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Methionine Task Force

c/o Coleman Natural Foods
PO Box 7368
Petaluma, CA 94955-7368

April 8, 2011

National Organic Standards Board
c/o Lisa M. Brines, Ph.D.
National List Manager
USDA National Organic Program, Standards Division
1400 Independence Ave. SW
Room 2945-S, STOP 0268
Washington, D.C. 20250

Dear NOP Staff and NOSB Chair,

Attached you will find a petition from the Methionine Task Force (MTF) for continued use of Synthetic Methionine (MET) in organic poultry feed. The MTF would like to address the allowance of MET beyond October, 2012, specifically by adding the following language to the annotation:

> **Effective October 1, 2012, the allowed maximum <u>average pounds per ton</u> of 100% synthetic methionine (MET) in the diet over the life of the bird will be at the following levels:**
>
> **Laying chickens---2.5 lbs;**
> **Broiler chickens – 3 lbs;**
> **Turkeys and all other poultry – 3 lbs.**

Additional specifics about our petition can be found in the body of the document itself. You will find that much of the background information on the petition is unchanged from our prior petition in 2009. However the justification statement has been updated to reflect our current research and a discussion of the viable alternatives.

The MTF membership represents the majority of organic broiler and laying hen operations in the United States. This group represents a cross section of long time organic producers, many of whom are considered pioneers within the organic industry.

As a group, we are extremely concerned about the end of the current annotation to use Synthetic Methionine in October 2012. The Step Down levels recommended at the last meeting were expressed as **maximum** allowances, not **averages** over the life of the bird. As such, they are inadequate to meet the needs of young birds and broilers, as we show in our petition.

By agreeing to calculate methionine allowances **<u>average over the life of the bird</u>**, the following will result:

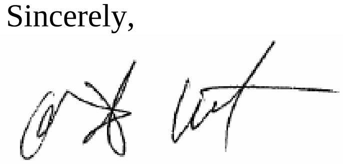
- Feed rations can better adjust to the naturally changing demands of the bird. Poultry farmers will have more flexibility to appropriate adjust diets for stage of life, seasonality, breed, etc.
- Overall usage of MET will be lowered. Producers can only add methionine to the average cap, not consistently add methionine at the maximum rate
- Farmers and nutritionists will still be only marginally capable of meeting the bird's basic needs. The organic poultry industry will continue to have a tremendous incentive to actively evaluate novel sources of methionine. The MTF members remain united in the belief that MET can eventually be eliminated from organic production.

The methionine allowances recommended in this petition are less than half the maximum amounts recommended by the NRC and are roughly equivalent to the Step Down levels recommended previously by the NOSB. These recommendations are based on the amounts needed by birds for maintenance, not growth enhancement or production maximization.

The MTF has funded a significant body of research on MET alternatives. The Task Force will continue to actively support the development of an array of the most viable alternatives in the hopes that a satisfactory alternative can be developed on a commercial scale as quickly as possible.

We again want to emphasize the importance of this issue to our industry. In addition to our MTF members, which are principally chicken farmers, we want to speak for other organic poultry producers (turkey, duck, goose, game bird, and ratite). We have all worked long and hard to raise our animals humanely and in accordance with organic principles. Over the years, we have developed a significant network of production to meet ever growing consumer demand. Given the current state of the alternatives, all of this is jeopardized without the continued allowance for synthetic MET.

Sincerely,



Dave Martinelli
Chair, Methionine Task Force
dmartinelli@colemannatural.com
(707) 283-2219

Petition for Amending the National List of the USDA's National Organic Program

<u>DL- Methionine, ML-Methionine Hydroxy analog, and DL-Methionine-hydroxy analog calcium-for use only in organic poultry production</u>

A Synthesized Essential Amino Acid

Submitted April 8, 2011 by:
Dave Martinelli, on behalf of the Methionine Task Force
(Please refer to the first attachment to this petition for a list of Task Force representatives.)

Introduction:

The Methionine Task Force requests that 7 CFR §205.603(d)(1) be amended, as follows:

> DL–Methionine, DL–Methionine—hydroxy analog, and DL–Methionine—hydroxy analog calcium (CAS #–59–51–8; 63–68–3; 348–67–4)—for use only in organic poultry production until October 1, 2012**,** at the following maximum levels of synthetic methionine per ton of feed:
> laying chickens---4 lbs; broiler chickens – 5 lbs; turkeys and all other poultry – 6 lbs.
> **Effective October 1, 2012, the allowed maximum <u>average pounds per ton</u> of 100% synthetic methionine (MET) in the diet over the life of the bird will be at the following levels:**
> **Laying chickens---2.5 lbs;**
> **Broiler chickens – 3 lbs;**
> **Turkeys and all other poultry – 3 lbs.**

Further Clarification of the Proposed Amendment
Under this recommendation, producers would be able to exceed the above levels on a particular formulation, provided that there was an offsetting formulation below the level, such that the **<u>average</u>** inclusion rate of 100% synthetic methionine

over the entire life cycle of the bird was below the allowed maximum level.

Reference is specifically made to 100% synthetic methionine as some forms of synthetic methionine (e.g. the liquid form Alimet) are not 100% methionine. The maximum pounds as shown above is based on the 100% synthetic methionine equivalent so that a consistent standard can be applied to all organic operations, irrespective of the form of MET they are using (e.g. wet vs. dry).

All of the above proposed maximum levels represent extremely small percentages of the overall diet. When expressed as percentages, the amounts are:

Laying chickens	0.20%
Broiler chickens	0.25%
Turkeys and all other poultry	0.30%

Petitioners are required to provide the following information as applicable:

Category for inclusion on the National List:

- *This request is to change the annotation date currently listed on synthetic methionine [MET] in Section 205.603 of the National List as a synthetic substance allowed for use in organic poultry production subject to the limitations on usage as defined above.*

Common name:

- *The three names in the title of this document are the most widely used common names. They are however the common names associated with specific manufacturers. Throughout this petition the term "Methionine" will be used to refer to the natural form of the amino acid and the term MET will be used to refer to any synthetic analog.*

Chemical Structure:

- *The formula for Methionine is* $H_2NCH_3SCH_2CH_2COOH$.
  Other Chemical Structures:
  DL-Methionine CH3-S-CH2-CH2-CH(NH2)-COOH
  Methionine Hydroxy Analog CH3-S-CH2-CH2-CH(OH)-COOH

Manufacturers name, address and telephone number

- *There are three major manufacturers of MET world wide:*

Adisseo
3480 Preston Ridge Road, Suite 375. Alpharetta, GA 30005 Phone 678-339-1513

Degussa Feed Additives
1701 Barrett Lakes Blvd., Suite 340 Kennesaw, GA 30144 Phone 800-955-3114

Novus International, Inc.
530 Maryville Centre Drive St. Louis, MO 63141 888-906-6887

List of uses, rates and applications for crops and livestock uses, mode of action for handling uses:

- *Typically added to poultry feed rations at the following rates:*
  - *Laying Hens: 0.20% (maximum 4 lbs per ton of feed)*
  - *Broilers: 0.25% (maximum 5 lbs per ton of feed)*
  - *Turkeys: 0. 30% (maximum 6 lbs per ton of feed)*

Sources and detailed description of manufacturing procedures:

The following text is taken from the May 21, 2001 Methionine Livestock TAP Review:

Methionine may be isolated from naturally occurring sources, produced from genetically engineered organisms, or entirely synthesized by a wide number of processes. While methionine has been produced by fermentation in laboratory conditions, racemic mixtures of D- and L- methionine (DL-Methionine) are usually produced entirely by chemical methods (Araki and Ozeki, 1991). Methionine can be produced from the reaction of acrolein with methyl mercaptan in the presence of a catalyst (Fong, et al., 1981). Another method uses propylene, hydrogen sulfide, methane, and ammonia to make the intermediates acrolein, methylthiol, and hydrocyanic acid (Degussa). The Strecker synthesis can be used with α-methylthiopropionaldehyde as the aldehyde (Fong, et al., 1981). A recently patented process reacts 3-methylmercaptopropionaldehyde, ammonia, hydrogen cyanide, and carbon dioxide in the presence of water in three reaction steps (Geiger et al., 1998). Other methods are discussed in the 1999 Crops Amino Acid TAP review. DL-methionine hydroxy analog calcium and DL-methionine hydroxy analog forms are considered to be alpha-keto acid analogues in which the amine group has been replaced by a hydroxy (OH) group. These forms are converted to the amino form in the bird by transamination in the liver, using non-essential amino acids such as glutamic acid (Cheeke 1999; Leeson 1991). These forms are produced by reacting hydrogen cyanide with an aldehyde that has been treated with a sulfite source to form a cyanohydrin. The aldehydes used are prepared from either hydrogen sulfide or an alkyl mercaptan with an aldeyhde such as acrolein and are then hydrolyzed using sulfuric or hydrochloric acid (USPO 1956).

Regulatory status with EPA, FDA or state authorities:

The following text is taken from the May 21, 2001 Methionine Livestock TAP Review:

Regulated as a nutrient / dietary supplement by FDA (21 CFR 582.5475). The Association of American Feed Control Officials (AAFCO) set the standard of identity for DL-methionine as containing a minimum of 99% racemic 2-amino-4-methylthiobutyric acid (AAFCO, 2001). The AAFCO model regulation states that "the term Methionine Supplement may be used in the ingredient list on a feed tag to indicate the addition of DL-Methionine" (AAFCO, 2001). AAFCO also lists a feed definition for DL-Methionine hydroxy analogue calcium (min. 97% racemic 2-amino-4-methylthiobutyric acid, 21 CFR 582.5477) and DL-Methionine hydroxy analogue, (min. 88% racemic 2-amino-4-methylthiobutyric acid, 21 CFR 582.5477).

Chemical Abstract Service (CAS) number or other product number, samples of labels:

The following text is taken from the May 21, 2001 Methionine Livestock TAP Review:

Chemical Names:
2-amino-4-methylthiobutyric acid and
α-amino-©-methylmercaptobutyric acid

Other Names:
DL-methionine, D-methionine, L-methionine,
Met, Acimethin

CAS Numbers:
59-51-8 (DL-methionine)
63-68-3 (L-methionine)
348-67-4 (D-methionine)

Other Codes:
International Feed Names (IFN):
DL-methionine: 5-03-86
DL-methionine hydroxy analog calcium: 5-03-87
DL-methionine hydroxy analog: 5-30-28

Physical properties of the substance and chemical mode of action: including environmental impacts, interactions with other materials, toxicity and persistence, effects on human health, effects of soil organisms, crops or livestock:

The following text is taken from the May 21, 2001 Methionine Livestock TAP.

Properties:
L-Methionine: Colorless or white lustrous plates, or a white crystalline powder. Has a slight, characteristic odor. Soluble in water, alkali solutions, and mineral acids. Slightly soluble in alcohol, insoluble in ether. MP 280-82°C. It is assymetric, forming both an L- and a D- enantiomer. Methionine hydroxy analog (MHA) is available in liquid form.

Action:
Amino acids form protein. Of the 22 amino acids found in body proteins, the National Research Council lists 13 as essential in poultry diets, and these must be consumed in feed. These 13 are: arginine, glycine, histidine, isoleucine, leucine, lysine, methionine, cystine, phenylalanine, proline, threonine, tryptophan, and valine (NRC 1994). Five that are deemed critical in poultry rations are methionine, cystine, lysine, tryptophan, and arginine (North, 1990).

Animals convert dietary protein into tissue protein through digestive processes. Proteins are metabolized by animals through two phases: catabolism (degradation from body tissue to the free amino acid pool) and anabolism (synthesis into body tissue). Amino acids utilized as proteins are primary constituents of structural and protective tissues, including skin, feathers, bone, ligaments, as well as muscles and organs.

Combinations:
Amino acids are combined in feed rations of grains, beans, oilseeds, and other meals with antioxidants, vitamins, minerals, antibiotics, and hormones (Pond, Church, and Pond, 1995). Methionine is a precursor in the diet to cystine, and the amount needed in the diet depends on the amount of cystine also present. Requirements for methionine are frequently cited in terms of methionine plus cystine, because methionine converts to cystine as needed.

OFPA 2119(m) Criteria:

(1) The potential of such substances for detrimental chemical interactions with other materials used in organic farming systems.

The primary chemical interaction is the dietary intake by animals. While many of the interactions may be regarded as beneficial, excess methionine in a diet may cause deficiencies in other amino acids and induce toxicity (D'Mello, 1994). Methionine, while often one of the most limiting amino acids, is also one that readily goes to toxic excess. Small excesses of methionine can be deleterious (Buttery and D'Mello, 1994). Errors in feed formulation or excess supplemental methionine can actually depress growth and development at levels of 40 g/kg (4.0%) (Baker, 1989, NRC 1994). Excess methionine exacerbates deficiencies of vitamin B-6, which results in depressed growth and feed intake (Scherer, 2000). Growth depressions resulting from excess supplemental amino acids include lesions in tissues and organs (D'Mello, 1994). Methionine is "well established as being among the most toxic of all amino acids when fed at excess levels in a diet" (Edmonds and Baker, 1987 cited in Scherer, 2000).

(2) The toxicity and mode of action of the substance and of its breakdown products or any contaminants, and their persistence and areas of concentration in the environment.

While it is nutritionally essential, methionine excesses are far more toxic to poultry than similar excesses of tryptophan, lysine, and threonine (National Research Council, 1994). Force feeding methionine to excess can result in death to chicks (National Research Council, 1994). However, NRC acknowledges that such toxicities are unlikely in practical circumstances for poultry, in that an amino acid toxicity requires a particularly high level of an amino acid relative to all others. Supplemental levels fed to poultry are usually fed at lower levels, ranging from 0.3 - 0.5% of the diet. Susceptibility of an animal to imbalances and excesses is influenced by the overall protein supply, and animals that are fed relatively high levels of protein are more tolerant (Buttery and D'Mello, 1994).

A dosage of 2 g / mature cat / day (20 to 30 g / kg dry diet) for 20 days induces anorexia, ataxia, cyanosis, methemoglobinemia and Heinz body formation resulting in hemolytic anemia (Maede, 1985). Rat studies of methionine is significantly toxic in excess (Regina, et al., 1993). High levels of methionine were found to be toxic to hepatic cells and liver function of the rat models. The results of this study indicated that the Biochemical reason for the extreme sensitivity of mammals to excess dietary methionine is thought to be due to the accumulation of toxic catabolites, most notably, S-adenosylmethione, resulting in liver dysfunction. L-methionine has an acute LD_{50} of 4,328 mg/kg (rat) (NIEHS, 1999b). NIEHS carcinogenicity and teratogenicity are not available, but reports positive mutagenicity (NIEHS, 1999b). Methionine is stable in crystalline form at standard temperature and pressure.

(3) *The probability of environmental contamination during manufacture, use, misuse or disposal of such substance.*
Synthetic production of DL-methionine involves a number of toxic source chemicals and intermediates. Each of the several manufacturing processes used to produce DL-methionine was rated as either "moderately heavy" to "extreme" (Fong, et al., 1981). Newer processes have not replaced many of the feedstocks. Several of the feedstocks are likely to result in ruptured storage tanks, leaking chemicals, and releases into the environment. The methionine production process is listed by EPA as a hazardous air pollutant (40 CFR 63.184).
Methyl mercaptan can react with water, steam, or acids to produce flammable and toxic vapors (Sax, 1984). The EPA rates methyl mercaptan fires as highly hazardous and can cause death by respiratory paralysis (EPA, 1987). Acrolein has a toxicity rating of 5 (on a scale of 1 to 6 with 6 being most toxic) (Gosselin, 1984) and it is also an aquatic herbicide (Meister, 1999). The acrolein process involves several steps that render it synthetic as well (1994). Acrolein itself is an extreme irritant.

Hydrogen cyanide is produced by further processing of methane and ammonia. Hydrogen cyanide is a gas that is highly toxic. Hydrogen cyanide has a toxicity rating of 6 and is one of the fastest acting poisons known to man (Gosselin, 1984). Exposure causes paralysis, unconsciousness, convulsions, and respiratory arrest. Death usually results from exposure at 300 ppm concentrations for a few minutes. Manufacture of hydrogen cyanide is a significant source of atmospheric release of cyanide (Midwest Research Institute, 1993). Ammonia is a corrosive agent. Methane is a central nervous system depressant (Gosselin, 1984).

(4) The effect of the substance on human health.
Methionine is essential in small amounts in the human diet, and is sold over-the-counter as a dietary supplement. The L- form of methionine is used extensively in human medicine for a variety of therapeutic purposes, including pH and electrolyte balancing, parenteral nutrition, pharmaceutical adjuvant, and other applications. It is in fact one of the top 800 drugs in human medicine (Mosby, 1997). Methionine may cause nausea, vomiting, dizziness, and irritability and should be used with caution in patients with severe liver disease (Reynolds, 1996).

The D- form of methionine is not well utilized by humans (Lewis and Baker, 1995). Individuals may have allergic reactions to the D- isomers or a racemic mixture of DL-methionine. While a number of amino acids are considered GRAS for human consumption and as feed supplements, DL-methionine is not (see 21 CFR 172, 21 CFR 184, and 21 CFR 570.35). DL-methionine is unique among amino acids cleared for food use in that it is the only one listed that explicitly says it is not for use in infant feed formulas (21 CFR 173.320). When heated to decomposition, methionine emits dangerous and highly toxic fumes (NIEHS, 1999).

(5) The effects of the substance on biological and chemical interactions in the agroecosystem, including the physiological effects of the substance on soil organisms (including the salt index and solubility of the soil), crops and livestock.
Interactions and Imbalances
Protein is required for body development in all growing birds, and layers also need a good proportion since eggs consist of 13-14% protein. Broilers also need high energy diets as they are commercially raised to grow rapidly to reach about 4.4 lbs in 8 week at a desired food conversion rate of 1.8 (consuming less than 8 lbs of feed total) (Sainsbury, 2000). This is a 50-55 fold increase in body weight by 6 weeks after hatching, which leads to a high amino acid requirement to meet the need for active growth (NRC 1994). The dietary requirement for protein is actually a requirement for the amino acids contained in the dietary protein. Protein quality is related to the proper balance of essential amino acids in the diet. The presence of nonessential amino acids in the diet also reduces the necessity of synthesizing them from the essential amino acids (NRC 1994).

Amino acids in the body are constantly in flux between three states: stored in tissue, oxidized from tissue to free amino acids, and digested and excreted as uric acid. If some nonessential amino acids are low, they may be synthesized from others in the free amino acid pool, or degraded from those stored in tissue. Deficiencies or excesses of a particular amino acids can cause problems in availability of other amino acids (Buttery and D'Mello 1994; Baker 1989). Intact proteins (as in natural grains) are more slowly available in the digestive system, while pure sources of amino acids are more bioavailable than intact-protein sources (Baker, 1989). Excesses of some amino acids in an unbalanced source of crude protein can reduce feed intake and depress amino acid utilization (Pack, 1995). Depressed feed intake and growth at excess intake levels of protein has recently been attributed to insufficient supply of vitamin B-6 which is required to metabolize the sulfur amino acids (Scherer and Baker 2000).

The requirement for sulfur containing amino acids of methionine, cystine, and cysteine can be misjudged due to inaccurate accounting for the availability of cystine in the diet (NRC, 1994).

Other cases have shown significantly higher weights and faster gains from amino acid (lys+met) supplementation (Slominski et al, 1999). Also, the digestibility of practical ingredients, such as corn and soybeans, appears to be on the order of 85% or more (NRC, 1994).

Amino acid requirements may be affected by environmental temperature extremes, basically because of the effect on feed intake, but amino acid supplementation will only affect weight gain if it improves feed intake (Baker 1989; NRC 1994). Interactions between deficiencies of methionine and several vitamins and minerals have also been documented, and suggest that other dietary factors in addition to total protein have an effect on the efficiency of amino acid utilization (Baker, D.H. et al, 1999).

<u>Environmental Impact</u>

Managing the nitrogen cycle is seen as a challenge to livestock producers (Tamminga and Verstegen, 1992; Tamminga, 1992; Morse, no date). Poultry layer operations are experiencing increased costs and regulations for manure management (Sloan, et al., 1995). Supplementation with amino acids may allow dietary protein and excretory nitrogen levels to be reduced with a minimum reduction in egg output and no loss in weight gain in broilers (Summers, 1993; Sloan et al., 1995, Ferguson, et al 1998). Excess ammonia build up in poultry houses can be a hazard to workers and birds if not properly ventilated (Ferguson, 1998).

Feeding systems that reduce levels of protein fed using amino acid supplementation are not the only means identified to reduce nitrogen pollution from animal manure. Other potential solutions include lower animal densities; more frequent rotations; better manure storage, handling, and application techniques; use of enzymes; improved processing of the feed; and selection of more appropriate land and locations to graze and shelter animals (Archer and Nicholson, 1992; Tamminga, 1992; Tamminga and Verstegen, 1992). Increased digestibility of protein in feeds supplemented with microbial phytase provided better availability of most of the amino acids other than lysine and methionine and allowed for reduced P and Ca levels in feed, a goal in reducing phosphorus overload from poultry manure (Sebastian 1997). Another study found that reduced crude protein and energy content were needed in enzyme supplemented broiler diets, although availability of individual amino acids were not improved equally and were still deemed to need balancing (Zanella, et al 1999).

One grower reported success with innovative housing design that allows twice daily cleanout of manure, combined with a commercial composting operation (La Flamme, 2001). Manure from organic operations has potential added value to organic crop farmers seeking to avoid manure from conventional operations. Some markets in the EU require that imported crops are documented to be grown free of "factory farm" manure, requiring additional verification from U.S. certifiers (McElroy, 2001).

<u>Impacts on Bird Health</u>

A number of reports cite a benefit of methionine supplementation on reduced immunologic stress (Klasing, 1988; Tsiagbe et al, 1986). Immunologic stress is considered to be a response to microbial challenges, in these experiments due to injections of E. coli and Salmonella and other pathogens. This causes decreased feed rates and lower rates of growth. Chicks that received deficient levels of methionine were more subject to an impaired immune response. These experiments seem to be more applicable to a high density confinement system or high density production system in terms of bird treatment, and may not be very relevant to an organic system approach.

A problem exacerbated by excess methionine is hepatic lipidosis, a condition of excessive fat in the liver commonly associated with caged birds and is related to the fact that wild diets are much lower in fat than seed diets fed to captive species (Aiello, 1998). This can be managed by a well balanced diet, and is reportedly not a problem in free range birds in organic systems (Krengel, 2001). Enteritis is a disease frequently observed in poultry that do not have access to the soil and green growing plants (Titus, 1942). Well managed pasture would prevent this cause of the disease.

Reduced feathering has been reportedly linked to lack of methionine and cystine (Elliott, no date). Many other factors are also involved, including deficiencies of other amino acids, vitamins, zinc, feather pecking in cage systems, and cannibalism (Elliot, NRC 1994). Increased protein level is correlated with reduced feather loss and cannibalism (Ambrosen, 1997).

(6) The alternatives to using the substance in terms of practices or other available materials.
Birds raised on pasture with access to insects and worms historically did not need supplementation (Morrison, 1951), and smaller scale pastured operations have success without the need for synthetic supplements (Salatin, 1993). Pasture quality will vary according to field conditions and the season. However, free range poultry on well managed pasture are able to supplement their diets with insects, annelids, and fresh green forage (Smith and Daniel, 1982). The two most limiting amino acids, methionine and lysine, are found in richest sources in proteins of animal origin. Common natural sources of these amino acids have traditionally been fish meal and meat meal, especially for starter chicks and broilers (Sainsbury, 2000). The USDA organic program final rules do not allow the use of meat meal as feed for poultry or mammals and may or may not allow fish or crab meal (7CFR 205).

Diets can be formulated without supplemented synthetic acids to meet the objective of adequate methionine percentages, but this usually requires an increase in crude protein level of the diet (Hadorn, 2000). Many studies have been done to identify a cost effective method of lowering protein content by supplementing with methionine and lysine. Often the control treatments are non-supplement grain based diets. A comparison study using supplemented and non-supplemented diets found that adequate dietary methionine can be attained, at a cost of higher intake of protein and less protein efficiency ratio (Emmert 2000). Another study fed a control diet using only corn and soy to satisfy amino acid levels compared to reduced protein supplemented with methionine and lysine, and these treatments were considered successful because performance was not lowered in 4-5 experiments (Harms, 1998).

Rice and casein offer potential novel available sources of methionine (Lewis and Bayley, 1995). Yeast protein has long been known as a rich protein source relatively high in methionine+cystine (Erbersdobler, 1973; National Research Council, 1994), as well as phosphorous and B-complex vitamins (Morrison, 1951). As a natural feed supplement, NOSB should advise whether yeast is considered agricultural and required from organic sources or permitted as a natural substance. Other potential sources of available methionine for poultry appear to be sunflower meal and canola meal (Waibel et al., 1998). These natural sources are all currently of limited availability in organic forms. Alfalfa meal is reported to be a good additional protein source, though difficult to blend in commercial formulations. Optimally balancing these nutrients may be challenging to feed processors and livestock producers.

Feed sources with high percentages of methionine are bloodmeal, fish meal, crab meal, corn gluten meal, and sunflower seed meal (National Research Council, 1994). If fish meal were permitted, there is a lack of supply that does not contain ethoxyquin, a synthetic antioxidant not permitted under the final rules. A limited supply of fish meal preserved with natural tocophorols has gone mostly into the pet food market (Mattocks, 2001). Corn gluten and sunflower seed meal are not currently very available in organic form, and feed formulators and nutritionists have reported difficulty in meeting NRC requirements for methionine based on currently available organic plant protein sources (Mattocks, Morrisson, Simmons, 2001). One feed mill operator feels he can meet even broiler needs with a combination of crab meal (at 75 lbs/ton or 3.75%) and organic corn gluten (Martens, 2001). Crab meal is a by-product of crab processing and not treated with preservatives but has limitations due to salt content. Another certified feed mill produces layer and range broiler rations without synthetic amino acids based only on plant products, including corn, soy, barley, oats, wheat, field peas, and flaxmeal. These products are labeled at a minimum of 0.3% met and 0.6% lysine, but reportedly achieve good results (White 2001, VOG).

NRC requirements for amino acids and protein are designed to support maximum growth and production. The recommended levels for methionine in poultry depend on species, stage, and level of feed consumption. For chickens, recommendations for layers range from 0.25% to 0.38% and for broilers 0.32 - 0.50%. NRC notes that maximum growth and production may not always ensure maximum economic returns when protein prices are high, and that if decreased performance can be tolerated, dietary concentrations of amino acids may be reduced somewhat to maximize economic returns (NRC, 1994). Methionine is known to have a direct effect on egg weight (size) and rate of lay, and is used by some producers to manipulate egg production to meet market needs, such as to increase egg size in younger birds, reduce it in older birds, or produce more eggs in off peak market periods (NRC 1994; Harms 1998; Simmons 2001). A reduction in rate of gain in broilers (longer time to finish) would be an outcome of lower than optimal methionine levels. Unless the diet contained other forms of sulfur containing amino acids (cystine or cysteine), problems with inadequate feathering might be encountered (Simmons, 2001).

Temporarily confined poultry can be fed a practical organic corn / soybean ration. Depending on market conditions and on how other parts of the standards evolve, novel organic products can be developed as supplements. Among the potential alternative sources include organic dairy products such as casein (National Research Council, 1982 and 1994).

Macroorganisms commonly found in healthy pasture soils cannot be discounted as a source of nutrient cycling in free-range poultry systems. Given the natural feeding habits of poultry and other birds, the use of earthworms is a logical source of protein in chicken feed (Fisher, 1988). Earthworm populations in a pasture depends on a number of factors (Curry, 1998). The amino acid content of earthworms will vary depending on species and food source. However, earthworms have been found to accumulate and concentrate methionine found in the ecosystem in proportions greater than for other amino acids (Pokarzhevskii, et al., 1997). As a feed supplement, earthworms have been found to equal or surpass fish meal and meat meal as an animal protein source for poultry (Harwood and Sabine, 1978; Toboga, 1980; Mekada et al., 1979; and Jin-you et al., 1982 all cited in Edwards, 1998).

Earthworms can play a role in moderating nitrogen losses as well. Enzyme treatment of feedstuffs can improve amino acid availability and also reduce nitrogen pollution (Tamminga and Verstegen, 1992), as can changes in stocking density, rotations, and manure handling.

(7) Its compatibility with a system of sustainable agriculture.
In 1994 the NOSB recommended that feed and feed supplements be produced organically. When considering the review of feed additive vitamins and minerals, an NOSB statement of principles advised that non-synthetic vitamins and mineral sources are preferable when available. The NOSB also advised that a farm plan should reflect attempts to decrease or eliminate use of feed additives when possible (NOSB, 1995).

A constraint to optimal production in modern organic systems that are not able to utilize pasture based systems appears to be adequate organic sources of the first limiting amino acid, methionine. The allowance of isolated amino acids facilitates the use of the lowest cost, non-diverse corn-soy ration. It is the basis of conventional confinement animal production systems which may be considered as antithetical to the principals of organic livestock production. The source and method of production of synthetic amino from non-renewable fossil fuels and toxic chemicals is also questionable in compatibility with system of sustainable agriculture.

The use of synthetic amino acids increases animal production by increased efficiency of protein conversion, which lowers feeding costs and reduces nitrogen content of the waste output. While this is not by itself unsustainable, synthetic amino acids discourage the integration of a whole-systems approach to cycling nutrients, particularly nitrogen, as part of an integrated crop-livestock production system. Allowance of synthetic sources of amino acids may discourage market development of organic plant sources, such as seed meals.

Increased efficiency of protein conversion reduces the amount of nitrogen excreted (Summers, 1993; deLange, 1993). The cycling of nutrients from animals is part of an integrated farming system, and the environmental effects of manure management requires looking at the big picture (Archer and Nicholson, 1992). What is viewed as a liability in confinement animal systems—nitrogen production—is seen in cropping systems as a limiting factor resource. Reduction of nitrogen pollution may require improved range or pasture management, and with that either more frequent rotations or lower stocking rates

Safety information, including a MSDS (Material Safety Data Sheet) and report from National Institute of Environmental Health Studies (NIEHS):

A MSDS for DL Methionine is attached to this petition. A search of the National Institute of Environmental Health Sciences (NEIHS) website for Methionine and DL Methionine yielded no Substance Reports.

Petition justification statement which states why the synthetic substance is necessary, alternatives that could be used, beneficial effects to the environment, etc:

Justification Statement

Reference is made to the previous MTF petition on Methionine (July 31, 2009), which provides significant background information on this material. The following information in this Justification Statement represents only new information or updates to previous data submitted.

In this petition, a number of new points will be discussed:

1. **NOSB Step Down Recommendation**
2. **Methionine Demand Changes with Stage of Life**
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1. NOSB Step Down Recommendation

The current organic standards allow for the use of synthetic methionine in organic poultry diets up to the following maximum levels until October 1, 2012:

a) Laying chickens - 4 pounds per ton;
b) Broiler chickens - 5 pounds per ton;
c) Turkeys & all other poultry - 6 pounds per ton

The most recent NOSB recommendation is that after October 1, 2012, the maximum allowance will be reduced to "Step Down" levels. The NOSB has recommended Step Down levels at the following amounts:

a) Laying chickens and Broiler chickens - 2 pounds per ton;
b) Turkeys & all other poultry - 3 pounds per ton

One important point is that the NOSB recommendation is expressed as a total maximum limit of lbs of MET per ton of feed. The MTF petition from July 2009 recommended that the maximum allowance for Methionine be expressed as an average over the life of the bird.

The MTF petition was phrased in this way, because Methionine demand is not consistent over the life of the bird. In fact Methionine demand is subject to a wide range of variables:

- Type of bird (e.g. laying chickens, broiler chickens, turkeys)

- Breed of bird (e.g. Hyline, Bovans Browns, Cobb, Ross)
- Stage of life (methinione demand is higher with younger birds)
- Environment (e.g. time of year, weather, condition of housing)
- Quality of pasture (seasonally dependent)
- Diet / Feed Ration

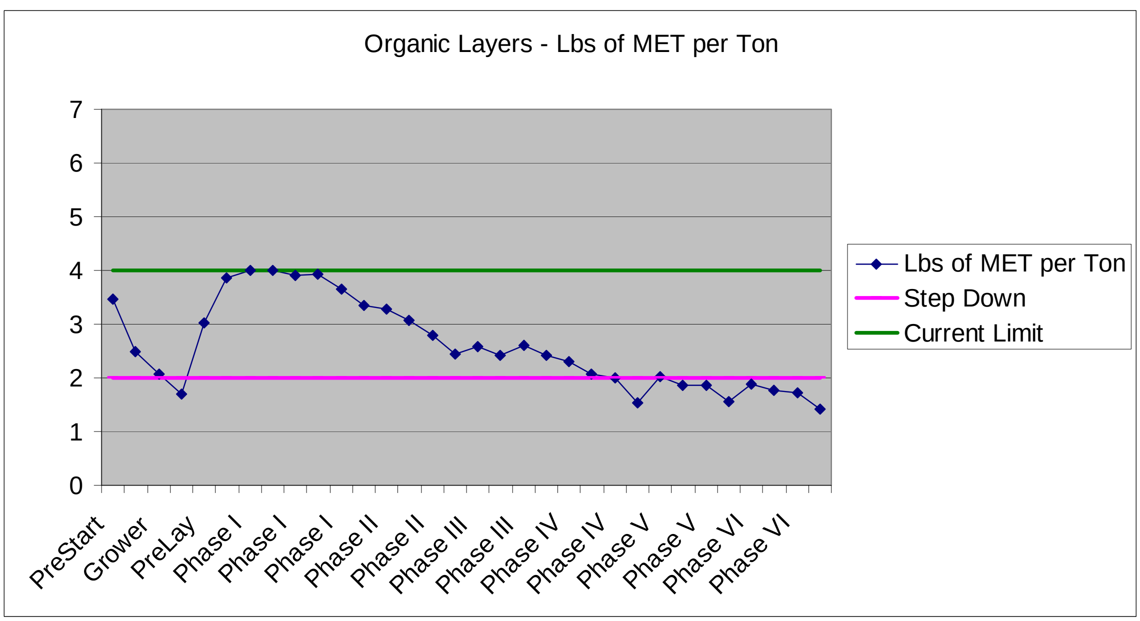
As previously approved by the NOSB, the Step Down levels create significant feed formulation, animal welfare, and environmental challenges, as will be discussed in this petition. They are not reflective of the birds actual methionine needs and are not supported by the nutritionist community, as discussed below.

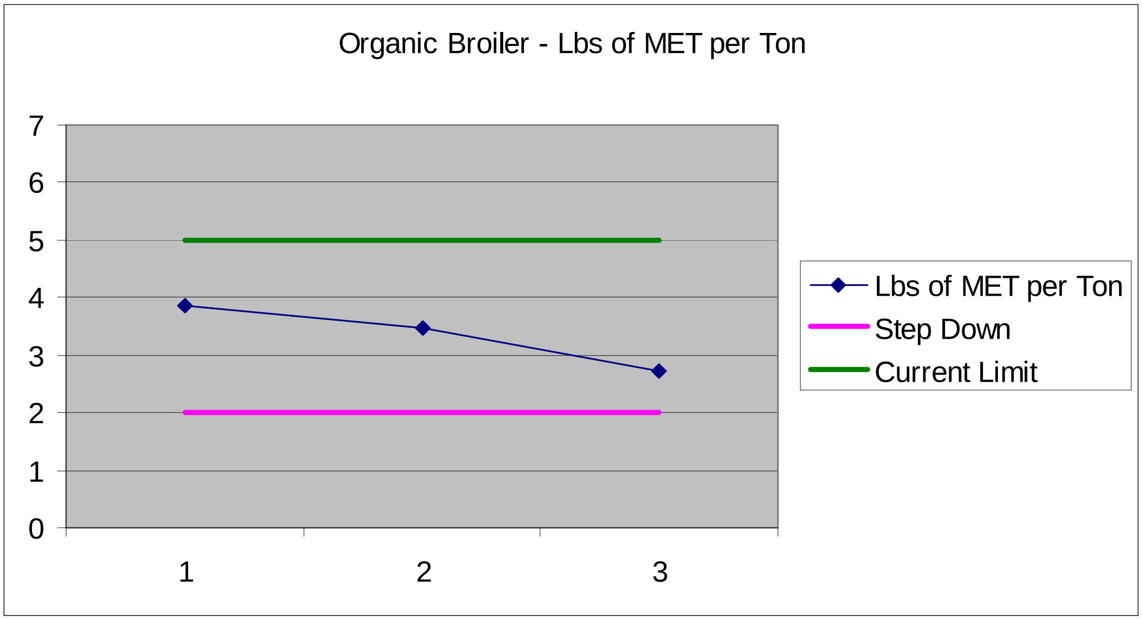
2. Methionine Demand Changes with Stage of Life

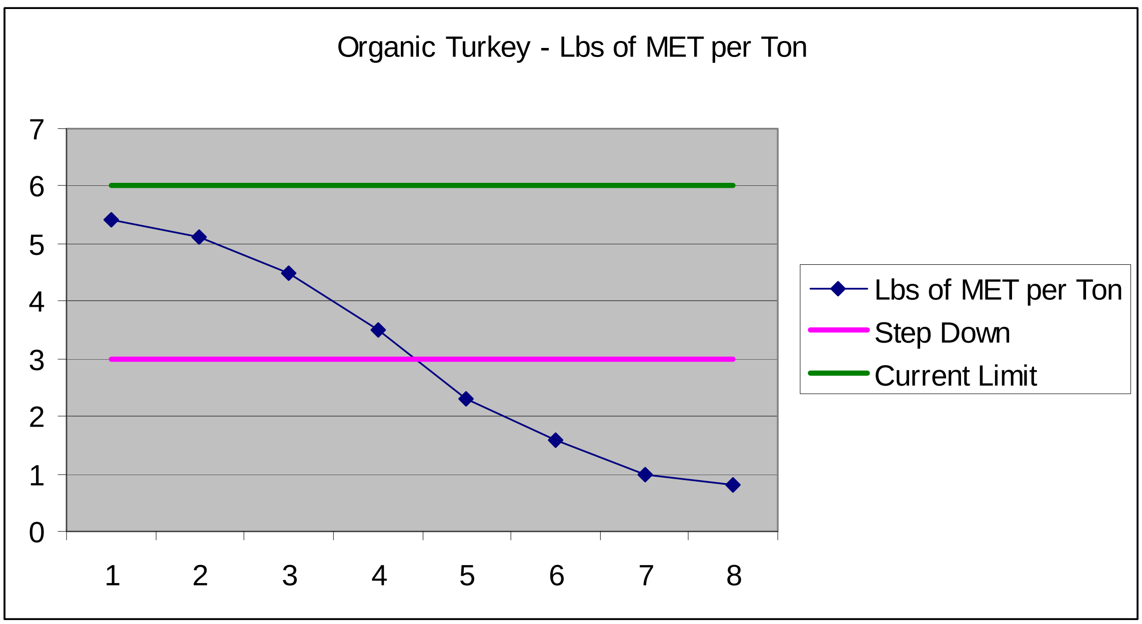
In all birds, Methionine demand is highest with chicks. Young birds are growing rapidly and adequate methionine is critical to develop proper bone structure, muscle, and feathering. Methionine needs generally decline as the birds hit maturity, although there is a short term spike in Methionine demand when laying chickens first come into production.

Following are charts which graphically show the lbs of MET per ton in some sample organic diets formulated by Akey (a leading poultry nutrition company). These graphs illustrate two main points:

- MET inclusion changes with the stage of life of the bird
- While nutritionists are able to adequately formulate within the current maximum allowance, the Step Down levels previously recommended by the NOSB are inadequate







3. Increasing Crude Protein to meet Methionine Demand

In order meet the bird's nutritional demands while formulating diets that would meet the step down limitations, most nutritionists have increased the percentage of crude protein in the diet. This is typically done by increasing the percentage of Soybean Meal (SBM), as SBM carries the highest levels of protein and methionine of the major feed ingredients. The TAP Review shown above acknowledges that methionine demand can be met in this fashion, but it is very important to note that increasing crude protein several problems.

Proper formulation requires an appropriate balance of dietary inputs against nutritional needs. By increasing the crude protein / SBM percentage, the bird's methionine demand may be met, but the diet is fundamentally unbalanced. The bird receives an excess of protein and other amino acids. Because these proteins are greater than the bird's nutritional needs, they pass through the bird unused and are excreted out into the litter in the poultry house. This high protein excretion takes the form of uric acid, which in turn breaks down into water and ammonia, leading to elevated levels of ammonia in the litter.

In order to meet the proposed animal welfare standards for organic poultry production, farmers must maintain acceptable nitrogen levels in the house. But when diets are formulated with excessive crude protein, the ammonia levels in the poultry house increase.

The imbalanced diet not only results in significant unnecessary cost for the poultry farmer, but also leads to animal welfare and environmental concerns.

4. Current Situation in the EU

Synthetic amino acids are not allowed in EU organic production. Since January 1, 2010, EU producers have also been limited to no more than 5% non organic feed in their diets. Effective January 1, 2012, EU producers will be required to use 100% organic feed.

For now, EU producers have responded by maximizing the utilization of the 5% non-organic allowance. Corn Gluten Meal is an important alternative that can close the methionine gap, even when limited to the 5% allowance. However, CGM is not available in organic form, and therefore not available to US producers.

Similarly, Betaine, a by-product of sugar beets, has the ability to partially spare the bird's methionine demand. This product is currently used by EU producers and MTF trials showed good results from this product. Betaine can be derived from organic beets, and has previously been approved for organic production. However, the world supply of organic beets is currently inadequate to support the amount of Betaine needed and this product is not currently allowed under the USDA organic standards.

MTF correspondence with EU producers indicates that most producers are simply feeding excessive amounts of crude protein to meet the bird's amino acid requirements. The implications of this have been discussed previously. There is also discussion in the EU about delaying the 100% organic feed requirement for some additional time.

5. Discussion of Alternatives

It is important to understand that the Stepped Down averages recommended in this petition are still only <u>marginally adequate</u> to meet the bird's methionine demand. Poultry farmers still will be continually faced with the threat of insufficient methionine in the diets. Consequently, producers still need to maintain an active and well funded Methionine Task Force that continues to evaluate new potential methionine sources and sponsors research into the most promising alternatives.

Included in this petition are various updates on specific alternatives that continue to be investigated by the MTF.

- **Pasture**

As discussed in the previous petition, plant based feed ingredients tend to be lower in protein and MET than animal sources. Chickens are omnivores who depend upon animal protein sources in their natural diet in order to meet their MET demand. With respect to pasture poultry, Dr. Anne Fanatico notes that "foraging should be encouraged", but also states that "the MET level of forage is generally low to moderate".

Moritz et al. (2005) concluded that "the ability of forage to meet the MET requirement depends on environmental conditions and subsequent feed intake." Additionally, Moritz found that forage digestibility varies over the seasons. In most parts of the US, pasture poultry operations are either limited or completely impractical in winter months.

- **High Methionine Corn**

High Methionine (HM) corn research is being led in the US by Dr. Walter Goldstein of the Michael Fields Agricultural Institute. Over the past several years, the MTF has sponsored hybrid development, seed multiplication, and feed stock trials with the MFAI.

In short, there are two main types of HM corn: hard endosperm and soft endosperm (or floury) corn. The crop yields from hard endosperm hybrids approximate 80-90% of standard control varieties. However, the hard endosperm hybrids tend to have less consistent MET levels, and generally lower levels, when compared to the floury strains. In addition, the MET:Protein ratio is the same as standard corn varieties.

The MET:Protein ratio is an important point to understand when formulating to offset methionine deficiencies. For feed ingredients to be viable as a methionine source, <u>they must have higher levels of methione without pro rata increases in Protein</u>. The feed ingredient is only of value in this equation if there is an incremental increase in Methionine---increasing Protein and MET will simply lead to over-feeding of crude protein, which has already been discussed.

The soft endosperm or floury hybrids can have higher MET levels and increased MET:protein ratios. However, the crop yields are disappointing---below 70% according to MFAI trials. Feed corn trials done by MTF members (Kreamer Feeds) and SunOpta resulted in yields well below 50% of the control groups.

Development of high methionine corn on a commercial scale will require the following:

- Improved agronomics, including more consistent methionine levels and yields equivalent to standard organic hybrids. It is hoped that Dr. Goldstein's continued research with the soft endosperm opaque varieties can work to close the yield gap.

- Verification, through replicated poultry feeding trials, that the methionine content is sufficient to meet the needs of all poultry. Feeding trials should also consider the effectiveness of high methionine corn when used in conjunction with other feed ration additives (e.g. Canola Meal, Pearl Millet).

- Significant additional time to conduct farming and feeding experimentation. It is believed that commercial scale implementation is <u>at least</u> five years away.

- **<u>Naturally Produced Methionine</u>**

The MTF has been working with Dr. Steve Ricke at the University of Arkansas (Department of Poultry Science and Center for Food Safety) and a copy of his research to date is attached as an exhibit to this petition. In his recent study, yeast strains were isolated and evaluated for their potential to over-produce methionine. One of the yeast strains identified was non pathogenic (GRAS) and as having potential for methionine production at an industrial level. Dr. Ricke is in the process of submitting a research request to the MTF for a further round of study to evaluate the viability of this yeast strain as a methionine source on a commercial scale.

- **<u>Canola Meal</u>**

There is anecdotal evidence that Canola Meal is being used in the EU to help meet MET demand. Canola Meal has a better MET:Protein ratio than SBM. However, since it has lower protein overall, there is a limited inclusion rate in a balanced diet. Additionally, it is lower in Lysine than SBM, with lysine being the second most limiting amino acid for poultry diets. Consequently, this product does not appear viable to offset an MET deficiency.

- **<u>Pearl Millet</u>**

The MTF has also investigated Pearl Millet as a potential MET source. Pearl Millet could potentially be used in the place of corn in the diet, although Pearl Millet has lower energy level than corn. If all corn in the diet was replaced by Pearl Millet, synthetic MET inclusion could be reduced by approximately 25%. However, similar to Canola Meal, a Lysine deficiency would result. As a more practical matter, Pearl Millet could be used as a partial substitute for corn, up to perhaps 10% of the diet. And while it would certainly provide some incremental methionine and start to “close the gap”, it would not eliminate the need for supplemental synthetic MET.

6. <u>Conclusions and Recommendations</u>

The MTF has been unable to develop scientific support for the current Step Down proposed by the NOSB. Moreover, the move from the prior MTF petition (asking for an Average allowance) to the current NOSB recommendation (requiring a lower maximum cap) creates some very significant environmental, economic, and animal welfare issues.

The MTF had represented to the NOSB that it would work with its group of nutritionists to determine the absolute minimum number for acceptable MET inclusion in organic poultry diets. Unfortunately, it is extraordinarily difficult to develop a single “number” that takes into account all the variables of bird type, breed, stage of life, environment, diet, etc. Consequently, if the discussion is limited to a maximum allowance of MET, the number is larger, similar to the cap currently in place.

By agreeing to calculate methionine allowances average over the life of the bird, the following will result:

- Feed rations can better adjust to the naturally changing demands of the bird. Poultry farmers will have more flexibility to appropriate adjust diets for stage of life, seasonality, breed, etc.
- Overall usage of MET will be lowered. Producers can only add methionine to the average cap, not consistently add methionine at the maximum rate
- Farmers and nutritionists will still be only marginally capable of meeting the bird's basic needs. The organic poultry industry will continue to have a tremendous incentive to actively evaluate novel sources of methionine.

This petition by the MTF seeks to align the interests of the NOSB (setting a cap, lowering the MET allowance, incenting the poultry industry to explore alternatives) with that of poultry farmers (allowing flexibility in formulations, providing time to bring alternatives to market, minimizing excess crude protein in the diets). The MTF makes this petition with the intent of resolving this issue once and for all for the benefit of all concerned parties.

Commercial Confidential Information Statement - describing information that is considered to be confidential business or commercial information:

- *None of the information submitted in this report is considered confidential.*

LIST OF ATTACHMENTS AND STATUS

As of April 8, 2011

- **Methionine Task Force Members** –*attached*
- **MSDS Sheet for DL Methionine** –*attached*
- **Screening and Identification of Natural Microbial Sources for Methionine Production for Organic Poultry Feed**–*attached*
- **Poultry Diets from the 1940's** –*attached*
- **Summary of Methionine Sources** –*attached*

Methionine Task Force Members

Company Name	***Representatives***
Eberly Poultry	Bob Eberly
Kreamer Feed	Bob Schwartz
	Steve Smelter
	Billy Robinson
Heritage Poultry	Chris Pierce
	Mel Gehman
Organic Valley / CROPP Cooperative	David Bruce
Chino Valley Ranchers / MCM Poultry	David Will
	Steve Nichols
Free Bird Chicken / Hain Celestial	Joe DePippo
Organics Unlimited	Ken Rice
Foster Farms	Dr. Alfonso Mireles
	Shivi Rao
Coleman Natural Foods	Dave Martinelli
	Mike Leventini
Hidden Villa Ranch	Mike Sencer
Pete and Gerry’s Organic Eggs	Jesse Laflamme
Herbruck’s	Greg Herbruck
SonCrest Eggs	Randy Boone
Delta Egg Farm	Sherman Miller
Nature Pure, LLC	Kurt Lausecker
Centurion Poultry	Gijs Schimmel

Material Safety Data Sheet (MSDS) for DL Methionine

SIGMA-ALDRICH

Material Safety Data Sheet

Date Printed: 03/AUG/2005
Date Updated: 16/MAR/2004
Version 1.1
According to 91/155/EEC

1 - Product and Company Information

Product Name	DL-METHIONINE
Product Number	M9500
Company	Sigma-Aldrich Pty. Ltd. 12 Anella Avenue Castle Hill NSW 2154
Technical Phone #	+61 2 9841 0555 (1800 800 097)
Fax	+61 2 9841 0500 (1800 800 096)
Emergency Phone #	+44 8701906777 (1800 448 465)

2 - Composition/Information on Ingredients

Product Name	CAS #	EC no	Annex I Index Number
DL-METHIONINE	59-51-8	200-432-1	None

Formula: $C_5H_{11}NO_2S$
Molecular Weight: 149.21 AMU
Synonyms: Acimetion * alpha-Amino-gamma-methylmercaptobutyric acid * DL-2-Amino-4-(methylthio)butyric acid * Banthionine * Cynaron * Dyprin * Lactet * Lobamine * Meonine * Methilanin * (+-)-Methionine * DL-Methionine (9CI) * Metione * Mertionin * Neston * Racemethionine

3 - Hazards Identification

SPECIAL INDICATION OF HAZARDS TO HUMANS AND THE ENVIRONMENT
Not hazardous according to Directive 67/548/EEC.

4 - First Aid Measures

AFTER INHALATION
If inhaled, remove to fresh air. If breathing becomes difficult, call a physician.

AFTER SKIN CONTACT
In case of contact, immediately wash skin with soap and copious amounts of water.

AFTER EYE CONTACT
In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. Call a physician.

AFTER INGESTION
If swallowed, wash out mouth with water provided person is conscious. Call a physician.

5 - Fire Fighting Measures

EXTINGUISHING MEDIA
Suitable: Water spray. Carbon dioxide, dry chemical powder, or appropriate foam.

SPECIAL RISKS
Specific Hazard(s): Emits toxic fumes under fire conditions.

SPECIAL PROTECTIVE EQUIPMENT FOR FIREFIGHTERS
Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

6 - Accidental Release Measures

PROCEDURE(S) OF PERSONAL PRECAUTION(S)
Exercise appropriate precautions to minimize direct contact with skin or eyes and prevent inhalation of dust.

METHODS FOR CLEANING UP
Sweep up, place in a bag and hold for waste disposal. Avoid raising dust. Ventilate area and wash spill site after material pickup is complete.

7 - Handling and Storage

HANDLING
Directions for Safe Handling: Avoid inhalation. Avoid contact with eyes, skin, and clothing. Avoid prolonged or repeated exposure.

STORAGE
Conditions of Storage: Keep tightly closed.

8 - Exposure Controls / Personal Protection

ENGINEERING CONTROLS
Safety shower and eye bath. Mechanical exhaust required.

GENERAL HYGIENE MEASURES
Wash thoroughly after handling.

PERSONAL PROTECTIVE EQUIPMENT
Respiratory Protection: Wear dust mask.
Hand Protection: Protective gloves.
Eye Protection: Chemical safety goggles.

9 - Physical and Chemical Properties

Appearance — Physical State: Solid
Color: White
Form: Powder

Property	Value	At Temperature or Pressure
pH	N/A	
BP/BP Range	186 °C	
MP/MP Range	280 °C	
Flash Point	N/A	
Flammability	N/A	
Autoignition Temp	N/A	

Oxidizing Properties	N/A
Explosive Properties	N/A
Explosion Limits	N/A
Vapor Pressure	N/A
SG/Density	1.34 g/cm3
Partition Coefficient	N/A
Viscosity	N/A
Vapor Density	N/A
Saturated Vapor Conc.	N/A
Evaporation Rate	N/A
Bulk Density	N/A
Decomposition Temp.	N/A
Solvent Content	N/A
Water Content	N/A
Surface Tension	N/A
Conductivity	N/A
Miscellaneous Data	N/A
Solubility	Solubility in Water:soluble

10 - Stability and Reactivity

STABILITY
Stable: Stable.
Materials to Avoid: Strong oxidizing agents.

HAZARDOUS DECOMPOSITION PRODUCTS
Hazardous Decomposition Products: Carbon monoxide, Carbon dioxide, Nitrogen oxides, Sulfur oxides.

HAZARDOUS POLYMERIZATION
Hazardous Polymerization: Will not occur

11 - Toxicological Information

RTECS NUMBER: PD0456000

SIGNS AND SYMPTOMS OF EXPOSURE
To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

ROUTE OF EXPOSURE
Skin Contact: May cause skin irritation.
Skin Absorption: May be harmful if absorbed through the skin.
Eye Contact: May cause eye irritation.
Inhalation: May be harmful if inhaled. Material may be irritating to mucous membranes and upper respiratory tract.
Ingestion: May be harmful if swallowed.

CHRONIC EXPOSURE - REPRODUCTIVE HAZARD

Species: Rat Rat
Dose: 40 GM/KG 40 GM/KG
Route of Application: Oral Oral
Exposure Time: (1-20D PREG) (1-20D PREG)
Result: Effects on Fertility: Other measures of fertility Effects on Embryo or Fetus: Extra embryonic structures (e.g., placenta, umbilical cord). Effects on Embryo or Fetus: Fetotoxicity (except death, e.g., stunted fetus). Effects on Fertility: Other measures of fertility Effects on Embryo or Fetus: Extra embryonic structures (e.g., placenta, umbilical cord). Effects on Embryo or Fetus: Fetotoxicity (except death, e.g., stunted fetus).

12 - Ecological Information

No data available.

13 - Disposal Considerations

SUBSTANCE DISPOSAL

Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber. Observe all federal, state, and local environmental regulations.

14 - Transport Information

RID/ADR

Non-hazardous for road transport.

IMDG

Non-hazardous for sea transport.

IATA

Non-hazardous for air transport.

15 - Regulatory Information

Not hazardous according to Directive 67/548/EEC.

COUNTRY SPECIFIC INFORMATION

Germany

WGK: 1

16 - Other Information

WARRANTY

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Isolation of methionine-producing microorganisms and methionine quantification

Suwat Saengkerdsub [1, 2], Jody M. Lingbeck [1], Debabrata Biswas [1],
Intrariya Saengkerdsub [1, 2], Si Hong Park [1, 2], Heather H. Wilkinson [3],
Arunachalam Muthaiyan[1], and Steven C. Ricke [1, 2 *]

[1] Center for Food Safety-IFSE, and Department of Food Science, University of Arkansas, Fayetteville, AR, 72704, USA.
[2] Department of Poultry Science, University of Arkansas, Fayetteville, AR 72701, USA ,
[3] Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas 77845

Introduction

In the U.S., approximately 90 % of poultry feed is composed of corn and soybean; however, methionine is one of the first limiting amino acids in chicken feed. Currently, methionine is produced by either a chemical process or protein hydrolysis. These reactions are expensive and require hazardous compounds. In 1996, methionine production was 350,000 tons; however, it was produced by chemical synthesis . Currently, organic poultry industry still uses methionine from chemical synthesis. The aims of this study were the isolation of methionine-producing microorganisms from environments and quantification of methionine production from these strains.

Materials and Methods

Methionine-producing yeast isolation: In this study, yeast samples were Caucasian Kefir grains and an air-trapped sample. Yeast cells were trapped from air by mixing all purpose flour and water stand at opened-environment for 10 hours. Kefir grains and air-trapped sample were inoculated into yeast minimal medium . The cultures were maintained at 30 ^{o}C, 200 rpm and were transferred to fresh medium for eight consecutive days.

Methionine feedback effect assay: For screening methionine-overproducing yeast, each strain was assayed on yeast minimal medium agar containing norleucine up to 2 % (w/v). The plates were incubated under aerobic atmosphere at 30 ^{o}C for two days.

Identification of potential methionine-producing microorganisms: The large subunit rRNA (LSU rRNA) genes of yeast strains were amplified by using primers NL-1 and NL-4. The thermal cycling profile was as follows: 2 min at 95 ^{o}C, followed by 30 cycles of 30 s at 95 ^{o}C, 30 s at 52 ^{o}C, 1 min at 72 ^{o}C, followed finally by 10 min at 72 ^{o}C. The PCR products were sequenced by DNA Resource Center, University of Arkansas.

Yeast growth measurement: The potential methionine-overproducing yeast strain was cultured in Lactose medium at 30 ^{o}C, 200 rpm for 12 hours. The 1/100 volume of yeast cells was transferred into 50-ml fresh medium in three-baffled 500-ml flask and was maintained at the same condition for 14 hours. The turbidity of the culture read at 580 nm (OD580).

Quantification of amino acids by using thin layer chromatography (TLC): Cells were grown in minimal medium at 37 ^{o}C for 1 to 3 days and 10 µl volumes of cultures were spotted on thin layer chromatography. Thin layer chromatography was then carried out with n-butanol/formic acid/methanol/water (4:1:2:0.5 by vol.) (Kase and Nakayama,1975). Amino acids were detected with ninhydrin reagent.

Methionine quantification by HPLC: Yeast strain K1 was cultured in Lactose medium until the late log phase. Yeast cells were collected by centrifugation at 14,000 x g, 4 ^{o}C for 10 min and were washed three times with the same volume of 0.9 % NaCl solution. For cell digestion, 100 µl of 6 N HCl was added into each sample and the samples stand at 110 ^{o}C for 1 hour. Methionine and norleucine were dissolved in 0.1 N HCl and were kept at 4 ^{o}C. Norleucine was added into each sample as internal standard. Methionine standard solution and yeast lysates were prepared with some modification. Standards or samples (10 µl each) plus 10 µl of 10 mg/ml norleucine solution were mixed in screw-cap tubes with 40 µl of 500 mM sodium bicarbonate pH 9.0 and 95 µl of dabsyl chloride reagent (4 mM in acetonitrile). The tubes were sealed with Teflone-lined caps and heated at 70 ^{o}C for 10 min. After cooling, the mixture was diluted with 1 ml of 70 % ethanol and filtered through 0.22-µm-pore-size Millipore filter.

Results and Conclusions

Identification for potential methionine-overproducing microorganism by molecular method: The LSU rRNA sequences of strain K1 and Y1 were matched with *Kluyveromyces marxianus*, and *Candida* sp. Since *Pantoea* sp., *P. aeruginosa*, and *Candida* sp. may potentially be pathogens, only yeast strain K1 was used in future steps. Two yeast strains were identified by sequencing rRNA genes as the standard method. Strain A171 originated from soybean field at the University of Arkansas belongs to the genus *Pantoea*. This result was in agreement that the genus *Pantoea* are generally associated with plants. Three yeast species have been isolated in Taiwanese kefir grains and one of them is *K. marxianus* which was isolated from kefir gains in our study.

Methionine quantification: Since only yeast strain K1 was a non-pathogenic microorganism, the growth of this strain was measured (Fig.1). The lag phase was 4 hours and the late exponential phase was 14-hour incubation. Yeast cells were collected at 14 hours incubation and methionine was quantified by HPLC. The methionine amount was 8.46 mg per gram dry cell. The amount of methionine produced by *K. marxianus* in this study coincides with the results reported in the previous investigations for other yeast. One previous study showed that methionine was one of the main amino acids from *K. marxianus* and this might explain the higher methionine found in this study than other reports.

Conclusion: Since *K. marxianus* is recognized as a “generally recognized as safe” (GRAS) organism, strain K1 in this study might be potential for methionine production at industrial level. Also, it is possible to culture yeast strain K1 as the single cell protein in organic waste

Acknowledgments

This work was supported by the Methionine Task Force, Coleman Natural Foods, Petaluma, CA. We thank Rohana Liyanage at Chemistry and Biochemistry dept, University of Arkansas for technical assistance.

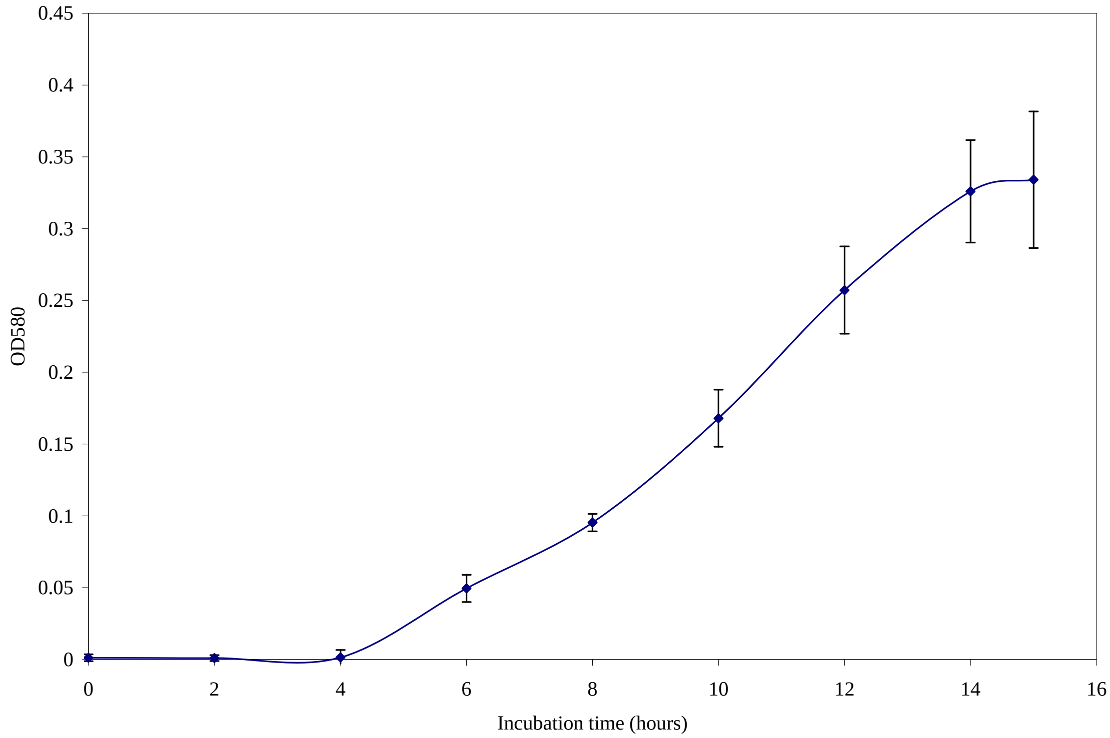

Fig. 1 Growth of yeast strain K1.

Poultry Diets from the 1940's

		Weaning to 50 lbs.	50 to 75 lbs.	75 to 125 lbs.	125 to 175 lbs.	Over 175 lbs.
		Lbs.	Lbs.	Lbs.	Lbs.	Lbs.
4.	Barley	79	86	92	99	99
	Tankage, meat scraps, or fish meal	8	6	3.5	..	..
	Linseed meal	13	8	3.5	..	..
	Bone meal	..	..	0.6	0.6	0.6
	Ground limestone	..	..	0.4	0.4	0.4
	Total	100	100	100	100	100
5.	Grain sorghum	78	83	91	95	96
	Tankage, meat scraps, or fish meal	8	6	4	..	..
	Soybean oil meal	14	11	5	4	3
	Bone meal	..	..	..	0.6	0.6
	Ground limestone	..	..	..	0.4	0.4
	Total	100	100	100	100	100

POULTRY*

Laying hens and laying pullets

In addition to the mash and scratch grain in these rations, oyster shell or another source of calcium should be available at all times in hoppers. Plenty of fresh water should also be furnished. Cod-liver oil or other suitable vitamin-rich fish oil should be added to the mash when the hens are confined. To insure an ample supply of manganese, 0.25 lb. of manganese sulfate should be added to each ton of mash.

A. Laying mashes with which an equal weight of grain is to be fed

1. Ground yellow corn, 20 lbs.; ground heavy oats, 12.5 lbs.; wheat bran, 20 lbs.; wheat flour middlings, 20 lbs.; dried skimmilk or buttermilk, 2.5 lbs.; meat scraps, 17.5 lbs.; alfalfa meal (low fiber), 5 lbs.; ground limestone or oyster shell flour, 2 lbs.; salt, 0.5 lb. Add 0.4 lb. vitamin A and D feeding oil when hens are not on range.

2. Ground yellow corn, 25 lbs.; ground oats, 10 lbs.; wheat standard middlings, 10 lbs.; soybean oil meal, 24 lbs.; peanut oil meal or other suitable plant-protein supplement, 5 lbs.; meat scraps, 4 lbs.; alfalfa meal, 9 lbs.; distillers' dried solubles, 2 lbs.; ground limestone, 5.5 lbs.; bone meal, 3.5 lbs.; salt, 1.6 lbs.; vitamin A and D feeding oil, 0.4 lb.

3. Ground barley, 30 lbs.; ground wheat, 12 lbs.; soybean oil meal, 20 lbs.; corn gluten meal or soybean oil meal, 7.5 lbs.; peanut oil meal or other suitable plant-protein supplement, 4 lbs.; meat scraps, 3.5 lbs.; dried whey, 2 lbs.; alfalfa meal, 10 lbs.; ground limestone, 5.5 lbs.; bone meal, 3.5 lbs.; salt, 1.6 lbs.; vitamin A and D feeding oil, 0.4 lb.

4. Ground grain sorghum, 30 lbs.; ground barley, 13 lbs.; soybean oil meal, 22.5 lbs.; peanut oil meal or other suitable plant-protein supplement, 7.5 lbs.; meat scraps, 4 lbs.; alfalfa meal, 10 lbs.; distillers' dried solubles, 2 lbs.; ground limestone, 5.5 lbs.; bone meal, 3.5 lbs.; salt, 1.6 lbs.; vitamin A and D feeding oil, 0.4 lb.

5. For hens on good pasture: ground yellow corn, 100 lbs.; ground oats, 100 lbs.; wheat bran, 100 lbs.; wheat middlings, 100 lbs.; meat scraps, 100 lbs.; salt, 5 lbs.

*Unless otherwise indicated, these rations are chiefly from: Titus, Hammond, Whitson, and Bird, U. S. Dept. Agr., Special Cir., Dec., 1946; Heuser, Feeding Poultry, 1946.

B. Grain mixtures to be fed with laying mash

Almost any mixture of grain that does not contain too large a proportion of oats is suitable. Popular combinations are:

1. Equal weights of yellow corn (cracked or whole) and wheat.
2. Yellow corn, 40 lbs.; wheat, 40 lbs.; oats, 20 lbs.
3. Grain sorghum, 30 to 50 lbs.; yellow corn, 25 to 40 lbs.; wheat, 20 lbs.

C. All-mash rations for laying hens and laying pullets

1. Ground yellow corn, 50.5 lbs.; wheat bran, 10 lbs.; wheat flour middlings, 20 lbs.; meat scraps, 7.5 lbs.; dried skimmilk, 2.5 lbs.; alfalfa meal, 5 lbs.; salt, 0.5 lb.; oyster shell meal or ground limestone, 3 lbs.; cod-liver oil, 1.0 lb.
2. Ground yellow corn, 42.5 lbs.; ground oats, 10 lbs.; wheat standard middlings, 15 lbs.; wheat bran, 5 lbs.; soybean oil meal, 12 lbs.; peanut oil meal or other suitable plant-protein supplement, 3 lbs.; meat scraps, 2 lbs.; alfalfa meal, 4 lbs.; ground limestone, 3 lbs.; bone meal, 2.5 lbs.; salt, 0.8 lb.; vitamin A and D feeding oil, 0.2 lb.
3. Ground barley, 30 lbs.; ground wheat, 36.5 lbs.; soybean oil meal, 12 lbs.; corn gluten meal or soybean oil meal, 4 lbs.; peanut oil meal or other suitable plant-protein supplement, 3 lbs.; meat scraps, 2 lbs.; alfalfa meal, 6 lbs.; ground limestone, 2.5 lbs.; bone meal, 3 lbs.; salt, 0.8 lb.; vitamin A and D feeding oil, 0.2 lb.
4. Ground grain sorghum, 40 lbs.; ground barley, 28 lbs.; soybean oil meal, 12.5 lbs.; peanut meal or other suitable plant-protein supplement, 5 lbs.; meat scraps, 2 lbs.; alfalfa meal, 6 lbs.; ground limestone, 2.5 lbs.; bone meal, 3 lbs.; salt, 0.8 lb.; vitamin A and D feeding oil, 0.2 lb.

D. Mashes for breeding hens

For hens producing eggs for hatching, the same sort of mashes are satisfactory, except that a mash to be fed with grain should contain at least 7 to 9 per cent of protein supplements of animal origin (including meat scraps, fish meal, and dried dairy by-products). Also, the content of riboflavin should be increased. (1409)

Chicks and growing chickens

A. All-mash chick starters

1. Ground yellow corn, 20 lbs.; ground wheat, 32 lbs.; soybean oil meal, 21 lbs.; other suitable plant-protein supplement, 10 lbs.; dried skimmilk or buttermilk, 5 lbs.; alfalfa meal, 7.7 lbs.; bone meal, 2 lbs.; ground limestone or oyster shell, 1.2 lbs.; salt, 1 lb.; vitamin A and D feeding oil, 0.1 lb.
2. Ground barley or grain sorghum, 42 lbs.; ground wheat, 10 lbs.; soybean oil meal, 21 lbs.; other suitable plant-protein supplement, 10 lbs.; meat scraps, 2.5 lbs.; dried whey, 5 lbs.; alfalfa meal, 6 lbs.; bone meal, 1.1 lbs.; ground limestone or oyster shell, 1.3 lbs.; salt, 1 lb.; vitamin A and D feeding oil, 0.1 lb.
3. Ground yellow corn, 42 lbs.; ground oats or wheat middlings, 10 lbs.; soybean oil meal, 24 lbs.; other suitable plant-protein supplement, 10 lbs.; fish meal, 2 lbs.; dried whey, 5 lbs.; distillers' dried solubles, 2.7 lbs.; bone meal, 2 lbs.; ground limestone or oyster shell, 1.2 lbs.; salt, 1 lb.; vitamin A and D feeding oil, 0.1 lb.
4. Ground wheat, 52 lbs.; soybean oil meal, 21 lbs.; other suitable plant-protein supplement, 10 lbs.; meat scraps, 3 lbs.; alfalfa meal, 8 lbs.; distillers' dried solubles, 2.6 lbs.; bone meal, 1 lb.; ground limestone or oyster shell, 1.3 lbs.; salt, 1 lb.; vitamin A and D feeding oil, 0.1 lb.

SUMMARY OF METHIONINE SOURCES

Alternative	Approx. Methionine %	Synthetic	Natural	Organic	Commercially Available	Other Comment
Dl Methionine	99.00	Yes				Included as 0.001
Corn	0.18			Yes	Yes	*Currently in Poul*
Hi Methionine Corn	0.36			Yes	No	In R&D Phase
Soybean Meal	0.60			Yes	Yes	*Currently in Poul*
Sesame Meal	1.26			Yes	No	Deficient in Lysin
Sunflower Meal	0.72			Yes	No	Relatively Low M
Canola Meal	0.75			Yes	No	Relatively Low M
Alfalfa Meal	0.20			Yes	No	Very Low Methio
Corn Gluten Meal	1.50		Yes			Not available in o
Flax Meal	0.50			Yes	No	Relatively Low M
Blood Meal	1.00			No		Not allowed: Anin
Insects	Unknown				No	Pilot project unde
Earthworm Meal	1.60				No	Not being produc
Crab Meal	0.50					Not available in o
Meat Meal	0.81					Not allowed: Anin
Rice	0.22			Yes	No	Very Low Methio
Casein	2.56			Yes	No	Ratio of methioni
Milk Powder, Skim	0.79			Yes	Yes	Relatively Low M
Potato Protein	1.60		Yes		No	Not available in o
Fish Meal	1.68		Yes	??		Not available in o methionine to cys