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Improvement of the Immune System with Two Types of Emergency Rations in the Murine Animal Model

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ABSTRACT

Introduction: Rescue and preservation of refugees and disaster victims depend on delivering cost-effective, nutritionally sound food options. Utilizing food items enriched with vital nutrients and immune system fortifiers is imperative to bolster and sustain proper immune system functionality. This study explores the immunomodulatory impacts of two emergency rations on the immune system using a murine animal model.

Methods: In this study, four sets of ten Balb/c strain mice aged between 4 and 6 weeks, weighing 17.8 to 18.9 grams, were handpicked. Two of these groups were subjected to treatment diets designated as 1 and 2, while the other two groups were provided with control diets numbered 1 and 2 administered at 3 to 4 grams daily over eight weeks. Following the 8-week dietary intervention, blood samples were collected to evaluate interleukin-4 (IL-4), interferon-gamma (IFN-γ), immunoglobulin G 1 (IgG1), and IgG2 levels.

Results: The outcomes revealed that the treatment groups exhibited significantly higher IFN-γ levels than their control counterparts. Additionally, the IFN-γ/IL-4 ratio was consistently elevated within the treatment groups as opposed to the control groups. There was a significant enhancement in cellular immune responses within the treatment group, as indicated by an increase in Th1/Th2 cell ratios. Moreover, in the treatment group, there was a significant increase in IgG2 antibodies and a corresponding decrease in IgG1 antibodies compared to the control group .

Conclusions: Based on the results, using emergency rations in mice increased cellular immune responses in both treatment groups.

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Introduction

Every year, natural or human-made disasters affect large human populations. Examples of such disasters are earthquakes, floods, wildfires, hurricanes, chemical spills, nuclear plant accidents, and wars. Anxiety, stress, and severe tension resulting from disasters or emergencies often lead to a lack of appetite in affected individuals. Thus, survivors cannot use many common foods due to power outages and the destruction of warehouses and refrigeration facilities, which prevent the distribution of regular food by relief organizations. Iran is a disaster-prone country, ranking fourth in Asia and sixth globally in natural disasters [1]. Furthermore, a military crisis is not out of the question, considering its geopolitical position and the presence of external enemies.

As natural and human-induced catastrophes become more frequent, developing emergency food provisions for relief missions has become a top priority for crisis management. Specialized rations and emergency sustenance solutions have been meticulously crafted within the United States to address these pressing needs.

The emergency ration is considered in classical and guerrilla wars and military maneuvers alongside operational rations to support forces that have not supplied food for more than 24 hours [3]. Various rations, including biscuit-like

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and bar-like forms, are produced for these purposes [3,6].

In the formulation of these food products, the synthesis of five pivotal factors takes precedence: (1) paramount product safety, (2) an element of palatability, (3) streamlined distribution logistics, (4) user-friendliness, and (5) comprehensive nutrient content [5]. Emergency food products should furnish the essential energy, proteins, vitamins, minerals, and other nutrients indispensable for sustaining human life during brief periods of crisis. Preserving microbial safety, nutritional value, and oxidation resistance emerge as quintessential traits for a long-lasting, shelf-stable product under adverse environmental conditions [2]. Furthermore, the sensory attributes of these products should resonate with diverse cultural norms and preferences [7]. Thus, the harmonious amalgamation of these elements becomes pivotal in designing an ideal emergency ratio.

The composition, encompassing macronutrients and such products' sensory and physical properties, has been meticulously expounded in antecedent reports and research endeavors [2]. These rations' moisture content and water activity should hover around 9.5% and 0.6% to prevent microbial spoilage, nutrient degradation, and oxidative degradation [5]. Ideally, an emergency ration should boast a shelf life of no less than 36 months, even when stored at 21℃. Furthermore, each serving of these rations ought to yield approximately 233kcal. As a result, adults consume roughly 9 to 10 servings per day, equating to about 2100 kilocalories daily. The constitution of these rations becomes paramount, considering that these products are expected to serve as an individual's exclusive source of sustenance for up to 15 days.

A myriad of ingredient combinations have been explored across various studies to create these emergency rations, including soy products (ranging from flour and concentrate to isolate) as primary protein sources, low-fat or fat-free powdered egg and milk, semi-hydrogenated soybean oil, vegetable oils, and hydrogenated fats for the requisite fat content. Additionally, grain-based mixtures, vitamin and mineral premixes, sugar, and potentially cooking agents have been employed as complementary components [3,5]. Harmonizing these constituents is essential, allowing the resultant product to withstand diverse distribution methods, including airdropping, even under the most challenging conditions.

The characteristics of emergency food products have been studied using various formulations with different technologies. Additionally, affordability and the potential to enhance the consumer's immune system have received less attention. Stress levels during crises and wartime conditions weaken the immune system, making individuals more susceptible to diseases. Therefore, producing rations that can improve individuals' immune status during crises can provide the necessary nutrients and make them more resistant to potential illnesses. Moreover, food items such as grains, their products, and their abundant nutrients have functional properties in foods, which are readily available and cost-effective, making them suitable for various food formulations [4].

An exemplary emergency ration should encompass adequate proportions of essential components, including plant oils, proteins, carbohydrates, and vitamin/mineral premixes. The immune system's role, particularly innate and cell-mediated immunity, holds paramount importance in preventing and managing microbial infections. Incorporating foods enriched with immune-boosting nutrients becomes imperative to fortify and sustain optimal immune system performance. Nutrition deficiencies often affect the immune system, particularly cell-mediated immunity, phagocyte function, cytokine production, and antibody secretion. Malnutrition stands as a pervasive driver of immune system impairments on a global scale [13,16].

Therefore, this study aimed to unveil the immunomodulatory impacts of two distinct emergency rations through the lens of a murine animal model.

Materials and Methods

This study used a diet with formulation number 1, developed by Dehghani Moghadam and colleagues. The only change in the diet was the variation in zinc, selenium, and vitamin D levels to investigate their immunomodulatory effects. In the Dehghani Moghadam and colleagues' study, diet formulation number 1 had the highest acceptability regarding sensory characteristics, primarily composed of wheat flour and powdered milk. Furthermore, none of the tested microorganisms, including Klebsiella,

Salmonella, molds, yeast, and *E. coli*, were detected in any treatment group [8].

Composition of Treatment Diet 1

Wheat flour (25g), powdered milk (5g), canola oil (8g), sugar (7g), and, in equal proportions, lecithin (0.05g), vanilla (0.05g), cocoa powder (0.5g), coconut powder (0.75g), BHT (as an antioxidant) (0.005g), salt (0.2g), water (4 to 6mL), 0.3 tablets of Imustim containing dried extract of Echinacea purpurea (as an immune system enhancer), vitamin/mineral premix (3.5g), and maltodextrin (0.25g) were added. The micronutrients for the emergency diet are determined based on Table 1.

Table 1. Emergency feed micronutriensts for treatment group 1

Micronutrient type	The amount in each approximately 50 gr of bar
Vitamin A (In the form of capsulated palmitate)	116.55 µg
Vitamin D_3 (In the form of cholecalciferol)	1.11 µg
Vitamin E (In the form of acetate)	3.33 UI
Vitamin K_1 (In the form of phytonadione)	0.011 mg
Vitamin C (In the form of capsulated ascorbic acid)	31.10 mg
Vitamin B_1 (In the form of capsulated thiamine mononitrate)	0.19 mg
Vitamin B_2 (In the form of riboflavin)	0.20 mg
Niacin (In the form of niacinamide)	1.33 mg
Vitamin B_6 (In the form of pyridoxine hydrochloric acid)	0.22 mg
Folic Acid	0.044 mg
Vitamin B_{12} (In the form of cyanocobalamin)	2.78 µg
Biotin	5.56 µg
Pantothenic Acid (In the form of D-Calcium pantothenate)	0.78 mg
Calcium (In the form of tricalcium phosphate or calcium carbonate)	66.67 mg
Phosphorus (In the form of dipotassium phosphate or tricalcium phosphate)	111.11 mg
Magnesium (In the form of magnesium oxide)	22.2 mg
Zinc (In the form of zinc oxide or zinc sulfate)	2.06 mg
Copper (In the form of copper oxide or copper gluconate)	0.10 mg
Manganese (In the form of manganese sulfate)	0.056 mg
Selenium (In the form of sodium selenate or selenomethionine)	4.44 µg
Chromium (In the form of chromium chloride ($6H_2O$))	2.78 µg
Iodine (In the form of potassium iodide)	0.011 mg
Iron (In the form of ferric EDTA or chelated iron)	1.89 mg
Potassium (In the form of dipotassium phosphate)	204.44 mg
Choline (In the form of lecithin)	769 mg

Composition of Treatment Diet 2

Wheat flour (25g), powdered milk (5g), canola oil (8g), sugar (7g), and, in equal proportions, lecithin (0.05g), vanilla (0.05g), cocoa powder (0.5 grams), coconut powder (0.75 grams), BHT (as an antioxidant) (0.005g), salt (0.2g), water (4 to 6mL), 0.3 tablets of Imustim containing dried extract of Echinacea purpurea (as an immune system enhancer), vitamin/mineral premix (3.5g), and maltodextrin (0.25g) were added. The micronutrients for the emergency diet are determined based on Table 2, based on which This diet's vitamin D, selenium, and zinc content were increased.

Composition of Control Diet 1

The diet used for the control group was provided by Javaneh Khorasan Company and included the following components: Protein: 20-21%, Fat: 2-3%, Energy: 2750kcal/kg, Crude fiber: 5-6%, Methionine: 0.05%, Lysine: 0.05%, Salt: 0.5%, Calcium-to-phosphorus ratio: 1.5-2.5%, Ash: 4%, Soybean meal, canola meal, cottonseed meal, molasses, salt, phosphate, methionine, vitamin and mineral supplement, fish meal, and wheat bran.

Composition of Control Diet 2

Wheat flour (25g), powdered milk (5g), canola oil (8g), sugar (7g), and, in equal proportions, lecithin (0.05g), vanilla (0.05g), cocoa powder (0.5g), coconut powder (0.75g), BHT (as an antioxidant) (0.005g), salt (0.2g), water (4 to 6mL).

In pursuit of pioneering research, ten cohorts of Balb/c mice, aged between 4 and 6 weeks and exhibiting a weight range of 17.8-18.9g, were assembled. In this study, two of these groups were entrusted with treatment diets denoted as 1 and 2, while an additional two groups were provided with control diets marked as 1 and 2. These diets were diligently administered at a daily rate of 3-4g over eight weeks. The vigilant observations encompassed the mice's overall

well-being, water consumption, and food intake throughout this duration. Blood samples were harvested to meticulously gauge the concentrations of IL-4, IFN-γ, IgG1, and IgG2 after this 8-week dietary intervention, as detailed in reference [15].

Table 2. Emergency feed micronutriensts for treatment group 2

Micronutrient type	The amount in each approximately 50 gr of bar
Vitamin A (In the form of capsulated palmitate)	116.55 µg
Vitamin D_3 (In the form of cholecalciferol)	5 µg
Vitamin E (In the form of acetate)	3.33 UI
Vitamin K_1 (In the form of phytonadione)	0.011 mg
Vitamin C (In the form of capsulated ascorbic acid)	31.10 mg
Vitamin B_1 (In the form of capsulated thiamine mononitrate)	0.19 mg
Vitamin B_2 (In the form of riboflavin)	0.20 mg
Niacin (In the form of niacinamide)	1.33 mg
Vitamin B_6 (In the form of pyridoxine hydrochloric acid)	0.22 mg
Folic Acid	0.044 mg
Vitamin B_{12} (In the form of cyanocobalamin)	2.78 µg
Biotin	5.56 µg
Pantothenic Acid (In the form of D-Calcium pantothenate)	0.78 mg
Calcium (In the form of tricalcium phosphate or calcium carbonate)	66.67 mg
Phosphorus (In the form of dipotassium phosphate or tricalcium phosphate)	111.11 mg
Magnesium (In the form of magnesium oxide)	22.2 mg
Zinc (In the form of zinc oxide or zinc sulfate)	500 mg
Copper (In the form of copper oxide or copper gluconate)	0.10 mg
Manganese (In the form of manganese sulfate)	0.056 mg
Selenium (In the form of sodium selenate or selenomethionine)	2 mg
Chromium (In the form of chromium chloride ($6H_2O$))	2.78 µg
Iodine (In the form of potassium iodide)	0.011 mg
Iron (In the form of ferric EDTA or chelated iron)	1.89 mg
Potassium (In the form of dipotassium phosphate)	204.44 mg
Choline (In the form of lecithin)	769 mg

Selection of Primary Ingredients

The choice of key constituents for formulating dietary compositions was rooted in a multifaceted evaluation, encompassing nutritional excellence, local availability in Iran, economic viability, widespread cultural endorsement among the Iranian populace, and alignment with prevailing dietary inclinations. Most of the ingredients for diet production were sourced from Shahrvand chain stores in Tehran, while some were sourced from confectionery ingredient distributors in the city. Wheat flour, soy flour, powdered milk, and canola oil were considered sources of carbohydrates, protein, and fat, respectively. Lecithin, vanilla, cocoa powder, coconut powder, BHA, salt, water, and vitamin/mineral premixes were also added to the formulations. The ratios of these ingredients in the formulations are listed in Table 1. Only the antioxidant butylated hydroxyanisole (BHA), lecithin (as an emulsifier), and maltodextrin (as a bulking agent) were procured from Merck (Germany). The vitamin premix for diets 1 and 2 was formulated by Eswe Iran Pharmaceutical Company, considering the recommended amounts by the Institute of Medicine.

Diet Preparation

A progressive approach was employed for diet preparation. Initially, canola oil was introduced into a beaker and subjected to gentle heating in an oven set at 112°C, maintaining the process until complete liquefaction ensued. Simultaneously, the dry constituents of each formulation underwent meticulous blending for 5 minutes, utilizing a mixer of Tefal makes, hailing from France. Subsequently, lecithin was judiciously incorporated into the molten oil, ensuring thorough dissolution. This amalgamation was introduced into the preceding mixture with 5 minutes of rigorous mixing. Water was added at the end of the process to facilitate the creation of the definitive dough-like substance, which required another five minutes of comprehensive blending. This resultant amalgam was adeptly poured onto aluminum foil and sculpted to dimensions measuring 7.6 ×4.4 cm. During baking, the molds were carefully placed in an oven set at 150°C for exactly 20 minutes. Following baking, the diets were carefully enclosed in polyethylene packaging and meticulously stored at 38°C, awaiting subsequent experiments, as detailed in reference [8].

Modification of Diets for Immunomodulation

Iron was included as iron oxide or sulfate to transform the diets into immunomodulatory agents with immune-enhancing properties and infection resistance, and selenium and vitamin D were added to each tablet or dough. Only in diet two the levels of iron, selenium, and vitamin D were increased. These diets were available to the mice for an 8-week, during which no other food except water was provided (Tables 1 and 2).

ELISA Procedure

Dilution of Standard Solutions

The standard solutions were diluted uniformly in 5.1mL microtubes, following the kit instructions. The ELISA procedure, including the addition of blood serum samples, was carried out as follows:

1. **Control Wells**: These wells served as blanks and received only a combination of chromogen solution A, B, and the stop solution.
2. **Standard Solution Wells**: 50µL of standard solution and 50µL of streptavidin-HRP were meticulously introduced into these wells.
3. **Sample Wells**: The sample wells initiated with the addition of 40µL of blood serum samples, followed by the sequential introduction of 10µL of interleukin-4, interferon-gamma, IgG1, IgG2 antibodies, and 50µL of HRP-streptavidin. Subsequently, the plate was securely covered, gently agitated, and incubated at 37°C for 60 minutes.
4. **Wash Solution Preparation**: A potent wash solution (30X) was expertly crafted by judiciously diluting it with distilled water, paving the way for upcoming crucial steps.
5. **Thorough Washing**: The plate cover was adroitly removed, and the liquid contents were judiciously discarded from each well. Subsequently, each well was diligently flooded with the prepared wash solution. The solution was swiftly drained after a brief 30-second interval. This crucial step was repeated five times to ensure thorough washing, followed by careful blotting of the plate until drying.
6. **Chromogenic Reaction**: The plate was gently agitated to foster a harmonious blend of the contents, commencing with the introduction of 50µL of chromogen solution A, followed by 50µL of chromogen solution B into each well. The plate then embarked on a controlled incubation journey, held at a consistent temperature of 37°C for precisely 10 minutes, shrouded in darkness to facilitate optimal color development.
7. **Reaction Termination**: About 50µL of the stop solution was systematically introduced into every well with precision. This judicious addition transformed from the initial blue hue to a resplendent yellow.
8. **Assessment**: This pivotal phase was conducted with meticulous timing, within a strict 10-minute window post-addition of the stop solution. The absorbance (OD) measurement for each well was exactingly executed at a wavelength of 450nm, employing a state-of-the-art ELISA reader (EL Bioteck, 800X). The blank served well as the reference point, assigning it a zero value for accurate comparisons.

Based on the standards and the optical density (OD) readings of the samples, the concentrations of the factors were calculated in pg/mL [9].

Statistical Analysis

Microsoft Excel software (Macintosh version 2016) was harnessed as the analytical tool of choice to analyze all the data gathered, encompassing sensory analysis scores and microbial counts of the samples. A rigorous statistical evaluation was undertaken to assess potential disparities between treatment-related mean results, employing a one-tailed, one-way analysis of variance (ANOVA) methodology executed using SPSS IBM software (version 24). A significance threshold of 5% ($P<0.05$) was diligently adhered to throughout the analytical process.

Results

The outcomes pertaining to alterations in interleukin-4, gamma interferon, IgG1, and IgG2a concentrations are elucidated as follows:

In interleukin-4, the treatment group exhibited a notably lesser increment than the control group. A significant difference was observed in the concentration of this cytokine regarding treatment group 1 in comparison to both control group 1 ($P=0.001$) and control group 2 ($P=0.003$), as well as in treatment group 2 compared to control group 1 ($P=0.001$) and control group 2 ($P=0.018$).

Conversely, the level of gamma interferon in the treatment group displayed a significantly more pronounced elevation when contrasted with the control group. Significant concentration shifts were established in treatment group 1 about control group 1 ($P=0.012$) and control group 2 ($P=0.03$), as well as in treatment group 2

compared to control group 1 (P=0.001) and control group 2 (P=0.018).

On a divergent note, the concentration of IgG1 exhibited a reduction within the treatment group as opposed to the control group. Significant differences in concentration were observed in treatment group 1 compared to control group 1 (P=0.002) and control group 2 (P=0.03), as well as in treatment group 2 in contrast to control group 1 (P=0.006) and control group 2 (P=0.01).

Furthermore, the level of IgG2a within the treatment group demonstrated a marked escalation when juxtaposed with the control group. Concentration fluctuations of statistical significance were discerned in treatment group 1 when compared to control group 1 (P=0.014) and control group 2 (P=0.02), as well as in treatment group 2 vis-à-vis control group 1 (P=0.001) and control group 2 (P=0.04) (Table 3).

Table 3. The results of changes in the concentration (pg / mL) of blood IL4, IF Gamma, IgG1.IgG2 (standard deviation±mean) at different times before and after eating two practical emergency food rations in the treatment group and before and after eating two normal food rations in control group

		Time	
Measured factors	**Group**	**Before receiving the emergency food ration**	**8 weeks after receiving food ration**
IL4 (Pg/ml)	Treatment 1	1.2830±..23712	42.35±#10.7*×
	Treatment 2	1.2820±23701	46.88±4.83 #*×
	Control 1	1.2848±.24712	78.3509±9.97
	Control 2	1.3348±.28708	65.18±11.67
IF Gamma (Pg/ml)	Treatment 1	152.10±26.06	4.92±# 32.67*
	Treatment 2	150.30±23.01	-30.97±×#46.44*
	Control 1	149.40±25.01	24.27±47.15
	Control 2	152.31±27.20	25.96±65.69
IgG1 (Pg/ml)	Treatment 1	1.2848±.24	.396±.646 *×#
	Treatment 2	1.1938±.18	.476±# .264*×
	Control 1	1.2520±.26	1.28±.24
	Control 2	1.2638±.25	.831 ±.493
IgG2a (Pg/ml)	Treatment 1	152.10±26.06	#-188.3±9.61*
	Treatment 2	150.19±19.9	171.21±17.87
	Control 1	148.34±30.03	132.1026.06
	Control 2	149.07±24.04	126.70±35.01

*: Significant changes compared to before receiving food ration in the same group.
#: Significant changes compared to the control group 1 in 8 weeks after receiving food ration
×: Significant changes compared to the control group 2 in 8 weeks after receiving food ration

Discussion

Iran, a country susceptible to various disasters, is frequently hit by substantial financial and human losses caused by natural calamities. A staggering 31 of the 40 recognized categories of natural disasters worldwide occur in Iran. Furthermore, the prospect of military crises looms ominously, given Iran's pivotal strategic and geopolitical positioning, coupled with the persistent specter of external threats. Emergency diets often emerge as the sole lifeline in these dire scenarios, providing sustenance in the early throes of catastrophes like earthquakes, hurricanes, and the difficulties of war zone evacuations. Within this context, emergency diets' nutritional potency, sensory appeal, and immune system fortification offer paramount significance in meeting the acute dietary needs of those thrust into these harrowing circumstances [10,11].

Mice consuming the treatment diets exhibited elevated levels of gamma interferon compared to their counterparts in the control groups. Moreover, the IFNγ/IL4 ratio within the treatment groups exceeded that within the control groups, associated with an increase in the Th1/Th2 cell ratio and a stronger cellular immune response within the cohort. Accordingly, the treatment group showed an increase in IgG2 antibodies and a decrease in IgG1 antibodies compared to the control group. This shift in IgG2 and IgG1 isotypes among the mice is underpinned by the influence of IFNγ and IL4, aligning seamlessly with the cytokine analysis results, thereby substantiating the heightened

Th1 cell ratio in the treatment-receiving group [15].

Protein malnutrition, a profound concern, exerts significant deleterious effects on cellular immune response, phagocytic function, complement system activity, secretory immunoglobulin A antibody concentrations, and cytokine production. Even relatively mild nutrient deficiencies can lead to tangible alterations in immune responses. Among the crucial micronutrients, zinc, selenium, iron, copper, and vitamins A, C, E, B, and folic acid profoundly influence immune responses [15].

Alberts et al. 2003 delved into the immunomodulatory potentials of dietary supplements incorporating vitamins A, E, C, selenium, and zinc using a mouse model. Researchers investigated phagocytic activity, oxidative responses, gamma interferon, interleukin 4, and immunoglobulin G after sensitizing mice with dinitrochlorobenzene. In this study, dietary vitamin A supplementation induced heightened inflammatory responses decreased Th1 responses, and increased mucosal responses. Young mice who received insufficient nourishment in the form of vitamin C, E, selenium, or zinc exhibited no discernible impact on their immune systems, which resonates with the results from the present study [12].

Kiremidjian et al. (1990) examined the effects of selenium-containing dietary supplements in mice for eight weeks and investigated the effects of these supplements on interleukin 1 and 2. In this study, a selenium diet significantly affected lymphocyte proliferation. Furthermore, selenium supplementation increased interleukin levels considerably. Therefore, mechanisms responsible for the effects of immune responses through lymphocyte proliferation are independent of IL-2 or IL-1 levels [14].

Ramiro-Puig et al. (2007) explored the influence of cocoa-rich diets on the intestinal immune system of desert mice. Over three weeks, these intrepid mice were nourished with cocoa-rich diets, and their immune system dynamics underwent thorough scrutiny to assess critical factors such as immunoglobulin A, interleukins 2, 4, 10, and gamma interferon. Astonishingly, the findings unveiled a transformative impact that consumption of cocoa-rich diets culminated in the proliferation of mesenteric lymph nodes and amplification of Peyer's patches. Furthermore, the T-cell ratio and antigen receptor activity within both lymphatic tissues was augmented. This groundbreaking study unequivocally validated that cocoa-rich diets served as catalysts for heightened production of immunoglobulin A and gamma interferon while concurrently suppressing interleukin ten levels. In essence, cocoa consumption was shown to profoundly influence the modulation of the intestinal immune response, particularly in young mice [23].

These intriguing revelations serve as poignant reminders of nutrition's pivotal role in fortifying immune responses, a factor of paramount significance, especially in the difficulties of emergencies and disasters. Specifically, tailored diets enriched with specific nutrients can profoundly impact immune system performance. Further exploration and in-depth research in this field promise to unveil novel avenues to enhance immunity and resilience in diverse crises and disasters.

Conclusion

Based on the results, considering all aspects, including sensory evaluation, microbial tests, total fat percentage, fat oxidation levels, water activity, and production economics, the three-year stability at a temperature of 21°C and, most importantly, the enhanced immune response in the mouse model, these diets can be regarded as an acceptable regimen for boosting the immune system for use in emergency conditions in the country. Therefore, further investigation and validation in humans are required. Compared with foreign emergency diets, this diet costs half to one-third less, and bulk ingredient procurement can make it even cheaper.

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