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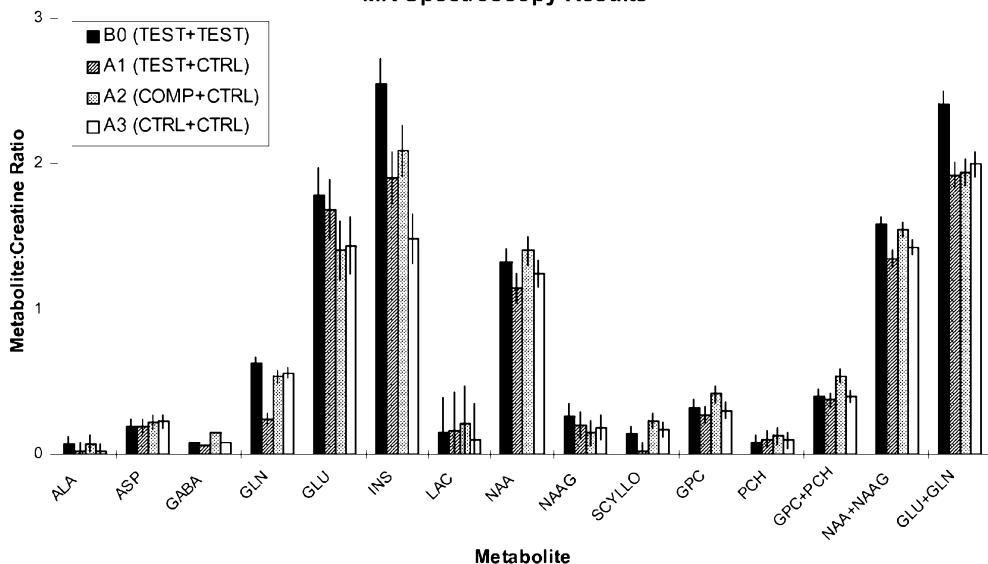
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(54) Title: COMPOSITIONS AND METHODS FOR ENHANCING NEUROLOGIC DEVELOPMENT

FIGURE 1

MR Spectroscopy Results



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(57) Abstract: Compositions and methods useful to enhance neurologic development of a growing animal are disclosed.

**COMPOSITIONS AND METHODS FOR ENHANCING
NEUROLOGICAL DEVELOPMENT
CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims priority to U.S. Provisional Application No. 60/891,171, filed on February 22, 2007 the contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The present invention relates to compositions and methods for enhancing the neurological development of mammals.

BACKGROUND OF THE INVENTION

[0003] Commercially available pet foods, e.g., cat food, include compositions specially formulated to address many different nutritional needs. These include, for example, formulations designed for different breed types, sizes and body conditions. They also include formulations designed to address the nutritional needs of animals in the different stages of their life cycle. Despite the availability of such pet food formulations, however, there is a need to develop formulations and methods to address other aspects of an animal's health.

[0004] Nootropic agents are known in the art, and generally include drugs and compounds which improve cognitive and neurological development. Although nootropic agents have been used for many years, some of the agents are toxic or expensive to use in food products. Thus, it is desirable to develop compositions and methods which may aid in neurological development of animals without increasing costs for manufacturing. Preferably, compositions containing materials which are readily available in the art may be utilized to improve neurological development, however, specific formulations need to be developed before their advantages may be realized and appreciated.

SUMMARY OF THE INVENTION

[0005] In certain aspects, the present invention relates to compositions that are useful to enhance the neurological development of an animal.

[0006] The present invention includes Composition 1.0, a pet food composition useful to neurological development in an animal comprising:

about 0.1% to about 0.7 % DHA;
about 2400 ppm to about 7500 ppm choline; and

about 100 ppm to about 500 ppm carnitine.

[0006A] The present invention also provides a pet food composition comprising: about 0.1% to about 0.7 % DHA; about 2400 ppm to about 7500 ppm choline; and about 100 ppm to about 500 ppm carnitine.

5 [0007] The present invention also includes the following compositions:

1.1 Composition 1.0 comprising about 0.1% to about 0.50% DHA, e.g., about 0.1% to about 0.4%, e.g., about 0.2, about 0.3%, or about 0.4%;

1.2 Composition 1.0 or 1.1 comprising about 200 to about 1200 IU/kg vitamin E, e.g., about 500 IU/kg to about 1100 IU/kg, about 700, about 800, about 900, or about 10 1000 IU/kg;

1.3 Any of the preceding compositions comprising about 50 to about 500 ppm vitamin C, e.g., about 100 to about 400 ppm Vitamin C, e.g., about 150, about 175, about 200, or about 225 ppm;

1.4 Any of the preceding compositions comprising about 200, about 300, or 15 about 400 ppm carnitine;

1.5 Any of the preceding compositions comprising about 2.5 g/1000 kcal to about 7 g/1000 kcal lysine;

1.6 Any of the preceding compositions comprising about 2500 ppm to about 7500 ppm choline, e.g., about 3000, about 4000, about 4500, about 4600, about 4625, 20 about 4650, about 4700, about 5000, or about 6000 ppm;

1.7 Any of the preceding compositions comprising about 0.1 % to about 0.7 % EPA, e.g., about 0.2%, about 0.3%, about 0.4%, or about 0.5%;

1.8 Any of the preceding compositions comprising about 50 ppm to about 200 ppm manganese;

25 1.9 Any of the preceding compositions comprising about 0.50% to about 1.6% methionine, e.g., about 0.8% to about 1.6% methionine, e.g., about 1.3 or about 1.4% methionine.

1.10 Any of the preceding compositions further comprising:

0 to about 90% by weight of carbohydrates;

30 about 5% to about 70% by weight of protein;

about 2% to about 50% by weight of fat;

about 0.1% to about 20% by weight of total dietary fiber;

0 to about 15%, preferably about 2% to about 8%, by weight of vitamins, minerals, and other nutrients, in varying percentages which support the nutritional needs of the animal.

5 1.11 Composition 1.10 comprising about 5% to about 55%, by weight of carbohydrates;

1.12 Composition 1.10 or 1.11 comprising about 20% to about 60%, by weight of protein, e.g., about 30 – about 55%;

1.13 Any one of compositions 1.10 – 1.12 comprising about 5% to about 40%,

10 by weight of fat, e.g., at least about 8% or about 9% to about 40% fat;

1.14 Any one of compositions 1.10 – 1.13 comprising about 1% to about 11%, by weight of total dietary fiber;

1.15 Any of the preceding compositions comprising about 1000 to about 4000 ppm taurine;

15 1.16 Any of the preceding compositions comprising about 0.5% to about 6 % linoleic acid, e.g., about 2.5% to about 5%;

1.17 Any of the preceding compositions comprising about 1% to about 3% total n-3 fatty acids, e.g., about 1.3%, about 1.4%, about 1.5%, or about 1.6%.

1.18 Any of the preceding compositions comprising about 1% to about 6% total

20 n-6 fatty acids, e.g., about 3% to about 5%, about 3.5%, or about 4%.

[0008] The compositions of the present invention may be a wet, dry, or semi-dry food.

[0009] The present invention includes Method 2.0, a method to enhance the neurological development of a feline comprising administering to the feline any one of compositions 1.0 – 1.18.

25 [0009A] The present invention provides a method to enhance the neurological development of a feline comprising administering to the feline or to the mother of the feline while the feline is *in utero* the pet food composition according to the invention.

[0010] The present invention also includes the following methods:

2.1 Of method 2.0 wherein the feline is a kitten

30 2.2 Of method 2.0 or 2.1 wherein the feline is born of a queen fed any one of compositions 1.0 – 1.15 during pregnancy.

- 2.3 Of method 2.2 wherein the feline is *in utero*.
- 2.4 Of method 2.2 wherein the queen is fed any one of compositions 1.0 – 1.18 prior to pregnancy.
- 2.5 Of method 2.2 or 2.4 wherein the queen is fed any one of compositions 1.0 – 1.18 for a majority of the pregnancy duration.
- 2.6 Of any one of methods 2.2 – 2.5 wherein the queen is fed compositions consisting essentially of any one of compositions 1.0 – 1.18 prior to and during pregnancy.
- 2.7 Of any one of the preceding methods wherein the kitten is fed any one of compositions 1.0 – 1.18 prior to weaning, e.g., while still nursing.
- 2.8 Of any one of the preceding methods wherein the kitten is fed any one of compositions 1.0 – 1.18 post weaning.
- 2.9 Of method 2.8 wherein the kitten is fed food compositions consisting of any one of compositions 1.0 – 1.18.
- 2.10 Of any one of the preceding methods wherein an effective amount of the composition is administered to the animal.
- 2.11 Of any one of the preceding methods wherein the composition is administered to the animal for an effective amount of time.

[0011] It is also contemplated that, in addition to administering the compositions disclosed herein directly to a growing animal, e.g., to a growing puppy or kitten, the compositions may be administered to the dam of the animal while the animal is still *in utero* or while the animal is a nursing.

[0011A] The present invention further provides a pet food composition suitable for enhancing the neurological development of a feline comprising:

- about 0.1% to about 0.7 % DHA;
- about 2400 ppm to about 7500 ppm choline; and
- about 100 ppm to about 500 ppm carnitine;
- about 200IU/kg to about 1200IU/kg vitamin E;
- about 50 to about 500 ppm vitamin C;
- about 2.5 g/1000 kcal to about 7 g/1000 kcal lysine;
- about 0.1 % to about 0.7 % EPA;
- about 50 ppm to about 200 ppm manganese; and

about 0.5% to about 1.6% methionine.

[0012] Other features and advantages of the present invention will be understood by reference to the detailed description of the examples that follow.

5 [0012A] Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

10 [0012B] Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

15 BRIEF DESCRIPTION OF THE DRAWINGS

[0013] Figure 1 is magnetic resonance spectroscopy data relevant to Example 1.

DETAILED DESCRIPTION OF THE INVENTION

20 [0014] It is contemplated that the invention described herein is not limited to the particular methodology, protocols, and reagents described as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention in any way.

25 [0015] Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. In addition, all references cited herein are hereby incorporated by

reference in their entireties. In the event of a conflict in a definition in the present disclosure and that of a cited reference, the present disclosure controls.

[0016] As used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise.

[0017] The present invention relates to any animal, preferably a mammal, more preferably a companion animal. The term "companion animal" refers to any animal that lives in close association with humans and includes, but is not limited to, canines and felines of any breed. It is contemplated herein, however, that any animal whose diet may be controlled by humans may benefit from feeding the formulations disclosed herein. These animals may include, for example, domesticated farm animals (e.g., cattle, horses, swine, etc.) as well as undomesticated animals held in captivity, e.g., in zoological parks and the like. Preferably, the animal is a feline, either a kitten, or adult cat.

[0018] As used herein, "an amount effective to" or "an effective amount" to achieve a particular result, and like terms, refer to that amount of a compound, material or composition as described herein that may be effective to achieve a particularly desired biological result. As contemplated herein, such results include, for example, enhancement of neurological development, bone and joint health, immune function and/or promotion of a healthy body composition of an animal, either while developing *in utero* and/or during its growth stage after birth, e.g., up to 6 months, 9 months, 12 months, or 15 months after birth. Such effective activity may be achieved, for example, by administration of compositions of the present invention to the dam of said animal while the animal is *in utero* or nursing, as well as by direct administration to the animal during its growth stage.

[0019] As used herein, the "enhancement" of a particular biological process or body condition in a growing animal such as described herein refers to an improvement in the biological process or body condition of a growing animal compared to a control animal. Improvement in such a process or condition may be determined by one of skill in the art.

[0020] As used herein, "enhancement of the development of a growing animal" or "enhanced growth" and like terms refer to an overall improvement in one or more biological processes and/or the body condition of a growing animal, including but not limited to, biological processes central to the growth and development of an organism, including, but not limited to, the biological processes described herein, e.g., bone and joint health,

neurological and immune system development and body weight gain (e.g., increase in lean muscle mass instead of adipose tissue).

[0021] The “growth” life stage of an animal refers to the period from birth or weaning (approximately 8 weeks of age) to about 1 year of age.

[0022] As used herein, the term “kitten” refers to an immature feline, typically between the ages of birth and 12 months.

[0023] “Essential amino acids” as used herein refers to those amino acids that cannot be synthesized *de novo* by an organism and thus must be supplied in the diet. It is understood by one of skill in the art that the essential amino acids vary from species to species, depending upon the organism’s metabolism. For example, it is generally understood that the essential amino acids for dogs and cats (and humans), are phenylalanine, leucine, methionine, lysine, isoleucine, valine, threonine, tryptophan, histidine and arginine. In addition, taurine, while technically not an amino acid but a derivative of cysteine, is an essential for cats. A balanced diet can provide all the essential amino acids, however, there are certain essential amino acids that are more critical, as a diet deficient in one of them will limit the usefulness of the others, even if the other essential amino acids are present in sufficient quantities.

[0024] As understood by one of skill in the art, a “limiting amino acid” refers to an amino acid which if present in insufficient quantities in a diet, results in the limitation in usefulness of other essential amino acids, even if the other essential amino acids are present in otherwise large enough quantities. Lysine is the limiting essential amino acid in the compositions disclosed herein. Thus, the remaining essential amino acids are quantitatively formulated or “balanced” in relationship to the amount of lysine determined critical to affect the desired biological result. As used herein, “balanced amino acids” refers to the relationship of the essential amino acid lysine to energy to assure optimal animal growth and development.

[0025] “Essential nutrients” as used herein refers to nutrients required for normal body functioning that cannot be synthesized by the body. Categories of essential nutrient include vitamin dietary minerals, fatty acid, and amino acid. It is understood by one of skill in the art that the nutrients deemed essential varies from species to species, depending upon the organism’s metabolism. For example, essential nutrients for dogs and cats include Vitamins A, D, E, K, B1, B6, B12, riboflavin, niacin, pantothenic acid, folic acid, calcium,

phosphorous, magnesium, sodium, potassium, chlorine, iron, copper, zinc, manganese, selenium and iodine. Choline, generally regarded as a B complex vitamin, may be included among the semi-essential nutrients.

[0026] Carnitine, also known as L-carnitine, (levocarnitine) is a quaternary ammonium compound synthesized from the amino acids lysine and methionine and is responsible for the transport of fatty acids from the cytosol into the mitochondria.

[0027] Without being limited to any theories or particular modes of action, the present invention is based on the surprising discovery that the addition of certain ingredients to pet food compositions and administration of these compositions to animals can enhance the development of a growing animal. Data indicates that animals fed the compositions of the present invention (or those whose dams were fed the compositions during gestation and prior to weaning but continued throughout growth of their litters), demonstrate enhanced neurological development. In one aspect, the invention relates to a method to enhance the neurological development of a growing animal comprising administering to said animal a composition of the present invention. An enhancement in neurological development may be indicated by better brain development, as measured by MRI spectroscopy, and/or by better cognitive and motor skill scores, compared to control animals. For instance, the state of neurological development may be assayed by measuring the level of trainability in a growing animal to which is administered a composition of the present invention and comparing levels to a suitable control animal.

[0028] As contemplated herein, the compositions of the present invention comprise nutritionally complete and balanced animal feed compositions. Such compositions include, among other nutrients and ingredients, recommended healthful amounts of protein, carbohydrate and fat.

[0029] "Nutritionally complete and balanced animal feed compositions", as well as nutrients and ingredients suitable for animal feed compositions, and recommended amounts thereof, are familiar to one of skill in the art (see, for example, National Research Council, 2006 Nutritional Requirements for Dogs and Cats, National Academy Press, Washington D.C. or the Official Publication of the Association of American Feed Control Officials, Inc. Nutrient Requirements for Dogs and Cats 2006).

[0030] It is contemplated herein that the compositions disclosed herein may also comprise antioxidants, additives, stabilizers, thickeners, flavorants, palatability enhancers and colorants in amounts and combinations familiar to one of skill in the art. “Antioxidants” refers to a substance that is capable of reacting with or decreasing the production of free radicals and neutralizing them. Examples include, but are not limited to, beta-carotene, selenium, coenzyme Q10 (ubiquinone), lutein, tocotrienols, soy isoflavones, S-adenosylmethionine, glutathione, taurine, N-acetylcysteine, vitamin E, vitamin D, vitamin C, flavanoids, anthocyanindins, and lipoic acid.

[0031] While foods of any consistency or moisture content are contemplated, preferably the compositions of the present invention may be, for example, a wet, semi-dry or dry animal food composition. “Wet” food refers to food which is sold in cans or foil bags and has a moisture content of about 70 to about a 90%. “Dry” food refers to compositions with about 5 to about 15% moisture content and is often manufactured in the form of small bits or kibbles. Semi-dry compositions refer to compositions having about 15% to about 70% moisture. Also contemplated herein are compositions of intermediate moisture consistency and those that may comprise components of various consistency as well as components that may include more than one consistency, for example, soft, chewy meat-like particles as well as kibble having an outer cereal component and an inner cream component as described in, e.g., US Patent 6,517,877.

[0032] The following examples further illustrate the present invention and are not intended to limit the invention. As used throughout, ranges are used as shorthand for describing each and every value that is within the range. Any value within the range can be selected as the terminus of the range. It is understood that when formulations are described, they may be described in terms of their ingredients, as is common in the art, notwithstanding that these ingredients may react with one another in the actual formulation as it is made, stored and used, and such products are intended to be covered by the formulations described.

[0033] The following examples further describe and demonstrate illustrative embodiments within the scope of the present invention. The examples are given solely for illustration and are not to be construed as limitations of this invention as many variations are possible without departing from the spirit and scope thereof. Various modifications of the

invention in addition to those shown and described herein should be apparent to those skilled in the art and are intended to fall within the appended claims.

Example 1

[0034] Formulations to enhance the development of growing animal are disclosed here-in-below. These compositions are developed taking into account the “ideal protein concept” (Baker and Czarnecki-Maulden, 1991 Annu. Rev. Nutr. 11:239-63).

[0035] Foods are developed for the “growth” life stage. These foods include formulations for canine growth and feline growth. The minimum nutrient recommendations for these foods, as well as the targeted values for a prototype food, are listed below in Table 1.

Table 1: Key Nutrients for Kitten Formula

Nutrient	Target	Minimum	Maximum
Protein, %	45.5	30	55
Methionine, %	1.4	0.8	1.5
Manganese, ppm	90	50	200
DHA, %	0.21	0.1	0.5
EPA, %	0.31	0.1	0.70
Choline, ppm	4880	2500	7500
Taurine, ppm	2380	1000	4000
Linoleic acid, %	3.8	2.5	6.0
Total n-3 fatty acids, %	1.35	1.0	3.0
Vitamin E, IU/kg	900	200	1200
Vitamin C, ppm	90	50	500
Lysine, g/1000 kcal	4	2.5	7

Example 2

[0036] Four foods are used for the study, experimental Formulation X, Commercial A, Commercial A1, and Commercial B. The composition of the foods is presented in Table 2. Commercial A, A1, and B foods are available from commercial sources. Commercial A and A1 are the same brand of food, but produced in different lots.

Table 2 Analyzed nutrients of foods fed to queens and kittens

Nutrient,	Formula X	Commercial A	Commercial A1	Commercial B
Crude Protein, %	41.63	41.2	36.09	35.47
Fat, %	23.15	14.47	12.43	22.94
Ca, %	1.23	1.12	1.50	1.06
P, %	1.11	1.11	1.19	0.96
DHA, %	0.22	0.06	0.04	<0.01
EPA, %	0.32	0.06	0.04	<0.01
Linoleic Acid, %	3.79	1.59	1.96	1.37
Total n-3 fatty acids, %	1.47	0.34	0.25	0.53
Total n-6 fatty acids, %	3.86	1.88	1.91	1.44
Taurine, %	0.24	0.17	0.23	0.20
Methionine, %	1.3	0.76	0.62	0.99
Cystine, %	0.49	0.51	0.44	0.35
Manganese, ppm	78	63	77	56
Vitamin E, IU/kg	914	35	76	138
Vitamin C, ppm	183	--	--	--
Choline, ppm	4624	3010	2807	3331

[0037] 19 queens are fed Formulation X or Commercial A for at least 2 weeks prior to conception. Queens are maintained in group lodging until they are confirmed pregnant via palpation, and are then moved to maternity lodging. 48 kittens are produced from queens fed Commercial A, and 16 kittens are produced from queens fed Formulation X. Following birth of kittens, the kittens from queens are kept on same foods until the kittens are weaned.

[0038] Following weaning, the 48 kittens produced from queens fed Commercial A were divided as follows: 16 kittens are fed Commercial A1 (Group A3 in Figure 1); 16 kittens are fed Commercial B (Group A2 in Figure 1); and 16 kittens are fed Formulation X (Group A1 in Figure 1). Following weaning, the 16 kittens produced from the queens fed Formulation X are maintained on Formulation X (Group B0 in Figure 1).

[0039] Brain imaging studies are performed when kittens are 7 months old to assess neurological growth and development. Brain metabolites are assessed with magnetic resonance imaging using single-voxel spectroscopy. Each subject is pre-medicated with a single bolus injection of atropine and acepromazine administered intramuscularly or subcutaneously. Anesthesia is induced using an appropriate injectable anesthetic and is maintained using an isoflurane-oxygen mixture.

[0040] Magnetic imaging procedures are conducted using a GE-LX 1.5T MRI scanner, LX hardware configuration, 9.0 software level. The subject is placed into the magnet in a prone position with the head inside a quadrature transmit-receive knee coil for imaging. The specific parameters for each acquisition type are: APRESS, or point-resolved spectroscopy (Probe-P) with pulse sequence (TR = 1500 msec; TE = 102 msec; single voxel placed in centre of brain = 8 ml; NEX = 256) is performed for each kitten. Total acquisition time is approximately 7 minutes. The concentrations of metabolites in Table 3 are determined using the LC Model fitting procedure.

Table 3: Spectroscopy metabolites and their abbreviations

Abbreviation	Metabolite
Ala	L-alanine
Asp	Aspartate
Cr	Creatine
GABA	gamma-aminobutyric acid
Gln	Glutamine
Glu	Glutamate
Ins	myo-inositol
Lac	L-lactate
NAA	N-acetylaspartate
NAAG	N-acetylasparylglutamate
Scyllo	scyllo-inositol
GPC	glycerophosphocholine
PCh	phosphocholine

[0041] A separate analysis of the ratios of each metabolite concentration to the creatine level for the same group is conducted, with a square root transformation to reduce heterogeneity of variance and non-normality. Results are presented in Figure 1 and Table 4.

Table 4: Summary of MR spectroscopy ANOVA results - metabolite:creatinine ratios

Variable	Effect	Ratio
Scyllo	Food	3.21
INS	Food	3.54
NAAG	Sex	3.99
NAA + NAAG	Sex	3.71
NAAG	Food + Sex	2.87
NAA + NAAG	Food + Sex	4.00

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A pet food composition suitable for enhancing the neurological development of a feline comprising:
 - 5 about 0.1% to about 0.7 % DHA;
 - about 2400 ppm to about 7500 ppm choline;
 - about 100 ppm to about 500 ppm carnitine;
 - about 200IU/kg to about 1200IU/kg vitamin E;
 - about 50 to about 500 ppm vitamin C;
- 10 about 2.5 g/1000 kcal to about 7 g/1000 kcal lysine;
- about 0.1 % to about 0.7 % EPA;
- about 50 ppm to about 200 ppm manganese; and
- about 0.5% to about 1.6% methionine.
2. The composition of claim 1 comprising about 0.1% to about 0.5% DHA.
- 15 3. The composition of any one of the preceding claims comprising about 3000 ppm to about 5000 ppm choline.
4. The composition of any one of the preceding claims further comprising:
 - 0 to about 90% by weight of carbohydrates;
 - about 5% to about 70% by weight of protein;
- 20 about 2% to about 50% by weight of fat;
- about 0.1% to about 20% by weight of total dietary fiber; and
- 0 to about 15% by weight of vitamins, minerals, and other nutrients.
5. A method to enhance the neurological development of a feline comprising administering to the feline or to the mother of the feline while the feline is *in*
25 *utero* the pet food composition of any one of claims 1 – 4.
6. The method of claim 5 wherein the feline is a kitten.
7. The method of claim 5 or 6 wherein the feline is born to a queen fed the composition of any one of claims 1 – 4.
8. The method of claim 7 wherein the queen is fed a composition according to any

one of claims 1 – 4 prior to pregnancy.

9. The method of claim 7 or 8 wherein the queen is fed a composition according to any one of claims 1 – 4 for a majority of the pregnancy duration.
10. The method according to claims 7 – 9 wherein the queen is fed compositions consisting essentially of any one of the compositions according to any one of claims 1 – 4 prior to and during pregnancy.
11. The method of any one of claims 6 – 10 wherein the kitten is fed a composition according to any one of claims 1 – 4 prior to weaning.
12. The method of any one of claims 6 – 10 further comprising feeding the kitten a composition according to any one of claims 1 – 4 post weaning.
13. A pet food composition comprising: about 0.1% to about 0.7 % DHA; about 2400 ppm to about 7500 ppm choline; and about 100 ppm to about 500 ppm carnitine, substantially as hereinbefore described with reference to any one of the Examples, excluding the comparative Examples.
14. A method to enhance the neurological development of a feline, substantially as hereinbefore described with reference to any one of the Examples, excluding the comparative Examples.

FIGURE 1

MR Spectroscopy Results

