on the nitrogen metabolic pathway.

Fat is an energy source complementary to carbohydrates. Because of the difficulty in defining requirements for fat uptake, we used standard recommendations for the ratio of energy uptake from fat. In Japan, the recommended energy intake from fats is 20–50% for adults, 45% for infants under six months, and 30–40% for pregnant and lactating women.

It is important to keep the balance of fatty acid groups within an appropriate range. Firstly, the best composition of fat intake occurs when the ratio of fat sources is mammalian meat:plant:fish = 4:5:1. Secondly, the ratio among saturated fatty acid (SFA), mono-ununsaturated fatty acid (MUSFA), and polyunsaturated
fatty acid (PUSFA) should ideally be SFA:MUSFA: 
PUSFA = 3:4:3. Thirdly, PUSFA is divided to two 
categories: n-6 fatty acid from plants, which are rich 
in linoleic acid, and n-3 fatty acid from fish, which are 
rich in linolenic acid, eicosa pentaenoic acid (EPA) or 
decosahexaenoic acid (DHA). The ratio between n-6 
and n-3 is best at 4:1.

For cholesterol uptake, the Japanese reference defines 
only its daily upper limit of 600 and 750 mg/day for 
women and men, respectively. Cholesterol is an essential 
chemical family, necessary for the synthesis of bile 
acid, adrenal cortex hormone, and sex hormones. At 
the same time, its excessive intake induces hardening of 
arteries. The upper consumption limit is defined to avoid 
this kind of negative effect. On the other hand, insuffi-
cient cholesterol intake results in imperfect immune 
function and menstrual irregularity. Phospholipids are 
another important group of nutrient chemicals. Their 
chemical structure consists of two fatty acids bound to 
glycerin, and a third hydroxy group of glycerin forms 
ester with phosphate. These phospholipids are the main 
constituent of the double lipid layer found in cell mem-
branes. Both cholesterol and phospholipids are essential 
for the function of the bio-membrane.

Eighty percent of the fat in our body can be reused. 
The other 20% of fat must be obtained from foods. A 
group of PUSFAs, such as linoleic acid or linolenic acid, 
are essential because they cannot be synthesized in our 
body. These essential fatty acids are required for growth 
regulation of the human body. Their deficiency causes 
retardation of growth, pathological skin changes, and 
degradation of immune function. On the other hand, n-6 
and n-3 fatty acids, PUSFAs with longer chains, can be 
synthesized in our body.

There are some items, for which the upper or lower 
intake limit is not defined in the reference. If excess-
ive intake of that item never results in negative effects, 
then the upper limit is not defined for it. In case our de-
signed menu cannot provide a certain nutrient at its rec-
ommended level, its impact shall be carefully evaluated 
at either its deficiency or excessiveness. We may also 
consider intake of trace elements or chemicals at their 
appropriate level, if they are essential for our health. 
Other indices may come to light, based on studies that 
will be conducted on human nutrition, especially those 
specific to the space environments.

Even though the nutritional value of a proposed space 
menu is high, we must take into consideration individual 
and cultural food preferences when designing it. When 
we propose exotic food materials or cooking methods, 
we shall take those aspects into account, in order to 
make it acceptable to humans.
Ordinary plants are rich in potassium, but low in sodium. Under stressful situations, demand for sodium salt increases. In the Japanese standard of 2005, a daily intake of 9 g of sodium salt (NaCl) is recommended. Because ordinary food materials of our proposed daily menu contain about 2 g of sodium salt, an addition of 7 g of sodium salt is required in the form of cooking or table salt. The American standard recommendation for maximum sodium chloride intake is 5.8 g. If the American standard is used, 3.8 g of added salt is enough. When humans are exposed to low gravity on Mars, body fluid distribution shifts to the upper body, and excess water is lost as urine. When overall body fluid volume is reduced, there is increased load on the kidneys [20].

For kidney patients, salt uptake shall be reduced. In this context, we decided on the addition of 3 g of sodium salt in the form of cooking or table salt, for a total of 5 g in our space menu.

It is difficult to keep the PFC ratio in its preferred range, i.e. 15% protein, 15–20% fat, and 55–65% carbohydrate, when designing a menu with plants as the major food members. Plant materials are quite diverse in terms of nutrition, ranging from root crops characterized by their high carbohydrate content, to soybeans characterized as rich in protein. In general, vegetables are low in fat. This causes a poor PFC ratio in the vegetarian menu, and necessitates the intake of animal originated food materials to supplement fat. In terms of amino acids composition, lysine is deficient in many plant food materials. Addition of animal foods greatly increases the amino acids score.

Cholesterol is not available from plants, but can be obtained by adding appropriate animal origin food. V-D can be obtained from mushroom, egg, animal meat, or fish. Candidate source of V-B12 and cholesterol is mammalian meat or fish. Marine algae and shellfish, such as green string lettuce and clam, contain V-B12.

6. Addition of insect to the basic space menu

In order to supplement the deficient nutrients of the core vegetarian menu, we considered insects. In our space agriculture concept, tree cultivation is implemented. Plant photosynthesis produces biomass and oxygen. If this biomass is oxidized or metabolized, oxygen is stoichiometrically consumed. By utilizing wood lumber for living space and furniture, excess oxygen can be stored and utilized. Insect rearing is also possible on tree leaves. Silkworm is our candidate insect. It has been domesticated for 5000 years. As a model animal for scientific research, the rearing protocol for silkworms is well established. In addition to this, the mulberry tree has been extensively studied for feeding silkworms. Many cultivars of mulberry are available. Furthermore, it is possible to make silkworm polytrrophic. They can eat plant leaves other than mulberry. Artificial feeding materials that taste like mulberry have also been developed. Silkworms at the developmental stage of either pupa metamorphosed in cocoon or adult moth might be appropriate for utilization as a food source.

7. Loach and Azolla to the basic space menu

Silkworm pupa does not meet all of the nutritional requirements of our animal meat addition to the basic vegetarian space menu. Fish contains both V-D and V-B12. Herring, pacific saury, red salmon, tilapia, and loach are species that are rich in vitamins. Tilapia has been included in the space menu of NASA. The vitamins in loach are similar to those in tilapia, except that V-D is less, at 4 mg per 100 g. We selected loach based on the following reason. Loach is a traditional fish species cultured in rice paddies in East Asia. Loach is a tough fish species. In poor quality water, such as low dissolved oxygen, loach will swim up to the water surface, gulp air into the digestive tube, and expel air bubbles from the anus after making gas exchange along the gut. Thus, culture conditions are quite permissive for loach. Since loach abdominal organs are rich in vitamins, it is beneficial to eat the whole body.

We decided to add Azolla, and aquatic fern, to our space menu that has rice as its core grain. Simultaneous production of plant crop and animal was proposed by the International Rice Research Institute, IRRI [15]. Azolla is cultivated on the water surface of rice paddies for the purpose of nitrogen fixation by symbiotic cyanobacteria. This provides “green fertilizer” for rice production, and biomass other than rice is fed to water birds or fish. In our space agriculture concept, rice, Azolla, and loach will be co-cultured in rice paddies in order to greatly enhance productivity under limited available resources. The symbiotic cyanobacteria in Azolla quickly fix nitrogen if there is a shortage of nitrogen fertilizer. Azolla is an effective green manure, and also suppresses growth of weeds by covering the water surface. The further advantage of co-culturing fish is upgrading biomass to edible food by converting Azolla to animal meat. Swimming of fish in rice paddies brings other positive effects on rice production. We also examined the feasibility of adding Azolla to the human diet during space habitation, and analyzed the nutritional value of the Azolla menu.
Table 1
Nutritional Comparison of Sprouts, Algae, and Azolla, composition per 100 g of each food material

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Unit/100 g</th>
<th>Azolla</th>
<th>Soybean sprout</th>
<th>Alfalfa sprout</th>
<th>Marine macro-alga</th>
<th>Ulva sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>kJ</td>
<td>29</td>
<td>155</td>
<td>50</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>Protein</td>
<td>g</td>
<td>1.1</td>
<td>3.7</td>
<td>1.6</td>
<td>1.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Potassium</td>
<td>mg</td>
<td>92.8</td>
<td>160</td>
<td>43</td>
<td>167</td>
<td>131</td>
</tr>
<tr>
<td>Calcium</td>
<td>mg</td>
<td>34.6</td>
<td>23</td>
<td>14</td>
<td>3.2</td>
<td>20.0</td>
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<tr>
<td>Magnesium</td>
<td>mg</td>
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<td>23</td>
<td>13</td>
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<td>131</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>mg</td>
<td>22.5</td>
<td>51</td>
<td>37</td>
<td>19.7</td>
<td>6.54</td>
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<tr>
<td>Iron</td>
<td>mg</td>
<td>0.98</td>
<td>0.5</td>
<td>0.5</td>
<td>1.8</td>
<td>0.21</td>
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<tr>
<td>Zinc</td>
<td>mg</td>
<td>0</td>
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<td>0.4</td>
<td>0.1</td>
<td>0.049</td>
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<tr>
<td>Copper</td>
<td>mg</td>
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<td>0.12</td>
<td>0.09</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Manganese</td>
<td>mg</td>
<td>0.98</td>
<td>0.3</td>
<td>0.1</td>
<td>0.06</td>
<td>0.69</td>
</tr>
<tr>
<td>Retinol/β-Carotene equiv</td>
<td>mg</td>
<td>0.102</td>
<td>0.0</td>
<td>0.056</td>
<td>28.0</td>
<td>2.7</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>mg</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>0.11</td>
<td>0.41</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>g</td>
<td>1.6</td>
<td>2.3</td>
<td>1.4</td>
<td>1.4</td>
<td>1.2</td>
</tr>
</tbody>
</table>

8. Chemical composition of Azolla and its cooking methods

Chemical analysis of Azolla filiculoides, cultivated on the IRRI medium, indicated 29 kJ, 1.1 g protein, 0.1 g lipid, 0.4 g sugar, 1.6 g dietary fiber, 35.3 mg Na, 22.5 g P, 0.98 mg Fe, 34.6 mg Ca, 92.8 mg K, 23.7 mg Mg, 0.04 mg Cu, trace Zn, 0.98 mg Mn, 0.103 mg V-A, and 4 mg V-C per 100 g of plant body. Nutritional value of Azolla is similar to that of alfalfa sprouts, or typical marine macro-algae, as shown in Table 1.

One important factor when designing a menu is whether visual presentation and quantity are acceptable as a meal for ordinary people. It is true that including large quantities of Azolla in a human diet requires certain treatment. Azolla were cut to leaves and roots. Azolla roots taste similar to Alfalfa sprouts, and the leaves resemble moss. However, the smell of Azolla might cause a problem of acceptance as food material. However, boiling Azolla reduces its smell to an acceptable level. Boiling is also important for storage of Azolla. Azolla contains 96.4% water, and its taste is crisp. Roots of Azolla can be cooked in many different ways, for example fried, sautéed, and baked or added to soup and salad. Leaves of Azolla are green or red in color. Red-colored Azolla leaves contain anthocyanins, which are known to show anti-oxidative function. This is good for healthy life in space.

9. Model menu and its nutrition

Nutritional evaluation of the three menus is shown in Table 2. Vitamin D, B12 and cholesterol, for instance, were not available in the basic vegetarian menu. The sufficiency ratio of amino acids was also found to be insufficient, with only 10% of the required lysine per day. Menu of both “insect and loach” and “insect, loach, and Azolla,” has no shortage of essential amino acids. Fat ratio (plant and fish) is evidently out of the recommended range. Other indices, n-6/n-3 fatty acid ratio, and cholesterol are not far from recommended values.

The PFC ratio is 19.3% protein, 16.4% fatty acid, and 64.3% carbohydrate, for the combination of 300 g rice, 120 g soybean, 200 g sweet potato, 300 g Komatsuna, 50 g insect (represented by grasshopper, of which nutritional value including minor component is known), 120 g loach, 100 g Azolla, and 3 g sodium salt. This ratio for fat is slightly lower than the recommendation. The ratios of protein and carbohydrate are within the recommended range. The PFC ratio is greatly improved from the basic vegetarian menu by the addition of insect, loach, and Azolla.

Both the “insect and loach”, and “insect, loach, and Azolla” menus meet human nutritional requirements. To efficiently use biomass energy produced by plants, we decided to use the menu consisting of the basic vegetarian menu plus insect, loach, and Azolla for our conceptual design of space agriculture.

10. Space agriculture based on the menu with Azolla

We estimated the farming area for our four major plant species will be 200 m² per person, considering that solar light intensity on Mars is about half of that on Earth [1,2]. Annual consumption of the four major crops per person is 110 kg rice, 44 kg soybean, 73 kg sweet potato, and 110 kg Komatsuna, postulating that
Table 2
Nutritional evaluation of model menu for space habitation

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Unit (2005)/day person</th>
<th>Basic vegetarian</th>
<th>Basic menu + insect, loach</th>
<th>Basic menu + insect, loach, Azolla</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>kJ</td>
<td>8380</td>
<td>7777</td>
<td>8426</td>
</tr>
<tr>
<td>Protein</td>
<td>g</td>
<td>55.0</td>
<td>69.7</td>
<td>96.1</td>
</tr>
<tr>
<td>Potassium</td>
<td>mg</td>
<td>1800</td>
<td>5410</td>
<td>5758</td>
</tr>
<tr>
<td>Calcium</td>
<td>mg</td>
<td>800</td>
<td>905</td>
<td>2242</td>
</tr>
<tr>
<td>Magnesium</td>
<td>mg</td>
<td>310</td>
<td>680</td>
<td>730</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>mg</td>
<td>1000</td>
<td>1793</td>
<td>2621</td>
</tr>
<tr>
<td>Iron</td>
<td>mg</td>
<td>9.0</td>
<td>27.4</td>
<td>34.1</td>
</tr>
<tr>
<td>Zinc</td>
<td>mg</td>
<td>8.0</td>
<td>10.24</td>
<td>13.7</td>
</tr>
<tr>
<td>Copper</td>
<td>mg</td>
<td>0.8</td>
<td>2.5</td>
<td>2.6</td>
</tr>
<tr>
<td>Manganese</td>
<td>mg</td>
<td>4.0</td>
<td>9.7</td>
<td>10.16</td>
</tr>
<tr>
<td>Retinol equivalent</td>
<td>µg</td>
<td>700</td>
<td>785</td>
<td>803</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>µg</td>
<td>5.0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>mg</td>
<td>9.0</td>
<td>11.7</td>
<td>12.4</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>µg</td>
<td>70</td>
<td>652</td>
<td>653</td>
</tr>
<tr>
<td>Vitamin B1</td>
<td>mg</td>
<td>1.0</td>
<td>2.7</td>
<td>2.8</td>
</tr>
<tr>
<td>Vitamin B2</td>
<td>mg</td>
<td>1.3</td>
<td>0.93</td>
<td>2.2</td>
</tr>
<tr>
<td>Niacin</td>
<td>mg</td>
<td>12</td>
<td>26.1</td>
<td>30.9</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>mg</td>
<td>1.3</td>
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<td>3.0</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>µg</td>
<td>2.4</td>
<td>0</td>
<td>10.2</td>
</tr>
<tr>
<td>Folic acid</td>
<td>µg</td>
<td>240</td>
<td>785</td>
<td>804</td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td>mg</td>
<td>6.0</td>
<td>8.78</td>
<td>9.58</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>mg</td>
<td>100</td>
<td>175</td>
<td>176</td>
</tr>
<tr>
<td>Cholesterol (upper limit)</td>
<td>mg</td>
<td>700</td>
<td>0</td>
<td>252</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>g</td>
<td>21</td>
<td>39.8</td>
<td>40.1</td>
</tr>
<tr>
<td>Sodium salt (upper limit)</td>
<td>g</td>
<td>9.0</td>
<td>5.9</td>
<td>8.5</td>
</tr>
<tr>
<td>n-3 Fatty acid (lower limit)</td>
<td>g</td>
<td>2.4</td>
<td>2.4</td>
<td>2.5</td>
</tr>
<tr>
<td>n-6 Fatty acid (upper limit)</td>
<td>g</td>
<td>11</td>
<td>13.1</td>
<td>13.2</td>
</tr>
</tbody>
</table>

multiple cropping is possible. Estimation of planting area required for mulberry trees to rear 18 kg of silkworm pupa is 64 m². Maximum production of loach per unit water surface area is 10 kg/m², and it takes about 1 year to rear adult loach from the egg stage [10]. In order to produce 44 kg of loach per year, the minimum area for aquaculture is 4.4 m². Since loach will be cultured in rice paddies with Azolla on their surface, we can manage all these items within the original farming area. The water surface area will be sufficient for Azolla as well.

Azolla will be used not only for food material, but also for some other applications. For example, Azolla is able to remove heavy metals and antibiotics from waste water. As well, collection of trace elements by Azolla may prevent fish diseases caused by lack of those elements.

11. Concluding remarks

We confirmed that our proposed model diet of basic vegetarian foods with insects, loach and Azolla fills the nutritional requirements of humans. Based on this menu, we determined the specific requirements for the component system of space agriculture. Use of the agricultural resource on Mars could be optimized by the combination of crop production with chosen species, sericulture, and raising fish. Space agriculture is designed under the severe constraint of resources, with a materials recycle loop as closed as possible. We could apply this knowledge to the support and development of a sustainable civilization on Earth.

References


