TECHNICAL REPORT for VETERINARIAN USE ONLY

Robert J. Silver, MS, DVM
RxVitamins for Pets Formulator and Clinical Consultant

ULTRA EFA FORMULA - New Higher Potency

THERAPEUTIC STRATEGY:

1) Provides nutritional support for a variety of active tissues such as the epidermis and adnexal structures, kidney, bowel, immune system, heart, brain, endocrine system

2) Dampens pro-inflammatory eicosanoid cascade via two eicosanoid pathways (now has GLA from borage seed oil to enhance PG1 cascade, as well as EPA’s beneficial influence on PG3 pathways)

3) Provides balanced amounts of the essential fatty acid, linoleic acid, an omega 6 oil, in fresh, unadulterated form, and also provides the essential membrane nutrients, zinc, biotin and lecithin

4) Omega-3 to Omega-6 ratio of this custom formulation dietary oil is: 2:1. This makes this oil highly anti-inflammatory by its regulation of eicosanoid cascade. Suggested tissue ratio of Omega-3 to Omega-6 is 1:4. The higher level of omega-3 provided by UltraEFA™ Formula is designed to counter-balance the ordinarily very high level of omega-6’s found in grain-based and grain-fed meat based diets. The addition of fresh omega-3 and omega-6 fatty acids in this formula is designed to help bring the body’s balance of omega3:6 fatty acids closer to the optimal 1:4-5 ratio recommended by nutritionists.

INGREDIENT LIST: (per level teaspoon)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marine Lipid Concentrate</td>
<td>2000 mg</td>
</tr>
<tr>
<td>Eicosapentaenoic acid (18% EPA)</td>
<td>360 mg</td>
</tr>
<tr>
<td>Docosahexaenoic acid (12% DHA)</td>
<td>240 mg</td>
</tr>
<tr>
<td>Borage seed oil (contains 22% GLA)</td>
<td>500 mg</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>500 mg</td>
</tr>
<tr>
<td>Supplies 300mg Linoleic Acid</td>
<td></td>
</tr>
<tr>
<td>Lecithin</td>
<td>1265 mg</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>304 mg</td>
</tr>
<tr>
<td>Phosphatidylinositol</td>
<td>240 mg</td>
</tr>
<tr>
<td>Zinc gluconate</td>
<td>2.3 mg</td>
</tr>
<tr>
<td>Biotin</td>
<td>23 mcg</td>
</tr>
<tr>
<td>Rosemary Extract</td>
<td>2.3 mg</td>
</tr>
<tr>
<td>Mixed tocopherols</td>
<td>45 IU</td>
</tr>
</tbody>
</table>
POTENTIAL UltraEFAs USES:

1. Conditions of the Skin (Chung S, 2002)
2. Renal Disease (Plotnick 1996)
3. Inflammatory diseases
   a. IBD (Belluzi, Brignola et al. 1996).
   b. Asthma (Thien and al. 1996)
   c. Epilepsy
   d. Rheumatoid (Kremer 1996 (Suppl.)
   e. Discoid Lupus (Chung S, 2002)
   f. Amyloidosis (Thornhill, 2003)
3. Autoimmune disease (Harbige LS, 2001), (Furse RK, 2002)
4. Hepatitis (Choi and al. 1992)
5. Acute Pancreatitis (Foitzik, et al. 2002)
6. Cardiovascular Disease
   a. Arrhythmias (McLennan 1993)
   b. Hypertension (Schoene and Fiore 1981)
   c. CHF
   d. Cardiomyopathy
3. Oncology patient support (Chamras H, 2002)

INGREDIENTS INFORMATION:

1.) Marine Lipid Concentrate (2000mg/teaspoon) containing 360mg EPA/tsp and 240 mg DHA/tsp; is the purified extract of oils derived from cold water fish, containing high assays of eicosapentaenoic acid (EPA: C20:5-ω3), docosahexaenoic acid (DHA; C22:6-ω3) and low levels of lipid peroxidation verified by certificate of analysis. Supplementation with EPA competes with arachidonic acid for the common eicosanoid producing enzymes, 5-lipoxygenase and 15-lipoxygenase, and produces leukotriene B₅ and 15-hydroxyeicosapentaenoic acid (15-HPTE).

Both of these EPA metabolites inhibit the production of the pro-inflammatory eicosanoid, leukotriene B₄. Phospholipase A₂, which is found in cell membranes, is released following physical or biological stimuli, such as hypersensitivity reactions, or tissue damage. Phospholipase A₂ precipitates the release of arachidonic acid from the cell membranes. Arachidonic acid is then acted upon by either the lipoxygenase or cyclooxygenase pathways, resulting in a pro-inflammatory response. EPA will dampen that response (White 1993).

Logas, 1993, determined in a double-blinded placebo controlled cross-over study that approximately 180 mg of EPA per 10 pounds of body weight was sufficient to significantly reduce dermal inflammation causing pruritis.
Other studies have determined the value of supplementation with EPA for conditions of renal failure (Plotnick 1996), liver disease (Choi and al. 1992), immune disorders (Berger and al. 1993), neurologic development (improved by DHA) (Nettleton 1993), cardiac arrhythmias (McLennan 1993), hypertension (Schoene and Fiore 1981), and inflammatory disorders such as rheumatoid arthritis (Kremer 1996 (Suppl.)), asthma (Thien and al. 1996), and inflammatory bowel disease (Belluzi, Brignola et al. 1996).

2.) Borage seed oil (22% gamma linolenic acid (GLA)) (Supplying 110 mg/tsp of GLA and 390 mg Linoleic acid/tsp)

Gamma-linolenic acid (GLA) is an important fatty acid that is found primarily from plant sources. Linoleic acid, which is found in cooking oils and processed foods, is converted into GLA in the body. GLA supplements are available in the form of evening primrose oil, black currant seed oil, and borage oil, which also provide linoleic acid. For example, evening primrose oil is 72 percent linoleic acid. The average North American diet provides more than 10 times the necessary amount of linoleic acid.

Diabetics are less able than “sugar-healthy” individuals to convert linoleic acid to GLA. Other conditions that appear to reduce the body's ability to convert linoleic acid to GLA include aging, atopic dermatitis, and other inflammatory conditions such as, rheumatoid arthritis, cancer, auto-immune disease, and cardiovascular disease. Aging also appears to reduce conversion of linoleic acid to GLA.

Research has shown that GLA may help prevent cardiovascular disease by dilating blood vessels, normalizing blood pressure, and preventing cardiovascular disease in humans.

Cancer is another condition where GLA serves an important function. Studies in people with colon cancer, breast cancer, and melanoma show that GLA inhibits the growth of tumors and the spread of cancer.

For human hemodialysis patients with uremia with associated skin symptoms, studies demonstrated that patients improved when the subjects used evening primrose oil supplements. Studies suggest that GLA is also helpful in increasing bone density and calcium absorption in people who have osteoporosis.

3.) Sunflower seed oil: (Supplying 300 mg linoleic acid and 200 mg of the omega 9 fatty acid.) The oil in this formula provides a pure fresh source of this essential fatty acid without over-supplying the already abundant omega 6 fatty acids as fed in most pets’ diets. The precise proportion of Omega3:Omega6 in the diet that would produce the desired 1:4-5 ratio in the tissues is not known. Temperatures greater than 250° F. will disrupt the molecular integrity of linoleic acid.

4.) Lecithin (1265 mg) is a palatable source of phospholipids, which are a structural component of cell membranes as well as being essential metabolic intermediaries for a number of biological processes including the synthesis of the neurotransmitter, acetylcholine (Rosenberg and Davis 1982). Phosphatidylcholine (PC), is considered to be
the active ingredient in lecithin and assists in the transport and utilization of fatty acids and lipoproteins. Large concentrations of PC in the body are found in combination with lipoproteins in the cell membrane, where this combination facilitates the passage of fat in and out of the cell. PC in the blood assists in the transport of lipids. (Wojcicki and al 1995) PC is essential for the proper metabolism of fats; its absence allows the deposition of fat in the liver leading to hepatic lipidosis. In this formulation, lecithin assists with fatty acid metabolism, emulsifies the EFA’s in this formula, and ensures healthy cell membrane structure and function.

5.) Zinc gluconate (2.3 mg/tsp) is a chelated form of elemental zinc, which delivers this metal more efficiently to the tissues than other metallic forms of zinc such as zinc oxide or sulfate (Cunliffe and al. 1979). Zinc is involved in over 300 enzymatic reactions in the body. Zinc is necessary for proper immune system function (Bogden and al. 1987); it is required for protein synthesis, cell growth and wound healing (Tuormaa 1995); zinc is also important for normal skin function (Loeffel and Koya 1978).

6.) Biotin (23 mcg/tsp) is a B vitamin that has been determined to play a significant role in the healthy formation of skin, hair and nails (Murray 1996). Biotin deficiency results in dry, scaly skin, seborrhea and alopecia. (Nisenson 1972) Biotin plays a role in the hardening of nails and hooves (Hochman and al. 1993). Biotin is necessary for long-chain fatty acid synthesis (Murray 1996).

7.) Vitamin E (45 IU) has been included in this formula as an antioxidant for its ability to reduce lipid peroxidation, an important consideration when dealing with fragile omega 3 fatty acids. Lipid peroxidation can be an important source of oxidant stress, leading to a variety of degenerative clinical conditions. (Zapsalis and Beck 1985) Vitamin E is a lipid-phase antioxidant due to its prevalence in lipid-soluble body compartments such as cell membranes and fatty molecules. (Krummel 1996) Supplementation with polyunsaturated fatty acids increases vitamin E requirements. (Codner and Thatcher 1993) Recent studies have pointed out the limitations of using Vitamin E alone as a lipid anti-oxidant (Allard and al. 1997).

8.) Rosemary Extract (23 mg) has been included in this formula based on its superlative properties as a preservative and anti-oxidant. (Halliwell and al. 1995) Rosemary extract has also found to have potent anti-microbial activity. (Panizzi and al. 1993)
ULTRAEFAs USES:

1. Conditions of the skin: Atopy (Soyland, Funk et al. 1993), non-specific dermatitis, dry hair coat, seborrhea (Ackerman 1995), food allergy, keratinization disorders (Kettler and al. 1988), miliary dermatitis (Harvey 1993), hot spots, staph dermatitis, pododermatitis, sebaceous adenitis, follicular dysplasia (Kuhl 1996)

2. Conditions responsive to omega 3 fatty acid supplementation: Renal disease (Plotnick 1996), inflammatory diseases (arthritis (Fortin, Lew et al. 1995), and inflammatory bowel disease (Lorenz and al. 1989)), autoimmune disease (SLE) (Mohan and Das 1997), liver disease (hepatic lipidosis, cholangiohepatitis, liver failure), respiratory diseases (asthma (Knapp 1995), and COPD (Britton 1995)), cardiovascular disease (atherosclerosis, arrhythmias (Endres, Catarina et al. 1995)), and reproduction (Olsen, Sorenson et al. 1992)

3. Healthy maintenance of nutritional balance, prevention of diseases related to long term suboptimal dietary intake of omega 3 fatty acids and accessory nutrients.

RECOMMENDED DOSAGES:

DOG: Low Maintenance: 1 tsp/10.0 kg body weight daily
     Maintenance: 1 tsp/7.0 kg body weight daily
     High Maintenance: 1 tsp/2.5 kg body weight daily

Total daily dosage can be split into two doses each day

360 mg EPA per 10kg of body weight daily (Logas, 1993)

CAT: Low Maintenance: ½ tsp daily
     Maintenance: 1 tsp daily
     High Maintenance: 1 tsp twice daily
APPENDIX Papers:

Recent Research on EPA:

Eicosapentaenoic acid and gamma-linolenic acid induce apoptosis in HL-60 cells.
Gillis RC, Daley BJ, Enderson BL, Karlstad MD.
Division of Trauma and Critical Care, Department of Surgery, Graduate School of Medicine, University of Tennessee Medical Center, Knoxville 37920, USA.
Enteral nutrition with eicosapentaenoic acid (EPA; 20:5 n-3) and gamma-linolenic acid (GLA; 18:3 n-6) decreased pulmonary inflammation by reducing neutrophil counts and chemotactic factors in bronchoalveolar lavage fluid during acute respiratory distress syndrome (ARDS). We hypothesize that the anti-inflammatory effects of EPA and GLA may be due, in part, to induction of neutrophil apoptosis. Apoptosis was verified by DNA fragmentation as assessed by agarose gel electrophoresis. EPA, GLA, and various combinations of EPA and GLA significantly induced apoptosis and reduced cell viability in HL-60 cells. Viability was significantly reduced to the same extent with the combination of 50 micromol/L EPA/20 micromol/L GLA compared with 100 micromol/L EPA. These data indicate that EPA and GLA, alone or in combination, reduce cell survival by induction of apoptosis. Thus, induction of apoptosis by select dietary n-3 (EPA) and n-6 (GLA) polyunsaturated fatty acids may be the mechanism of the resolution of pulmonary inflammation in ARDS.

2) Nutr Biochem 2002 Dec;13(12):711-716
Fatty acid modulation of MCF-7 human breast cancer cell proliferation, apoptosis and differentiation.
Chamras H, Ardashian A, Heber D, Glaspy JA.
Division of Hematology/Oncology and Center for Human Nutrition, Department of Medicine, UCLA, Los Angeles, California, USA.
Epidemiological studies suggest that dietary polyunsaturated fatty acids (PUFA) may influence breast cancer progression and prognosis. In order to study potential mechanisms of action of fatty acid modulation of tumor growth, we studied, in vitro, the influence of n-3 and n-6 fatty acids on proliferation, cell cycle, differentiation and apoptosis of MCF-7 human breast cancer cells. Both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) inhibited the MCF-7 cell growth by 30% and 54%, respectively, while linoleic acid (LA) had no effect and arachidonic acid (AA) inhibited the cell growth by 30% (p < 0.05). The addition of vitamin E (10uM) to cancer cells slightly restored cell growth. These observations suggest that fatty acids may influence cellular processes at a molecular level, capable of modulating breast cancer cell growth.

3) Am J Physiol Heart Circ Physiol 2003 Jan 9;
Dietary n-3 PUFAs affect the blood pressure rise and cardiac function modulation in a hyperinsulinemia rat model in vivo.
Rousseau D, Helies-Toussaint C, Moreau D, Raederstorff D, Grynberg A.
The cardiovascular consequences of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid specific intake were evaluated in vivo in a hyperinsulinemia (HI) model, induced by dietary fructose intake. Wistar rats were fed a diet containing (or not for control) either EPA or DHA. The rise in BP, heart rate, and ECG were continuously monitored using an intra-abdominal telemetry system. The myocardial phospholipid fatty acid profile was significantly affected by DHA intake, less by EPA intake. The data indicate a reduced rise in
BP in both DHA and EPA hyperinsulinemic groups as compared to control. This result was confirmed by tail-cuff measurement after 5 weeks (133.3+/−1.67 and 142.5+/−1.12 mmHg in n-3 PUFA and control groups, respectively), whereas n-3 PUFA did not affect BP in non-HI rats (116.3+/−3.33 mmHg). It is concluded that both EPA and DHA were efficient to prevent the hyperinsulinemia-induced rise of blood pressure, and that DHA exhibited a double effect directly exerted on the heart, through a mechanism that may involve the cardiac adrenergic system.

4) Surg Infect (Larchmt) 2002 Summer;3(2):145-9

**Omega-3 fatty acid lipid emulsion reduces LPS-stimulated macrophage TNF-alpha production.**

Babcock TA, Helton WS, Hong D, Espat NJ.

Department of Surgery, University of Illinois at Chicago, 840 South Wood Street, Chicago, IL 60612, USA.

BACKGROUND: Omega-3 (omega-3) fatty acids (FA), specifically eicosapentaenoic acid (EPA), attenuate cytokine-mediated inflammation. Currently, in the United States, there is no commercial source of omega-3 lipid for clinical use. A clinically used European lipid emulsion, Omegaven, has been shown to have beneficial anti-inflammatory effects; however, the mechanisms of its action are not well defined. In the present work, this omega-3 FA emulsion has been evaluated in order to define its effects on TNF-alpha production in a model of LPS-stimulated macrophages CONCLUSION: Four-hour omega-3 FA emulsion pretreatment significantly attenuated LPS-stimulated macrophage TNF-alpha production. These data support the contention that anti-inflammatory effects of omega-3 FA occur at least in part through the inhibition of macrophage TNF-alpha production in response to endotoxin.


**Omega-3 fatty acid supplementation increases anti-inflammatory cytokines and attenuates systemic disease sequelae in experimental pancreatitis.**

Foitzik T, Eibl G, Schneider P, Wenger FA, Jacobi CA, Buhr HJ.

Department of Surgery, Benjamin Franklin Medical Center, Freie Universitat Berlin, Germany. thomas.foitzik@med.uni-rostock.de

BACKGROUND: The cytokines involved in the systemic inflammatory response in acute pancreatitis (AP) comprise lipid mediators (e.g., prostanoids, thromboxanes, leukotrienes) generated from arachidonic acid (AA) and eicosapentaenoic acid (EPA). The AA-derived mediators are generated from omega-6-fatty acid (FA) and have strong proinflammatory effects and the EPA-derived mediators generated from omega-3-fatty acid are less active or even exhibit anti-inflammatory effects. Basic parenteral nutrition delivers omega-6-FA and omega-3-FA at a ratio of approximately 7:1. AIM: To investigate whether altering the FA composition by fish oil supplementation (omega-3-FA) affects cytokine production and the parameters reflecting systemic disease severity in experimental AP. CONCLUSIONS: Altering eicosanoid mediator precursor availability by infusion of omega-3 fatty acid increases anti-inflammatory cytokines in this model of AP. This together with improved renal and respiratory function suggests that the systemic response to pancreatic injury is attenuated.


**Effects of purified eicosapentaenoic and docosahexaenoic acids on glycemic control, blood pressure, and serum lipids in type 2 diabetic patients with treated hypertension.**

Woodman RJ, Mori TA, Burke V, Puddey IB, Watts GF, Beilin LJ.

Department of Medicine, The University of Western Australia, Perth, Australia. rwoodman@cyllene.uwa.edu.au

BACKGROUND: n-3 Fatty acids lower blood pressure, improve lipids, and benefit other cardiovascular disease risk factors. Effects on glycemia in patients with type 2 diabetes are uncertain. OBJECTIVE: We determined whether purified eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have differential
effects on glycemic control, including insulin sensitivity and stimulated insulin secretion; 24-h ambulatory blood pressure; and serum lipids in type 2 diabetic patients with treated hypertension. CONCLUSIONS: EPA and DHA had similar benefits on lipids but adverse effects on short-term glycemic control in hypertensive diabetic patients. The overall implications for cardiovascular disease require long-term evaluation.

Recent Research On GLA:

Dietary fatty acid modulation of mucosally-induced tolerogenic immune responses.
Harbige LS, Fisher BA.
School of Chemical and Life Sciences, University of Greenwich, London, UK.
L.Harbige@gre.ac.uk

Immunological unresponsiveness or hyporesponsiveness (tolerance) can be induced by feeding protein antigens to naïve animals. Using a classical oral ovalbumin gut-induced tolerance protocol in BALB/c mice we investigated the effects of dietary n-6 and n-3 polyunsaturated fatty acids (PUFA) on high-and low-dose oral tolerance (and in non-tolerised animals, i.e. effects of antigen challenge alone) in relation to lymphoproliferative, cytokine and antibody responses.

Fish oil rich in long-chain n-3 fatty acids decreased both T-helper (Th) 1- and Th2-like responses. In contrast, borage (Borago officinalis) oil rich in n-6 PUFA, of which gamma-linolenic acid is rapidly metabolised to longer-chain n-6 PUFA, increased Thl-like responses and decreased Th2-like responses, and possibly enhanced suppressor cell or Th3-like activity.

These findings are in general agreement with other studies on the effects of long chain n-3 PUFA on immune system functions, and characterize important differences between long-chain n-3 and n-6 PUFA, defining more precisely and broadly the immunological regulatory mechanisms involved. They are also discussed in relation to autoimmune disease.


Gamma-linolenic acid in borage oil reverses epidermal hyperproliferation in guinea pigs.
Chung S, Kong S, Seong K, Cho Y.
Department of Medical Nutrition, Graduate School of East-West Medical Science, Kyung Hee University, Seoul, Korea.

As dietary sources of gamma-linolenic acid [GLA; 18:3(n-6)], borage oil (BO; 24-25 g/100 g GLA) and evening primrose oil (PO; 8-10 g/100 g GLA) are efficacious in treating skin disorders. The triglycerol stereospecificity of these oils is distinct, with GLA being concentrated in the sn-2 position of BO and in the sn-3 position of PO.

To determine whether the absolute level and/or the triglycerol stereospecificity of GLA in oils affect biological efficacy, epidermal hyperproliferation was induced in guinea pigs by a hydrogenated coconut oil (HCO) diet for 8 wk. Subsequently, guinea pigs were fed diets of PO, BO or a mixture of BO and safflower oil (SO) for 2 wk.

The mixture of BO and SO (BS) diet had a similar level of GLA as PO but with sn-2 stereospecificity. As controls, two groups were fed SO and HCO for 10 wk. Epidermal hyperproliferation was reversed by all three oils in the order of BO > BS > PO. However, proliferation scores of group PO were higher than of the normal control group, SO.
The accumulations of dihomo-gamma-linolenic acid [DGLA; 20:3(n-6)], an elongase product of GLA, into phospholipids and ceramides, of 15-hydroxyeicosatrienoic acid (15-HETrE), the potent antiproliferative metabolite of DGLA, and of ceramides, the major lipid maintaining epidermal barrier, in the epidermis of group BO were greater than of groups BS and PO. Group BS had higher levels of DGLA, 15-HETrE and ceramides than group PO.

With primary dependence on absolute levels, our data demonstrate that the antiproliferative efficacy of GLA in the epidermis is preferably exerted from sn-2 stereospecificity of GLA in BO.


**Oral administration of gamma linolenic acid, an unsaturated fatty acid with anti-inflammatory properties, modulates interleukin-1beta production by human monocytes.**

Furse RK, Rossetti RG, Seiler CM, Zurier RB.

University of Massachusetts Medical School, Department of Medicine, Worcester 01655, USA.

Administration of gamma linolenic acid (GLA), an unsaturated fatty acid, reduces joint inflammation in patients with rheumatoid arthritis. Addition of GLA in vitro suppresses release of interleukin-1beta (IL-1beta) from human monocytes stimulated with lipopolysaccharide (LPS). LPS-induced IL-1beta release is followed by IL-1-induced IL-1beta release, an amplification process termed "autoinduction."

We show here, using IL-1alpha stimulation to simulate autoinduction, that administration of GLA to healthy volunteers and to patients with inflammatory arthritis reduces LPS-induced IL-1beta secretion mainly by reducing autoinduction of IL-1beta.

GLA reduces LPS-induced pro-IL-1beta mRNA modestly and IL-la-induced pro-IL-1beta gene expression markedly. In addition to reducing amplification of IL-1beta, GLA increases the amount of IL-1 receptor antagonist (IL-1Ra) secreted from stimulated cells, thereby facilitating an increase in the secreted IL-1Ra/IL-1beta ratio. IL-1beta is important to host defense, but the amplification mechanism may be excessive in genetically predisposed individuals.

Thus, reduction of IL-1beta autoinduction may be protective in some patients with endotoxic shock and with diseases characterized by chronic inflammation.
REFERENCES:


