RHODOSPIRILLUM RUBRUM FOOD ACCEPTABILITY STUDY FOR MELISSA

Technical note on Work package 30.1

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WORK PACKAGE 30.1
Literature Survey on *Rhodospirillum rubrum* in diets

MELISSA contains food and oxygen reprocessing compartments, one of them growing *Rhodospirillum rubrum*. As *R.rubrum* is to be used as a food source, a literature survey was carried out to evaluate all the known data concerning *R.rubrum* as a single-cell protein (SCP) source. The term "SCP" refers to the dried matter of micro-organisms such as algae, bacteria, yeasts and fungi, grown in large scale culture systems for use in human foods or animal feed (1). A previous literature survey has been conducted on the use of *Spirulina* as a SCP source in diets (2).
No information was found relating directly to the use of *R.rubrum* as a SCP source in animal (or human) feed. However, the growth of the photosynthetic bacteria *Rhodopseudomonas capsulata* (3,4) and *Rhodopseudomonas gelatinosa* (5) has been investigated as sources of SCP production from agricultural by-products. Kobayashi and Kurata (6) have reported that the addition of *R.capsulata* cells to hen feed (0.01 to 0.04%) increased the egg laying rate of the hens by 10% and also improved the quality of the egg yoke.

Though nothing is known about *R.rubrum* as a source of SCP, extensive research has been done on SCP and guidelines for its use. Hoogerheide (7) proposed guidelines for testing SCP as a major protein source for animal feed and its standards of identity. De Groot (8) discussed the minimal tests necessary to evaluate the nutritional qualities and the safety of SCP, while Calloway (9) discussed the place of SCP in man's diet.

Tests using *Hydrogenomonas eutropha* as a SCP source have shown that though rodents thrive on relatively high doses of bacterial matter (10), men fed 15-25 grams of *H.eutropha* suffered mild symptoms such as bulky stools and flatulence and, more seriously, vertigo, nausea, vomiting, diarrhea and headaches. Symptoms appeared 1-2 hours after eating and subsided in 24 hours (11). *Aerobacter aerogenes* also produced similar reactions in man (11). It was surprising that killed and purified bacterial cells caused these adverse reactions in man but not in any of an array of animal species tested (9,11).
Bacterial SCP have a high nucleic acid content of up to 16% of cell dry weight, and this, if not reduced, could lead to kidney stone formation and gout if more than 2 grams of nucleic acid equivalent per day is consumed. Calloway et al. have studied in detail the high levels of uric acid formation from dietary nucleic acid (12,13,14,15). Although not of immediate relevance to this study, this information should prove useful in future investigations.

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WORK PACKAGE 30.1
Acceptance of polyhydroxybutyric acid

Introduction

MELISSA, a model of a future biological life support system for manned missions to space, contains four compartments: a liquefying, a phototrophic, a nitrifying and a photosynthetic compartment. A previous food acceptability study was conducted to determine the suitability of *Spirulina*, growing in the photosynthetic compartment, as a component of the consumer diet in the MELISSA ecosystem model (Tranquille and Emeis 1991 ESA Contract 113088; Tranquille et al. Adv Space Res 1994; 14(11): 167-170).

The aim of this food acceptability study was to test the suitability of *Rhodospirillum rubrum*, in the second MELISSA (phototrophic) compartment, as a component of the consumer diet in the Melissa model. These data will be presented and discussed in Technical Note 30.2. However, as *R.rubrum* contains a high content of polyhydroxybutyric acid (PHB), initial acceptability tests were conducted to see if rats will accept and endure PHB in their diet.

Set-up of the PHB pilot study

PHB ws a gift of Dr P. Brunker (Zeneca, UK). It was 95-97 % pure; its molecular weight averaged 550,000.
PHB was added to a normal powdered semi-synthetic rat diet, to a final concentration of 1% (w/w). The powdered rat diet (a product of Hope Farms, Woerden, the Netherlands) was a wholemeal flour containing all the carbohydrates, proteins, vitamins and salts found in normal pelleted rat food. A similar semisynthetic diet was used in the *Spirulina* acceptability study (Tranquille and Emeis, ESA Contract 113088, 1991). The PHB/wholemeal flower mixture was made into a dough with water; the water content in the prepared rat diet was 46% of the total weight. The diet was frozen at -20°C until it was used.
Six male SPF Wistar rats were used; three consumed the semisynthetic diet containing PHB, and three consumed a normal rat pellet diet.
The animals were housed under normal animal house conditions and were assessed three times a week (Mondays, Wednesdays, and Fridays): they were weighed, the water intake measured and the food intake weighed.

Results of PHB pilot study

The six rats used for the study were adult male SPF Wistar rats, weighing at the start of the pilot experiment 364, 332 and 360 grams in the PHB diet group, and 362, 320 and 348 grams in the control group. The rats were fed their respective diets for 44 days. The results of the rats weight increase over the duration of the experiment can be seen in Figure 1, which shows the average weight increase for the three animals in each group of rats. No differences were noted between the rats consuming the PHB diet and the rats consuming the normal control diet, both sets of rats following the same growth curve. Figure 2 shows the water and food intake of both groups of rats. The rats on the PHB diet consumed more food, but less water, than the control group. This is due to the fact that the PHB diet contains 46% water. Taking the water content of the diet itself into consideration, both sets of rats consumed similar amounts of food and water.

After 44 days the animals general health was assessed and all the major organs were inspected. Abnormal findings were not noted in any animal (except for a white speck seen on the left kidney capsule of diet rat nr 3; this was not thought to be connected to the diet). A series of clinical-chemical tests were conducted on blood and plasma: glucose, creatinine, GOT, GPT and haematocrit. Haematocrit and glucose were tested to ascertain the general status of the rats; creatinine was tested to check kidney function; GOT and GPT, two hepatic enzymes, were measured to check liver function. The results of these tests are shown in Table 1. No significant difference was noted between the animals eating the control diet and the animals consuming the PHB-containing diet.

Conclusion

From the data collected in this experiment one can conclude that adding 1% (w/w) polyhydroxybytyric acid to a normal rat diet is not harmful in the short term (45 days). Nothing unusual was noted in growth, food and water intake, or behaviour of the PHB-consuming rats compared to the control rats, nor in the metabolic functions tested.

Table 1
Clinical-chemistry variables

	PHB diet	**Control diet**
Plasma glucose (mmole/liter)		
Rat 1	8.4	10.6
Rat 2	9.6	9.0
Rat 3	10.3	8.7
	9.4 ± 1.0	9.4 ± 1.0
Plasma creatinine (μmole/liter)		
Rat 1	<44	<44
Rat 2	<44	<44
Rat 3	<44	<44
Plasma GPT (U/liter)		
Rat 1	24.4	19.2
Rat 2	25.2	34.9
Rat 3	28.4	33.4
	26.0 ± 2.1	29.2 ± 8.7
Plasma GOT (U/liter)		
Rat 1	69.5	51.5
Rat 2	63.9	76.3
Rat 3	55.5	61.5
	63.0 ± 7.0	63.1 ± 12.5
Haematocrit (%)		
Rat 1	41	43
Rat 2	44	43
Rat 3	45	45
	43 ± 2	44 ± 1

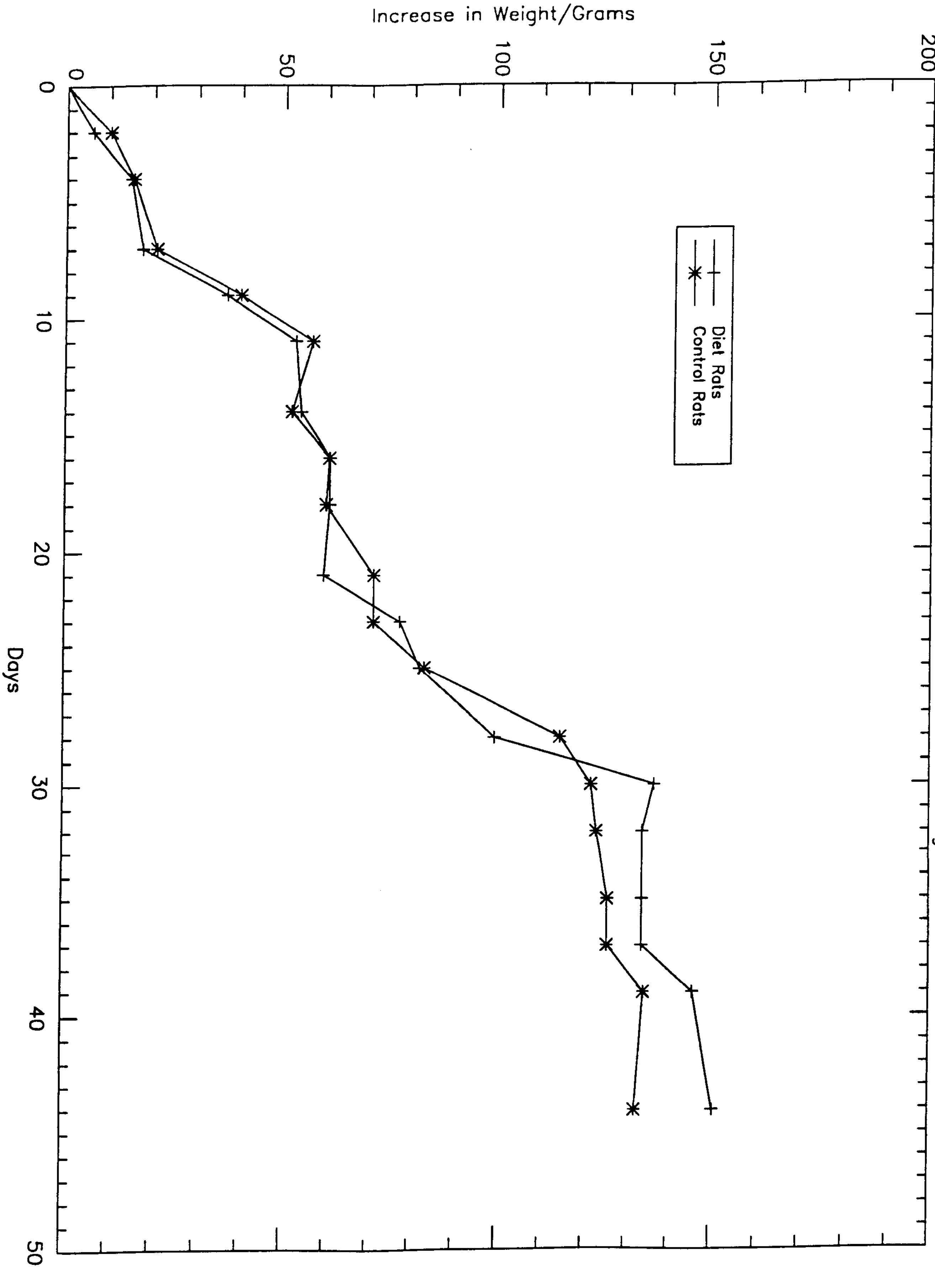

FIGURE 1: Increase in Rat Weight

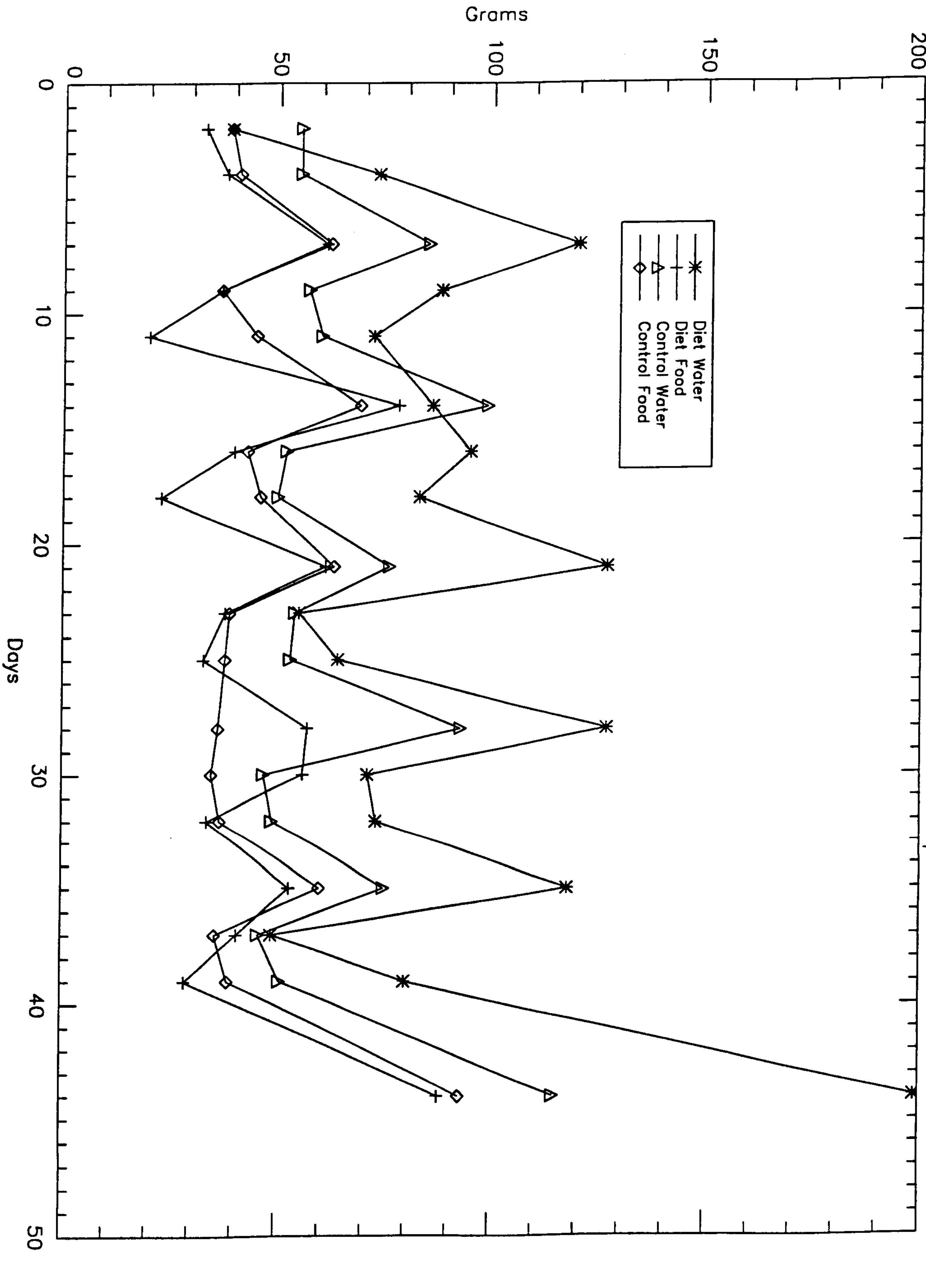

FIGURE 2: Water and Food Consumption