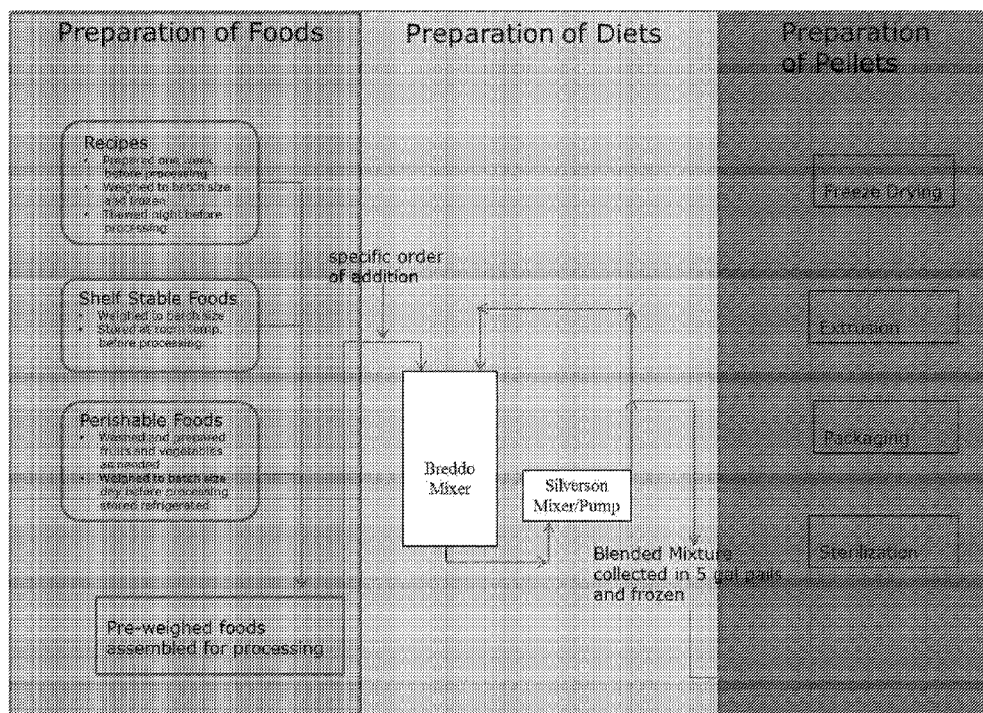




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(57) Abrégé/Abstract:

The present invention provides methods for creating a human diet equivalent for use in animal feed, animal feed compositions comprising a human diet equivalent, and methods for processing human foods into an animal feed ration.

ABSTRACT

The present invention provides methods for creating a human diet equivalent for use in animal feed, animal feed compositions comprising a human diet equivalent, and methods for processing human foods into an animal feed ration.

HUMAN DIET EQUIVALENT ANIMAL FEED

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority of US provisional application number 61/841,786, filed July 1, 2013, and US provisional application number 61/869,047, filed August 22, 2013.

FIELD OF THE INVENTION

[0002] The present invention provides animal feed compositions comprising a human diet equivalent, and methods for processing human foods into an animal feed ration.

BACKGROUND OF THE INVENTION

[0003] When animal models are employed in the study of human disease, they are frequently selected because of their similarity to humans in terms of genetics, anatomy, and physiology. Also, animal models are often preferable for experimental disease research because of their ease of manipulation. For example, to obtain scientifically valid research, the conditions associated with an experiment must be closely controlled. This often means manipulating only one variable while keeping others constant, and then observing the consequences of that change. In addition, to test hypotheses about how a disease develops, an adequate number of subjects must be used to statistically test the results of the experiment. Therefore, scientists cannot conduct research on just one animal or human, and it is easier for scientists to use sufficiently large numbers of animals (rather than people) to attain significant results.

[0004] Diet and nutritional statuses are among the most modifiable determinants of human health. The nutritional value of food is influenced in part by a person's gut microbial community (microbiota) and its component genes (microbiome). Unraveling the interrelations among diet, the structure and operations of the gut microbiota, and nutrient and energy harvest is confounded by variations in human environmental exposures, microbial ecology and genotype. Animal models of the human gut ecosystem have helped to advance our understanding of these complex relationships. Similar advances in animal diets are also needed.

[0005] Historically, studies seeking to understand effects of diet have relied either on traditional animal chow supplemented with one or more nutrients or a cafeteria diet approach (Free Radical Biology and Medicine (2013) 65: 254-261; Physiology and Behavior (1977) 18: 1021-1026; Obesity (2011) 19: 1109-1117)., Both approaches fail to reflect human consumption patterns, nor do they rigorously ensure nutritional adequacy and consistency. Thus, there remains a need in the art for animal feed rations that more closely mirror human food consumption patterns in a format that allows for the animal to get a controlled amount and ratio of foods equivalent to a representative diet of a specific human sub-population.

SUMMARY OF THE INVENTION

[0006] In an aspect, the present disclosure encompasses an animal feed composition. The animal feed composition comprises a human diet equivalent. The human diet equivalent consists of 45- 53% by wet weight carbohydrates, 1- 4% by wet weight total dietary fiber, 20- 25% by wet weight fat, 7- 9% total saturated fatty acids of fat, 83- 88 mg cholesterol/100g total diet (TD), 19- 23% by wet weight protein, 800- 1000 mg sodium/100g TD, 300- 500 mg potassium/100g TD, 4- 8% by wet weight moisture, and 4.6- 4.9 Kcal/g TD. The human diet equivalent is made from a defined ratio of eight food groups. The eight food groups consist of food items identified by the first three or four digits of an eight digit food code as represented in Table A. Milk and milk products have a food code with a first digit of 1 and are 5- 25% by wet weight. Meat, poultry, fish, egg, nuts, seeds, and legumes have a food code with a first digit selected from the group consisting of 2, 3, and 4 and are 10- 25% by wet weight. Grain products have a food code with a first digit of 5 and are 10- 20% by wet weight. Fruits have a food code with a first digit of 6 and are 0- 1% by wet weight. Vegetables have a food code with a first digit of 7 and are 0- 1% by wet weight. Fats and oils have a food code with a first digit of 8 and are 0.5-5.0% by wet weight. Sugars and sweets have a food code with a first digit of 9 and a second digit of 1 and are 0.5-5.0% by wet weight. Beverages have a food code with a first digit of 9 and a second digit of 2 or 3 and are 0.5-5.0% by wet weight.

[0007] In another aspect, the present disclosure encompasses an animal feed composition. The animal feed composition comprises a human diet equivalent. The human diet equivalent consists of 57- 64% by wet weight carbohydrates, 3- 7% by wet weight total dietary fiber, 10- 15% by wet weight fat, 2.0- 3.5% total saturated fatty acids of fat, 35-40 mg cholesterol/100g TD, 13- 17% by wet weight protein, 300- 500 mg sodium/100g TD, 500- 700 mg potassium/100g TD, 4- 8% by wet weight moisture, and 4.0- 4.4 Kcal/g TD. The human diet equivalent is made from a defined ratio of eight food groups. The eight food groups consist of food items identified by the first three or four digits of an eight digit food code as represented in Table A. Milk and milk products have a food code with a first digit of 1 and are 5- 25% by wet weight. Meat, poultry, fish, egg, nuts, seeds, and legumes have a food code with a first digit selected from the group consisting of 2, 3, and 4 and are 10- 25% by wet weight. Grain products have a food code with a first digit of 5 and are 10- 20% by wet weight. Fruits have a food code with a first digit of 6 and are 10- 25% by wet weight fruits. Vegetables have a food code with a first digit of 7 and are 10- 25% by wet weight. Fats and oils have a food code with a first digit of 8 and are 0.5- 5% by wet weight. Sugars and sweets have a food code with a first digit of 9 and a second digit of 1 and are 0.5- 5% by wet weight sugars and sweets. Beverages have a food code with a first digit of 9 and a second digit of 2 or 3 and are 10- 60% by wet weight beverages.

[0008] In yet another aspect, the present disclosure encompasses a process to prepare pelletized animal feed. The process comprises (i) introducing two or more food items independently selected from the group consisting of frozen food items, refrigerated food items, and room temperature food items into a first mixer and blending the food items, wherein the first mixer is a high shear liquid mixer; (ii) passing and/or recirculating the blended food items into a second mixer to form a homogenous composite, wherein the second mixer is a rotor-stator mixer and the homogenized composite is evenly blended by visual inspection; (iii) freeze drying the homogenous composite and milling the dehydrated composite to a 20 mesh size, wherein the moisture content of the dehydrated composite is less than 5%; and (iv) adding water to the milled composite and extruding into pellets, wherein the moisture content of the composite at the end of the extrusion process is less than 10%.

[0009] In still yet another aspect, the present disclosure encompasses a method of determining the effect of at least one dietary supplement on a subject. The method comprises administering or feeding a composition of any of the compositions described herein further comprising at least one dietary supplement to a subject and determining the presence or absence of a difference in the subject after administering the composition comprising the dietary supplement. The presence or absence of a difference in the subject represents the effect of the dietary supplement on the subject.

[0010] In still yet another aspect, the present disclosure encompasses a method of determining the effect of at least one dietary supplement on a subject. The method comprises (1) administering or feeding a first composition to a subject for a first period of time, wherein the first composition is a composition disclosed herein, (2) administering or feeding a second composition to the subject for a second period of time, wherein the second composition consists of the first composition and at least one dietary supplement, and (3) determining the presence or absence of a difference in the subject after administering or feeding the second composition. The presence or absence of a difference in the subject represents the effect of the dietary supplement on the subject.

[0011] In still yet another aspect, the present disclosure encompasses a method of determining the effect of at least one dietary supplement on a subject. The method comprises (1) administering or feeding a first composition to a subject for a first period of time, wherein the first composition is a composition disclosed herein and at least one dietary supplement, (2) administering or feeding a second composition to the subject for a second period of time, wherein the second composition consists of the first composition without the at least one dietary supplement, and (3) determining the presence or absence of a difference in the subject after administering or feeding the second composition. The presence or absence of a difference in the subject represents the effect of the dietary supplement on the subject

BRIEF DESCRIPTION OF THE FIGURES

[0012] The application file contains at least one drawing executed in color. Copies of this patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee

[0013] **FIG. 1** depicts a production process flow diagram.

DETAILED DESCRIPTION OF THE INVENTION

[0014] The present invention encompasses methods for creating a human diet equivalent for use in animal feed, animal feed compositions comprising human diet equivalents, and methods for processing human foods into a homogenous animal feed ration. Critically, the processes of the invention that convert human foods into homogenous animal feed rations provide an even mix of foods in the right quantitative portions while retaining nutrients. This approach also overcomes any taste issues that can be associated with eating particular bitter and sour foods by creating a homogenous composite of foods that is palatable to the animals. As a result, the animal feed compositions accurately model the human diet, deliver known amounts and ratios of nutrients, and can be processed into a structurally stable form. Animal feed compositions differing in the amounts of one or more food items may be used to test the impact of diet on various facets of a mouse model, and are particularly well suited to test how gut microbes adapt to dietary perturbations. Methods for deriving an animal feed composition from a human diet, animal feed compositions comprising human diet equivalents, and processes for producing said compositions are described in further detail below.

I. METHODS FOR CREATING A HUMAN DIET EQUIVALENT FOR USE IN ANIMAL FEED

[0015] The present invention encompasses methods for creating a human diet equivalent for use in an animal feed ration. Generally speaking, a human diet equivalent is constructed from one or more foods that characterize a particular human diet. Stated another way, a human diet equivalent comprises at least one food item typically consumed by a human. The terms “food” and “food items” are defined below. A method for creating a human diet equivalent typically comprises (i) collecting information on what people eat, (ii) analyzing the data in order to identify one or more

subpopulations, (iii) identifying one or more foods that characterize a subpopulation, and (iv) determining the proportion of each characterizing food item to be included in the human diet equivalent.

[0016] In an aspect, the present invention utilizes methods for collecting information on what people eat. Such information is needed in order to determine the foods a person typically eats. Suitable methods for collecting information on what people eat are known in the art, and may include, but are not limited to, 24-hour recalls and food diaries recorded by an individual. A skilled artisan may conduct the food survey or may rely on a previously conducted food survey(s). Such surveys are well known to a skilled artisan and may include, but are not limited to, What We Eat in America (WWEIA), which is the dietary intake interview component of the National Health and Nutrition Examination Survey (NHANES), United States Department of Agriculture (USDA) Nationwide Food Surveys, and World Food Surveys conducted by the Food and Agricultural Organization of the United Nations.

[0017] After data collection, a skilled artisan needs a way to translate the food intake data into a form that can be used for analysis. In some embodiments, the USDA Food and Nutrient Database for Dietary Studies (FNDDS) is used. The USDA FNDDS is a resource that is used to code dietary intakes and to calculate nutrients for WWEIA and other food surveys. The FNDDS is based on nutrient values in the USDA National Nutrient Database for Standard Reference, Release 22 (Agricultural Research Service, Nutrient Data Laboratory, 2009). Other suitable food coding schemes may be used, provided the scheme provides nutrient values and weights for typical food portions and food items.

[0018] The USDA FNDDS provides a unique eight digit food code for specific foods. This food coding scheme provides an outline of the major food groups and subgroups by the first 1 to 4 digits of the food code. The coding scheme is represented in **Table A**. The first digit in the food code identifies one of nine major food groups: (1) milk and milk products, (2) meat, poultry, fish, and mixtures, (3) eggs, (4) legumes, nuts and seeds, (5) grain products, (6) fruits, (7) vegetables, (8) fats, oils and salad dressings, and (9) sugars, sweets and beverages. The second, third and (sometimes) fourth digits of the food code identify increasingly more specific subgroups

within the nine major food groups. Most subgroups are identified by the first three digits, except for some subgroups in the Meat, Poultry, Fish and Mixtures, and Sugar, Sweet and Beverages section. A “food item”, as used herein, refers to a food that is represented by a complete eight-digit FNDDS code or equivalent thereof. The term “food”, as used herein, may refer to a food item, a food subgroup (i.e. the second, third, or fourth digit of an eight digit FNDDS food code, or the equivalent thereof), or a major food group (i.e. the first digit of an eight digit FNDDS food code, or the equivalent thereof). The term “food” includes both foods and beverages. Further details regarding the USDA FNDDS may be found in The USDA Food and Nutrient Database for Dietary Studies, 4.1- Documentation and User Guide (2010. Beltsville, MD: U.S. Department of Agriculture, Agricultural Research Service, Food Surveys Research Group), incorporated herein by reference in its entirety.

[0019] In an aspect, the present invention utilizes an approach for identifying one or more subpopulations within a total population by characterizing one or more diet patterns of the population. A subpopulation may be identified based on an analysis of the consumption of the total daily intakes of foods. For example, a subpopulation may be identified based on an analysis of all foods consumed, an analysis of the amount of the types of foods consumed, an analysis of the calories derived from one or more types of food consumed, or a combination thereof. Other suitable subpopulations for analysis will also be apparent to a skilled artisan and are contemplated by the invention. In some embodiments, a subpopulation may be identified based on an analysis of the consumption of at least one food. In other embodiments, a subpopulation may be identified based on an analysis of the consumption of at least two foods. In still other embodiments, a subpopulation may be identified based on an analysis of the consumption of at least three foods. In yet other embodiments, a subpopulation may be identified based on an analysis of the consumption of at least four foods. In different embodiments, a subpopulation may be identified based on an analysis of the consumption of at least five foods. In each of the above embodiments, the analysis may occur at the major food group level (i.e. the first digit of an eight digit FNDDS food code, or the equivalent thereof), at a food subgroup level (i.e. the second, third, or fourth digit of an eight digit FNDDS food code, or the

equivalent thereof), or at the food item level (i.e. a complete eight-digit FNDDS code, or equivalent thereof).

[0020] Subpopulations may also be identified based on a nutritional analysis of the foods consumed. Suitable nutrients and food associated compounds may include, but are not limited to total fat, saturated fat, monounsaturated fat, polyunsaturated fat, trans saturated fat, cholesterol, carbohydrate, total sugars, dietary fiber, Vitamin A, Vitamin B6, Vitamin B12, Vitamin C, Vitamin D, Vitamin E, Vitamin K1, Thiamin, Riboflavin, Niacin, Folate, Pantothenic acid, Calcium, Phosphorus, Magnesium, Manganese, Iron, Zinc, Copper, Selenium, Sodium, Potassium, Fructose, Sucrose, Glucose, Lactose, Maltose, Fatty acid distribution, amino acid distribution, betacarotene, retinol, alphotocopherol, betatocopherol, gammatocopherol, deltatocopherol, alphotocotrienol, betatocotrienol, gammatocotrienol, deltatocotrienol, apo-8-carotenal, trans-lycopene, cis-lycopene, trans-beta-carotene, cis-beta-carotene, caffeine, and combinations thereof. In some embodiments, a subpopulation may be identified based on an analysis of the consumption of at least one nutrient. In other embodiments, a subpopulation may be identified based on an analysis of the consumption of at least two nutrients. In still other embodiments, a subpopulation may be identified based on an analysis of the consumption of at least three nutrients. In yet other embodiments, a subpopulation may be identified based on an analysis of the consumption of at least four nutrients. In different embodiments, a subpopulation may be identified based on an analysis of the consumption of at least five nutrients.

[0021] In preferred embodiments, a subpopulation may be identified based on saturated fat intake. In other preferred embodiments, a subpopulation may be identified based on total fruit intake. In still other preferred embodiments, a subpopulation may be identified based on total vegetable intake. In yet other preferred embodiments, a subpopulation may be identified based on total fruit and vegetable intake. In yet other preferred embodiments, a subpopulation may be identified based on saturated fat intake and either total vegetable intake or total fruit intake. In yet other preferred embodiments, a subpopulation may be identified based on saturated fat intake and total fruit and vegetable intake.

[0022] Once the basis of each grouping has been determined, as described above, the total sample may be divided into two or more subpopulations. For example, the total sample may be divided into groups of two, groups of three, groups of four, groups of five, groups of six, groups of seven, groups of eight, groups of nine, or groups of ten or more. Alternatively, the total sample may be divided into groups of 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 or more. Groups may or may not be split equally, depending on the type of statistical analysis performed. Suitable methods are well known in the art. The terms “high” and “low” may represent the highest and lowest group in the distribution, or some combination of lowest groups and highest groups in the distribution. In some embodiments, the total sample may be divided into quintiles. In preferred embodiments, the total sample may be divided in tertiles.

[0023] In preferred embodiments, a first subpopulation comprises the highest quintile saturated fat intake and lowest quintile fruit and vegetable intake, and a second subpopulation comprises the lowest quintile saturated fat intake and highest quintile fruit and vegetable intake. In another exemplary embodiment, a first subpopulation consists of the highest quintile saturated fat intake and lowest quintile fruit and vegetable intake, and a second subpopulation consists of the lowest quintile saturated fat intake and highest quintile fruit and vegetable intake. In an exemplary embodiment, the quintile is a tertile.

[0024] In another aspect, the present invention provides methods for identifying foods that characterize the diet of a subpopulation. The method comprises identifying food groups and subgroups that characterize the diet of a subpopulation (i.e. a “key food group” or “key food subgroup”) and then selecting one or more food items that characterize the key food group or key food subgroup.

[0025] Generally speaking, for each subpopulation, key food subgroups may be identified by determining the relative energy intakes by major food group and food subgroup and the proportion of the subpopulation consuming foods within individual food subgroups. Preferably, the following criteria are used to identify key food subgroups: (1) Food subgroups providing less than 20 kcal/1000 kcal total diet (i.e., less than 2.0% of energy) are excluded from consideration; (2) Within each food subgroup providing from 20 to 50 kcal/1000 kcal total diet (i.e., 2.0 to 5.0% of energy), the single

food subgroup representing the largest source of energy is selected as a key food subgroup; (3) Within each food group providing more than 50 kcal/1000 kcal total diet (i.e., over 5.0% of total energy), all food subgroups meeting at least one of the following two criteria were considered key food subgroups (a) Contributing at least 10% of kcal per 1000 kcal for the food group *and* consumed by at least 10% of the subpopulation, or (b) Consumed by 20% or more of the subpopulation. Finally, once each key food group or key food subgroup has been identified, the food code with the greatest weighted proportion of members of the subpopulation reporting consumption on the day of recall is selected to be the key food item characterizing the entire food group or key food subgroup.

[0026] In another aspect, the present invention utilizes methods for determining the proportion of each characterizing food to be included in an animal feed composition. The method comprises determining the relative energy contributions from key food subgroups to the total energy intake from the food group (per 1000 kcal total diet) and then determining the weight of the food item (in g) required to deliver the calculated energy contribution from the relevant food group per 1000 kcal total diet.

II. ANIMAL FEED COMPOSITIONS COMPRISING HUMAN DIET EQUIVALENTS CONSTRUCTED FROM ONE OR MORE FOODS THAT CHARACTERIZE A HISF-LOFV DIET OR LOSF-HIFV DIET

[0027] In another aspect, the present invention encompasses an animal feed composition comprising a human diet equivalent that is constructed from one or more foods that characterize either a high saturated fat, low fruit and vegetable diet (HiSF-LoFV diet) or a low saturated fat, high fruit and vegetable diet (LoSF-HiFV diet). Methods for constructing a human diet equivalent are described above.

[0028] A human diet equivalent that is constructed from one or more foods that characterize either a HiSF-LoFV diet or a LoSF-HiFV diet has the right proportions of nutrients for each particular diet. In some embodiments, a human diet equivalent consists of 45- 53% by wet weight carbohydrates, 1- 10% by wet weight total dietary fiber, 20- 25% by wet weight fat, 7- 9% total saturated fatty acids of fat, 83- 88 mg cholesterol /100g total diet (TD), 19- 23% by wet weight protein, 800- 1000 mg

sodium/100g TD, 300- 500 mg potassium/100g TD, 4- 8% by wet weight moisture, and 4.6- 4.9 Kcal/g TD. In other embodiments, a human diet equivalent consists of 45- 53% by wet weight carbohydrates, 1- 4% by wet weight total dietary fiber, 20- 25% by wet weight fat, 7- 9% total saturated fatty acids of fat, 83- 88 mg cholesterol/100g TD, 19- 23% by wet weight protein, 800- 1000 mg sodium/100g TD, 300- 500 mg potassium/100g TD, 4- 8% by wet weight moisture, and 4.6- 4.9 Kcal/g TD. In still other embodiments, a human diet equivalent consists of 57- 64% by wet weight carbohydrates, 1- 10% by wet weight total dietary fiber, 10- 15% by wet weight fat, 2.0- 3.5% total saturated fatty acids of fat, 35-40 mg cholesterol/100g TD, 13- 17% by wet weight protein, 300- 500 mg sodium/100g TD, 500- 700 mg potassium/100g TD, 4- 8% by weight moisture, and 4.0- 4.4 Kcal/g TD. In yet other embodiments, a human diet equivalent consists of 57- 64% by wet weight carbohydrates, 3- 7% by wet weight total dietary fiber, 10- 15% by wet weight fat, 2.0- 3.5% total saturated fatty acids of fat, 35- 40 mg cholesterol/100g TD, 13- 17% by wet weight protein, 300- 500 mg sodium/100g TD, 500- 700 mg potassium/100g TD, 4- 8% by wet weight moisture, and 4.0- 4.4 Kcal/g TD.

[0029] Preferably, a human diet equivalent of the invention is made from a defined ratio of eight food groups selected from the groups consisting of (i) milk and milk products, (ii) meat, poultry, fish, egg, nuts seeds and legumes, (iii) grain products, (iv) fruits, (v) vegetables, (vi) fats and oils, (vii) sugars and sweets, and (viii) beverages. In some embodiments, the eight food groups consist of food items identified by the first three or four digits of an eight digit food code as represented in **Table A**, wherein (i) milk and milk products have a food code with a first digit of 1, (ii) meat, poultry, fish, egg, nuts seeds and legumes have a food code with a first digit selected from the group consisting of 2, 3, and 4, (iii) grain products have a food code with a first digit of 5, (iv) fruits have a food code with a first digit of 6, (v) vegetables have a food code with a first digit of 7, (vi) fats and oils have a food code with a first digit of 8, (vi) sugars and sweets have a first food code with a first digit of 9 and a second digit of 1, and (h) beverages have a food code with a first digit of 9 and a second digit of 2 or 3. In other embodiments the eight food groups consist of food items defined by a different food scheme.

[0030] Constructing a human diet equivalent from a defined ratio of the eight food groups insures the nutrients present in the animal feed mirror the proportion of nutrients in the equivalent human diet. In embodiments where a human diet equivalent is constructed from one or more foods that characterize a HiSF-LoFV diet, the ratio of eight food groups may be about 5- 25% by wet weight milk and milk products, about 10- 25% by wet weight meat, poultry, fish, egg, nuts, seeds, and legumes, about 10- 20% by wet weight grain products, about 0- 1% by wet weight fruits, about 0- 1% by wet weight vegetables, about 0.5-5.0% by wet weight fats and oils, about 0.5- 5.0% by wet weight sugars and sweets, and about 10- 60% by wet weight beverages. In a preferred embodiment, the ratio of eight food groups may be about 18.9% by wet weight milk and milk products, (b) about 18.0% by wet weight meat, poultry, fish, egg, nuts, seeds, and legumes, (c) about 16.3% by wet weight grain products, (d) about 0.0% by wet weight fruits, (e) about 0.8% by wet weight vegetables, (f) about 0.6% by wet weight fats and oils, (g) about 1.2% by wet weight sugars and sweets, and (h) about 44.2% by wet weight beverages.

[0031] In embodiments where a human diet equivalent is constructed from one or more foods that characterize a LoSF-HiFV diet, the ratio of eight food groups may be about 5- 25% by wet weight milk and milk products, (b) about 10- 25% by wet weight meat, poultry, fish, egg, nuts, seeds, and legumes, (c) 1 about 0- 20% by wet weight grain products, (d) about 10- 25% by wet weight fruits, (e) 1 about 0- 25% by wet weight vegetables, (f) about 0.5- 5% by wet weight fats and oils, (g) about 0.5- 5% by wet weight sugars and sweets, and (h) about 10- 60% by wet weight beverages. In a preferred embodiment, the ratio of eight food groups may be about 10.2% by wet weight milk and milk products, (b) about 15.7% by wet weight meat, poultry, fish, egg, nuts, seeds, and legumes, (c) about 12.7% by wet weight grain products, (d) about 19.5% by wet weight fruits, (e) about 18% by wet weight vegetables, (f) about 0.9% by wet weight fats and oils, (g) about 0.6% by wet weight sugars and sweets, and (h) about 22.2% by wet weight beverages.

[0032] The number of food items that comprise a human diet equivalent can vary provided that the human diet equivalent has the right proportion of nutrients and/or the correct ratio of eight food groups. Generally speaking, a human diet

equivalent is comprised of at least 6 food items. For example, a human diet equivalent may be comprised of at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 food items.

[0033] In a preferred embodiment, an animal feed composition comprises a human diet equivalent, the human diet equivalent consisting of 45- 53% by wet weight carbohydrates, 1- 4% by wet weight total dietary fiber, 20- 25% by wet weight fat, 7- 9% total saturated fatty acids of fat, 83- 88 mg cholesterol/100g TD, 19- 23% by wet weight protein, 800- 1000 mg sodium/100g TD, 300- 500 mg potassium/100g TD, 4- 8% by wet weight moisture, and 4.6- 4.9 Kcal/g TD; wherein the human diet equivalent is made from a defined ratio of eight food groups, the eight food groups consisting of food items identified by the first three or four digits of an eight digit food code as represented in **Table A**, wherein (a) milk and milk products have a food code with a first digit of 1 and are 5- 25% by wet weight; (b) meat, poultry, fish, egg, nuts, seeds, and legumes have a food code with a first digit selected from the group consisting of 2, 3, and 4 and are 10- 25% by wet weight; (c) grain products have a food code with a first digit of 5 and are 10- 20% by wet weight; (d) fruits have a food code with a first digit of 6 and are 0- 1% by wet weight; (e) vegetables have a food code with a first digit of 7 and are 0- 1% by wet weight; (f) fats and oils have a food code with a first digit of 8 and are 0.5-5.0% by wet weight; (g) sugars and sweets have a food code with a first digit of 9 and a second digit of 1 and are 0.5-5.0% by wet weight; and (h) beverages have a food code with a first digit of 9 and a second digit of 2 or 3 and are 0.5-5.0% by wet weight.

[0034] In another preferred embodiment, an animal feed composition comprises a human diet equivalent, the human diet equivalent consisting of 57- 64% by wet weight carbohydrates, 3- 7% by wet weight total dietary fiber, 10- 15% by wet weight fat, 2.0- 3.5% total saturated fatty acids of fat, 35-40 mg cholesterol/100g TD, 13- 17% by wet weight protein, 300- 500 mg sodium/100g TD, 500- 700 mg potassium/100g TD, 4- 8% by wet weight moisture, and 4.0- 4.4 Kcal/g TD; wherein the human diet equivalent is made from a defined ratio of eight food groups, the eight food groups consisting of food items identified by the first three or four digits of an eight digit

food code as represented in Table A, wherein (a) milk and milk products have a food code with a first digit of 1 and are 5- 25% by wet weight, (b) meat, poultry, fish, egg, nuts, seeds, and legumes have a food code with a first digit selected from the group consisting of 2, 3, and 4 and are 10- 25% by wet weight, (c) grain products have a food code with a first digit of 5 and are 10- 20% by wet weight, (d) fruits have a food code with a first digit of 6 and are 10- 25% by wet weight fruits, (e) vegetables have a food code with a first digit of 7 and are 10- 25% by wet weight, (f) fats and oils have a food code with a first digit of 8 and are 0.5- 5% by wet weight, (g) sugars and sweets have a food code with a first digit of 9 and a second digit of 1 and are 0.5- 5% by wet weight sugars and sweets, and (h) beverages have a food code with a first digit of 9 and a second digit of 2 or 3 and are 10- 60% by wet weight beverages.

[0035] In an embodiment, the composition may further comprise a dietary supplement. As used herein, a “dietary supplement” is any component added to the compositions described herein. A dietary supplement may be an amount of a food or food item, as defined herein. Non-limiting examples of a dietary supplement may be milk or milk product, meat, poultry, fish, egg, nuts, seeds, legumes, grains, fruits, vegetables, fats, oils, sugars, sweets, beverages, vitamins, minerals, herbs, botanicals, and amino acids. For example, a dietary supplement may be acai, aloe vera, anabolic steroids, astragalus, bilberry, bitter orange, black cohosh, butterbur, calcium, carnitine, cartilage (bovine and shark), cat's claw, chamomile, chasteberry, chondroitin, chromium, cinnamon, coenzyme Q10, colloidal silver, cranberry, vitamin C, candelion, echinacea, ephedra, essiac/lor-essence, European elder, evening primrose oil, fenugreek, feverfew, fish oil, flaxseed, folate, garlic, ginger, ginkgo, ginseng, glucosamine, goldenseal, grape seed extract, green tea, hawthorn, hoodia, horse chestnut, iodine, iron, kava, lavender, licorice root, magnesium, melatonin, milk thistle, mistletoe, noni, omega-3 fatty acids, PC-SPES, peppermint oil, red clover, sage, SAME (S-adenosyl-L-methionine), saw palmetto, selenium, soy, St. John's wort, tea, thunder God vine, turmeric, valerian, vitamin A, vitamin B12, vitamin B6, vitamin C, vitamin D, vitamin E, vitamin K, yohimbe, and zinc. Additionally, a dietary supplement may be derived from a food or food item. For example, a dietary supplement may be derived from cereal grains, legumes, nuts, seeds, fruits and/or vegetables. A dietary supplement

may be high in non-digestible carbohydrates and antioxidants. Suitable examples of a dietary supplement high in non-digestible carbohydrates and antioxidants may include agave, chicory, banana, barley, buckwheat, canary seed, chia, cocoa, coffee, corn, fonio, grape, guar, job tears, millet, millet seed, oat, quinoa, rice, rye, sorghum, soy, spelt, teff, tigernut, triticale, wheat bran, acacia and citrus fruits. A dietary supplement may be about 0-10% by wet weight of the compositions described above. For example, a dietary supplement may be about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1% wet weight of the compositions described above. Alternatively, a dietary supplement may be about 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10% wet weight of the compositions described above. For a dietary supplement that is a milk or milk product, meat, poultry, fish, egg, nuts, seed, legume, grain, fruit, vegetable, fat, oil, sugar, sweet, and/or beverage, the dietary supplement may be about 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10% wet weight of the compositions described above. For a dietary supplement that is a vitamin, mineral, herb, botanical, and/or amino acid, the dietary supplement may be about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1% wet weight of the compositions described above.

[0036] In each of the embodiments above, a composition of the invention may be processed into a form for animal consumption. In preferred embodiments, compositions of the invention are formulated as chow for a laboratory animal. Non-limiting examples of a laboratory animal may include rodents, canines, felines, and non-human primates. In some embodiments, the animal is a mouse. In other embodiments, the animal is a rat. In still other embodiments, the animal is a guinea pig. In yet other embodiments, the animal is a hamster. Methods for processing human foods into an animal feed ration are described below in Section III and further detailed in the Examples.

III. PROCESSES FOR PRODUCING AN ANIMAL FEED COMPOSITION COMPRISING A HUMAN DIET EQUIVALENT

[0037] In another aspect, the present invention encompasses processes for producing an animal feed composition comprising a human diet equivalent. Suitable human diet equivalents and animal feed compositions are described above in Section I and II. The process typically comprises (i) introducing one or more food items selected

from the group consisting of a frozen food item, a refrigerated food item, a room temperature food item, and a combination thereof into a first mixer and blending the food items, (ii) passing the blended food items into a second mixer to form a homogenous composite, (iii) dehydrating the homogenous composite and milling the dehydrated composite to a 20 mesh size, and (iv) processing the milled composite into its final form. Step (ii) can be optionally incorporated into step (i) through a recirculation loop that is connected to the vessel carrying out the first mixing step. In certain embodiments, the composition in its final form may then be packaged and/or sterilized by gamma irradiation. Sterilization by gamma irradiation may occur before or after packaging, or a combination thereof.

[0038] A process of the invention generally starts with frozen, refrigerated and/or room temperature food items. Generally, the food items will characterize a diet of a subpopulation and, in total, will provide the right proportions of nutrients for each particular diet. The food items, or the ingredients to make a food item, may be purchased from a grocery store, a farmers market or any other vendor that sells food items or the ingredients to make them. A food item may be used as is or may be prepared from ingredients. Typical preparation methods may include, but are not limited to, peeling, coring, cutting, chopping, dicing, grating, shredding, mincing, de-seeding, kneading, milling, mixing, blending, thawing, boiling, blanching, braising, coddling, infusing, simmering, steaming, steeping, stewing, grilling, broiling, frying, sautéing, microwaving, roasting, searing, pickling, salting, seasoning, marinating, brining, and drying. Recipes for preparing food items that require preparation are known in the art. Food items that require preparation may be prepared as needed, or may be made days, weeks or months in advance, optionally weighed into batches, and frozen until needed.

[0039] Steps (i) and (ii) of the process of the invention comprise intimate mixing. Intimate mixing, as used herein, refers to high shear mixing, homogenization, such as through a homogenizer, sonification, or through ultrasonification. Intimate mixing disperses food items (present as liquid, solid and/or gas) into a main continuous liquid phase, with which it would normally be immiscible. A rotor or impellor, alone or together with a stationary component known as a stator, or an array of rotors and stators, is used either in a tank containing the solution to be mixed, or in a pipe through

which the solution passes, to create shear. The end result of steps (i) and (ii) is a homogeneous composite mixture. As used herein, the term “homogenous composite mixture” or “homogenous composite” refers to a mixture that is an emulsion, a suspension, or an emulsion and a suspension. Intimate mixing may or may not occur in combination with other kinds of mixing, for example, static mixing. Non-limiting examples of intimate mixers includes a rotor-stator type homogenizer or mixer, a high pressure homogenizer, a high shear granulator, a batch high-shear mixer, and an impeller mixer (including both an axial flow impeller and a radial flow impeller). In some embodiments, mixing is provided by a high shear mixer. In other embodiments, mixing is provided by a rotor-stator mixer. In still other embodiments, mixing is provided by a homogenizer. In alternative embodiments, mixing is provided by more than one mixer. In preferred embodiments, mixing is provided by a first mixer and a second mixer, wherein the first mixer is a high shear mixer and the second mixer is a rotor-stator type mixer. In an exemplary embodiment, a first mixer is a Breddo mixer and a second mixer is a Silverson Mixer/Pump.

[0040] In some embodiments, a process of the invention comprises introducing one or more food items selected from the group consisting of a frozen food item, a refrigerated food item, a room temperature food item, or a combination thereof into a first mixer. For example, a process of the invention may comprise introducing 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 or more food items into a first mixer. In embodiments where more than one food item is added to a first mixer, each food item is independently selected from the group consisting of a frozen food item, a refrigerated food item, and a room temperature food item.

[0041] Food items may or may not be added to a first mixer in a particular order. In some embodiments, food items are added to a first mixer in a particular order. In other embodiments, food items are added to a first mixer in a random order. A suitable order for the addition of food items to a first blender for a HiSF-LoFV and LoSF-HiFV diet is provided in the Examples. Those skilled in the art should, however, in light of the present disclosure, appreciate that changes can be made to the order disclosed

and still obtain a like or similar result without departing from the spirit and scope of the invention.

[0042] In a preferred embodiment, a first mixer is a high shear mixer. For example, a first mixer may be an impeller mixer. The type of impeller can vary, provided the impeller type produces sufficient shear for the blending process to achieve a homogenous emulsion and/or suspension. The size of the impeller can and will vary depending on other parameters. In some embodiments, the impeller diameter is about 5 to about 36 inches. For example, the impeller diameter may be about 5 inches, about 6 inches, about 7 inches, about 8 inches, about 9 inches, about 10 inches, about 11 inches, about 12 inches, about 14 inches, about 15 inches, about 16 inches, about 17 inches, about 18 inches, about 19 inches, about 20 inches, about 21 inches, about 22 inches, about 23 inches, about 24 inches, about 25 inches, about 26 inches, about 27 inches, about 28 inches, about 29 inches, about 30 inches, about 31 inches, about 32 inches, about 33 inches, about 34 inches, about 35 inches, or about 36 inches. In other embodiments, the impeller diameter is about 5 to about 10 inches. In still other embodiments, the impeller diameter is about 10 to about 15 inches. In different embodiments, the impeller diameter is about 15 to about 20 inches. In still different embodiments, the impeller diameter is about 20 to about 25 inches. In alternative embodiments, the impeller diameter is about 35 to about 30 inches. In additional embodiments, the impeller diameter is about 30 to about 36 inches. The rotations per minutes (rpm) of the impeller can vary in each of the above embodiments. In some embodiments, the speed ranges from about 1500 rpm to about 3500 rpm. In certain embodiments the speed is 1500 rpm, 1600 rpm, 1700 rpm, 1800 rpm, 1900 rpm, 2000 rpm, 2100 rpm, 2200 rpm, 2300 rpm, 2400 rpm, 2500 rpm, 2600 rpm, 2700 rpm, 2800 rpm, 2900 rpm, 3000 rpm, 3100 rpm, 3200 rpm, 3300 rpm, 3400 rpm, or 3500 rpm. In preferred embodiments, the speed does not exceed 2000 rpm.

[0043] To facilitate blending, food items added to a first mixer may be optionally recirculated or passed through a second mixer until evenly blended by visual inspection. In a preferred embodiment, the second mixer is a rotor-stator type mixer. The size of the rotor can and will vary depending on other parameters. In some embodiments, the rotor diameter is about 1 to about 24 inches. For example, the rotor

diameter may be about 1 inch, about 2 inches, about 3 inches, about 4 inches, about 5 inches, about 6 inches, about 7 inches, about 8 inches, about 9 inches, about 10 inches, about 11 inches, about 12 inches, about 14 inches, about 15 inches, about 16 inches, about 17 inches, about 18 inches, about 19 inches, about 20 inches, about 21 inches, about 22 inches, about 23 inches, or about 24 inches. In other embodiments, the rotor diameter is about 1 to about 5 inches. In still other embodiments, the rotor diameter is about 5 to about 10 inches. In yet other embodiments, the rotor diameter is about 10 to about 15 inches. In different embodiments, the rotor diameter is about 15 to about 20 inches. In still different embodiments, the rotor diameter is about 20 to about 24 inches. The rotations per minutes (rpm) of the rotor can vary in each of the above embodiments. In some embodiments, the speed ranges from about 1500 rpm to about 3500 rpm. In certain embodiments the speed is 1500 rpm, 1600 rpm, 1700 rpm, 1800 rpm, 1900 rpm, 2000 rpm, 2100 rpm, 2200 rpm, 2300 rpm, 2400 rpm, 2500 rpm, 2600 rpm, 2700 rpm, 2800 rpm, 2900 rpm, 3000 rpm, 3100 rpm, 3200 rpm, 3300 rpm, 3400 rpm, or 3500 rpm. In preferred embodiments, the speed does not exceed 2000 rpm.

[0044] In a preferred embodiment, the first mixer is attached through a recirculating loop to the second mixer that is in-line. The recirculating loop may be chosen from any suitable material including, but not limited to, tubing or piping. The second mixer may be optionally employed when, following blending with the first mixer, the mixture comprises a visible distribution of particle sizes in order to create an even particle size distribution.

[0045] Temperature may also be controlled during mixing. The temperature of the mixture is generally maintained as cool as possible while maintaining the mixture in a liquid phase. Preferably, the maximum temperature during steps (i) and (ii) is about 60°F.

[0046] The residence time from the introduction of the food items to the end of step (ii) may range from about 10 minutes to about 120 minutes, and is dependent, on multiple factors related to process design. In some embodiments, residence time is about 10 minutes, about 15 minutes, about 20 minutes, about 25 minutes, about 30 minutes, about 35 minutes, about 40 minutes, about 45 minutes, about 50 minutes, about 55 minutes, about 60 minutes, about 65 minutes, about 70

minutes, about 75 minutes, about 80 minutes, about 85 minutes, about 90 minutes, about 95 minutes, about 100 minutes, about 105 minutes, about 110 minutes, about 115 minutes, or about 120 minutes. In other embodiments, residence time is about 10 to about 40 minutes. In still other embodiments, residence time is about 20 to about 50 minutes. In still other embodiments, residence time is about 30 to about 60 minutes. In still other embodiments, residence time is about 40 to about 70 minutes. In still other embodiments, residence time is about 50 to about 80 minutes. In still other embodiments, residence time is about 60 to about 90 minutes. In still other embodiments, residence time is about 70 to about 100 minutes. In still other embodiments, residence time is about 80 to about 110 minutes. In still other embodiments, residence time is about 90 to about 120 minutes. In a preferred embodiment, residence time does not exceed 2 hours.

[0047] A homogenous composite produced by the process of the invention is collected and dehydrated. In certain embodiments, the homogenous composite may be optionally frozen and/or stored prior to dehydration. Suitable dehydration processes are known in the art. In some embodiments, the homogenous composite is freeze dried. Briefly, a homogenous composite is frozen in an appropriate container, finely shredded and placed in a vacuum chamber until available moisture was reduced. The moisture content of the freeze dried composite is generally reduced to less than 10%. For example, the moisture content of the freeze dried composite may be reduced to less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, or less than 1%. In some embodiments, the moisture content of the freeze dried composite is about 1% to about 5%. In other embodiments, the moisture content of the freeze dried composite is about 5% to about 10%. In different embodiments, the moisture content of the freeze dried composite is about 1% to about 3%. In still different embodiments, the moisture content of the freeze dried composite is about 3% to about 6%. In alternative embodiments, the moisture content of the freeze dried composite is about 6% to about 9%.

[0048] The temperature and pressure of freeze drying can and will vary in order to achieve the described moisture content. In some embodiments, the temperature is about -20°C to about -60°C. For example, the temperature may be about

-20°C, about -21°C, about -22°C, about -23°C, about -24°C, about -25°C, about -26°C, about -27°C, about -28°C, about -29°C, about -30°C, about -31°C, about -32°C, about -33°C, about -34°C, about -35°C, about -36°C, about -37°C, about -38°C, about -39°C, about -40°C, about -41°C, about -42°C, about -44°C, about -44°C, about -45°C, about -46°C, about -47°C, about -48°C, about -49°C, about -50°C, about -51°C, about -52°C, about -53°C, about -54°C, about -55°C, about -56°C, about -57°C, about -58°C, about -59°C, or about -60°C. In some embodiments, the pressure of the vacuum chamber is about 0.001 mbar to about 30 mbar. For example, the pressure of the vacuum chamber may be about 0.001 mbar to about 0.010 mbar, about 0.010 mbar to about 0.100 mbar, about 0.100 mbar to about 1.000 mbar, about 1.000 mbar to about 10.000 mbar, or about 10.000 mbar to about 30.000 mbar. In another example, the pressure of the vacuum chamber may be about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30 mbar.

[0049] Dehydrated composite may then be milled to a suitable size for processing to a final form. The type of 'grind' (i.e. coarse, medium or fine) plays an important part in press output and quality of final form. Very coarse grinds have the added disadvantage of providing breaking points in the cubes or pellets. Medium and fine grinds generally will result in higher pelleting capacity than a coarse grind. In some embodiments, dehydrated composite is ground to a uniform size. In other embodiments, dehydrated composite is ground to a mixture of grinds of differing fineness. Dehydrated composite of the invention is generally ground to a mesh size of about 5 to about 100. In some embodiments, dehydrated composite is milled to about a 5 mesh size to about a 20 mesh size. In other embodiments, dehydrated composite is milled to about a 15 mesh size to about a 30 mesh size. In still other embodiments, dehydrated composite is milled to about a 25 mesh size to about a 40 mesh size. In yet other embodiments, dehydrated composite is milled to about a 35 mesh size to about a 50 mesh size. In still other embodiments, dehydrated composite is milled to about a 45 mesh size to about a 60 mesh size. In still other embodiments, dehydrated composite is milled to about a 55 mesh size to about a 70 mesh size. In still other embodiments, dehydrated composite is

milled to about a 65 mesh size to about a 80 mesh size. In still other embodiments, dehydrated composite is milled to about a 75 mesh size to about a 90 mesh size. In still other embodiments, dehydrated composite is milled to about a 85 mesh size to about a 100 mesh size. In different embodiments, dehydrated composite is milled to at least a 40 mesh size. In alternative embodiments, dehydrated composite is milled to at least a 30 mesh size. In a preferred embodiment, dehydrated composite is milled to at least a 20 mesh size. In another preferred embodiment, dehydrated composite is milled to a 20 mesh size. Methods for milling are well known in the art.

[0050] The milled composite is then processed into a final form. In some embodiments, the milled composite is processed into a liquid. In other embodiments, the milled composite is processed into a dried form. Non-limiting examples of suitable dried forms include powders, agglomerates, granules and pellets. In a preferred embodiment, the milled composite is processed to a granule. In another preferred embodiment, the milled composite is processed to a pellet. When processed into a dried form, moisture may be added to the milled composite to facilitate processing. Generally, the moisture content of the final form is generally less than 10%. For example, the moisture content of the may be less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, or less than 1%. In some embodiments, the moisture content of the freeze dried composite is about 1% to about 5%. In other embodiments, the moisture content of the freeze dried composite is about 5% to about 10%. In different embodiments, the moisture content of the freeze dried composite is about 1% to about 3%. In still different embodiments, the moisture content of the freeze dried composite is about 3% to about 6%. In alternative embodiments, the moisture content of the freeze dried composite is about 6% to about 9%. In a preferred embodiment, the milled composite is processed to a pellet and the moisture content at the end of the extrusion process is less than 7%. Methods for producing animal feed ration into powder, granules and pellets are known in the art.

[0051] In some embodiments, a dietary supplement may be added during the processing of the composition. In an embodiment, a dietary supplement may be added during steps (i) or (ii). In another embodiment, a dietary supplement may be added after step (iii) prior to step (iv). For example, a dietary supplement may be added

to a milled composite prior to processing into a final form. In an embodiment where a dietary supplement is added to a milled composite, the dietary supplement may be dehydrated. The dehydration of the dietary supplement may enable homogenous distribution of the dietary supplement and the milled composite. In a preferred embodiment, a dietary supplement may be added to a milled composite that has been processed into a dried form. In other preferred embodiments, a dietary supplement may be added to a milled composite that has been processed into a powder. The milled composite comprising the dietary supplement may then be processed to a pellet. In an embodiment where a dietary supplement is added to a milled composite that has been processed into a powder, the dietary supplement may be dehydrated and of an equivalent particle size to the particles of the powder. The milled composite comprising the dietary supplement may then be processed to a pellet.

IV. METHODS

[0052] In another aspect, the invention encompasses a method to determine the effect of at least one dietary supplement on a subject. More specifically, the invention encompasses a method to determine the effect of at least one dietary supplement on a gut microbiota of a subject. The method generally comprises evaluating the subject before and after consumption of at least one dietary supplement, wherein the presence or absence of a difference in the subject represents the effect of the dietary supplement on the subject. More specifically, the method generally comprises evaluating the gut microbiota of a subject before and after consumption of at least one dietary supplement, wherein the presence or absence of a difference in the gut microbiota represents the effect of the dietary supplement on the gut microbiota of the subject.

[0053] For example, the method may comprise administering or feeding a composition of the invention comprising at least one dietary supplement to a subject and determining the presence or absence of a difference in the subject after administering or feeding the composition comprising the dietary supplement(s), wherein the presence or absence of a difference in the subject represents the effect of the dietary supplement(s) on the subject. Alternatively, the method may comprise (1)

administering or feeding a first composition of the invention to a subject for a first period of time, (2) administering or feeding a second composition to the subject for a second period of time, wherein the second composition consists of the first composition and at least one dietary supplement, and (3) determining the presence or absence of a difference in the subject after administering or feeding the second composition, wherein the presence or absence of a difference in the subject represents the effect of the dietary supplement on the subject. In another alternative, the method may comprise (1) administering or feeding a first composition to a subject for a first period of time, wherein the first composition is a composition of the invention further comprising at least one dietary supplement, (2) administering or feeding a second composition to the subject for a second period of time, wherein the second composition consists of the first composition without the at least one dietary supplement, and (3) determining the presence or absence of a difference in the subject after administering or feeding the second composition, wherein the presence or absence of a difference in the subject represents the effect of the dietary supplement on the subject. As a non-limiting example, the method may comprise comparing the gut microbiota of the subject before and after administration or feeding of the two compositions to determining the presence or absence of a difference, wherein the presence or absence of a difference represents the effect of the dietary supplement(s) on the subject.

[0054] Alternatively, the method may comprise (1) administering or feeding to a first subject or first group of subjects a composition comprising at least one dietary supplement and administering or feeding to a second subject or second group of subjects the same composition without said dietary supplement(s), (2) comparing the first subject or groups of subjects to the second subject or group of subjects, and (3) determining the presence or absence of a difference between the first and second subjects or group of subjects, wherein the presence or absence of a difference represents the effect of the dietary supplement(s) on the subject or group of subjects. As a non-limiting example, the method may comprise comparing the gut microbiota of the first subject or groups of subjects to the second subject or group of subjects and determining the presence or absence of a difference between the first and second

subjects or group of subjects, wherein the presence or absence of a difference represents the effect of the dietary supplement(s) on the subject.

[0055] The effect of one dietary supplement, or a combination of more than one dietary supplement (e.g. 2, 3, 4, 5, 6, 7, 8, 9, 10 or more supplements), on a subject may be determined by quantifying any measurable change in a subject. Without wishing to be bound by theory, the effect of one or more dietary supplements on a subject may be determined by measuring a change in gut microbiota, a change in weight, a change in body fat, a change in energy consumption, a change in health, a change in inflammation, a change in immune function, a change in metabolism, or a combination thereof. Non-limiting examples of indicators of health may include low birth weight, obesity, arthritis, diabetes, asthma, allergies, high blood pressure, autoimmune disease, heart disease, respiratory disease, cancer incidence, disease resistance, disease susceptibility, pathogen shedding, chronic pain, depression, hospital visits due to injury, foodborne illnesses, mood, mental health, smoking habits, alcohol consumption, physical exercise habits, and breastfeeding. Methods of measuring indicators of health are known in the art. Further, methods of measuring energy consumption, weight, body fat, inflammation, immune function and metabolism are known in the art. Inflammation or an inflammatory response is achieved by increased movement of plasma and leukocytes from the blood to the region of interest. Inflammation may be acute or chronic. Signs of acute inflammation may include, but are not limited to, pain, heat, redness, swelling, and loss of function. Signs of chronic inflammation may include, but are not limited to, allergies, hay fever, asthma, periodontitis, atherosclerosis, rheumatoid arthritis and cancer. As such, a change in inflammation may be a change in one or more signs of acute inflammation or a change in one or more signs of chronic inflammation. Alternatively, inflammation may be measured by erythrocyte sedimentation rate or C-reactive protein. Other methods of measuring inflammation are known in the art and may also be used. Immune function refers to the functioning of the immune system. The immune system protects against disease. Disorders of the immune system may result in autoimmune disease, inflammatory diseases and cancer. As such, a change in immune function may be measured as a change in susceptibility to disease or a change in the signs or symptoms

associated with disorders of the immune system. Alternatively, immune function may be measure by quantitation of cell-mediated immunity. Other methods of measuring immune function are known in the art and may also be used. Metabolism is enzyme-catalyzed reactions that allow organisms to grow and reproduce, maintain their structures, and respond to their environments. Metabolism may include digestion and the transport of substances into and between different cells. Metabolism may be measured as a metabolic rate. Metabolic rate is the rate of energy expenditure by a subject. As such, a change in metabolism may be measured by a change in digestion, a change in metabolic rate, a change in energy consumption, or a change in energy expenditure. Alternatively, other methods of measuring metabolism are known in the art and may be used.

[0056] The effect of a dietary supplement on a gut microbiota may be measured as change in relative and/or absolute abundance of microbes at any taxonomic level, a change in phylotypic composition, a change in expression of nucleic acids or nucleic acid products, or a change in metabolite profile. Methods to determine the relative or absolute abundance of microbes are known in the art and may include culturing or sequencing. The term “phylotypic composition,” as used herein, refers to the composition of a gut microbiota as defined by phylotypes. A phylotype is a biological type that classifies an organism by its phylogenetic, e.g. evolutionary, relationship to other organisms. The term phylotype is taxon-neutral, and therefore, may refer to the species composition, genus composition, class composition, etc. Methods to determine expression of nucleic acids are known in the art and may include northern blot, PCR, RT-PCR, qRT-PCR, array, or microarray. Methods to determine expression of nucleic acid products are known in the art and may include western blot, array, microarray, antibody- or epitope binding agent-based methods, mass spectrometry, or peptide sequencing. Methods of measuring a change in metabolite profile are also known in the art and are common in the field of metabolomics.

[0057] As used herein, “change” means more than about 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1% difference between the subject before and after consumption of the dietary supplement. Alternatively, “change” means more than about 30, 29, 28, 27, 26, 25, 24,

23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1% difference between the subject consuming the dietary supplement compared to the subject not consuming the dietary supplement. A change may be positive or negative (e.g. increase or decrease).

[0058] In the embodiments above, a subject may be a laboratory animal. Non-limiting examples of a laboratory animal may include rodents, canines, felines, and non-human primates. In some embodiments, the animal is a mouse. In other embodiments, the animal is a rat. In still other embodiments, the animal is a guinea pig. In yet other embodiments, the animal is a hamster. The effect of the dietary supplement on a subject may be used as a surrogate to determine the effect of a dietary supplement on a human.

TABLE A. USDA Food and Nutrient Database for Dietary Studies Food Coding Scheme (The USDA Food and Nutrient Database for Dietary Studies, 4.1-Documentation and User Guide (2010) Beltsville, MD: U.S. Department of Agriculture, Agricultural Research Service, Food Surveys Research Group)

DESCRIPTION	FOOD CODE (1 st four digits)
Milk and Milk Products	1XXX
Milks and milk drinks	11XX
Milk, human	110X
Milk, fluid (regular; filled; buttermilk; and dry reconstituted)	111X
Milk, fluid, evaporated and condensed	112X
Milk, fluid, imitation	113X
Yogurt	114X
Yogurt, baby food	1148
Flavored milk and milk drinks, fluid	115X
Milk-based meal replacements, fluid	116X
Infant formulas, fluid, reconstituted concentrate, reconstituted dry, and ready-to-feed (milk-based formulas; soy-based formulas; therapeutic formulas)	117X
Milk, dry, and powdered mixtures with dry milk, not reconstituted	118X
Creams and cream substitutes	12XX
Sweet dairy cream	121X
Cream substitutes	122X
Sour cream	123X
Milk desserts, sauces, gravies	13XX

Milk desserts, frozen	131X
Puddings, custards, and other milk desserts	132X
Milk desserts baby food	133X
White sauces and milk gravies	134X
Cheeses	14XX
Cheese, NS6 as to type	140X
Natural cheeses	141X
Cottage cheeses	142X
Cream cheeses	143X
Processed cheeses and cheese spreads	144X
Imitation cheeses	145X
Cheese mixtures	146X
Cheese soups	147X
Meat, Poultry, Fish, and Mixtures	2XXX
Meat, NS as to type	20XX
Meat, NS as to type	200X
Beef	21XX
Beef, NFS7	210X
Beef steak	211X
Beef oxtails, neckbones, short ribs, head	213X
Beef roasts, stew meat, corned beef, beef brisket, sandwich steaks	214X
Ground beef, beef patties, beef meatballs	215X
Other beef items (beef bacon; dried beef; pastrami)	216X
Beef baby food	217X
Pork	22XX
Pork, NFS; ground, dehydrated	220X
Pork chops	221X
Pork steaks, cutlets	222X
Ham	223X
Pork roasts	224X
Canadian bacon	225X
Bacon, salt pork	226X
Other pork items (spareribs; cracklings; skin; miscellaneous parts)	227X
Pork baby food	228X
Lamb, veal, game, other carcass meat	23XX
Lamb, NFS	230X
Lamb and goat	231X
Veal	232X
Game	233X
Lamb or veal baby food	234X
Poultry	24XX
Chicken (breast; leg; drumstick; wing; back; neck or ribs; misc,)	241X
Turkey	242X
Duck	243X

Other poultry	244X
Poultry baby food	247X
Organ meats, sausages and lunchmeats, and meat spreads	25XX
Organ meats and mixtures	251X
Liver	2511
Hearts	2512
Kidney	2513
Sweetbreads	2514
Brains	2515
Tongue	2516
Other variety meats	2517
Frankfurters, sausages, lunchmeats, meat spreads	252X
Frankfurters	2521
Sausages	2522
Luncheon meats (loaf)	2523
Potted meat, spreads	2524
Fish and shellfish	26XX
Finfish	261X
Other seafood	262X
Shellfish	263X
Meat, poultry, fish with nonmeat items	27XX
Meat, poultry, fish in gravy or sauce or creamed	271X
Beef in gravy or sauce (tomato-based sauce; gravy; cream, white, or soup-based sauce; soy-based sauce; other sauce; Puerto Rican)	2711
Pork with gravy or sauce	2712
Lamb and veal with gravy or sauce	2713
Poultry with gravy or sauce (tomato-based sauce; gravy; cream, white, or soup-based sauce; soy-based sauce; other sauces; Puerto Rican)	2714
Fish, shellfish with gravy or sauce	2715
Miscellaneous meats with gravy or sauce	2716
Meat, poultry, fish with starch item (include white potatoes)	272X
Beef with starch item (potatoes; noodles; rice; bread; Puerto Rican)	2721
Pork with starch item	2722
Lamb, veal, game with starch item	2723
Poultry with starch item (potatoes; noodles; rice; bread)	2724
Fish, shellfish with starch item	2725
Miscellaneous meats with starch item	2726
Meat, poultry, fish with starch item and vegetables	273X
Beef with starch and vegetable (potatoes; noodles; rice; bread; Puerto Rican)	2731
Pork with starch and vegetable	2732

Lamb, veal, game with starch and vegetable	2733
Poultry with starch and vegetable (potatoes; noodles; rice; bread; Puerto Rican)	2734
Fish, shellfish with starch and vegetable	2735
Miscellaneous meats with starch and vegetable	2736
Meat, poultry, fish with vegetables (excluding white potatoes)	274X
Beef with vegetable, no potatoes	2741
Pork with vegetable, no potatoes	2742
Lamb, veal, game with vegetable, no potatoes	2743
Poultry with vegetables, no potatoes	2744
Fish, shellfish with vegetables, no potatoes	2745
Miscellaneous meats with vegetable, no potatoes	2746
Sandwiches with meat, poultry, fish	275X
Beef sandwiches	2751
Pork sandwiches	2752
Poultry sandwiches	2754
Fish, shellfish sandwiches	2755
Frankfurters, luncheon meat, potted meat sandwiches	2756
Hors d'oeuvres, finger sandwiches	2757
Meat, poultry, fish with nonmeat items baby food	276X
Beef mixtures baby food	2761
Poultry mixtures baby food	2764
Frozen and shelf-stable plate meals, soups, and gravies with meat, poultry, fish base; gelatin and gelatin-based drinks	28XX
Frozen or shelf-stable plate meals with meat, poultry, fish as major ingredient	281X
Beef frozen or shelf-stable meals	2811
Pork or ham frozen or shelf-stable meals	2812
Veal frozen or shelf-stable meals	2813
Poultry frozen or shelf-stable meals	2814
Fish, shellfish frozen meals	2815
Miscellaneous meat frozen or shelf-stable meals	2816
Soups, broths, extracts from meat, poultry, fish base	283X
Beef soups	2831
Pork soups	2832
Lamb soups	2833
Poultry, soups	2834
Fish, shellfish soups	2835
Puerto Rican soups	2836
Gelatin and gelatin-based meal supplements	284X
Gravies from meat, poultry, fish base	285X
Eggs	3XXX
Eggs	31XX

Chicken eggs	311X
Other poultry eggs	312X
Egg mixtures	32XX
Egg dishes	321X
Egg sandwiches	322X
Egg soups	323X
Meringues	324X
Egg substitutes	33XX
Egg substitute, NS as to form	330X
Egg substitute, from powdered mixture	331X
Egg substitute, from frozen mixture	332X
Egg substitute, from liquid mixture	333X
Frozen plate meals with egg as major ingredient	35XX
Frozen plate meals with egg as major ingredient	350X
Dry Beans, Peas, Other Legumes, Nuts, and Seeds	4XXX
Legumes	41XX
Dried beans	411X
Dried beans mixtures	412X
Dried peas, lentils, and mixtures	413X
Soybean derived products (excluding milks)	414X
Frozen plate meals with legumes as major ingredient	415X
Soups with legumes as major ingredient	416X
Meat substitutes, mainly legume protein	418X
Meat substitute sandwiches	419X
Nuts, nut butters, and nut mixtures	42XX
Nuts	421X
Nut butters	422X
Nut butter sandwiches	423X
Coconut beverages	424X
Nut mixtures	425X
Seeds and seed mixtures	43XX
Seeds	431X
Carob products	44XX
Carob powder, flour	441X
Carob chips, syrup	442X
Grain Products	5XXX
Flour and dry mixes	50XX
Flour and dry mixes	500X
Yeast breads, rolls	51XX
Breads, rolls, NFS	510X
White breads, rolls	511X
Whole wheat breads, rolls	512X
Wheat, cracked wheat breads, rolls	513X
Rye breads, rolls	514X
Oat breads	515X
Multigrain breads, rolls	516X
Other breads	518X

Quick breads 521 Biscuits	52XX
Cornbread, corn muffins, tortillas	522X
Other muffins, popovers	523X
Other quick breads	524X
Cakes, cookies, pies, pastries 531 Cakes	53XX
Cookies	532X
Pies (fruit pies; pie tarts; cream, custard, and chiffon pies; miscellaneous pies; pie shells)	533X
Cobblers, eclairs, turnovers, other pastries	534X
Danish, breakfast pastries, doughnuts, granola bars	535X
Coffee cake, not yeast	536X
Cookies and bars, baby food	538X
Crackers and salty snacks from grain products	54XX
Crackers, NS as to type	540X
Sweet crackers	541X
Low sodium crackers	542X
Nonsweet crackers	543X
Salty snacks from grain products	544X
Pancakes, waffles, french toast, other grain products	55XX
Pancakes	551X
Waffles	552X
French toast	553X
Crepes	554X
Flour-water patties	555X
Flour-milk patties	556X
Rice flour cakes	557X
Funnel cakes	558X
Pastas, cooked cereals	56XX
Pastas	561X
Cooked cereals, rice	562X
Cereals, not cooked or NS as to cooked	57XX
Cereal, NS as to cooked	570X
Ready-to-eat cereals	571X
Ready-to-eat cereals	572X
Ready-to-eat cereals	573X
Ready-to-eat cereals	574X
Cereal grains, not cooked	576X
Cereals baby food	578X
Grain mixtures, frozen plate meals, soups	58XX
Mixtures, mainly grain, pasta, or bread	581X
Mixtures, mainly grain, pasta, or bread	582X
Frozen plate meals with grain mixture as major ingredient	583X
Soups with grain product as major ingredient	584X
Grain mixtures baby food	585X
Meat substitutes, mainly cereal protein	59XX
Meat substitutes, mainly cereal protein	590X
Fruits	6XXX

Citrus fruits, juices	61XX
Citrus fruits	611X
Citrus fruit juices	612X
Dried fruits	62XX
Dried fruits	621X
Other fruits	63XX
Fruits, excluding berries	631X
Berries	632X
Mixtures of two or more fruits	633X
Mixtures of fruits and nonfruit items	634X
Fruit juices and nectars excluding citrus	64XX
Fruit juices, excluding citrus	641X
Nectars	642X
Vinegar	644X
Fruits and juices baby food	67XX
Fruits and fruit mixtures baby food	671X
Fruit juice and fruit juice mixtures baby food	672X
Fruits with cereal baby food	673X
Fruit desserts and fruit-flavored puddings and yogurt desserts baby food	674X
Fruits with meat or poultry baby food	675X
Fruits and vegetables mixtures baby food	676X
Vegetables	7XXX
White potatoes and Puerto Rican starchy vegetables	71XX
White potatoes, NFS	710X
White potatoes, baked and boiled	711X
White potatoes, chips and sticks	712X
White potatoes, creamed, scalloped, au gratin	714
White potatoes, fried	713X
White potatoes, fried	714X
White potatoes, mashed, stuffed, puffs	715X
Potato salad	716X
Potato recipes	717X
Potato soups	718X
Puerto Rican starchy vegetables	719X
Dark-green vegetables	72XX
Dark-green leafy vegetables	721X
Dark-green nonleafy vegetables	722X
Dark-green vegetable soups	723X
Deep-yellow vegetables	73XX
Carrots	731X
Pumpkin	732X
Squash, winter	733X
Sweet potatoes	734X
Deep-yellow vegetable soups	735X
Tomatoes and tomato mixtures	74XX
Tomatoes, raw	741X
Tomatoes, cooked	742X

Tomato juices	743X
Tomato sauces	744X
Tomato mixtures	745X
Tomato soups	746X
Tomato sandwiches	747X
Other vegetables	75XX
Other vegetables, raw	751X
Raw vegetable mixtures	7514
Other vegetables, cooked	752X
Other vegetable mixtures, cooked	753X
Other cooked vegetables, cooked with sauces, batters, casseroles	754X
Olives, pickles, relishes (excluding tomatoes)	755X
Vegetable soups	756X
Vegetables and mixtures mostly vegetables baby food	76XX
Dark-green vegetables baby food	761X
Deep-yellow vegetables baby food	762X
Vegetables other than dark-green, deep-yellow, and tomato baby food	764X
Vegetables with grain baby food	765X
Vegetables with meat baby food	766X
Vegetables with meat, poultry, fish	77XX
White potato with meat, poultry, fish (mixtures)	771X
Puerto Rican starchy vegetable (viandas) mixtures	772X
Other vegetable mixtures	773X
Puerto Rican stews or soups with starchy vegetables (viandas)	775X
Mixtures mostly vegetables without meat, poultry, fish	78XX
Vegetable and fruit juice blends, 100% juice	781X
Fats, Oils, and Salad Dressings	8XXX
Fats	81XX
Table fats	811X
Cooking fats	812X
Other fats	813X
Oils	82XX
Vegetable oils	821X
Salad dressings	83XX
Regular salad dressings	831X
Low-calorie and reduced calorie salad dressings	832X
Sugars, Sweets, and Beverages	9XXX
Sugars and sweets	91XX
Sugars and sugar-sugar substitute blends	911X
Sugar replacements or substitute	912X
Syrups, honey, molasses, sweet toppings	913X
Jellies, jams, preserves	914X
Gelatin desserts or salads	915X
Ices or popsicles	916X
Candies	917X

Chewing gums	918X
Nonalcoholic beverages	92XX
Coffee	921X
Coffee substitutes	922X
Tea	923X
Soft drinks, carbonated	924X
Fruit drinks	925X
Fruit juice drinks and fruit flavored drinks	9251
Group Discontinued as of 12/31/2004; previously described as "Fruitades and drinks, low calorie, NS as to vitamin C content"	9252
Fruit juice drinks and fruit flavored drinks with high vitamin C	9253
Fruit flavored drinks, made from powdered mix	9254
Fruit juice drinks and fruit flavored drinks, low calorie	9255
Sports drinks and thirst quencher beverages	9256
Beverages, fluid replacement	9257
Fruit juice drinks and fruit flavored drinks, fortified with calcium	9258
Beverages, nonfruit	926X
Beverages, nonfruit, fortified (include energy drinks)	9265
Group Discontinued as of 12/31/2004; previously described as "Beverages, noncarbonated, without vitamin C, made from powdered mixes"	927X
Nonalcoholic beers, wines, cocktails	928X
Beverage concentrates, dry, not reconstituted	929X
Alcoholic beverages	93XX
Beers and ales	931X
Cordials and liqueurs	932X
Cocktails	933X
Wines	934X
Distilled liquors	935X
Water, noncarbonated 940 Water, not bottled	94XX
Water, bottled	941X
Water, bottled, fortified	942X

Note: X represents a place holder, and may be any digit from 1-9.

EXAMPLES

[0059] The following examples illustrate various iterations of the invention.

Example 1. Development of Human Food-Based Diets, Based on Two “Typical” U.S. Adult Diets Differing in Saturated Fat and Fruit/Vegetable Consumption

[0060] Dietary recalls collected on Day 1 of the National Health and Nutrition Examination Surveys (NHANES) administered in 2003-2004, 2005-2006 and 2007-2008 were used: 1) to identify healthy, non-pregnant, non-lactating American adults, aged 20-65 yrs, who are highly differentiated relative to two aspects of dietary guidance, specifically saturated fat and fruit and vegetable intakes, and 2) to characterize key foods in the diets for these two populations. The two subpopulations identified within the larger dataset are HiSF-LoFV and LoSF-HiFV (**Table 1**). The HiSF-LoFV (High Saturated Fat, Low Fruit and Vegetable) group included individuals in the highest tertile of saturated fat and lowest tertile of fruit and vegetable intakes. In contrast, the LoSF-HiFV group included individuals in the lowest tertile of saturated fat and highest tertile of fruit and vegetable intakes. Key characterizing foods consumed by the HiSF-LoFV and LoSF-HiFV subpopulations were then identified in a two-step process using eight broad food groups and 63 food subgroups expressed on a kcal food/1000 kcal total diet basis (**Table 2**). A detailed description of the methodology is provided below.

TABLE 1. HiSF-LoFV and LoSF-HiFV subpopulations among healthy adults 20-65 yrs of age in the National Health and Nutrition Examination Surveys (NHANES) conducted between 2003-2008 (n=10,190 non-pregnant, non-lactating).

Population	n	Cut-point intakes (g) per 1000 kcal	
		Saturated Fatty Acids	Total Fruit and Vegetables
Total population	10,190	12.4 ± 0.1 (mean ± SEM)	ND
HiSF-LoFV	1203	≥ 14.1	≤ 65
LoSF-HiFV	1503	≤ 10.3	≥ 181

TABLE 2. Characterizing foods consumed by the HiSF-LoFV (A) and LoSF-HiFV (B) subpopulations.

(A) HiSF-LoFV characterizing foods

Key Food Group/Food Name (Food Code)	kcal/ 1000 kcal total diet	% weight
<u>Milk & Milk Products</u> - Milk, cow's fluid, 2% fat (11112110) - Ice cream, regular, flavors other than chocolate (13110100) - Cheese, processed, American or Cheddar (14410200)	49 40 54	13.9 2.7 2.3
<u>Meat, poultry, Fish; Eggs; Nuts and Seeds; Legumes</u> - Ground beef or patty, cooked (21500100) - Ham, sliced, prepackage or deli, luncheon meat (25230210) - Cheeseburger (2 patties), with tomato/catsup, bun (27510330) - Eggs, whole, fried (31105000)	41 59 131 37	2.2 5.9 7.3 2.6
<u>Grain Products</u> - Roll, white soft (51150000) - Tortilla, wheat (52215200) - Cookie, chocolate chip (53206000) - Tortilla chips (54401080) - Pizza with meat, thin crust (58106520)	64 26 58 33 193	3.3 1.2 1.7 0.9 9.2
<u>Fruits</u> --	--	--
<u>Vegetables</u> - White potato, chips (71201010)	32	0.8
<u>Fats and Oils</u> Mayonnaise, regular (83107000)	29	0.6
<u>Sugars and Sweets</u> Milk chocolate candy, plain (91705010)	46	1.2
<u>Beverages</u> - Beer, lite (9310200) - Fruit flavored drink, powdered mix (92541010) - Soft drink, cola type (92410310)	20 18 71	9.9 7.2 27.1
<u>Total Characterizing Diet (18 foods)</u>	1,000	100

(B) LoSF-HiFV characterizing foods

Key Food Group/Food Name (Food Code)	kcal/1000 kcal total diet	% weight
<u>Milk & Milk Products</u> - Milk, cow's, fluid, skim or nonfat, 0.5% or less butterfat (11113000) - Ice cream, regular, flavors other than chocolate (13110100) - Cheese, processed, American or Cheddar (14410200)	35 14 15	9.2 0.6 0.4
<u>Meat, poultry, Fish; Eggs; Nuts and Seeds; Legumes</u> - Chicken, breast, roasted, broiled or baked (24122120) - Tuna salad (27450060) - Egg omelet or scrambled egg (32105000) - Peanut butter (42202000) - Milk, soy, ready to drink (11320000)	54 93 20 35 32	3.0 4.5 1.1 0.5 6.6
<u>Grain Products</u> - Roll, white soft (51150000) - Cheerios (57123000) - Rice, white, cooked (5605010) - Tortilla, corn (52215100) - Cookie, chocolate (53209000) - Tortilla chips, corn (54401080) - Spaghetti with tomato sauce (58132110)	76 28 35 36 54 32 61	2.4 0.7 2.4 1.5 1.0 0.6 4.1
<u>Fruits</u> - Orange juice (61210220) - Apple, raw (63101000) - Banana, raw (63107010) - Grapes, raw (63123000) - Apple juice (64104010)	39 15 22 24 22	7.2 2.7 2.2 3.2 4.2
<u>Vegetables</u> - White potato, chips (71201010) - White potato, mashed (71501000) - Tomatoes, raw (75113000) - Lettuce, raw (75113000) - Onion, raw (75117020)	27 24 11 4 33	0.4 2.3 5.5 2.3 7.5
<u>Fats and Oils</u> - Italian dressing, vinegar and oil (83106000)	29	0.9
<u>Sugars and Sweets</u> - Hard candy (91745020)	27	0.6
<u>Beverages</u> - Beer (93101000) - Fruit flavored drink (92511010) - Soft drink, cola type (92410310)	33 26 44	6.8 4.8 10.6
<u>Total Characterizing Diet (30 foods)</u>	1,000	100

[0061] *Data Source and Sample* NHANES utilizes in-person, 24-hr dietary recalls using an automated, multiple-pass method. This method guides the respondent

through the dietary intake period more than once and consequently provides multiple opportunities to identify foods and specific details about the foods consumed during the recall period (Raper et al. *J Food Compos Anal* 2004; 17(3-4): 545-555). Diet recalls were analyzed by the United States Department of Agriculture (USDA) Food Surveys Research Group (FSRG) to estimate the types and amounts of foods, as well as their nutrient composition, consumed by each respondent (CDC 2007, 2008, 2010). Additional information on foods reported by NHANES respondents in 24-hour recalls has been provided by USDA-FSRG in their Food and Nutrition Database for Dietary Studies (FNDDS); FNDDS data include a food coding scheme, “recipes” used in deriving nutrient composition data, and additional detail on foods categorized by specific food codes. USDA-FSRG has developed a grouping scheme for the survey food codes to aid in reporting food intake estimates. In the USDA-defined food grouping system, NHANES food codes (excluding plain water) are typically grouped into 71 food groups or subgroups.

[0062] Selected data from NHANES Food Frequency Questionnaires (FFQs) administered in 2003-2004 and 2005-2006 were also used in the analyses in order to collect additional information regarding frequency of consumption of caloric beverages. The FFQ, developed by the National Cancer Institute (NCI), is based on the NCI Diet History Questionnaire, a 124-item food frequency instrument that is widely used in nutritional epidemiology research (Subar et al. *Am J Epidemiol* 2001;154:1089-99).

[0063] Food Categories The 71 USDA-defined food groups and subgroups represent hierarchical groupings of food codes within broad food categories, and consequently the USDA hierarchy provides a food coding scheme that can be used to identify key characterizing foods within groups of similar foods. This food grouping scheme was used to identify key foods consumed by the HiSF-LoFV and LoSF-HiFV subpopulations. In the analyses, the Meat, Poultry and Fish; Eggs; Nuts and Seeds; and Legumes groups were combined into one major food group. The total fluid milk subgroup was used to assess all fluid milk intake and separate subgroups for “total fruit juice drinks and fruit flavored drinks” and “total carbonated soft drinks” were used to

assess total intakes of these beverages. After modification, the food grouping system included 63 food subgroups within eight broad food groups.

[0064] Subpopulations Based on Saturated Fat and Total Fruit and Vegetable Consumption Among the total sample (n=10,190), tertiles were determined based on g saturated fat/1000 kcal and g fruit+vegetable/1000 kcal (NHANES food codes starting with 6 for fruit and 7 for vegetables), with >14.1 g saturated fat/1000 kcal and <10.3 g saturated fat/1000 kcal defining the top and bottom tertiles, respectively. For combined fruit and vegetable intakes, ≤ 65 g fruits + vegetables/1000 kcal defined the lowest tertile and >181 g fruits + vegetables/1000 kcal defined the top tertile. Using these tertile cutpoints, 11.8% of individuals were in the HiSF-LoFV group (n=1203) and 14.7% were in Low Saturated Fat, High Fruit and Vegetable (LoSF-HiFV) group (n=1503).

[0065] Foods that Characterize HiSF-LoFV and LoSF-HiFV Diets Key characterizing foods consumed by the HiSF-LoFV and LoSF-HiFV subpopulations were identified in a two-step process using eight broad food groups and 63 food subgroups expressed on a kcal food/1000 kcal total diet basis.

[0066] In the first step, key food subcategories were identified. Relative energy intakes by major food group and food subgroup, and the proportion of the subpopulation consuming foods within individual food subgroups were used to identify the key food subgroups consumed by the HiSF-LoFV subpopulation, and separately by the LoSF-HiFV subpopulation. The specific criteria used were as follows:

[0067] • Food subgroups providing less than 20 kcal/1000 kcal total diet (i.e., less than 2.0% of energy) were excluded from consideration.

[0068] • Within each food subgroup providing from 20 to 50 kcal/1000 kcal total diet (i.e., 2.0 to 5.0% of energy), the single food subgroup representing the largest source of energy was selected as a key food subgroup.

[0069] • Within each food group providing more than 50 kcal/1000 kcal total diet (i.e., over 5.0% of total energy), all food subgroups meeting at least one of the following two criteria were considered key food subgroups: 1) Contributing at least 10% of kcal per 1000 kcal for the food group AND consumed by at least 10% of the subpopulation; 2) Consumed by 20% or more of the subpopulation.

[0070] Coffee and tea were excluded because mice are sensitive to caffeine and because these beverages contribute little to energy intake.

[0071] In the second step, food(s) that best characterized each identified key food group/subgroup were selected. For each identified key food group/subgroup, the food code with the highest weight of food consumed per 1000 kcal in the subpopulation reporting consumption on the day of recall was selected to be the key food characterizing that entire food group/subgroup.

[0072] *Proportions of Key Foods in HiSF-LoFV and LoSF-HiFV Diets* The proportions of key foods to be included in mouse diets (wet weight) were calculated by first determining the relative energy contributions from key food subgroups to the total energy intake from the USDA-based food group (per 1000 kcal total diet) and then determining the weight of the food (in g) required to deliver the calculated energy contribution from the relevant food group per 1000 kcal total diet (**Table 1**). Once the characterizing foods were identified for the two diets, calculated nutrient intakes were compared to 24 hr recall data as an indicator of how well the characterizing foods reflect reported intakes. Results indicated that cholesterol was low in the LoSF-HiFV diet and therefore egg was added to the LoSF-HiFV diet. **Table 3** shows that mean nutrient content of animal chow made from key characterizing foods were similar to intakes reported from the 24-h recall for both the resulting HiSF-LoFV and LoSF-HiFV diets.

TABLE 3. Comparison of nutrient content per 1000 kcal from intake based on a 24-hour diet recall (24 hr Recall); calculated diet based on key characterizing foods (Calculated Diet); or chemical analysis of the Animal Chow (Animal Chow).

Nutrient per 1000 kcal	HiSF-LoFV			LoSF-HiFV		
	24 hr Recall	Calculated Diet	Animal Chow	24 hr Recall	Calculated Diet	Animal Chow
Protein, g	39.9	42.0	45.8	41.0	38.9	38.3
Total Fat, g	45.3	47.0	47.2	28.6	28.0	33.2
Total Saturated Fat, g	17.6	18.6	17.7	7.6	6.5	7.0
Total Monounsaturated Fat, g	16.4	16.4	13.6	10.5	9.0	12.8
Total Polyunsaturated Fat, g	7.4	7.7	8.5	7.7	10.0	9.3
Cholesterol, mg	163	191	179	109	91	84
Carbohydrate, g	105	99	97	142	148	139
Total Sugars, g	48	47	50	66	72	71
Dietary Fiber, g	5.1	3.5	5.7	11.0	9.8	13.7
Vitamin A, mcg RAE	252	201	188	385	286	370

Vitamin E, mg alpha-toc	2.7	2.5	0.7	1.1	0.9	0.7
Vitamin C, mg	14.3	10.4	8.0	88.5	61	23
Thiamin, mg	0.7	0.8	0.9	0.9	0.7	0.8
Riboflavin, mg	1.1	0.9	0.7	1.1	0.9	0.7
Niacin, mg	10.2	8.9	11.4	14.3	16.1	14
Folate, mcg DFE	211	169	131	331	334	250
Vitamin B6, mg	0.7	0.6	0.4	1.3	1.2	0.9
Vitamin B12, mcg	2.6	2.8	2.8	2.4	2.9	3.3
Calcium, mg	480	523	601	418	439	500
Phosphorus, mg	652	672	764	647	613	617
Magnesium, mg	113	90	99	171	158	161
Iron, mg	6.5	5.9	8.8	8.7	8.1	8.4
Zinc, mg	6.0	6.2	6.6	5.8	4.5	5.1
Copper, mg	0.5	0.5	0.5	0.8	0.7	0.8
Selenium, mcg	52	54	101	55	61	100
Sodium, mg	1676	1975	2021	1621	1279	804
Potassium, mg	1076	899	821	1704	1581	1550

Example 2. Preparing and Processing Animal Chow

[0073] Characterizing foods were processed into dried animal chow pellets using methods to achieve an even mix of foods in quantitative proportions and to retain nutrients. The approach of blending all the diverse food items into one mixture and freeze drying the resulting mixture was experimental. Due to the unique nature of this mixture it was unknown if the freeze dried material would have the correct physicochemical characteristics to be extruded/molded into a structurally stable granular or pellet form. The blending of all the food items that represent a diet into a homogeneous mixture distributed the water, fat, oil and low molecular weight solutes (sugars and minerals) into an emulsion and suspension that was amiable to freezing and freeze drying. It is hypothesized that the freezing point of the blended mix was raised compared to some of the individual food items, making the homogenous composite a feasible approach to freeze drying food items that have a much lower freezing points and difficult to freeze dry. Food items with low freezing points and potentially more difficult to freeze dry would be those high in simple sugars, salt and fat (e.g. salad dressing, chocolate, candy). The distribution of the water in this complex food matrix was such that when removed via the freeze drying process the resulting dry powder had the correct characteristics for extrusion and molding into an appropriate form.

[0074] Homogenous food composites for the two diets were prepared as follows. Characterized foods for each diet were purchased from Chicago area stores

and/or prepared using standard recipes from the USDA FNDDS or from standard authoritative cookbooks. Mixed dishes (e.g. pizza) were produced over a one week timeframe, weighed into batches, and then frozen until they were thawed prior to compositing. Perishable foods were prepared (e.g., peeled, cored), weighed out the day before blending and stored refrigerated or at room temperature as appropriate. To composite the diets, foods were added in quantitative proportions into a Breddo Likwifer mixer (model LOR 25) and recirculated through a Silverson high energy mixer until evenly blended by visual inspection in single batches to ensure a homogeneous mixture (**Tables 4 and 5**). Homogenized mixtures were collected in 5 gallon pails and then frozen at -20°C. **FIG. 1** illustrates the flow of diet manufacture.

[0075] Homogenous composite formulas were maintained at -20°C. Frozen blocks of each composite diet were finely shredded and then placed in a vacuum chamber set at 1 mb until available moisture was removed (approximately 27 hours). Freeze dried material was then milled to a 20 mesh size and packed into 40 lb. bags (stored at room temperature). Water was added to the freeze dried formula to reach an acceptable visual viscosity for extrusion into pellets. Samples were collected from the beginning, middle and end of the extrusion process for compositional analysis based on AOAC methodology. The averages of those data are shown in **Table 3** and **Table 6**. Pellets were flushed with nitrogen and vacuum packed. Vacuum packed diets were sterilized via gamma irradiation.

[0076] Recipes: Reference numbers for the following recipes were from the USDA Food and Nutrient Database for Dietary Studies, 4.1.2010. Beltsville, MD; U.S. Department of Agriculture, Agriculture Research Service, Food Surveys Research Group; and USDA Food and Nutrient Database for Dietary Studies, 3.0.2008. Beltsville, MD; U.S. Department of Agriculture, Agriculture Research Service, Food Surveys Research Group (FNDDS3.0 data used only for Pizza). Pizza, thin crust meat: 58106520, recipe slightly modified, used premade crust and substituted tomato sauce for tomato juice and puree; Double Cheeseburger: 27510330; Egg fried: 31105000; Tuna Salad: 27450060; Spaghetti with tomato sauce: 58132110; White Rice: 56205010; Mashed Potato: 71501000; Scrambled Egg: 32105000; Baked Chicken

breast: 24122120; Italian Dressing recipe from Joy of Cooking (1975; 23rd printing 1981).

TABLE 4. High Saturated Fat Low Fruit Vegetable Processing Protocol

High Saturated Fat Low Fruit and Vegetable Diet			
Food Type	Order Added to Breddo	Process Adjustment	Comments
Soft Drink	1	Recirculation Off	Breddo at 600rpm split amount by half added second half after ground beef patty addition
Fruit Drink from powder	2		
Corn Snack	3		
Potato Chip	4		
White Roll soft	5		
Lite Beer	6		
Chocolate Chip Cookie	7		
Flour Tortilla	8	Pulse	Pulse Breddo at 2200 rpm for a few minutes
Milk Chocolate Candy	9		
Processed Cheese American	10	Pulse	Pulse Breddo at 2200 rpm for a few minutes
Ham sliced	11	Pulse, Recirculation On	Pulse Breddo at 2200 rpm for a few minutes, Recirculation started with Silversonmixer run at 3500rpm
Double Cheeseburger w/ tomato on bun	12	Pulse	Pulse Breddo at 2200 rpm for a few minutes
Egg Fried	13		
Ground Beef Patty	14		
Mayonnaise	15		
Pizza, thin crust meat	16	Pulse	Pulse Breddo at 2200 rpm for a few minutes
Ice Cream	17		
Milk 2%	18		
			Mix for approximately 5 minutes before collecting mixture

TABLE 5. Low Saturated Fat High Fruit Vegetable Diet Processing Protocol

Low Saturated Fat High Fruit and Vegetable Diet			
Food Type	Order Added to Breddo	Process Adjustment	Comments
Soft drink	1	Recirculation Off	Breddo at 600rpm
Hard Candy	2		
Cheese, Cheddar	3		
Chicken Breast baked no skin	4		
Potato Chips	5		
Corn Snack	6		
Ready to Eat Cereal	7		Increase Breddo Speed to approx 1,100
Orange Juice	8		
Raw Onion	9	Pulse	Pulse Breddo at 2200 rpm for a few minutes
Raw Banana	10		
Raw Lettuce	11	Pulse	Pulse Breddo at 2200 rpm for a few minutes
Raw Tomato	12		
Raw Grapes	13		
Raw Apple	14	Pulse	Pulse Breddo at 2200 rpm for a few minutes
Peanut Butter	15		
Chocolate cookie	16		
Corn Tortilla	17	Pulse	Pulse Breddo at 2200 rpm for a few minutes
Mashed Potatoes	18		
White Rice	19	Pulse	Pulse Breddo at 2200 rpm for a few minutes
Spaghetti with Tomato Sauce	20		
Scrambled Egg fat added in cooking	21	Recirculation On	Recirculation started with Silversonmixer run at 3500rpm
Roll White Soft	22	Pulse	Pulse Breddo at 2200 rpm for a few minutes
Apple Juice	23		
Ice Cream	24		
Milk Skim	25		
Soy Milk	26		
Beer	27		
Lemonade	28		Powdered Lemonade added to italian dressing
Italian Dressing vinegar and oil	28		
Tuna Salad*	29		*Stephan processing Mix for approximately 5 minutes before collecting mixture

TABLE 6. Formula Composition after Processing Steps: (A) Starting Weight and Final Weight, (B) Mixing, (C) Freeze Drying, (D) Pellet Extrusion

(A) Starting Weight and Final Weight

Diet Formula	Starting Weight Combined wt of batches (lbs)	Final Weight weight of pellets (lbs)
HiSF-LoFV	500	115
LoSF-HiFV	600	101

(B) After all Mixing

Analysis	Units	HiSF-LoFV	LoSF-HiFV
Moisture	% as is	73.14	81.22
Ash	% as is	1.28	0.64
Fat, g	per 1000 kcal	47.3	43.5
Protein, g	per 1000 kcal	57.8	53.5
Carbohydrate, g	per 1000 kcal	108.2	175.0
Vit C, mg	per 1000 kcal	9.0	63.0
Beta carotene, mg	per 1000 kcal	0.2	0.6

(C) After Freeze Drying

Analysis	Units	Freeze Dried	
		HiSF-LoFV	LoSF-HiFV
Moisture	% as is	2.13	2.62
Ash	% as is	3.31	3.10
Fat, g	per 1000 kcal	45.3	31.5
Protein, g	per 1000 kcal	46.4	38.2
Carbohydrate, g	per 1000 kcal	101.6	140.8
Vit C, mg	per 1000 kcal	11.8	43.0
Beta carotene, mg	per 1000 kcal	0.1	0.5

(D) Beginning (Beg), Middle (Mid) and End of Pellet Extrusion

Analysis	Units	HiSF-LoFV			LoSF-HiFV		
		Beg	Mid	End	Beg	Mid	End
Moisture	% as is	5.33	5.25	5.18	7.25	7.70	6.53
Ash	% as is	4.34	4.36	4.31	3.06	2.73	3.05
Fat, g	per 1000 kcal	47.2	47.4	47.7	32.9	33.0	33.0
Protein, g	per 1000 kcal	45.9	46.0	46.1	38.3	38.2	38.1
Carbohydrate, g	per 1000 kcal	97.5	97.3	97.2	137.1	136.9	138.9
Vit C, mg	per 1000 kcal	8.0	7.8	8.0	22.8	22.5	23.2
Beta carotene, mg	per 1000 kcal	0.1	0.1	0.1	0.4	0.4	0.4

CLAIMS

What is claimed is:

1. An animal feed composition consisting of a human diet equivalent, the human diet equivalent consisting of 57- 64% by wet weight carbohydrates, 3- 7% by wet weight total dietary fiber, 10- 15% by wet weight fat, 2.0- 3.5% total saturated fatty acids of fat, 35-40 mg cholesterol/100g total human diet equivalent (TD), 13- 17% by wet weight protein, 300- 500 mg sodium/100g TD, 500- 700 mg potassium/100g TD, 4- 8% by wet weight moisture, and 4.0- 4.4 Kcal/g TD; wherein the human diet equivalent is made from eight food groups, the eight food groups consisting of food items identified by the first three or four digits of an eight digit food code, wherein (a) milk and milk products have a food code with a first digit of 1 and are 5- 25% by wet weight, (b) meat, poultry, fish, egg, nuts, seeds, and legumes have a food code with a first digit selected from the group consisting of 2, 3, and 4 and are 10- 25% by wet weight, (c) grain products have a food code with a first digit of 5 and are 10- 20% by wet weight, (d) fruits have a food code with a first digit of 6 and are 10- 25% by wet weight, (e) vegetables have a food code with a first digit of 7 and are 10- 25% by wet weight, (f) fats and oils have a food code with a first digit of 8 and are 0.5- 5% by wet weight, (g) sugars and sweets have a food code with a first digit of 9 and a second digit of 1 and are 0.5- 5% by wet weight, and (h) beverages have a food code with a first digit of 9 and a second digit of 2 or 3 and are 10- 60% by wet weight.
2. The composition of claim 1, wherein the milk and milk products are 10.2% by wet weight; meat, poultry, fish, egg, nuts, seeds, and legumes are 15.7% by wet weight; grain products are 12.7% by wet weight; fruits are 19.5% by wet weight; vegetables are 18% by wet weight; fats and oils are 0.9% by wet weight; sugars and sweets are 0.6% by wet weight; and beverages are 22.2% by wet weight.

3. The composition of any one of claims 1 or 2, wherein the food items are blended into a homogenous mixture.
4. The composition of any one of claims 1 to 3, wherein the composition is processed into a granule form or a pellet form.
5. A process to prepare a pelletized animal feed composition of claim 1 or claim 2, the process comprising
 - (i) introducing two or more food items independently selected from the group consisting of frozen food items, refrigerated food items, and room temperature food items into a first mixer and blending the food items, wherein the first mixer is a high shear liquid mixer;
 - (ii) passing and/or recirculating the blended food items into a second mixer to form a homogenous composite, wherein the second mixer is a rotor-stator mixer and the homogenized composite is evenly blended by visual inspection;
 - (iii) freeze drying the homogenous composite and milling the dehydrated composite to a 20 mesh size, wherein the moisture content of the dehydrated composite is less than 5%;
 - (iv) adding water to the milled composite and extruding into pellets, wherein the moisture content of the composite at the end of the extrusion process is less than 10%.
6. The process of claim 5, wherein dehydrated composite is a freeze dried composite with a moisture content of less than 2.7%.
7. The process of claim 5 or claim 6, wherein the moisture content of the extruded pellet is less than 7%.

8. The process of any one of claims 5 to 7, wherein the total processing time of steps (i) and (ii) does not exceed two hours.
9. The process of any one of claims 5 to 8, wherein the pellets are packaged and sterilized by gamma irradiation.
10. The process of any one of claims 5 to 8, wherein the pellets are sterilized by gamma irradiation and packaged.
11. The process of any one of claims 5 to 10 further comprising adding a dietary supplement, wherein the dietary supplement may be added during step (i), step (ii), after step (iii) but prior to step (iv).
12. The process of any one of claims 5 to 10 further comprising adding a dietary supplement, wherein the dietary supplement is added to the milled composite of step (iii) prior to processing into a final form.
13. A method of determining the effect of at least one dietary supplement on a subject, the method comprising administering or feeding a composition of any one of claims 1 to 4 further comprising at least one dietary supplement to a subject and determining the presence or absence of a difference in the subject after administering the composition comprising the dietary supplement relative to a subject administered the composition without the dietary supplement, wherein the presence or absence of a difference in the subject represents the effect of the dietary supplement on the subject.
14. A method of determining the effect of at least one dietary supplement on a subject, the method comprising (1) administering or feeding a first composition to

a subject for a first period of time, wherein the first composition is a composition of any one of claims 1 to 4, (2) administering or feeding a second composition to the subject for a second period of time, wherein the second composition consists of the first composition and at least one dietary supplement, and (3) determining the presence or absence of a difference in the subject after administering or feeding the second composition, wherein the presence or absence of a difference in the subject represents the effect of the dietary supplement on the subject.

15. A method of determining the effect of at least one dietary supplement on a subject, the method comprising (1) administering or feeding a first composition to a subject for a first period of time, wherein the first composition is a composition of any one of claims 1 to 4 and at least one dietary supplement, (2) administering or feeding a second composition to the subject for a second period of time, wherein the second composition consists of the first composition without the at least one dietary supplement, and (3) determining the presence or absence of a difference in the subject after administering or feeding the second composition, wherein the presence or absence of a difference in the subject represents the effect of the dietary supplement on the subject.
16. The method of any one of claims 13, 14, and 15, wherein the difference is measured as a change in gut microbiota, a change in weight, a change in body fat, a change in energy consumption, a change in health, a change in inflammation, a change in immune function and/or a change in metabolism.
17. The method of any one of claims 13, 14, 15, and 16, wherein the dietary supplement is high in non-digestible carbohydrates and antioxidants.

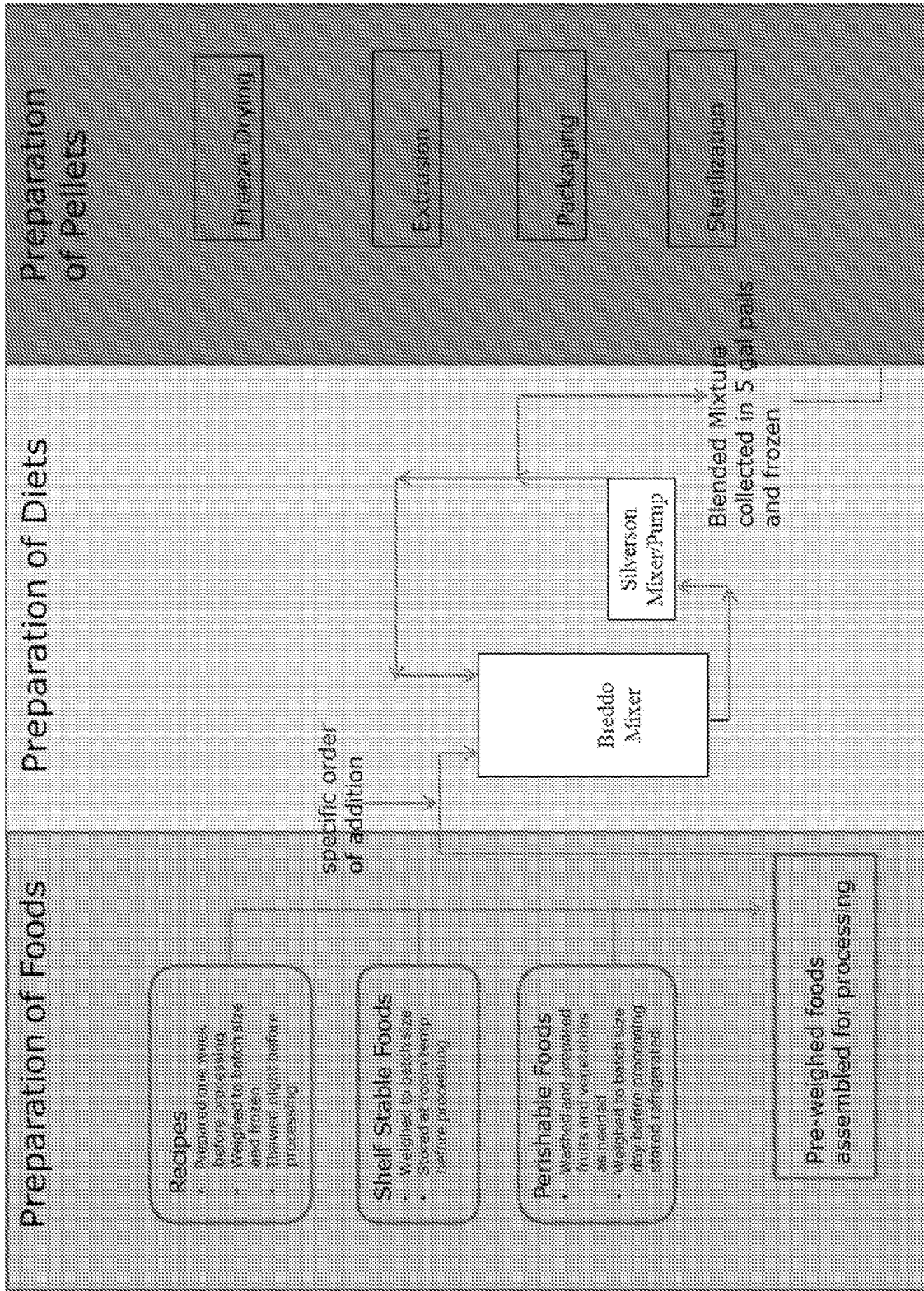


FIG. 1

Preparation of Foods

Recipes

- Proper to specific diets
- Before processing
- Weighed to proper size
- Not expired
- Fresh or slight before processing

Shelf Stable Foods

- Weighed to batch size
- Stored at room temp.
- Before processing

Perishable Foods

- Weighed and assembled
- Fruits and vegetables as needed
- Weighed to batch size
- dry before processing
- stored off to one side

Pre-weighed foods assembled for processing

Preparation of Diets

specific order of addition

Bredde Mixer

Silverson Mixer/Pump

Blended Mixture collected in 5 gal. pails and frozen

Preparation of Pellets

Freeze Drying

Extrusion

Packaging

Sterilization