EVALUATION OF NASA FOODBARS AS A STANDARD DIET FOR USE IN SHORT-TERM RODENT SPACE FLIGHT STUDIES

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Running Title: Foodbars as Standard Rodent Space flight Diet

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ABSTRACT

A standard rodent diet for space flight must meet the unique conditions imposed by the space environment and must be nutritionally adequate since diet can influence the outcome of experiments. This paper evaluates the use of National Aeronautics and Space Administration (NASA) developed Foodbars as a standard space flight diet for rats. The Foodbar's semi-purified formulation permits criteria such as nutrient consistency, high nutrient bioavailability and flexibility of formulation to be met. Extrusion of the semi-purified diet produces Foodbars with the proper texture and a non-crumbing solid form for use in space. Treatment of Foodbar with 0.1% potassium sorbate prevents mold growth. Irradiation (15-25 kGy) prevents bacterial growth and in combination with sorbate-treatment provides added protection against mold for shelf-stability. However, during the development process, nutrient analyses indicated that extrusion and irradiation produced nutrient losses. Nutrients were adjusted accordingly to compensate for processing losses. Nutrient analysis of Foodbars continues to be performed routinely to monitor nutrient levels. It is important that the standard rodent diet provide nutrients that will prevent deficiency but also avoid excess that may mask physiological changes produced by space flight. All vitamins levels in the Foodbars, except for vitamin K conformed to or exceeded the current NRC (1995) recommendations. All indispensable amino acids in Foodbar conformed to or exceeded the NRC nutrient recommendation for mice growth and rat maintenance. However, some indispensable amino acids were slightly below recommendations for rat reproduction/growth. Short-term (18-20 d) animal feeding studies indicated that Foodbars were palatable, supported growth and maintained health in rats. Results indicated that NASA rodent Foodbars meet both the physical and nutritional criteria required to support rodents in the space environment and thus, may be used successfully as a standard diet for short-term space

flight studies. However, nutritional adequacy of NASA Rodent Foodbars as a standard diet on longer duration (>20 d) space flight missions remains to be determined.

KEY WORDS: Foodbars, space flight, standard diet, nutrients, extrusion, irradiation

INTRODUCTION

Since the beginning of space exploration, animals have been an integral part of flight programs. Animal experimentation has permitted the collection of physiological data and contributed to the development of engineering design concepts required to support human space exploration[1]. In conducting animal studies, diet is an important factor to consider because nutrition affects all physiological systems. This may be particularly relevant to space flight studies because the space environment produces various physiological changes. Therefore, inappropriate diet selection may introduce nutritionally induced variables with the potential to confound results, influence interpretation of data and/or produce unintended adverse effects.

Standard diets have been developed for feeding rodents under laboratory research conditions but the space flight environment differs significantly from Earth-based laboratory research conditions. Various criteria must be met in the development of a standard diet to support rodents in the space environment. It is important that the diet be nutritionally adequate to maintain growth, health and disease resistance in space flight rodents. The diet must provide nutritional consistency yet at the same time be amenable to nutritional modifications. The texture of the diet should provide the proper density for teeth maintenance as well as prevent excessive crumbling for housekeeping reasons. The diet should have adequate shelf-stability for the mission duration. In addition, due to the constraints on crew time, the diet may be required to be compatible with automated feeding systems.

The National Aeronautics Space Administration (NASA) rodent Foodbar was specifically developed to meet space flight requirements and has been used in rodent experiments aboard space flight missions[2]. This paper assesses the use of the NASA developed rodent Foodbars as a standard diet in rodent space flight experiments of short duration. The existing data for short

duration space flight, in turn may provide valuable information towards the evaluation of Foodbars as a standard diet for a new generation of animal studies to be performed on extended space flight missions and the International Space Station. Providing a common diet in rodent experiments reduces variation and provides uniformity to allow comparisons between experiments. This has particular significance to the space life sciences program because it will allow data generated from the limited and costly space flight opportunities to be maximized.

MATERIALS AND METHODS

Diet Formulation

The ingredient composition of the powder diet used to manufacture the NASA Foodbar was developed at the Ames Research Center (ARC, Moffett Fields, CA) in collaboration with Harlan Teklad (Madison, WI) and Dr. J. J. Knapka at the National Institute of Health. The Foodbar powder diet ingredients were formulated according to the AIN-76A diet, a purified diet designed to meet the 1978 National Research Council (NRC)[3] guidelines for rodent maintenance. However, wheat flour, wheat gluten and corn syrup was required in the Foodbar powder diet formulation to prevent crumbing resulting in a semi-purified rather than purified diet formulation. The nutrients in the semi-purified Foodbar powder diet were adjusted accordingly to account for additional nutrients introduced by the addition of wheat flour, wheat gluten and corn syrup. The final nutrient concentration was a result of ongoing analyses and adjustments compensating for nutrient loss during the processing of semi-purified powder diet into Foodbars.

Since the initial development of the Foodbar powder diet formulation, the NRC has revised its recommendations for rodent nutrient requirements[4]. The Foodbar powder diet also has been reformulated several times to address concerns about specific diet components. Figure 1 shows the different Foodbar powder diet formulations. The current powder diet used in the formulation of Foodbars (TD 97071) was established in 1997. During the development of the current powder diet formulation, the powder diet underwent three different formulations. The changes included fortification of g/Kg Foodbar powder diet with 0.0031 menadione sodium bisulfite complex (K_3), 0.012 folic acid and 0.23 B_{12}. However, the major nutrient difference was a three fold increase in thiamin levels of 0.18 g/Kg diet.

Table I list the ingredients of the AIN-76A purified diet and of the current semi-purified Foodbar powder diet (TD 97071, Harlan Teklad, Madison, WI) used for the manufacture of NASA rodent Foodbars. The Foodbar powder diet ingredients differ from AIN-76A due to the addition of wheat flour, wheat gluten, corn syrup and the absence of dextrose. Also, the amount of cornstarch is higher while casein, sucrose and corn oil levels in Foodbar powder diet are lower than the AIN-76A diet. AIN-76A vitamin mix (TD 40077) is added in Foodbar powder diet at a higher concentration of 20 g/Kg compared to the 10 g/Kg used in the AIN-76A diet. The Foodbar powder is also fortified with thiamin, folic acid, B_{12} and menadione sodium bisulfite complex (vitamin K_3) to account for losses during processing of the powder diet into Foodbars (Table II). The AIN-76A mineral mix (TD 17915) is added to the Foodbar powder diet at the same level (35 g/Kg diet) as the AIN-76A diet except for the addition of calcium carbonate (5 g/Kg diet) to the Foodbar powder diet (Table II).

Foodbar Manufacturing Processes

Diets in solid and paste forms have been used for rodent space flight experiments. Use of solid diets for space flight was limited due to crumbing. A space flight paste diet has been used successfully,[5] however, shelf-stability was limited and sophisticated dispensing systems were required to provide timed feeding rather than *ad libitum* feeding.[6] Another potential problems that may develop with paste diets in long-term space flight missions is inadequate rodent teeth maintenance.[7]

Various processing steps are required to produce NASA rodent Foodbars with the appropriate physical form (e.g. non-crumbing solid), texture, density, dimensions and shelf-stability to meet the criteria of a space flight diet. The processes involved in the manufacture of Foodbar are illustrated in Figure 2.

Step 1 Extrusion. The powder diet (1 Kg) is mixed with water (400 ml) and extruded through a model TX-52 hot head twin screw extruder (Wenger Manufacturing, Sabetha, KS) maintained at a constant speed of 170 $\pm$2 rpm and head temperature of 102 $\pm 2^0$C. One long length of Foodbar is extruded and sliced into sections. Each extrusion run has the capability of producing a maximum of 1,200 Foodbars. Following extrusion Foodbars are tempered for 16-20 h at 20^{0}C and 40-70% humidity and loosely covered by a food grade plastic sheet to prevent contamination and moisture loss below the range of 26 $\pm$2%. The moisture content of extruded Foodbars are determined using a CENCO moisture analyzer (CSC Scientific Inc., Fairfax, VA).

Step 2 Milling. Foodbars are milled to the appropriate dimensions to permit fit into animal enclosure modules (AEMs) or NASA developed research animal holding facility (RAHF) automated feeding systems. Foodbars are kerfed on two sides to fit into the RAHF automated

feeder. Figure 3 shows the dimension of AEM and RAHF milled Foodbars. The final weight of RAHF milled Foodbars is approximately 300 g and 350 g for AEM milled Foodbars. The density of Foodbars is approximately 1.1 g/cm^3 and is based on a serving size that minimizes the need for changeout of feeders during flight.

Step 3 Sorbate Treatment. To inhibit mold growth that can occur in the rodent cage environment, Foodbars are treated with potassium sorbate. Foodbar are dipped in a 15% potassium sorbate solution for 1 min then dried at 20^{0}C for 2 h in a laminar flow hood so that the final concentration of the additive in Foodbars is 0.1% potassium sorbate. The level of potassium sorbate that was effective as a mold-inhibiting agent was determined by spreading 6 to 6x10^6 pencillium spores over a 4 cm^2 surface area of the Foodbar followed by incubation in humidified bags at 23^{0}C for 18 d. The experimental period of 18 d was equivalent to short-term space flight duration. Untreated Foodbars exposed to the maximum of 6x10^6/4 cm^2 spores had heavy mold growth by 7 d. Foodbars treated with 0.1% potassium sorbate demonstrated reduced mold growth at 7 d. At 0.1% potassium sorbate, mold growth in Foodbars treated with 6 x 10^4 spores was completely inhibited throughout the 18 d study duration.

Step 4 Vacuum Packaging. Foodbars are vacuum packaged 5 bars/bag using a KOCH X 1880 vacuum sealer (KOCH Supplies Inc., Kansas City, MO). Vacuum packaging reduces moisture loss, minimizes oxidation and protects against contamination. During shipment and handling the vacuum packaged Foodbars are placed in wooden crates (4 bags/crate) lined on all sides with corrugated paper to prevent damage.

Step 5 Irradiation. Irradiation treatment prevents bacterial growth. In addition, irradiation of sorbate-treated Foodbars prevented mold growth in Foodbars treated with the maximum number (6 x 10^6) spores throughout the study duration (18 d). Foodbars are sterilized

for safety and shelf-stability by irradiation of palletized crates of Foodbars with a gamma radiation dose of 15-25 kGy (Ion Beam Applications, Tustin, CA).

Step 6 Storage. Foodbars are stored in sealed wooden crates to reduce loss of light-sensitive vitamins and maintained at 4^0C to facilitate nutrient shelf-stability.

Nutrient Analysis

A Foodbar from each production run was randomly selected and sent to Medallion Laboratories (Minneapolis, MN) for analysis of vitamin A, vitamin E, thiamin, B_{12}, niacin, folic acid and all indispensable amino acids (aa) except tryptophan which was performed by Silliker Inc (Modesto, CA). In addition, Silliker Inc analyzed the total protein, vitamin D, vitamin K_3, riboflavin, B_6, biotin and pantothenic acid levels in Foodbars.

The Foodbar powder diet was reformulated to increase some vitamin levels (Figure 1). Except for thiamin, there were no significant differences in the nutrient levels of Foodbar manufactured from different diet formulations. Therefore, the mean, maximum and minimum for all nutrients, except thiamin were determined from values compiled from nutrient analysis of the different production runs irrespective of the diet formulation. However, separate compiled values for thiamin was determined. Thiamin (8.04 $\pm$2.0 mg/Kg diet) was higher ($p<0.05$) in Foodbars manufactured from production runs 1997-2002, which are based on the current formulation compared to thiamin (3.3 $\pm$0.5 mg/Kg diet) in Foodbars manufactured from production runs 1989-1996, which were based on the earlier formulations (Figure 1).

Rodent Feeding Studies

All procedures used in the animals studies conformed to the published guidelines of Animal Care and Use.[8] The animal protocols for these studies were approved by the IACUC.

Experiment I. Biocompatability of Sorbate-Treated Foodbars

To determine the biocompatibility of 0.1% potassium sorbate-treated Foodbars, male Sprague-Dawley rats (n=12) weighing (242 ±3 g) were individually housed in vivarium cages in a room maintained at 22 ±2^{0}C with a 12:12 h light/dark cycle (6:00 am light on/6:00 pm lights off). Rats were fed standard rodent chow throughout the 7 d acclimation. Following acclimation, the rats were randomly assigned (n=6 rats/group) to be fed either sorbate-treated or untreated Foodbars. Assigned diets and water were provided *ad libitum* throughout the 18 d study. Body mass, food intake and water consumption were determined biweekly. At the end of the 18 d experimental period, animals were weighed and then euthanized. Major organs were dissected, blotted and weighed.

Experiment II Biocompatability of Irradiated/ Sorbate-treated Foodbar

Irradiation (15-25 kGy) prevented bacterial growth and in combination with 0.1% sorbate treatment had increased effectiveness against mold growth. A rodent feeding study was performed to determine the biocompatibility of irradiated sorbate-treated Foodbars. Experimental design was similar to that described in Experiment I. Male Sprague-Dawley rats (n=10) weighing (247 ±1g) were randomly assigned (n=5/group) to be fed either non-irradiated/sorbate-treated Foodbars or irradiated/sorbate-treated Foodbars. Assigned diet and water were provided *ad libitum* throughout the 20 d study. Body mass, food intake and water consumption were measured biweekly.

Statistical Analysis

All statistical analyses were done using StatView 5.0.1 (Abacus Concepts, Berkeley, CA). For nutrient analysis, the mean, maximum and minimum concentrations were calculated by compiling values obtained from nutrient analyses of the different production runs. For thiamin, separate mean, maximum and minimum concentrations were calculated for 1989-1996 and 1997-2002. In animal feeding studies, t-tests were used to determine the effect of diet on body mass, mass gain, food consumption, water intake and organ weight. Differences were considered significant at $p < 0.05$. Results were expressed as means ± SEM.

RESULTS AND DISCUSSION

Diet Formulation

Purified rather than natural ingredient (e.g. cereal-based) chow diets are used in the manufacture of Foodbars due to the batch to batch variations that may occur in chow diets. Variations in natural ingredient diets occur depending on the soil condition, season, weather, fertilizers, harvest, milling and storage. Besides providing consistency, the purified diet formulation is readily altered to allow for the adjustment of an individual nutrient while continuing to provide all essential dietary components in balanced proportions.[9] Purified diets also provide greater nutrient bioavailability than chow diets. Various non-nutrients (e.g, phytate, lignin, tannins) present in chow diets can bind to nutrients thereby decreasing their availability.[10] In purified

diets containing 5-10% fat, the digestible energy ranges from 90-95% of the gross energy, and metabolizable energy ranges from 90-95% of digestible energy.[11-13]

The reduced caloric intake and weight loss experienced by astronauts [14-15] has not been observed in space flight rodents.[2] A reason for the absence of effects in space flight rodents may be the higher caloric density and metabolizable energy provided by the ingestion of a semi-purified diet. Since inadequate caloric intake exerts critical effects on endurance and performance, and may contribute to bone and/or muscle loss, humans traveling in space may benefit from similar readily digestible calorically dense diets. Another advantage of purified diets is they contain fewer undesirable contaminants than natural ingredient diets.[16]

Nutrient Analysis

Laboratory rodents require about 50 nutrients in appropriate dietary concentrations.[4] Nutrient requirements are not static and change according to the animal's developmental stage. Therefore, the NRC developed separate nutrient recommendations for adult maintenance, growth and reproduction in rats. For mice, the NRC established nutrient recommendations for growth but not maintenance. It is generally accepted that nutrient levels required for mouse growth also supports maintenance. In the formulation of a standard diet, not only must nutrient requirements be met, but, nutrient bioavailability and nutrient losses during manufacturing must be taken into account. In the manufacturing of Foodbars, *Step 1* (Extrusion) and *Step 5* (Irradiation) can result in substantial nutrient losses (Fig. 2).

Protein and Indispensable Amino Acids

During extrusion the powder diet is forced through a die under high temperature and pressure. The heat and shear force produced during extrusion may weaken chemical bonds and denature proteins.[17] However, analysis of total protein the NASA rodent Foodbars indicated levels conformed to the NRC recommendations for proteins (Table III). Ensuring that the standard diet formulated for space flight provides adequate protein is important because one of the most consistent and physiologically relevant findings from space flight studies has been the loss of body protein.[18] Protein is required for formation of various tissues, hormones and immune cells. Thus, providing a protein deficient diet may exacerbate the physiological disturbances of muscle atrophy, bone loss, hormone suppression and compromised immunity elicited by space flight.

In addition to maintaining health, increasing dietary protein may provide protection against muscle atrophy based on reports that oral ingestion of indispensable aa by human subjects stimulated net protein synthesis in skeletal muscle.[19] Reduced muscle mass in astronauts that occurs in the microgravity of space flight, has been reported to be partly offset by the consumption of protein.[20] Also, infusion of human subjects with a balanced aa mixture (approximately 0.15 g/kg/h for 3 h) has been shown to enhance the metabolic effect of exercise on muscle protein.[21] However, caution should be taken because conditions in space produce urine chemistries in astronauts that favor an increased risk of calcium oxalate and uric acid stone formation.[22-23] Similarly, rats fed 23% versus 15% protein diets have increased risk of renal disease.[24] The increased risk of kidney stone formation by feeding high protein diets in space may outweigh the benefits on muscle. Therefore, in determining protein levels in the standard

space flight diet, the diet must provide the optimal amount that will prevent deficiency but avoid excess.

In addition, the protein source must also be considered because depending on the source aa balance e.g. protein quality can differ. Suspension hypokinesia/hypodynamia studies in rats showed that the degree of muscle atrophy, especially soleus muscles of rats fed soy protein was smaller than that of rats fed casein diet.[25] Similarly, the activities of muscle protein degrading enzymes were significantly lower in rats fed soy protein compared to rats fed casein. The authors suggested that different proteins affected muscle atrophy differently.[25] Based on this finding the results of space flight rats may not be directly comparable to ground studies using chow diets since the protein source in chow diets is commonly derived from soy.

Dietary requirements for protein are actually the requirement for amino acids (aa). There are 10 aa that are regarded as indispensable because they cannot be synthesized and therefore must be provided by the diet. NRC indispensable aa requirements for maintenance, growth and reproduction of rats and growth of mice are listed in Table III. All indispensable aa measured in the Foodbars met or exceeded recommendations for rat maintenance and mouse growth. However, several indispensable aa were below NRC recommendations for rat growth and reproduction (Table III) due some loss during processing of the powder diet into Foodbars.

Processing losses of aa were likely due to *Step 1* (Extrusion) rather than *Step 5* (Irradiation) (Fig. 2). Ley et al.[26] reported protein quality and aa composition of rodent diets is unaltered by irradiation doses of up to 70 kGy which was much higher then the 15-20 kGy dose used to sterilize Foodbars. Lysine, the most sensitive aa to damage during processing and storage, was used to monitor processing losses. Lysine in powder diet (9.0 $\pm$0.5 g/Kg) was significantly (p=0.005) reduced following manufacture into Foodbars (7.7 $\pm$0.2 g/Kg). The

result was average lysine in the final Foodbar product conformed to recommendations for maintenance but was slightly below recommendations for rat reproduction and growth (Table III). Due to low sulfur aa, casein-based diets are supplemented with DL-methionine. However, processing of powder diet into Foodbars produced methionine losses. Initial methionine level (6.6 $\pm$0.05 g/Kg) in powder diet was reduced (p=0.008) following manufacture into Foodbars (5.4 $\pm$0.03 g/Kg). As a result methionine was below the NRC recommendations for rat growth and reproduction (Table III). Similarly, phenylalanine, tryptophan and threonine averages conformed to recommendations for maintenance but were slightly below recommendations for rat growth and reproduction (Table III).

Water-Soluble Vitamins

Vitamins are essential micronutrients that are especially sensitive to processing losses. The order of sensitivity of the water-soluble vitamins to irradiation is thiamin>vitamin C> B_6 >riboflavin>folic acid>B_{12} >niacin.[27-29] Therefore, the high processing losses of thiamin in Foodbars maybe due to *Step 5* (Irradiation). In addition, *Step 1* (Extrusion) of the powder diet into Foodbars may lead to significant losses, due to leaching of water-soluble vitamins during mixing of the power diet with water (Fig. 2). Slinger et al.[30] reported a 5-20% loss in pantothenic acid, 0-27% loss in folic acid, 0-17% loss in thiamin and a 3-13% loss in B_6 activity through leaching, after only a 10 second water immersion period. Although most of the water-soluble vitamins are relatively stable to heating, the high temperature produced during extrusion may also contribute to vitamin loss. Extrusion processing losses of 26% riboflavin and 20% for niacin have been reported for extruded pet foods.[31]

Thiamin is the water-soluble vitamin most susceptible to extrusion loss. Beetner et al.[32] reported thiamin retention was 21% lower for each 22^{0}C increase in extrusion discharge temperature between 150 and 190^{0}C and 15% lower for each 25 rpm increase in screw speed. Thiamin is a cofactor for several enzymes in energy metabolism and in the brain plays a role in nerve membrane function. Hence, a deficiency of thiamin rapidly results in anorexia, impairment of carbohydrate metabolism and eventually central nervous system dysfunction.[33] There is no convincing evidence that the requirements for B vitamins are altered in space.[34] However, a deficiency in the diet can be expected to have an important impact on body mass, activity and performance during space flight.

Significant thiamin loss led to reformulations of the diet with increasing levels of thiamin. The original 1985 Foodbar powder diet formulation (TD 85348) consisted of 0.6 g thiamin /Kg diet based on the AIN-76A vitamin mix (Fig. 1). No thiamin deficiency symptoms were observed in rodents but due to high thiamin losses during processing, the Foodbar powder diet was fortified with 0.02 g thiamin/Kg diet (TD 88179 formulation). In 1993, an additional 0.052 g thiamin /Kg diet was added to the Foodbar powder diet (TD 93062 formulation) due to concerns about thiamin loss during storage. The current Foodbar powder diet (TD 97071) was formulated in 1997 with an additional 0.18 g thiamin/Kg diet resulting in a three fold greater thiamin level than the original formulation. Thiamin levels (8.04 $\pm$2.0 mg/Kg diet) in Foodbars manufactured from the current powder diet formulation meet the NRC recommendations (Table IV).

Although the other water-soluble vitamins were more stable to processing, in 1988 the Foodbar powder diet formulation was fortified with an additional 0.23 g B_{12} /Kg diet and 0.012 g folic acid /Kg diet (Fig. 1). Of the B vitamins, niacin levels in Foodbar were highest at four

times the concentration of NRC recommendations. Toxicity associated with high consumption of water-soluble vitamins is mild because excess amounts are excreted. However, high dietary niacin levels can lead to fatty liver in rats.[4] Vitamin B_{12} levels in Foodbars were also high and may prevent the observation of some of the physiological disturbances produced by space flight. For example both folic acid and vitamin B_{12} prevent megaloblastic anemia while riboflavin enhances hematological responses to iron.[35] Therefore, in the presence of excess riboflavin, B_{12} and folic acid, the physiological changes in red blood cell mass and tissue iron availability produced by space flight may be masked. This illustrates the importance that the standard rodent diet used for space flight provide the optimal amount of water-soluble vitamins that will prevent deficiency and avoid excess.

Fat-Soluble Vitamins

Order of irradiation sensitivity of the fat-soluble vitamins is vitamin E>vitamin A>vitamin K>vitamin D.[36] Vitamin A is perhaps the most labile of the fat-soluble vitamins. Vitamin A level in powder diet was reduced by ≈50% ($p<0.001$) following processing into Foodbars. Losses of 20% vitamin A have been reported for extruded pet foods.[31] Deficiencies in vitamins A can lead to cessation of growth and render the organism more susceptible to infection.[37] During space flight, the absence of gravitational forces, the stress of living in a confined space, alterations in cell structure and function due to ionizing radiation or the combination of these factors can deteriorate immune defense mechanisms.[38-41]

In NASA rodent Foodbars, vitamin A levels exceed the NRC recommendations. Vitamin A supplementation has been shown to enhance immune responses.[37] Thus, increasing vitamin A

may ameliorate the negative effects of space flight on the immune system by enhancing immune cell function and by protecting against the oxidative stress that can result from increased radiation exposure in space. However, caution must be used when considering increased levels of fat-soluble vitamins because they are not readily excreted and may accumulate causing toxicity. In addition, excess vitamin A has been reported to stimulate bone resorption and to inhibit bone formation that over time may lead to bone loss and fracture.[37]

Average vitamin D levels in Foodbars also exceeded the NRC recommendation (Table IV). Similarly, the risk to benefit ratio of high dietary vitamin D intake must be considered because adequate quantities of vitamin D are needed to preserve bone but excessive vitamin D results in hypercalciuria. A problematic situation during space flight where increased risk of kidney stones is already of concern.

Vitamin E level in powder diet (60.1 $\pm$4.0 mg/Kg diet) was not significantly (p=0.06) reduced following processing into Foodbars (49.3 $\pm$3.4 mg/Kg diet) and levels in Foodbars exceeded the NRC recommendations. Although high levels of fat-soluble vitamins can cause toxicity, in general vitamin E is non-toxic. However, there is some concern that high vitamin E level in Foodbar may affect vitamin K because tocopheryl quinone, a main metabolic product of vitamin E, inhibits the normal metabolism of vitamin K.

Of the fat-soluble vitamins, only vitamin K levels in Foodbars was below the NRC recommendation despite fortification with 0.0031 menadione sodium bisulfite complex g/Kg diet (Table IV). Endogenous synthesis by microorganisms in the lower gastrointestinal tract does provide a source of vitamin K. Therefore, it is difficult to produce a vitamin K deficiency without reducing bacterial population and/or preventing coprophagy. However, vitamin K levels may need to be increased due to the potential of the high vitamin E content in Foodbars to

interfere with vitamin K metabolism. In addition, there are suggestions that increased intake of vitamin K may contribute to counteracting microgravity-induced loss of bone.[42] In considering fortification of vitamin K, other forms than the menadione sodium bisulfite complex may be used. The bioavailability of vitamin K in this form is limited because menadione sodium bisulfite complex contains 50% menadione sodium bisulfite which is equivalent to 33% menadione, a synthetic compound that must then be converted to vitamin K in the intestinal tract. The AIN-93 rodent diets formulated to improve the AIN-76A diet, incorporated increased vitamin K using the natural vitamin K_1 (phylloquinione) rather than the synthetic vitamin K_3 (menadione).[43]

In formulating a standard space flight rodent diet, it is important to establish the dose that will provide the most health benefits with the fewest risks. Meeting the NRC recommendations for fat-soluble vitamins and other nutrients ensures that experimental results will reflect the effects of microgravity and not the effects produced by nutritional deficiencies or supplementation.

Other Nutrients

The extent of mineral loss during the manufacture of powder diet into Foodbars is expected to be minor because there is no evidence that irradiation, regardless of dose, has any effect on the amount or bioavailability of minerals and trace elements.[43] Furthermore, only a limited amount of mineral loss may occur through leaching in water during *Step 1* Extrusion (Fig. 2). Mineral levels in the Foodbar are based on levels present in the AIN-76A mineral mix (Table II). The most significant problem related to AIN-76A has been the development of nephrocalcinosis in female rats in the long-term. To help eliminate this problem, the AIN-76A diet was reformulated

to increase the calcium to phosphorous ratio.[42] The current Foodbar powder diet formulation was supplemented with additional calcium carbonate at 5 g/Kg diet. Adequate quantities of dietary calcium preserves bone; however, excessive calcium intake may decrease absorption of important trace elements such as zinc and iron. Excess calcium also increases hypercalciuria, which may pose greater risks during space flight where renal stone formation is more common.

Current recommended mineral intakes for rodents are based on estimates of the amount of minerals that promote maximal growth in short-term studies. However, various minerals have been shown to exert health benefits, many of which may be of value for counteracting the various physiological changes caused by space flight. For example in humans iron supplementation may have use in preventing astronaut anemia. Similarly, mineral supplementation to the Foodbar powder diet formulation may benefit rodents. Zinc, copper, iron and selenium have antioxidant properties that may protect against the radiation encountered by astronauts and space flight rodents. Minerals also have immunomodulating functions that may help boost immune function in astronauts as well as space flight rodents. Other minerals such as sodium and potassium may be adjusted to counteract the shifts in cardiovascular function and the compartmentalization of body fluids encountered in astronauts and rodents during space flight.

In summary, nutrient analysis of the NASA rodent Foodbars indicated that the level of the indispensable aa analyzed in Foodbars conformed to the NRC recommendations for rat maintenance and mice growth. However, some of the indispensable aa levels were slightly below NRC recommendations for rat growth and reproduction. Analysis of particularly labile vitamins in the Foodbars showed levels conformed to the NRC recommendation. Further studies involving animal feeding studies were performed to determine Foodbar biocompatability (e.g. ability to support mass gain, food intake and animal health) and palatability.

Rodent Feeding Studies

Space flight creates an environment of high humidity and waste accumulation in the rodent cages that required adding an additive in Foodbars to prevent mold growth. Potassium sorbate was selected because it is an effective anti-mycotic agent that does not affect food flavor and has been used successfully in various baked goods consumed by humans.[44] As previously described 0.1% potassium sorbate in Foodbars was determined by microbiological tests to significantly reduce mold growth. In animal feeding Experiment I, male rats were fed non-sorbate or sorbate-treated Foodbars for 18 d (the duration of short-term space flight), to determine the safety and palatability of sorbate-treated Foodbars. The body mass, mass gain, food consumption, water intake and relative organ weights of rats fed sorbate-treated Foodbars were not significantly different compared to animals fed untreated Foodbars (Table V). The results suggested that the level of 0.1% potassium sorbate in Foodbars was safe and readily consumed by rats. Potassium sorbate was not expected to be toxic since it is metabolized in the same manner as the fatty acid caproic acid.[45-46]

Foodbars used in space flights studies are also irradiated. Irradiation of sorbate-treated Foodbars extends shelf-stability by providing added protection effect against mold growth. However, irradiation can produce organoleptic changes, destroy vitamins and lead to formation of free radicals and/or radiolytic products.[47] There was also concern about the effect irradiation would have on the potassium sorbate in the Foodbars, although, to our knowledge there is no scientific evidence to indicate health hazards associated with the irradiation of foods containing additives. In animal feeding Experiment II, male rats were fed non-irradiated versus irradiated/sorbate-treated Foodbars for 20 d to determine the safety and palatability of

irradiated/sorbate-treated Foodbars. There was no significant difference in body mass, mass gain, food consumption or water intake between rats fed either non-irradiated or irradiated/sorbate-treated Foodbars. The results indicated the safety and palatability of irradiated sorbate-treated Foodbars (Table VI).

The NASA rodent Foodbars are nutritionally adequate based on comparisons with the NRC (1995) recommendations and the accompanying animal feeding studies show that Foodbars are safe, palatable and successfully maintain rodent body mass, mass gain and health in short-term studies. However, the space environment has numerous conditions that may alter nutrient requirements such as stress, increased radiation exposure, group housing and gravitational changes. In addition, there are suggestions that the space environment may slow intestinal transit time and decrease gastrointestinal motility resulting in reduced absorption and bioavailability of nutrients.[48] Ground-simulated hindlimb unloading models that mimic the physiological effects of space flight were used to determine whether Foodbars could be utilized as a standard diet to supply adequate nutrition without masking potential environmental effects. Munoz and Tischler[49] reported normal body mass gains and atrophic response of skeletal muscle in hindlimb unloaded rats fed Foodbars versus standard chow diet. Munoz and Tischler[49] used Foodbars manufactured from an earlier 1988 powder diet formulation (TD 88179) in which thiamin was fortified at 0.02 g/Kg diet compared to the current higher fortification level of 0.18 g /Kg diet. Zerath et al.[50] showed that feeding Foodbar manufactured from the current formulation (TD 97071) did not affect normal body and skeletal growth and concluded that body mass and bone changes due to hindlimb unloading were not significantly different in male rats fed Foodbars versus animals fed standard chow diet.

Not simulated in ground studies was the increased radiation exposure experienced during space flight that may lead to free radicals and radiolytic products in food. Wade et al.[2] analyzed data from 15 different space flight missions where rats were fed Foodbars. No difference in body mass, mass gain, food consumption or water intake of rats exposed to space flight for up to 18 d was found compared to flight control animals fed either Foodbars or standard chow diet. Although the NASA rodent Foodbars has gained acceptance as a standard diet for use in short-term space flight studies, the duration of space flight missions is steadily increasing and the nutritional adequacy of Foodbars to support long-term space flight will need to be tested.

CONCLUSIONS

The NASA developed rodent Foodbar appears to meet the various criteria required of a standard rodent space flight diet. It's non-crumbling solid form minimizes food waste, allows easy measurement of food intake and fits in NASA designed automated feeders used during space flight. The nutrient levels provided in the Foodbars appear to be adequate to support health and maintenance of rodents in the space flight environment without producing nutrient excesses that will confound interpretation of results or lead to adverse effects. Although the NASA Foodbar is the accepted standard diet fed to rodents on short-term space flight, it is unrealistic to presume that any single diet can be formulated that satisfies all circumstances. For example nutrient requirements differ depending on rodent strain, gender and physiological state (e.g. pregnancy, growth, aging). Also, the nutritional sufficiency of Foodbars to support long-term space flight studies has not been explored and the physiological changes accompanying long-term space flight may require additional nutrients. The increasing sophistication of studies may also lead to

dietary changes. In the future, diet may be used to play a role in counteracting some of the disadvantageous physiological effects of space flight. Finally, nutrient requirements are based upon current knowledge in the field and may change with time resulting in a need to change the standard Foodbar formulation. In this respect the semi-purified Foodbar powder diet formulation makes it readily amenable to change. The Foodbar formulation has been successfully altered to assess a high versus low calcium diet.[51] The Foodbar has also been altered so that soy replaced casein as the protein source. The NASA rodent Foodbar has many advantages as a standard diet, although a somewhat limiting factor may be the high cost resulting from the various processing steps involved in the manufacture of Foodbars.

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REFERENCES

1. Ballard RW, Connolly JP. U.S./U.S.S.R. joint research in space biology and medicine on Cosmos biosatellites. FASEB J 1990; 4:5.
2. Wade CE, Baer LA, Moran MM, Steele MK, Stein TP. Body mass, energy intake, and water consumption of rats and humans during space flight. Nutrition 2002; 18:829.
3. National Research Council. Nutrient requirements of laboratory animals, 3rd revised edition. National Academy Press, Washington, DC. 1978.
4. National Research Council. Nutrient requirements of laboratory animals, 4th revised edition. National Academy Press, Washington, DC. 1995.
5. Pace NR, Rahlmann DF, Smith AH, Pitts GC. Effects of the Cosmos 1129 Soviet paste diet on body composition in the growing rat. NASA Technical Report 19810023267; 1981.
6. Vasques Mulenburg J, Gundo D, Griffith J. An automatic 14-day paste diet feeder for animals. NASA Technical Memorandum 108804; 1994.
7. Strength VE, Holley DC and Battles AH. Long-term growth, tooth development and reproduction of rodents fed purified paste diet. Gravitational and Space Biology Bulletin 1997; 11:51.
8. National Research Council. Guide for the care and use of laboratory animals. National Academy Press, Washington DC. 1996.
9. Borzelleca J. Macronutrient substitutes: safety evaluation. Regul Toxicol Pharmacol 1992; 16:253.
10. Keenan K, Ballam GC, Haught DG, Laroque P. Nutrition. In: Kinke GJ, ed. *The Laboratory Rat.* San Diego: Academic Press, 2000:57.

11. Hartsook EW, Hershberger T, Nee JC. Effects of dietary protein content and ratio of fat to carbohydrate calories on energy metabolism and body composition of growing rats. J Nutr 1973; 103:167.

12. McCraken K. Effect of feeding pattern on the energy metabolism of rats given low protein diets. Br J Nutr 1975; 33:277.

13. Deb S, Martin R, Hershberger TV. Maintenance requirement and energetic efficiency of lean and obese Zucker rats. J Nutr 1976; 106:191.

14. Da Silva MS, Zimmerman P, Meguid MM, Nandi J, Ohinata K, Xu Y, Chen C, Tada T, Inui A. Anorexia in space and possible etiologies: an overview. Nutrition 2002; 18:837.

15. Lane HW, LeBlanc A, Putcha L, Whitson PA. Nutrition and human physiological adaptations to space flight. Am J Clin Nutr 1993; 58:583.

16. Oller WL. Kendall D, Greenman DL. Variability of selected nutrients and contaminants monitored in rodent diets: a 6-year study. J Toxicol Environ Health 1989; 27:47.

17. Hayta M, Alpaslan M. Effects of processing on biochemical and rheological properties of wheat gluten proteins. Nahrung 2001; 45:304.

18. Ferrando AA, Paddon-Jones D, Wolfe RR. Alterations in protein metabolism during space flight and inactivity. Nutrition 2002; 18:837.

19. Volpi E, Mittendorfer B, Wolf SE, Wolfe. Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first-pass splanchnic extraction. Am J Physiol 1999; 277:E513.

20. Lane HW, Smith SM. Nutrition in Space. In: Shils ME, Olson, JA, Shike, M and Ross, AC, eds. *Modern Nutrition in Health and Disease.* Philadelphia, PA: Lea and Febiger, 1998.

21. Biolo G Tipton KD, Klein S, Wolfe RR. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. Am J Physiol 1997; 237: E122.

22. Whitson PA, Pietrzyk RA, Pak CY. Renal stone risk assessment during space shuttle flights. J Urol 1997; 158:2305.

23. Whitson PA, Pietrzyk RA, Sams CF. Space flight and the risk of renal stones. J Gravit Physiol 1999; 6:P87.

24. Rao GN, Edmondson J, Elwell MR. Influence of dietary protein concentration on severity of nephropathy in Fischer-344 (F-344/N) rats. Toxicol Pathol 1993; 21:253.

25. Tada O, Yokogoshi H. Effect of different dietary protein composition on skeletal muscle atrophy by suspension hypokinesia/hypodynamia in rats. J Nutr Sci Vitaminol 2002; 48:115.

26. Ley FJ, Bleby J, Coates ME, Patterson, JS. Sterilization of laboratory animal diets using gamma radiation. Lab. Anim 1969; 3:221.

27. Josephson ES, Thomas MH, Calhoun WK. Nutritional aspects of food irradiation: an overview. J Food Process Preserv 1978; 2:299.

28. Diehl J. Safety of Irradiated Foods. In: OR Fennema, GW Sanderson, P Walstra, M Karel, SR Tannenaum and J Whitaker, eds. *Food Sciences and Technology*. New York: Marcel Dekker, Inc, 1990:66.

29. Diehl JF Hasselmann C, Kilcast D. Regulation of food irradiation in the European community: is nutrition a food issue. Food Control 1991; 2:212.

30. Slinger, S.J., A. Razzaque and C.Y. Cho. Effect of feed processing and leaching on the losses of certain vitamins in fish diets. In: J.E. Halver and K. Tiews, eds. *Finfish Nutrition and Fishfeed Technology*, 1979:425.

31. National RC. Nutrient requirements of warmwater fishes and shellfishes. Washington, D.C., National Academy Press, 1983.

32. Beetner G, Tsao TT, Frey A and Harper J. Degradation of thiamin and riboflavin during extrusion processing. J. Food Sci 1974; 39:207.

33. Haas R. Thiamin and the brain. Annu Rev Nutr 1988; 8:483.

34. Volpe S, King JC, Coburn, SP. Micronutrients: trace elements and B vitamins. In: Lane, HW, Schoeller, DA, eds. *Nutrition in Space flight and Weightlessness Model,* Boca Raton: CRC Press, 2000:213.

35. Fishman SM Christian P, West KP. The role of vitamins in the prevention and control of anemia. Public Health Nutr 2000; 3:125.

36. Thomas M. Use of Ionizing Radiation. In: Karmas, E and Harris, RS, eds. *Nutritional Evaluation of Food Processing.* New York: Avi Book, 1988:457.

37. Sklan D. Vitamin A in human nutrition. Prog Food Nutr Sci 1987; 11:39.

38. Konstantinova IV Sonnenfeld G, Schaffar L, Mastro A. Results of immunological experiments aboard the Cosmos biosatellites and problems in space immunology. Physiologist 1992; 35 suppl: S220.

39. Lesnyak AT Sonnenfeld G, Rykova MP, Meshkov DO, Mastro A, Konstantinova I. Immune changes in test animals during space flight. J Leuk Biol 1993; 54:214.

40. Taylor G. Immune changes in humans concomitant with space flights of up to 10 days duration. Physiologist 1993; 36 suppl:S71.

41. Stein TP, Gaprindashvili T. Spaceflight and protein metabolism, with special reference to humans. Am J Clin Nutr 1994; 60:806S.

42. Reeves PG, Nielson F, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993; 123:1939.

43. WHO. High-dose irradiation: wholesomeness of food irradiated with doses above 10 kGy. Report of a joint FAO/IAEA/WHO Study Group. Technical report series no. 890. Geneva, Switzerland: World Health Organization , 1999.

44. Linsay K. Food Additives. In: Fennema, OR ed. *Food Chemistry 3rd.* New York: Marcel Dekker, Inc, 1996:767.

45. Quattrucci E, Masci V. Nutritional aspects of food preservatives. Food Addit Contam 1992; 9:515.

46. Daniel J. Metabolic aspects of antioxidants and preservatives. Xenobiotica 1986; 16: 1073.

47. Siddhuraju P Makkar H, Becker K. The effect of ionizing radiation on antinutritional factors and the nutritional value of plant materials with reference to human and animal food. Food Chemistry 2002; 78:187.

48. Rabot S, Szylit O, Nugon-Baudon L, Meslin JC, Vaissade P, Popot F, Viso M. Variations in digestive physiology of rats after short duration flights aboard the US space shuttle. Dig Dis Sci. 2000; 45:1687.

49. Munoz KA, Tischler TM. The effect of a space food bar diet on body and muscle mass in normal and hind-limb suspended rats. Aviat Space Environ Med. 1991; 62:875.

50. Zerath E, Holly X, Andre C, Renault S. Effect of space food bar feeding on bone mass and metabolism in normal and unloaded rats. Nutr Res 2002; 22:1309.

51. Hatton DC Yue Q, Chapman J, Xue H, Dierickx J, Roullet C, Coste S, Roullet JB, McCarron DA. Blood pressure and mesenteric resistance arterial function after spaceflight. J Appl Physiol 2002; 92:13.

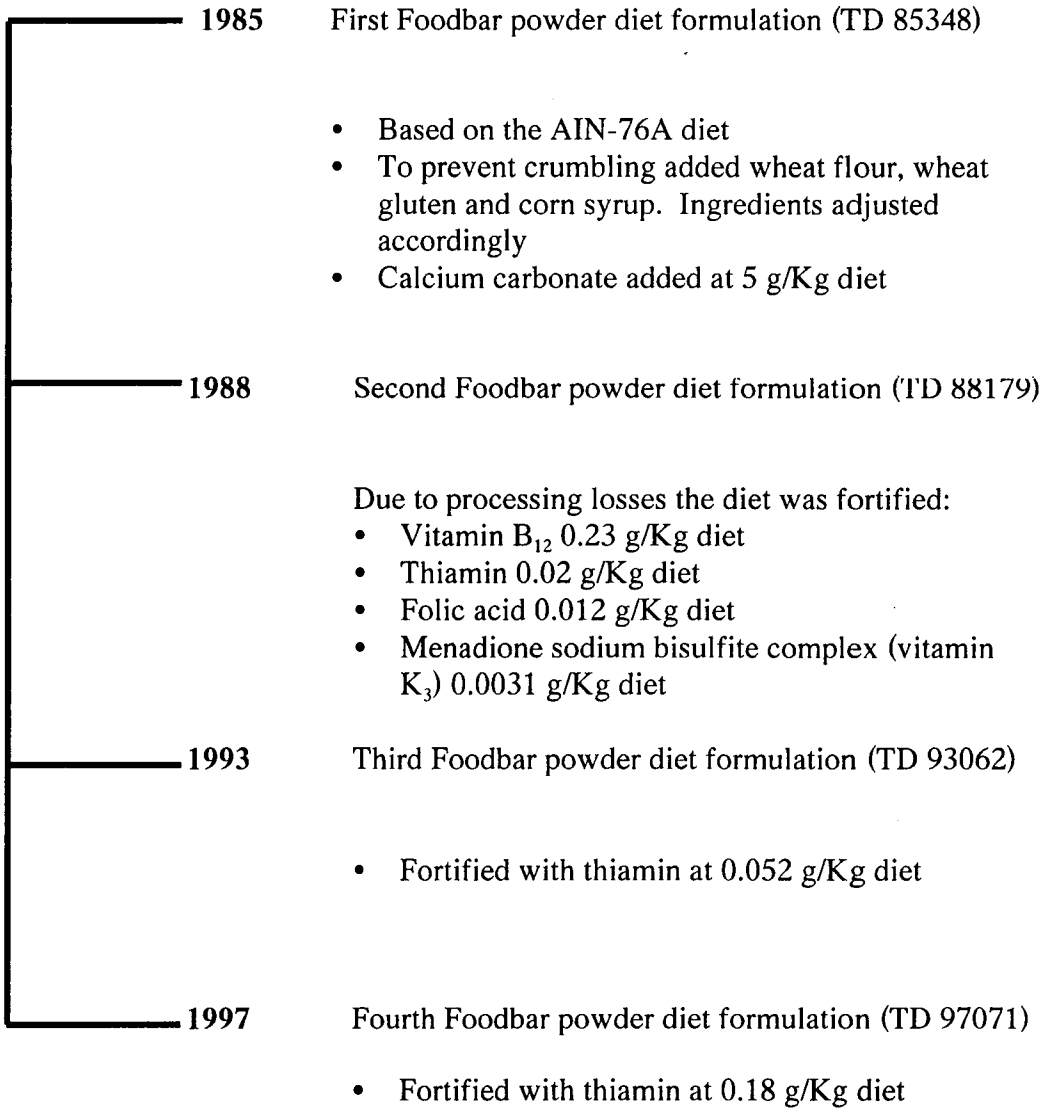


FIG 1. Development of the NASA Rodent Foodbar Powder Diet Formulation.

TABLE I

DIET COMPOSITION

Diet Ingredients	AIN-76A	Foodbar Powder Diet (TD 97071)
	g/Kg Diet	
Casein	200	100
DL-Methionine	3	3
Wheat Flour	—	225
Wheat Gluten	—	120
Corn Starch	150	199.575
Corn Syrup	—	100
Sucrose	250	100
Dextrose	250	—
Corn Oil	50	40
Fiber (cellulose)	50	50
Choline Bitartrate	2	2
Vitamin Mix (AIN-76A, TD 40077)	10	20
Mineral Mix (AIN-76A, TD 17915)	35	35

TABLE II

COMPOSITION OF VITAMIN AND MINERAL MIXES

Ingredients	AIN-76A	Foodbar Powder Diet (TD 97071)
AIN-76A Vitamin Mix (TD 40077)		
	g/Kg Mix	
Water-Soluble Vitamins		
Thiamin HCl	0.60	0.60 +fortification[1]
Riboflavin	0.60	0.60
Niacin	3	3
Folic Acid	0.20	0.20 + fortification[2]
Pyridoxine HCl (B_6)	0.70	0.70
Vitamin B_{12}	1.00	1.00 +fortification[3]
Biotin	0.02	0.02
Calcium Pantothenate	1.6	1.6
Fat-Soluble Vitamins		
Dry Vitamin A Palmitate	0.80	0.80
Dry Vitamin E Acetate	10	10
Vitamin D_3 Trituration	0.25	0.25
Menadione Sodium Bisulfite complex (Vitamin K)	0.15	0.15 + fortification[4]
Powdered Sucrose	981.08	982.08
AIN-76A Mineral Mix (TD 17915)		
Calcium Phosphate, dibasic	500	500
Calcium Carbonate	—	5[5]
Potassium Citrate, monohydrate	220	220
Potassium Sulfate	52	52
Sodium Chloride	74	74
Magnesium Oxide	24	24
Manganous Carbonate	3.5	3.5
Ferric Citrate	6	6
Zinc Carbonate	1.6	1.6
Chromium Potassium Sulfate	0.55	0.55
Cupric Carbonate	0.3	0.3
Potassium Iodate	0.01	0.01
Sodium Selenite	0.01	0.01
Powdered Sucrose	118.03	118.03

[1]Fortified with 0.18 g thiamin/Kg diet.

[2]Fortified with 0.012 g folic acid/Kg diet.

[3]Fortified with 0.23 g B_{12}/Kg diet.

[4]Fortified with 0.0031g Menadione sodium bisulfate complex/Kg diet.

[5]5 g calcium carbonate/Kg diet.

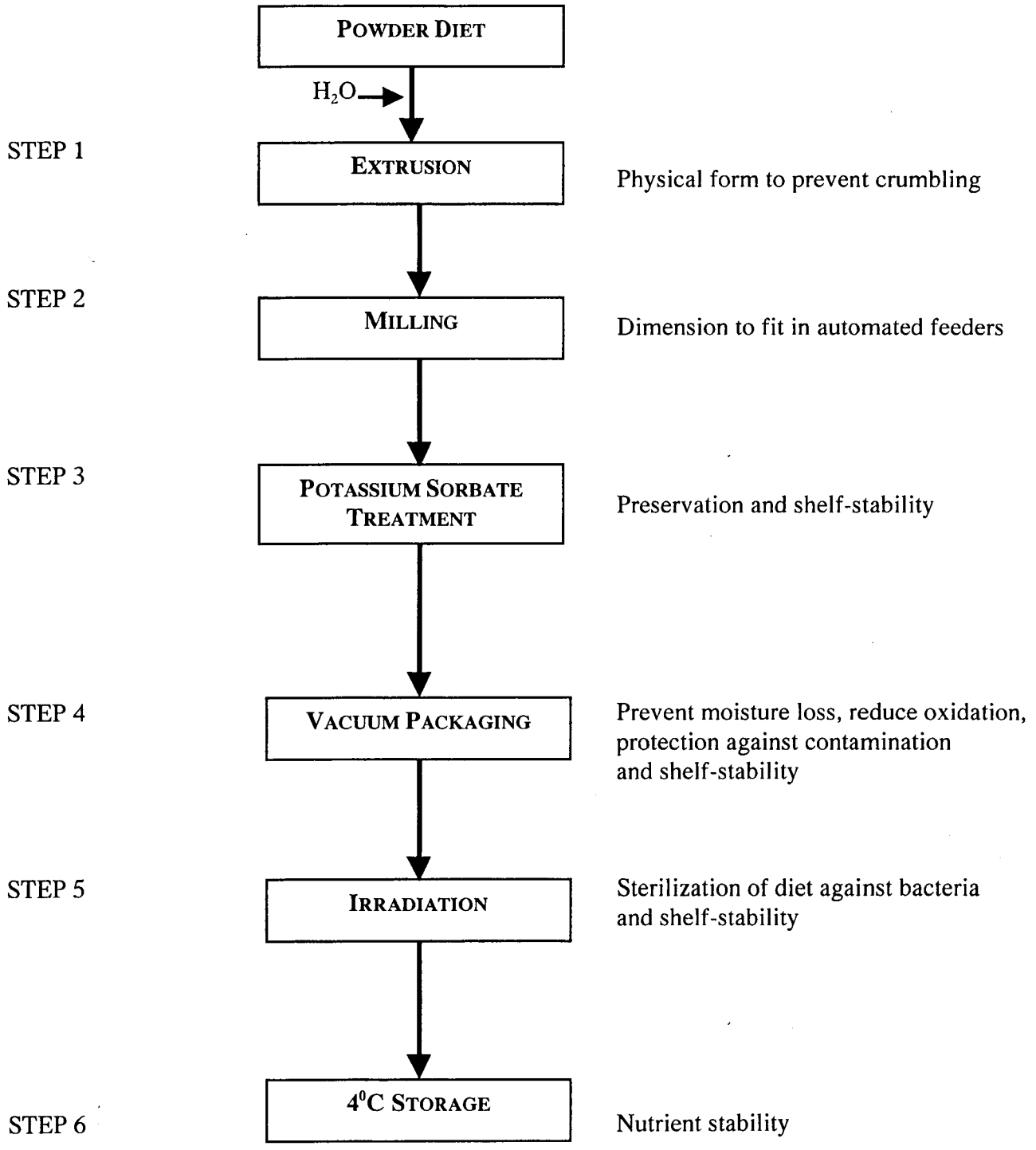
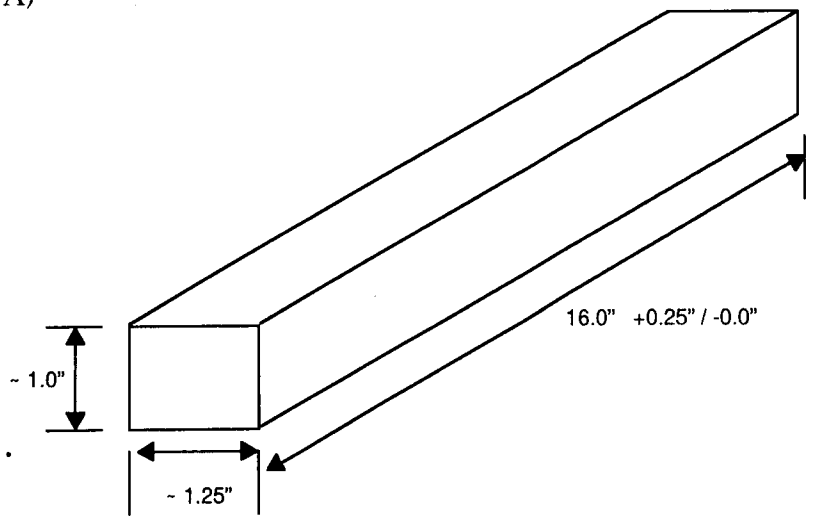


FIG 2. Flow Chart of Steps in the Manufacture of NASA Rodent Foodbars.

A)



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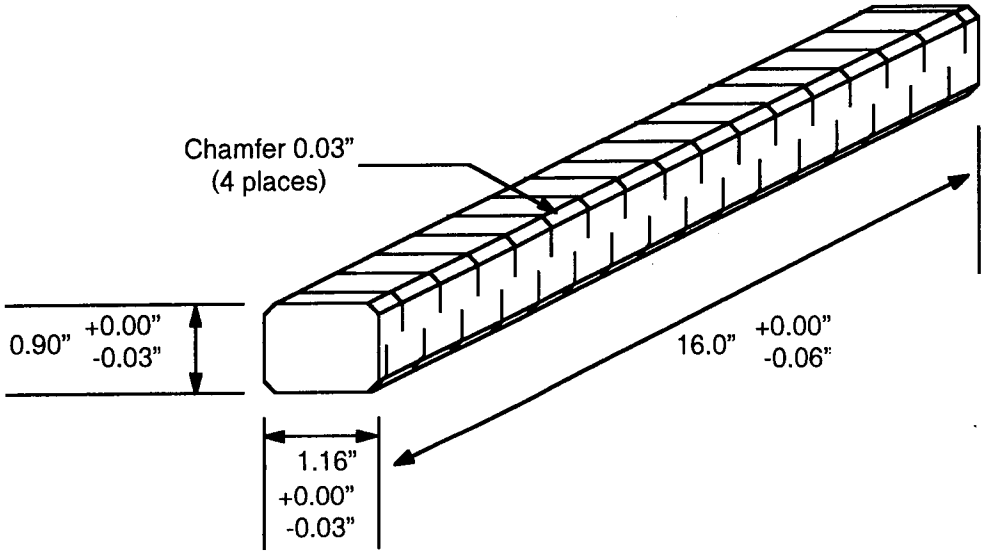

FIG 3. Dimension of the NASA Rodent Foodbars. Animal enclosure module (AEM) milled Foodbars. Dimensions shown in inches (equals 40.6 cm x 3.2 cm x 2.5 cm). B) Research animal holding facility (RAHF) milled Foodbars. Dimensions shown in inches for centimeters 40.6 cm x 3.0 cm x 2.3 cm.

TABLE III

INDISPENSABLE AMINO ACIDS IN NASA RODENT FOODBARS COMPARED TO THE NATIONAL RESEARCH COUNCIL (NRC) NUTRIENT RECOMMENDATIONS FOR RATS AND MICE

Nutrients	NRC for Mice (g/Kg diet)	NRC for Rats (g/Kg diet)		NASA Rodent Foodbars (g/Kg diet)			
	Growth	Maintenance	Reproduction/ Growth	Average[1]	n	Min	Max
Indispensable Amino Acids							
Arginine	3.0	ND	4.3	7.3	23	5.4	10.3
Phenylalanine	7.6	1.9	10.2	**9.6**	23	7.8	12.7
Histidine	2.0	0.8	2.8	4.6	23	3.2	7.2
Isoleucine	4.0	3.1	6.2	7.6	23	6.0	10.2
Leucine	7.0	1.8	10.7	14.8	23	12.9	19.1
Lysine	4.0	1.1	9.2	**7.7**	23	5.9	11.0
Methionine	5.0	2.3	9.8	**5.4**	23	0.0	9.1
Threonine	4.0	1.8	6.2	**6.1**	23	3.9	9.5
Valine	5.0	2.3	7.4	9.0	23	6.4	12.2

[1]Bolded values fall below the National Research Council (NRC, 1995) nutrient recommendations.

TABLE IV

VITAMINS LEVELS IN NASA RODENT FOODBARS COMPARED TO THE NATIONAL RESEARCH COUNCIL (NRC) NUTRIENT RECOMMENDATIONS FOR RATS AND MICE

Vitamins	NRC for Mice[1]	NRC for Rats		NASA Rodent Foodbars Average[3]	n	Min	Max
	Growth	Reproduction	Growth				
Water-Soluble							
Thiamin (mg/Kg diet)[2]	5.0	4.0	4.0	8.0	23	0.6	17.5
Niacin (mg/Kg diet)	15.0	15.0	15.0	65.0	23	31.2	84.9
Folic acid (mg/Kg diet)	0.5	1.0	1.0	3.0	15	1.6	5.6
B_{12} (µg/Kg diet)	10.0	50.0	50.0	113.5	11	46.0	187.3
Fat-Solubles							
Vitamin A (mg/Kg diet)	0.72	0.7	0.7	1.2	23	2.0	0.7
Vitamin E (mg/Kg diet)	22.0	18.0	18.0	49.3	15	20.1	59.6

[1]Separate recommendations for maintenance have not been determined by the NRC for vitamins.

[2]Mean value for Foodbars from production runs 1997-2002 using current powder diet formulation. Foodbars from production runs 1989-1996 using earlier powder diet formulations mean=3.3 ±0.5 mg/Kg, minimum=1.05, maximum=6.30.

TABLE V

THE EFFECT OF NON-SORBATE VERSUS SORBATE-TREATED FOODBARS ON BODY MASS, MASS GAIN, FOOD CONSUMPTION AND WATER INTAKE

Measurements[1]	Non-Sorbate-Treated Foodbars	Sorbate-Treated Foodbars
Initial Body Mass (g)	241.7 ±4.0	235.7 ±2.0
Final Body Mass (g)	365.3±7.9	364.5 ±3.7
Body Mass Gain (g/d)	7.2 ±0.2	6.9 ±0.3
Food Intake (g/d)	26.2 ±0.7	27.1 ±0.5
Water Intake (ml/d)	25.1 ±1.7	28.0 ±4.0
Liver (mg/100g)	4579.6 ±73.0	4796.0 ±72.0
Kidneys (mg/100g)	777.6 ±28.4	847.2 ±13.0
Heart (mg/100g)	353.3 ±11.5	356.2 ±14.3
Spleen (mg/100g)	267.2 ±6.1	287.4 ±14.3
Thymus (mg/100g)	213.1 ±16.3	228.6 ±6.9
Andrenal (mg/100g)	116.7 ±4.5	121.2 ±3.3

[1]Values are the means ±SEM of n=6 rats/group. Differences between groups was determined by paired t-test with significance at $p<0.05$.