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(54) **Titre : UTILISATION D'ACIDE EICOSAPENTAENOIQUE AFIN DE DIMINUER LES LESIONS CARTILAGINEUSES CHEZ LES CHIENS**
(54) **Title: USE OF EICOSAPENTAENOIC ACID FOR DECREASING CARTILAGE DAMAGE IN DOGS**

(57) **Abrégé/Abstract:**

A method for modulating enzymatic degradation of articular cartilage in a dog comprises administering to the dog an enzymatic degradation modulating effective amount of eicosapentaenoic acid (EPA), for example as a component of a food composition. By practice of the method in a dog having arthritis, mobility of the dog can be increased, weight bearing in an arthritic limb can be increased, and/or pain associated with arthritis can be reduced.

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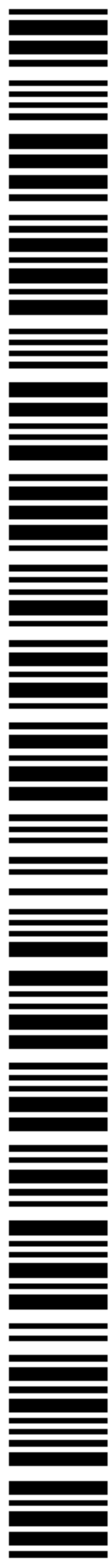
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(54) Title: METHOD FOR DECREASING CARTILAGE DAMAGE IN DOGS

(57) Abstract: A method for modulating enzymatic degradation of articular cartilage in a dog comprises administering to the dog an enzymatic degradation modulating effective amount of eicosapentaenoic acid (EPA), for example as a component of a food composition. By practice of the method in a dog having arthritis, mobility of the dog can be increased, weight bearing in an arthritic limb can be increased, and/or pain associated with arthritis can be reduced.



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USE OF EICOSAPENTAENOIC ACID FOR DECREASING CARTILAGE DAMAGE IN DOGS

BACKGROUND OF THE INVENTION

Field of the Invention

[0002] The present invention relates to methods of managing arthritis and arthritis-related conditions in companion animals, more particularly dogs.

Description of the Related Art

[0003] Arthritis, more particularly osteoarthritis, is a degenerative joint disease commonly occurring in humans and in companion animals. See for example Richardson *et al.* (1997), *Vet. Clin. North Amer. Small Animal Practice* 27, 883-911.

[0004] Osteoarthritis involves progressive deterioration of articular cartilage, with loss of proteoglycan and collagen and proliferation of new bone, accompanied by a variable inflammatory response within the synovial membrane. It is the most common form of joint and musculoskeletal disease affecting dogs but is relatively uncommon in cats. See for example Schoenherr *et al.* (2000) in Hand *et al.*, eds.: *Small Animal Clinical Nutrition*, 4th ed., 907-921, Walsworth Publishing Co., Marceline, MO; Hedborn *et al.* (2002) *Cell Mol. Life Sci.* 59, 45-53; Pool (1999) *Front. Biosci.* 4, D662-D670.

[0005] Management of osteoarthritis can include pharmacological treatments, surgery, nutraceutical administration and diet management. Such current management approaches have, however, focused on symptomatic relief and as such they have not been entirely successful in disease management or in treating the underlying pathologies. Hence there remains a continuing need for new approaches in managing osteoarthritis in companion animals, more particularly dogs.

[0006] Omega-3 (also known as n-3) fatty acids are needed in diets of mammals. They are naturally occurring materials in foods and have been used in dietary supplements. Schoenherr *et al.* (2000), *supra*, reviewed use of fatty acids including n-3 fatty acids in inflammatory disease including arthritis, and referenced a compilation by Miller *et al.* (1992) *Canine Practice* 17(6), 6-8, of observations of dog owners who perceived improvement in clinical signs of arthritis in their dogs when treated with fatty acids for dermatological problems.

[0007] Three omega-3 fatty acids are currently of most interest as dietary components: eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and alpha-linolenic acid (ALA). Hitherto no great distinction has been drawn among these three.

SUMMARY OF THE INVENTION

[0008] It has now been found that, in the canine, omega-3 fatty acids are not equal in their activity in arthritic conditions where cartilage is involved. In studies reported herein, only EPA, but not

DHA or ALA, was absorbed appreciably by canine cartilage, and cartilage damage as measured by glycosaminoglycan (GAG) release was significantly lowered after exposure to EPA, but not DHA or ALA. Benefits of administering the specific omega-3 fatty acid EPA to a dog having arthritis can include increased mobility of the dog, increased weight bearing in a limb of the dog, and reduced pain associated with the arthritis.

[0009] Accordingly there is now provided a method for increasing mobility of a dog having arthritis. The method comprises administering to the dog EPA in an amount effective to decrease cartilage damage in the dog.

[0010] There is further provided a method for increasing weight bearing in a limb of a dog having arthritis comprising administering to the dog EPA in an amount effective to decrease cartilage damage in the dog.

[0011] There is still further provided a method for reducing pain associated with arthritis in a dog comprising administering to the dog EPA in an amount effective to decrease cartilage damage in the dog.

[0012] In a further embodiment of the invention, there is provided a method for modulating enzymatic degradation of articular cartilage in a dog comprising administering to the dog an enzymatic degradation modulating effective amount of EPA.

[0013] In another embodiment, the invention provides a method for decreasing cartilage damage in a dog by administering to the dog a cartilage damaging decreasing amount of eicosapentaenoic acid.

[0014] According to the above methods, EPA can be administered by various routes, including orally as a component of a food composition or as a supplement.

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According to one aspect of the present invention, there is provided use of a dog food composition for reducing aggrecanase expression in a dog, wherein the composition comprises at least 0.4% of eicosapentaenoic acid and a total omega-3 fatty acid content of about 3.5 weight% to about 5 weight% on a dry matter basis, and at least one omega-6 fatty acid, wherein the ratio of total omega-3 fatty acids to total omega-6 fatty acids is about 1 to about 0.8 or less.

[0015] Further advantages and benefits of the present invention will be apparent to one skilled in the art from reading this specification.

DETAILED DESCRIPTION OF THE INVENTION

10 [0016] This invention involves administration of EPA as a method of managing osteoarthritic diseases and conditions, and symptoms thereof, in dogs.

[0017] Omega-3 fatty acids are a recognized group of polyunsaturated long-chain (generally 12-26 carbon atoms) carboxylic acids. The physiologically more important omega-3 fatty acids have unbranched chains that are 18-22 carbon atoms in length. All have a double bond between the 3rd and 4th carbon atoms as counted from the methyl (omega) end of the molecule. Eicosapentaenoic acid (EPA) has a chain length of 20 carbon atoms and has a total of five double bonds, including one at the omega-3 position.

[0018] When an omega-3 fatty acid, in particular EPA, is mentioned herein, it will be understood that derivatives thereof, known to those of skill in the art, can be substituted if desired. Examples of suitable derivatives include esters, such as branched or unbranched and/or saturated or unsaturated

C₁–C₃₀ alkyl or cycloalkyl esters, in particular C₁–C₆ alkyl esters, of omega-3 fatty acids, particularly EPA.

[0019] EPA can be administered to a dog by one or more of many routes of administration, such as, for example, oral, intranasal, parenteral (*e.g.*, intravenous or subcutaneous) routes and the like. The oral route is particularly suitable and EPA can be administered orally in a nutraceutical or pharmaceutical dosage form or as a component of a food composition.

[0020] When present in a food composition, which can be wet or dry, EPA can be incorporated therein, for example by any suitable mixing procedure, and/or distributed on the surface of food pieces, for example by spraying, agglomerating, dusting or precipitating on the surface. In particular embodiments EPA is present in a food composition providing the nutritional diet *per se*, in a snack, supplement or treat, or in a liquid portion of the diet such as water or another fluid.

[0021] EPA can alternatively be administered in solid form such as a powder, or in liquid or gel form, or in a nutraceutical or pharmaceutical dosage form such as a capsule, tablet, caplet, syringe or the like. Within such a dosage form the EPA can be present in solid, liquid or gel form. Any of the usual nutraceutical or pharmaceutical carriers can be employed together with the EPA, including water, glucose, sucrose and the like.

[0022] In certain embodiments, EPA-containing food compositions are administered that are essentially free of DHA and/or ALA. “Essentially free of DHA and/or ALA” is intended to mean that either or both of DHA and ALA are substantially absent or that there are only small and insignificant amounts of either or both of DHA or ALA present, for example, less than about 0.1%, less than about 0.03%, less than about 0.01%, less than about 0.003% or less than about 0.001%, by weight of the composition. In embodiments that are “essentially free of DHA and/or ALA” herein, any amount of DHA and/or ALA present is at a concentration sufficiently low so that no substantial incremental effect is produced in an osteoarthritic dog on osteoarthritis or the progression thereof or symptoms produced thereby.

[0023] In other embodiments, present with the EPA can be other omega-3 fatty acids such as DHA and ALA in significant quantities. In some embodiments, omega-6 fatty acids such as linoleic acid, gamma-linolenic acid (GLA) and/or especially arachidonic acid (AA), can also be present. Omega-3 and omega-6 fatty acids can be found in sources such as fish oils and fish meals in relatively large quantities. According to the present invention, the benefits in decreasing cartilage damage by administration of a mixture of omega-3 fatty acids, or a mixture of omega-3 and omega-6 fatty acids, are attributable largely or essentially wholly to EPA. In any such mixture, therefore, it is important that EPA be present in an amount effective to decrease cartilage damage in a dog.

[0024] EPA administered according to the present method is effective against various forms of osteoarthritis as well as other forms of arthritis including rheumatoid arthritis.

[0025] EPA acts to inhibit development of the degenerative process in joint cartilage or to diminish the degenerative process and thereby improve joint health in osteoarthritic dogs or in dogs that might otherwise develop osteoarthritis. This effect is in addition to any anti-inflammatory action of omega-3 fatty acids, which may be of less importance in canine osteoarthritis because of limited involvement of inflammation in the osteoarthritis.

[0026] Use of an *in vitro* explant procedure involving articular cartilage as shown in the examples below, demonstrated that, of EPA, DHA and ALA, the only omega-3 fatty acid to significantly decrease induced release of glycosaminoglycan (GAG) from the cartilage was EPA. GAGs are a structural component of proteoglycan, therefore, release of GAG indicates degradation of proteoglycan.

[0027] With respect to prevention of joint damage from osteoarthritis, a particular target group of dogs comprises those in need of such preventive care. For example, large breeds such as labrador retriever, rottweiler, German shepherd and the like are more susceptible to osteoarthritis as demonstrated by its greater occurrence in these breeds. Additionally, dogs above the age of about 6 years have a significantly greater occurrence of osteoarthritis. Active dogs, athletic dogs and obese dogs can also be at risk.

[0028] The quantity of EPA to be administered can vary substantially. As shown in examples herein, an actual dose response is observed – the greater the amount of EPA administered, the greater the anti-arthritic effect. Generally, a minimum of at least about 0.2% by weight of a nutritious diet satisfying ordinary daily requirements of a dog is required. In various embodiments, at least about 0.2%, at least about 0.25%, at least about 0.3%, at least about 0.4%, at least about 0.5% or at least about 0.6% by weight of the diet can be used. Suitably the diet can contain, in various embodiments, up to about 5%, up to about 4%, up to about 3%, up to about 2.5%, up to about 2.25% or up to about 2% by weight EPA. All percentages by weight herein, unless otherwise specified, are on a dry matter basis.

[0029] A specific amount of EPA can be included in the usual food ration on a daily basis, or the same amount can be provided to the animal in a treat or supplement on a daily basis. A combination of these or any other dosing means can be employed as long as an effective quantity of EPA is provided daily.

[0030] In mixtures of omega-3 and omega-6 fatty acids, the weight ratio of omega-3 to omega-6 fatty acid can vary significantly. In various embodiments, the omega-6 to omega-3 weight ratio can be about 1.1:1 to about 0.2:1 or about 1.08:1 to about 0.42:1; for example about 0.2:1, about 0.25:1, about 0.3:1, about 0.4:1, about 0.5:1, about 0.6:1, about 0.8:1, about 1.0:1, or greater. In various embodiments, the omega-6 to EPA weight ratio can be about 12.5:1 to about 1.0:1 or about 12.4:1 to about 1.12:1, for example about 0.2:1, about 0.25:1, about 0.3:1, about 0.4:1, about 0.5:1, about 0.6:1, about 0.8:1, about 1.0:1, about 1.5:1, about 2:1, about 2.5:1, about 3:1, about 4:1, about 5:1,

about 6:1, about 7.5:1, about 10:1, about 12.5:1, or greater. In various embodiments, the arachidonic acid (AA, an omega-6 fatty acid) to EPA weight ratio can be about 2.8:1 to about 0.01:1 or about 0.28:1 to about 0.08:1, for example about 0.01:1, about 0.02:1, about 0.04:1, about 0.06:1, about 0.08:1, about 1.0:1, about 1.5:1, about 2.8:1, or greater.

[0031] The EPA can be administered in amounts calculated as mg/kg body weight. Thus, for example, a 20 kg dog consumes a daily diet of about 275 g of food per day. Amounts of EPA in the diet of about 0.2%, about 0.3%, about 0.4%, about 0.5% or about 0.6% by weight would result in administering to such a dog about 27.5, about 41.25, about 55, about 68.75 or about 82.5 mg/kg body weight respectively. More particularly, EPA can be administered to a dog in an amount of about 20 to about 150 mg/kg body weight per day, for example about 20, about 28, about 30, about 40, about 41, about 50, about 55, about 60, about 69, about 70, about 80, about 82, about 90, about 100, about 120 or about 150 mg/kg body weight, or greater.

[0032] Foods are generally classified in the pet food industry as “wet” or “dry”. A wet food has a relatively high amount of water and is usually presented in a can or other container wherein air is substantially or totally excluded. Examples of such foods are “chunk and gravy” compositions, compositions having individual solid particles in the presence of a liquid gravy, and loaf-type compositions, which generally take the shape of the container. Dry foods are generally baked or extruded materials, the latter then cut into individual shaped portions, usually known as kibbles. EPA is readily incorporated into a wet food through conventional means. Encapsulation can be employed to protect EPA from air oxidation in a dry food. Additionally, use of antioxidants and nitrogen sweeps of packaging can also be employed. This is exemplified by U.S. Patent No. 4,895,725 which has special emphasis on micro-encapsulation of specific fish oils. Oils which have high levels of omega-3 fatty acids include oils of menhaden, salmon, cod and the like.

[0033] The present invention also provides, in various embodiments, methods involving administration of a composition comprising EPA to a dog for reducing the severity and frequency of clinical signs of osteoarthritis and the pain associated with this disease without substantial adverse reactions or side effects. Additionally, in various embodiments, the invention provides a method of slowing the clinical progression of an osteoarthritic condition of a dog comprising administration of a composition comprising EPA. In various embodiments, a method as described herein substantially improves the overall osteoarthritic condition of the dog so that this benefit can be objectively measured through increased weight bearing in osteoarthritic limbs. The present invention also provides methods involving administration of EPA in combination with other treatment modalities for osteoarthritis, including administration of various anti-arthritic medicaments and/or feeding the animal a weight management diet, both of which are known in the art.

[0034] It is believed that the effect of EPA in decreasing cartilage damage associated with osteoarthritis can occur at least in part through down-regulation of one or more genes responsible for

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cartilage degradation. In some cases, one or more genes responsible for cartilage degradation can be turned off. According to an embodiment of the invention, mRNA message expression in cartilage tissue of the dog for an enzyme causing cartilage degradation, for example an aggrecanase, is reduced. Decreased cartilage damage can be indicated by a decrease in induced release of glycosaminoglycan (GAG) from cartilage tissue. Thus the following are further embodiments of the invention.

[0035] A method of down-regulating one or more genes related to enzymatic degradation of articular cartilage in a dog comprising administering to the dog a gene down-regulating effective amount of EPA.

[0036] A method for turning off one or more genes related to enzymatic degradation of articular cartilage in a dog comprising administering to the dog a gene turning-off effective amount of EPA.

[0037] A method for reducing mRNA message expression in cartilage tissue of a dog for an enzyme, *e.g.*, an aggrecanase, causing cartilage degradation comprising administering to the dog an mRNA message expression reducing effective amount of EPA.

[0038] A method for decreasing induced release of GAG from cartilage tissue of a dog comprising administering to the dog a GAG release decreasing effective amount of EPA.

[0039] The invention is not limited to the particular methodology, protocols, and reagents described herein because they may vary. Further, 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. As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Similarly, the words "comprise", "comprises", and "comprising" are to be interpreted inclusively rather than exclusively.

[0040] Unless defined otherwise, all technical and scientific terms and any acronyms used herein have the same meanings as commonly understood by one of ordinary skill in the art in the field of the invention. Although any compositions, methods, articles of manufacture, or other means or materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred compositions, methods, articles of manufacture, or other means or materials are described herein.

[0041] The discussion of all patents, patent applications, publications, and other references cited or referred to herein is intended merely to summarize the assertions made therein. No admission is made that any such patents, patent applications, publications or references, or any portion thereof, is relevant prior art for the present invention and the right to challenge the accuracy and pertinence of such patents, patent applications, publications, and other references is specifically reserved.

EXAMPLES

[0042] The invention can be further illustrated by the following examples, although it will be understood that these examples are included merely for purposes of illustration and are not intended to limit the scope of the invention unless otherwise specifically indicated.

Example 1

[0043] This example illustrates the release of glycosaminoglycan (GAG) as effected by omega-3 fatty acids in cultured canine cartilage tissue.

[0044] Articular cartilage was obtained from left and right stifles (both femoral condyles and tibial plateau) of four dogs. Cartilage explants were cultured for 3 days in a medium with 10% fetal bovine serum, then washed 3 times in a serum-free medium. Explants were then cultured for 6 days in a serum-free medium containing 0, 100 or 300 µg/ml n-3 fatty acid (EPA, ALA or DHA). After this period, all explants were washed 3 times in a fatty acid-free, serum-free medium. Explants were then cultured individually in triplicate for 4 days in 1 ml fatty acid-free, serum-free medium containing no additives (control, C), 10^{-6} M retinoic acid (RA) or 50 ng/ml oncostatin M (OSM). Note that not all treatments were possible on all dogs because of cartilage availability. The release of GAG into the medium was measured (µg/mg wet weight) at the termination of culture. In the tables below, the mean and standard deviation (SD) of GAG release are given for the triplicate cultures from each of the four dogs. In addition, the media lactate concentrations (µg/mg wet weight) are given for each treatment.

Table 1: Results for Dog 1

Treatment	n	GAG release				lactate	
		mean	SD	min	max	mean	SD
C	3	1.36	0.50	0.85	1.84	26.07	33.7
C + carrier	3	1.63	0.31	1.31	1.92	21.95	22.6
C + 100 EPA	3	1.59	0.29	1.29	1.87	23.85	25.4
C + 300 EPA	3	1.04	0.53	0.57	1.61	NA**	NA**
RA	3	10.50	1.84	8.89	12.50	36.00	39.3
RA + carrier	3	7.15	4.53	2.00	10.50	33.07	45.4
RA + 100 EPA	3	8.68	2.00	6.61	10.60	29.37	34.8
RA + 300 EPA	3	1.59	1.70	0.44	3.54	26.40	39.1
OSM	3	13.60	1.56	12.60	15.40	25.37	30.8
OSM + carrier	3	14.25	6.44	7.35	20.10	27.40	33.8
OSM + 100 EPA	3	6.29	2.30	4.34	8.80	33.57	52.5
OSM + 300 EPA	3	2.17	1.93	0.93	4.39	20.05	23.8

** not analyzed

[0045] As shown in Table 1, significant decrease in GAG release occurred with 100 µg/ml EPA in OSM treated cultures and with 300 µg/ml in RA and OSM treated cultures. There was no significant decrease in media lactate concentrations with any dose of EPA.

Table 2: Results for Dog 2

Treatment	n	GAG release				lactate	
		mean	SD	min	max	mean	SD
C+ carrier	3	0.50	0.42	0.13	0.96	22.80	NA**
C+ 100 EPA	3	0.34	0.33	0.10	0.72	39.52	24.57
C+ 300 EPA	3	0.57	0.46	0.25	1.10	39.20	13.86
OSM+ carrier	3	11.70	5.11	7.10	17.20	26.90	4.77
OSM+ 100 EPA	3	5.25	3.00	2.19	8.19	21.70	9.84
OSM+ 300 EPA	3	2.83	0.23	2.66	3.09	16.23	3.60
C+ carrier	3	0.97	0.22	0.84	1.23	17.40	NA**
C+ 100 DHA	3	0.64	0.31	0.45	1.00	21.00	6.26
C+ 300 DHA	3	0.84	0.36	0.43	1.10	36.20	NA**
OSM+ carrier	3	8.7	0.78	8.10	9.60	25.33	7.11
OSM+ 100 DHA	3	8.57	4.22	3.70	11.20	28.13	2.72
OSM+ 300 DHA	3	6.07	4.03	3.18	10.70	24.80	1.95
C+ carrier	3	0.82	0.68	0.19	1.55	15.57	1.96
C+ 100 ALA	3	1.12	0.09	1.05	1.22	28.40	13.72
C+ 300 ALA	3	0.99	1.10	0.14	2.24	41.67	14.96
OSM+ carrier	3	7.81	7.47	0.26	15.20	51.70	28.49
OSM+ 100 ALA	3	8.50	4.36	4.09	12.80	28.80	4.96
OSM+ 300 ALA	3	6.42	2.73	3.44	8.80	55.23	30.31

** not analyzed

[0046] As shown in Table 2, EPA but not ALA or DHA significantly decreased GAG release in OSM treated cultures. There was no significant effect on media lactate concentration by any dose of any of the fatty acids.

Table 3: Results for Dog 3

Treatment	n	GAG release				lactate	
		mean	SD	min	max	mean	SD
C+ carrier	3	2.73	0.87	2.01	3.69	26.33	4.37
C+ 100 ALA	3	2.12	0.43	1.81	2.61	24.40	4.00
C+ 100 DHA	3	1.90	0.83	1.28	2.84	29.35	5.73
C+ 100 EPA	3	1.67	0.41	1.30	2.11	36.10	NA**
C+ 300 ALA	3	2.45	0.32	2.14	2.18	20.75	7.00
C+ 300 DHA	3	1.55	0.73	0.73	2.13	28.40	0.57
C+ 300 EPA	3	1.57	0.39	1.30	2.01	10.53	10.85
RA+ carrier	3	20.82	0.65	20.10	21.37	38.47	4.78
RA+ 100 ALA	3	20.44	0.90	19.40	21.02	43.23	2.28
RA+ 100 DHA	3	21.09	6.88	13.38	26.60	45.67	8.00
RA+ 100 EPA	3	16.22	6.65	8.61	20.93	41.53	2.52
RA+ 300 ALA	3	24.47	2.99	21.10	26.80	44.73	4.82
RA+ 300 DHA	3	19.46	2.39	17.28	22.00	47.97	9.14
RA+ 300 EPA	3	1.54	0.62	1.08	2.24	NA**	NA**
OSM+ carrier	3	12.77	5.85	6.36	17.80	37.87	11.55
OSM- 100 ALA	3	22.03	4.60	18.40	27.20	32.77	1.82
OSM- 100 DHA	3	11.67	6.01	5.50	17.50	32.27	11.47
OSM- 100 EPA	3	17.85	2.05	16.40	19.30	39.05	11.53
OSM- 300 ALA	3	23.47	3.10	20.30	26.50	34.03	1.38

Treatment	n	GAG release				lactate	
		mean	SD	min	max	mean	SD
OSM- 300 DHA	3	11.63	5.07	6.79	16.90	30.00	5.96
OSM- 300 EPA	3	8.10	6.77	3.79	15.90	21.47	1.93

** not analyzed

[0047] As shown in Table 3, none of the fatty acids significantly altered GAG release from RA- or OSM-stimulated cartilage in this particular animal. There was no change in media lactate associated with any dose of any fatty acid.

Table 4: Results for Dog 4

Treatment	n	GAG release				lactate	
		mean	SD	min	max	mean	SD
C+ carrier	3	1.96	0.53	1.51	2.55	22.93	4.75
C+ 100 ALA	3	2.10	0.11	1.98	2.17	20.53	3.48
C+ 100 DHA	3	2.34	0.33	2.00	2.66	19.10	2.35
C+ 100 EPA	3	2.69	1.00	1.72	3.71	23.00	6.18
C+ 300 ALA	3	1.53	1.24	0.13	2.50	29.17	22.07
C+ 300 DHA	3	2.31	0.36	1.93	2.65	24.93	3.40
C+ 300 EPA	3	2.10	0.45	1.64	2.55	24.77	13.00
RA+ carrier	3	14.11	3.89	9.64	16.70	34.53	12.37
RA+ 100 ALA	3	12.55	6.35	5.94	18.60	39.93	11.59
RA+ 100 DHA	3	11.28	7.12	4.79	18.90	25.60	11.77
RA+ 100 EPA	3	14.39	2.90	11.23	16.93	32.97	4.22
RA+ 300 ALA	3	14.09	6.14	8.98	20.90	59.37	31.17
RA+ 300 DHA	3	11.30	6.82	3.50	16.10	25.33	11.68
RA+ 300 EPA	3	9.09	1.32	8.26	10.61	25.10	4.67
OSM+ carrier	3	16.08	3.54	12.05	18.70	31.20	5.99
OSM+ 100 ALA	3	11.70	2.19	9.43	13.80	26.33	9.25
OSM+ 100 DHA	3	24.97	3.26	21.20	26.90	36.83	5.07
OSM+ 100 EPA	3	15.88	4.32	11.95	20.50	27.24	6.34
OSM+ 300 ALA	3	19.56	3.91	15.50	23.30	26.67	6.10
OSM+ 300 DHA	3	16.40	6.27	9.40	21.50	36.23	20.34
OSM+ 300 EPA	3	13.49	5.75	7.54	19.02	27.80	2.72

[0048] As shown in Table 4, EPA at 300 µg/ml, but not any other fatty acid at any dose, significantly decreased GAG release from RA treated cultures. There was a significant decrease in media lactate concentration in control, RA- and OSM- treated cultures with the 300 µg/ml OSM pre-treatment.

Example 2

[0049] This example illustrates the incorporation of omega-3 fatty acids into canine chondrocyte membranes.

[0050] The majority of these experiments were performed using monolayer cultures, however, in a single experiment, the incorporation of fatty acids into explant cultures of canine cartilage was analyzed.

[0051] Monolayer Cultures: Over 24 or 48 hours there was no incorporation of the 18:3 omega-3 fatty acid ALA into chondrocyte membranes from two dogs. The percentage of ALA in chondrocytes incubated in medium alone was <1% out of 5 (range 0.3–0.9%) and after 24 or 48 hours of incubation with 100 or 300 µg/ml ALA this percentage had not significantly changed (range 0.3–2.5%).

[0052] Over 48 hours there was significant incorporation of the 20:5 omega-3 fatty acid EPA into chondrocyte membranes from one dog. The percentage of EPA increased from <1% (range 0.2–0.6%) to approximately 7% (range 5.6–8%) when cultures were treated with 100 or 300 µg/ml EPA for 48 hours. The incorporation was not different when cultures were performed in the presence or absence of 5% fetal calf serum (FCS).

[0053] Over 48 hours there was significant incorporation of the 20:5 omega-3 fatty acid EPA but not the 18:3 omega-3 fatty acid ALA into chondrocyte membranes from one dog (doses of 300 µg/ml for each fatty acid). The percentage of EPA increased from <1% to approximately 15%.

[0054] Over 3 or 6 days there was significant incorporation of the 20:5 omega-3 fatty acid EPA into chondrocyte membranes from one dog (dose of 300 µg/ml EPA). The percentage of EPA increased from <1% to 16–18% with no difference between 3 and 6 days incubation.

[0055] Explant Culture: Over 6 days there was apparent incorporation of the 20:5 omega-3 fatty acid EPA, but not the 18:3 omega-3 fatty acid DHA or the omega-6 fatty acid AA into cartilage explants from one dog (dose of 300 µg/ml for each fatty acid). The percentage of EPA increased from 0% (none detectable) to approximately 2%.

[0056] Summary: These data indicated that EPA, but no other omega-3 fatty acid, was incorporated into canine chondrocyte membranes in either monolayer or explant cultures.

Example 3

[0057] This example illustrates the effect of omega-3 fatty acids on canine chondrocyte metabolism.

[0058] To assess the potential effect of omega-3 fatty acids on protein and proteoglycan metabolism in canine cartilage, cultures were set up as described in Example 1 except for the final 4 days of culture, when no catabolic stimuli were added (*i.e.*, all “control” cultures). During the final 24 hours of culture (i) ³⁵SO₄ (to measure proteoglycan synthesis) or (ii) ³⁵S-methionine and ³⁵S-cysteine (to measure protein synthesis) were added to the medium to radiolabel newly synthesized proteoglycans and proteins, respectively. The incorporation of radiolabel into the cartilage matrix was measured at the termination of culture. No attempt was made to quantitate loss of radiolabeled material from the cartilage over the 24-hour labeling period. The mean and standard deviation (SD) of the incorporation of ³⁵SO₄ (“PG”) or ³⁵S-methionine and ³⁵S-cysteine (“PROT”) as DPM/mg wet weight are shown in Table 5 below.

Table 5

Treatment	n	PG		PROT	
		mean	SD	mean	SD
Carrier	3	292.7	53.1	574.3	198.3
100 ALA	3	246.3	100.8	503.7	184.2
100 DHA	3	156.0	82.5	503.7	81.34
100 EPA	3	537.3	161.8	442.0	72.7
300 ALA	3	443.0	205.4	393.7	35.0
300 DHA	3	123.3	38.2	564.3	220.0
300 EPA	3	275.7	161.7	504.0	44.5

[0059] As shown in Table 5, there was no significant effect of any omega-3 fatty acid on protein synthesis and incorporation into the matrix. EPA at 100 µg/ml significantly increased proteoglycan synthesis and incorporation. No other dose or fatty acid significantly altered proteoglycan synthesis and incorporation into the cartilage matrix.

[0060] Reverse transcription-PCR was used to measure the mRNA message expression levels of matrix proteinases (aggrecanases-1 and -2), cyclooxygenases-1 and -2, lipoxygenases-5 and -12, and potential autocrine cytokines and their receptors (*e.g.*, IL-1, IL-6 and TNF).

[0061] The results of this study found that aggrecanase-1 and aggrecanase-2 mRNA messages were expressed in “normal” canine cartilage tissue. In addition, some dogs expressed mRNA message of cyclooxygenase-2 (COX-2) message although there were no signs of joint pathology in these animals. This enabled monitoring the effects of omega-3 and omega-6 fatty acid supplementation on mRNA expression of aggrecanases and COX-2 in unstimulated canine articular cartilage explants. EPA was the only fatty acid able to reduce the mRNA message for the degradative enzymes, aggrecanase-1 and aggrecanase-2, in canine articular cartilage. This demonstrated the ability of EPA to “turn off” the genes responsible for cartilage degradation.

Example 4

[0062] This example illustrates the effects of omega-3 fatty acids in canine osteoarthritis clinical studies.

[0063] Three clinical studies were conducted in pet dogs clinically diagnosed with osteoarthritis. Veterinary general practitioners and orthopedic specialists enrolled client owned dogs that met specific eligibility criteria. All patients were required to (i) have radiographic evidence of osteoarthritis with measurable clinical manifestations of disease, based on historical accounts by pet owners and physical examinations by veterinarians; (ii) be otherwise healthy and free of concurrent diseases based on physical exam, complete blood count (CBC), blood chemistry and urinalysis; and (iii) maintain regimen of therapy if receiving medications or supplements prescribed for osteoarthritis during the 30 days prior to enrolling in the study.

[0064] The following measurements were made. Serum fatty acid profile: This was determined by a gas chromatography method involving extraction of fatty acids by chloroform and methanol

mixture (2:1), methylation using boron trifluoride-methanol (BF₃:MeOH) reagent followed by flame ionization detection (FID). Fatty acid methyl esters were identified by comparison of retention times with those of known standards and quantitated using an internal standard. Veterinary clinical evaluation: Veterinarians conducted both a physical exam and a clinical evaluation of the patient's osteoarthritic condition during the screening phase and at the conclusion of each of the feeding intervals over the course of the clinical trial. Veterinarians assessed the severity of five osteoarthritic parameters: lameness, reluctance to bear weight, reduction in range of motion, reluctance to hold up contra-lateral limb, and pain on palpation of the joint. Changes in severity scores for these individual parameters were measured over the duration of the feeding period. A comprehensive veterinary clinical assessment of the impact of dietary intervention on the osteoarthritic condition of patients was derived by combining the changes in severity scores for all five individual parameters.

[0065] Pet owner subjective evaluation. Pet owners were required to complete an enrollment questionnaire prior to participating in the study and additional questionnaires at the conclusion of each of the feeding intervals over the course of the clinical trial.

[0066] Enrollment questionnaire. Pet owners rated the observed frequency and severity of the most common signs of canine osteoarthritis including difficulty rising from rest, limping, stiffness, soreness when touched, lagging behind during walks, yelping or whimpering in pain, aggressive behaviors, difficulty in running, difficulty in walking, difficulty in climbing steps, difficulty in jumping, difficulty in playing, impaired mobility, and overall activity level. In addition, owners rated the overall osteoarthritic condition of their pet.

[0067] Feeding questionnaire. Pet owners rated both the frequency and change in severity of the signs of canine osteoarthritis which were benchmarked during enrollment. In addition, the pet owners rated the severity of their animal's pain associated with osteoarthritis.

[0068] Force plate gait analysis. Dogs were evaluated at each institution using a computerized biomechanics force plate at 0, 6 and 12 weeks. The plate was mounted centrally in and flush with the surface of a 10 m walkway. A handler trotted dogs across the force plate and an observer evaluated each pass across the plate to confirm foot-strikes and gait. A trial was considered valid if there were distinct ipsilateral fore foot and hind foot strikes while the dog was trotted across the force plate at a velocity of 1.7–2.0 m/s, with an acceleration variation of -0.5–0.5 m/s². During each trial, the dog's forward velocity was measured, using a millisecond timer and two photoelectric switches. Each trial was videotaped for review and confirmation of valid foot-strikes. Care was taken to ensure that the dog triggered the timer and that a consistent speed (as perceived by the handler and observer) was maintained across the plate during each trial.

[0069] Five valid trials for each test period were obtained for each affected limb and each ipsilateral limb of each dog. Orthogonal ground reaction forces of peak vertical force, vertical impulse, braking and propulsive peak forces, and braking and propulsion impulses were measured

and recorded by a specialized software program. (Acquire, Sharon Software, DeWitt, MI), All forces were normalized with respect to body weight in kilograms. Data from the valid trial for each limb were averaged to obtain a mean value for each force or impulse at each time period.

[0070] Ground reaction force data were compared between treatment and placebo groups as a percentage difference between lame and ipsilateral limbs at each time period. Percentage change of ground force data on the lame limb were compared at the beginning and end of the feeding period.

[0071] Study Number 1: A canine study was conducted to evaluate the dietary effect of feeding increased levels of n-3 fatty acids to dogs diagnosed with osteoarthritis. Eighteen veterinary general practitioners were recruited to enroll patients in the study. A total of 131 dogs were randomly assigned to two dietary treatments and fed for 180 days. The test and control foods had similar macronutrient profile, but were significantly different in fatty acid composition (Table 6). The test diet contained high levels of ALA, EPA and DHA, and was formulated with a low n-6/n-3 ratio. The control diet was a leading selling commercially available dog food, with typical levels of n-3 fatty acids and an n-6/n-3 ratio characteristic for the industry.

Table 6

Dietary nutrient	Control food (%)	Test food (%)
Protein	23.2	19.9
Fat (total)	13.9	13.6
CHO ₂ (NFE*)	54.7	53.3
ALA (n-3)	0.12	2.8
AA (n-6)	0.03	0.06
EPA (n-3)	<0.01	0.38
DHA (n-3)	<0.01	0.31
Sum n-6	1.99	2.53
Sum n-3	0.09	3.48
n6/n3 ratio	22.8	0.7

*NFE = Soluble carbohydrate content as nitrogen free extract

[0072] Serum fatty acids and pet owner evaluations were recorded at 0, 45, 90 and 180 days. Serum fatty acid profiles were significantly modulated by the test food. The test group had significantly higher concentrations of n-3 fatty acids ($P<0.01$), specifically EPA, DHA and ALA, significantly lower concentrations of AA ($P<0.01$), and significantly lower n-6/n-3 ratios ($P<0.01$) as compared to the control group at the conclusion of each feeding interval (Table 7). The test group showed significant improvements for rising from rest, running and playing at day 45 and walking at days 90 and 180 as compared to the control group based on pet owner observations ($P<0.05$), even in the presence of a strong placebo effect (Table 8).

Table 7: Mean Serum Fatty Acid Levels (mg/dl)

	Group	Day 0	Day 45	Day 90	Day 180
ALA (n-3)	Control	1.10	0.89	0.52	0.53
	Test	1.05	5.61	6.51	7.13
AA (n-6)	Control	71.35	66.34	68.03	68.21

	Test	64.32	45.90	46.13	42.65
EPA (n-3)	Control	1.14	0.90	0.67	0.93
	Test	1.28	16.28	18.64	19.94
DHA (n-3)	Control	2.67	2.03	1.70	1.98
	Test	2.93	11.31	12.24	12.17
Sum n-6	Control	141.08	138.72	137.85	140.28
	Test	130.85	118.87	128.71	123.99
Sum n-3	Control	4.95	3.84	2.93	3.51
	Test	5.36	33.20	37.39	39.24
n-6/n-3 ratio	Control	33.33	37.95	51.59	51.39
	Test	33.90	7.47	8.63	6.92

Table 8: Pet Owner Observed Change in Severity of Osteoarthritis*

Osteoarthritic sign	Group	Day 0-45		Day 45-90		Day 90-180	
		mean	P value	mean	P value	mean	P value
Rising from rest	Control	1.77	.041	1.77	nsd**	1.93	nsd**
	Test	1.56		1.84		1.91	
Running	Control	1.81	.037	1.83	nsd**	1.94	nsd**
	Test	1.56		1.71		1.91	
Walking	Control	1.71	nsd**	2.00	.018	2.19	.002
	Test	1.69		1.71		1.75	
Playing	Control	1.83	.008	1.90	nsd**	2.06	nsd**
	Test	1.50		1.78		1.97	

* Osteoarthritis severity rating scale: 1 = better, 2 = no change, 3 = worsened.

** nsd = no significant difference.

[0073] Study Number 2: A canine study was conducted to evaluate the dietary effect of feeding increased levels of n-3 fatty acids to dogs diagnosed with osteoarthritis. Two veterinary orthopedic specialists enrolled patients in the study. A total of 38 dogs were randomly assigned to two dietary treatments and fed for 90 days. The test and control diets were manufactured from the same lots of foods as described above (Table 6).

[0074] Serum fatty acids, force plate gait analysis, and veterinary clinical assessments were recorded at 0, 45 and 90 days. Serum fatty acid profiles were significantly modulated by the test food. The test group had significantly higher serum concentrations of n-3 fatty acids ($P < 0.01$), specifically EPA, DHA and ALA, significantly lower concentrations of AA at day 90 ($P < 0.01$), and significantly lower n-6/n-3 ratios ($P < 0.01$) as compared to the control group at the conclusion of each feeding interval (Table 9).

Table 9: Mean Serum Fatty Acid Levels (mg/dl)

	Group	Day 0		Day 45		Day 90	
		mean	P value	mean	P value	mean	P value
ALA (n-3)	Control	0.89	0.7764	0.34	<0.0001	0.27	<0.0001
	Test	0.98		4.45		5.04	
AA (n-6)	Control	55.55	0.6880	50.78	0.0736	55.95	0.0001
	Test	57.13		41.94		38.01	
EPA (n-3)	Control	1.19	0.7000	0.34	<0.0001	0.20	<0.0001
	Test	1.54		11.52		11.89	

	Group	Day 0		Day 45		Day 90	
		mean	P value	mean	P value	mean	P value
DHA (n-3)	Control	4.30	0.4323	1.82	<0.0001	1.32	<0.0001
	Test	3.37		11.15		11.21	
Sum n-6	Control	122.85	0.2508	112.46	0.0148	114.60	0.0036
	Test	113.61		91.72		89.85	
Sum n-3	Control	6.36	0.8335	2.57	<0.0001	1.79	<0.0001
	Test	5.90		27.14		28.13	
n-6/n-3 ratio	Control	32.54	0.2521	66.66	<0.0001	75.90	<0.0001
	Test	45.90		8.48		3.59	

[0075] A biomechanical assessment of the dogs' most severe osteoarthritic limb was objectively evaluated using force plate gait analysis (Table 10). Vertical peak force is the key parameter measured to determine weight bearing of the affected limb. There was no significant change in mean vertical peak force over the duration of the 90 day feeding for the control group ($P=0.91$), while there was a significant increase in mean vertical peak force over time for the test group ($P=0.01$). The percent mean change in vertical peak force was also significantly different between groups ($P<0.05$), indicating that the test group increased weight bearing in the affected limb, while the control group displayed no change in weight bearing over the course of the study. Weight bearing ability can also be represented by displaying the frequency distribution of percent change in vertical peak for each dietary group. Only 31% of animals in the control group showed improvement in weight bearing after the 90 day feeding, while 82% of the dogs in the test group increased weight bearing over the course of the study.

Table 10: Vertical Peak Force

Group	Day 0		Day 90		Change (Day 0-90)			
	mean	P value	mean	P value	mean change	mean = 0 Pr > t	% mean change	Pr > t
Control	72.80	0.5981	72.63	0.9323	-0.17	0.9144	-0.58	0.0443
Test	69.51		73.21		3.71	0.0103	5.35	

[0076] The subjective clinical evaluations performed by the veterinary orthopedic surgeons provided additional support for the efficaciousness of the test diet. Based upon the comprehensive veterinary clinical assessment, a significantly greater percent of dogs were evaluated as improved that consumed the test food as compared to dogs that consumed the control food ($P<0.05$). The veterinary specialists also observed a greater percent of dogs in the test group displaying a reduction in pain on palpation of the joint as compared to the control group ($P=0.05$).

[0077] Study Number 3: A canine study was conducted to determine the dose effect of feeding increased levels of n-3 fatty acids to dogs diagnosed with osteoarthritis. Twenty-eight veterinary general practitioners enrolled patients in the study. A total of 177 dogs were randomly assigned to three dietary treatments and fed for 90 days. Approximately two-thirds of the dogs participating in the study were receiving medications and/or supplements prescribed for treating osteoarthritis, in

addition to consuming the therapeutic diets being evaluated. The three test foods had similar macronutrient profiles, but varied in composition of EPA and DHA, with variable A containing the lowest levels and variable C containing the highest levels (Table 11).

Table 11

Dietary nutrient	Test variable %		
	A	B	C
Protein	19.97	19.51	19.37
Fat (total)	13.78	15.34	19.55
CHO ₂ (NFE*)	53.92	52.34	47.66
ALA (n-3)	2.65	1.18	1.10
AA (n-6)	0.11	0.18	0.24
EPA (n-3)	0.50	1.18	1.69
DHA (n-3)	0.34	0.80	1.15
Sum n-6	2.70	2.45	2.14
Sum n-3	3.54	3.53	4.52
n-6/n-3 ratio	0.76	0.70	0.47

*NFE = Soluble carbohydrate content as nitrogen free extract

[0078] Serum fatty acids, pet owner evaluations, and veterinary clinical assessments were recorded at 0, 21, 45 and 90 days. Serum fatty acid profiles were significantly modulated by all dietary variables. The dogs fed test variables B & C had significantly higher serum concentrations of n-3 fatty acids ($P<0.01$), specifically EPA, DHA and ALA, significantly lower concentrations of n-6 fatty acids, specifically AA ($P<0.01$), and significantly lower n-6/n-3 ratios ($P<0.01$) as compared to the dogs fed test variable A at the conclusion of each feeding interval (Table 12).

Table 12: Mean Serum Fatty Acid Levels (mg/dl)

	Test variable	Day 0	Day 21	Day 45	Day 90
ALA (n-3)	A	1.34	5.65	5.29	5.63
	B	1.29	3.36	3.99	3.82
	C	1.25	2.92	3.32	3.29
AA (n-6)	A	76.37	51.10	47.54	47.77
	B	73.15	41.55	38.94	37.00
	C	70.05	37.35	36.86	34.73
EPA (n-3)	A	1.32	18.74	18.51	19.26
	B	1.54	26.14	29.87	30.03
	C	1.85	34.42	35.71	39.04
DHA (n-3)	A	3.50	13.75	13.84	13.88
	B	4.72	18.47	19.98	20.16
	C	3.91	21.01	21.47	22.49
Sum n-6	A	150.38	114.38	110.12	112.70
	B	143.93	93.83	95.87	92.10
	C	139.97	79.71	82.65	80.74
Sum n-3	A	6.16	38.14	37.65	38.77
	B	7.55	47.96	53.84	54.01
	C	7.01	58.35	60.50	68.83
n-6/n-3 ratio	A	29.99	5.65	3.48	3.75

	Test variable	Day 0	Day 21	Day 45	Day 90
	B	28.09	3.36	1.92	1.79
	C	32.30	2.92	2.02	1.73

[0079] Pet owners reported improvements in 13 of 14 individual osteoarthritic signs for dogs consuming any of the dietary variables for 21 days (Table 13). Additionally, pet owners reported a decrease in severity for 13 of 14 individual osteoarthritic signs for dogs consuming any of the dietary variables for 90 days (Table 14). Pet owners also reported a significant reduction in the frequency of observable osteoarthritic signs after the dogs consumed any of the dietary variables for 90 days (Table 15).

Table 13: Pet Owner Observed Improvements in Osteoarthritic Signs (Day 0–21)

Osteoarthritic Sign	Diet	Mean	Mean = 0 Pr > t
Rising from rest	A	-0.439	0.0002
	B	-0.738	<0.0001
	C	-0.763	<0.0001
Limping	A	-0.720	<0.0001
	B	-0.731	<0.0001
	C	-0.837	<0.0001
Stiffness	A	-0.537	<0.0001
	B	-0.783	<0.0001
	C	-0.627	<0.0001
Soreness	A	-0.750	0.0005
	B	-0.800	0.0002
	C	-0.379	0.0451
Lagging behind on walks	A	-0.564	0.0004
	B	-0.909	<0.0001
	C	-0.531	0.0022
Pain	A	-0.476	0.0245
	B	-0.478	0.0184
	C	-0.889	0.0002
Aggression	A	0.000	1.0000
	B	-0.313	0.1050
	C	-0.429	0.1401

Osteoarthritic Sign	Diet	Mean	Mean = 0 Pr > t
Running	A	-0.524	0.0004
	B	-0.682	<0.0001
	C	-0.674	<0.0001
Walking	A	-0.553	0.0007
	B	-0.750	<0.0001
	C	-0.667	<0.0001
Stair climbing	A	-0.449	0.0012
	B	-0.667	<0.0001
	C	-0.723	<0.0001
Jumping	A	-0.362	0.0049
	B	-0.600	<0.0001
	C	-0.542	<0.0001
Playing	A	-0.622	<0.0001
	B	-0.763	<0.0001
	C	-0.487	0.0014
Impaired mobility	A	-0.528	0.0005
	B	-0.700	<0.0001
	C	-0.564	0.0001
Activity level	A	-0.745	<0.0001
	B	-0.857	<0.0001
	C	-0.865	<0.0001

The above “p” values refer to the mean change from day 0 to day 21

Table 14: Difference in Pet Owners’ Severity Rating (Day 0-90)

Osteoarthritic Sign	Diet	Mean	Pr > t
Rising from rest	A	-0.463	<0.0001
	B	-0.633	<0.0001
	C	-0.518	<0.0001
Limping	A	-0.489	0.0003
	B	-0.588	<0.0001
	C	-0.681	<0.0001
Stiffness	A	-0.255	0.0420
	B	-0.483	<0.0001

Osteoarthritic Sign	Diet	Mean	Pr > t
Running	A	-0.579	<0.0001
	B	-0.558	<0.0001
	C	-0.605	<0.0001
Walking	A	-0.294	0.0358
	B	-0.643	<0.0001
	C	-0.595	<0.0001
Stair climbing	A	-0.419	0.0024
	B	-0.489	0.0002

	C	-0.589	<0.0001		C	-0.689	<0.0001
Soreness	A	-0.810	<0.0001	Jumping	A	-0.571	0.0003
	B	-0.920	<0.0001		B	-0.479	0.0011
	C	-0.926	<0.0001		C	-0.773	<0.0001
Lagging behind on walks	A	-0.657	<0.0001	Playing	A	-0.606	0.0002
	B	-0.531	0.0014		B	-0.571	0.0003
	C	-0.448	0.0094		C	-0.694	<0.0001
Pain	A	-0.684	0.0002	Lameness	A	-0.484	0.0045
	B	-0.571	0.0009		B	-0.778	<0.0001
	C	-0.667	0.0010		C	-0.667	<0.0001
Aggression	A	-0.750	0.0234	Activity level	A	-0.409	0.0009
	B	-1.000	0.0025		B	-0.704	<0.0001
	C	-1.000	0.0751		C	-0.551	<0.0001

The above “p” values refer to the mean change from day 0 to day 90.

Table 15: Difference in Pet Owners’ Frequency Rating (Day 0-90)

Osteoarthritic Sign	Diet	Mean	Pr > t	Osteoarthritic Sign	Diet	Mean	Pr > t
Rising from rest	A	-0.370	<0.0001	Limping	A	-0.239	<0.0165
	B	-0.467	<0.0001		B	-0.365	<0.0001
	C	-0.509	<0.0001		C	-0.396	<0.0001
Stiffness	A	-0.098	0.2929	Lagging behind on walks	A	-0.571	<0.0001
	B	-0.373	<0.0001		B	-0.643	<0.0001
	C	-0.421	<0.0001		C	-0.500	0.0004
Soreness	A	-0.381	0.0146	Aggression	A	-0.417	0.0536
	B	-0.680	<0.0001		B	-0.467	0.0175
	C	-0.821	<0.0001		C	-0.167	0.5741
Running	A	-0.447	0.0004	Walking	A	-0.206	0.0911
	B	-0.395	0.0009		B	-0.558	<0.0001
	C	-0.477	<0.0001		C	-0.447	0.0002
Jumping	A	-0.357	0.0027	Stair climbing	A	-0.302	0.0069
	B	-0.354	0.0015		B	-0.348	0.0014
	C	-0.467	<0.0001		C	-0.457	<0.0001
Playing	A	-0.455	0.0013	Impaired mobility	A	-0.250	0.0643
	B	-0.297	0.0238		B	-0.436	0.0005
	C	-0.667	0.0010		C	-0.667	<0.0001

[0080] Dogs consuming higher concentrations of n-3 fatty acids were reported to have more significant improvement in osteoarthritic condition and more significant reduction in the progression of osteoarthritis than those dogs receiving the lowest dosage, based on veterinarians clinical assessments (Table 16). There was no significant difference in improvement in osteoarthritic condition or reduction in the progression of osteoarthritis between the group receiving medications and/or supplements and the non-medicated group (Table 17). This indicates that the therapeutic diets work synergistically with other therapies or at least not withstanding other therapies by providing additional benefit to dogs suffering from osteoarthritis.

[0081] An extremely low incidence of adverse reactions or side effects was reported among dogs participating in this study. Only five dogs out of the 215 animals assigned to food were reported to

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have diarrhea and vomiting, which could possibly be attributed to consuming one of the dietary variables. Similar incidence of adverse reactions or side effects were reported for those dogs consuming the therapeutic diets in the previous two studies discussed (1/88 and 1/26 for examples 1 and 2 respectively).

Table 16

Progression of osteoarthritic condition					Overall change in osteoarthritic condition				
Diet	n	mean	P		Diet	n	mean	P	
A	55	2.327	0.2891	A vs B	A	54	3.148	0.1675	A vs B
B	62	2.177	0.1619	B vs C	B	62	2.871	0.0787	B vs C
C	59	1.983	0.0168	A vs C	C	59	2.525	0.0024	A vs C

Table 17

Progression of osteoarthritic condition					Overall change in osteoarthritic condition				
Diet	Medicated	n	mean	P	Diet	Medicated	n	mean	P
A	no	22	2.273	0.6665	A	no	21	3.143	0.9770
	yes	33	2.364			yes	33	3.152	
B	no	23	2.130	0.7109	B	no	23	2.696	0.3247
	yes	39	2.205			yes	39	2.974	
C	no	28	2.071	0.4003	C	no	28	2.750	0.1285
	yes	31	1.903			yes	31	2.323	

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CLAIMS:

1. Use of a dog food composition for reducing aggrecanase expression in a dog, wherein the composition comprises at least 0.4% of eicosapentaenoic acid and a total omega-3 fatty acid content of about 3.5 weight% to about 5 weight% on a dry matter basis, and at least
5 one omega-6 fatty acid, wherein the ratio of total omega-3 fatty acids to total omega-6 fatty acids is about 1 to about 0.8 or less.
2. The use of claim 1, wherein the composition decreases release of glycosaminoglycan from cartilage tissue of the dog.
3. The use of claim 1 or 2, wherein the composition is a component of a
10 nutritional diet, a snack, a supplement or a treat.
4. The use of claim 1, 2, or 3 wherein the composition is for administration of eicosapentaenoic acid to the dog in an amount of from about 20 to about 150 mg/kg body weight of the dog per day.