Methionine
Livestock

Identification of Petitioned Substance

Chemical Names:
Methionine
DL-2-amino-4-(methylthio)butyric acid
L-2-amino-4-(methylthio)butyric acid
(S)-2-amino-4-(methylthio)butanoic
Acid
D-2-amino-4-(methylthio)butyric acid

Other Names:
DL-methionine, L-methionine, D-methionine, racemethionine

Trade Names:
Mepron® and Alimet® (Evonik Degussa, Germany)

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

Other Codes:
International Feed Names (IFNs):
DL-methionine: 5-03-86
DL-methionine hydroxy analog calcium: 5-03-87
DL-methionine hydroxy analog: 5-30-281

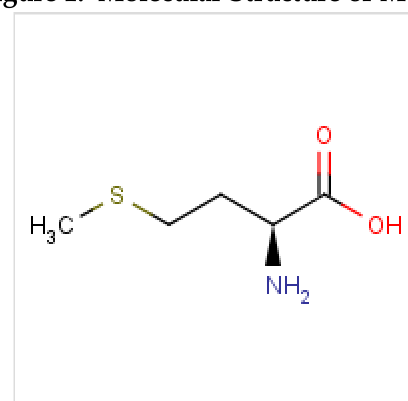
Characterization of Petitioned Substance

In preparing this technical report, ICF reviewed and updated the information presented in the Technical Advisory Panel (TAP) Review for methionine used in livestock (USDA, 2001). Details from the 2001 TAP Review that still reflect the best and most currently available information were used in preparing the document.

<u>**Composition of the Substance**</u>:

Amino acids have an amino group (NH_2) adjacent to a carboxyl (COOH) group on a carbon. Methionine, with an empirical formula of $C_5H_{11}NO_2S$, is a sulfur-containing essential amino acid. The molecular structure of methionine is shown as Figure 1.

Figure 1. Molecular Structure of Methionine



Source: ChemIDplus Lite (2011)

Properties of the Substance:

Methionine is typically found as a white solid or white crystalline powder. Methionine is asymmetric, forming both an L- and a D- enantiomer. Methionine hydroxy analog (MHA) is available in liquid form. It is soluble in water, methanol, alkali solutions, and mineral acids, and is slightly soluble in ether. Methionine is stable under normal temperature and pressure, but is incompatible with strong oxidizing agents (Acros, 2009). Hazardous decomposition products of methionine include nitrogen oxides, carbon monoxide, oxides of sulfur, and carbon dioxide (Pestell Minerals and Ingredients, 2008). Physical and chemical properties of methionine are summarized in Table 1.

Table 1. Physicochemical Properties of Methionine

Physical or Chemical Property	Value[a]
Physical state	Solid
Appearance	White crystalline powder
Odor	Characteristic
Molecular weight (g/mol)	149.21
Boiling point	NA
Melting point	281°C
Solubility in water (g/L)	30 (20°C)
Vapor pressure (hPa)	<0.0000001
Density (g/cm^3)	1.34

[a]Sources: ChemIDplus Lite (2011); Pestell Minerals and Ingredients (2008).

Specific Uses of the Substance:

In poultry production, methionine is used as a feed additive. For optimum health and performance, the animal's diet must contain adequate quantities of all nutrients needed, including amino acids. A shortage in the diet of one or more of the essential amino acids could constrain animal growth, reduce feed efficiency, and, in extreme cases, cause nutritional deficiency. Supplementation with isolated amino acids increases feed conversion efficiency, thus lowering feed costs per unit of weight gain or production (Pond et al., 1995). Methionine is considered to be the first limiting amino acid in corn-soy poultry diets, and the literature indicates that cysteine (specifically total cysteine + methionine, which are both sulfur amino acids) and lysine are the next limiting amino acids (NRC, 1994; Cheeke, 1999; Gehrke et al., 1987). Cyst(e)ine comes in two forms—Cys (cysteine) and Cys-Cys (cystine), which is comprised of two bonded cysteines. According to Dilger et al. (2007), both forms support animal growth equally when provided in a cyst(e)ine-deficient and methionine-adequate diet. Extensive literature has been published that documents the efforts to optimize the balance of amino acids in poultry diets in an effort to lower feed costs, reduce the need for animal or fish proteins, replace soy meal with less expensive or more locally available plant proteins, and utilize plant proteins more efficiently (Degussa, 1995, 1996; North and Bell, 1990; Neto et al., 2000; Cino et al., 1999; Emmert et al., 2000; D'Mello, 1994; Waibel et al., 2000).

Amino acids are also used in livestock healthcare. Methionine is used as a urine acidifier because excretion of its sulfate anion lowers urine pH. In lowering pH, the sulfate anion may also displace phosphate from the magnesium-ammonium-phosphate hexahydrate (struvite) crystals and uroliths, which form best at a pH above 6.4–6.6. As a result, methionine may assist in dissolving and/or preventing uroliths, kidney stones, bladder stones, or urologic syndromes thought to be caused by struvite crystals or uroliths (Lewis et al., 1987). Methionine, important in mobilizing fat and transporting fat from cells, is sometimes used to assist in the treatment and/or prevention of hepatic lipidosis, or fatty liver disease in livestock (USDA, 2001). However, it appears there are insufficient data to support its efficacy in treating this condition (Merck Veterinary Manual, 2011).

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 analog calcium (minimum of 97% racemic 2-amino-4-methylthiobutyric acid and calcium salt; 21 CFR 582.5477) and DL-methionine hydroxy analog (minimum of 88% racemic 2-amino-4-methylthiobutyric acid; 21 CFR 582.5477) (USDA, 2001). AAFCO has a memorandum of understanding with the FDA's Center for Veterinary Medicine that allows FDA to formally recognize the Association's list of feed ingredients (FDA, 2007).

Approved Legal Uses of the Substance:

Synthetic methionine is currently included on the National List of Allowed and Prohibited Substances (hereafter referred to as the National List), 7 CFR 205.603(d), for use in organic livestock production as a feed additive. Specifically, DL–methionine, DL–methionine–hydroxy analog, and DL–methionine–hydroxy analog calcium can be used in organic poultry production until October 1, 2012, at the following maximum levels of synthetic methionine per ton of feed: laying chickens–4 pounds; broiler chickens–5 pounds; and turkeys and all other poultry–6 pounds. After October 1, 2012, the allowed levels will be reduced to 2 pounds for laying chickens, 2 pounds for broiler chickens, and 3 pounds for turkeys and other poultry through October 1, 2015 (76 FR 13501; "see OFPA, USDA Final Rule" section).

Methionine is also regulated as a human nutrient/dietary supplement and is generally recognized as safe (GRAS) by the U.S. Food and Drug Administration (FDA) for humans (21 CFR 582.5475). *N*-acetyl-L-methionine (CAS Number 65-82-7) is cleared for use as a food additive, but regulations explicitly say it is not to be used in infant feed formulas (21 CFR 172.372).

Action of the Substance:

Methionine is classified as an essential amino acid because it is required in the diet for cell growth, but cannot be biologically produced. Of the 22 amino acids found in body proteins, the National Research Council (NRC) lists 13 as essential in poultry diets, and these must be consumed in feed: arginine, glycine, histidine, isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, proline, threonine, tryptophan, and valine (NRC, 1994). The status of methionine, methionine + cysteine (together known as sulfur amino acids, or SAA), and lysine as the most limiting amino acids is well established in the literature on traditional poultry diets (Gehrke et al., 1987). Others that are deemed "next limiting amino acids" in poultry rations include threonine, valine, isoleucine, tryptophan, and arginine (North and Bell, 1990). Poultry feed made of corn and soybean does not supply enough methionine to prevent deficiency symptoms that include curled toes, bare spots, and improper feathering (Hungerford, 2007). In addition, amino acids like methionine improve the efficiency of the production of animal protein. Adding amino acids to feed reduces the amount of protein sources like soybean meal needed to produce the same amount of animal protein (Toride, undated).

Combinations of the Substance:

Amino acids are combined in feed rations of grains, beans, oilseeds, and other meals with antioxidants, vitamins, and minerals (Pond et al., 1995). In conventional agricultural feed products, amino acids also are combined with antibiotics and hormones, which are not permitted to be fed to organic livestock. Methionine is a precursor in the diet to cysteine, and the amount needed in the diet depends on the amount of cysteine already present. Requirements for methionine are frequently cited in terms of methionine + cysteine because methionine converts to cysteine as needed. There are also producers of the feed additive zinc methionine, a liquid complex of the trace mineral zinc chemically bonded with methionine (Global Animal Products, 2001).

Status

Historic Uses:

Synthetic methionine was first licensed for poultry feed in the 1950s (Evonik Corporation, undated). As discussed in the 2001 TAP Review, the widespread use of crystalline (pure) amino acids in formulated rations has expanded greatly since 1980 for nonorganic poultry production. The largest methionine factory in the world was opened in Antwerp, Belgium in 2006 (Evonik, undated). Most current use in organic poultry production appears to be as a supplement for broilers (meat chickens) and turkeys as well as for laying hen feed rations (Fanatico, 2010).

OFPA, USDA Final Rule:

Synthetic methionine is currently allowed on the National List (7 CFR 205.603(d)) for use in organic livestock production. However, a "step-down" measure was established to reduce the amount of synthetic methionine allowed in feed. Until October 1, 2012, the following maximum levels of synthetic methionine per ton of feed are allowed—4 pounds for laying chickens, 5 pounds for broiler chickens, and 6 pounds for turkeys and all other poultry. The NOSB has recommended that, after October 1, 2012, the allowed levels of methionine be reduced to 2 pounds for laying chickens, 2 pounds for broiler chickens, and 3 pounds for turkeys and other poultry through October 1, 2015 (76 FR 13501). As of the date of this publication, the NOP has not published regulations to implement the stepdown provisions that will take effect after October 1, 2012.

International

According to the Canadian General Standards Board, organic operators may not use "feed and feed additives, including amino acids and feed supplements that contain substances not in accordance with CAN/CGSB-32.311, Organic Production Systems - Permitted Substances Lists (CAN/CGSB-32.310-2006)." However, on the Permitted Substances List, nonsynthetic amino acids are permitted and an exception was made for synthetic DL-methionine, DL–methionine hydroxy analog, and DL–methionine hydroxy analog calcium until October 1, 2010. No further amendments to this exception were identified.

The Codex Alimentarius Commission (2010) does not specifically address methionine use in organic production. However, the livestock nutrition section of the Codex states that for additives and processing aids, "antibiotics, coccidiostatics, medicinal substances, growth promoters or any other substance intended to stimulate growth or production shall not be used in animal feeding"(Codex Alimentarius Commission, 2010).

Furthermore, for feedstuffs and nutritional elements, the guidelines specify the following criteria.

> (1) Feedstuffs of mineral origin, trace elements, vitamins, or provitamins can only be used if they are of natural origin. In case of shortage of these substances, or in exceptional circumstances, chemically well-defined analogic substances may be used.
>
> (2) Synthetic nitrogen or nonprotein nitrogen compounds shall not be used.

The second point appears to also prohibit synthetic amino acids. Nonprotein nitrogen compounds include substances such as urea and ammoniated materials (AAFCO, 2001). In the technical literature, nonprotein nitrogen is considered to include "free amino acids, amino acid amides, glucosides containing nitrogen, nucleotides, urea, nitrates, ammonium salts and other low-molecular weight compounds containing nitrogen" (Boda, 1990).

The European Economic Community (EEC) Council Regulations, EC No. 834/2007 and 889/2008, state that "growth promoters and synthetic amino acids shall not be used" in animal feed in organic production. Copper substances (copper (II) oxide, basic copper (II) carbonate, monohydrate, copper (II) sulphate, and pentahydrate) are allowed as nutritional additives in organic livestock per Annex VI of EC 889/2008, but copper (or other metal) chelates of the hydroxy analog of methionine are not listed as allowed trace metals for organic animal nutrition. However, EC No. 1253/2008 authorizes the use of the copper chelate of hydroxy analog of methionine (17% copper and 78% [2-hydroxy-4-methylthio]butanoic acid) as a feed additive for the fattening of conventionally fed (i.e., not organically fed) chickens. The supplement is used primarily to provide copper, a necessary trace element. However, some research has shown that (2-hydroxy-4-methylthio)butanoic acid may be bioavailable as a methionine source to animals that are given the analog along with trace minerals such as copper, zinc, and manganese (Yi et al., 2007). Authors stated that these mineral supplements have high methionine activity: 80% by weight for Mintrex-Zn, 78% for Mintrex-Cu, and 76% for Mintrex-Mn. In fact, the zinc supplement provided an equivalent amount of methionine as Alimet, a DL-methionine-equivalent supplement commonly used in feed (Yi et al., 2007). Although not allowed in organic agriculture in Europe, amino acid chelates are allowed in organic livestock production in the United States as a source of trace metals (USDA, 2011).

The International Federation of Organic Agriculture Movements (IFOAM) prohibits the use of "amino-acid isolates" in their norms (IFOAM, 2010).

The Japan Agricultural Standard (JAS) for Organic Feed makes no mention of the allowed or prohibited status of amino acids, or methionine specifically, in livestock feed. However, the standard states the following as permitted (JMAFF, 2005):

> *Feed additives (except for those produced by using antibiotic and recombinant DNA technology), which are natural substances or those derived from natural substances without being chemically treated. In case of a difficulty to obtain feed additives listed in 8, the use of similar agents to the described food additives are permitted only for supplementing nutrition and effective components in feeds.*

This suggests that methionine may be allowed if natural substitutes are not available.

Evaluation Questions for Substances to be used in Organic Crop or Livestock Production

<u>Evaluation Question #1: What category in OFPA does this substance fall under:</u> (A) Does the substance contain an active ingredient in any of the following categories: copper and sulfur compounds, toxins derived from bacteria; pheromones, soaps, horticultural oils, fish emulsions, treated seed, vitamins and minerals; livestock parasiticides and medicines and production aids including netting, tree wraps and seals, insect traps, sticky barriers, row covers, and equipment cleansers? (B) Is the substance a synthetic inert ingredient that is not classified by the EPA as inerts of toxicological concern (i.e., EPA List 4 inerts) (7 U.S.C. § 6517(c)(1)(B)(ii))? Is the synthetic substance an inert ingredient which is not on EPA List 4, but is exempt from a requirement of a tolerance, per 40 CFR part 180?

Methionine is classified as an essential amino acid, and is a sulfur-containing substance.

Methionine appears on EPA's table of Inert Ingredients Permitted for Use in Nonfood Use Pesticide Products in conventional agriculture (U.S. EPA, 2011a). L-methionine (CAS Number 63-68-3) is listed on the August 2004 document "Other ingredients for which EPA has sufficient information to reasonably conclude that the current use pattern in pesticide products will not adversely affect public health or the environment" (historically referred to as lists "4A" and "4B") (U.S. EPA, 2004). When used as a feed additive, methionine is not considered to be an inert ingredient, as defined under 7 CFR 205.2 because it is not included in any pesticide product.

Evaluation Question #2: Describe the most prevalent processes used to manufacture or formulate the petitioned substance. Further, describe any chemical change that may occur during manufacture or formulation of the petitioned substance when this substance is extracted from naturally occurring plant, animal, or mineral sources (7 U.S.C. § 6502 (21)).

Methionine may be isolated from naturally occurring sources, produced from genetically engineered organisms, or synthesized by a wide number of processes. While methionine has been produced by fermentation in the laboratory, racemic mixtures of D- and L- methionine (i.e., 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).
- The reaction of propylene, hydrogen sulfide, methane, and ammonia to make the intermediates acrolein, methylthiol, and hydrocyanic acid (DeGussa, 1995, 1996).
- Use of the Strecker synthesis method with α-methylthiopropionaldehyde as the aldehyde (Fong et al., 1981).
- The reaction of 3-methylmercaptopropionaldehyde with 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 TAP review for use of amino acid in organic crop production.

DL-methionine hydroxy analog calcium and DL-methionine hydroxy analog forms are considered to be alpha-keto acid analogs 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 nonessential amino acids such as glutamic acid (Cheeke, 1999; Leeson and Summers, 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).

Evaluation Question #3: Is the substance synthetic? Discuss whether the petitioned substance is formulated or manufactured by a chemical process, or created by naturally occurring biological processes (7 U.S.C. § 6502 (21)).

The petitioned substance is synthetic methionine. It is manufactured by the chemical processes described in Evaluation Question #2.

The nonsynthetic amino acid methionine is found naturally in foods such as: rice; rapeseed; soybean meal; sunflower, safflower, and sesame seeds; flax; alfalfa; grass; corn; wheat; and peas (Fanatico, 2010). Levels of methionine vary by food. For example, corn has only 0.17% methionine while soybean meal has 0.64% methionine. Methionine is also found naturally in animal protein from insects, fish, and dairy products, which are permitted in organic agriculture. Thus, natural methionine can be obtained from high-methionine foods; however, these foods are also high in protein. High protein diets are not physiologically healthy for birds due to excess excretion of uric acid, which is broken down into water and ammonia in the environment (Fanatico, 2010).

Evaluation Question #4: Describe the persistence or concentration of the petitioned substance and/or its by-products in the environment (7 U.S.C. § 6518 (m) (2)).

Synthetic methionine used as a nutritional supplement in livestock production can enter the environment through waste streams from its production, use, and disposal. Methionine has a relatively low vapor pressure, indicating that methionine present in soil or water is not likely to evaporate into air. Methionine is highly mobile in soil, and research has shown that most of the methionine in soil breaks down in about 16 days. Methionine can exist as a vapor or particulate in the air. Airborne methionine vapor will be degraded in the atmosphere with a half-life of about 7.5 hours. Methionine is also found naturally in water from metabolism of proteins. The potential for bioconcentration of methionine in aquatic organisms is considered low due to its high water solubility. Methionine will degrade in water from exposure to sunlight (HSDB, 2010).

<u>Evaluation Question #5:</u> Describe the toxicity and mode of action of the substance and of its breakdown products and any contaminants. Describe the persistence and areas of concentration in the environment of the substance and its breakdown products (7 U.S.C. § 6518 (m) (2)).

Toxicity and Health in Poultry

While it is nutritionally essential, methionine excesses (≥2–4% of diet) are far more toxic to poultry than similar excesses of other amino acids such as tryptophan, lysine, and threonine. Force feeding methionine to excess can result in death to chicks. Errors in feed formulation or excess supplemental methionine can depress growth and development at levels of 40 g/kg (4.0%) (Baker, 1989; NRC, 1994). Growth depressions resulting from excess supplemental amino acids include lesions in tissues and organs (D'Mello, 1994). However, NRC acknowledges that such toxicities are unlikely in practical circumstances for poultry in that amino acid toxicity requires a particularly high level of an amino acid relative to all others (NRC, 1994). 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).

Adequate supplies of methionine are required to maintain health in chickens. A number of reports cite the benefit of methionine supplementation in reducing 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, which caused decreased feed rates and lower rates of growth. Chicks that received deficient levels of methionine were more subject to an impaired immune response. It should be noted that 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.

Reduced feathering has been reportedly linked to lack of methionine and cysteine (Elliott, undated). Many other factors are also involved, including deficiencies of other amino acids, vitamins, zinc, feather pecking in cage systems, and cannibalism (Elliot, undated; NRC, 1994). Increased protein level is correlated with reduced feather loss and cannibalism (Ambrosen, 1997).

Toxicity in Other Animals

In mature cats, a dosage of 2 g/day (20 to 30 g/kg dry diet) for 20 days induced anorexia, ataxia, cyanosis, methemoglobinemia, and Heinz body formation resulting in hemolytic anemia (Maede et al., 1985). Rat studies of methionine indicate that it is significantly toxic in excess (Regina et al., 1993). High doses may lead to homocysteinemia, which is the build-up of homocysteine due to impaired methionine metabolism (Brattström et al., 1984, in Garlick, 2004). High levels of methionine were found to be toxic to hepatic cells and liver function in rat models. The results indicated that the biochemical reason for the extreme sensitivity of mammals to excess dietary methionine is likely 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 reports suggest that methionine is mutagenic (NIEHS, 1999b). According to one reproductive study, female rats administered 5% methionine in diet had no successful pregnancies (Matsueda and Niiyama, 1982, in Garlick, 2004). No other reproductive or developmental toxicity data were identified.

It is unlikely that the use of methionine and its breakdown products will cause harm to the environment. As discussed in Evaluation Question #8, methionine supplementation can reduce environmental pollution from nitrogen-rich manure, a significant concern in poultry production.

<u>Evaluation Question #6:</u> Describe any environmental contamination that could result from the petitioned substance's manufacture, use, misuse, or disposal (7 U.S.C. § 6518 (m) (3)).

As described in the 2001 TAP Review, synthetic production of DL-methionine involves a number of toxic source chemicals and intermediates. Each of the manufacturing processes used to produce DL-methionine was rated as either "moderately heavy" or "extreme" (Fong et al., 1981) in terms of toxics production, and it appears that newer processes have not replaced them. Storage tanks of methionine or intermediate chemicals can rupture and/or leak, releasing these chemicals into the environment.

Methyl mercaptan, the chemical used as a catalyst in the production of methionine, can react with water, steam, or acids to produce flammable and toxic vapors (Sax, 1984). Methyl mercaptan fires are highly hazardous and can cause death by respiratory paralysis (U.S. EPA, 1987). Another potential component of methionine production is acrolein, which has a toxicity rating of 5 (on a scale of 1 to 6 with 6 being most toxic) by Gosselin et al. (1984), and it is also an aquatic herbicide (Meister, 1999). Acrolein is an eye and respiratory tract irritant (OEHHA, 2000) listed as a federal air pollutant by U.S. EPA and is 1 of 33 pollutants of "greatest concern for exposure and health effects" (U.S. EPA, 2003).

<u>Evaluation Question #7:</u> Describe any known chemical interactions between the petitioned substance and other substances used in organic crop or livestock production or handling. Describe any environmental or human health effects from these chemical interactions (7 U.S.C. § 6518 (m) (1)).

No interactions between methionine and other feed additives were identified.

The primary chemical interactions of methionine occur physiologically once inside the body. While many of the interactions may be regarded as beneficial, excess methionine in the diet can cause deficiencies in other amino acids and induce toxicity (D'Mello, 1994). Excess methionine exacerbates deficiencies of vitamin B6, which results in depressed growth and feed intake (Scherer and Baker, 2000).

<u>Evaluation Question #8:</u> Describe any effects of the petitioned substance on biological or chemical interactions in the agro-ecosystem, including physiological effects on soil organisms (including the salt index and solubility of the soil) crops, and livestock (7 U.S.C. § 6518 (m) (5)).

<u>Interactions and Imbalances</u>

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 particular amino acids can cause problems in availability of other amino acids (Buttery and D'Mello 1994; Baker, 1989). Amino acid requirements may be affected by environmental temperature extremes, mainly 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 et al., 1999). In addition, immunological stress, age, species, genetics, and gender can also affect the physiological response of poultry to amino acids (Fanatico, 2010).

<u>Environmental Impact</u>

Managing the nitrogen cycle is seen as a challenge to livestock producers (Tamminga and Verstegen, 1992; Tamminga, 1992; Morse, undated). 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 buildup 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 phosphorus and calcium levels in feed, a goal in reducing phosphorus overload from poultry manure (Sebastian, 1997).

<u>Evaluation Question #9:</u> Discuss and summarize findings on whether the petitioned substance may be harmful to the environment (7 U.S.C. § 6517 (c) (1) (A) (i) and 7 U.S.C. § 6517 (c) (2) (A) (i)).

The most likely source of possible environmental contamination associated with synthetic methionine is through waste streams from its production. Methionine is manufactured using a number of toxic intermediates including methyl mercaptan and acrolein. However, it is unlikely that the use of methionine and its breakdown products will cause harm to the environment. As discussed in Evaluation Question #8, methionine supplementation can reduce environmental pollution from nitrogen-rich manure.

<u>Evaluation Question #10:</u> Describe and summarize any reported effects upon human health from use of the petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (i), 7 U.S.C. § 6517 (c) (2) (A) (i)) and 7 U.S.C. § 6518 (m) (4)).

Methionine is essential in small amounts in the human diet, and is an over-the-counter 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, and as a pharmaceutical adjuvant. However, methionine has been called the most toxic of amino acids (Benevenga and Steele, 1984 in Garlick, 2004). Methionine may cause nausea, vomiting, dizziness, irritability, and liver dysfunction at high doses and should be used with caution in patients with severe liver disease (Reynolds, 1996). In volunteers given 10–20 g/d of methionine by mouth for 2 weeks, 7 of 11 patients with schizophrenia experienced functional psychosis (Antun et al., 1971 in Garlick, 2004). In addition, animal studies indicate methionine may cause homocysteinemia, which is correlated with cardiovascular disease. This may be a concern for long-term users of methionine as a supplement (Garlick, 2004). These adverse effects are thought to be associated with the production of methanethiol-cysteine–mixed disulfides in the body.

The D- form of methionine is not well utilized by humans (Lewis and Bayley, 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 172.372).

It should also be noted that when heated to decomposition, methionine emits dangerous and highly toxic fumes, which may be a hazard to occupationally exposed workers (NIEHS, 1999a).

<u>Evaluation Question #11:</u> Describe all natural (non-synthetic) substances or products which may be used in place of a petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (ii)). Provide a list of allowed substances that may be used in place of the petitioned substance (7 U.S.C. § 6518 (m) (6)).

There are reports of herbal supplements that mimic methionine activity (e.g., Methiorep and Herbomethione), which are made up of methionine-rich herbs such as *Cicer arienticum, Triticum sativum, Phaseolus mungo, Mucuna puriens*, and *Allium cepa*; however, the efficacy and commercial availability of these products is unclear. See Additional Question #1 for more information on these herbal methionine sources.

Another way to supplement natural methionine is through consumption of additional plant and animal proteins. Diets can be formulated without supplemented synthetic acids to meet the objective of adequate methionine percentages, but this usually requires an increase in the crude protein level of the diet (Hadorn, 2000). A comparison study in growing chicks using supplemented and nonsupplemented diets found that adequate dietary methionine can be attained at a cost of higher intake of protein and less weight gain for the amount of protein consumed (Emmert et al., 2000). Another study fed one group of chickens a control diet using only corn and soy to satisfy amino acid levels and gave another group reduced protein supplemented with methionine and lysine. Authors concluded that diets with low protein could be used effectively when supplemented with methionine and lysine and that supplemented diets reduced both required feed intake by hens and reduced nitrogen excretion compared with traditional corn and soy diets. (Harms and Russell, 1998). See Additional Question #2 for a discussion of dietary methionine sources.

__Evaluation Question #12:__ Describe any alternative practices that would make the use of the petitioned substance unnecessary (7 U.S.C. § 6518 (m) (6)).

Raising chickens with access to pasture is considered a possible alternative to synthetic methionine supplementation. Some sources indicate that they can adequately raise chickens without synthetic methionine as long as the birds have adequate access to pasture (Hungerford, 2007). Forage provides low to moderate levels of methionine and allows birds to obtain high-quality protein from insects and worms (Fanatico, 2010). However, foraging conditions change by season, affecting the pasture's ability to supplement the diet. During certain times of the year, it is difficult for methionine needs to be met from forage alone (Rack et al., 2009). See Additional Question #4 for more information on pasture-raised chickens.

Additional Questions for Substances to be used in Livestock Production

__Additional Question #1:__ When would naturally extracted methionine (non-synthetic processing) be commercially available?

Research indicates that the organic poultry industry has not been able to develop a commercially viable, nonsynthetic form of methionine extract for use in organic poultry diets. While methionine can be extracted from intact proteins or proteins partially hydrolyzed to isolate it, there are still no commercially available forms of naturally extracted methionine (Fanatico, 2010).

Although almost all other amino acids can be developed using fermentation, while attempted, there is no commercial bioproduction of methionine (Kumar and Gomes, 2005; Usuda and Kurahashi, 2005). This is likely partially due to the complexity of the methionine biosynthetic pathway and, because methionine is vital to cellular function, it is highly regulated by microorganisms that produce it. The realization of large-scale fermentation production of methionine will likely require genetic modifications of microorganisms to deregulate some of these controls and allow for significant excretion of methionine (Usuda and Kurahashi, 2005). The yields from nonmodified bacteria would be too low for this to be a viable methionine source, but it is unclear if the genetically modified bacteria could produce commercially viable supplies of methionine. A recent patent application for a method to produce L-methionine by fermentation was available online (Usuda and Kurahashi, 2010); however, actual use of this method could not be confirmed.

There are reports of herbal supplements that mimic methionine activity (e.g., Methiorep and Herbomethione). These supplements are made up of herbs such as *Cicer arienticum, Triticum sativum, Phaseolus mungo, Mucuna puriens*, and *Allium cepa*. It appears that some herbs and spices contain S-adenosyl-l-methionine, the bioactive form of methionine, and this is the main ingredient in herbal methionine supplements (Salome et al., 2010). Two studies regarding the efficacy of herbal methionine were somewhat conflicting. Salome et al. (2010) indicated that Methiorep was not an effective substitute for methionine when administered in broiler chicken diets at 0.25, 0.5, and 1% Methiorep (compared to 0.25% methionine). While chickens had improved growth performance with Methiorep compared with controls, the chickens performed better when given 0.25% DL-methionine. On the contrary, Halder and Roy (2007) indicated that herbal methionine could be used more efficiently by broiler chickens than synthetic DL-methionine. They concluded that it could be substituted in an equal amount (e.g., 1.2 kg Herbomethionine instead of 1.2 kg DL-methionine) and be just as effective, if not more so, than the synthetic counterpart. These authors also found that the herbal methionine facilitated efficient lipid metabolism and may reduce the incidence of fatty liver disease in broiler chickens (Halder and Roy, 2007). It should be noted that both of these herbal methionine studies were conducted under conventional poultry raising systems. While the safety of these herbal supplements is not well documented, one study on herbal Methiorep found no adverse effects on growth, behavior, mortality, or biochemical parameters in rats (Rajurker et al., 2009).

<u>Additional Question #2</u>: To what degree would the current alternative methionine sources listed below, or any others, contribute to meeting the methionine needs of growing and egg producing birds without negatively affecting flavor of eggs and meat, palatability of complete feed, and overall health and well-being of birds?

High methionine corn: High methionine corn has been used in feed without the need for synthetic methionine. One variety, 3-floury-2-MF hybrid, contained 0.32% methionine on average compared with 0.15% in regular corn. However, this corn also is higher in protein, and as explained previously, high protein diets are not physiologically healthy for birds and lead to excess excretion of uric acid, which is broken down into water and ammonia in the environment. This corn is also high-moisture and results in lower yields, so it is also less desirable to corn growers (Fanatico, 2010).

Fish and crab meal: Fish and crab meal are good sources of methionine; however, the supply is limited. Fish meal fed to organic livestock cannot contain ethoxyquin, an antioxidant with preservative properties. Producers add natural preservative ingredients, but the meal will usually spoil more rapidly than nonorganic fish meal. Furthermore, high levels of fish meal can reduce palatability to the chickens and to the consumers eating the meat (Rack et al., 2009).

Alfalfa concentrate: Dehydrated alfalfa meal (17%) contains approximately 0.18% methionine. This is a relatively low percentage of methionine (Fanatico, 2010). According to producers, Vitalfa (an alfalfa nutrient concentrate) contains 1.14% methionine, 0.47% cysteine, 3.38% lysine, and 52% crude protein without added synthetic methionine. The company has been trying to get these alfalfa pellets certified organic for a number of years, but they have not yet been successful (Brewster, 2011). The protein level of this feed is quite high compared with diets supplemented with synthetic methionine, so the concerns related to increased nitrogen excretion and the physiological health of the birds remain.

In a study in which Sprague-Dawley rats were administered diets of 10 or 20% commercial alfalfa protein concentrate (APC; 38% protein), growth was poor when APC was the only protein supplement. However, when supplemented with soybean or herring meal and lysine and methionine, maximum growth weights were achieved. This study indicates that protein, methionine, and lysine levels are not sufficient when alfalfa concentrate is used alone (Myer and Cheeke; 1975). It is important to note that this alfalfa concentrate differed slightly in formulation from the Vitalfa brand; while it appears Vitalfa contains only extracted alfalfa, the supplement in the Myer and Cheeke study contained alfalfa in addition to ryegrass straw, casein, and lard.

Corn gluten meal: Corn gluten meal is not available in organic form in the United States. Unlike traditional corn meal, it is high in methionine (approximately 1.46% methionine) (Fanatico, 2010). Corn gluten meal can be contaminated with alflatoxin (a naturally-occurring, carcinogenic fungal toxin) from residue in the raw material and mold when it is stored (Swick, 1999).

Canola meal: Canola meal has less methionine than soybean meal, which is currently used in most poultry feeds; therefore, it has no advantages over conventional feed ingredients (Fanatico, 2010).

Sunflower meal: Sunflower meal has a methionine content on par with soybean meal (~0.6%). Like sesame and safflower, supply is inadequate in the United States (Hungerford, 2007). Research indicates that sunflower meal can have negative impacts on chickens due to difficulty with digestibility, and Swick (1999) indicated that higher levels of methionine and choline are required (compared to soybean meal) to counteract the effect of chlorogenic acid in the sunflower meal, a tannin-like compound that inhibits digestive enzymes. Sunflower meal may perform better when in a processed, pelleted form (Senkoylu and

Dale, 2006). High-oil sunflower meal (containing about 1.25% methionine and 0.68% cysteine) has a higher protein level than regular sunflower meal. Research indicates that this dehulled, pelleted oil meal can be used in up to 28% of the diet without adversely impacting broiler chicken performance. Nonetheless, available pelleted diets formulated with high-oil sunflower meal still contained added methionine in the form of synthetic DL-methionine (Senkoylu and Dale, 2006).

Quinoa: Quinoa is a grain similar to the protein profile for casein. It contains 12.2% crude protein, of which 6.7% is lysine and 2.9% is methionine. However, quinoa contains antinutrients such as saponins, phytic acid, tannins, and trypsin inhibitors. These substances can negatively impact performance and survival of animals when it is used as the primary energy source in feed (University of Kentucky, 2011).

Casein: Casein has about 2.56% methionine, but it is also very high in crude protein (80%). Unfortunately, organic casein is not commercially available for use as poultry feed (Fanatico, 2010).

Meal worms: Like insect meal, meal worms are high in protein. No specific information regarding the successful use of meal worms for protein supplementation in poultry diets could be obtained; however, given that worms such as earthworms are palatable to poultry, it is likely meal worms would also be palatable. It is unclear whether or not meal worms alone would provide a sufficient amount of methionine without exceedingly high protein levels.

Insect Meal: Earthworm and insect meal are high in protein and methionine. There is only limited commercially available insect meal. However, at least one company (Neptune Industries) has an insect meal available for use in livestock feed. It should be noted that worms may contain heavy metals at higher concentrations than in the environment, which could transfer to the meat and eggs of chickens that consume them (Fanatico, 2010). At least one study concluded that insect meal containing acridids (grasshopper, crickets, and similar insects) was a viable protein supplement in livestock feed with 60-66% protein content and appropriate balances of calories, fat, and carbohydrates. However, these authors did not measure or discuss amino acid balance (Anand et al., 2008).

Sesame meal: Sesame meal is high in methionine (1.06%). However, it is also low in lysine and is not well digested (Fanatico, 2010). In addition, sesame meal is high in antinutritive substances such as oxalic and phytic acid, which can interrupt mineral metabolism and reduce the availability of calcium, phosphorus, magnesium, zinc, and iron from the diet. Oxalic acid also causes palatability issues (Swick, 1999).

Pearl millet: Pearl millet is one of several types of millet crops. Compared with corn, the protein content is typically higher and the amino acid content is usually more balanced although these will vary depending upon environmental conditions during cultivation. Between 1999 and 2001, the methionine content of pearl millet ranged from 0.26 to 0.37% (Davis et al., 2003). It is difficult to grind and thus must be rolled, which requires specific equipment. It is also susceptible to rust disease, which has limited its production. Although pearl millet has successfully been substituted for the conventional corn-soybean diet in broiler chickens, feed formulations still contained added synthetic methionine (Davis et al., 2003).

Potato Protein: Potato protein is not available in organic form in the United States. In Europe, however, this high-methionine (1.64%) feed is often used because a small percentage of feed ingredients do not need to be organic (Fanatico, 2010).

Meat and bone meal: As animal byproducts, meat and bone meals are not currently allowed in organic feed. Crude protein levels in the meal range from 47.7–51%, and the average levels of methionine and cysteine in the crude protein are 1.4% and 0.7%, respectively. The bone in the meal provides a good source of calcium and phosphorus. FAO (undated) reported conflicting information related to the food efficiency of meat and bone meal compared with other meal types. Some sources indicated that using meat and bone meal instead of fish meal results in a lower food efficiency, while others found no differences in food efficiency between chickens fed with up to 10% meat and bone meal and chickens fed soybean meal (FAO, undated).

<u>Additional Question #3</u>: Methionine is an essential amino acid and is listed in the NRC manual. Would it be considered a growth promoter or production enhancer at any time? If so, at what levels? For various types of birds (layers, broilers) and for the most widely used commercial breeds, what is the level of supplemental methionine recommended by NRC (expressed either as an absolute maximum or range of values based on stage of life)?

The levels of supplemental methionine recommended by NRC for egg-laying and broiler chickens are presented in Tables 2 and 3, respectively. Some manufacturers market their methionine product as a "performance enhancer" for optimum growth and higher egg production. One study indicated that methionine supplements can increase egg production (number of eggs) and egg mass (CJBIO, 2010). In another study, doses of 413 mg/hen/day of methionine supplement resulted in increased albumen total solids and protein and yolk crude protein (CP), which resulted in an increased amount of liquid per egg and thus increased productivity at liquid egg processing plants (Schafer et al., 1998).

Recent work suggests that the NRC recommendations for methionine supplementation may not be adequate in poultry that are challenged by vaccines or other stresses. Maroufyan et al. (2010) studied the effect of methionine and threonine supplementations on the immune responses of broiler chickens given a vaccine for live-infectious bursal disease (IBD), a common disease in poultry. Results indicated that threonine and methionine requirements based on NRC recommendations were not sufficient to meet the requirements in commercial poultry operations that are not operating with healthy birds under "ideal conditions." Authors indicated that methionine is more critical for optimum immunity than it is for growth or feed conversion. In addition, Moritz et al. (2005) stated that broiler chickens may require methionine at levels higher than those recommended by NRC during times of moderate heat stress (≥80°F), and organic broilers tend to be subjected to more variable temperatures than conventional broilers. Furthermore, methionine requirements may be affected by additional substances added to feed, including copper (copper sulfate is a feed additive to supply copper), and monensin sodium (used to control parasites). These data indicate that methionine supplementation is vital beyond "growth enhancement" purposes (Moritz, 2005).

Table 2. Methionine Requirements of Egg-Laying Chickens as Percentages of Diet[a]

Amino Acid	Percent of Diet for Leghorn-Type White Egg-Laying Strains by Age					Percent of Diet for Leghorn-Type Brown Egg-Laying Strains by Age				
	0-6 wks	6-12 wks	12-18 wks	18 wks-first egg	Mature egg-layers (100 g food/hen)	0-6 wks	6-12 wks	12-18 wks	18 wks-first egg	Mature egg-layers (100 g food/hen)
Methionine	0.30	0.25	0.20	0.22	0.30	0.28	0.23	0.19	0.21	ND
Methionine + cystine	0.62	0.52	0.42	0.47	0.58	0.59	0.49	0.39	0.44	ND

[a]Source: NRC (1994)
ND = no data

Table 3. Methionine Requirements of Broiler Chickens as Percentages of Diet[a]

Amino Acid	Percent of Diet for Broiler Chickens by Age[b]		
	Starter (0-3 wks)	Grower (3-6 wks)	Finisher (6-8 wks)
Methionine	0.50	0.38	0.32
Methionine + cystine	0.90	0.72	0.60

[a]Source: NRC (1994)
[b]Broiler chickens are those used for meat production, most commonly Cornish cross chickens in the United States.

Additional Question #4: What contribution to methionine needs could be expected from forage plants in the outdoor living space? Would there still be a need for supplemental methionine in a pasture situation? And if so, at what levels?

Forage provides low to moderate levels of methionine. Foraging allows birds to obtain high-quality protein from insects and worms (Fanatico, 2010). However, foraging conditions change with season, affecting a pasture's ability to supplement the diet. During certain times of the year, it is difficult for methionine needs to be met from forage alone. There is some debate about the amount of pasture considered adequate for chickens at various life stages. Rack et al. (2007) recommended that 27 ft^2 per bird is adequate to supply the required amount of methionine via forage; however, it is unclear how this number was calculated. This much space may be economically impractical for many commercial poultry operations (Rack et al., 2009). Recently, the NOSB Livestock Committee proposed a minimum space of 2.0 ft^2 per bird of indoor space and 5.0 ft^2 per bird for outdoor runs/pens for laying hens and breeders and 1.0 ft^2 of indoor space and an additional 1.0 ft^2 of outdoor space for pullets and broilers (NOSB, 2011). The Government of Manitoba recommends similar space requirements; they suggest 1.0 ft^2 of indoor space for birds up to 6 weeks of age, 1.5 ft^2 per bird for small broilers (4 lb live weight), and 2 ft^2 for roasters (6 lb live weight) (MAFRI, undated). Similarly, the nonprofit organization, American Pastured Poultry Producers Association (APPPA), recommends that each bird have at least 1.5 ft^2 of space in a pen as it matures (Padgham, 2005). However, these organizations likely created pasture access recommendations assuming chickens are supplied with adequate methionine in the diet (via methionine supplementation or feed formulation), rather than relying on the pasture area to supply adequate methionine.

In one study, pasture access did not affect the growth of slow-growing breeds of broilers, but decreased growth in fast-growing broilers. Animals were given 1.5 ft^2 of space/bird indoors, and had access to a 21 × 25-foot fenced pasture. The chickens not given synthetic methionine supplements (both with and without pasture access) did not experience increased illness or death, and authors concluded that synthetic methionine was not required for rearing organic broiler chickens to achieve market weight (Rack et al., 2009).

In another study, Moritz et al. (2005) concluded that the ability of forage to meet methionine requirements in 3–6-week-old broilers depended on a combination of environmental conditions and feed intake. Measured methionine levels were 0.36% in diets not supplemented by synthetic methionine (containing mostly corn and soybean meal) and 0.40% in diets with synthetic methionine added; cysteine content was about 0.35% for both feeds. Authors found that if adequate forage was provided (in this study, space was 1.5ft^2/bird inside the house, with access to 6.1× 9.1 m^2 paddock with tall fescue, orchard grasses, and clover), organic broilers provided diets without synthetic methionine could largely overcome growth deficiencies by increasing feed intake. Authors reported that in the summer, forage accounted for approximately 0.33%of dietary methionine (short of the NRC guideline of 0.36%) and 0.23% cysteine in diet, while fall forage accounted for about 0.15% methionine and 0.20% cysteine. Authors suggested that additional methionine from insects and earthworms added to intake during foraging (levels from insects not estimated) (Moritz et al., 2005).

Additional Question #5: Would heritage breeds differ from current hybrid production hens in methionine requirements? To what extent could methionine demand be reduced through genetic selection? If feasible, what would be the timeline to accomplish this?

According to Fanatico (2010), several studies have shown that slower-growing meat chickens (often heritage breeds) have the same general methionine requirements as faster-growing breeds (e.g., Cornish cross-hens commonly found in the United States) when they are young animals and during the grower phase. Another study found that methionine requirements were the same for slow- and fast-growing birds during the grower life stage, but cystine requirements were lower for the slow-growing breeds (Kalinowski et al., 2003). Rack et al. (2009) indicated that different breeds might have different methionine needs due to differences in the rate of feathering or in the rate of conversion of methionine to cystine, but evidence to support this is not clear.

There are some data indicating that genetic selection of poultry species may improve production. Although authors did not say that methionine demand would be reduced, it is possible that the requirements for all amino acids may be reduced in animals bred for high feed efficiency and high body weight. According to authors, "Selection for growth rate improves utilization of energy and amino acids in growing animals" (European Commission, 2000). However, genetic selection can cause birds with higher fat content, which can result in an increased susceptibility to infectious disease, cardiac problems, and leg (tibial dyschondroplasia) problems. These problems result in weekly mortality rates of 4 or 7 times the mortality in slower-growing heritage breeds or normal young female chickens (pullets), respectively (European Commission, 2000).

The NRC (1975) reported that there have been limited studies on the methionine requirements of different strains bred using genetic selection; however, they stated that the issue of the ability to alter the methionine requirement of chickens through selection was "unresolved." NRC noted, however, that such a selection program would not be productive for egg-laying hens due to the time and resources that would be required (NRC, 1975).

According to Hoste (2007, cited in Chadd, 2007), due to the high cost of poultry nutrition, the optimization of feed utilization by birds will remain a priority to geneticists working on genetic selection in poultry species. Although there seems to be interest in further developing genetic selection in poultry, no other current sources of information were identified.

Additional Question #6: Would methionine requirements be satisfied by a wide range or mix of small grains in the ration?

There are no published data indicating that methionine requirements can be met with an all-grain diet without supplementation or allowed foraging. Most grains (e.g., rye, barley, oats, corn, buckwheat, rice grain, soft winter wheat, bran, rice millet) contain about 0.15–0.2% methionine compared with 0.42% for crab meal, 0.6% for expelled soybean meal, 1.48% for potato protein, and 1.6% for fishmeal (Fanatico, 2010); and thus, grain diets do not contain adequate methionine. An Australian company (Avi Grain, 2011) produces an all-grain (wide range of whole and cracked grains), supplement-free poultry feed suitable for free-range chickens. Chickens fed this diet may be able to meet methionine requirements through the combination of feed and insects foraged by the chickens. However, the supplier did not provide methionine levels in the feed on its website.

Other alternative diets formulations (containing mixtures of different proteins and grains) may be able to replace the use of synthetic methionine in traditional corn and soybean feed mixes. Fanatico (2010) calculated methionine content in traditional, grain-based feeds (with added DL-methionine) and several alternatives in which no synthetic methionine was added. Measurements indicated that alternate diets meeting methionine requirements contained levels of protein that could be physiologically harmful to the chicken (protein increased from 21 to 38% over traditional feed). Diets included combinations of fishmeal, cornmeal, potato protein, earthworm meal, and sesame meal. Nutritionists who reviewed these data did not feel protein increases of more than 5% were sustainable. The author indicated that the primary constraint of formulating a sufficient diet with alternative ingredients was the lack of or limited availability of appropriate alternatives that met organic ingredient standards (Fanatico, 2010). Some have advocated that the balance of feed be shifted to meet methionine requirements. Currently, feed for chickens is about 90% corn and 10% soy; if it were changed to 70% corn and 30% soybean, methionine requirements could be met without synthetic sources. However, this would require increased feed (due to food conversion inefficiencies) and longer growth time, and would create more manure (Hungerford, 2007).

Additional Question #7: Provide the balance of amino acids recommendations, which are essential, from NRC for Cornish cross broilers at weekly intervals, layers at 1 week, 10 weeks, 20 weeks, and 40 weeks, and broad breasted and heritage turkeys at 1 week, 10 weeks, and 25 weeks.

Tables 4 and 5 provide the NRC recommendations of amino acid balance for chickens, and Table 6 provides recommendations for turkeys.

Table 4. Amino Acid Requirements of Male Broiler Chickens as Percentages of Diet[a]

Amino Acid	Percent of Diet for Male Broiler Chickens by Age[b]		
	0–4 wks	4–20 wks	20–60 wks
Protein	15.00	12.00	12
Arginine	***-***	***-***	***680***
Lysine	***0.79***	***0.64***	***475***
Methionine	***0.36***	***0.31***	***340***
Methionine + cystine	***0.61***	***0.49***	***490***

[a]Adapted from NRC (1994)
[b]Where experimental data are lacking, values typeset in bold italics represent an estimate based on values obtained for other ages or related species.

Table 5. Amino Acid Requirements of Female Broiler Chickens as Percentages of Diet[a]

Amino Acid	Percent of Diet for Female Broiler Chickens by Age[b,c]		
	0–3 wks	3–6 wks	6–8 wks
Crude protein	23.00	20.00	18.00
Arginine	1.25	1.10	1.00
Glycine + serine	1.25	***1.14***	***0.97***
Histidine	0.35	***0.32***	***0.27***
Isoleucine	0.80	***0.73***	***0.62***
Leucine	1.20	***1.09***	***0.93***
Lysine	1.10	1.00	0.85
Methionine	0.50	0.38	0.32
Methionine + cystine	0.90	0.72	0.60
Phenylalanine	0.72	***0.65***	***0.56***
Phenylalanine + tyrosine	1.34	***1.22***	***1.04***
Proline	0.60	***0.55***	***0.46***
Threonine	0.80	0.74	0.68
Tryptophan	0.20	0.18	0.16
Valine	0.90	***0.82***	0.70

[a]Adapted from NRC (1994)
[b]Where experimental data are lacking, values typeset in bold italics represent an estimate based on values obtained for other ages or related species.
[c]No data presented for ages over 8 wks.

Table 6. Amino Acid Requirements of Turkeys as Percentages of Diet[a]

Amino Acid	Percent of Diet for Turkeys by Age							
	0-4 wks (M and F)	4-8 wks (M and F)	8-12 wks (M); 8-11 wks (F)	12-16 wks (M); 11-14 wks (F)	16-20 wks (M); 14-17 wks (F)	20-24 wks (M); 14-20 wks (F)	Holding Breeder	Laying Hens
Arginine	1.6	1.4	1.1	0.9	0.75	0.6	0.5	0.6
Glycine + serine	1.0	0.9	0.8	0.7	0.6	0.5	0.4	0.5
Hitidine	0.58	0.5	0.4	0.3	0.25	0.2	0.2	0.3
Isoleucine	1.1	1.0	0.8	0.6	0.5	0.45	0.4	0.5
Leucine	1.9	1.75	1.5	1.25	1.0	0.8	0.5	0.5
Lysine	1.6	1.5	1.3	1.0	0.8	0.65	0.5	0.6
Methionine	0.55	0.45	0.4	0.35	0.25	0.25	0.2	0.2
Methionine + cystine	1.05	0.95	0.8	0.65	0.55	0.45	0.4	0.4
Phenylalanine	1.0	0.9	0.8	0.7	0.6	0.5	0.4	0.55
Phenylalanine + tyrosine	1.8	1.6	1.2	1.0	0.9	0.9	0.8	1.0
Threonine	1.0	0.95	0.8	0.75	0.6	0.5	0.4	0.45
Tryptophan	0.26	0.24	0.2	0.18	0.15	0.13	0.1	0.13
Valine	1.2	1.1	0.9	0.8	0.7	0.6	0.5	0.58

[a]Adapted from NRC (1994)

<u>Additional Question #8</u>: Provide the balance of amino acids in vegetarian diets for poultry, with and without synthetic methionine, and with fish and/or crab meal.

Amino acids must be in an ideal balance to one another because chickens are unable to synthesize some amino acids in their bodies. Lysine is used as the reference amino acid (Fanatico, 2010); it is chosen as a reference amino acid because its only major function in the animal body is for protein tissue deposition; therefore, its requirement is not influenced by other metabolic functions (Miles and Chapman, undated). Furthermore, lysine is considered one of the limiting amino acids for poultry (Toride, undated).

The amount of total sulfur amino acids (methionine and cysteine; the ratio should be 60:40, respectively), rather than methionine amounts alone, need to be considered for adequate diets. If an animal is deficient in cysteine, some of the methionine in the body will be used as compensation (Fanatico, 2010). In vegetarian diets, other protein must be provided to compensate for the lack of animal protein. Commercial broiler feed (predominantly corn and soybean meal) with synthetic methionine added contain 0.49% methionine, 21% protein, and 0% excess lysine. In broiler diets identical to conventional feeds, but with synthetic methionine supplement removed, available methionine was 0.45%, protein was 28% of the diet, and excess available lysine was 100%. When fishmeal was added to this diet, available methionine was 0.49%, but protein was 39% of the diet and excess available lysine was 108%. The lysine levels in the no-added-methionine and fishmeal diets were considered “unacceptable,” and nutritionists indicated that the protein levels were too high (Fanatico, 2010).

<u>Additional Question #9</u>: Provide an explanation of bird feed metabolism when the balance of amino acids deviates from recommendations.

If a bird’s diet is deficient in essential amino acids, feed intake will often increase in an effort to obtain the required amino acids. Increased feed intake compensates for marginal deficiencies in amino acids (particularly the sulfur amino acids), preventing significant effects on body weight, sexual maturity, egg production and size, or other ill effects associated with amino acid deficiency (Fanatico, 2010). Increased consumption of less nutritious feed reduces feed efficiency (the calories ingested via food divided by weight gain, or in other words, how much the chicken is fed compared to its weight gain) and protein conversion efficiency (the amount of animal protein yielded per unit of protein in feed) in the body. Nutrient balance can also impact the chicken's body composition. Diets high in energy produce fat carcasses while lean diets high in protein lead to lean carcasses. If a bird's diet is deficient in protein, the bird will overeat to get enough protein (Fanatico, 2010).

Diets deficient in methionine can lead to specific problems in chickens. In egg-laying hens, Elwinger and Tausen (2009, in Fanatico, 2010) found that insufficient methionine in the diet decreased feather cover and egg weight, but did not affect the production of eggs. The authors also reported that food intake increased as feather cover decreased, which indicates decreased food efficiency.

Studies also indicate that feeding chickens excesses of lysine, methionine, or phenylalanine influences glucose metabolism. Chickens fed excess levels of these amino acids had higher blood glucose levels after being given excess amino acids than chickens not supplemented with amino acid. The blood levels also took longer to return to normal than in the chickens that were not supplemented (Anderson and Combs, 1951).

Additional Question #10: The Committee has noted that excess protein fed to the birds, in an effort to meet the methionine requirement, could contribute to the amount of ammonia produced in the house. The Committee is concerned that this could affect animal welfare, since high ammonia levels result in damage and discomfort to the eyes and lungs of birds and people during time periods when houses may be closed up (e.g. cold weather, winter season, etc.). The Committee would like the report to include any technical information which may address the excess feeding of protein to poultry to meet methionine requirements, which could include information on recommended ammonia level standards for poultry housing and ventilation options to manage.

In addition to being physiologically unhealthy for birds, high protein diets lead to high levels of ammonia in poultry houses. This occurs because excess protein results in excess nitrogen in the chicken's body, which is eliminated in the feces in the form of uric acid. Uric acid is then broken down into water and ammonia. In addition to increased ammonia levels, high protein diets create wetter feces and thus litter that can harbor more bacterial and fungal pathogens (Fanatico, 2007).

Ammonia levels should not exceed 25 parts per million (ppm) in the poultry housing. Testing should be done regularly at the level of the birds; ammonia testing strips are an economical way to test (Fanatico, 2007). Natural ventilation is usually sufficient in the warmer months by opening windows and/or using roof vents or "whirly bird" vents to allow rising warm air to escape. Because pens are usually closed up entirely in the winter, mechanical ventilation may be needed. Mechanical ventilation involves positive and negative pressure systems; fans direct air into the house (positive) or exhaust air from the house (negative). The cold, dry air that is brought into the house will warm to room temperature and, when it is exhausted out, will take out some of the excess moisture, ammonia, and carbon dioxide that builds up in the house. However, mechanical ventilation is usually not suitable for free-range chicken houses due to the "static pressure" they must maintain. When houses have low static pressure (usually because they are not well sealed), incoming air moves too slowly, causing cold, heavy incoming air to drop to the floor, causing water vapor to pool in the litter (Fanatico, 2007). One option for pasture-raised birds is using movable pens, which have floorless bottoms for grazing. They are moved daily (or more infrequently) so the chickens have new grazing land and there is no manure buildup. These pens can be insulated in the winter with aluminized bubble insulation. Floorless houses are cooler in the summer and warmer in the winter compared with houses that have floors (Plamonden, 2003).

Additional Question #11: How pure is synthetic methionine and what might be in the product if it is not 100% pure?

Several Material Safety Data Sheets (MSDS) specify that the purities of their products are between 98 and 100% (Fischer Scientific, 2007; Acros, 2009; Sciencelab, 2010), but none provide details on the other ingredients in their products. Another source indicated that both liquid and dry methionine feed supplements usually contain water (12% and 1%, respectively) (Goodson and Payne, 2007). No other information on the purity of synthetic methionine products could be found.

Additional Question #12: How many manufacturers of methionine are there? What by-products are a result of methionine manufacture and how are the by-products disposed?

There are four major producers of synthetic methionine—Evonik Degussa (Germany), Novus International (United States), Adisseo (France), and Sumitomo (Japan)—along with a number of minor producers in China (SRI Consulting, 2009). Evonik Degussa was fined by the European Commission in 2002 for running a price-fixing cartel for methionine (along with Nippon Soda Company, Ltd. of Japan and Aventis SA of France) for almost 13 years (European Commission, 2008). According to a study by Liske et al. (2000), millions of tons of ammonium sulfate ($(NH_4)_2SO_4$) and several thousand tons of ammonium bisulfate (NH_4HSO_4) are produced in the manufacture of various feed additives, including methionine. Some ammonium sulfate is repurposed for fertilizer. Various thermal and combustion-based processes may be used to convert byproducts like ammonium bisulfate to sulfur dioxide or hydrogen sulfide for recovery (Liske et al., 2000). It is unclear, however, if companies such as Evonik Degussa use these processes to break down their waste byproducts. Reports from the EPA Biennial Reporting System show that sulfuric acid was also reported as a hazardous waste product of methionine production by Degussa (RTKnet, 1999). This waste may have been shipped elsewhere for treatment or disposal, as required by law (U.S. EPA, 2011b).

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